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On the early onset of vascular stiffening and sexual dimorphism of sympathetic control in the spontaneously hypertensive rat

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Kinesiology

On the early onset of vascular stiffening and sexual dimorphism of sympathetic control in the spontaneously hypertensive rat

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Abstract and Keywords

The purpose of this thesis was to explore the role of the sympathetic nervous system (SNS) in hindlimb vasomotor control during the development of hypertension (HT). Using an animal model of essential HT (the spontaneously hypertensive rat [SHR]) we demonstrated that neuropeptide Y (NPY) and the Y_1 receptor (Y_1R) play a greater role in modulating hindlimb hemodynamics in the early stages of HT compared to normotensive controls (Wistar Kyoto [WKY]). Hindlimb vascular mechanics (compliance [C] and viscoelasticity [*K*]) were assessed using a modified Windkessel model developed in our laboratory. The hindlimb mechanics did not appear to be regulated by NPY or the Y_1R specifically, but the SNS did appear to regulate the hindlimb mechanics in both SHRs and WKY animals. The use of female animals in physiologic research is limited, thus the role of the SNS in developing HT in females is unknown. Finally, differences in the hemodynamic and hindlimb vascular mechanics between male and female animals were examined. Female animals exhibited augmented MAP and HR relative to males in conjunction with greater stiffness and viscoelasticity in both SHR and WKY animals. Female SHRs also appeared to lose SNS control over the stiffness and viscoelastic properties of the hindlimb vascular bed, while male SHRs maintained this control. These sexually dimorphic characteristics provide evidence for a novel proposed mechanism of cardiovascular regulation in young female rats.

Keywords: Compliance, Conductance, Hexamethonium Bromide, Modified Windkessel Model, Pulse Wave Velocity, Resistance, Sexual Dimorphism, Spontaneously Hypertensive Rats, Viscoelasticity, Wistar-Kyoto Rats

Epigraph

‘I love fools’ experiments. I am always making them.’

Charles Darwin
(1809 – 1882)

‘It is not the mountain we conquer but ourselves.’

Sir Edmund Hillary
(1919 – 2008)

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List of Abbreviations

AR (α- or β-)	–	Adrenergic receptor
μ	–	Viscosity (Pa·s or kg/(s·m))
AC	–	Adenylyl cyclase
AFB	–	Advential fibroblasts
AOC	–	Area over the curve
APP	–	Aminopeptidase P
ATP	–	Adenosine triphosphate
BIBP3226	–	N^2-(Diphenylacetyl)-N-[(4-hydroxyphenyl) methyl]-D-arginine amide
C	–	Compliance (mL/min/mmHg)
cAMP	–	Cyclic-adenosine monophosphate
$Cond_{Caud}$	–	Caudal conductance (μL/min/mmHg)
Cond	–	Hindlimb conductance (μL/min/mmHg)
C_{Local}	–	Local aortic compliance (mL/mmHg)
CoV	–	Coefficient of variation
DAG	–	Diacyl glycerol
DPPIV	–	Dipeptidyl Peptidase IV
EC	–	Endothelial cells
EJP	–	Excitatory junctional potential
ER (α- or β-)	–	Estrogen receptor
ERK 1/2	–	Extracellular signal-regulated kinase 1 and 2
GPR30	–	Estrogen GPCR
G-Protein	–	Guanine nucleotide binding protein
GPCR	–	G-Protein coupled receptor
GRK	–	GPCR kinase
GTP	–	Guanosine triphosphate
h	–	wall thickness
Hex	–	Hexamethonium bromide
HR	–	Heart rate (beats/min)

HT	–	Hypertension
IP_3	–	Inositol 1,4,5-triphosphate
JNK	–	c-Jun N-terminal kinase
K	–	Viscoelasticity (mmHg/mL/min)
L	–	In Poiseuille equation = length (usually in cm)
L	–	In *RCKL* model = inertial effects of blood and vessel tissue ($mmHg/mL/min^2$)
MAP	–	Mean arterial pressure (mmHg)
MLC_{20}	–	Myosin light chain 20
MLCK	–	Myosin light chain kinase
MLCP	–	Myosin light chain phosphatase
NIBP	–	Non-invasive blood pressure
NE	–	Norepinephrine
NO	–	Nitric oxide
(e)NOS	–	(endothelial) Nitric oxide synthase
NPY	–	Neuropeptide Y
OVX	–	Ovariectomy
OVX+E2	–	Ovariectomy with estrogen replacement
P	–	Pressure (usually in mmHg)
P2X	–	ATP gated ion channels
P2Y	–	ATP GPCR
PE	–	Phenylephrine
PI3	–	Phosphoinositide 3-kinase
PIP2	–	Phosphatydlinositol 4,5-bisphosphate
PKA	–	Protein kinase A (cAMP dependent protein kinase)
PKC	–	Protein kinase C
PLC	–	Phospholipase C
PWV	–	Pulse wave velocity
Q	–	Volume flow (mL/min)
Q_{Aorta}	–	Aortic blood flow (mL/min)
Q_{Fem}	–	Femoral blood flow (mL/min)

R	–	Vascular resistance (mmHg/mL/min)
$r_{(i)}$	–	Radius (internal (usually in cm))
SD	–	Sprague dawley rat
SERCA	–	Sarco/endoplasmic reticulum Ca^{2+} ATP-ase
SHR	–	Spontaneously hypertensive rat
SNP	–	Single nucleotide polymorphism
SNS	–	Sympathetic nervous system
T	–	Wall tension
TH	–	Tyrosine Hydroxylase
VSMC	–	Vascular smooth muscle cell
WKY	–	Wistar-Kyoto rat
Y_1R	–	NPY Y_1 receptor
Z	–	Impedance

Chapter 1 – Review of the Literature

1.1 Introduction

The mechanisms governing the distribution of blood flow throughout the body are numerous and complex. The methods utilized for blood flow distribution include short-term (e.g., beat-to-beat) fluctuations in heart rate and cardiac contractility, to more continuous changes in the diameter of blood vessels and, in some cases, morphologic changes in the mechanics and structure of vascular tissue. In most cases the sympathetic nervous system (SNS) is essential in regulating these processes.

Hypertension (HT) is a chronic medical condition that is pervasive in many industrialized nations (145). The complex etiology of the condition makes it difficult to manage with medications, and chronic HT is an independent risk factor for many cardiovascular diseases including myocardial infarction, atherosclerosis, and stroke (13; 58; 136; 156). Hypertension has also been implicated in other pathological conditions such as metabolic syndrome (11; 79). The physiologic mechanisms contributing to HT are numerous, and an important role for the SNS is clear; however, the reasons why the SNS, and other systems, propagate HT remain unclear.

This thesis focuses on the integration of the nervous and cardiovascular systems and how the two are regulated in normotensive and hypertensive rats. It is important to note that most of the comparisons made here have been done in very young animals (7 weeks of age). This is a critical point to emphasize, as it exposes differences in neurovascular coupling *as* the HT is developing (91; 132). Much of the work describing alterations in HT has been done in *established* HT; when the blood pressure has been elevated for a time. Examining differences in neurovascular regulation *as* HT develops may expose novel mechanisms which act to augment the blood pressure. Emphasis will

be placed on the mechanical properties of the vasculature in normotension and developing HT, and sex differences in neurovascular regulation of both normotensive and hypertensive animals will be examined.

1.2 Outline of Thesis

This thesis outlines our attempts to understand the integration of the sympathetic nervous system (SNS) and cardiovascular systems in the early stages of hypertension (HT). The experiments outlined were designed to examine **the working hypothesis** that the sympathetically-mediated mechanical and biochemical properties of the vasculature would be altered very early in the development of HT, with specific changes in neuropeptide Y (NPY) signaling through the Y_1 receptor (Y_1R). It has been demonstrated that although female rats possess NPY and the Y_1R, this pathway is not utilized in the control of hindlimb hemodynamics such as it is in male animals (74; 75). Therefore, it was hypothesized further that alterations in sympathetic control in female hypertensive rats would be different than those observed in males. To address these hypotheses, the integration of the SNS and cardiovascular systems was examined by combining physiological data in the form of systemic and regional hemodynamics, molecular markers of sympathetic signaling, and mathematical modeling of the mechanical properties of the hindlimb. This section outlines the rationale for each of the subsequent chapters, and, hopefully, bridges them creating a more cohesive document.

Chapter 2 entitled "Enhanced regulation of hindlimb vascular control by neuropeptide Y during the development of hypertension" was designed to assess the role of the SNS in the hemodynamic responses of exogenous adrenergic receptor (α-AR) and Y_1R agonists in 3-7 week old *male* SHR animals and WKY controls. It was

hypothesized that altered neurovascular regulation would occur early in the development of HT with changes that include modifications to the Y_1R and/or α-AR regulation. If so, then any differences observed in SHR animals in the response to exogenous sympathetic agonists would reflect alterations to the cellular signaling which might contribute to the development of HT.

Chapter 3 entitled "Y_1 receptor versus sympathetic control of hindlimb vascular stiffness in spontaneously hypertensive rats" utilized a modeling approach to quantify the mechanical properties of the vasculature in both normotension and hypertension. We employed this technique to supplement data from chapter 2 in order to examine the role of the Y_1R in particular, and the SNS in general, in mediating vascular stiffness in HT. We tested the **specific hypothesis** that Y_1R activation, and the **general hypothesis** that sympathetic activation, would contribute to vascular stiffness in the developmental stages of HT. Should the SNS regulate the stiffness of the vasculature, increased stiffness would augment blood pressure and exacerbate HT. Increased vascular stiffness is a hallmark of *established* HT (81); however, whether the stiffness contributes to, or is a consequence of, HT is debated (65). Examining the mechanical properties of the vasculature during the early stages of HT should provide a more complete understanding of the role of vascular mechanics in augmenting blood pressure.

Sex-based differences exist in sympathetic cardiovascular regulation (74-76) and the prevalence and severity of HT (5; 67). Chapter 4, entitled "Sympathetic contribution to peripheral vascular stiffness in young female spontaneously hypertensive rats", was designed to examine the role of the SNS in regulating the mechanical properties of the hindlimb in female animals. Detailed examinations of specific agonist/antagonist control

were not performed as previous research indicates females do not utilize the NPY system in vascular regulation (74; 75), and chapter 3 suggests that the regulation of the mechanical properties are not under Y_1R control but, rather, are related to sympathetic neural activity *per se*. Therefore, we tested the **hypothesis** that vascular stiffness and viscoelasticity are under neurogenic control in females as they appear to be in males. But, because females appear to rely on different mechanisms of cardiovascular control, differences in HT would be manifest in the mechanical properties of the vasculature.

Finally, chapter 5 "Evidence for sexual dimorphism in sympathetic control of vascular function" explores the sex-associated differences observed in hemodynamic and hindlimb vascular mechanics in both normotensive and hypertensive rats. These differences were evident during data analysis and warrant some discussion. This chapter also hypothesizes a mechanism as to why these differences may exist.

The dissertation concludes in chapter 6 with a general discussion about the implications for the body of work as a whole. The role of NPY and the SNS in cardiovascular regulation are highlighted, as are the differences in cardiovascular control between male and female animals. Limitations of the research are addressed, and areas where this line of research can be continued are proposed.

This dissertation embodies a series of studies which examine the mechanical and/or biochemical alterations that occur in the early stages of HT. The role of the SNS in general, and NPY in particular, are investigated as is the role of sex in moderating differences observed.

1.3 Mechanisms of Sympathetic Control

The SNS relies on three chemical transmitters to communicate with peripheral tissues. Adenosine triphosphate (ATP), norepinephrine (NE) and neuropeptide Y (NPY) are synthesized, stored and released from neurons of the SNS and act via specific receptors to elicit discrete responses. Of the sympathetic neurotransmitters, NE has received the most consideration, but a role for NPY in baseline vasomotor control has emerged (74-76). ATP, NE and NPY exert both acute and chronic effects on the vasculature. Acutely, all three transmitters elicit vasoconstriction of vascular smooth muscle cells (VSMCs), which acts to increase vascular resistance (18; 103; 179). Chronically, these molecules are mitogenic and stimulate VSMCs growth and proliferation also known as vascular remodeling (121; 170; 176). Vascular remodeling is a characteristic of HT, typically seen as a thickening and stiffening of the vascular wall (see below) (34; 48; 104).

The SNS regulates the distribution of blood throughout the body by acting on a level of the vasculature known collectively as resistance vessels. By altering the diameter of the resistance vessels the SNS increases the resistance to flow, and therefore limits tissue perfusion (118). Tissues that require an increase in blood flow, due to increased metabolic demand, release a host of metabolic vasodilators that compete with, or directly inhibit, the constrictor effect of the SNS (60; 83).

ATP, a purine nucleotide primarily known for its role as a co-enzyme, fueling metabolic processes through the cleavage of its high energy phosphate bonds (84), is released from synaptic vesicles with NE and NPY and is responsible for excitatory junction potentials (EJPs). EJPs are minute depolarizations that occur in a postjunctional

cell (often VSMCs) following a single 'pulse' from a prejunctional neuron. These EJPs are rapid and can summate to initiate a mechanical contraction. However, EJPs are rapid and transient, and are often followed by a slow depolarization that is mediated by NE ((144) see below). ATP acts through two types of receptors ($P2X_{1\text{-}7}$ and $P2Y_{1, 2, 4, 6, 11, 12, 13,14}$). P2X receptors are fast inotropic ligand-gated ion channels, and P2Y receptors are part of the G protein-coupled receptor (GPCR) superfamily, which are described below. P2X receptors are found on neurons and smooth muscle cells; while, P2Y receptors can be found on most cells in the body (21; 22).

The archetypal neurotransmitter of the SNS is NE. NE is a catecholamine that is synthesized from the amino acid tyrosine through a series of enzymatic reactions. NE is released from sympathetic varicosities in a Ca^{2+} dependent manner following the depolarization of the membrane by an action potential. NE exerts its actions on post synaptic cells through α- and β-adrenergic receptors (AR) (52). There are two types of α-ARs; α_1- and α_2-ARs. α_1-ARs are primarily found on VSMCs and elicit cellular contraction (tissue vasoconstriction) through a GPCR mechanism. α_2-AR are found primarily pre-junctionally on nerve terminals, where they inhibit vesicle release through a negative feedback mechanism. Post-junctionally α_2-ARs signal vasoconstriction (53).

While three β-ARs have been identified, cloned and classified as GPCRs (97), the majority of NEs effect in the vascular system is done through the β_2-AR. Stimulation of the β_2-ARs, which are primarily found in arterioles of skeletal muscle, will result in vasodilation (14). It should be noted, that the β_1-AR is found in the heart, and increases heart rate and contractility. While these inotropic and chronotropic effects will factor into

vascular regulation, it is the direct role of the α_1-AR on the diameter of the blood vessels supplying a vascular bed that is the primary determinant of tissue perfusion (148).

NPY exerts it actions through a family of GPCRs called Y-receptors. Six members of the Y-receptor family have been cloned or postulated; however, the dominant receptors mediating cardiovascular responses are the Y_1 and Y_2 receptors (Y_1R and Y_2R, respectively) (3; 66; 120). The Y_1R is primarily found on VSMCs and preferentially binds NPY (30; 107; 183) eliciting vasoconstriction, potentiation of NE, and mitogenesis (2; 76; 105; 181). Y_2Rs are found in abundance on sympathetic and parasympathetic nerve terminals (146; 157) and preferentially bind an enzymatically truncated NPY molecule. NPY is degraded by aminopeptidase P (APP) and dipeptidyl peptidase IV (DPP IV) in the neurovascular cleft to produce the biologically active NPY_{3-36} and NPY_{2-36}, respectively, both of which act via the Y_2R to reduce vesicular release in a negative feedback manner (3; 120; 124; 125).

Many of the vascular receptors mentioned above are classified as G-protein coupled receptors, or GPCR. GPCR are members of the rhodopsin-like superfamily of receptors characterized by a 7-transmembrane receptor linked to a guanine nucleotide-binding protein (G-protein) (101). The G-protein is comprised of α-, β-, and γ-subunits each of which have isoforms resulting in immense diversity (20; 45). Ligand specificity occurs at the 7-transmembrane receptor domain where activation (from ligand binding) produces a conformational change in the Gα-subunit resulting in the substitution of a guanosine diphosphate (GDP) molecule for guanosine triphosphate (GTP). GTP binding releases the tightly associated Gβ- and Gγ-subunits (the βγ-complex) allowing both the

Gα-subunit and the βγ-complex to activate targets, thus commencing intracellular signaling (59; 78).

GPCRs are classified by the actions of the Gα-subunit; however, signaling by the βγ-complex is now accepted (129). Four main classes of Gα-subunits have been identified. They include, $G\alpha_s$ which stimulates Adenylyl Cyclase (AC), $G\alpha_i$ which inhibits AC, $G\alpha_q$ which increases the activity of phospholipase C (PLC), and $G\alpha_{12/13}$ which activates the Rho Family of GTP-ases (101; 129).

α_1-ARs and some P2Y ($P2Y_{1, 2, 4, 6, 11 \text{ and } 14}$) receptors contain $G\alpha_q$-subunits culminating in the activation of PLC. PLC enzymatically cleaves the phospholipid phosphatydlinositol 4,5-bisphosphate (PIP2) into the biologically active diacyl glycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) (114). IP_3 binds to the $InsP_3$-receptor on the sarcoplamsic reticulum resulting in the mobilization of Ca^{2+} (53). DAG, in turn, activates Protein Kinase C (PKC) which, in VSMCs, leads to the phosphorylation of myosin light chain phosphatase (MLCP). Phosphorylation of MLCP reduces its activity, resulting in sustained Ca^{2+} sensitization of myosin light chain 20 (MLC_{20}). The sustained Ca^{2+} sensitization of MLC_{20} allows for continued actomyosin coupling, and in conjunction with increased Ca^{2+} mobilization (from IP_3), leads to cellular contraction.

α_2-AR's, some P2Y ($P2Y_{4, 12, 13}$) receptors and all Y-receptors contain the $G\alpha_i$-subunit. The β-ARs contain $G\alpha_s$, which acts in opposition to $G\alpha_i$. $G\alpha_s$ activates AC (26) which converts ATP into cyclic-adenosine monophosphate (cAMP). Increases in the concentration of cAMP activate cAMP-dependent protein kinase (protein kinase A; PKA). PKA phosphorylates a myriad of cellular targets including the sarco/endoplasmic reticulum Ca^{2+} ATP-ase (SERCA) and myosin light chain kinase (MLCK). Activation of

SERCA reduces intracellular Ca^{2+}, while phosphorylation of MLCK reduced its activity allowing MLCP to reduce the affinity of MLC_{20} for Ca^{2+}. Both of these events will lead to reduced actomyosin coupling and relaxation of VSMCs. Inhibition of AC (by $G\alpha_i$) prevents the activation of PKA, ultimately leading to increased intracellular Ca^{2+} concentrations and contraction of VSMCs (2; 20; 86; 115).

The cellular pathways outlined above are required to elicit acute sympathetically mediated vasoconstriction in blood vessels. Other more chronic sympathetically mediated GPCR actions have been identified (40; 41). Both NE and NPY have been shown to activate a variety of mitogen activated protein kinases (MAPKs) which play an important role in mitogenesis, proliferation and migration of VSMCs (40; 87; 116; 120; 127; 134; 170; 170; 183). Because the targets of second messenger cascades are numerous, cross talk between many GPCRs signaling cascades occurs, and in many cases leads to the activation of various MAPK pathways including extracellular signal-regulated kinases (ERK) 1 and 2 (ERK1/2), p38, and c-Jun N-terminal kinases (JNK) (12; 41; 128; 177). These pathways lead to gene transcription, cellular proliferation and ultimately vascular remodeling (see below).

1.4 The Sympathetic Nervous System and Hypertension

Hypertension is a multi-faceted condition that has many complex and diverse etiologies. Heightened SNS activity is a prominent feature of HT (25; 104; 139; 178) that begins early in life (24; 48; 169). The underlying cause of augmented SNS activation leading to HT remains unknown (54); however, the mechanisms involved include defective baroreceptor autoregulation, increased hypothalamic response to environmental stimuli, stimulation of renal afferent sympathetics, and elevated thoracolumbar

sympathetic activity (79). Regardless of the cause, the heightened SNS activity produces chronically elevated vascular resistance (*R*) (10; 71; 132) and exposes the vasculature to trophic factors which can cause inappropriate tissue remodeling (10; 168). This thesis examines the impact of heightened SNS activity on vascular structure and function as a critical feature in the early stages of HT.

Detailed examinations of the hemodynamic and phenotypic alterations during the development of HT have been made (4; 122). Use of the spontaneously hypertensive rat (SHR) has identified many underlying pathologies that lead to the development of HT, including alterations in the Renin-Angiotensin System, renal physiology and the SNS (110; 155; 156). HT develops early in the life of SHR animals (approximately 5-weeks of age) and elevated sympathetic outflow has been implicated in this early pathology. In the SHR model, sympathetic innervation of several vascular beds occurs very soon after birth, before the development of hypertension (64) and neonatal sympathectomy consistently prevents the development of high blood pressure in SHR (90; 153). Thus, pre-hypertensive SHR animals at age 4-5 weeks have higher interstitial NE levels in muscle and fat than their age-matched Wistar-Kyoto (WKY) controls (24). However, evidence suggests that this innervation pattern is tissue-specific with little difference in the sympathetic activation of the heart in young SHR and WKY animals (64).

Similarly, evidence indicates that hindlimb vascular remodeling occurs as early as 4-5 weeks of age in the SHR, and may precede the blood pressure augmentation (85; 132; 140). This remodeling displayed regional specificity and debate exists as to the specific involvement of sympathetic stimuli (91; 140). Furthermore, these studies do not address the role of NPY in mediating the vascular remodeling associated with HT at this young

age. Indeed, the roles that NPY plays in the regulation of blood pressure, vasomotor control and/or vascular mechanics at this critical age are not well understood.

1.5 Signaling Alterations in Hypertension

As mentioned, sympathetic hyperactivity is seen regularly in HT (25; 104; 139; 178). The influence of augmented innervation can be compounded by genetic modifications which have been identified in HT (33; 44; 56; 143). These genetic modifications lead to an imbalance between constrictor and dilatory signals, and lead to increased constriction, augmented vascular resistance and arterial pressure (45; 46; 56; 143).

An extremely important step in GPCR signaling is the process of desensitization. Desensitization is the mechanism whereby a signaling 'memory' is formed (20). If, for instance, a cell is exposed to NE, GPCR activation and 2nd messenger cascades are initiated (as outlined above). In addition to the target molecules which regulate vasoconstriction, another a group of molecules termed GPCR kinases (GRKs), are activated which lead to the phosphorylation of the GPCR. A phosphorylated GPCR is targeted by β-Arrestin molecules which internalize the GPCR. If the cell is repeatedly exposed to a ligand, desensitization is a means by which the cell expresses fewer receptors (of that specific ligand). Alternatively, the same mechanism allows a cell to express more receptor when low levels of a specific ligand are present (i.e. sensitization) (49; 92). A variety of alterations in GRK activity/expression have been associated with HT (46; 142). For example, increased expression of GRK2 is often seen in hypertensive models. Increased GRK2 leads to increased desensitization of the β_2-AR resulting in reduced vasodilation, blunted AC coupling and vascular remodeling (36; 61).

Additionally, transgenic mice expressing a GRK4 gene isolated from human hypertensive patients demonstrated alterations in dopamine stimulated sodium excretion from the kidneys, impairments in GRK4 activity and HT (43).

Genetic alterations in HT have also been observed in the β_2-AR (44; 45). The primary role of the β_2-AR on VSMCs is vasodilation, mediated by $G\alpha_s$ and the activation of AC. A number of single nucleotide polymorphisms (SNPs) of the β-AR are associated with HT. Both the Arg16Gly and Gln27Glu SNPs have little effect on receptor binding affinity or AC activation, but prevent receptor desensitization compared with wildtype receptors (17; 55). Additionally, the Thr164Ile SNP results in reduced AC activity and impaired $G\alpha_s$ coupling (16). Ultimately, the physiological consequence of these SNPs is a blunted vasodilatory response in VSMCs.

Another type of molecular alteration that occurs in HT is known as signal switching (33). The β_2-AR typically couples with the $G\alpha_s$ subunit (as described above) to elicit vasodilation. In some cases the β_2-AR has been shown to couple to $G\alpha_i$ leading to MAPK activation (89; 171). The mechanisms that result in signal switching may not be pathophysiological, but could be a secondary signaling mode of the β_2-AR. If this is the case, it underscores our limited understanding of the molecular mechanisms of cellular regulation (143).

1.6 Vascular Mechanics

The control of blood flow distribution and blood pressure maintenance in the body has many levels of regulation. At the systemic level the SNS plays a vital role in distributing blood away from areas of low metabolic demand, while local metabolic factors allow for increased blood supply to places where demand is high (7; 31; 113;

151). The large elastic arteries in the trunk of the body branch and become muscular resistance vessels in the limbs and visceral organs. Distribution of blood flow is primarily done through manipulation of the resistance in the vessels feeding a given tissue. Resistance to flow is determined by the pressure change across a vascular bed and the flow entering the system (117). The relationships between flow, pressure gradients and the dimensions of a capillary tube were first established by Jean Leonard Poiseuille (1797 – 1869). Poiseuille's equation (126) relates a pressure gradient across a tube with the flow, viscosity of the fluid, length and radius of the tube:

$$Q = \frac{\pi r^4 (P_1 - P_2)}{8\mu L} \qquad \text{Equation 1.1}$$

where Q is the volume flow per unit time, r is the internal radius , $P_1 - P_2$ is the pressure drop, μ is the viscosity of the blood, and L is the length of the of the tube.

To use Poiseuille's equation, knowledge of the architecture of the vessels is needed. This is not always possible to ascertain; however, when considering the vascular system as a whole, or one of its regions, the formula can be simplified to relate the pressure difference (i.e. arterial – venous pressure) as the product of the flow (Q; i.e. cardiac output) and the resistance to flow (R) (117). As the pressure in the venous system is sufficiently low, R can be calculated as:

$$R = \frac{MAP}{Q} \qquad \text{Equation 1.2}$$

where R is the resistance, MAP is the mean arterial pressure and Q is the flow (cardiac output systemically, or volume flow in a large peripheral artery). Notice that an increase in resistance will cause a drop in flow (if pressure is held constant). In the vascular system, the most effective way to change the resistance of a vessel is through the

manipulation of the vessel diameter. Poiseuille's equation dictates that flow (Q) is proportional to the radius (r) to the 4^{th} power, so a small change in the diameter of a vessel will have profound effects on flow through that vessel.

A number of assumptions must be made in order to use Poiseuille's equation in physiologic situations so that flow, pressure and/or resistance can be derived from the equations outlined. These assumptions relate to the properties of the blood, blood flow and the 'tube' in which the flow is traveling. First, it is assumed that the fluid is homogeneous and its viscosity is the same at all rates of shear. Blood is, of course, composed of particulate matter (cells) suspended in a fluid (plasma). In arteries where the radius is sufficiently large compared to the size of red cells, blood behaves as a Newtonian fluid, and this assumption holds true. However, these fluid characteristics change as the vessels become smaller at the arteriolar level. The second assumption is that the liquid does not slip at the wall. This is universally true for liquids, as the friction between the wall and the flowing liquid makes the velocity at the wall zero. Assumption three dictates that the flow is laminar with the liquid moving parallel to the wall of the tube. This assumption is not satisfied in some large vessels where turbulent flow is possible (i.e. the aorta). Assumption four is also invalid in large arteries, because the flow is not 'steady' but is pulsatile and is subject to acceleration and deceleration. The final two assumptions deal with the tube in which the flow is occurring. Assumption five states that the tube is long compared with the region being studied. This is required to allow for laminar flow to develop (assumption three). The final assumption dictates that the tube is rigid and the diameter does not vary with internal pressure. Blood vessels are viscoelastic structures which distend with an increase in arterial pressure during each heart beat.

Some have argued that in many vascular beds the walls are muscular and blood flow is sufficiently steady so that the internal diameter does not distend and the application of Poiseuille's equation is permitted (118). This speculation may apply to microvascular beds. Yet, the pulsatile mechanics of microvascular beds have not been studied, particularly in the context of hypertension. Our laboratory has developed methods to study the oscillatory stiffness, viscoelasticity and inertial elements of flow in a vascular bed (172-175) and these methods will be used, for the first time, in a rodent model of hypertension in the ensuing studies.

