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Conductance and Capacitance Effects of Acute, Electrical, Carotid Baroreflex Stimulation

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By

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Abstract

Baroreflex activation therapy (BAT) is effective in resistant hypertension; we hypothesized that BAT increases both venous capacitance and arterial conductance. We measured aortic pressure and flow and inferior vena caval flow and used a modified Brooksby-Donald technique to evaluate sub-diaphragmatic blood volume. Data were recorded with BAT, and with sodium nitroprusside (SNP) and Angiotensin II (Ang II) infusions, alone and with BAT.

BAT substantially increased venous capacitance and arterial conductance. SNP also increased venous capacitance and arterial conductance. During SNP infusion BAT remained effective, further decreasing blood pressure and increasing capacitance and conductance. Ang II decreased both capacitance and conductance. During Ang II infusion, increased BAT reversed the decrease in venous capacitance while restoring BP completely and conductance to 80% of baseline.

BAT decreases blood pressure and increases arterial conductance and venous capacitance, even when combined with powerful vasoactive drugs. These may be important effects in hypertension.

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List of Symbols and Abbreviations

ρ	Density
ACE	Angiotensin-converting enzyme
Ang II	Angiotensin II
ANS	Autonomic nervous system
$Area_{LVED}$	Left-ventricular end-diastolic area
BAT	Baroreceptor activation therapy
CO	Cardiac Output
CVLM	Caudal ventrolateral medulla
CVP	Central venous pressure
d	Diameter
E	Elastic modulus
EF	Ejection fraction
g	Gravity
G_{ao}	Aortic/arterial conductance
h	Wall thickness
HR	Heart Rate
I.V.	Intra-venous
IVC	Inferior vena cava
LV	Left ventricle
MCFP	Mean circulatory filling pressure
mQdia	Mean aortic diaphragmatic flow
mQivc	Mean inferior vena caval flow
NA	Nucleus ambiguous
NE	Norepinephrine
NTS	Nucleus tractus solitarii
P	Pressure
P_{ao}	Aortic pressure
P_{LVED}	Left-ventricular end-diastolic pressure
P_{RA}	Right atrial pressure
P_{RAED}	Right-atrial end-diastolic pressure

P-V	Pressure-Volume
PWV	Pulse wave velocity
$Q_{ao\text{-}asc}$	Ascending aortic flow
r	Radius
RA	Right atrium
RV	Right ventricle
SD	Standard deviation
SE	Standard error
SNP	Sodium nitroprusside
SV	Stroke volume
SVR	Systemic vascular resistance
$V_{abdominal}$	Abdominal blood volume

1 Introduction

CVRx Inc., a medical device company, has developed an implantable device to provide electrical stimulation to the carotid artery baroreceptor. The device is intended to treat patients with chronic high blood pressure for whom traditional drug therapy is unsuccessful. Treatment with the device is referred to as 'Baroreceptor Activation Therapy' (BAT).[1]

Baroreceptors are stretch receptors that respond to blood pressure changes by eliciting a response of the autonomic nervous system. High-pressure baroreceptors are located in the aortic arch and carotid arteries. Increased blood pressure stimulates an autonomic nervous system response, decreasing heart rate and reducing peripheral vascular resistance, thus decreasing arterial blood pressure. This negative-feedback system allows for variations in blood pressure to be attenuated and a steady-state maintained.

Peripheral vascular resistance is controlled by vascular smooth muscle. Relaxation of smooth muscle cells reduces the resistance to flow through the vasculature and increases the compliance of the vasculature. Modification of venous tone can have two fundamental effects: the venous compliance can be changed, indicated by a change in the slope of the pressure-volume relationship, and the venous capacitance can be changed, indicated by a change in the unstressed volume of the vein.

The application of this device in an experimental setting provides a novel method for investigating hemodynamic changes due to artificial stimulation of the baroreflex. An intervention can affect arterial and venous properties to different degrees. Identifying the relative magnitude of these effects can increase the applicability of a therapy in disease states, such as hypertension and heart failure.

2 Literature Review

2.1 Baroreceptors

Baroreceptors exist in four locations in the vascular system with arterial baroreceptors in the carotid and aortic arch. Cardiopulmonary baroreceptors exist in the atria and ventricles of the heart and the pulmonary vasculature. While both arterial baroreceptors respond in the same manner to attenuate changes in blood pressure, their effects are not equipotent. The carotid artery baroreceptor is more sensitive and induces greater systemic responses.[2]

Baroreceptors are mechano-sensitive receptors, responding to the stretch of the artery. Carotid artery baroreceptors are located in the carotid artery sinus – a widened portion of the carotid artery near the bifurcation of the artery into the internal and external carotid arteries.[2] As blood pressure increases, the artery is stretched more. The response depends on the absolute pressure as well as the rate of pressure/stretch change in the artery.[3;4] Baroreflex dysfunction can be an effect of various disease states, including multiple forms of hypertension[5;6] and heart failure.[7] Rare cases exist where the baroreflex itself is the source of failure as a consequence of neck injury, tumor development, or neck irradiation, among others. Baroreceptor dysfunction in such cases often results in a wide range of blood pressure and heart rate surges.[8]

2.1.1 Sympathetic & Parasympathetic Activity

The sympathetic and parasympathetic nervous systems are divisions of the autonomic nervous system (ANS) and contribute to the control of the circulation. Comprising two independent neural pathways, the sympathetic and parasympathetic system are often described as having opposing effects on organs, though their work together is important in maintaining homeostasis. Sympathetic nerve activity is frequently referred to as the 'flight-or-fight'[9] response due to its effects of increasing heart rate (HR), dilating pupils and decreasing gastric motility, among others. The parasympathetic division of the ANS is associated with a 'rest-and-digest'[9] response, decreasing heart rate, constricting pupils, and increasing gastric motility.

2.1.2 Signalling

Afferent nerve fibres carry carotid artery baroreceptor signals to the nucleus tractus solitarus (NTS), located in the medulla oblongata.[10] The NTS receives afferent signals from the arterial baroreceptors as well as vagal afferents from chemoreceptors in the heart and venous system, and is the principal centre of integration for circulatory system control.[11]

Baroreceptor signals arriving in the NTS stimulate interneurons located in the caudal ventrolateral medulla (CVLM). Stimulation of the CVLM inhibits stimulation of the rostral ventrolateral medulla, the location of sympathetic premotor neurons,[10] thus decreasing sympathetic outflow from the brainstem.

Baroreceptor signals arriving in the NTS also excite preganglionic vagal motor neurons in the nucleus ambiguus (NA).[10] From the NA, parasympathetic nerve signals are carried to organs through cranial nerve X (vagus nerve).[10] This includes innervations of the pacemaker and atria cells of the heart. Parasympathetic stimulation of the heart causes heart rate to decrease.

2.1.3 Frequency of Baroreceptor Stimulation

Studies of artificial stimulation of the carotid baroreceptors have helped map the peak vagal and sympathetic response for varying frequencies of stimulation. Electrical stimulation has been shown to have the greatest heart rate effect, increasing the R-R interval, at frequencies between 80 and 120 Hz.[12] In a study of normotensive patients by Borst et al.,[13] the blood-pressure decrease from bilateral electrical stimulation of carotid sinus nerves was frequency-dependent, with the effect increasing to a peak at a stimulating frequency of 80 Hz. Stimulation beyond 80 Hz, tested to a maximum of 200 Hz, yielded blood-pressure decreases that were gradually compensated by baroreflex adaption.

For our study, based on the literature and the recommendation of experienced operators at CVRx. Inc., we kept frequency constant at 80 Hz for all device activations in all experiments.