The assumptions underlying Poiseuille only allow us to approximate the physiology of the circulation. Historically, *R* has been the primary indicator of vascular regulation, as a change in resistance is indicative of vessel caliber (23; 135). This however, is an estimate at best, as *R* only reflects the steady state component of blood flow and neglects the pulsatile nature of blood flow which violates the assumptions listed above. The analogue of *R* in a pulsatile system is termed impedance (*Z*) and is given by (173; 174):

$$Z = \frac{R}{1+i\omega RC} \qquad \text{Equation 1.3}$$

where *R* is resistance, $i = \sqrt{-1}$, ω is the frequency of oscillation and *C* is the compliance of the vessel given by:

$$C = \frac{\Delta V}{\Delta(\Delta P)} \qquad \text{Equation 1.4}$$

where ΔV is a change in volume and Δ(ΔP) is a change in the pressure gradient. Compliance reflects the change in the pressure difference needed to change the volume of a system. Increases in *C* allow for greater volume changes for a given change in the

pressure gradient (173). *C* is the inverse of stiffness, and in many disease states, stiffness is used as a clinical marker of disease severity (i.e. pulse wave velocity) (112; 137).

To better understand the oscillatory nature of the vascular system it is typically modeled as a Windkessel. Windkessel in German means 'air chamber' but in respect to the vascular system it implies the elastic reservoir created by the aorta and other large elastic arteries with increasing pressure from each heart beat. The principle of the Windkessel effect is simply the stretching of the walls of large elastic arteries due to the ejection of blood from the heart. The stretch of the vessel walls store potential energy during systole, allowing for a passive recoil of the elastic lamella of the tunica media creating blood flow during diastole (when the heart is relaxed) (108; 118).

Windkessel models are typically lumped models of the vasculature. Lumped models are useful tools for studying the vasculature because vascular networks are immensely complex. By representing the entire structure of a vascular network as a single tube with the properties of the network as a whole, a "black-box" is created, where the relationship between inputs and output is easily understood (173). To adequately model the pulsatile nature of skeletal muscle blood flow, it is necessary to include additional parameters to Equation 1.3. Equation 1.5 incorporates Equation 1.3 with the addition of the viscoelastic properties of the vessel (*K*), and the inductance (*L)*, representing the effects of fluid and vessel wall inertia, both of which are required in series with *C* (Figure 1.1) (174):

$$Z = \frac{R[\omega KC + i(\omega^2 LC - 1)]}{\omega C(K+R) + i(\omega^2 LC - 1)} \qquad \text{Equation 1.5}$$

This model (termed the '*RCKL*' model) uses measured pressure and flow waveforms (Figure 1.2) which are decomposed and averaged into pressure (*P*; Figure 1.3), and flow

harmonics (*Q*; Figure 1.4) using fast Fourier transformations (15; 158), which can be used in Equation 1.6 to calculate impedance:

$$Q = \frac{P}{Z} \qquad \text{Equation 1.6}$$

This is analogous to Equation 1.2 and when combined with Equation 1.5 we are able to approximate *Q* using the measured pressure waveform (Figure 1.3) and Equation 1.7:

$$Q = \frac{P\omega C(K+R)+i(\omega^2 LC-1)}{R[\omega KC+i(\omega^2 LC-1)]} \qquad \text{Equation 1.7}$$

RCKL predicts a flow waveform based on the measured pressure waveform. By manipulating the parameters *C*, *K*, and *L*, the calculated flow waveform can be compared to a measured flow waveform. When congruency between the measured and calculated waveforms is found, the values for *C*, *K*, and *L* represent the properties of the lumped vascular bed (Figure 1.5).

The lumped properties obtained using the *RCKL* model, while providing useful information about the mechanical properties of a given vascular bed, do have inherent limitations which need to be addressed. Primarily, because these properties represent the vasculature as a whole, we are unable to ascribe a specific level of the vascular tree to a given component of the model (174). Additionally, a large proportion of the resistance in the model is generated by resistance arterioles, but it is unclear from the model whether or by how much these vessels also contribute to the compliance of the vascular bed. The model as it is applied in this thesis only reflects the pressure-flow relationships of the rat hindlimb being studied, and do not reflect the vasculature as a whole. Further, as surgical interventions were required to obtain the pressure and flow measurements used, the effect of anaesthesia and/or the surgery itself in altering the mechanical properties of the

vasculature cannot be ruled out. Finally, the four components of this model; resistance, compliance, viscoelasticity and inertia, represent an over simplification of a complex physiologic system giving us a method to conceptualize the mechanical properties (174).

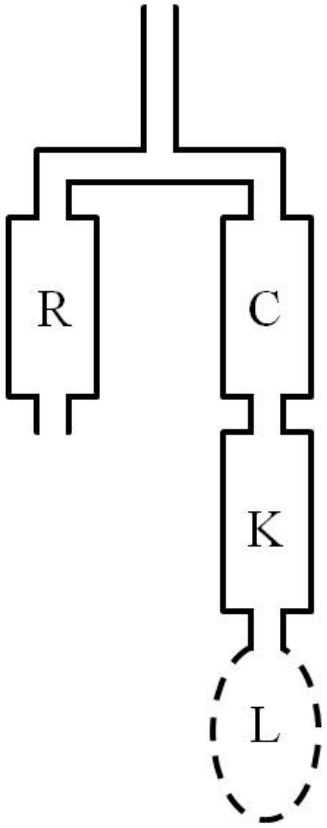

Figure 1.1 – Representation of the *RCKL* model. Resistance (*R*) in this lumped modified Windkessel model is in parallel with compliance (*C*), the viscoelastic properties of the vessel wall (*K*), and inductance (*L*) representing the inertial effects of the blood and vessel walls.

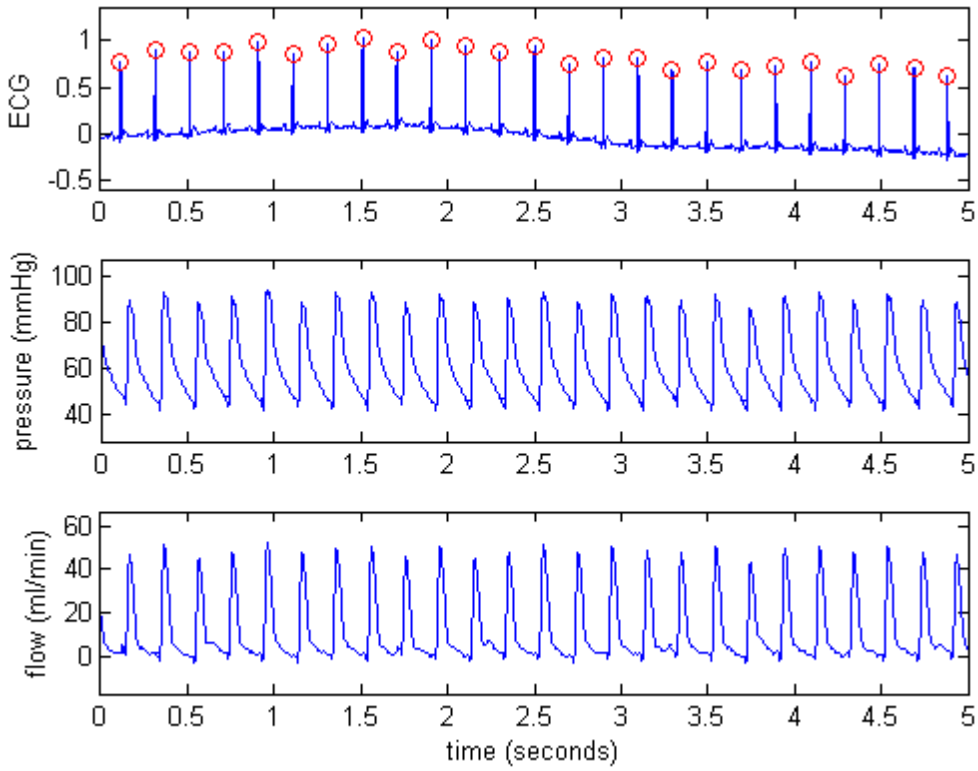

Figure 1.2 – Data selection used to estimate the mechanical properties of the vasculature of the rat hindlimb. ECG (top) arterial blood pressure (middle) and arterial blood flow (bottom) are obtained during a representative period of baseline or during a reflex maneuver.

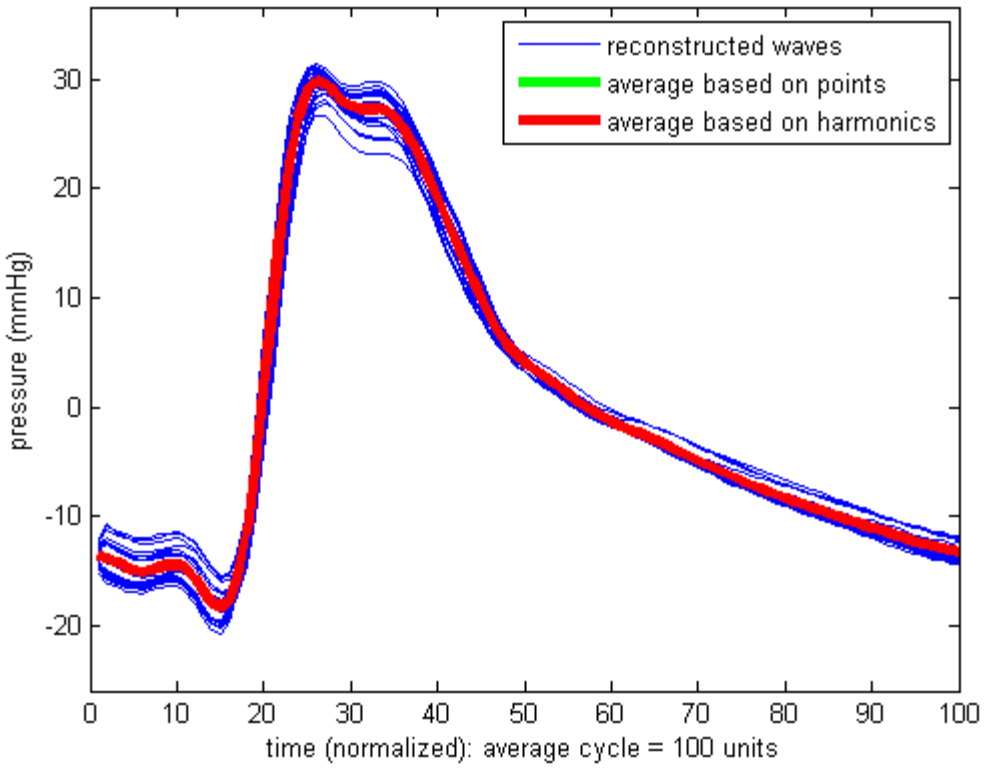

Figure 1.3 – Averaged pressure waveform from data selection in Figure 1.2. Waveforms are averaged in both the time and frequency domain to obtain an average pressure waveform.

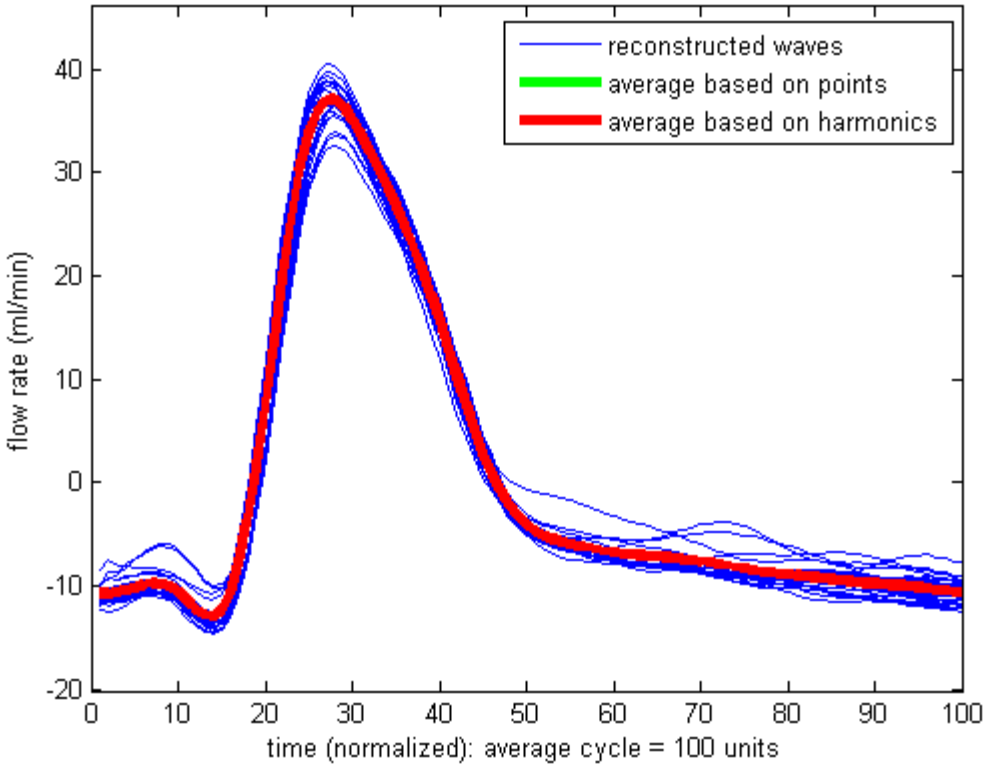

Figure 1.4 – Averaged flow waveform from data selection in Figure 1.2. Waveforms are averaged in both the time and frequency domain to obtain an average flow waveform.

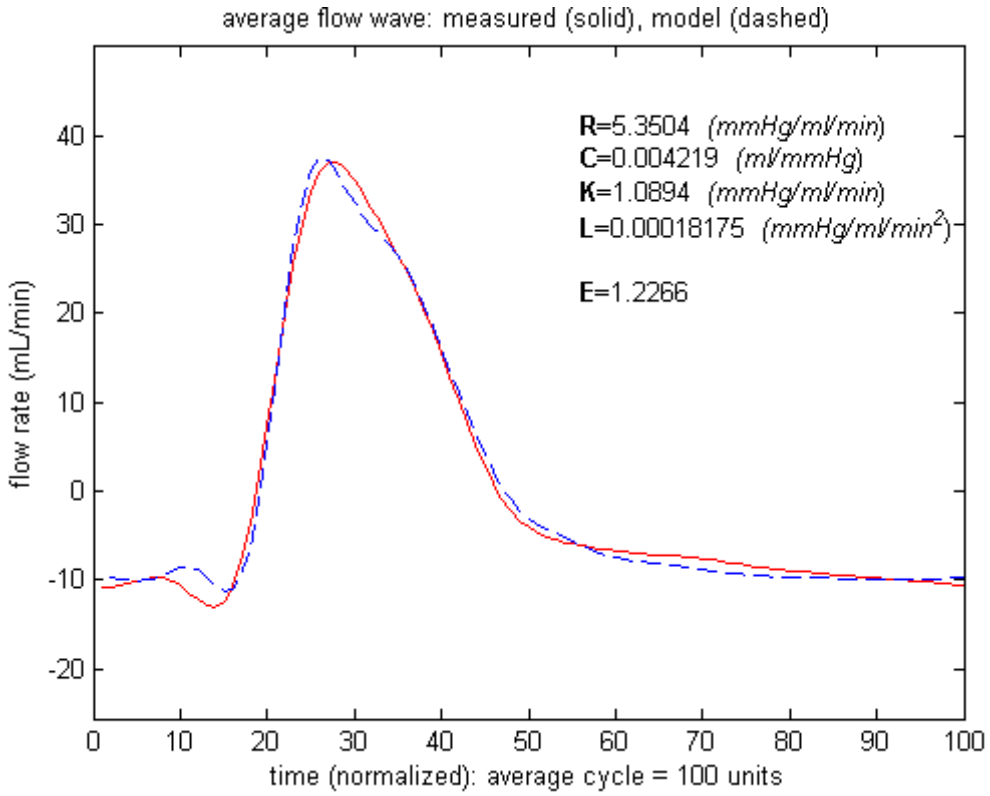

Figure 1.5 – Measured (solid) and calculated (dashed) flow waveform from the *RCKL* model. The value for resistance (*R*) is calculated as the mean pressure divided by the mean flow. The values for compliance (*C*), viscoelasticity (*K*), and inertia (*L*) shown are those that produced the best agreement between the averaged pressure (Figure 1.3) and flow (Figure 1.4) waveforms. These values represent the lumped properties of a vascular bed. 'E' as outlined above represents the error (least square difference) between the measured and calculated waveforms.

1.7 Vascular Remodeling and Mechanical Alterations in Hypertension

Increased blood pressure and SNS activity both contribute to the characteristic vascular remodeling seen in HT (34; 48; 104; 164). Both hemodynamic forces acting from within the vessel and extrinsic factors that act on the vessel wall factor into the remodeling process (180). Debate exists as to whether vascular remodeling occurs before blood pressure rises, or occurs as a consequence of HT (65). Many factors contribute to vascular remodeling; however, this section outlines only those aspects which are mediated by mechanical or sympathetic mechanisms. For detailed reviews of different aspects of vascular remodeling see (94; 131; 180).

Mechanical stresses have the ability to alter the composition of biological tissues. Changes in volume, shear stress and stretch have all been documented to alter the structure of the vessel wall (69). This section will highlight augmented blood pressure causing an increase the circumferential stress within the wall of the vessel. The increased wall stress is sensed by vascular cells (likely via integrins linked to cytoskeletal components (106)), and stimulates a phenotypic change in VSMCs in an attempt to normalize the wall tension (described below).

The relationship between arterial pressure and the architecture of a blood vessel is defined by the Law of Laplace (23; 163):

$$\boldsymbol{T = \frac{P \times r_i}{h}} \qquad \text{Equation 1.8}$$

where T is the wall tension (wall stress), P is the distending (arterial) pressure, r_i is the internal radius and h is the wall thickness. Using the Law of Laplace we are able to conceptualize the physiologic mechanisms at work in the vessel wall that act to counter the increased wall tension created by augmented pressure. Acutely, an increase in

distending pressure will cause an increase in myogenic constriction, thereby reducing r_i in an attempt to restore the wall stress; but, at the same time increasing vascular resistance (see Poiseuille's equation) resulting in a positive-feedback mechanism which ultimately increases blood pressure (65; 73).

Chronically, vascular tissue remodels as a compensatory mechanism to normalize wall stress. Hypertrophy and hyperplasia occur in VSMCs following a phenotypic shift from one of constriction to synthesis. VSMCs begin to divide and excrete extracellular matrix. The excretion of extracellular matrix proteins alters the proportions of components of the vessel wall and can perpetuate the remodeling process, as it has been shown *in vitro* that VSMCs are sensitive to the makeup of the medium on which they are grown (68; 116; 152). In this instance, vascular remodeling is a consequence of HT (1; 19).

Extrinsic regulators of vascular remodeling are numerous and include angiotensin II, nitric oxide, matrix metalloproteinases, inflammatory mediators, sex hormones, and sympathetic neurotransmitters (2; 40; 50; 131; 147; 167). The vasculature is chronically exposed to these factors, with the exception of sex hormones which have a more pronounced role following puberty. The cumulative activation of vascular cells is thought to initiate vascular remodeling in the absence of augmented blood pressure. As outlined in Section 1.3 activation of MAPKs can lead to gene transcription, protein translation and ultimately vascular remodeling. The processes which result in vascular remodeling are not exclusive to VSMCs, as endothelial cells (ECs) and adventitial fibroblasts (AFB) express a number of the same receptors as VSMCs. Importantly for the remodeling process AR and Y-receptors on AFB stimulate cell growth and protein synthesis, further

altering the makeup of the extracellular matrix and facilitating VSMC activation and migration (42; 68; 116; 152). The activation of vascular cells can occur independently of arterial pressure causing a remodeling that occurs before the pressure is augmented (85; 132; 140).

Both of the mechanisms described independently cause vascular remodeling, but they can also act in conjunction with one another to accelerate the remodeling process. Thus, the study of vascular remodeling in HT is immensely complex. Multiple processes converge to elicit the transformation of the vessel wall (65; 133; 180). Compounding the situation in HT is the continual augmentation of blood pressure, and associated co-morbidities. Functionally, it is the narrowing of the lumen of blood vessels and the thickening of the vessel wall which promote HT. A narrower lumen increases the resistance to flow thus adding to the increased blood pressure (63; 165). Thickening of the arterial wall acts to stiffen the vessel which adds to HT by increasing the afterload on the left ventricle (119). Afterload is dependent on systemic pressure and reflects the force needed to open the aortic valve. Afterload is augmented by the summation of systolic pressure (generated by the left ventricle) and pressure waveforms reflected from the periphery. Reflected pressure waveforms arise from sites of incongruity (i.e. bifurcations) in the peripheral vasculature. In a healthy arterial system, wave reflections reach the aorta during diastole which adds little to the workload of the heart (as the aortic valve is closed). Stiffer blood vessels transmit both outgoing and reflected pressure waveforms faster, allowing the reflected waveform to return to the aorta during systole and forces the heart to work harder to expel blood (81; 119; 161; 162).

Details of the mechanisms responsible for vascular remodeling are being elucidated to determine whether augmented pressure leads to vascular remodeling, or the remodeling initiates the rise in pressure (35; 65; 94; 164). Indeed both scenarios are evident (63; 70), and likely occur in concert to generate cardiovascular dysfunction. Clinical assessment of vascular stiffening is typically measured using either pulse wave velocity, and/or arterial compliance (8; 72). Pulse wave velocity and arterial compliance are useful tools to assess either 'central' or 'point' arterial stiffness, respectively, but neglect the contribution of the microcirculation in mediating stiffness. An emerging hypothesis suggests that the microvasculature is the site where remodeling begins leading to changes in central arteries, and ultimately HT or cardiovascular disease (88; 96), although this has not been established. Thus, the functional and morphologic bases of the vascular properties in the developmental stages of HT are required. The studies in this dissertation emphasize vascular remodeling using both functional and morphologic measurements, with emphasis on peripheral hindlimb vascular beds and systemic vascular measures that directly relate to stiffness.

1.8 Estrogen, the Vascular System and Hypertension

Estrogens, androgens and progestogens, the sex hormones, are groups of structurally-related steroid hormones which exert distinct actions throughout the body. Estrogens have received the most attention in the literature and have been linked to both beneficial *and* adverse cardiovascular outcomes (111; 119). Of the three estrogens: estradiol, esterone, and estriol, estradiol (E2) is the most biologically available and is has the greatest influence on tissue function and structure (141). This section examines the

physiologic role of E2 in vascular tissue, and will examine the evidence for and against protective effects of E2.

A myriad of discrete physiologic effects have been identified for E2 outside of reproductive tissues. Specifically in vascular tissue (predominantly ECs and VSMCs) both rapid (acute) and genomic (chronic) effects have been identified and warrant some discussion (29; 154). Because estrogen is derived from cholesterol, it readily diffuses through the plasma membrane and can bind to the two related estrogen receptors (ER) that have been identified and cloned. The ERα and ERβ are members of a nuclear receptor superfamily of ligand- or hormone-dependent transcription factors. Both receptors display high homology, similar affinity for E2, and coexpression in many tissues (32; 109). However, distinct expression patterns have been documented, and where co-expression exists, ERβ has been suggested to modulate the actions of ERα (159).

Estradiol exerts a host of acute effects in vascular tissues. These effects occur in the order of seconds to minutes and are initiated far too quickly to involve gene transcription (102; 130). These effects were initially attributed to a membrane bound subpopulation of the ERα (38), but many of the acute responses to E2 have now been ascribed to a novel GPCR known as GPR30. It should be noted that debate as to whether GPR30 is in fact an estrogen receptor exists (95; 123). Acutely E2 typically elicits endothelial dependant vasodilation. The mechanism has been linked to GPR30 stimulation of endothelial nitric oxide synthase (NOS). Additionally, acute activation of GPR30 by E2 has been linked to activation of PKA, and phosphoinositide 3-kinase (PI3)

kinase. Concurrent activation of MAPKs, Raf and Src kinases leads to genomic translation and thereby linking the acute and chronic effects of E2 (47; 102).

As with many GPCRs, 2nd messenger signaling cascades initiated by GPR30 can activate pathways that promote both constriction *and* relaxation depending on the cell type on which they are expressed. For example, E2 stimulated activation of PI3 kinase in ECs leads to the induction of endothelial NOS which produces the potent vasodilator nitric oxide. In VSMCs PI3 kinase activation leads to the phosphorylation of MLCK which promotes constriction (101; 166). Furthermore, both ECs and VSMCs express ERα and ERβ which, when activated, lead to the transcription of a host of proteins associated with vascular cell signaling (39). Many of these transcripts confer beneficial phenotypic alterations in both ECs and VSMCs resulting in reduced leukocyte adhesion, inhibition of VSMC proliferation, and cell survival (38). On the other hand, HT has been associated with a genetic variant of the ERβ and it has been clearly documented that E2 taken as an oral contraceptive augments blood pressure (27; 93; 149). Additionally, the discontinued use of hormone replacement therapy due to *increased* risk of cardiovascular disease and some forms of cancers, shed doubt on the beneficial effects of E2 (9; 82; 98).

Evidence suggests that male and female animals may utilize different strategies to regulate a variety of processes in the body. From cellular mechanisms responsible for cell survival (99), to blood pressure regulation by the SNS (57; 62; 138), it appears that females may rely on different mechanisms to maintain cardiovascular homeostasis. For example, female rats possess NPY and the Y_1R, but unlike males, this pathway contributes little to baseline blood flow control (74-76). Further, E2 has been shown to down-regulate NPY mediated control of hindlimb blood flow in females (77).

Moreover, in humans, E2 is the leading candidate behind the so-called ‘female advantage’ in cardiovascular disease (80). The ‘female advantage’ refers to the epidemiologic observations that prior to menopause, the prevalence and severity of a number of cardiovascular diseases, including HT, are markedly lower in females than age matched males. Following menopause, the ‘protection’ is lost, and the rates and severity of HT and other cardiovascular diseases including atherosclerosis, myocardial infarction and coronary artery disease (among others), in women surpass those found in age matched males (Figure 1.6). The differences between males and females in figure 1.6 reflects the prevalence in the population, and while E2 is has been identified as a contributor to the dimorphism, little is known about the mechanisms responsible.

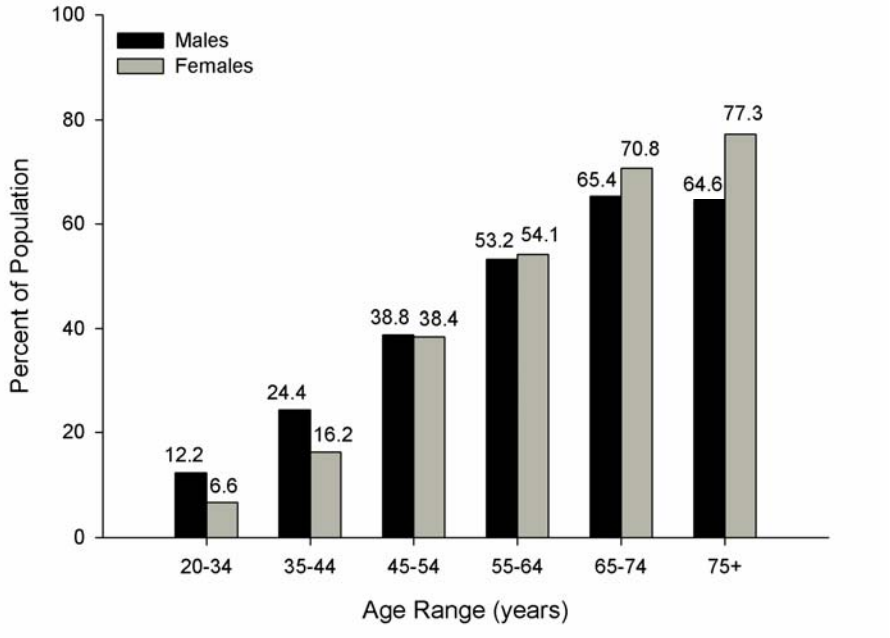

Figure 1.6 – Prevalence of high blood pressure in adults age 20 and older, by age and sex. Rates of hypertension among males are greater than those found in females up until 45years of age, at which point females surpass males in prevalence. Data from (5).