2.1.4 Sympathetic Innervation of Vasculature

Smooth muscle cells, which control the tone of the vasculature, are contained within all blood vessels, with the exception of capillaries.[14] Cardiovascular smooth muscle cells contain

adrenoreceptors, which are receptors for the catecholamine norepinephrine (NE).[15] Sympathetic outflow releases NE, which binds to the smooth muscle cell receptors. Binding causes smooth muscle cell contraction, which maintains or increases vascular tone.

2.1.5 Alpha-1 and Beta-1 Adrenergic Receptors

Sympathetic stimulation of the vasculature has more functional influence on the capacitance vessels as compared to the resistance vessels; capacitance vessels reach their maximum constriction at a lower frequency of sympathetic firing versus resistance vessels.[2] While all vessels exhibit properties of resistance, in terms of cardiovascular control, "resistance vessel" refers to small arteries. Local and systemic control of resistance vessels allows for blood flow to be directed based on demand. "Capacitance vessel" conventionally refers to the venous system, which holds 70-80% of blood.[16]

2.2 Arterial Stiffness

Arterial stiffness is an important parameter in the efficient delivery of blood throughout the body. Increased arterial stiffness, especially in the aorta, can alter the pulse-wave shape and velocity, as well diminish the capacitive ability of the aorta, essential to diastolic flow. Passive arterial stiffness is mainly governed by the elastin and collagen content, with greater elastin content resulting in lower arterial stiffness.

The ability of the aorta to stretch is governed by its mechanical properties. The proportions of elastin and collagen in the aorta, along with the presence of smooth muscle and its tone affect the ability of the aorta to stretch. These properties provide the aorta with a passive pressure-volume relationship, relating the distensibility of the wall to the pressure generated within the aorta. This relationship can be altered by changes to the aorta's mechanical properties, including local aortic stiffness, with varying age, disease state, and remodeling.

Pulse wave velocity (PWV) is a function of arterial geometry and stiffness and average PWV can be measured by determining the transmission time of the pulse wave through a known

distance. For this reason PWV has been extensively used to examine arterial stiffness in various populations and disease states.

2.2.1 Arterial Stress and Strain Basics

A standard clinical PWV measurement is the carotid-femoral PWV.[17] This test involves the non-invasive measurement of pulse waves from both sites to provide a length of time for the pulse wave to travel the given distance, with distance taken as the distance between the sites on the surface of the body. The average pulse wave velocity is then given as PWV = distance / time. In a clinical setting, PWV is used almost interchangeably with arterial stiffness. This is due to the relationship between PWV and elastic modulus, as defined by the Moens-Korteweg equation.[18]

2.2.1.1 Pulse Wave Velocity

Moens-Korteweg Equation

$$Pulse\ Wave\ Velocity = \sqrt{\frac{E\ h}{2r\rho}}$$

E = elastic modulus h = wall thickness

r = radius ρ = fluid density

Elastic modulus is the primary variable of interest in the PWV equation. Elastic modulus represents the ability of a material to deform elastically when subjected to a stress. A stiffer artery will deform less under the same stress, and therefore has a higher elastic modulus. This higher arterial elastic modulus is typically determined clinically by the observation of a higher PWV.

2.2.1.2 Pressure-Diameter Loops

While PWV is the most common measure of arterial stiffness used in the clinical setting, the results of a PWV test provide average values for the length of an examined arterial segment, and do not provide information on stiffness at a specific aortic location.[19] Local aortic stiffness can be determined by creating a pressure – diameter loop for the location under investigation. An aortic pressure-diameter loop is a more direct representation of the mechanical properties

of the artery and can be used to calculate arterial stiffness (elastic modulus or pressure-strain elastic modulus) as follows:[19]

Aortic Distensibility: $\frac{2\Delta d}{d\Delta P}$

Where d = diastolic aortic diameter

Δd = difference between systolic-diastolic diameter (pulse diameter)

ΔP = difference between systolic and diastolic pressure (pulse pressure)

Pressure – Strain Elastic Modulus: $E_p = \frac{\Delta P d}{\Delta d}$

Pulse wave velocity: $\sqrt{\frac{E_p g}{2\rho}}$

Where g = gravity

ρ= blood density

2.2.2 Elastin and Collagen

The main determinants of passive properties of the arteries and aortic stiffness are the elastin and collagen content at a given location. Elastin is the primarily responsible for the control of arterial dilation, and can be stretched by 50% to 70% of resting lengths.[20] At low pressure, depending on the location, collagen fibres are inactive. The arrangement of collagen in this condition has been described as coiled[21] or folded.[22;23] While unfolding, the stretch of elastin is governed by an immeasurably small Hooke's constant. Only when they become uncoiled and reach their maximum unstressed length do they become engaged, displaying a high Hooke's constant, and begin to contribute to the stress-strain characteristics of the artery.[24] They serve mainly to ensure the structural integrity of the artery wall at higher pressure. Collagen can be stretched by 2% to 4% of its resting length.[20] Within the arterial system, the ratio of elastin to collagen is greatest in the thorax, and is lower outside the thorax.[25] The engagement of collagen fibres as the artery wall is stretched accounts for the non-linearity of the stress-strain

relationship of the arterial wall. This allows the artery to be characterised as a "two-phase" material, with elastin describing the behaviour of the lower, physiological range, of the stress-strain curve. Collagen is responsible for the high portion of the stress-strain curve, where the artery is much less distensible.[26] Studies have suggested that at physiological blood pressures less than ten percent of collagen fibres are active in the arterial wall.[27]

2.2.3 Arterial Stiffness vs. Blood Pressure

Arterial stiffness increases with increased blood pressure. This is due to the engagement of more collagen fibres as the limits of elastin are exhausted.[28] This relationship makes the interpretation of anti-hypertensive treatments more challenging, as the passive changes to stiffness due to blood pressure reduction need to be accounted for while determining if any active changes to the artery wall have occurred.[29]

A study by Hayoz et al.[30] found no evidence to conclude that arterial compliance and distensibility are necessarily reduced in hypertensive patients. Arterial compliance and distensibility are reciprocal measures of elastance, and they relate more specifically to a diameter versus pressure relationship. Elastance increases with the higher pressures associated with hypertension, as expected from the non-linear relationship of stiffness and pressure. When Hayoz et al.[30] plotted arterial diameter against blood pressure for hypertensive and normotensive rats there was little difference between the curves at near-matching pressures. They gathered their observation from pressure-diameter data collected at both the radial artery and carotid artery. Data collected from the medium-sized muscular radial artery exhibits more scatter than elastic arteries, such as the carotid artery, due to additional vasoactive stimulation, emphasizing that changes to the mechanical properties of elastic arteries are likely to be more easily measured.

2.2.4 Sympathetic Effects on Arterial Stiffness

A nervous system response resulting in sympathetic outflow will alter vascular tone to ensure proper blood supply to organs as required in a flight-or-fight response. This would include dilation of skeletal muscle arteries and constriction in many other arterial beds. In an investigation on the effects of noradrenaline on ascending aortic diameter, only small changes

in diameter were observed.[31] While diameter changes were small (6%), changes to the dynamic stiffness of the ascending aorta due to the constriction were more meaningful, showing increases in pressure-strain elastic modulus of 30%-67%.[31] While the ascending aorta is less innervated than some peripheral arteries, the highly-sensitive vascular smooth muscle and excess of alpha1-adrenoreceptors can explain the responsiveness to adrenergic stimulation.[32] Direct electrical stimulation of the sympathetic trunk has been shown to cause a reduction in the diameter of the abdominal aorta.[33] Similar control of hemodynamic properties in the aorta can be observed when sympathetic outflow is modified by manipulation of the carotid sinus baroreceptor reflex.[34]

Conclusions from studies on the effects of vascular tone on arterial stiffness have been mixed, with results showing both increases and decreases in stiffness.[32] This has been attributed to the variations in methods of comparison for varying pressures, transmural pressures, diameters, strains, and stresses.[32] Results showing reduced radial artery compliance for a constant diameter indicate that vasomotor tone has a key role in controlling arterial stiffness, along with the passive properties of the artery.[35]

2.3 Cardiac Output

Cardiac output (CO) is determined by cardiac contractility, heart rate, preload, and afterload.[36] CO can be calculated by multiplying heart rate and LV stroke volume. Contractility is the ability of the heart to generate force during contraction, and is related to the level of actin and myosin binding in cardiac muscle. Cardiac contractility, as assessed by the left-ventricular end-systolic pressure-volume relationship, has been shown to be unchanged with baroreceptor activation.[1] Heart rate is reduced with baroreflex activation via vagal pathways.[1] If there are no changes to peripheral circulation to alter preload and afterload, a decrease in heart rate will decrease CO.

Cardiac output is both a determinant of preload and afterload and is determined by preload and afterload.[2] Preload is the amount the ventricles are stretched before contraction begins, and is quantified by ventricular end-diastolic volume, or to a potentially less accurate

ventricular end-diastolic pressure.[37] Preload can decrease with decreased central venous pressure (CVP) and reduced venous return, which in turn will decrease CO via the Frank-Starling mechanism. With CVP measured as the dependent variable, increasing CO will draw more blood from the venous system, draining the reservoir and lowering both CVP and likely preload.

The relationship between CVP and CO can be described with cardiac and vascular function curves.[38] On a cardiac function curve, CO and CVP are proportionally related, with CO being the dependent variable. An increase in CVP will result in an increase in CO. On a vascular function curve, where CVP is the dependent variable, the relationship of CO to CVP is inversely proportional. With an increase in CO and no change to SVR, the venous reservoir will be depleted and CVP will decrease.[2] If CO could be decreased to zero, the value reached for CVP is referred to as the mean circulatory filling pressure (MCFP), and reflects the total blood volume and compliance of the system.[2]

Afterload is the force that the ventricle has to work against in order to eject blood. It relates the pressure in the aorta to the tension developed in the ventricular walls. Decreasing aortic pressure will decrease afterload, allowing for a greater volume of blood to be ejected, therefore increasing CO. This increased CO is likely to increase aortic pressure and therefore increase afterload, as blood is shifted from the venous to the arterial circulation.

2.4 Venous Capacitance

With the veins containing 70% of blood volume[16] any change in venous tone and the capacity of the venous system will have a significant effect on hemodynamic characteristics and cardiac function. An increase in the capacitance of the venous system due to the sympathetic inhibition provided by BAT could be a significant factor in BAT's ability to decrease blood pressure.

Veins are referred to as capacitance vessels due to their large compliance relative to arteries. Compliance is a measure of distensibility, represented as change in volume per change in pressure. Venous compliance is approximately 20 times greater than arterial compliance.[2] Therefore, if a volume of blood is shifted from the venous system to the arterial system the pressure change in the arterial system would be 20 times greater than the pressure change in the venous system. This is important to consider during pharmacological interventions. While a vasodilator may achieve a goal of lowering arterial blood pressure, it is important to distinguish its mechanism of action, whether acting primarily on the venous or arterial circulation, or having a relatively equal effect on both.[39] When venous capacitance is altered the compliance (slope of the pressure-volume relationship) is left relatively unchanged, while the entire P-V relationship is shifted to lower or higher volumes. Evidence suggests that changes in venous capacitance can be of relatively greater importance than changes in venous compliance, with changes to venous smooth muscle activity not necessarily affecting both properties to the same degree.[40] It is therefore important to distinguish between the two measures when describing changes to venous characteristics.

Unstressed volume refers to the capacity of the veins when venous transmural pressure is zero.[38] Alterations to this volume through venous constriction or dilation result in changes in venous capacitance. Venous capacitance can also change through alterations to the properties of the stressed wall and volume. When the venous transmural pressure is greater than zero, the additional volume of blood in the vein is referred to as the stressed volume. Increases in stressed volume are governed by venous compliance, with an incremental increase in volume associated with an incremental increase in pressure.

2.4.1 Evaluating Circulating Blood Volume

Due to systemic changes that occur to maintain homeostasis, CVP is not an adequate measure of venous blood volume status.[41] This can be observed when changes in blood volume buffer changes to CO during volume loading or hemorrhage.[38] Determining MCFP would provide a measure for volume status.[41] Most techniques for its measurement involve challenging experimental methods requiring temporary circulatory or cardiac arrest to obtain curves for venous and arterial pressure decay.[16] An additional technique for evaluating venous tone is the constant CO technique, in which venous return is fed into an external reservoir while CO is held constant. Changes to the external reservoir and venous pressure can yield information on venous compliance and unstressed volume.[16]

Several techniques exist for evaluating capacitance changes in an organ or region. A fluid-filled plethysmograph can be used on a limb or organ to measure changes in volume.[42] Blood-pool scintigraphy involves i.v. injection of a radioactive tracer which can be measured or imaged in the organ or region of interest.[16] The activity of the radioactive element in an organ is proportional to the blood volume and thus capacity can be evaluated.

Brooksby and Donald adopted a different approach to quantify changes to the blood volume of the splanchnic circulation.[43] Blood flow in and out of the vascular bed was recorded and the integration of the flow difference allowed for a change in blood volume to be determined. The subsequent use of the technique has been referred to in literature as the "Brooksby-Donald" technique, which can be applied to any organ or vascular bed.[44;45]

Brooksby and Donald's initial experiment[43] was an investigation of acute blood volume storage and mobilization, with several cannulations of the aorta and inferior vena cava (IVC). Balloon catheters inserted via the femoral arteries were advanced into the aorta and vena cava in an effort to isolate splanchnic flow. A shortcoming of this experiment is that neither pressure nor flow to the splanchnic bed was controlled.[42] Without keeping either the flow or pressure constant it is not possible to distinguish between active and passive capacitance changes.

Brooksby and Donald addressed this in a subsequent experiment[46] where pressure was controlled and the passive effect in capacitance change was quantified.

Our experimental setup more closely resembles the original Brooksby and Donald setup, which was adapted by Wright et al.[44] to investigate both venous capacitance and systemic conductance changes. Their goal was to evaluate transient capacitance response in a chronic setting and they thus used a modified Brooksby-Donald method. In our experiment we also wished to evaluate a transient response due to BAT. Along with capacitance changes, we gathered data to evaluate changes in the heart (preload) and aorta (afterload), and the modified Brooksby-Donald[44] setup was better suited for this purpose.

2.4.2 Mechanical Effects on Venous Capacitance

Manipulation of carotid artery pressure is a common experimental method to elicit an autonomic nervous system response. This can include occlusion of the carotid artery or perfusing the artery with fluid at known pressures. Shoukas and Sagawa[47] employed this technique to evaluate a total systemic vascular capacity change and reported an increase in systemic unstressed volume of 7.5 mL/kg for a carotid artery pressure increase from 75 mmHg to 200 mmHg. Carneiro and Donald also employed carotid artery occlusion to observe the changes to different vascular beds due to changes to sympathetic outflow. Blood volume decreased in the intestine, liver, and spleen, with the spleen showing the greatest ability to store or release blood.[48]

The intention of controlling carotid artery pressure is to trigger the baroreceptor reflex. Electrical stimulation can accomplish the same effect. Carneiro and Donald used this technique to analyze changes in blood volume in response to changes in sympathetic outflow.[49] They also quantified volume changes within an individual organ, the liver, by electrically stimulating the hepatic nerve.