As mentioned, E2 has the ability to both dilate *and* constrict blood vessels (101; 166), and can confer both beneficial *and* adverse effects in systemic vascular regulation (27; 38; 93; 149). Further, E2 has also been shown to both augment *and* reduce vascular stiffness which can have direct effects on blood pressure (37; 51; 100; 150). Additionally, E2 is known to influence the development of the SNS (6; 28; 182) and can modulate its activity (160). Therefore the role of E2 in regulating sympathetic cardiovascular control in both health and disease remains unclear.

1.9 Summary

Hypertension is a multifactorial condition with much pathology. It manifests clinically as augmented arterial pressure and a clear role for the SNS is evident. The role of the sympathetic nervous system in *established* HT has been extensively studied but the contribution it plays in the early stages of HT is less well understood. Both the regulation of hemodynamics and the mechanical properties of the vasculature are altered in HT. This thesis examines the role of NPY in particular, and the SNS in general in regulating the changes observed in males and contrasts them with observations made in female animals. Furthermore, the ability of the SNS to regulate vascular mechanics in females is unknown and the role of the animal's sex in regulating hemodynamics, vascular mechanics and sympathetic control in normotension and HT, will be addressed.

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Chapter 2 – Enhanced regulation of hindlimb vascular control by neuropeptide Y during the development of hypertension

2.1 Introduction

Hypertension (HT) develops early in the life of the spontaneously hypertensive rat (SHR; approximately 5-weeks of age). Elevated sympathetic nervous system (SNS) activity is often observed in the pathology of hypertension (2; 14; 15; 25) and neonatal sympathectomy consistently prevents the development of high blood pressure in the SHR (11; 24). Thus, the development of HT is related to sympathetic activation. However, the role of specific sympathetic neurotransmitters and post-junctional receptor control in the development of HT remains unclear.

Sympathetic neurotransmitters responsible for vascular control in skeletal muscle include norepinephrine (NE) and neuropeptide Y (NPY). Acutely, these neurotransmitters signal vasoconstriction (26) leading to increased peripheral vascular resistance. Chronically, these same mediators can exert trophic effects (4), which can lead to inappropriate tissue remodeling with outcomes such as wall thickening, wall stiffening and lumen narrowing. Of the sympathetic neurotransmitters, NE has received most attention as an etiology of HT, and evidence is mounting which suggests a role for NPY in vasomotor control (7; 8) and, in particular, HT (5; 16).

NPY acts via a variety of Y-receptors on vascular smooth muscle cells (VSMC) to elicit diverse cardiovascular effects (1; 17). A possible role for NPY in the development of HT was shown by Shin et al. (21) who, in a two-kidney, one-clip renovascular model of HT, blunted the rise in blood pressure via NPY Y_1 receptor (Y_1R) antagonism, a mechanism that was independent of renin release. Thus, it is possible that NPY exerts an important influence on hindlimb vasomotor control in the developing stages of HT. The purpose of this experiment was to test the hypothesis that altered

neurovascular regulation occurs early in the development of HT with changes that include modifications to Y_1R and/or α-adrenergic receptor (AR) regulation. Our approach was to assess the hindlimb hemodynamic responses of exogenous α-AR and Y_1R agonists in the early stages of HT and to combine these integrated responses with an analysis of receptor protein expression in young hypertensive (SHR) and normotensive (Wistar Kyoto; WKY) controls.

2.2 Methods

Experimental Animals

The experimental protocol was approved by the Animal Use Subcommittee at The University of Western Ontario. Male Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats were grouped according to age with emphasis on 3-5 weeks of age (SHR, n=10, 108±28 g; WKY, n=11, 89±27 g), 7 weeks of age (SHR, n=16, 199±24 g; WKY, n=17, 195±6 g) and 9 weeks of age (WKY only, n=5, 260±8 g).

Data collection and analysis

Non-invasive blood pressure (NIBP) was measured in a subset of 3-5 (SHR, n=6; WKY, n=7) and 7 week-old (SHR, n= 8; WKY, n=8) animals with a volume pressure recording sensor and an occlusion tail-cuff (CODA System, Kent Scientific, Torrington, CT). Measures of systolic, diastolic, and mean arterial pressures (MAP), as well as heart rate (HR) were obtained. The animals were familiarized with the protocol prior to data collection.

All animals underwent an acute *in vivo* vasomotor control study. Animals were anesthetized with an intraperitoneal injection of α-chloralose (80 mg/kg) and urethane (500 mg/kg). This mixture was used to ensure minimal impact on reflex cardiovascular

control (12; 13). Once surgical depth was achieved, the right jugular vein was cannulated with PE50 tubing for the administration of a dilute mixture of α-chloralose (8-16 mg/kg/hr) and urethane (50-100 mg/kg/hr) to maintain anesthesia throughout the protocol. A fluid filled PE50 cannula was inserted into the right common carotid artery to record arterial blood pressure using a pressure transducer (model MLT844; ADInstruments Colorado Springs, CO) and the signal was amplified using a bridge amplifier (model ML118 PowerLab Quad Bridge Amplifier; ADInstruments). HR was calculated online from the systolic peaks of the pressure signal and was used in conjunction with blood pressure and temperature (rectally) to ensure that the animal remained at a stable surgical depth of anaesthesia throughout the experiment. All data was collected using Chart 5 (ADInstruments) with a sampling rate of 4000Hz.

A midline incision was made to expose the visceral organs which were repositioned to reveal the bifurcation of the descending aorta into the iliac arteries. The right iliac artery was cannulated with PE50 tubing inserted rostrally to the bifurcation of the aorta for the delivery of vasoactive compounds (see below). Femoral blood flow (Q_{Fem}) was measured in the intact (left) hindlimb with a Transonic flow probe (0.7 PSB and model TS420 Perivascular Flowmeter Module; Transonic Systems, Ithaca, NY) positioned approximately 3mm distal to the femoral triangle. Care was taken to ensure that nerves and vessels were not damaged during this process. The area was covered with an innocuous water-based gel.

The animals were allowed to recover from the surgery for 1 hour. Baseline values were measured over a 10 minute period after which a 160 μL bolus of the agonists phenelypherine (PE; 2.5 μg/kg) or neuropeptide Y (NPY; 15 μg/kg) were administered to

all of the animals via the iliac artery. To test the influence of the Y_1R in baseline cardiovascular control, bolus' of the selective Y_1R antagonist N-[(1R)]-4-[(Aminoiminomethyl) amino-1-[[[(4-hydroxyphenyl) methyl] amino] carbonyl] butyl-a-phenylbenzeneacetamide trifluoroacetate (BIBP3226; 100 μg/kg) were infused into the hindlimb of a subset of 7-week-old animals (SHR n, = 10; WKY, n = 9). The peak response to BIBP3226 was measured approximately 10 minutes following the infusion. The ganglionic antagonist hexamethonium bromide (Hex; 25 mg/kg intravenously) was infused systemically in all animals to determine the viability of the sympathetic nervous system in the preparation. The dose of Hex that was used is higher than that of previously published reports (9; 22), in which 20 mg/kg of intravenous Hex abolished (renal) SNA in both SHR and WKY animals between 15 and 20 weeks of age. All drugs were purchased from Sigma-Aldrich (St. Louis, MO) with the exception of BIBP3226 (Tocris Bioscience; Ellisville, MO).

Changes in femoral vascular conductance (Cond = Q_{Fem}/MAP x 1000; μL/min/mmHg) were used as an indicator of vasomotor contractile state. Drug responses were compared using the 5-second average at the nadir and at 360 seconds post infusion, and these were compared to a 5-second baseline collected immediately prior to the start of the infusion. Additionally, the area over the curve (AOC; arbitrary units, A.U.) was calculated using the trapezoid method (20).

Western blot analysis

Following the infusion protocol, the red portion of the vastus lateralis muscle from the left hindlimb of 7 week-old animals was extracted and frozen for protein analysis. Briefly, equal amounts of total protein (60 μg for Y_1R and 120 μg for tyrosine

hydroxylase (TH) and α-AR) were assessed by western blot. Membranes were incubated overnight in primary antibody specific to either rat, human or mouse anti-Y_1R (1:300, affinity purified rabbit anti-mouse Y_1R IgG, Alpha Diagnostic International, Cat. No. NPY1R11-A, San Antonio, TX, USA), anti-α_1-AR (1:400 affinity isolated rabbit, Sigma-Aldrich Cat. No. A270) or anti-TH (1:1000 purified rabbit polyclonal antibody, Millipore, Cat. No. AB152, Billerica, MA, USA) in TTBS with 2% non-fat milk. Membranes were washed 3 times in TTBS then incubated in secondary antibody (goat anti-rabbit, or anti-mouse; 1:3000) conjugated to horseradish peroxidase (HRP; Bio-Rad, Cat. No. 170-6518) and Precision Protein StrepTactin-HRP Conjugate (1:10000; Bio-Rad, Cat.No. 161-0380) in TTBS with 2% non-fat milk for 1 hour and developed using Immun-Star WesternC chemiluminescent (Bio-Rad, Cat. No. 170-5070) and detected using a Bio-Rad ChemiDoc XRS system (Bio-Rad, Cat. No. 170-8070) with a supersensitive 16-bit CCD. Images were analyzed using Quantity One 1-D Analysis Software (Bio-Rad, Cat. No. 170-9600).

Statistics

The effect of age and strain on the progression of hypertension (NIBP measures) was assessed with a mixed two-way ANOVA (Statistical Analysis System (SAS) 9.1, SAS Institute Inc., Cary, NC, USA). The effects of group and the infused vasoactive agonists were assessed by the AOC as well as the difference from baseline at the nadir and 360 seconds post infusion using a mixed two-way ANOVA, again, on age and strain. The hemodynamic response to BIBP3226 infusion was compared to baseline using a paired Student's t-test within a strain (SigmaStat 3, Systat Software Inc., San Jose, California, USA). Western blot data were compared between strains of the 7 week-old

animals using an equal variance Student's t-test (SigmaStat 3). Differences were considered significant at $P<0.05$ and Tukey's post hoc analysis was used to identify where differences occurred in the ANOVAs. Data are represented as mean ± standard deviation.

2.3 Results

Non-invasive blood pressure

As expected, SHRs had higher arterial pressure as measured by NIBP versus WKYs (Table 2.1). Additionally, 7 week-old animals (of both strains) had higher pressure than 3-5 week-olds. Anesthetic administration dropped blood pressure, however, data from our lab indicates that reflex cardiovascular control using this anesthetic is maintained (Usselman et al., *In Press*)

In Vivo vasomotor control study

Hemodynamic values during baseline and following Hex administration can be found in Table 2.1. Table 2.2 depicts the hemodynamics following bolus infusions of both PE and NPY. Figure 2.2 shows the change in Cond in response to PE in 3-5 week and 7 week-old SHR and WKY animals (Figure 2.2; top and bottom respectively). In response to PE infusion, Cond in 3-5 week-old WKY animals fell by 2.8±2.9 μL/min/mmHg ($P<0.05$ versus baseline) and returned to baseline by 360s (*not significant* (*N.S.*) versus baseline). The femoral vascular conductance of 3-5 week-old SHR animals fell by 2.9±2.8 μL/min/mmHg ($P<0.05$ versus baseline) in response to PE and returned to baseline at 360s (*N.S.* versus baseline). In 7 week-old WKY animals, PE did not significantly reduce Cond (*N.S.*) but in 7 week-old SHR animals Cond fell by 8.1±6.3

μL/min/mmHg (*P<0.05* versus baseline) and remained depressed past 360s (4.7±3.4 μL/min/mmHg; *P<0.05* versus baseline).

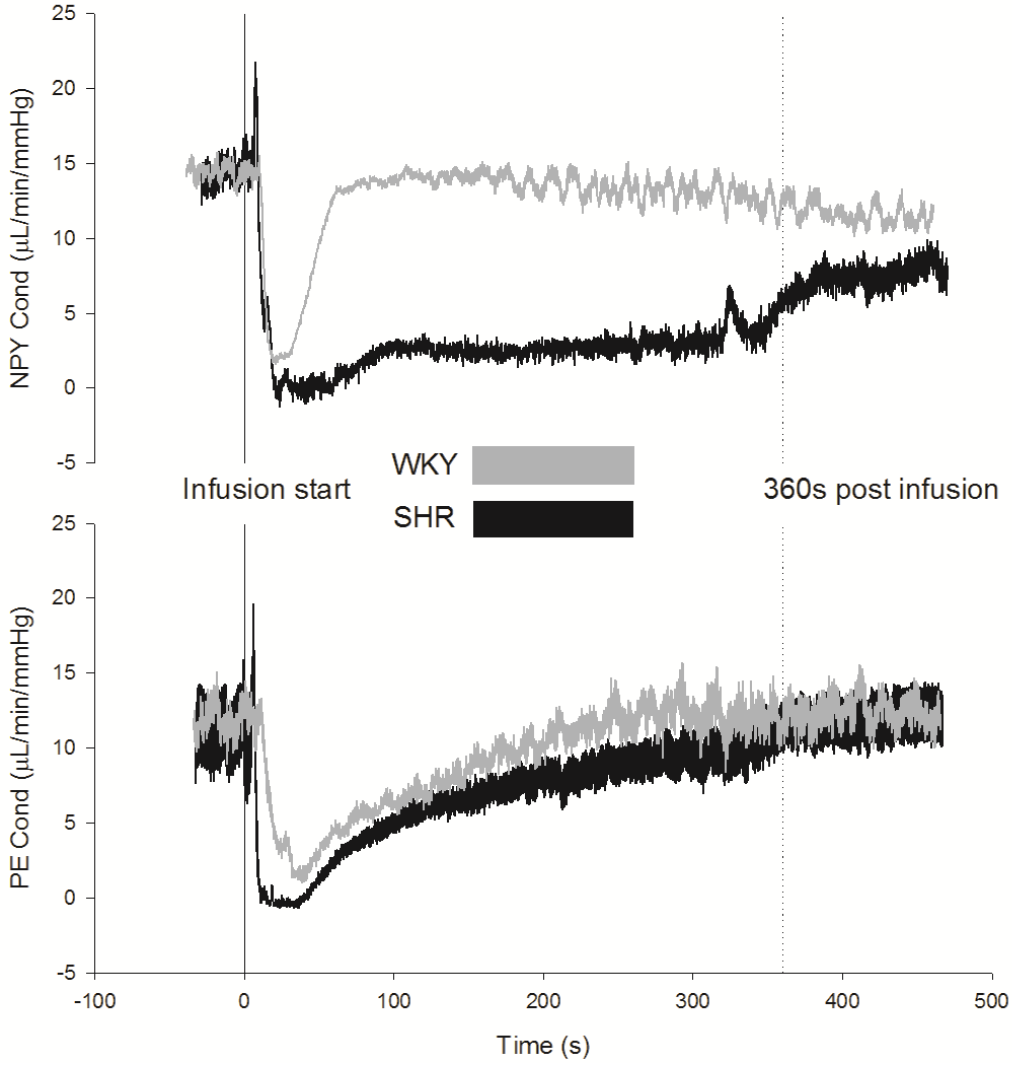

Figure 2.1 – Representative raw data tracings from 7 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats in response to both neuropeptide Y (NPY; 15µg/kg) and phenylephrine (PE; 2.5µg/kg). Solid line represents the start of drug infusion (0 seconds) and dashed line represents 360 seconds post infusion.

Table 2.1 – Non-invasive blood pressure and *in vivo* hemodynamic variables at baseline and following ganglionic blockade.

		NIBP	Anesthetized Baseline				Hexamethonium (25mg/kg)			
Strain	Age Group	MAP (mmHg)	MAP (mmHg)	HR (BPM)	Q_{Fem} (mL/min)	Cond (µL/min/mmHg)	MAP (mmHg)	HR (BPM)	Q_{Fem} (mL/min)	Cond (µL/min/mmHg)
WKY	3-5	102±21	68±12	391±52	0.49±0.35	7.5±6.2	59±6*	355±25	0.80±0.18*	13.6±2.8*
	7	128±19‡	72±13	314±73	0.69±0.35	9.9±5.6	67±2	348±51	0.91±0.37*	13.7±5.6*‡
	9	--	73±11	300±30‡	0.57±0.32	7.5±3.4	61±4*	365±22*	0.69±0.19	11.2±2.9
SHR	3-5	141±20†	89±19†	462±28†	0.57±0.42	6.7±4.7	64±9*	405±24†	0.70±0.15*	11.2±3.2*
	7	157±22†	94±21†	377±59‡†	0.97±0.64†	10.2±5.4	79±5*†	372±15‡	1.06±0.44‡	14.1±6.3†

Values are mean ± standard deviation. Spontaneously hypertensive rat = SHR; Wistar-Kyoto = WKY; non-invasive blood pressure = NIBP; mean arterial pressure = MAP, heart rate = HR, femoral blood flow = Q_{Fem} and femoral vascular conductance = Cond. * denotes $P<0.05$ versus baseline; † denotes $P<0.05$ versus age-matched WKY; ‡ denotes $P<0.05$ versus 3-5 week-old within the same strain.

Table 2.2 – Hemodynamic response prior to and following phenylephrine and neuropeptide Y.

Strain	Age (Weeks)	MAP (mmHg)	HR (BPM)	Q_{Fem} (mL/min)
Baseline (Prior to Drug Infusion)				
SHR(n=10)	3-5	85±17*	460±14*	0.50±0.34
WKY(n=11)	3-5	71±13	369±30	0.47±0.37
SHR(n=8)	7	97±11*	453±8*	1.32±0.51*
WKY(n=8)	7	80±18	351±46	0.68±0.56
PE				
SHR	3-5	98±18[a]	455±16*	0.29±0.22[a]
WKY	3-5	90±16[a]	366±22	0.33±0.33[a]
SHR	7	110±8[a]	448±8*	0.52±0.12[a]
WKY	7	99±22[a]	363±57	0.43±0.49[a]
NPY				
SHR	3-5	104±16[a]	452±15*	0.33±0.38[a]
WKY	3-5	95±18[a]	368±25	0.31±0.26[a]
SHR	7	120±14[a]	457±16*	0.34±0.25[a]
WKY	7	109±10[a]	332±13	0.45±0.55[a]

Values are mean ± standard deviation. Spontaneously hypertensive rat = SHR; Wistar-Kyoto = WKY; mean arterial pressure = MAP, heart rate = HR and femoral blood flow = Q_{Fem}; PE = phenylephrine (2.5µg/kg); NPY = neuropeptide Y (15µg/kg). * denotes $P<0.05$ versus baseline (of same age group). Main effect statistics: a denotes $P<0.05$ versus baseline.

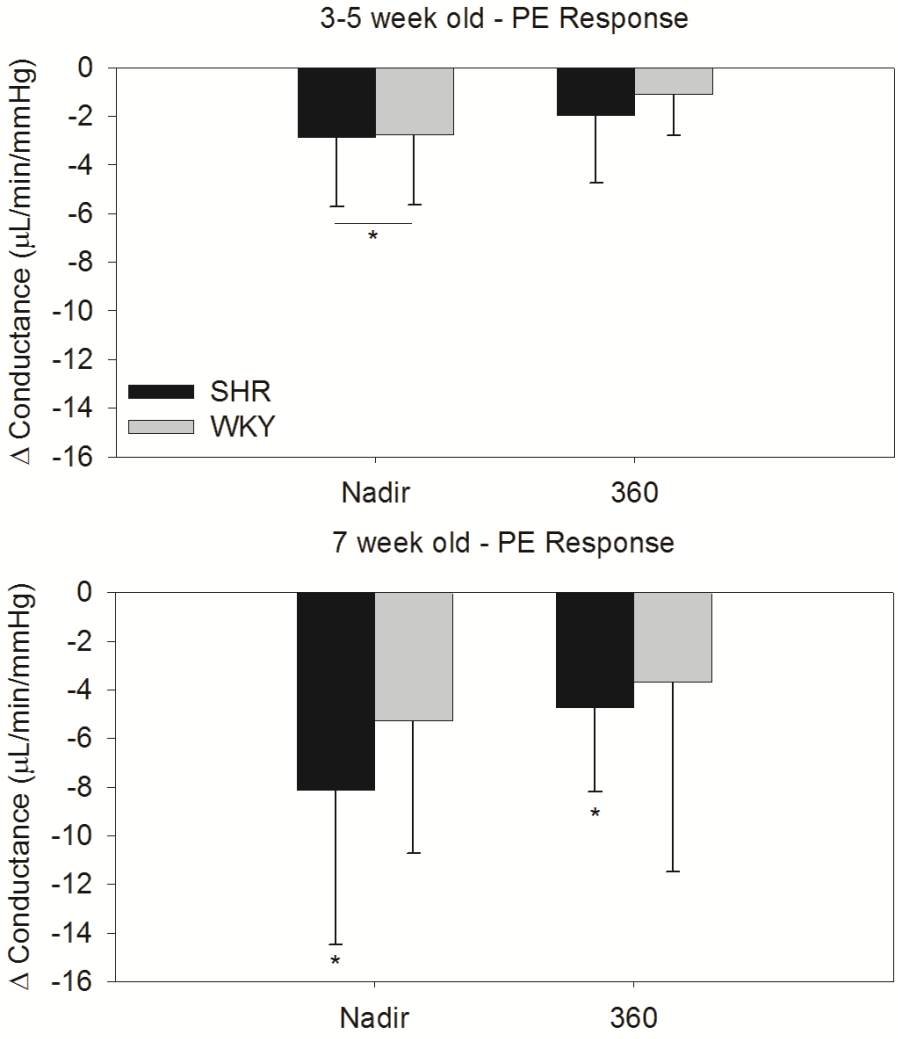

Figure 2.2 – The change in hindlimb conductance to phenylephrine (PE; 2.5µg/kg) in 3-5 week-old (top) and 7 week-old (bottom) spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. Nadir represents the minimum conductance reached and 360 represents 360 seconds post infusion. Values are mean ± standard deviation. * denotes $P<0.05$ versus baseline. Refer to Table 2.1 for baseline values.

Figure 2.3 depicts change in Cond in responses to NPY in both 3-5 and 7 week-old SHR and WKY animals (Figure 2.3; top and bottom respectively). In 3-5 week-old WKY animals, NPY reduced Cond by 4.0±4.1 μL/min/mmHg (*P<0.05* versus baseline) which returned to baseline by 360s (*N.S.*). With NPY infusion, femoral vascular conductance fell in the 3-5 week-old SHR animals by 2.5±2.3 μL/min/mmHg (*P<0.05* versus baseline) and returned to baseline by 360s (*N.S.*). In 7 week-old WKY animals, Cond was not significantly reduced (*N.S.* versus baseline), but fell by 5.9±3.4 μL/min/mmHg (*P<0.05* versus baseline) in 7 week-old SHR animals and remained depressed at 360s (2.8±2.7 μL/min/mmHg; *P<0.05* versus baseline).

The areas over the curve (AOC) for both NPY and NE in SHR and WKY animals were not different between either strain or drug at 3-5 week of age (Figure 2.4; top, *N.S.*). At 7 weeks of age, the AOC in response to PE was not different between the strains; however, the response to NPY was greater in SHR animals versus WKY animals (Figure 2.4; bottom; *P<0.05* versus WKY in NPY).

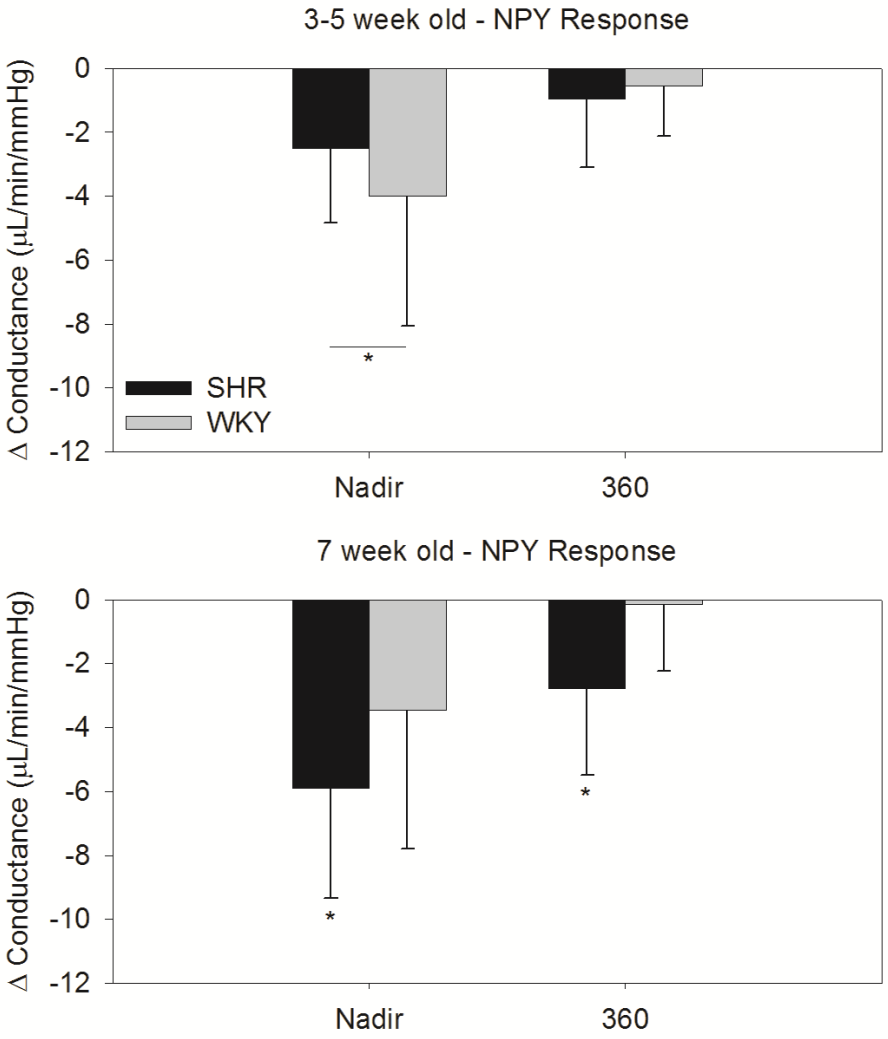

Figure 2.3 – The change in femoral vascular conductance to neuropeptide Y (NPY; 15 µg/kg) in 3-5 week-old (top) and 7 week-old (bottom) spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. Nadir represents the minimum conductance reached and 360 represents 360 seconds post infusion. Values are mean ± standard deviation. * denotes $P<0.05$ versus baseline. Refer to Table 2.1 for baseline values.

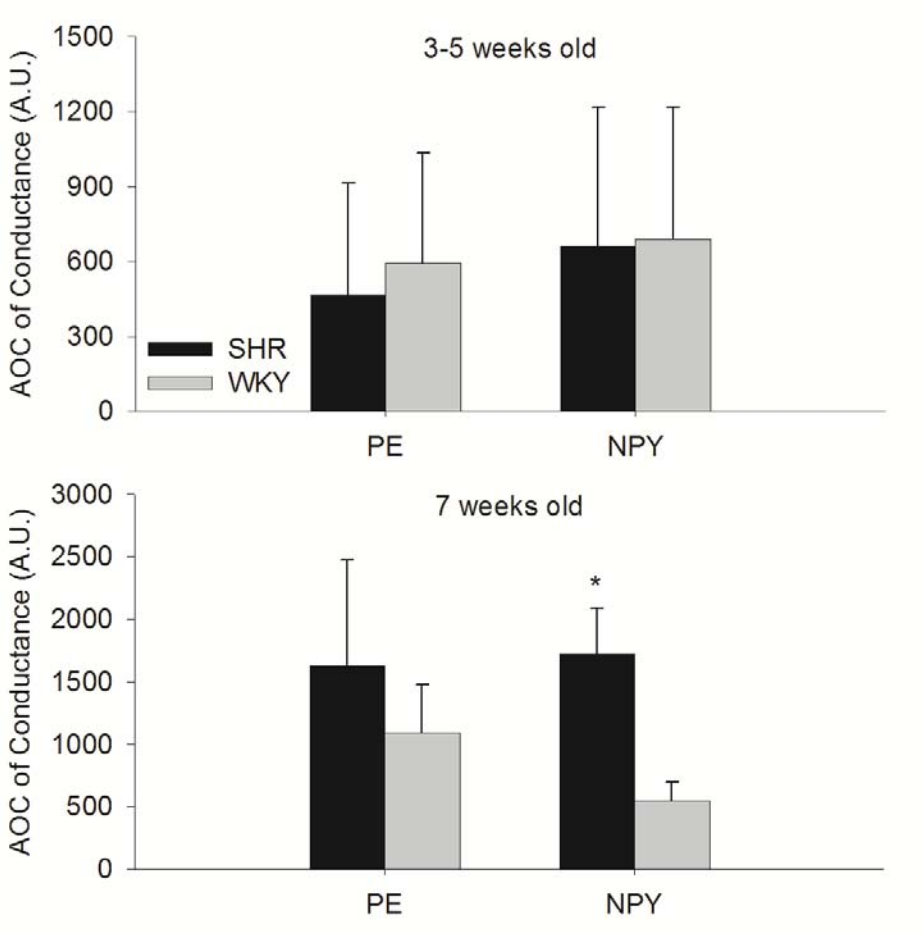

Figure 2.4 – Area over the curve (AOC) of femoral vascular conductance in response to phenylephrine (PE; 2.5 μg/kg) and neuropeptide Y (NPY; 15 μg/kg) in spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. Values are mean ± standard deviation. * denotes *P<0.05* versus WKY.