The human spleen is relatively small in comparison to the spleens of animals including horses, cats, and dogs and its role in storage of blood is considerably less.[50] The effects of a vasodilator in splenectomized dogs indentified the spleen as the primary reservoir for changes to vascular capacitance for the given intervention (SNP).[51] The capacitive capability of the spleen is

similarly demonstrated during alterations to sympathetic outflow. Hainsworth et al.[45] measured changes in abdominal blood volume with the splenic pedicle ligated. Without the splenic storage capabilities the changes in blood volume from electrical stimulation of the splanchnic nerve were attenuated.

2.4.3 Pharmacological Effects on Venous Capacitance

Pharmacological methods of autonomic nervous system control have been extensively studied for their effects on venous characteristics.[16] This typically involves drugs that either block or stimulate adrenergic receptors, which are activated during a sympathetic response. Results are similar to those observed with sympathetic activation through mechanical means: adrenergic receptor agonists increase MCFP and reduce unstressed venous volume, while adrenergic antagonists decrease venous tone, with the magnitude of the effect dependent on the baseline level of sympathetic tone.[16]

The effects of vasoactive agents on blood distribution have been investigated less extensively than for autonomic drugs. Vasoactive drugs can alter blood pressure through varying means, with the effects on resistance and capacitance vessels varying between drugs.[52] A distinction can therefore likely be made between the responses of venous capacitance changes under the influence of different vasoactive drugs. This was observed in a study by Ito and Hirakawa[53] where dilation of resistance and capacitance vessels varied between vasodilators and was dose-dependent. An important observation was that in their experimental setup it was only possible to dilate capacitance vessels when the level of vascular tone had been previously elevated by norepinephrine.

SNP is expected to increase the capacitance of the vasculature as veins and arteries dilate. Several different methods have been employed to demonstrate and quantify this change. Blood pool scintigraphy was used by Smiseth et al.[54] to identify an increase in blood volume of the mesentery. Anderson et al.[55] used blood density and plasma measurements to identify increases in blood volume in both macrocirculation and microcirculation with a bolus dose of SNP (and nitroglycerin). The importance of endogenous nitric oxide (NO) has been

demonstrated through the use of a nitric oxide synthase inhibitor (L-NAME),[56] demonstrating a dose-dependent decrease in vascular capacitance and compliance.

Keeping flow or volume constant allows for active and passive changes to be separated. In this case an SNP infusion is associated with a large active increase in vascular volume.[39] The experimental setup that we are using to measure changes in abdominal volume and venous capacitance is similar to that used by Wright et al.[44] to validate the Brooksby-Donald method. They used several vasoactive agents, including nitroglycerin, which demonstrated an increased in venous capacitance. It is expected that with our similar experimental setup, the use of SNP will yield a similar result, increasing venous capacitance.

In the experiment by Wright et al.,[44] the infusion of vasoconstrictors had the effect of reducing capacitance and conductance. Angiotensin II (Ang II), a vasoconstrictor, has been shown to decrease mesenteric blood volume.[54] While Ang II does not necessarily change venous unstressed volume,[57] reduction in venous compliance would be represented as a decrease in abdominal blood volume when used in a Brooksby-Donald setup.

Recent studies suggest that Ang II may be upregulated in hypertension, including salt-sensitive hypertension, to increase sympathetic nervous system activity to the splanchnic region.[58] The mobilization of blood from the splanchnic vascular bed to the central circulation due to increased tone and reduced venous capacitance may be a critical mechanism of increased blood pressure in hypertension.

2.5 Arterial Conductance

While alterations to venous properties are primarily quantified as changes in capacitance and compliance, the arterial system is much less compliant and is primarily considered to be a resistance network. The resistance of the entire system can be approximated as systemic vascular resistance (SVR), and is generally calculated as the difference between arterial pressure and right atrial pressure divided by flow. The inverse of SVR is systemic conductance,

which can be thought of as the amount of flow that the systemic circulation or a vascular bed will accept at a given pressure difference.

Sympathetic innervation of small arteries and arterioles allows for resistance, and therefore conductance to be altered with baroreflex activation and consequent changes of sympathetic tone.[2] With the level of innervation varying throughout the vasculature, interventions, including baroreflex activation and vasoactive pharmacological agents can elicit different levels of response in different vasculature. Semeniuk et al.[59] measured both venous capacitance and arterial conductance changes in order to determine the relative effects of the vasodilator toborinone on the venous and arterial system. A plot of venous capacitance versus systemic (arterial) conductance allows for a quantitative comparison of the effects.[38;59-61] In an induced heart failure canine model, Semeniuk et al. demonstrated a dose-dependent capacitance and conductance effects of toborinone, with higher doses yielding greater conductance effects.[59] Similar comparisons have been done to quantify venous and arterial effects of other vasodilators, including nitroglycerin[60] and amlodipine.[61] Understanding the relative effect of an intervention on venous versus arterial properties allows the intervention to be applied better as a therapy.

2.6 Background on Pharmacological Interventions

2.6.1 Vasodilation and Sodium Nitroprusside (SNP)

Sodium nitroprusside is a vasodilator and acts by breaking down to nitric oxide (NO) and diffusing into vascular smooth muscle, causing muscle relaxation and vascular dilation. SNP primarily dilates arterioles and venules. Nitric oxide does not have a direct effect on the heart, but the vascular relaxation reduces both cardiac preload and afterload. SNP degrades when exposed to light (20% degradation under four hours of fluorescent light exposure[62] and therefore should be wrapped in foil.

2.6.2 Angiotensin II (Ang II)

Angiotensin II is a vasoconstrictor produced naturally in the body and is activated through the renin-angiotensin-aldosterone system. A reduction in renal perfusion pressure signals the release of renin, which stimulates the release of angiotensinogen. Angiotensinogen is broken down in the circulation to angiotensin and subsequently converted by angiotensin-converting enzyme (ACE) to Angiotensin II.

Ang II acts through several different means to increase blood pressure. Ang II receptors on vascular resistance vessels cause vasoconstriction, increasing resistance and raising blood pressure. Ang II stimulates the release of aldosterone from the adrenal glands, which acts on the kidneys to increase sodium reabsorption and water retention. Stimulation of thirst and antidiuretic hormone release also work to increase fluid intake and retention. Sympathetic adrenergic function is upregulated through increased norepinephrine release and re-uptake at nerve endings.[63]

The effects of Ang II are a common target for therapy in hypertensive patients.[64] ACE inhibitors and Ang II receptor blockers are intended to reduce the amount of circulating Ang II or inhibit its ability to bind to receptors and increase systemic vascular resistance.

2.7 Baroreflex Stimulation as a Therapy in Hypertension and Heart Failure

CVRx baroreceptor stimulation therapy is currently intended as a treatment for resistant hypertension.[65] The concept of activating the baroreflex as a therapy for hypertension is not new, and can be traced back to observations by Moller in 1942, where carotid sinus massage in hypertensive patients acutely lowered blood pressure.[66] In 1958, Carlsten et al.[67] used direct electrical stimulation of the carotid sinus nerve to activate the baroreflex and decrease heart rate and blood pressure. Clinical investigations of implantable devices soon followed.[66] However, promising results yielded to a long list of side effects, many due to peripheral nerve stimulation, as well as surgical challenges and technological limitations of an implantable device.[68]

2.7.1 Venous Capacitance in Hypertension

Many types of hypertensive animal models are associated with reduced venous capacitance and increased MCFP.[69;70] In a canine hypertension model by Young et al.,[71] dogs on an eight-day i.v. Ang II drip and a high-salt diet developed hypertension with an increased MCFP but no change in blood volume, indicating that venous compliance and/or unstressed volume had decreased.

In patients, evaluation of venous compliance is generally limited to limb plethysmography, due to the invasiveness of other techniques.[16] Studies in hypertensive patients have observed reduced venous compliance and capacitance.[72;73] Similar results were observed in normotensive men with family history of hypertension.[74]

2.7.2 Venous Capacitance in Heart Failure

Chronic baroreflex activation is being presented as a possible treatment not only for hypertension, but is seen as a potential therapy in heart failure as well.[65] Heart failure is the inability of the heart to pump sufficiently to meet demand. Cardiac output decreases and the subsequent neurohumoral response increases sympathetic outflow and sodium and water retention through the renin-angiotensin-aldosterone system. Blood pressure increases and can eventually lead to ventricular remodeling.[65] The CVRx Rheos device is currently being investigated in 540 randomly-assigned heart failure patients with preserved ejection fraction (EF≥40%). Primary results are due in late 2013 (Clinical Trial Identifier NCT00957073).

Animal models with coronary artery ligation[75] and microsphere embolization[59;61;76] have examined the ability to alter vascular capacitance during heart failure. While fluid overload is associated with acute heart failure, a distinction between fluid accumulation and fluid redistribution can be made,[77] with the latter more likely and a result of vascular constriction, leading to increased cardiac preload.

Similar to hypertensive studies, the use of plethysmography has been able to identify reduced limb capacitance in subjects with congestive heart failure.[78] Blood-pool scintigraphy was employed by Thomson et al.[79] to identify decreased splenic contraction during exercise testing in subjects with hypertrophic cardiomyopathy. This is an indication that the storage capability

of the spleen is reduced, with blood mobilized to the central circulation, increasing cardiac preload.

2.8 Experimental Limits and Controversy

Due to the challenges associated with capturing physiological changes due to a reflex, the experimental protocol and setup were kept to a minimum. While there has been proven success in measuring abdominal blood volume change with sympathetic stimulation,[45;49] measuring change while trying to inhibit sympathetic stimulation through BAT could prove more challenging. The resting level of sympathetic outflow is affected by anesthesia and the invasiveness of the experiment and a baseline can be difficult to ascertain. For this reason only changes in abdominal volume have been examined, with no effort made to establish the baseline venous tone. Similar to other applications of the modified Brooksby-Donald method,[44] because we did not measure venous pressure we could not distinguish between changes in stressed or unstressed volume. Additionally, the method measures the capacitance of the entire sub-diaphragmatic vasculature, and any small capacitance effect of sub-diaphragmatic arteries is included in the results.

In this study, the "doses" of BAT were systematically chosen based on the drop in mean arterial pressure that they effected. The doses were not varied independently, as would be done to create a dose-response curve similar to what has been done with an earlier generation of the device (Rheos, CVRx Inc.).[80] For simplicity in our study we only varied the stimulating current even though frequency and pulse width might have also been varied, as is done when the device is applied clinically.[81]

2.9 Conclusions

The primary objective of this experiment is to evaluate changes to the blood capacity of the abdominal vasculature due to electrical stimulation of the carotid artery baroreceptor. While the control of venous capacitance by the sympathetic nervous system has been well

established, the effects of the CVRx device have not been determined, nor has the modified Brooksby-Donald method been used to evaluate this type of intervention.

Measurement of venous capacitance changes with BAT adds experimental results from a clinically used medical device to the large existing body of research on baroreceptor and sympathetic control of the veins. This experimental protocol should also further the application of the modified Brooksby-Donald technique and directly add to the knowledge gathered by Wright et al.'s[44] results from intra-venous injections of pharmacological agents.

The pharmacological interventions of SNP and Ang II will alter venous capacitance and allow for attenuation of the device's capabilities or synergistic effects to be identified. Contrasting BAT with the vasodilator SNP will provide a reference for the magnitude of the changes and additional insight into the location of changes (arterial versus venous). The results of applying BAT during an infusion of Ang II will help reveal the therapy's effectiveness at higher blood pressure and in the presence of circulating Ang II – a potentially important result for the application of the therapy in hypertension.

Similarly, these interventions will allow the device's effects on greater hemodynamic measures, including mean aortic pressure, left-ventricular end-diastolic pressure and volume, heart rate, and CO to be evaluated. The attenuation or amplification of the device's effects by these pharmacological interventions may provide insight into the mechanisms by which it lowers blood pressure.

3 Preliminary Studies

3.1 Introduction

A series of 10 experiments was performed as a preliminary study into invasive hemodynamic experiments in a dog model. The carotid artery baroreceptor was stimulated using Rheos,[1;80] an earlier-generation CVRx device. With this device, several electrodes were wrapped around the artery. The current device employs a single 1 mm^2 electrode. Additionally, the Rheos device was voltage controlled, while the latest device, used for our primary study, is current-controlled.

The preliminary protocol was extensive, including a high-level of instrumentation, a lengthy experimental protocol, and attempts to induce heart failure through microsphere injection into the coronary artery. The limited success of these experiments was taken into consideration when determining the instrumentation and protocol for the principal study.

3.2 Experimental Methodology

3.2.1 Animal Preparation

Data were collected from 10 mongrel dogs of both genders, weighing between 17 kg and 24 kg. The experimental protocol was approved by the faculty animal care committee and conformed to the "Guiding Principles of Research Involving Animals and Human Beings" of the American Physiological Society. Dogs were anesthetized with thiopental sodium (25 mg/kg, intravenous) and maintained with fentanyl citrate (0.2 mg/kg/hour intravenous, adjusted as necessary throughout the experiment). Animals were intubated and ventilated with a constant-volume respirator and a closed rebreathing system in order to maintain normal blood gas and pH. Body temperature was maintained between 36.5°C and 37.5°C through use of a warming blanket. An ECG was recorded throughout.

3.2.2 Instrumentation

With dogs in the supine position a midline sternotomy was performed and the ventral surface of the pericardium was opened with a base-to-apex incision. Sonomicrometry crystals (Sonometrics Corp., London, ON), were implanted in the LV endocardium and the midwall of the septum to provide septum-to-LV freewall and antero-posterior dimensions. A 3.5-Fr Mikro-tip catheter (model SPR-524, Millar Instruments, Houston, TX) was advanced to the right atrium via the jugular vein. A 7-Fr, pig-tailed, catheter-tipped manometer (model FTM-7011B-048A, Scisense Inc., London, ON) was inserted into the LV via the apex. A pig-tailed catheter was used to reduce the occurrence of catheter-tip contact with the LV wall. The pericardium was loosely re-approximated with sutures to avoid excessive constraint.[82]

A 3.5-Fr Mikro-tip catheter (model SPR-524, Millar Instruments, Houston, TX) was advanced to the ascending aorta via the right brachial artery. A 7-Fr catheter-tipped manometer (model FTM-7011B-0048C, Scisense Inc.) with a fluid-filled reference was introduced via the femoral artery and advanced in the aorta to the level renal arteries. All pressures were referenced to the mid-level of the right atrium.

Ultrasonic flow probes (Transonic Systems Inc., Ithaca, NY) were implanted on the ascending aorta, the descending aorta, the aorta above the diaphragm, the aorta at the iliac bifurcation, the inferior vena cava above the diaphragm, the coronary artery, the renal artery, and the portal vein.

The right carotid artery was dissected and the CVRx Rheos electrode (CVRx Inc., Minneapolis, MN) was implanted on the artery with a wrap-around band at the level of the carotid sinus.

3.2.3 Experimental Protocol

BAT was controlled through a digital interface where voltage, frequency, and pulse width were programmed. Frequency was held constant at 75 Hz. After instrumentation, voltage and pulse width were adjusted to elicit the maximum blood pressure reduction. A 15-minute stabilization interval was then allowed to elapse before the protocol began. Control data were collected before every intervention, as well as transiently with device activation and at steady-state.