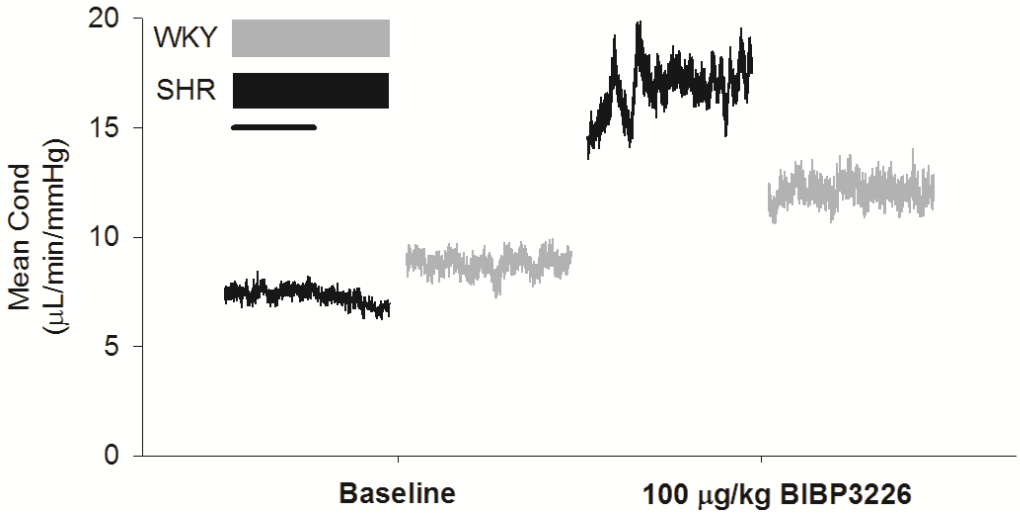

Figure 2.5 – Representative raw data tracing from 7 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats in response to BIBP3226 infusion. BIBP3226 data were taken approximately 10 minutes post infusion. Bar represents 50 seconds.

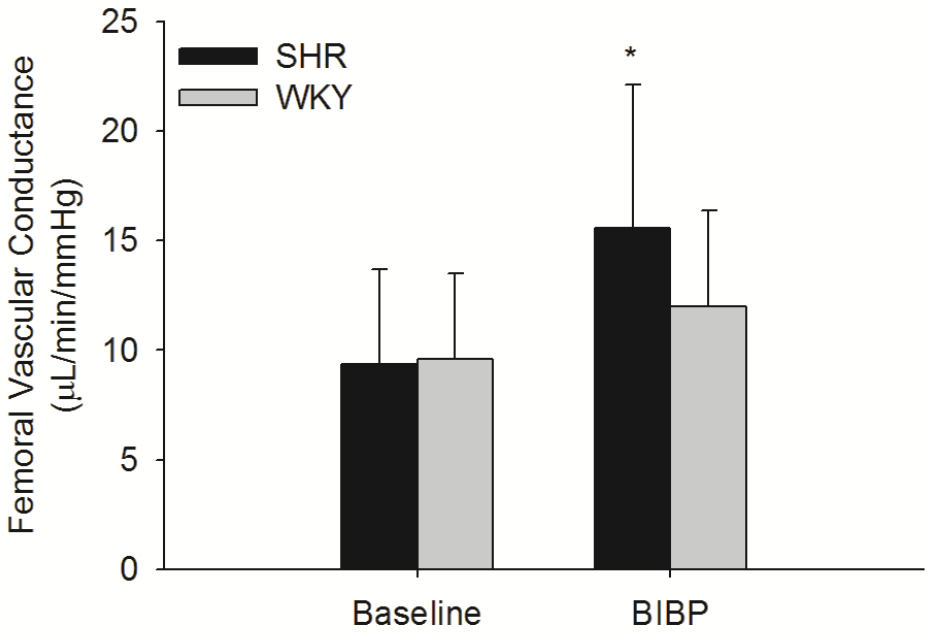

Figure 2.6 – Femoral vascular conductance in 7 week-old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats at baseline and following BIBP3226 (100µg/kg). Peak response occurred approximately 10 minutes after infusion. Values are mean ± standard deviation. * depicts *P<0.05* versus SHR baseline.

In the 7 week-old WKY animals, hindlimb conductance was 9.6±3.9 at baseline and 12.0±4.4 μL/min/mmHg (*N.S.* versus baseline) following BIBP3226 infusion. However, Cond in the 7 week-old SHR animals increased from 9.4±4.3 μL/min/mmHg at baseline to 15.5±6.5 μL/min/mmHg following BIBP3226 ($P<0.05$ versus baseline; Figure 2.6).

Following Hex administration, the hemodynamic responses in the 3-5 week-old group from both strains were similar whereby MAP decreased, HR decreased and Cond increased (Table 2.1). However, divergent responses were observed between the SHR and WKY animals at 7 weeks of age in response to Hex. Most notably, MAP was reduced in the SHR animals, while no changes were observed in 7 week-old WKY animals. It is noted here that the fall in MAP with Hex in the SHR group was not accompanied by a rise in heart rate which stands in contrast to the WKY group which experienced tachycardia. This observation raised the question as to whether the divergent responses between strains at 7 weeks were due to animal maturation and not hypertension *per se*. To address this question, the Hex approach was repeated in a subset of 9 week-old WKY animals. In this group ganglionic blockade evoked reductions in MAP and a rise in HR following Hex (Table 2.1). These responses were closer to those of the 7 week-old SHR animals than 7 week-old WKY animals.

Western Blot Analysis

Distinct bands of chemiluminescence were observed for the Y_1R, α-AR and TH. The Y_1R expression in SHR red vastus was 1.7±0.5 A.U. compared to 1.2±0.6 A.U. in the WKY animals ($P<0.05$; Figure 2.7, top). There were no differences between the strains in either α-AR (Figure 2.7, middle) or TH expression (Figure 2.7, bottom).

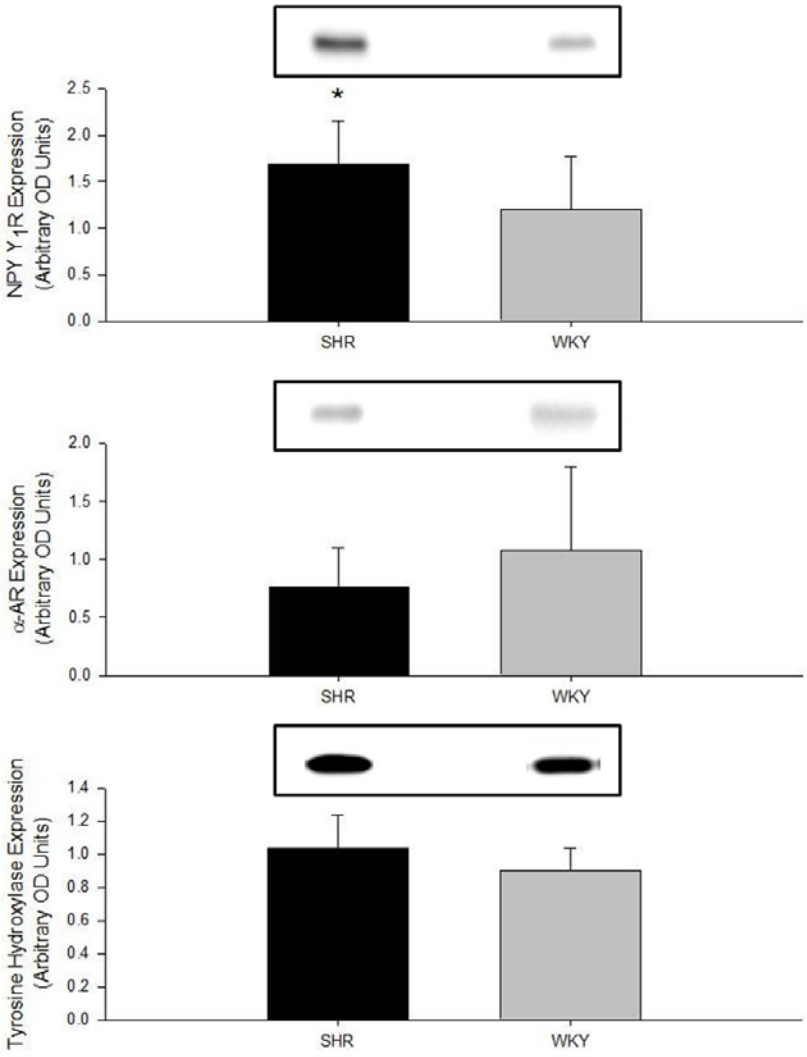

Figure 2.7 – Western blot analysis of the neuropeptide Y (NPY) Y_1 Receptor (Y_1R; top), α-adrenergic receptor (α-AR; middle) and tyrosine hydroxylase (TH; bottom) in the red portion of the vastus lateralis from spontaneously hypertensive (SHR; black bar) and Wistar-Kyoto (WKY; grey bar) rats. Insert in box - representative western blot for each protein. Values are mean ± standard deviation.* denotes *P<0.05* versus WKY.

2.4 Discussion

The main finding of the present study is that following a bolus of NPY, but not PE, 7 week-old SHR animals exhibited greater and prolonged reductions in Cond compared to age-matched WKY animals. Additionally, 7 week-old SHR animals displayed a greater dilatory response to Y_1R antagonism with BIBP3226 versus WKY animals, and the expression of the Y_1R in skeletal muscle was greater in SHR versus WKY rats. These data suggest that the involvement of the NPY system in cardiovascular regulation is upregulated in the early stages of hypertension.

The currently accepted role of the SNS in hypertension is heightened sympathetic activity leading to increased vascular resistance (3; 10). While it is traditionally thought that norepinephrine and the α-AR are primarily responsible for these effects (6; 23), the current data highlight an augmented role for the NPY system in the early stages of the condition. The divergent responses observed between the strains to the exogenous agonists (PE and NPY) only emerged at 7 weeks of age. The lack of differentiation between the strains to either PE or NPY at 3-5 weeks of age indicates that there appears to be a temporal onset of modified neurovascular coupling. In this context, it appears that the NPY system develops earlier in SHR animals than in the WKY strain.

Regulation of the NPY system could be altered in a number of ways: 1) enhanced NPY bioavailability following either increased neurotransmitter release and/or reduced neurovascular proteolytic breakdown, 2) modified Y_1R expression, and/or 3) altered post-receptor regulation of receptor sensitivity or intracellular contractile machinery. Here we examined the second option with emphasis on adrenergic and NPY-mediated neurovascular regulation. SHR and WKY animals displayed similar reductions in Cond

to exogenous PE and showed no difference in the expression of TH, the rate limiting enzyme in the production of norepinephrine, or in the α-AR expression, the primary receptor governing the VSMC response to norepinephrine. Therefore, we conclude that the adrenergic system is not modified between SHR and WKY animals at 7 weeks of age. In contrast, the increased expression of Y_1R in skeletal muscle observed in the SHR coincided with the heightened or prolonged *in vivo* responses to Y_1R activation or blockade. These data indicate that the NPY system of 7 week-old SHR animals plays a larger role in sympathetic control of hindlimb vasculature compared to age matched WKYs.

The main purpose of the Hex condition was to determine if ganglionic control over the cardiovascular system was maintained under anaesthesia. To this end, the current Hex results indicate that residual autonomic control was present whereby ganglionic blockade produced a reduction in blood pressure in both strains. This hypotensive response tended to be greater in the SHR (-28 and -16% change from baseline in 3-5 and 7 week old SHRs respectively) than WKY (-14, -6 and -16% change from baseline in 3-5, 7 and 9 week old WKYs respectively) suggesting a greater role for autonomic cardiovascular regulation in hypertensive animals. This is consistent with previous reports (2) of elevated sympathetic activation in SHR. The new observation of this study was that these differences are evident as early as 7 weeks of age.

However, an unexpected observation of the current study was the age-associated difference in the response to ganglionic blockade. While determining the mechanistic detail of neurovascular control in developing HT was not the purpose of the Hex trials, the observations deserve consideration. In both strains at 3-5 weeks of age, Hex caused

reductions in MAP and HR. The bradycardia *and* Hex-induced hypotension suggest immature reflexive cardiac control in 3-5 week-old animals. This pattern was modified by 7 weeks of age. The 7 week-old WKY animals demonstrated a smaller hypotensive response to Hex which was accompanied by the expected tachycardia, both of which were augmented to statistically significant levels by 9 weeks of age. In contrast, the SHR animals failed to mount a tachycardic response to Hex despite a marked fall in blood pressure. To examine whether the altered response to Hex by 7 weeks in SHR versus WKY animals was simply due to a more rapid maturation of neurovascular coupling in the SHR strain, a separate group of 9 week-old WKY animals was studied to see if they would mimic the 7 week-old SHR group. In this group, Hex induced a marked fall in MAP with the expected increase in HR. Thus, there appears to be important age-dependent alterations in neurovascular coupling in both strains, with the potential for aberrant development in the SHR strain that contributes to hypertension.

The augmented hindlimb vascular conductance to BIBP3226 infusion suggests the intriguing possibility that the heightened Y_1R regulation in 7 week old SHR animals may contribute to the early stages of HT. This observation is counter to reports from Y_1-receptor knockout mice (18) or from studies reporting an acute infusion of BIBP3226 does not affect basal blood pressure in normotensive or SHR rats (27). However these reports were conducted in older animals, and the response here may be specific to the initial stages of HT. Nonetheless, chronic activation of the Y_1R, independent of changes in blood pressure, is mitogenic resulting in vascular wall thickening and stiffening (19) which would have a concurrent impact on basal blood pressure and its long-term regulation.

2.5 Conclusion

The prolonged reduction in femoral vascular conductance observed in response to exogenous infusions of NPY in SHR but not WKY support the hypothesis that altered neurovascular regulation occurs in HT. Specifically, SHR animals appear to emphasize enhanced Y_1R regulation of hindlimb vascular conductance versus WKY animals. These *in vivo* observations were supported by an increased expression of Y_1R in skeletal muscle extracts from SHR while α-AR content was similar to WKY control animals.

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Chapter 3 – Y_1 Receptor versus sympathetic control of hindlimb vascular stiffness in spontaneously hypertensive rats.

3.1 Introduction

Chapter 2 examined the reaction of the vasculature to the sympathetic agonist's phenylephrine (PE) and neuropeptide Y (NPY). The vasculature of young spontaneously hypertensive rats (SHR) was more responsive to exogenously administered NPY versus the α-adrenergic receptor agonist PE when compared with normotensive Wistar-Kyoto rats (WKY). These differences were associated with an increased expression of the NPY Y_1 receptor (Y_1R) in SHR animals versus WKYs, while markers of adrenergic signaling (i.e. α-adrenergic receptor and tyrosine hydroxylase) were unchanged between the strains. Thus, it was concluded that enhanced Y_1R activation may influence the development of HT in the SHR animal.

Infusing an agonist can expose differences between strains in the responsiveness of the vasculature to a stimulus through alterations in receptor activation, signal transduction and/or second messenger signaling. However, this approach provides no information on the role of that receptor system on baseline or chronic receptor activation. Alternatively, by antagonizing a receptor, one can prevent signal transduction into the cell and uncover the role that a specific system (i.e. NPY and the Y_1R) plays in both normal and abnormal conditions. Whereas much emphasis has been placed on norepinephrine as the major sympathetic neurotransmitter affecting the development of HT, a growing body of evidence suggests that NPY plays an important role in HT (17; 23; 28; 29). The attention given to NPY is due, in part, to its physiologic actions which include potent and long-lasting vasoconstriction (27), potentiation of norepinephrine induced contraction (10), and mitogenesis (1). Each of these actions parallel a characteristic of established HT (25).

In 1989, Adams and colleagues (2) reported changes in perfusion pressure across a range of flow rates that suggested hindlimb vascular remodeling had occurred as early as 4-6 weeks of age in the SHR; vascular changes that may have even preceded the rise in blood pressure. This functional evidence of vascular remodeling occurred in concert with elevated tissue norepinephrine supporting the hypothesis that sympathetic hyperactivity early in the lifespan of SHR animals is an important contributor to the remodeling process (2; 16). The mechanism governing this sympathetic hyperactivity in HT is not clear but probably involves the potentiating impact of sympathetic neurotransmitters on local vascular growth factors (2; 6). It is notable that vascular remodeling will affect not only the long-term regulation of blood pressure but also the acute responsiveness of a vascular bed to neural inputs (22).

Evidence to date has emphasized the role of vascular resistance and the narrowing of blood vessels as a primary long-term determinant of HT (8; 9; 18). In addition, vascular stiffness is an important contributor to blood pressure and cardiac work as stiffer vessels transmit pressure waveforms to and from peripheral reflection points faster than normal. This causes the reflected pressure waveforms to return to the aorta during systole, therefore increasing systolic pressure and cardiac work, as opposed to diastole which actually aides in coronary perfusion (19; 20). Moreover, the role of the heightened sympathetic outflow on the stiffness and/or resistance of the intact hindlimb vascular bed in the early stages of HT have not been studied.

Therefore, the purpose of this study was to explore the acute roles of both the Y_1R in particular, and the sympathetic nervous system (SNS) in general, in regulating the mechanical properties of the rat hindlimb and how each might be modified by HT. We

tested the specific hypothesis that Y_1R activation contributed to vascular stiffness in the developmental stages of HT in the SHR model. Furthermore, we also tested the more general hypothesis that sympathetic activation per se (regardless of the neurotransmitters involved) affected vascular stiffness and viscoelasticity in the SHR hindlimb.

3.2 Methods

Experimental Animals

The following protocol was approved by the Animal Use-Subcommittee at The University of Western Ontario. Seven week old male Wistar-Kyoto (WKY; 207±16g, n = 7) and spontaneously hypertensive (SHR; 214±15g, n=10) rats were used in this experiment. Rats were housed in a light (12 h cycle) and temperature (22°C) controlled room in Plexiglas cages. Rats were allowed to eat (Prolab Rat chow, Mouse and Hamster 3000 Diet) and drink water *ad libitum.*

Data collection

All animals underwent an acute *in vivo* vasomotor control study. Animals were anesthetized with an intraperitoneal injection of α-chloralose (80 mg/kg) and urethane (500 mg/kg). The mixture of urethane and α-chloralose was used to minimize the impact of anesthetic on reflex cardiovascular control (14; 15). At a surgical depth of anesthesia, the right jugular vein was cannulated (PE50 tubing) so as to administer a dilute mixture of α-chloralose (8-16 mg/kg/hr) and urethane (50-100 mg/kg/hr). Mean arterial pressure (MAP; mmHg) was measured in the right common carotid artery using a fluid filled cannula (PE50 tubing) connected to a pressure transducer (model MLT844; ADInstruments Colorado Springs, CO) and bridge amplifier (model ML118 PowerLab Quad Bridge Amplifier; ADInstruments). Heart rate (HR; beats/min) was calculated

online using R-R peaks from sub-dermal ECG electrodes (model FD-E2; Grass Technologies West Warwick, RI) and a vital signs monitor (model 78353B; Hewlett Packard Palo Alto, CA) was used in conjunction with blood pressure and temperature (rectally) to ensure that the animal remained at a stable surgical depth of anaesthesia throughout the experiment.

The visceral organs were exposed via a midline incision in the abdomen. The right iliac artery was cannulated (PE50 tubing) to measure blood pressure at the level of the bifurcation of the aorta. Aortic blood flow (Q_{Aorta}) was measured just superior to the bifurcation of the aorta using a flowmeter (model TS420 Perivascular Flowmeter Module; Transonic Systems, Ithaca, NY) and Transonic flow probe (2.5 PSB). Pulse wave velocity (PWV; cm/s) was measured as described by Cosson et al. (4). Briefly, the time difference (T; seconds) between the diastolic foot of the pressure waveform at the carotid (proximal) and iliac (distal) arteries were measured. The distance (D; cm) between the catheter tips was measured post mortem, and PWV was calculated as (D/T; cm/s).

Baseline values were measured during a 10 minute period. To test the influence of the Y_1R in baseline cardiovascular control and vascular stiffness, a bolus of the selective Y_1R antagonist N-[(1R)]-4-[(Aminoiminomethyl) amino-1-[[[(4-hydroxyphenyl) methyl] amino] carbonyl] butyl-a-phenylbenzeneacetamide trifluoroacetate (BIBP3226; 100 µg/kg) was infused into the hindlimb. The peak response to BIBP3226 was measured approximately 10 minutes following the infusion. Once baseline values were re-established the neuronal nicotinic acetylcholine receptor agonist hexamethonium bromide (Hex; 25 mg/kg, Sigma-Aldrich, St. Louis, MO) was introduced systemically to abolish post-ganglionic nerve activity. Once a new hemodynamic baseline was achieved, 10

minutes of data were recorded. All drugs were purchased from Sigma-Aldrich (St. Louis, MO) with the exception of BIBP3226 (Tocris Bioscience; Ellisville, MO).

Data Analysis

MAP (mmHg), HR (beats/min), Q_{Aorta} (mL/min), PWV (cm/s), and compliance of the distal aorta ($C_{Local} = [\Delta Q x \Delta T]/\Delta P$; mL/mmHg, Figure 3.1) were calculated. C_{Local} is a surrogate of the local compliance at the level of the distal aorta. The traditional method for assessing cardiovascular regulation is by examining changes in vascular resistance (*R*) which, fundamentally, reflects a steady-state level of vascular diameter. Thus, by itself, *R* relates information regarding the steady component of blood flow alone (13; 21). Since blood flow is pulsatile, it incorporates both steady state and oscillatory blood flow (19). In contrast with *R*, the stiffness of a vessel, or vascular bed, is related to the oscillatory nature of blood flow in distensible vessels. In a pulsatile system, it is necessary to examine the *impedance* which reflects the frequency-specific resistance and is expressed in the compliance (*C*) and viscoelasticity (*K*) of the vessel wall, and the inertia (*L*) of blood and vessel tissue (30). Thus, the vascular resistance (*R*) and mechanical properties (*C*, *K* and *L*) of the hindlimb vascular bed were estimated using the aortic flow and pressure waveforms measured at the distal end of the aorta using a modified Windkessel model (30; 31). Briefly, the model modifies the values of *C*, *K* and *L* to predict a flow waveform that would be produced from the measured pressure waveform. The predicted waveform is then compared iteratively with the measured flow waveform until the best agreement is achieved, providing the values of *C*, *K* and *L*.

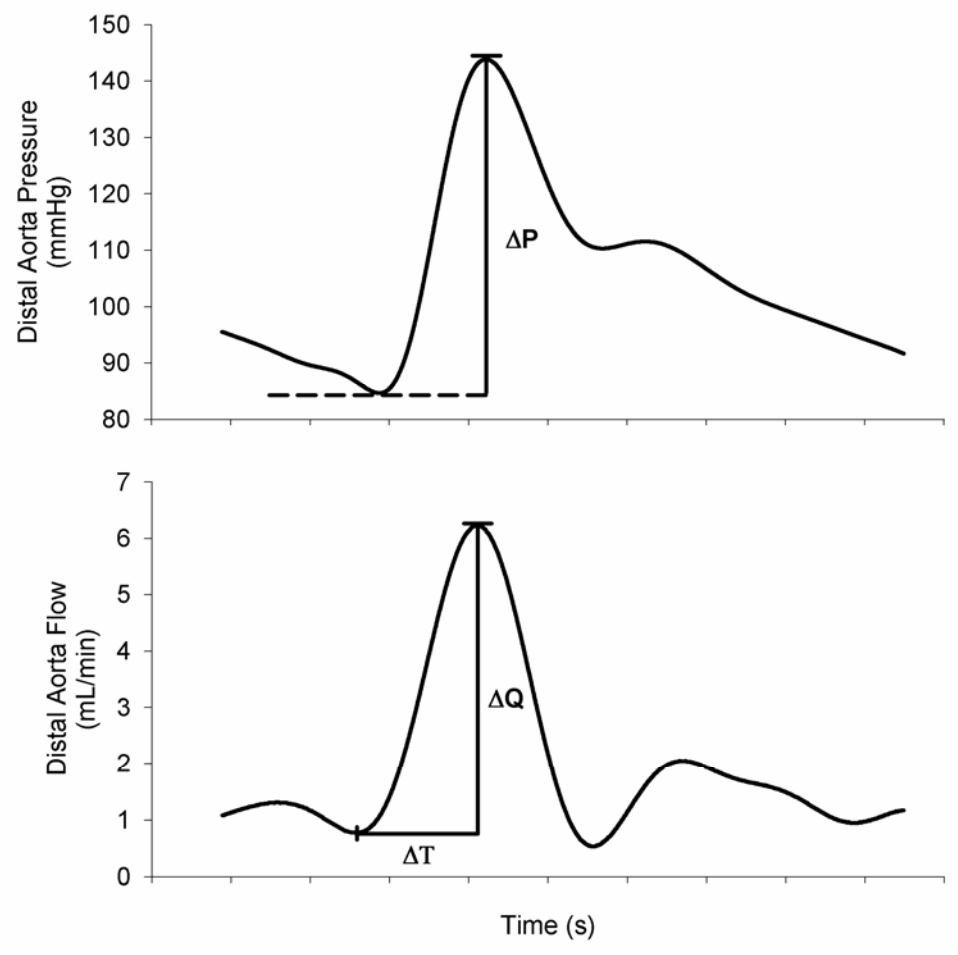

Figure 3.1 – Calculation of local aortic compliance (C_{Local}). Beat-by-beat averages of 10 consecutive distal aortic pressure (P, top) and flow (Q, bottom) waveforms were averaged. C_{Local} was calculated as the change in flow (ΔQ; mL/min) multiplied by the time to peak flow (ΔT; min) divided by the pulse pressure (ΔP; mmHg). Thus, C_{Local} = [ΔQxΔT]/ΔP; mL/mmHg.

Statistics

The effects of rat strain (SHR/WKY) and drug (baseline/BIBP3226/Hex) were compared using a mixed two way analysis of variance (ANOVA; SAS 9.1, Cary, NC, USA). Statistical significance was accepted at $P<0.05$. Tukey's *post hoc* analysis was used to identify differences in main effects and interactions. All data are expressed as mean ± standard deviation.

3.3 Results

Hemodynamics

SHR animals had higher MAP compared to WKYs (Figure 3.2 I; main effect of strain, $P<0.05$). BIBP3226 had no effect on MAP in either strain; however following Hex, MAP fell (main effect of drug, $P<0.05$ versus baseline and BIBP3226). Q_{Aorta} was higher in the WKY animals (data not shown; main effect of strain, $P<0.05$) and neither BIBP3226 nor Hex had any effect on Q_{Aorta}. Vascular resistance (R = MAP/Q_{Aorta}) was greater in SHRs compared to the WKYs at baseline and during BIBP3226, but not during Hex infusion (Figure 3.2 II; strain x drug interaction, $P<0.05$). BIBP3226 infusion had no effect on R in either strain, but following Hex R fell in the SHRs only ($P<0.05$ versus baseline and BIBP3226 in SHR only).

Systemic Vascular Stiffness

At baseline, PWV was greater in the SHRs compared to the WKYs (Figure 3.2 III; main effect of strain, $P<0.05$). Compared with baseline, PWV was unaltered following BIBP3226 infusion but was decreased following Hex in both strains (main effect of drug, $P<0.05$ versus baseline and BIBP3226).