3.2.4 Analytical Methodology

Conductance, which is the amount of flow a vascular bed will accept per unit of driving pressure and is the reciprocal of resistance, was calculated as mean $Q_{ao\text{-}asc}$ divided by mean P_{Ao} (venous pressure was not measured). SV was calculated as the integral of $Q_{ao\text{-}asc}$ during systole. CO is the product of SV and HR. End-diastolic LV area ($Area_{LVED}$), the product of LV freewall-to-septum and LV anterior-posterior dimensions, was used as an index of LV end-diastolic volume.[83]

Data were collected at a constant sampling rate of 200 Hz and recorded using data acquisition software Sonosoft (Sonometrics Corp., London, ON). Sonosoft is used for initial analysis of the data, including any relevant channel selection and data cleaning or filtering where necessary. More detailed analysis is performed with CVWorks (Advanced Measurements Inc., Calgary, AB). CVWorks can be used to calculate heart rate, adjust pressure measurements to their reference values, and establish cycle delineators, including markers for end diastole, peak systole, and end systole.

3.2.5 Statistical Analysis

Absolute values for hemodynamic measures were used for comparison except for conductance (baseline set at 100%). Results are presented as means ± standard errors (SE). The Student's paired *t*-test was used to determine statistical significance: a value of $P < 0.05$ was considered significant.

3.3 Results

Table 1– Results of BAT (n=9)

	Baseline	BAT	P Value
Mean P_{Ao} (mmHg)	84.6±3.4	59.2±4.7*	0.0007
HR (bpm)	92.8±4.7	75.8±6.4*	0.01
SV (mL)	20.6±2.5	19.2±2.5*	0.005
CO (mL/min)	1920±274	1512±305*	0.002
P_{LVED} (mmHg)	9.3±0.9	8.3±0.9*	0.02
$Area_{LVED}$ (mm^2)	1566±169	1478±169*	0.0005
P_{RAED} (mmHg)	6.6±0.8	6.8±0.8	0.59
Conductance (mL/min/mmHg)	24.3±3.0	27.9±3.4*	0.003

Mean aortic pressure (P_{Ao}), Heart Rate (HR), Stroke Volume (SV), Cardiac Output (CO), Left-Ventricular End-Diastolic Pressure (P_{LVED}), Left-Ventricular End-Diastolic Area ($Area_{LVED}$), Right-Atrial End-Diastolic Pressure (P_{RAED}). *$P < 0.05$ (interventions vs. baseline)

While BAT was able to achieve a reduction in blood pressure in most animals (9 out of 10), the limited control of the dosage via manipulation of the pulse width, frequency and, primarily, the voltage resulted in a wide range of blood pressure decreases among animals. The mean decrease in aortic pressure was 25.4±4.9 mmHg, but ranged from 10 mmHg to 54 mmHg. Results from instrumentation that was not successfully applied in each animal, including coronary and portal flow, were not included in Table 1.

3.4 Discussion

Most of the results from were also recorded during the more recent experimental series and will be discussed in detail in the experimental results section. However, the wide range of BP effect due to BAT does provide some useful insight into the relationship between right atrial pressure (used as an approximation for CVP), cardiac output, and mean arterial pressure.

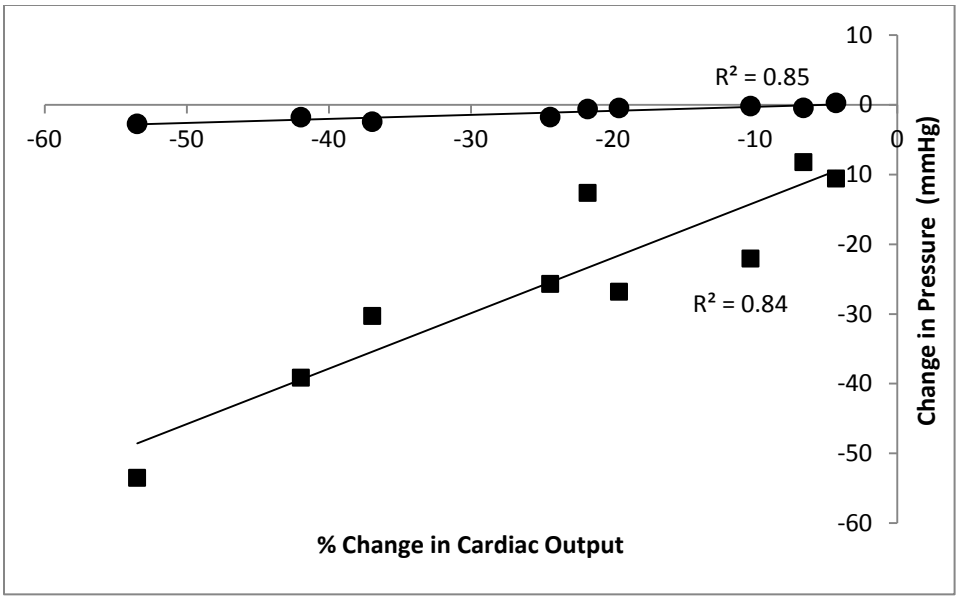

Figure 1 - Change in mean aortic and right atrial pressure vs. cardiac output.

Circles represent mean right atrial pressure and squares represent mean aortic pressure. Baseline is represented as the intersection of the axes, 0,0.

Figure 1 contains a data set (an atrial pressure and an aortic pressure) from each animal in the experimental series for activation of the device. While not as ideal as measuring pressure at varying cardiac outputs within each animal, the pooled data do provide a reasonable representation of the direct relationship between decreasing CO and decreasing arterial pressure.

The relationship between mean right atrial pressure and CO shown in Figure 1 is more complex than the relationship between CO and arterial pressure. From the perspective of a vascular function curve, where a reduction in CO results in sudden imbalance of blood flow to and from the veins and an increase in venous pressure, values do not fit.[2] This is likely due to the alteration of venous properties by BAT. Any increases to venous capacitance and/or compliance that have occurred are reflected in the new (or unchanged) values of venous pressure.

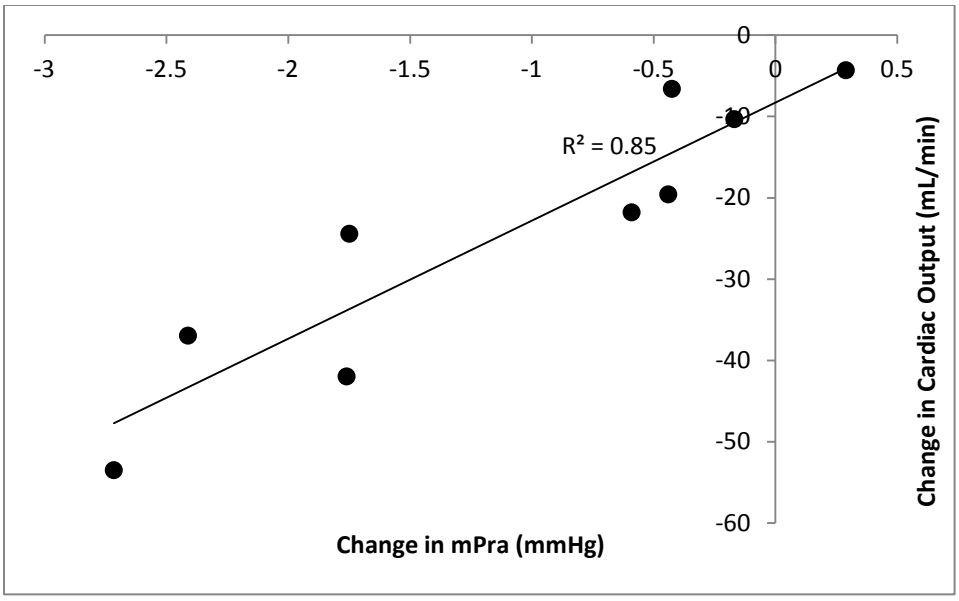

Figure 2 - Change in cardiac output vs. change in mean right atrial pressure.

Baseline is represented as the intersection of the axes, 0,0.

The relationship can also be viewed from the perspective of a cardiac function curve. When plotted with CO as the dependent variable, CVP is considered the independent variable.[38] Figure 2 shows the Figure 1 values re-plotted in this manner and the steady-state data points are useful in demonstrating the relationship.

Cardiac output is the product of stroke volume (SV) (measured as the cycle-integral of aortic flow) and heart rate. In these results changes in HR were the primary factor responsible for changing CO. While SV decreased 6.8 ± 1.7% with device activation, HR decreased 18.3 ± 3.3%.

3.5 Conclusions

The results of this preliminary study suggest that a BAT-induced reduction in HR explains most of the reduction in CO. Observation of a lack of increase in venous pressure associated with the reduction in CO suggests that BAT may have a considerable affect on venous properties. We went on to study the contribution of a putative increase in venous capacitance.

4 Hypothesis

1. The application of carotid artery baroreceptor stimulation will increase abdominal blood volume, as measured with a modified Brooksby-Donald technique.

2. The magnitude of abdominal blood volume change due to carotid artery baroreceptor stimulation will be diminished when the vasculature has already been dilated by sodium nitroprusside.

3. A reduction in abdominal blood volume due to an infusion of Angiotensin II will be reversed with carotid artery baroreceptor stimulation.

4. Changes to aortic stiffness will be passive, with stiffness increasing with increased arterial pressure and decreasing with decreased arterial pressure.

5 Experimental Methodology

5.1 Animal Preparation

Data were collected from 6 mongrel dogs of both genders, weighing between 17 kg and 24 kg. The experimental protocol was approved by the faculty animal care committee and conformed to the "Guiding Principles of Research Involving Animals and Human Beings" of the American Physiological Society. Dogs were anesthetized with thiopental sodium (25 mg/kg, intravenous) and maintained with fentanyl citrate (0.2 mg/kg/hour intravenous, adjusted as necessary throughout the experiment). Animals were intubated and ventilated with a constant-volume respirator and a closed rebreathing system in order to maintain normal blood gas and pH. Body temperature was maintained between 36.5°C and 37.5°C through use of a warming blanket. An ECG was recorded throughout.

5.2 Instrumentation

With dogs in the supine position a midline sternotomy was performed and the ventral surface of the pericardium was opened with a base to apex incision. Sonomicrometry crystals (Sonometrics Corp., London, ON), were implanted in the LV endocardium and the midwall of the septum to provide septum-to-LV freewall and antero-posterior dimensions. A 7-Fr, pig-tailed, catheter-tipped manometer (model FTM-7011B-048A, Scisense Inc., London, ON) was inserted into the LV via the apex. A pig-tailed catheter was used to reduce the occurrence of catheter-tip contact with the LV wall. The pericardium was loosely re-approximated with sutures to avoid excessive constraint.[82]

A 3.5-Fr Mikro-tip catheter (model SPR-524, Millar Instruments, Houston, TX) was advanced to the ascending aorta via the right brachial artery. Likewise, a 3.5-Fr Mikro-tip catheter (model SPR-524, Millar Instruments, Houston, TX) was introduced into the left brachial artery and advanced to the aorta to measure pressure at the descending aorta. A 3.5-Fr Mikro-tip catheter (model SPR-524, Millar Instruments, Houston, TX) was introduced via the femoral artery and advanced in the aorta to the level of the diaphragm. A 7-Fr catheter-tipped

manometer (model FTM-7011B-0048C, Scisense Inc.) with a fluid-filled reference was introduced via the tail artery and advanced in the aorta to the level of the iliac bifurcation. A vascular occluder (In Vivo Metric, Healdsburg, CA), appropriately sized to fit loosely, was affixed around the inferior vena cava (IVC).

Ultrasonic flow probes (Transonic Systems Inc., Ithaca, NY) were implanted on the ascending aorta, the descending aorta, the aorta above the diaphragm, the aorta at the iliac bifurcation, and the inferior vena cava above the diaphragm. Pairs of ultrasonic crystals were affixed to the aorta near the pressure measurement locations at the ascending aorta and proximal to the iliac bifurcation.

The right carotid artery was dissected and the CVRx electrode (CVRx Inc., Minneapolis, MN) was implanted on the artery with a wrap-around band at the level of the carotid sinus.

5.3 Calibration

Before animal instrumentation, all pressure catheters were calibrated using a mercury manometer (Baumanometer, W.A. Baum Co. Inc., New York). After instrumentation, all pressures were zeroed to the mid-level of the right atrium. Catheter-tipped manometers are highly precise, with Millar and Scisense device specifications reporting device sensitivities in the range of 5 μV/V/mmHg to 10 μV/V/mmHg. These catheters are, however, subject to electrical drift, with Millar specifications reporting a drift of up to 6 mmHg in 12 hours. Therefore, catheter-tipped manometers are referenced to a fluid-filled catheter system that displays a stable signal and can be referenced frequently. Fluid-filled catheters record acceleration due to catheter motion and vibration and therefore, in this way, are less acccurate than the high-fidelity pressure measurements.

Transonic flowsensors (Transonic Systems Inc., Ithaca, NY) are pre-calibrated for blood flow at body temperature. Flow probes much be sized appropriately to ensure appropriate vessel contact, while fitting loosely so as to minimally affect flow. Application of ultrasonic gel enables transmission of the ultrasonic signal between the vessel and the sensor. A low-pass filter on

the Transonic data acquisition system eliminates high-frequency noise. Based on the Transonic Flowmeter Operator's Manual, the low-pass filter frequency was set at 30Hz – a value at least ten times greater than the frequency of flow pulsation. The zero offset of the flow probes increases with increased size, ranging from ± 3 mL/min for the 2-mm flow probe, to ± 200 mL/min for the 16-mm flow probe. For all flow probes, regardless of size, the absolute and relative accuracies are ± 15% and ± 2% respectively.

5.4 Experimental Protocol

BAT was controlled through a digital interface where current, frequency, and pulse width were programmed. Pulse width and frequency were held constant at 60 μs and 80 Hz, respectively. After instrumentation, current was adjusted to reduce mean blood pressure by ~20 mmHg, thereafter defined as Dose 1. A 15-minute stabilization interval was then allowed to elapse before the protocol began. Control data were collected before every intervention. For each intervention data were recorded transiently as well as with the respirator turned off for 15 seconds at steady-state periods.

Intervention 1

The IVC occluder cuff is inflated and maintained to effect a blood pressure decrease of ~20 mmHg.

Intervention 2

The device (at Dose 1 current setting) was activated for a period of 5 minutes, after which blood pressure was allowed to return to baseline

Intervention 3

SNP was infused at 14 ug/kg/min. When steady state was reached (approximately 3 minutes) BAT at Dose 1 was applied. A washout period for the drug of 15 minutes was provided before proceeding to the next intervention.

Intervention 4

Ang II was infused at 0.05 ug/kg/min. When steady state was reached (approximately 3 minutes) BAT was applied at Dose 1. The device was subsequently activated at two increased settings (referred to as Doses 2 & 3), with the intention of having Dose 2 high enough to return mean aortic pressure half-way to the pre-Ang II level, while Dose 3 would return mean aortic pressure to pre-Ang II levels.

5.5 Analytical Methodology

Conductance, which is the amount of flow a vascular bed will accept per unit of driving pressure and is the reciprocal of resistance, was calculated as mean $Q_{ao\text{-}asc}$ divided by mean P_{Ao} (venous pressure was not measured). SV was calculated as the integral of $Q_{ao\text{-}asc}$ during systole. CO is the product of SV and HR. End-diastolic LV area ($Area_{LVED}$), the product of LV freewall-to-septum and LV anterior-posterior dimensions, was used as an index of LV end-diastolic volume.[83]

Data were collected at a constant sampling rate of 200 Hz and recorded using data acquisition software Sonosoft (Sonometrics Corp., London, ON). Sonosoft is used for initial analysis of the data, including any relevant channel selection and data cleaning or filtering where necessary. More detailed analysis is performed with CVWorks (Advanced Measurements Inc., Calgary, AB). CVWorks can be used to calculate heart rate, adjust pressure measurements to their reference values, and establish cycle delineators, including markers for end diastole, peak systole, and end systole.