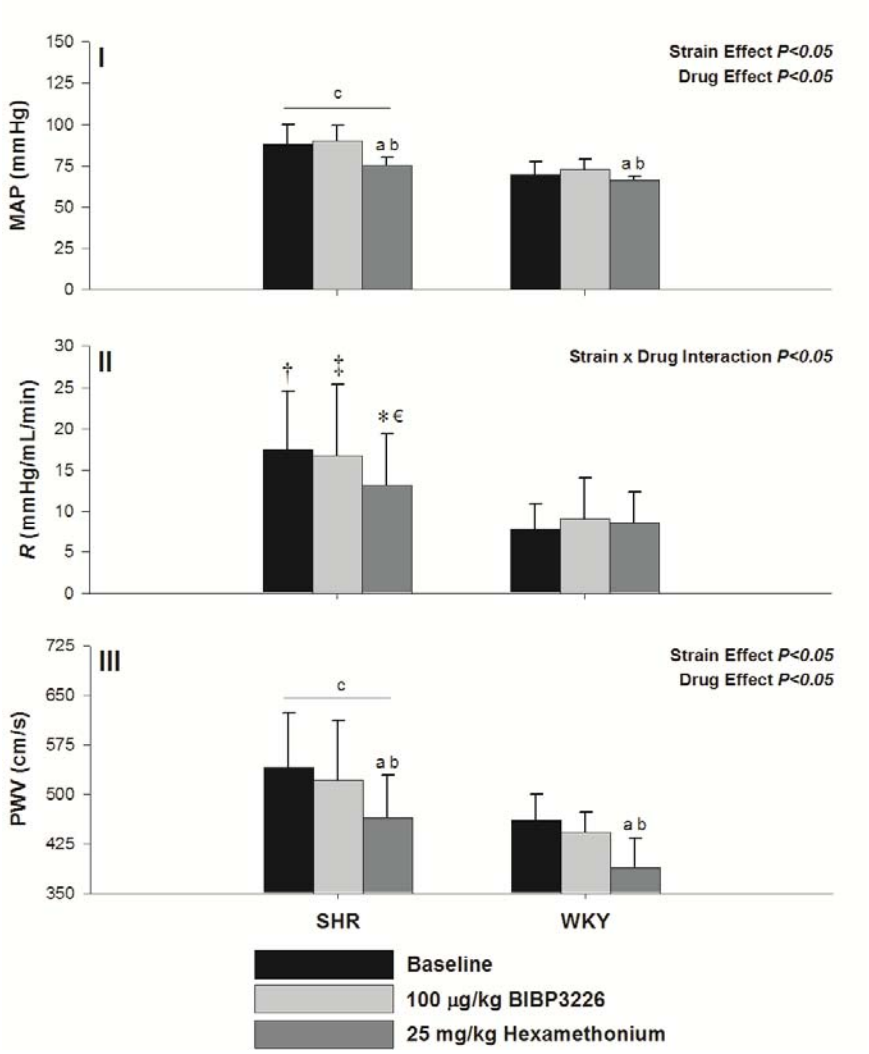

Figure 3.2 – Mean arterial pressure (MAP; I), caudal aortic vascular resistance (*R*; II) and pulse wave velocity (PWV; III) from young hypertensive (SHR) and normotensive (WKY) rats. Values are mean ± standard deviation. *, significantly different from baseline (within a strain); €, significantly different from BIBP3226 (within a strain); †, significantly different from WKY baseline; ‡, significantly different from WKY BIBP3226. Main effect statistics – a, significantly from baseline; b, significantly different from BIBP3226; c, significantly different from WKY. All statistics $P<0.05$

Hindlimb Vascular Mechanics

When compared with WKY, *C* and C_{Local} were lower in SHR (Figure 3.3 I and II respectively; main effect of strain, $P<0.05$). BIBP3226 infusion had no effect on hindlimb vascular compliance in either strain (*N.S.* versus baseline) but both *C* and C_{Local} were increased in both strains following Hex (main effect of drug, $P<0.05$ versus both baseline and BIBP3226). *K* was greater in SHRs compared with WKYs (Figure 3.3 III; main effect of strain, $P<0.05$) and *K* was reduced in both strains following Hex only (main effect of drug, $P<0.05$ versus baseline and BIBP3226).

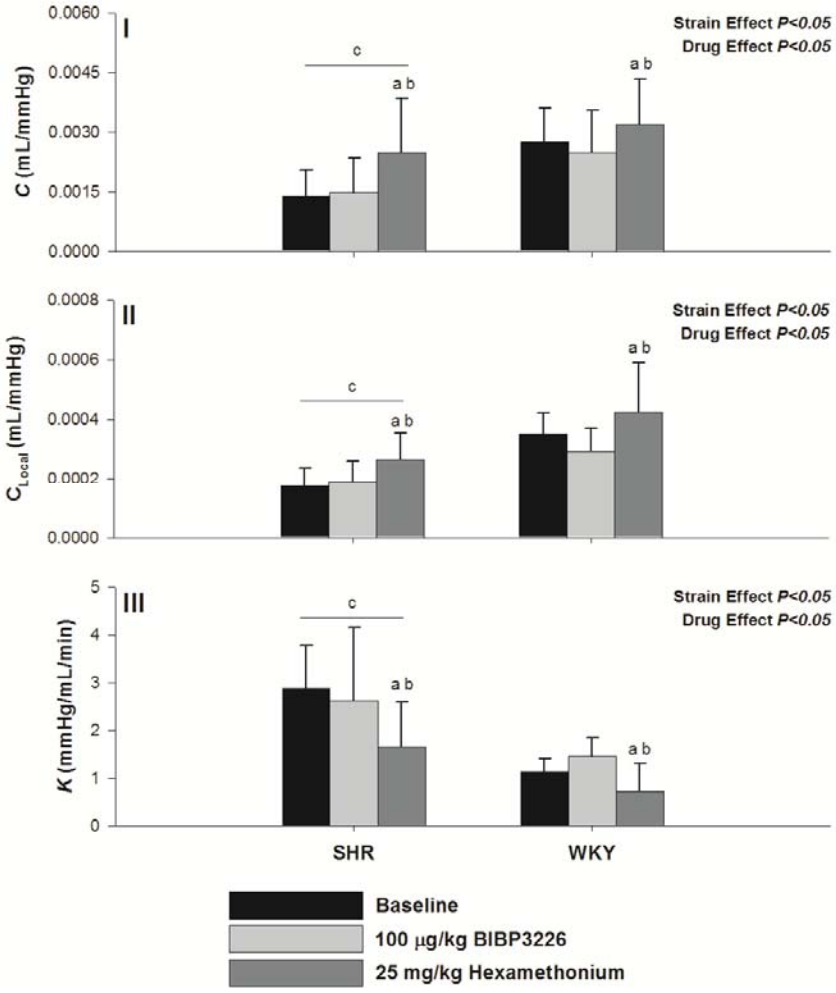

Figure 3.3 – Hindlimb vascular bed compliance (*C*; I), local aortic compliance (C_{Local}; II) and viscoelasticity (*K*, III) of the lumped hindlimb vascular beds of young hypertensive (SHR) and normotensive (WKY) rats. Values are mean ± standard deviation. Main effect statistics – a, significantly from baseline; b, significantly different from BIBP3226; c, significantly different from WKY. All statistics $P<0.05$

3.4 Discussion

The purpose of this study was to assess the role of the NPY Y_1R specifically, and the SNS generally, in regulating the mechanical properties of the rat hindlimb, and whether this regulation is modified in the early stages of HT. In particular, emphasis was placed on the impact of Y_1R under baseline conditions. As expected, vascular stiffness and resistance were greater in the SHR versus WKY animals. These differences were present regardless of whether the measurements were made locally (e.g., *C* or C_{Local} in the hindlimb) or systemically (e.g., PWV). Contrary to our specific hypothesis the NPY Y_1R has little regulatory control of hindlimb vascular mechanics during developing HT. Therefore, the SNS does exert some control over the hemodynamic and mechanical properties of the rat hindlimb, which are presumably mediated by adrenergic and/or purinergic mechanisms.

Vascular Resistance

Changes in *R* have been used to reflect vasomotor contractile state and sympathetic neurovascular control in cardiovascular research for many years (12). Enhanced SNS activity is routinely seen in HT, and it is suspected that increased *R* accounts for some of the augmented pressure (3; 5). This study confirms that the SNS is responsible for the increased *R* seen in HT at 7 weeks of age. *R* was unaffected by Hex in the normotensive WKY animals which is surprising considering other groups have reported Hex induced falls in regional vascular resistances in a variety of vascular beds in WKYs (26). These regional reductions in *R* were measured in conscious unrestrained animals therefore; the effect of anaesthesia in reflex cardiovascular control must be addressed. Data from our lab indicates that the urethane/α-chloralose cocktail used here

does not impair autonomic reflexes in response to lower body negative pressure (Usselman et al., *In Press*). Thus, *R* in the WKYs appears to be independent of sympathetic activity at 7 weeks of age.

Vascular Stiffness

A hallmark of HT is vascular stiffening which leads to faster pressure wave reflections and increased afterload on the left ventricle (20). Here, we have shown that such stiffening occurs early in the development of HT, namely, in 7-week old SHR animals, and this stiffening is not restricted to a particular region or level of the vascular tree but appears to be a generalized feature of SHR at 7 weeks of age. Moreover, changes in *C*, C_{Local}, PWV and *K* were observed in WKY animals following Hex, without a change in *R*. Therefore, the mechanics of the vasculature can be studied independently from vascular resistance, supporting earlier observations from our lab (30).

Y_1R antagonism resulted in little change in PWV, C_{Local} or hindlimb *C* in either strain. Therefore, the current results do not support the hypothesis that Y_1R activation is, by itself, an important contributor to vascular stiffening in SHR. However, ganglionic blockade did affect hindlimb vascular properties. Nonetheless, an important unresolved question in the study of the developing of HT is whether the observed stiffening of the vasculature is due to chronic remodeling or to more acute changes in sympathetic activation. One interpretation of the current results is that baseline levels of SNS activation contribute to vascular stiffness in both SHRs and WKYs. However, it is noted that, following Hex, *C* and C_{Local} increased whereas PWV and *K* fell with a concomitant fall in MAP. Therefore, the involvement of myogenic contributions to these variables cannot be ruled out. Additionally, changes in *C* may reflect the compliance of the

vascular bed at a different point on the same pressure-volume curve. As such, the changes in compliance observed may not indicate an actual shift in the mechanical properties of the vasculature.

Viscoelasticity

To our knowledge, this is the first attempt to examine the impact of sympathetic control on the viscoelastic properties of the vasculature and how it is affected by HT. The first observation was that *K* was much greater in SHR animals compared with WKY controls at baseline. Thus, viscoelastic resistance to vascular distension is greater in SHR and could contribute to augmented blood pressure. Second, *K* was unaffected by Y_1R antagonism but was lower following ganglionic blockade in both strains. Thus, the augmented *K* in young SHR appears to be due primarily to neural regulation and not chronic changes in the vascular wall matrix as would be predicted by vascular remodeling. In other words, it is the contractile component of the vascular bed that is causing the elevated value of *K* in SHR. Thus, the neurogenic regulation of *K* may be treated as an abnormality associated with the HT. This supposition awaits further experimentation as altered vascular wall structure may also affect its contractile properties (22).

Limitations

A major limitation to this study is the absence of measured sympathetic nerve activity. Thus, it is assumed that sympathetic outflow was elevated in the SHR animals, an assumption that has been validated previously (see (7) for a detailed review).

Using a chemical agent to antagonize ganglionic neural transmission, we can reasonably assume that complete ganglionic blockade occurred with Hex as previously

published reports (11; 24) indicate that 20mg/kg was a sufficient dose to completely block post-ganglionic neural transmission as measured in the renal nerve in both SHR and WKY animals.

3.5 Conclusion

The current results suggest that the increased vascular resistance in 7-week old SHR animals is neurally mediated, but does not involve the NPY Y_1R. Markers of vascular stiffness (*C*, C_{Local}, *K*, and PWV) displayed some neural control in both strains, but again, the Y_1R did not participate. Thus, it appears that neural activation contributes to baseline vascular stiffening and resistance in early stages of hypertension. However, this neural involvement does not include Y_1R activation. Nonetheless, levels of hindlimb *R* in 7-week old WKY animals appear to be independent of neural activation. These observations contrast with the prolonged Y_1R activation in young SHR in response to agonist infusion and highlight the importance of non-NPY sympathetic neurotransmitters in chronic vasomotor control in young SHR. Therefore, the current results suggest that increased vascular stiffness is evident early in HT, and this stiffening appears to be under neural control.

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Chapter 4 – Sympathetic Contribution to Peripheral Vascular Stiffness in Young Female Spontaneously Hypertensive Rats.

4.1 Introduction

The etiology of essential hypertension (HT) is complex and includes both a sustained increase in sympathetic nervous system (SNS) activity (4; 10; 22; 34; 44) that begins early in life (3; 8; 38), and a remodeled vascular bed (25). Evidence is growing which indicates that male and female animals differ in a number of physiological parameters (12; 13; 24; 26; 35), but the reason for these differences remains unknown. This chapter explores the mechanical properties of the vasculature during development of HT in young female animals. As figure 1.6 indicates, the prevalence of HT in humans displays sexual dimorphism. These differences are evident in the youngest age bracket (20-34 years) which indicates that the processes responsible are present throughout life, but appear to change with age (1). Unlike chapters 2 and 3, this chapter does not include agonist or antagonist interventions, but focuses solely on hemodynamic and mechanical properties of the vasculature at baseline and following the administration of hexamethonium bromide. Agonist stimulation was not included in the analysis because stable hindlimb blood flow values were unattainable in young female animals (See Appendix A.1). Also, antagonism of the neuropeptide Y (NPY) Y_1 receptor (Y_1R) was not performed, because, the NPY and Y_1R system contributes little to baseline blood flow control in female rats (13-15), and also because chapter 3 suggests that the Y_1R does not regulate the mechanical properties of the vasculature.

It is generally accepted that enhanced SNS activity and vascular remodeling, combine to enhance blood pressure through changes in vascular resistance (*R*) and stiffness (2; 6; 27; 32) (Figure 4.1). However, *R* reflects vascular resistance to only the steady component of blood flow (17; 31) and, therefore, has limited diagnostic function

in the context of the oscillatory component of blood flow (29). To better understand the mechanisms responsible for controlling pulsatile (steady plus oscillatory) blood flow, it is necessary to consider the *impedance* rather than *R* (40). The impedance in pulsatile blood flow involves *R*, but also the compliance (*C*) and viscoelasticity (*K*) of the vessel wall, and the inertia (*L*) of blood and vessel tissue (41). These properties are involved in the regulation of blood flow, yet the role that the SNS plays in regulating *C* and *K* has yet to be elucidated (Figure 4.1). Previously, it was shown that the brachial artery (33) and the forearm vascular beds (41) of humans stiffen during reflex maneuvers that elevate sympathetic outflow. The particular impact of sympathetic neural control of vascular mechanics was not assessed in these earlier studies. Thus, the neurogenic regulation of vascular mechanics is not known in either central or peripheral vascular beds, particularly in the context of developing HT. Also, little is known about the role of vascular remodeling in modifying neurogenic vasomotor control. Vascular remodeling is typically manifest as vascular smooth muscle hypertrophy (11), leading to wall thickening and increased arterial stiffness. In common practice, vascular stiffness is determined by measuring pulse wave velocity (PWV) along an arterial segment (9; 21) and is then used as a *global* measure of vasular stiffness. In this process, the measure of PWV does little to determine whether the stiffness is located centrally or peripherally, or what the root cause of the stiffness is in terms of mechanical properties of the vessel wall that affect its stiffness.

Hypertension develops early in the life of spontaneously hypertensive rats (SHR; approximately 5-weeks of age) where an elevated sympathetic outflow is often observed (23). Also, neonatal sympathectomy is known to consistently prevent the development of

high blood pressure in SHR (18; 37). Thus, while the involvement of the sympathetic nervous system in HT is generally suspected, little is known about how the SNS actually participates in the development of the disease. Furthermore, in general, more is known about HT in male rats, but little is known about the disease in young female rats.

The aim of our study was to explore the role of the SNS in both central arteries and peripheral vascular beds of young normotensive and hypertensive female rats. In conjunction with measures of PWV, we quantify *C*, *K*, and *L* in the hindlimb vascular bed of the spontaneously hypertensive rat (SHR) and thus examine the role of the SNS in regulating their values. With this approach we tested the hypotheses that a) vascular stiffness and viscoelasticity are under neurogenic regulation, and b) that this regulation is modified during the development of HT.

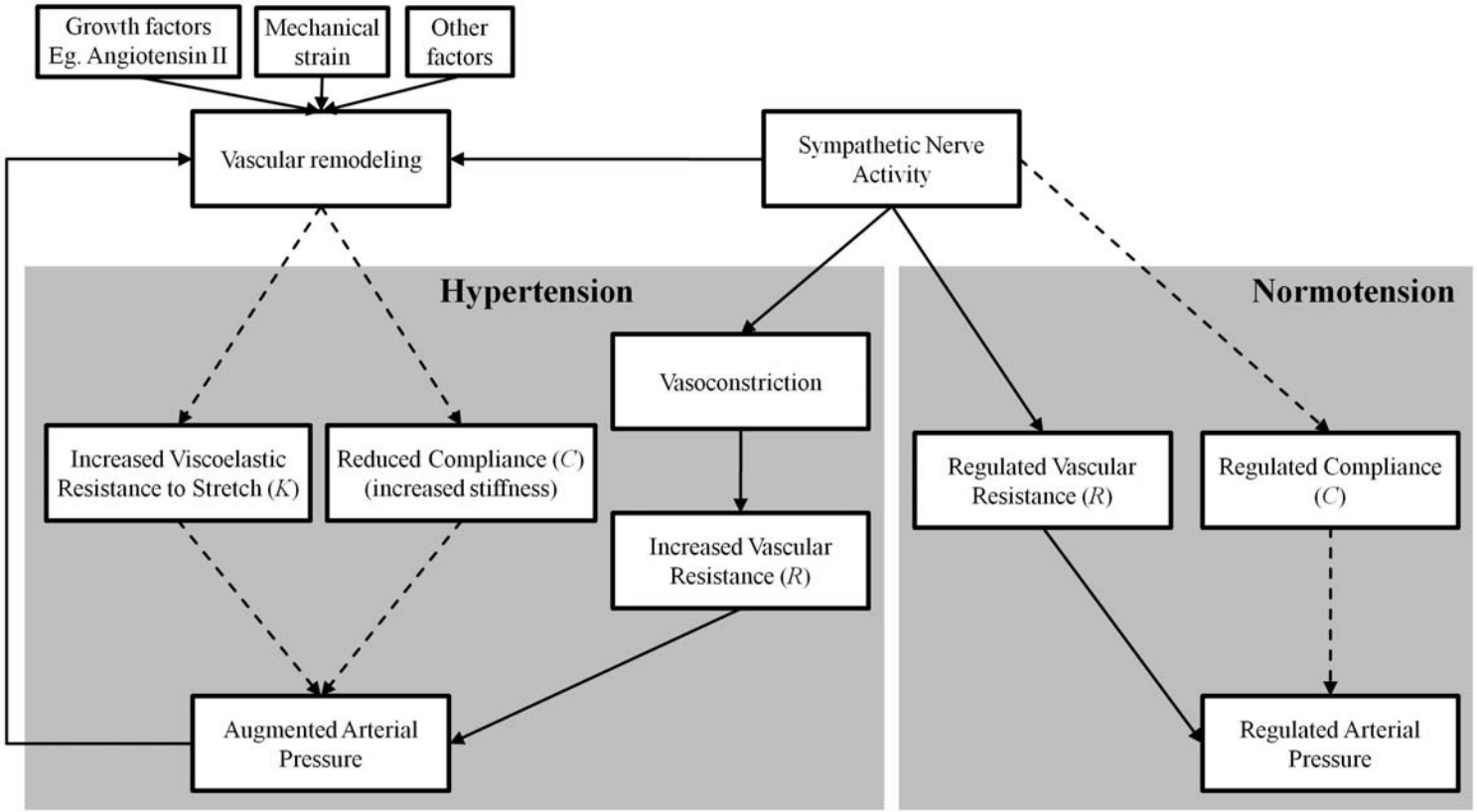

Figure 4.1– Schematic of currently accepted (solid arrows) and proposed (dashed arrows) pathways for the effects of sympathetic nerve activity on mean arterial pressure.

4.2 Methods

Animals

This protocol was approved by the Animal Use Subcommittee at the University of Western Ontario. Young female Wistar-Kyoto (WKY; 148±7g, n=7) and Spontaneously Hypertensive (SHR; 134±6 g, n=6) rats were used in this experiment (Charles River Laboratories, Montreal, Canada). Rats were housed in a light (12 h cycle) and temperature (22°C) controlled room in Plexiglas cages. Rats were allowed to eat (Prolab Rat chow, Mouse and Hamster 3000 Diet) and drink water *ad libitum.*

Data Acquisition

At approximately 7 weeks of age animals were anesthetized by an intraperitoneal cocktail of α-chloralose (80 mg/kg) and urethane (500 mg/kg) to ensure minimal impact on reflex cardiovascular control (19; 20). Once a surgical depth of anaesthesia was achieved, the right jugular vein and common carotid artery were exposed and cannulated with PE50 tubing to allow the delivery of dilute anaesthetic (α-chloralose 8-16 mg/kg/hr and urethane 50-100 mg/kg/hr) and measure arterial blood pressure (pressure transducer model MLT844, quad bridge amplifier model ML118; ADInstruments Colorado Springs, CO), respectively. Heart rate (HR; beats/min) was calculated online using R-R peaks from sub-dermal ECG electrodes (model FD-E2; Grass Technologies West Warwick, RI) and a vital signs monitor (model 78353B; Hewlett Packard Palo Alto, CA) was used in conjunction with blood pressure and temperature (rectally) to ensure that the animal remained at a stable surgical depth of anaesthesia throughout the experiment.

A midline incision was made in the abdomen to expose the visceral organs. The right iliac artery was cannulated with PE50 tubing. The cannula was inserted rostrally to

measure blood pressure at the bifurcation of the aorta into the two iliac arteries. Aortic blood flow (Q_{Aorta}) was measured just superior to the bifurcation of the aorta using a flowmeter (model TS420 Perivascular Flowmeter Module; Transonic Systems, Ithaca, NY) and Transonic flow probe (2.5 PSB). PWV was measured as described by Cosson et al. (5). Briefly, the time difference (T; seconds) between the diastolic foot of the pressure waveform at the carotid (proximal) and iliac (distal) arteries were measured. The distance (D; cm) between the catheter tips was measured post mortem, and PWV was calculated as (D/T; cm/s).

The animals were allowed to recover from surgery for 1 hour. Baseline values were measured during a 10 minute period after which the neuronal nicotinic acetylcholine receptor agonist hexamethonium bromide (Hex; 25 mg/kg, Sigma-Aldrich, St. Louis, MO) was introduced systemically to abolish post-ganglionic neural activity. Once a new hemodynamic baseline was achieved, 10 minutes of data were recorded.

Following data acquisition, animals were euthanized with an overdose of anaesthetic. Femoral arteries were harvested distal to the inguinal crease, cleaned of excess connective tissue, stabilized overnight in 15% sucrose, and then fixed overnight in 10% neutral buffered formalin (HT501320, Sigma-Aldrich). Fixed arteries were mounted in clear frozen section compound (VWR, Cat. No. 95057-838) and snap frozen. Mounted arteries were sectioned (7μm) using a Lauda-Leitz (model 1720) digital kryostat, mounted on Fisherbrand Superfrost/plus microscope slides (Cat. No. 12-550-15), and stained using a Modified Verhoeff Van Gieson Elastic Stain Kit (HT25A, Sigma-Aldrich).

Data Analysis

Mean arterial pressure (MAP; mmHg), HR (beats/min), Q_{Aorta} (mL/min), pulse wave velocity (PWV; cm/sec), and local compliance of the aorta ($C_{Local} = [\Delta Q x \Delta T]/\Delta P$ of the distal aorta; mL/mmHg, See Figure 3.1) were calculated. C_{Local} was used as a local measure of compliance at the level of the distal aorta. The vascular resistance (*R*) and mechanical properties (*C*, *K* and *L*) of the hindlimb vascular bed were assessed using the measured aortic flow and pressure waveforms in conjunction with a modified Windkessel model (41; 42). Briefly, the model predicts the flow waveform that would be produced by the measured pressure waveform. The predicted waveform is then compared with the measured flow waveform to determine the values of *C*, *K* and *L* (see Figure 1.5).

Sections of femoral artery were visualized using a Zeiss Axiovert S100 microscope, with a Sony Power HAD 3CCD colour video camera. Femoral artery wall thicknesses were measured using Northern Eclipse V8 (Empix Imaging Inc., Cheektowaga, NY, USA).

Statistics

The effects of rat strain (SHR/WKY) and drug (baseline/Hex) were compared using a mixed two way analysis of variance (ANOVA; SAS 9.1, Cary, NC, USA). Main effects of the ANOVA (SHR/WKY and baseline/Hex) were considered significant at $P<0.05$, and where interactions in the ANOVA were significant, again at $P<0.05$, Tukey's *post hoc* analysis was used to identify differences. Wall thicknesses were compared using an unpaired, equal variance Student's T-Test. Data are presented as mean ± standard deviation.

4.3 Results

Hemodynamic alterations following Hex

The results confirm that the hypertensive animals used in the experiment had higher baseline MAP compared to WKY animals (Figure 4.2 I; $P<0.05$ at baseline). Following Hex, MAP fell in both strains ($P<0.05$) but to a larger extent in SHRs, resulting in similar MAP between strains (*not significant*; *N.S.* following Hex). Q_{Aorta} was higher in the WKYs, (Figure 4.2 II; $P<0.05$); however, some of this difference can be attributed to the larger size of the WKYs compared to the SHRs (148±7g versus 134±6g, respectively; $P<0.05$). Systemic vascular resistance (R = MAP/Q_{Aorta}) was greater in SHRs compared to the WKYs (Figure 4.2 III; main effect of strain; $P<0.05$), and Hex lowered R in both strains (main effect of drug; $P<0.05$). PWV was higher in SHR animals at both baseline and following Hex (Figure 4.2 IV; both $P<0.05$), but Hex caused a reduction in PWV in both strains ($P<0.05$).

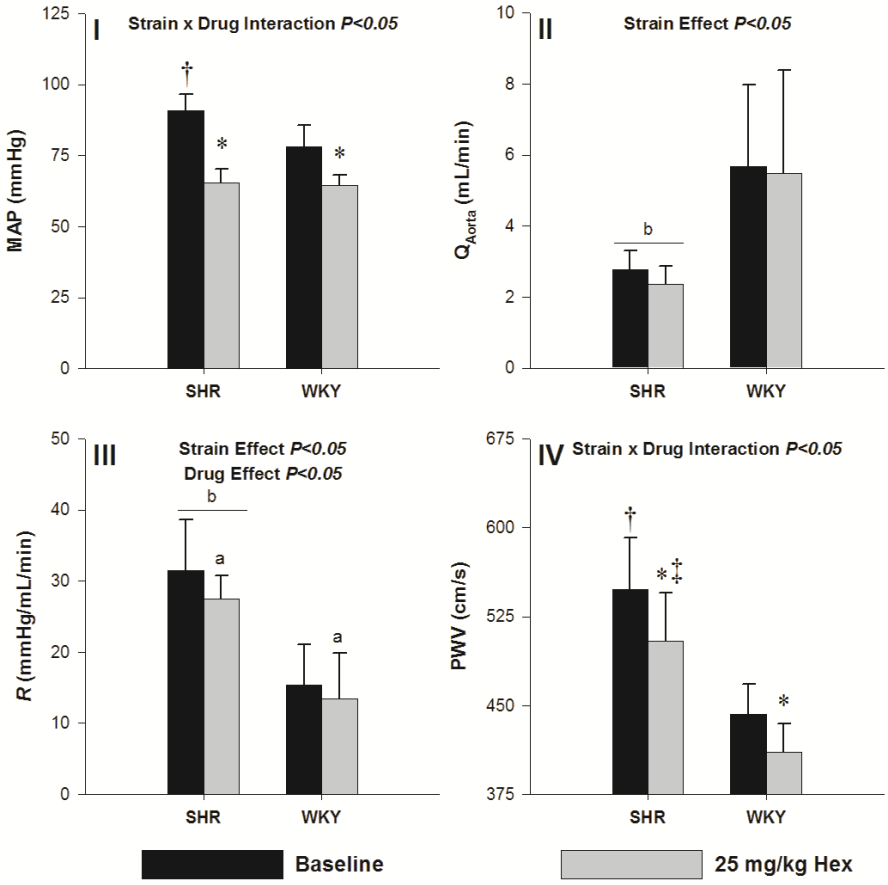

Figure 4.2 – Mean arterial pressure (MAP; I), caudal aortic flow (Q_{Aorta}; II), caudal aortic vascular resistance (*R*; III) and pulse wave velocity (PWV; IV) from young female hypertensive (SHR) and normotensive (WKY) rats. Hex, hexamethonium. Values are mean ± standard deviation. *, Significantly different from baseline, *P<0.05;* †, Significantly different from WKY baseline, *P<0.05*; ‡, Significantly different from WKY Hex, *P<0.05.* Main effect statistics: a, denotes *P<0.05* versus baseline; b, denotes *P<0.05* versus WKY.

Mechanical alterations following Hex

When compared with WKY, the SHR animals had lower *C* and C_{Local} (Figure 4.3 I and 4.3 II respectively; main effect of strain *P<0.05*) and higher *K* values (Figure 4.3 III; main effect of strain *P<0.05*). Compared with baseline, both *C* and C_{Local} increased only in the WKYs following Hex (Figure 4.3 I and 4.3 II respectively; *P<0.05*). However, Hex had little effect on *C* or C_{Local} in SHRs and failed to modify *K* in either strain (Figure 4.3 III; *N.S.*). Wall thickness for WKY (n=4) animals was 33.5±6.3μm versus 48.9±7.4μm in the SHR (n=4) (Figure 4.4; *P<0.05*; *β=0.930*).

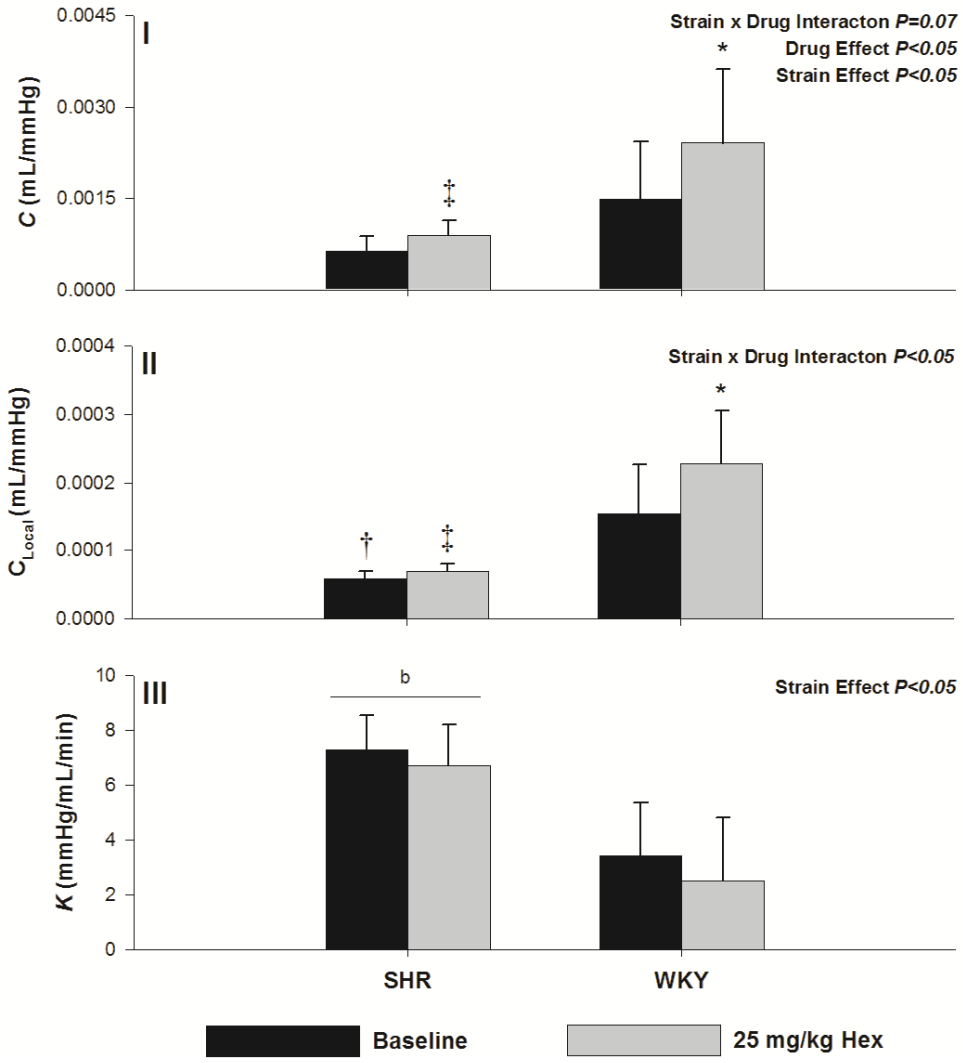

Figure 4.3 – Hindlimb vascular bed compliance (*C*; I), local aortic compliance (C_{Local}; II) and viscoelasticity (*K*, III) of the lumped hindlimb vascular beds of young female hypertensive (SHR) and normotensive (WKY) rats. Hex, hexamethonium. Values are mean ± standard deviation.*, Significantly different from baseline, *P<0.05;* †, Significantly different from WKY baseline, *P<0.05* ‡, Significantly different from WKY Hex *P<0.05*. Main effect statistics: b, denotes *P<0.05* versus WKY

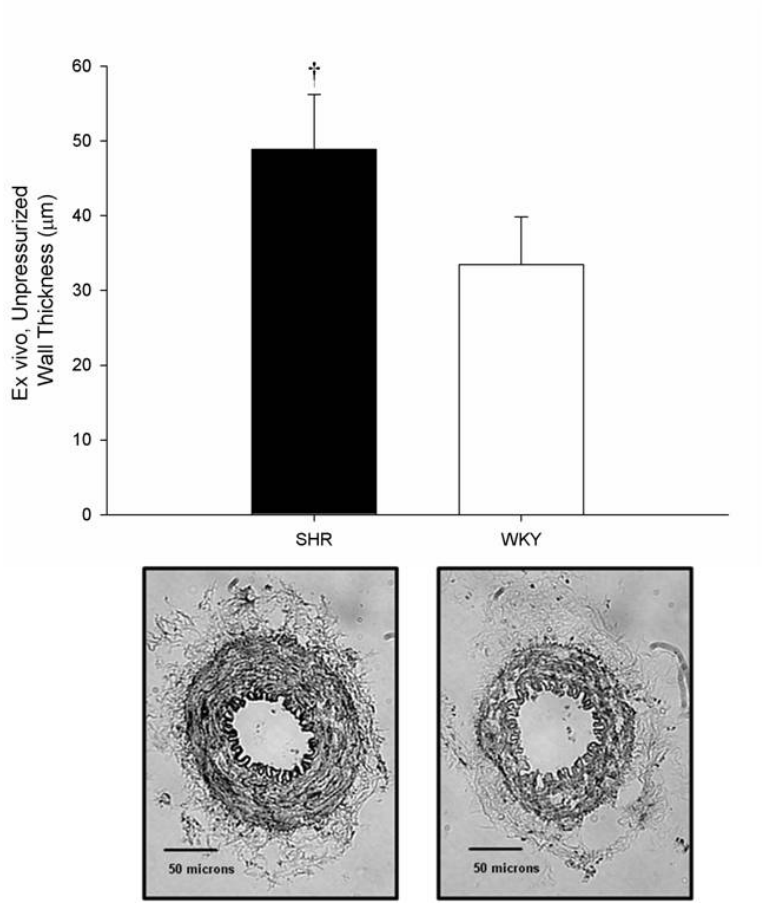

Figure 4.4 – Ex vivo, unpressurized femoral artery wall thickness from young female hypertensive (SHR) and normotensive (WKY) rats. Top: group averages of SHR and WKY animals. Bottom: representative SHR (left) and WKY (right) ex vivo unpressurized femoral arteries at 20X magnification. †, Significantly different from WKY, $P<0.05$.

4.4 Discussion

The purpose of this study was to examine the role of the SNS in the central arteries and peripheral vascular beds of young normotensive and hypertensive female rats. It was observed that the lumped compliance of the hindlimb vasculature of young female WKY animals increased following ganglionic blockade, indicating that peripheral vascular stiffness was affected by SNS input. By contrast, it was observed that SHRs had lower *C* and higher *K,* meaning stiffer peripheral vascular beds with higher resistance to stretch, both before and following ganglionic blockade. Thus, it appears that the normal neurogenic control of vascular stiffness is lost in the developmental period of SHR (i.e., within 7 weeks of age). This loss of neurogenic control was associated with vascular remodeling in the SHR, again, at the early age of 7 weeks. These data support the overall hypothesis that vascular stiffness is under some level of neurogenic control but that this control is modified in the developmental stages of HT in females.

Previously, it was demonstrated that brachial artery compliance of humans (33) and the lumped compliance of the entire human forearm vascular bed (41) are under some neural control, where sympathoexcitatory reflex maneuvers consistently reduced *C*. The current results from a rodent model provide new experimental evidence that the compliance (*C* and C_{Local}) of peripheral vascular beds are (normally) under some neural control as well, supporting these earlier results from humans. However, the current results show further that this neural control of vascular stiffness and viscoelasticity appears to be "lost" in HT. This observation supports the scenario proposed in Figure 4.1 whereby reduced compliance and increased viscoelasticity, possibly due to the trophic actions of neurotransmitters (7; 43), add to hypertension by means of faster wave

reflections reaching the aorta during systole which increases the afterload of the left ventricle (30).

It is particularly important that the value of *K* in SHR was found to be higher than that observed in the WKY group because this property represents viscoelastic resistance to stretch within the vessel wall which is over and above that of pure elasticity. While the concept of viscoelasticity is not new (28), the components of the vessel wall that account for changes in this important mechanical property, or differences among populations, remain largely unknown. Yin and colleagues (39) observed differences in the viscoelastic properties such as the stiffness modulus and phase lag (force-length relationships) in young and senescent arterial strips from dogs. They noted that although the stiffness was increased in the senescent animals, the differences observed could not be accounted for by changes in the content of elastin, collagen, or the ratio of the two. Our results suggest that vascular remodeling (at least in Ex vivo, unpressurized femoral arteries) may play a role in augmenting *K*.

Finally, the dichotomy between the roles of central arteries versus peripheral vasculature in the control of blood pressure deserves emphasis. It is well accepted that the resistance of the central vessels makes up a negligibly small part of total resistance because of their relatively large diameters. On that basis, it is reasonable to suspect that central vascular radii are not under acute SNS control, although it is known that the SNS exerts chronic (trophic) effects on all vascular beds, which can lead to vascular remodeling (7). The question then arises as to whether the compliance and viscoelasticity of the central arteries are also under these trophic effects or under SNS control. The wall thickening observed in the femoral artery, the higher PWV following Hex, and the

increased stiffness in the distal aorta, as measured by $C_{Local,}$ all point to a remodeled vascular system that is not necessarily confined to the peripheral vasculature. Thus, by 7 weeks of age, there is progressive remodeling present in the SHR model. It is likely that the mechanisms of such remodeling include pressure-induced hypertrophy and the trophic effects of chronic SNS activity. The way in which these factors combine to induce the stiffening and remodeling observed in HT remains unknown. However, our study supports the potential role of the SNS in the pathogenesis of HT.

Limitations

The interpretation of the results obtained here is based on the assumption of complete neural blockade by Hex. The fall in *R* and MAP following ganglionic blockade with Hex provide evidence of ganglionic antagonism. Also, the dose of Hex used here was higher than that of previously published reports (16; 36) in which 20mg/kg of intravenous Hex abolished (renal) sympathetic nerve activity in both SHR and WKY animals between 15 and 20 weeks of age.

Additionally, because of the observed falls in MAP following Hex, changes in *C* might reflect a shift along the same pressure-volume curve, and may not be indicative of an actual change in the mechanical properties of the vasculature (i.e. shift of the pressure-volume curve).

4.5 Conclusions

The current results indicate that vascular compliance of the peripheral vascular bed is regulated in part by the nervous system but that this control is lost in SHR in conjunction with vascular remodeling. The way in which the remodeled vascular bed and

aorta affect this neurogenic regulation is not known, but it was noted that this altered neurovascular control was evident already by 7 weeks of age in the SHR group.

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Chapter 5 – Evidence for sexual dimorphism in sympathetic control of vascular function.

5.1 Introduction

The preceding chapters have outlined studies which examined the role of the sympathetic nervous system (SNS) in the early stages of hypertension (HT). The designs of the studies were limited to either male or female animals as there was no initial intention to include male/female comparisons. However, during data collection and analysis of the studies, striking differences were observed in the hemodynamic and hindlimb mechanical properties of the male and female rats. In particular, female animals exhibited higher baseline heart rate (HR) and mean arterial pressure (MAP), but the most striking differences were lower levels of hindlimb compliance (*C*), and local aortic compliance (C_{Local}), with much higher viscoelasticity (*K*). In essence, female animals appeared to have much stiffer peripheral vascular beds compared to their age matched counterparts.

Sex differences have been reported in a number of (patho-) physiologic conditions including incidence of cardiovascular disease, systemic inflammation, systemic lupus erythematosus and type-2 diabetes (3; 4; 7; 21). Recently, Hart et al. (11) demonstrated that the relationships between muscle sympathetic nerve activity, cardiac output and vascular resistance, relationships that are often seen in males, break down in females. Similarly, females exhibit less vascular responsiveness to intra-arterial infusions of norepinephrine compared to males (18), and observations from rodent studies suggest that, in contrast to males, females lack baseline neuropeptide Y (NPY)-mediated control of hindlimb vascular conductance (12-14). Additionally, chapters 3 and 4 indicate that the mechanical properties of the rat hindlimb are regulated by the SNS, but the magnitude of the differences observed in the values *C*, C_{Local} and *K* between males and females are

striking. These examples of sexually dimorphic regulation by the SNS suggest that male and female animals may utilize different mechanisms of vascular control. Therefore the purpose of this study was to explore the differences observed in the systemic hemodynamics and hindlimb vascular mechanics of male and female rats, and how neural cardiovascular regulation might differ between the sexes.

5.2 Methods

A retrospective analysis of data from chapters 3 and 4 was performed in which the hemodynamic and lumped hindlimb vascular mechanics of male and female rats was assessed for sex-based differences. Spontaneously hypertensive (SHR; male n=10, female n=7) and Wistar-Kyoto (WKY; male n=6, female n=7) rats from both sexes underwent identical anaesthesia and surgical procedures as previously outlined (chapters 3 and 4). Briefly, the animals were anesthetized with an intraperitoneal injection of urethane (500mg/kg) and α-chloralose (80mg/kg). Animals were instrumented to measure blood pressure at the carotid artery, and at the bifurcation of the aorta into the iliac arteries. Blood flow was measured just superior to the bifurcation of the aorta.

Mean arterial pressure (mmHg), HR (beats/min), aortic blood flow (Q_{Aorta}, mL/min), caudal conductance ($Cond_{Caud}$, mL/min/mmHg), pulse wave velocity (PWV, cm/s) and C_{Local} (mL/mmHg) were calculated (see chapters 3 and 4). The vascular resistance (*R*) and mechanical properties (*C*, *K* and *L*) of the hindlimb vascular bed were also assessed (see chapters 3 and 4). *C* and C_{Local} were normalized to body weight to account for differences in the volume of vasculature tissue between the sexes. All variables were measured or calculated at baseline and following the administration of

hexamethonium bromide (Hex; 25mg/kg) to abolish post-ganglionic neural transmission. All animals were 7 weeks of age at the time of the surgical interventions.

Statistics

Data were analyzed at baseline and during Hex using a mixed two-way Analysis of Variance (ANOVA; SAS 9.1, Cary, NC, USA) on sex (male/female) and strain (SHR/WKY). The response to Hex was compared to baseline using a paired Student's t-test. Main effects of the ANOVAs and t-test were considered significant at $P<0.05$, and Tukey's *post hoc* analysis was used to identify where differences occurred in the ANOVA. Data are presented as mean ± standard deviation.

5.3 Results

Baseline hemodynamics and body weight can be found in Table 5.1. Main effects of sex and strain were observed for MAP, HR, Q_{Aorta}, $Cond_{Caud}$, and *R* (all $P<0.05$). MAP, HR and *R* were higher in SHRs versus WKYs and in females compared to males. Aortic blood flow and $Cond_{Caud}$ were lower in SHRs versus WKYs and were higher in males. Figure 5.1 depicts PWV (Figure 5.1, I), weight normalized *C* and C_{Local} (Figure 5.1 II and III, respectively) and lumped hindlimb *K* (Figure 5.1, IV). Compared with WKY PWV was higher in SHRs (main effect of strain, $P<0.05$) but was not different between the sexes. Females displayed lower *C* and C_{Local} compared to males (main effect of sex, $P<0.05$); however, the difference in *C* was abolished when normalized to body weight (C_{Local} remained lower in females). *C* and C_{Local} were lower in SHRs (main effect of strain, $P<0.05$) and this difference remained following normalization. Females had higher *K* values compared to males (interaction effect, $P<0.05$) and in SHRs were higher than WKYs (interaction effect $P<0.05$).

Response to Hex

The percent change from baseline for MAP, HR, Q_{Aorta}, $Cond_{Caud}$ and *R* can be found in Table 5.2. Mean arterial pressure fell in response to Hex in all groups except male WKYs (t-test, $P<0.05$). Heart rate rose in all groups except female SHRs following Hex (t-test, $P<0.05$). Aortic flow fell in female SHRs only (t-test, $P<0.05$), while $Cond_{Caud}$ rose and *R* fell in both male and female SHRs (t-test, $P<0.05$).

Pulse wave velocity fell in all groups following Hex (t-test, $P<0.05$; Figure 5.2 I). The weight corrected hindlimb *C* and C_{Local} rose in all groups except Male WKYs (t-test, $P<0.05$; Figure 5.2 II and III respectively) and *K* fell in all groups except female SHRs (t-test, $P<0.05$; Figure 5.2 IV).

Comparisons in Hex

Following Hex administration the SHRs animals had a larger change in MAP compared to WKYs (main effect of strain, $P<0.05$) and female animals had a greater fall compared to males (main effect of sex, $P<0.05$).The increase in HR was greater in male animals compared to females (main effect of sex, $P<0.05$). A significant interaction of sex and strain was seen in Q_{Aorta}, $Cond_{Caud}$ and *R* following Hex, in that male SHRs and WKYs were significantly different from one another in each variable. Additionally, Q_{Aorta} was significantly different between male and female SHRs, and *R* was significantly different between male and female WKYs (all $P<0.05$). PWV fell more in males than in females (main effect of sex, $P<0.05$) and there were no differences in the response to Hex in any group for *C*, C_{Local}, or *K*.

Table 5.1 – Weight (g), mean arterial pressure (MAP), heart rate (HR), aortic flow (Q_{Aorta}), caudal conductance ($Cond_{Caud}$) and caudal resistance (*R*) in male and female spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats.

	Male		Female	
	SHR (n=10)	WKY (n=7)	SHR (n=6)	WKY (n=7)
Weight (g)	207±16 [a]	214±15	134±6 [a,b]	148±7 [b]
MAP (mmHg)	86±13 [a]	64±5	91±6 [a,b]	78±7 [b]
HR (beats/min)	339±29 [a]	302±81	388±24 [a,b]	333±25 [b]
Q_{Aorta} (mL/min)	5.7±2.0 [a]	9.5±3.2	2.7±0.6 [a,b]	5.7±2.3 [b]
$Cond_{Caud}$ (μL/min/mmHg)	68±26 [a]	147±43	30±6 [a,b]	75±35 [b]
R (mmHg/mL/min)	17.6±7.0 [a]	7.7±3.3	31.5±7.1 [a,b]	15.4±5.7 [b]

All of the variables listed have significant main effects of sex and strain (both $P<0.05$). Values are mean ± standard deviation. Main effect statistics: a, $P<0.05$ versus WKY; b, $P<0.05$ versus male.

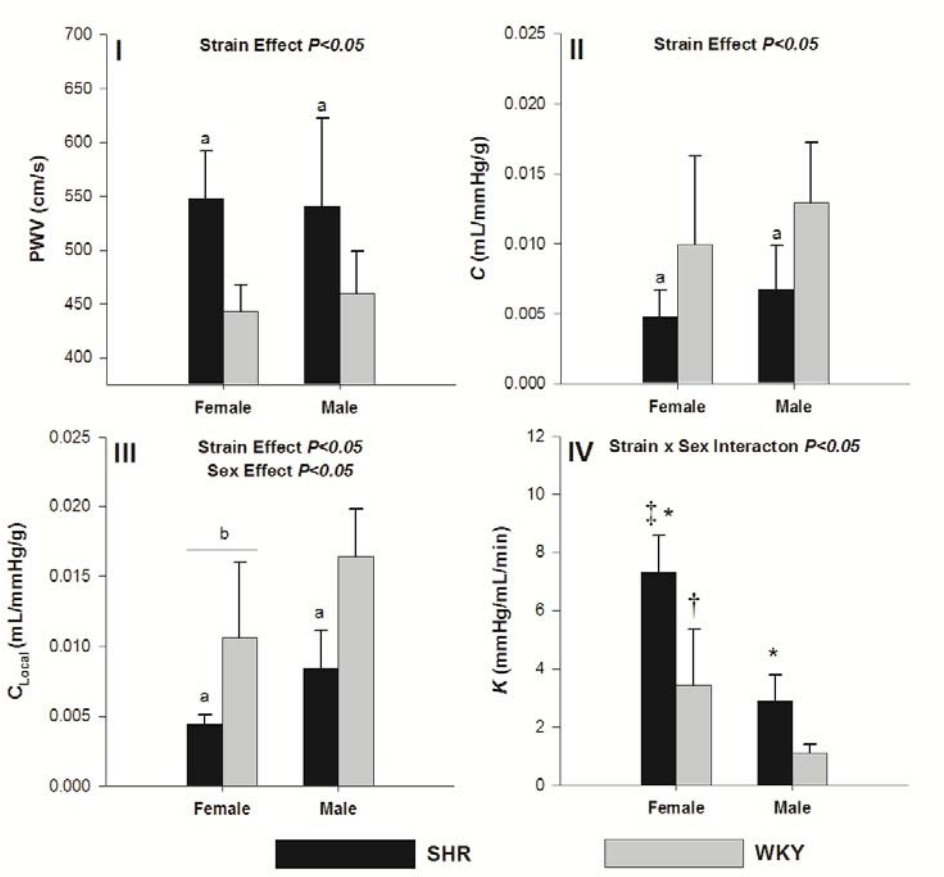

Figure 5.1– Pulse wave velocity (PWV; I), weight normalized hindlimb compliance (*C*; II), weight normalized local aortic compliance (C_{Local}; III) and lumped hindlimb viscoelasticity (*K*; IV) from male and female spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. Values are mean ± standard deviation. * denotes *P<0.05* versus WKY (within a sex), † denotes *P<0.05* versus male WKY; ‡ denotes *P<0.05* versus male SHR. Main effect statistics: a, *P<0.05* versus WKY; b, *P<0.05* versus male.

Table 5.2 – Percent change from baseline following ganglionic blockade with hexamethonium bromide (Hex; 25mg/kg) on mean arterial pressure (MAP), heart rate (HR), aortic flow (Q_{Aorta}), caudal conductance ($Cond_{Caud}$) and caudal resistance (*R*) in male and female spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats.

	Male		Female	
	SHR	WKY	SHR	WKY
MAP (% Δ)	-11±13†[a]	4±9	-28±7†[a,b]	-17±7†[b]
HR (% Δ)	9±7†	26±12†	-4±7[b]	7±6†[b]
Q_{Aorta} (% Δ)	22±36†	-5±28	-14±13†	-6±18
$Cond_{Caud}$ (% Δ)	36±37†*	-9±24	19±17†	14±23
R (% Δ)	-24±16†*	14±28	-11±14†	-14±17

Main effect of sex and strain for MAP, main effect of sex for HR and interaction effect for $Cond_{Caud}$, Q_{Aorta} and *R* (all $P<0.05$). Values are mean ± standard deviation. † denotes significantly different from baseline (t-test, $P<0.05$). * denotes $P<0.05$ versus WKY (within a sex). Main effect statistics: a, $P<0.05$ versus WKY; b, $P<0.05$ versus male.

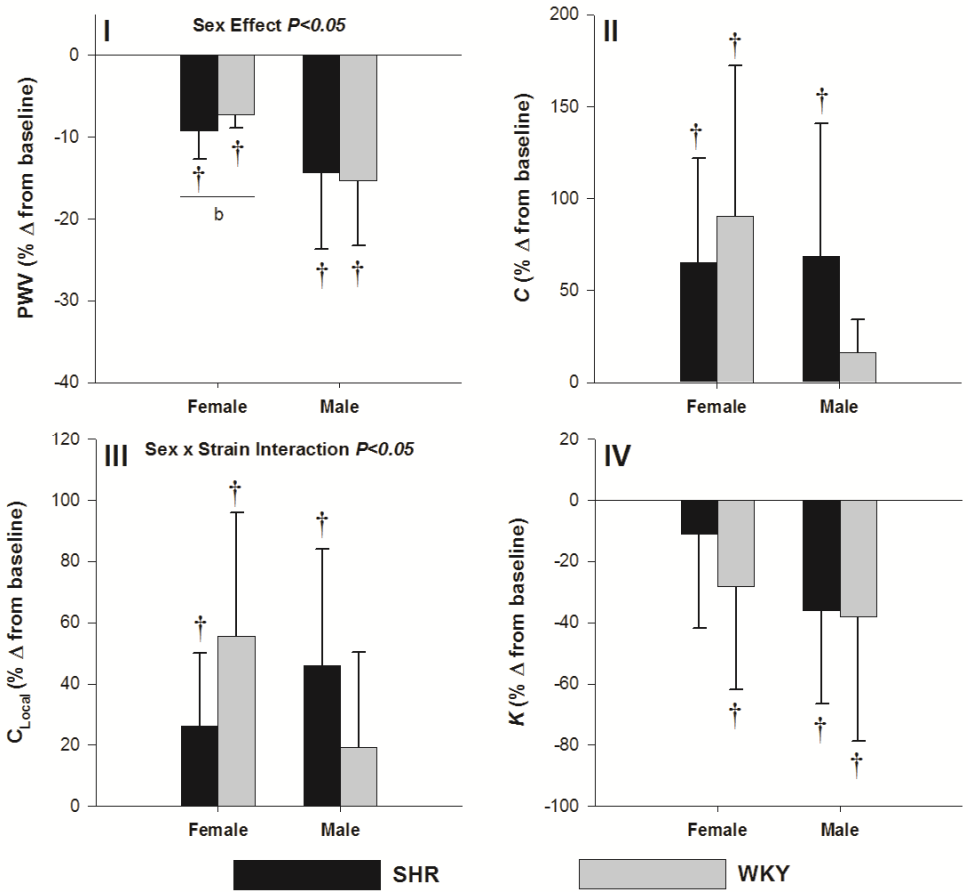

Figure 5.2 – Percent change from baseline of pulse wave velocity (PWV; I), weight normalized hindlimb compliance (*C*; II), weight normalized local aortic compliance (C_{Local}; III) and lumped hindlimb viscoelasticity (*K*; IV) from male and female spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats following ganglionic blockade with hexamethonium bromide (Hex; 25mg/kg). Values are mean ± standard deviation. † denotes significantly different from baseline (t-test, *P<0.05*). Main effect statistics: b, *P<0.05* versus male. Note that C_{Local} had a significant interaction, however no group differences were identified in the *post hoc* analysis.

5.4 Discussion

The main finding of the current study was that female rats have higher resistance (*R*) and viscoelasticity (*K*) in peripheral vascular beds relative to age-matched males. Some of the dimorphism was driven by the SNS, but other factors also play a role. Sexual dimorphism was particularly evident in baseline MAP, HR, *R*, C_{Local} and *K*. These sex-dependent differences were apparent in both normotension and HT indicating that they are not strain dependent.

Higher MAP in female animals, compared with male animals, was present in both SHRs and WKYs and was largely mediated by neural input in females and in HT. Male WKYs showed no change in MAP following Hex, but showed marked tachycardia. Conversely, female SHRs had a large fall in MAP with no change in HR following Hex. These divergent responses suggest different control mechanisms in male and female rats, which become amplified in female HT. Specifically, male normotensive rats display parasympathetic (PNS) control of the heart at baseline as ganglionic ablation increased HR in accordance with PNS withdrawal (10; 19). In contrast, female hypertensive rats appear to have altered autonomic control of the heart at baseline, in that ganglionic blockade had no effect on HR. The large fall in MAP with Hex in female SHRs was not accompanied by a large fall in *R* as Q_{Aorta} declined modestly. This latter observation suggests reduced cardiac contractility with Hex in the female SHR animals, rather than a PNS withdrawal mechanism.

The observed high *R* and low C_{Local} in female rats mirror human data from our lab (37). In this earlier study, using the same mathematical model, young subjects and HT patients were analyzed. The females (regardless of group) displayed higher *R* and lower

C relative to males. Also, *K* was markedly higher in the human HT patients (37) which parallels the results presented here in the SHR model. To date, no one has reported sex differences in vascular viscoelastic properties. The results presented here show striking sex differences in the viscoelasticity, which represents the resistance to stretch in the blood vessels. This property of the vessel wall is not well understood in either health or disease. It is apparent that viscoelasticity is calcium dependent (28; 29) indicating that the cross-bridge attachment within vascular smooth muscle cells (VSMC) is a key factor in its regulation. The current study also emphasizes that the SNS can influence *K* and that the control is modified by sex and in disease (i.e. female SHR). Such differences may be influenced by sex-dependent differences in VSMC structure and signaling cascades (16; 22). Specifically, reduced Ca^{2+} handling and a blunted expression and activation of protein kinase C (PKC) in VSMCs from female animals, leads to less contraction relative to male animals. How these alterations affect *K*, or its regulation, remains unclear.

Estrogen is a likely candidate mediating the sexual dimorphism seen here. The role of estrogen in regulating vascular stiffness is complex. Reports of both increased and reduced levels of vascular stiffness by estrogen supplementation have been reported (25; 26; 32; 35). Further, estrogen manipulation does have the ability to alter resting VSMC structure, intracellular Ca^{2+} concentrations and PKC expression to levels that are similar to males (16; 22; 24). Indeed these results are further confounded, as the type of estrogen used, the method of delivery and previous estrogen exposure all influence the outcome (8; 31). Despite these conflicting reports, estrogen *does* impact vascular stiffness, and does impact sympathetic vascular control (36; 38; 39).

To further address the direct effect of estrogen, a secondary study was performed on small groups of female animals in which estrogen levels were manipulated by ovariectomy (OVX; n = 6) and ovariectomy + estrogen replacement (OVX+E2; 0.25mg 17β-estradiol [3-week release]; n = 6). The data are provided in Appendix A.2. Briefly, a main effect of estrogen manipulation was observed in *C* and a strain x estrogen interaction was observed for PWV. Neither OVX, nor OVX+E2, had altered hindlimb mechanics relative to intact controls in either strain of rat. However, point-wise comparisons between OVX and OVX+E2 suggest that OVX tended to *reduce* indices of stiffness (*C* and PWV), while OVX+E2 tended to *increase* stiffness, particularly in HT. A main effect of strain was observed for MAP and again when point wise comparisons were made, OVX had lower MAP compared to OVX+E2 in SHRs. These results suggest that estrogen is an important factor in regulating the increased stiffness seen in female rats, at least in SHRs. However, it is clear that dosages of estrogen replacement must be titrated carefully in order to properly mimic the intact animal.

5.5 Perspectives

The sexual dimorphism of vascular bed mechanics reported here, combined with reports of similar sex-specific differences in vascular reactivity to sympathetic stimuli (12; 13; 18), suggest that female animals may rely on different mechanisms to regulate vascular function. Males appear to sympathetically control vessel diameter (i.e. alterations in vascular resistance) to regulate blood pressure, while the stiffer vasculature seen in female animals suggests a greater reliance on central (or cardiac) mechanisms. This mechanism has been observed previously, when, in response to lower body negative pressure (5) and progressive tilt (27), females demonstrate an exaggerated heart rate

response, and lower sympathetic nerve activation, despite the same level of hemodynamic stress. Additionally, females have shown greater cardiac responsiveness versus peripheral vascular reactivity to a variety of stressors (1; 9), and exhibit exaggerated vascular contraction to non-adrenergic/sympathetic antagonists (6; 30; 33) compared to males.

Female rats appear to lack the NPY mediated control of baseline hindlimb conductance which is present in males (12; 13). Estrogen appears to be involved in regulating the (lack of) NPY response in females. Recent work by Jackson et al. (15) examined the sympathetic hindlimb conductance response in female Sprague Dawley rats that were either intact, were ovariectomized, or were ovariectomized but had supplemental estrogen. They reported that following ovariectomy, the expression of both NPY and the NPY Y_1 receptor (Y_1R) were increased, as was the Y_1R control of hindlimb vascular conductance. These adaptations were prevented by the supplementation of estradiol (15) suggesting that estradiol plays a critical role in regulating sympathetic cardiovascular control.

In the vasculature NPY produces a potent and long lasting vasoconstriction and potentiates the effects of norepinephrine (20; 34). In male animals, based on the increased hindlimb conductance response to Y_1R antagonism, we can presume that these contractions are present at baseline (14) and may act to stabilize the vasculature over many cardiac cycles. The stiffer vasculature observed in females would work in much the same fashion, buffering beat-to-beat fluctuations, with little need for neural input. Thus, the need for NPY mediated baseline control of the hindlimb is absent and the system is down regulated (12; 13), and may account for the lower level of baseline sympathetic

nerve activity that is commonly observed in females (Figure 5.3), as stiffer arteries would require less neural input.

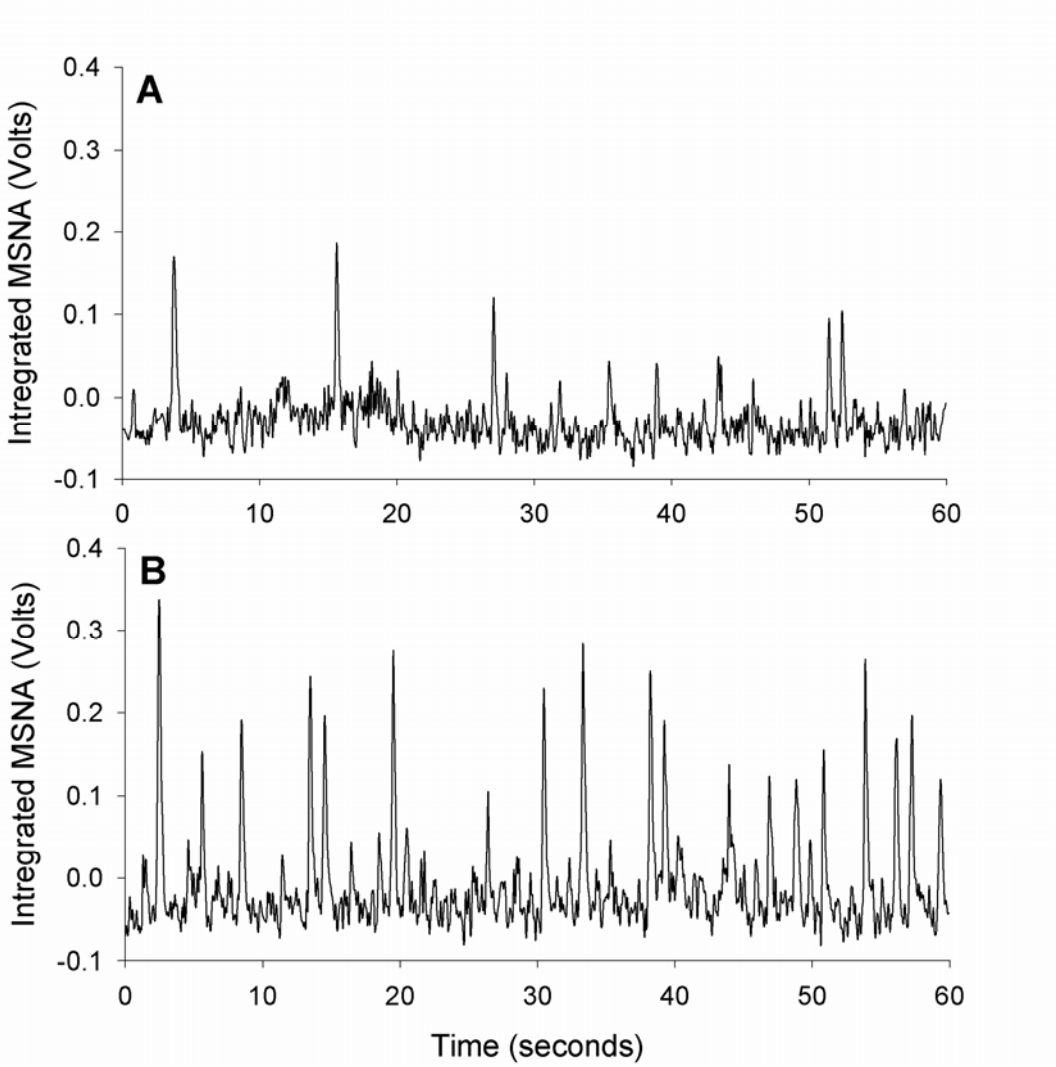

Figure 5.3 – Matched female (A) and male (B) baseline neurograms of muscle sympathetic nerve activity (MSNA) measured in the peroneal nerve. Data generously provided by Ruma Goswami.

Limitations

The dichotomous mechanisms of cardiovascular regulation proposed here are based on a limited number of observations in a young rodent population. Thus, the phenomenon mentioned may be the result of differences of the natural development of male and female animals, and may not reflect a distinct sex difference. Indeed, increased levels of endogenous estrogen and testosterone in males and females, respectively, have been associated with altered vascular stiffness in pre- versus post-pubertal humans (2). As the animals here were post-pubescent (17; 23) it is unlikely that the endogenous levels of estrogen and/or testosterone factored into the differences seen, although measurement of endogenous estrogen and testosterone is required for confirmation. Additionally, as the animals were measured at the same age (instead of weight), were housed in the same location and exposed to the same diet, it is unlikely that environmental or developmental factors can account for the increased stiffness observed in female animals. Much more work is required in order to ascertain whether the increased stiffness in female animals is a physiologically relevant mechanism of cardiovascular control.

5.6 Conclusions

This retrospective analysis of data presented in chapters 3 and 4 suggests that dichotomous mechanisms of cardiovascular control exist between male and female animals. Female animals exhibited augmented MAP and HR relative to males in conjunction with greater stiffness and viscoelasticity in both SHR and WKY animals. The impact of K on blood pressure control is unknown, but this parameter may be a critical feature in female hypertensive animals.

Differential control of the heart in male and female animals is suggested following ganglionic blockade and an increase in compliance and reduced *K* following Hex suggest that both of these variables are affected by agonist-induced intracellular calcium levels. The more compliant vascular beds of male animals may require greater neural input to maintain vascular homeostasis compared to the stiffer female animals. Females apparently rely on central (cardiac) mechanisms to regulate blood pressure, while males would rely on sympathetically mediated changes in vessel caliber to alter vascular resistance. It may be that these differences are due to altered vascular mechanics (or vice versa).

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Chapter 6 – General Discussion and Conclusions

6.1 Overall Summary

The primary aim of this body of research was to explore the role of the sympathetic nervous system (SNS) in regulating the cardiovascular system during the development of hypertension (HT). Through the actions of both norepinephrine (NE) and neuropeptide Y (NPY), the SNS elicits both acute and chronic effects on the vasculature. Much is known about how these neurotransmitters interact with the vasculature during both normotension and *developed* HT, but the way in which these critical neurotransmitters control the vasculature *as* HT develops remains unclear.

The SNS's involvement in HT fundamentally occurs through heightened sympathetic activity leading to increased vascular resistance (3; 18). NE and the α-adrenergic receptor (α-AR) are the classic mediators of the augmented resistance (10; 32). Data presented in chapter 2 highlight an exaggerated role for the NPY-mediated signaling in the early stages of the HT (at least in male animals). This enhanced NPY-mediated control was absent at 3-5 weeks of age, but present at 7 weeks, suggesting that alterations in sympathetic signaling mechanisms occur rapidly in developing HT. Additionally, greater expression of the NPY Y_1 receptor (Y_1R) was observed in skeletal muscle extracts from 7-week old SHRs, with no change in markers of adrenergic innervation (α-AR and tyrosine hydroxylase). These data suggest that adrenergic mechanisms do not contribute to HT at this age. The enhanced regulation of hindlimb conductance by NPY in SHRs suggests that NPY and the Y_1R are involved in the development of HT.

A hallmark of hypertension is vascular remodeling which is characterized by vascular stiffening and hypertrophy (3; 11; 24). NPY has been linked to vascular

remodeling (1); yet the role of the SNS in regulating vascular stiffness remains speculative. Using a modified Windkessel model, chapters 3 and 4 demonstrate that the SNS is actively involved in the regulation of hindlimb vascular stiffness in male and female rats. This stiffness was augmented in HT in both sexes; however, antagonism of the Y_1R produced no appreciable change in the measured stiffness in males, while ganglionic ablation reduced stiffness in both sexes. Assessment of the Y_1R control of vascular stiffness in females was not performed for two reasons. First, research from our lab has demonstrated that NPY plays little role baseline cardiovascular control in females (12; 13). Secondly, no effect was observed by Y_1R antagonism in males; therefore, it was deemed unnecessary to examine the response in females. It is important to point out that the regulation of the mechanical properties of the rat hindlimb appear to be different between males and females in both normotension and hypertension (Figure 6.1). Specifically, normotensive males (at least at 7 weeks of age) appear to regulate *C and K* through the sympathetic nervous system, but not *R*. In contrast, sympathetic mechanisms affect *R* and *C* in normotensive females *without* affecting *K*. Hypertension alters the control in both males and females but in different ways. Male hypertensive animals retain the neural control of *C* and *K* but gain control of *R*, while female animals lose the ability to regulate *C* likely because of vascular remodeling, but retain control of *R*. These differences highlight the dimorphism between males and females in vascular regulation in both normotension and HT, but also underscore our limited understanding of its complexity.

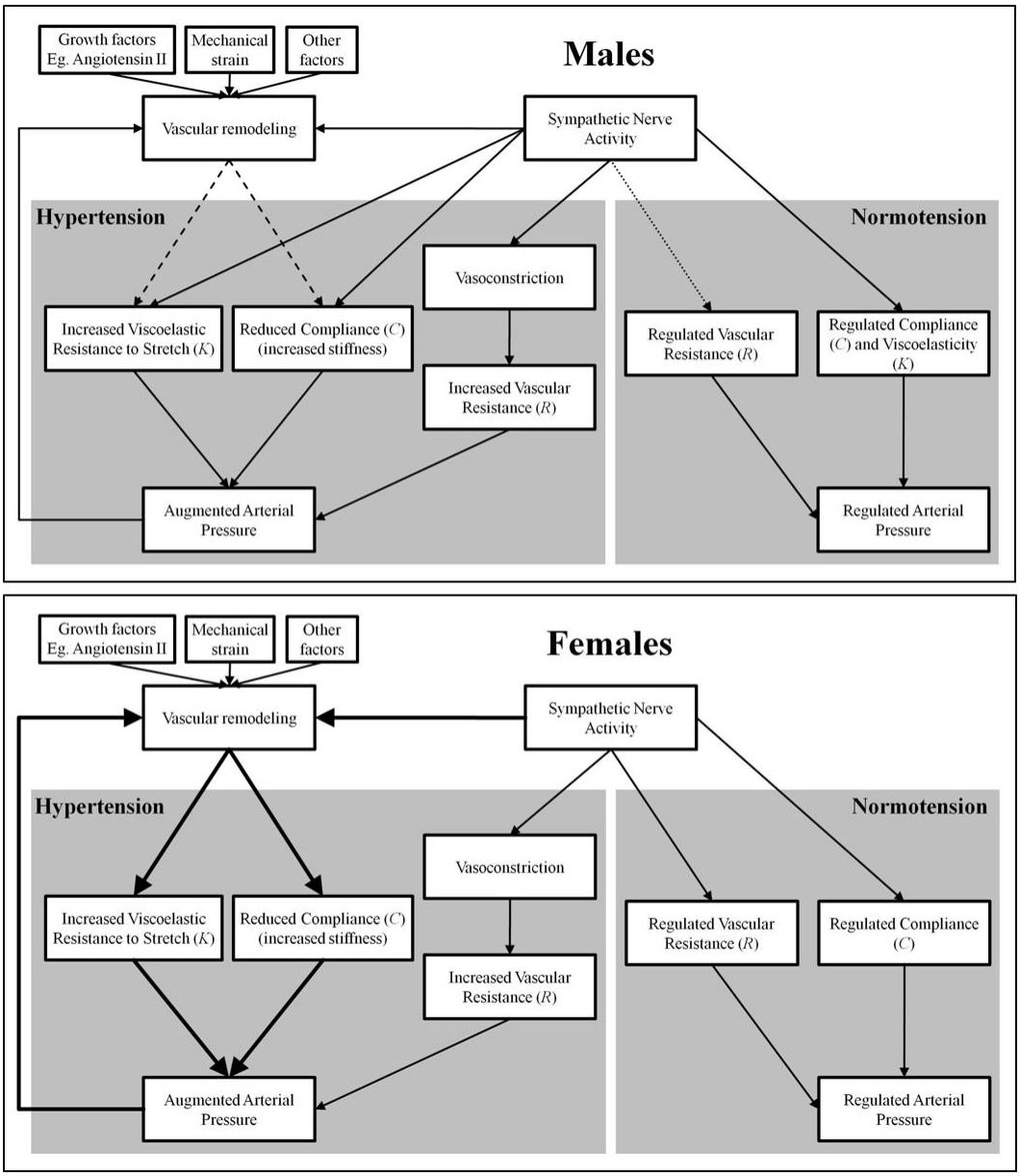

Figure 6.1 – Updated schematic depicting the mechanisms of control in both male and female normotensive and hypertensive rats. See text for details. Dotted line represents apparent lack of control (in males), dashed lines are speculated, but were not measured (in males), and bolded appear to be augmented/dysregulated (in females).

Neuropeptide Y and the Y_1R influence baseline vascular control in males (14), as antagonism of the Y_1R increased hindlimb vascular compliance. Chapter 3 demonstrated that Y_1R blockade did not alter vascular resistance. However, the measurements of arterial pressure and flow here were made at the level of the aorta, versus the femoral artery as in chapter 2. Vascular resistance reflects only the steady state component of blood flow and acts through alterations in vessel diameter (25). Thus, the resistance of the central vessels makes up a negligible part of total resistance, because of their relatively large diameters, and it is reasonable to suspect that central vascular radii are under less SNS control (31). Therefore, alterations in aortic flow following Y_1R antagonism were not expected.

The augmented vascular stiffness seen in female animals forms the basis for the proposed sex-associated differences in cardiovascular regulation outlined in chapter 5. The stiffer vascular beds of female animals suggests that female animals may rely on mechanical rather than chemical regulation of the vasculature. Alteration of the stiffness of the vasculature rather than the diameter of the blood vessels may increase the contribution of changes in heart rate and cardiac contractility to regulate blood pressure. This cardiac mechanism has been suggested previously. Using both lower body negative pressure (7) and progressive tilt (29), females preferentially induce tachycardia rather than increased vascular resistance. Additionally, reflexive increases in muscle sympathetic nerve activity are blunted relative to males, which is consistent with parasympathetic withdrawal occurring prior to sympathetic activation in the heart (8; 19; 28).

Finally, hypertensive animals, both male and female, exhibited markedly stiffer vessels compared to their normotensive counterparts. This observation was expected. However, the fact that only the female hypertensive animals appeared to have lost the neurogenic control over the vascular stiffness, is intriguing. Perhaps the augmented stiffness imposed by the hyper-adrenergic state that characterizes hypertension (10; 32; 36) irreversibly stiffens the vasculature through remodeling, thereby preventing it from relaxing. The mechanistic and teleology for this sex-based difference in SHR animals are not known. Regardless, these data clearly demonstrate that in hypertension sex differences in cardiovascular control are present.

In conclusion, two major themes are evident from this body of work. First, altered neurovascular regulation during the early stages of HT is evident. Secondly, the ways in which male and female animals regulate the cardiovascular system appear to be fundamentally different. Expanding on the first finding, enhanced NPY mediated hindlimb vascular conductance was observed in 7-week old male SHRs. These *in vivo* observations were supported by an increased expression of Y_1R in red vastus homogenates from SHR while α-AR mechanisms were comparable to WKY control animals. The increased vascular resistance and stiffness in the SHR were sympathetically-mediated in both sexes, but NPY and the Y_1R do not appear to be involved. The augmented vascular stiffness in females relative to males was the first indicator of the sexual dimorphism of cardiovascular control. Coupled with the lack of baseline NPY/Y_1R control (12; 13) and the modulation of sympathetic control with ovariectomy (15), a situation arises whereby the stiffer vasculature of female animals

would allow changes in heart rate to maintain blood pressure, while male animals utilize increases in vascular resistance.

6.2 Limitations

A major limitation of this dissertation is the absence of measured sympathetic nerve activity. Measurement of either renal or lumbar sympathetic nerve activity is routinely done in the literature. Much of this dissertation assumes that sympathetic outflow is elevated in the SHR animals. This assumption has been validated previously (see (9) for a detailed review), yet direct measurement here would strengthen our conclusions. Additionally, a direct measurement of sympathetic nerve activity would have allowed us to verify that the hexamethonium produced a complete ganglionic blockade. We can reasonably assume that complete ganglionic blockade occurred with hexamethonium as previously published reports (16; 30) indicate that 20mg/kg was a sufficient dose to completely block ganglionic neural transmission as measured in the renal nerve in both SHR and WKY animals.

A second limitation here is rooted in the fact that the dichotomous mechanisms of cardiovascular regulation being proposed are based on a finite number of observations from different data sets. While precautions are taken to ensure consistency between experiments, random variations are assured. The stiffness data presented were collected such that experiments were run on half of the female animals followed by all of the male animals, and finally the second half of the females. The unique timing of data collection reduces the likelihood of bias in data collection (i.e. surgical precision) and bolsters our confidence in the observations.

Third, there is a possibility that the responses seen in an anaesthetized rat are not physiological, due to blunted reflex control. This is a valid argument, and with many barbiturate and gas forms of anaesthesia this holds true (23; 34). Here, a mixture of urethane and α-chloralose was utilized as this cocktail has been shown to maintain reflex cardiovascular control (20; 21). Additionally, data from our lab (Usselman et al., *In Press*) indicates that this cocktail does not impair autonomic reflexes, as both light and deep levels of anaesthesia did not impair sympathetic reflexes in response to lower body negative pressure in rats.

As outlined in chapter 1, the *RCKL* model relies on pressure-volume relationships to estimate the mechanical properties of the vasculature. Primarily, because these properties represent the vasculature as a whole, we are unable to ascribe a specific level of the vascular tree to a given component of the model (35). Additionally, a large proportion of the resistance in the model is generated by resistance arterioles, but it is unclear from the model how these vessels also contribute to the compliance of the vascular bed. Further, the four components of this model represent an over simplification of a complex physiologic system giving us a method to conceptualize the mechanical properties. Additionally, because of the observed falls in MAP following Hex, changes in *C* might reflect a shift along the same pressure-volume curve, and may not be indicative of an actual change in the mechanical properties of the vasculature (i.e. shift of the pressure-volume curve).

Finally, as the majority of the data were collected from adolescent animals, it can be argued that the differences observed are due to variations in the growth patterns of the animals and are therefore artifacts of the normal growth process. It is unlikely that the

differences are artifacts, as the majority of animals tested were measured at the same absolute age of 7 weeks. This age was chosen for two specific reasons. First, at the same absolute age, WKY animals are heavier than SHR animals. Therefore had the animals been matched by weight, as is often done, the WKY animals would have represented a younger population of animals compared to the SHRs. Similarly, comparisons between the sexes would be invalid, as weight matched females would be older relative to the males. Secondly, at 7 weeks of age mild HT is evident in SHRs while other co-morbidities, such as heart disease, dyslipidemia or insulin resistance, are absent (4; 6; 27). Thus, matching the age of the animals allows for between group comparisons with little chance of growth artifact or co-morbidities factoring into the observations.

6.3 Future Directions

This dissertation outlines our attempts to understand the role of the SNS during the development of HT. It highlights some key alterations in the vascular response to sympathetic innervation, yet much work needs to be completed to broaden our understanding of how these two critically integrated systems are regulated.

A critical first step needs to be to integrate neural recordings into the methodologies described herein. Technological advances have led to the development of in-dwelling telemetric devices, which allow for the continuous measurement of two separate variables (i.e. blood pressure and sympathetic nerve activity). Using these devices tocollect data from conscious animals eliminates any anesthetic effects and allows for a longitudinal assessment of sympathetic hyperactivity and arterial pressure augmentation. Furthermore, the effectiveness of interventions designed to either raise or lower sympathetic activity (or blood pressure) can be evaluated.

A role for NPY and the Y_1R in the early stages of HT is implicated (chapter 2), yet much work is required to ascertain the mechanisms responsible for this enhanced regulation. To gain better insight into the roles of NPY and the Y_1R in HT, chronic receptor agonism/antagonism studies are required. Examining the molecular, cellular and hemodynamic responses to chronic agonism/antagonism will help expose some the mechanisms governing the development of HT.

The involvement of eatradiol (E2) in both sympathetic and cardiovascular development is known (2; 5; 33). Characterizing E2's role in regulating the mechanical properties of the vascular wall and how the SNS functions differently during chronic (8-12 week) E2 manipulation, is unknown. Additionally, E2 supplementation in the form of a continuous release pellet does not mimic the natural 4 day cycle of the rat (17; 26). Thus, a more physiologic study of E2 should be made, which accounts for the cyclic nature of this important hormone. Further, exploring how E2 and androgen manipulation in male rats affects both the sympathetic and cardiovascular systems is required.

Chapter 5 of this dissertation proposes a novel mechanism of cardiovascular control in young female rats. This supposition was based on a limited number of observations; therefore a thorough investigation of this phenomenon should be made. In order to ascertain whether female animals rely on central (cardiac) versus peripheral (vascular resistance) mechanisms to maintain blood pressure during times of stress, selective blockade of the heart and blood vessels is required. Selectively antagonizing different aspects of the autonomic nervous system will allow for clarification of the precise roles they have in cardiovascular regulation.

6.4 Final Remarks

The involvement of the SNS in the development of HT is known and accepted (9; 22), yet the alterations in sympathetic control that occur early in the pathogenesis are less well understood. We have shown that alterations in NPY signaling and the Y_1R precede changes in the adrenergic system. Additionally, the increased stiffness that is observed in HT begins *very* early in the process, does not involve the Y_1R and is a regulated property of the vasculature that appears to be defective in female HT animals. Finally, the sex-associated differences in vascular stiffness and the regulation of the mechanical properties of the vasculature may highlight a novel mechanism of vascular control in young male and female animals. Evidence supporting this theory is mounting; however, much work needs to be done to confirm these suppositions.

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Appendix A – Supplementary Data

Chapter 3– Femoral Flow Variability

Figure A.1 depicts the fluctuations in femoral flow observed in the female animals compared to male animals. The fluctuations were independent of changes in pressure, heart rate and temperature. The flow would rise for 2-3 minutes and would then fall. There was never a prolonged period of stability which prevented a stable baseline from being collected.

Figure A.2 illustrates the variability among intact female animals, female animals that were ovariectomized (OVX), ovariectomized + estrogen replacement (OVX+E2; 0.25mg 17β-estradiol [3-week release]) and male animals. The coefficient of variation (CoV = standard deviation/mean) for hindlimb conductance (C_{Fem}=femoral flow/mean arterial pressure) was calculated using 1000 consecutive heart beats at baseline and following ganglionic antagonism with hexamethonium bromide (Hex; 25 mg/kg). The variability was modulated by both E2 and the sympathetic nervous system, but the reason for this variability is unknown.

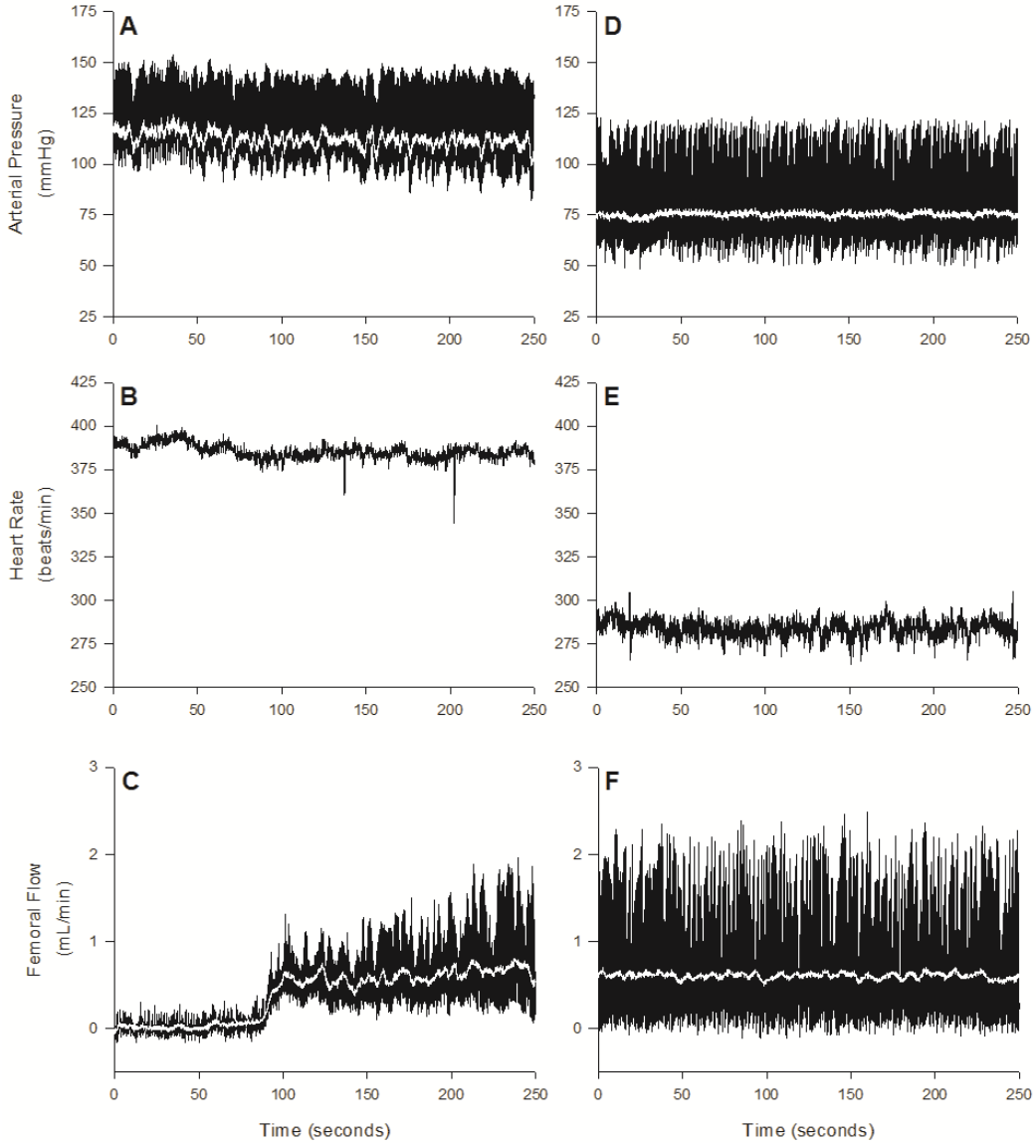

Figure A.1 – Representative raw data tracings of female (A, B, C) and male (D, E, F) spontaneously hypertensive rats. Note the large fluctuations in femoral flow (C) of female rat, with little or no change in arterial pressure (A), or heart rate (B).

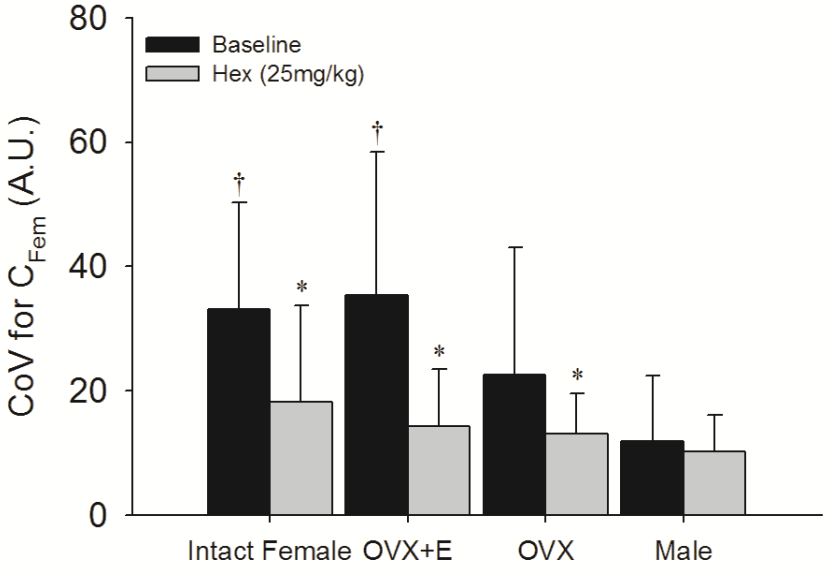

Figure A.2 – Coefficient of variation (CoV) for femoral conductance (C_{Fem}) amongst the experimental groups at baseline and following sympathectomy with hexamethonium bromide (Hex). * denotes *P<0.05* versus baseline within-group; † denotes *P<0.05* versus intact male at baseline.

Chapter 5 – Estrogen manipulation and vascular mechanics.

Table A.1 – The absolute *in vivo* hemodynamic variables from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) that were either ovariectomized (OVX) or ovariectomized and supplemented with estrogen (OVX+E2).

Strain	Variable	Intact	OVX	OVX+E
WKY	HR (beats/min)	345±29	399±20	327±23
	MAP (mmHg)	78±5	75±10	76±6
	Q_{Caud} (mL/min)	5.0±2.0	6.6±3.9	4.0±2.0
	C_{Cond} (mL/min/mmHg)	65±28	94±61	54±31
SHR	HR (beats/min)	381±38	340±43	390±33*
	MAP (mmHg)	103±19*	86±13	109±27*
	Q_{Caud} (mL/min)	3.5±1.6*	4.4±1.3	3.4±1.2
	C_{Cond} (mL/min/mmHg)	33±11*	51±12†	31±8‡

Values are mean ± Standard Deviation. HR = Heart Rate; MAP = Mean Arterial Pressure; Q_{Cond} = Caudal Flow; C_{Cond} = Caudal Conductance; R = Resistance; *C* = Compliance; *K* = Viscoelasticity; PWV = Pulse Wave Velocity. * denotes $P<0.05$ versus WKY (of same group); † denotes $P<0.05$ versus Intact; ‡ denotes $P<0.05$ versus OVX.

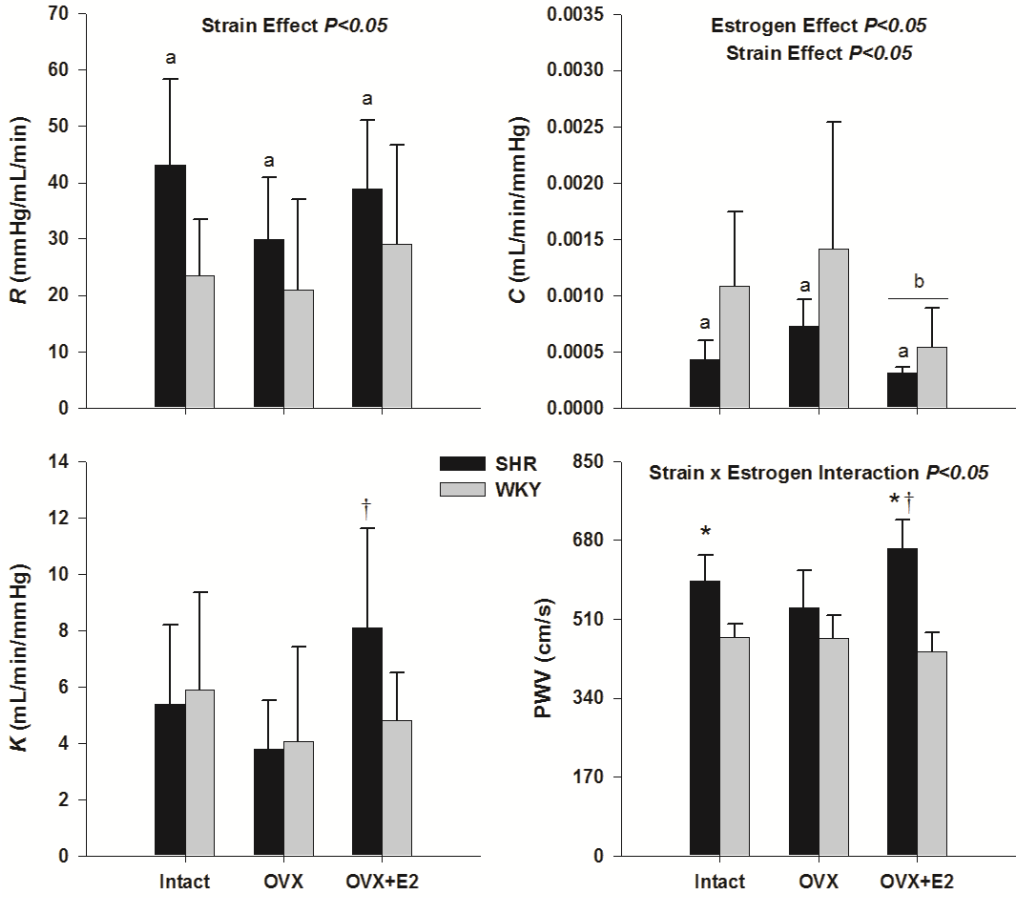

Figure A.3 – Resistance (*R*), compliance (*C*), viscoelasticity (*K*) and pulse wave velocity (PWV) in intact, ovariectomized (OVX) and ovariectomized supplemented with estrogen (OVX+E2) Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). Values are mean ± standard deviation. * denotes *P<0.05* versus WKY of same group, † denotes *P<0.05* versus OVX of same strain (both point to point comparisons). Main effect statistics: a, *P<0.05* versus WKY; b, *P<0.05* versus OVX.

Appendix B – Ethics Approvals

Western

06.01.2010
*This is the 3rd Renewal of this protocol
*A Full Protocol submission will be required in 05.31.2011

Dear Dr. **Shoemaker**

Your Animal Use Protocol form entitled:

Associating NPY and Y1 Receptor Changes with Blood Pressure and Vascular Mechanics in Developing Hypertension

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **05.01.2010 to 05.31.2011**

The protocol number for this project remains as **2007-046**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this *Animal Use Protocol* is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. L. Mattar

***The* University *of* Western Ontario**
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, • London, Ontario • CANADA – N6A 5C1
PH: 519-661-2111 ext. 86770 • FL 519-661-2028 • www.uwo.ca / animal

Western

11.01.08
*This is the 2nd Renewal of this protocol
*A Full Protocol submission will be required in 2010

Dear Dr. **Shoemaker**

Your Animal Use Protocol form entitled:

Physical Activity, Estrogen and Peptidase Control of Neurovascular Function in Skeletal Muscle

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **11.01.08** to **10.31.09**

The protocol number for this project remains as **2006-109**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. W Lagerwerf

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
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Appendix C – Details of Western Blot Protocol

Approximately 70mg of frozen tissues were homogenized in 15 volumes (approximately 1 mL) of extraction buffer (10mM Tris, 100mM NaCl, 1mM EDTA, 1mM EGTA Tetra Sodium, 1% Triton X-100, 10% Glycine, 0.1% SDS, 0.5% Sodium Deoxycholate, 1% protease inhibitor cocktail (Sigma-Aldrich, Cat. No. P8340); pH = 7.4), and centrifuged at 16000g for 20 min in order to collect the supernatant as the tissue extract. Sample homogenates were then stored at −70°C until the time of total protein concentration determination (Bradford protein assay (1)) and electrophoresis. Equal amounts of total protein (60 μg for Y_1R and 120 μg for Tyrosine Hydroxylase (TH) and α-AR) were run on 12% sodium dodecyl sulfate polyacrylamide gel (SDS PAGE) overlaid with a 4% acrylamide stacking gel. A protein standard (Bio-Rad Cat. No. 161-0376) was run along with the tissue homogenate. Following electrophoresis, the proteins were transferred at constant voltage in cold transfer buffer (10% running buffer, 20% methanol in ddH2O) to a polyvinylidene fluoride membrane (PVDF; Bio-Rad Cat. No. 162-0177). The membranes were wetted with methanol prior to protein transfer, and subsequently blocked in a 5% non-fat milk solution in Tris buffered saline + 0.05% Tween 20 (TTBS) (80mM Tris Base, 0.5M NaCl). Membranes were then incubated overnight in primary antibody specific to either rat, human or mouse anti-Y_1R (1:300, affinity purified rabbit anti-mouse Y_1R IgG, Alpha Diagnostic International, Cat. No. NPY1R11-A, San Antonio, TX, USA), anti-α_1-AR (1:400 affinity isolated rabbit, Sigma-Aldrich Cat. No. A270) or anti-TH (1:1000 purified rabbit polyclonal antibody, Millipore, Cat. No. AB152, Billerica, MA, USA) in TTBS with 2% non-fat milk. Membranes were washed 3 times in TTBS then incubated in secondary antibody (goat

anti-rabbit, or anti-mouse; 1:3000) conjugated to horseradish peroxidase (HRP; Bio-Rad, Cat. No. 170-6518) and Precision Protein StrepTactin-HRP Conjugate (1:10000; Bio-Rad, Cat.No. 161-0380) in TTBS with 2% non-fat milk for 1 hour. After washing (TTBS; 3 times), the blots were developed using Immun-Star WesternC chemiluminescent (Bio-Rad, Cat. No. 170-5070) and detected using a Bio-Rad ChemiDoc XRS system (Bio-Rad, Cat. No. 170-8070) with a supersensitive 16-bit CCD. Images were analyzed using Quantity One 1-D Analysis Software (Bio-Rad, Cat. No. 170-9600).

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Curriculum Vitae

Louis Mattar

PhD Candidate
Neurovascular Research Lab
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Education

2006–Present	Ph.D., Neurovascular Research Laboratory, The School of Kinesiology, Faculty of Health Sciences, The University of Western Ontario. Supervisor: Dr. J. Kevin Shoemaker. Doctoral Thesis: *On the early onset of vascular stiffening and sexual dimorphism of sympathetic control in the spontaneously hypertensive rat*
2004–2006	M.Sc., Cardiorespiratory and Vascular Dynamics Laboratory, The Department of Kinesiology, The Faculty of Applied Health Sciences, University of Waterloo. Supervisor: Dr. Richard L. Hughson. Masters Thesis: *The Effects of 60 days of Head Down Bed Rest on Vascular Health.*
2000–2004	Honours B.Sc., Kinesiology, Pre-Health Professional Option, University of Waterloo.

Specific Training

a) Specific Training Certifications

2010	*Certificate in University Teaching and Learning*, Teaching Support Center, The University of Western Ontario.
2008	*Rodent Hemodynamics Workshop*, By: ADInstruments, Transonic Systems Inc., and Gwathmey Inc., Boston, USA.
2008	*Advanced Teaching Program*, The Teaching Support Center, The University of Western Ontario.
2006	*Certificate in animal care*, The University of Western Ontario.

b) Training Statement

During my Master's degree, the bulk of my training focused on the quantification of blood borne proteins associated with cardiovascular health/disease. Measurement of these factors were completed using a variety of techniques including: enzyme-linked immunosorbent assay (ELISA), radioimmuno assays (RIA), and immunofluorescence. Through this experience, I became proficient in the set up, calibration, and collection of non-invasive blood pressure (Finometer/Finapres), and echo Dopplar ultrasound measurements of arterial structure and blood velocity (various ultrasound machines/Multigon). Data was obtained using a Powerlab and Chart system (ADInstruments).

My Doctoral research shifted my study from Human to animal (rat) research. Training in animal care, handling, husbandry, anesthesia, and aseptic surgical techniques (tracheal intubation, arterial and venous canulation, and perivascular flow probe placement) were required, obtained, and mastered. I also became proficient in a variety of non-invasive measurement techniques including: non-invasive blood pressure (CODA System, Kent Scientific) and micro ultrasound imaging (Visual Sonic). Additionally, I have become comfortable with the preparation, procedure, and analysis of western blotting techniques.

The aforementioned techniques from my Master's and Doctoral degrees are ones that I have performed, and am proficient in. I have been fortunate enough to become familiar with a variety of other techniques that, while I have not done them personally, I feel confident in my ability to explain both their theory, practice and in many cases, their analysis. These techniques include: microneurography, phlebotomy, functional magnetic resonance imaging (fMRI) and wire myography.

Honours and Awards

2011 *Graduate Thesis Award.* The University of Western Ontario. $795.

2011 *Ontario Exercise Physiology Student Presentation Award Finalist*, Ontario Exercise Physiology. $100.

2010 *Kinesiology Travel Award*. From The School of Kinesiology, The University of Western Ontario. $700.

2010 *Faculty of Health Sciences Travel Award*. From The Faculty of Health Sciences, The University of Western Ontario. $500.

2010 *Carolyn tum Suden Professional Opportunity Award*. From The American Physiological Society. $500.

2009–10 *Ontario Graduate Scholarship*. From the Government of Ontario. $15,000/year.

2009 *American Physiological Society Travel Award*. From The American Physiological Society. $625

2009 *Faculty of Health Sciences Travel Award*. From The Faculty of Health Sciences, The University of Western Ontario. $500.

2008–09 *Ontario Graduate Scholarship*. From the Government of Ontario. $15,000/year.

2008 *Faculty of Health Sciences Travel Award*. From The Faculty of Health Sciences, The University of Western Ontario. $500.

2008 *Kinesiology Travel Award*. From The School of Kinesiology, The University of Western Ontario. $625.

2008 *Society of Graduate Students Travel Subsidy.* The Society of Graduate Students, The University of Western Ontario. $400.

2008 *Graduate Thesis Award.* The University of Western Ontario. $544.

2008 *Mercury Printing Poster Award*. From the Western Research Forum, The University of Western Ontario. $50.

2004–06 *Master's Studentship Award*. From The Heart and Stroke Foundation of Ontario. $18,000/year.

Professional Membership

The American Physiological Society

The Canadian Society for Exercise Physiology

Professional Service

a) Conference Organization

2007 Canadian Society for Exercise Physiology (CSEP). The University of Western Ontario.

2007 Ontario Exercise Physiology (OEP). The University of Western Ontario.

2005 Ontario Exercise Physiology (OEP). University of Waterloo.

b) Committee Involvement

Graduate Student Level

2009–10 President, The Kinesiology Graduate Board, The University of Western Ontario.

2009–10 Member, Graduate Student Teaching Assistant Nomination Committee, The University of Western Ontario.

2009–10 Member, Western Research Forum Committee, The University of Western Ontario.

2008–09 Treasurer, The Kinesiology Graduate Board, The University of Western Ontario.

Department/School Level

2009–10 Graduate Student Member, Kinesiology Graduate Affairs Committee, The University of Western Ontario.

2009–10 Graduate Student Member, Kinesiology Executive Management Committee, The University of Western Ontario.

2007–10 Graduate Student Member, Kinesiology School Affairs Counsel, The University of Western Ontario.

2005–06 Member, Kinesiology Graduate Student Association, University of Waterloo.

Faculty Level

2009–10 Graduate Student Member, Faculty of Health Sciences Faculty Counsel, The University of Western Ontario.

University Level

2008–10 Counsellor, The Society of Graduate Students, The University of Western Ontario.

2005–06 Member-At-Large, Graduate Student Association, University of Waterloo.

Scholarly Activity

Summary:

Articles in Progress/Submitted: **5**
Articles in peer-reviewed journals: **6**
Published abstracts from professional meetings: **13**
Poster Presentations at Professional Meetings: **10**
Oral Presentations at Professional Meetings: **7**
Other Oral Presentations: **4**

a) Articles in Progress/Submitted:

1. **Mattar, L.** D.N. Jackson and Shoemaker, J.K. (**Submitted**). Estrogen and sympathetic modulation of hindlimb blood flow variability in rats. To be submitted to Applied Physiology Nutrition and Metabolism APNM 11-4.
2. **Mattar, L**., Gimon, T., Zamir, M., and J.K. Shoemaker (**submitted previously**). Sympathetic Contribution to Peripheral Vascular Stiffness in Young Female Spontaneously Hypertensive Rats.
3. **Mattar, L**., Jackson, D.N., Ellis, C.G., Noble, E. and Shoemaker, J.K. (**submitted previously**). Age-related changes in sympathetic control of hindlimb conductance and blood pressure in young hypertensive rats.
4. **Mattar, L.** and Shoemaker, J.K. (**In Progress**). Increased vascular stiffness in spontaneously hypertensive rats following estrogen supplementation. To be submitted to Clinical and Experimental Pharmacology and Physiology.
5. **Mattar, L**., Zamir, M., and J.K. Shoemaker (**In Progress**). Y1 Receptor versus sympathetic control of hindlimb vascular stiffness in spontaneously hypertensive rats. To be submitted to AJP - Reg. Int. Comp. Phys.

b) Articles in peer-reviewed journals:

1. Usselman, C.W., **Mattar, L.**, Junuzovic, J., Welch, I., and Shoemaker, J.K. (**Accepted**). Intra-strain baroreflex variability in urethane/α-chloralose anaesthetized male Sprague-Dawley rats. Applied Physiology Nutrition and Metabolism, APNM-10-264.
2. Hodges, G.J., **Mattar, L.**, Zuj, K, Greaves, D., Arbeille, P., Hughson, R.L., and Shoemaker, J.K. (2010). WISE 2005: Prolongation of left ventricular pre-ejection period with 56 days head-down bed rest in women. Experimental Physiology, 95(11): 1081.

3. Hodges, G.J., Jackson, D.N., **Mattar, L.**, Johnson, J., and Shoemaker, J.K. (2009). Neuropeptide Y and neurovascular control in skeletal muscle and skin. AJP – Regulatory, Integrative and Comparative Physiology. 297: R546-R555.
4. Arbeille, P., Kerbeci, P., **Mattar, L.**, Shoemaker, J.K., and Hughson, R.L. (2008). Insufficient flow reductions during LBNP in both splanchnic and lower limbs is associated with orthostatic intolerance after bedrest. AJP – Heart and Circulatory Physiology. 295: H1846 - H1845.
5. Arbeille, P., Kerbeci, P., **Mattar, L.**, Shoemaker, J.K., and Hughson, R.L. (2008). WISE-2005 – Tibial and Gastrocnemius Vein, and Calf Tissue Response to LBNP after a 60 day Bedrest with and without Counter-measures. Journal of Applied Physiology. 104: 938-943.
6. Shoemaker, J.K., **Mattar, L.**, Kerbeci, P., Trotter, S., Arbeille, P., Hughson, R.L. (2007). WISE 2005: Stroke Volume Changes Contribute to the Pressor Response During Ischemic Handgrip Exercise in Females. Journal of Applied Physiology. 103: 228 - 233.

c) Published abstracts from professional meetings:

1. **Mattar, L**., Gimon, T., Zamir, M., and J.K. Shoemaker (2010). Vascular stiffness and the sympathetic nervous system in young female rats. *APNM,* 35:S65.
2. **Mattar L**., Noble, E., and Shoemaker, J.K. (2010). Estrogen and sympathetic modulation of hindlimb blood flow variability in rats. *FASEB Journal.* 24:1039.13.
3. **Mattar L**., Jackson, D.N., Ellis, C.G., Noble, E., and Shoemaker, J.K. (2009). Beta-arrestin and vasomotor control in the spontaneously hypertensive rat. *FASEB Journal*. 23:1017.45.
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d) Poster Presentations at Professional Meetings:

1. **Mattar, L**., Gimon, T., Zamir, M., and J.K. Shoemaker (2010). Vascular stiffness and the sympathetic nervous system in young female rats. *The Canadian Society for Exercise Physiology; Toronto, ON;* November 2010.
2. **Mattar L**., Noble, E., and Shoemaker, J.K. (2010). Estrogen and sympathetic modulation of hindlimb blood flow variability in rats. *Experimental Biology; Anaheim, CA;* April 2010.
3. **Mattar L.**, Jackson, D.N., Ellis, C.G., Noble, E., and Shoemaker, J.K. (2009). Beta-arrestin and vasomotor control in the spontaneously hypertensive rat. *J. Allen Taylor International Prize in Medicine: Cardiovascular Research; London, Ontario;* November 2009.
4. **Mattar**, L., Noble, E., Shoemaker, J.K. (2009). Estrogen and the development of hypertension in the female spontaneously hypertensive rats. *APS Conference: Sex and Gender in Cardiovascular-Renal Physiology and Pathophysiology; Broomfield, CO;* July 2009.
5. **Mattar L**., Jackson, D.N., Ellis, C.G., Noble, E., and Shoemaker, J.K. (2009). Beta-arrestin and vasomotor control in the spontaneously hypertensive rat. *Experimental Biology; New Orleans, LA;* April 2009.
6. **Mattar L**., Jackson, D.N. and Shoemaker, J.K. (2008). Neuropeptide Y And Age-Related Development of Hypertension in the Rat. *Experimental Biology; Dan Diego, CA;* April 2008.
7. **Mattar L.**, Jackson, D.N. and Shoemaker, J.K. (2008). Neuropeptide Y And Age-Related Development of Hypertension in the Rat. *Western Research Forum; London, ON;* February 2008.
8. **Mattar L.**, Jackson, D.N. and Shoemaker, J.K. (2008). Enhanced Constrictor Response to Neuropeptide Y in the 7 week old Rat. *ARGC/FHS Symposium on aging; London, ON;* February 2008.
9. **Mattar, L.**, Rush, J.W.E., Shoemaker, J.K., Arbeille, P., and Hughson., R.L. (2006). WISE-2005: 60 days head-down bed rest causes a shift towards a pro-artherosclerotic environment. *Experimental Biology; San Francisco, CA;* April 2006.
10. **Mattar, L**.; Rush, J.W.E., Shoemaker, J.K., Arbeille, P., and Hughson, R.L. (2005). WISE-2005: 60 Days Head-Down Bed Rest Causes and Increase in Angiotensin II. *Canadian Society for Exercise Physiology; Gatineau, QC;* November 2005.

e) Oral presentations at Professional Meetings:

1. **Mattar, L.** and Shoemaker, J.K. (2011). Sexual dimorphism and cardiovascular regulation in rats. *Western Research Forum; London, ON;* February 2011.
2. **Mattar, L.** and Shoemaker, J.K. (2011). Evidence for sexual dimorphism in cardiovascular regulation in rats. *Ontario Exercise Physiology; Barrie, ON;* January 2010.
3. **Mattar, L.,** Jackson, D.N., Noble, E., and Shoemaker, J.K. (2010). Modulation of hindlimb blood flow variability by estrogen and the sympathetic nervous system in rats. *Ontario Exercise Physiology; Barrie, ON;* January 2010.
4. **Mattar, L.**, Jackson, D.N., Ellis, C.G., Noble, E., and Shoemaker, J.K. (2009). Beta-arrestin and vascular control in hypertension. *Western Research Forum; London, ON;* January 2009.
5. **Mattar, L.**, Jackson, D.N., and Shoemaker, J.K. (2009). Neuropeptide Y And Age-Related Development of Hypertension in the Rat. *Ontario Exercise Physiology; Barrie, ON;* January 2009.
6. **Mattar, L**., Jackson, D.N., and Shoemaker, J.K. (2008). Enhanced Constrictor Response to Neuropeptide Y in the Spontaneously Hypertensive Rat. *Ontario Exercise Physiology; Barrie, ON;* January 2008.
7. **Mattar, L.**, Rush, J.W.E., Shoemaker, J.K., Arbeille, P., and Hughson, R.L. (2006). WISE 2005: 60 Days Head down bed rest causes an increase in oxidative stress. *Ontario Exercise Physiology; Barrie, ON;* January 2006.

f) Other Oral Presentations:

1. Presentation for Mrs. Kelly Sauve Grade 12 University Biology Preparation class. "A career in school: Options for after University". *Dorchester High School; Dorchester, ON;* December 2008.
2. Lecture for Dr. H. Prapavessis 2nd/3rd year statistics (KIN 332a). *The University of Western Ontario. London, ON*; October 2007.
3. Presentation for Ms. Jan Farquhar Grade 8 gifted class. "Why I like science". *Charles R. Beaudoin Public School; Oakville, ON;* June 2007.
4. Lecture for Dr. Timothy Wilson 2nd/3rd year anatomy. Anatomy of the Heart and Lungs. *The University of Western Ontario; London, ON;* November 2006.