CVWorks can also be used to perform various mathematical operations on collected data channels. This includes the mathematical steps required to perform the modified Brooksby-Donald analysis.[44] To determine a change in abdominal blood volume, the difference between diaphragmatic inflow (mL/s) and inferior vena caval outflow (mL/s) is integrated with respect to time. At baseline mean values for both flows are established for a 30 second time period ($mQdia_{(30s)}$ and $mQivc_{(30s)}$). The ratio of these mean values is used to adjust mean

diaphragmatic flow (per beat) to match mean IVC flow to establish a baseline where the flows are equal and abdominal volume is therefore constant. The integration of abdominal flow with respect to time provides a change in abdominal volume.

$$mQdia\ corrected = mQdia\ /\left(\frac{mQdia(30s)}{mQivc(30s)}\right) \quad \textit{(Equation 1)}$$

$$mQdia\ corrected - mQivc = \Delta abdominal\ flow \quad \textit{(Equation 2)}$$

$$\int \Delta abdominal\ flow = \Delta abdominal\ volume \quad \textit{(Equation 3)}$$

5.6 Stiffness and Pulse Wave Velocity

Stiffness was calculated as the slope of a linear regression for a pressure – diameter loop. Proximal aortic stiffness was determined at the aortic root, where ultrasonic crystals were implanted radially. Crystals provided a measurement of aortic diameter, while a catheter in the artery at the same location provided pressure. Abdominal aortic stiffness was calculated by the same method, from diameter and pressure measurements of crystals implanted on the abdominal aorta near the iliac bifurcation and a pressure catheter at the same location.

PWV was calculated as the distance between pressure catheters in the aorta divided by the time difference between the pressure waveforms. Proximal PWV was calculated between the ascending aorta and diaphragmatic aorta pressure catheters. Abdominal PWV was calculated between the diaphragmatic aorta and iliac bifurcation pressure catheters. This form of PWV calculations provides an average value for the artery. This is in contrast to our method of stiffness calculation, which represents only the local diameter and pressure measurement. Total PWV refers to an average PWV for the entire aortic measurement range, from the ascending aorta to the iliac bifurcation. This type of measurement is more comparable to how PWV might be measured in a clinical setting.[84]

5.7 Statistical Analysis

Absolute values for hemodynamic measures were used for comparison except for conductance (baseline set at 100%) and capacitance (volume change during first minute of the intervention (mL/kg/min)). Results are presented as means ± standard errors (SE). The Student's paired *t*-test was used to determine statistical significance: a value of $P < 0.05$ was considered significant.

6 Principal Study Results

The current required to lower mean P_{Ao} by ~20 mmHg (Dose 1) was 6.75 ± 3.43 (SD) mA. Doses 2 and 3 were, respectively, 2.6 ± 1 mA and 4.4 ± 1.5 mA greater than Dose 1.

Baseline inferior vena caval flow was 0.91 ± 0.22 [SD] times the aortic flow at the diaphragm.

Results from measurements of aortic stiffness and PWV were less consistent than other hemodynamic measurements, providing fewer cases with statistically significant change. While statistical results are shown for abdominal aortic stiffness, measurements were only obtained from three experiments. This is due to the limitations of ultrasonic crystals at low amplitude changes, as well as the challenge associated with abdominal aorta crystal implantation.

6.1 Primary Hemodynamics

6.1.1 Effects of BAT

The effects of BAT are shown in Table 2 and Figure 3. BAT decreased mean P_{Ao} by 22.5 ± 1.5 mmHg. This was associated with a 16.0 ± 4.9% increase in arterial conductance and a 14.3 ± 2.6% decrease in HR. Abdominal blood volume increased at a rate of 2.2 ± 0.6 mL/kg/min. SV remained unchanged but there was a trend for CO to decrease (-11.0 ± 3.2%, P = 0.06). $Area_{LVED}$ decreased by 5.5 ± 0.8% and P_{LVED}, by 2.5 ± 0.8 mmHg. The effects of BAT on aortic stiffness and PWV are shown in Table 3. BAT increased proximal aortic stiffness by 8.4 ± 2.7% and decreased abdominal aortic stiffness by 19.8 ± 2.9%. The decrease in proximal aortic PWV

was not significant, while abdominal aortic PWV decreased by 24.7 ± 2.9% and total aortic PWV decreased by 13.9 ± 0.9%.

6.1.2 Effects of SNP and BAT (n=5)

As shown in the middle column of Figure 3 and listed in Table 2, SNP decreased mean P_{Ao} by 17.4 ± 0.8 mmHg. Arterial conductance increased by 76.0 ± 22.0% and HR increased by 44.9 ± 10.4%. Abdominal blood volume increased at a rate of 2.9 ± 0.8 mL/kg/min. SV did not change significantly, and the increase in CO was not statistically significant (54.7 ± 24.4% P = 0.12). $Area_{LVED}$ decreased by 13.1 ± 2.7% and P_{LVED}, by 3.7 ± 0.9 mmHg. The effects of SNP on aortic stiffness and PWV (Table 3) were similar to the effects of BAT, with no statistical difference between the two. SNP increased proximal aortic stiffness 11.4 ± 2.8% and decreased abdominal aortic stiffness 19.3 ± 2.6%. Abdominal aortic PWV decreased 21.4 ±3.3% and total PWV decreased 13.9 ± 0.9%

During SNP administration, BAT decreased mean P_{Ao} by 26.0 ± 2.1 mmHg, which was similar to the change due to BAT during the control state (22.5 ± 1.5 mmHg). BAT increased arterial conductance further to 116.0 ± 16.5% of baseline. The rate of change of abdominal blood volume tended to increase further relative to baseline, to 5.1 ± 2.2 mL/kg/min (P=0.06). BAT did not change $Area_{LVED}$ and P_{LVED}, while it tended to decrease CO (-20.8 ± 10.1%, P = 0.09). SV did not change significantly, and the decrease in HR was not statistically significant (-17.2 ± 3.6%, P = 0.12). All measures of stiffness and PWV changed significantly. BAT during SNP administration increased proximal aortic stiffness 30.3 ± 1.3% and decreased abdominal aortic stiffness 35.5 ± 7.2%. Proximal aortic PWV decreased 5.4 ± 1.8%, abdominal aortic PWV decreased 24.2 ± 2.3%, and total PWV decreased 15.6 ± 0.7%.

Table 2 – Results of BAT during the control state, SNP, and BAT during SNP administration (n=5)

	Baseline	BAT (Dose 1)	Baseline	SNP	SNP + BAT (Dose 1)
Mean P_{Ao} (mmHg)	95.5±5.1	73.0±5.8*	92.4±6.7	75.0±7.2*	49.0±5.1*†
HR (bpm)	71.4±5.7	61.2±6.4*	73.1±9.7	105.9±10.6*	87.7±9.7
SV (mL)	15.7±2.0	16.4±2.0	14.8±2.3	15.5±3.3	14.6±1.9
CO (mL/min)	1106±154	984±151	1069±197	1652±432	1309±295
P_{LVED} (mmHg)	9.1±0.4	6.6±0.7*	8.8±0.6	5.1±0.7*	4.4±0.8*
$Area_{LVED}$ (mm^2)	1275±102	1205±100*	1282±126	1114±119*	1074±115*
Conductance (% control)	100	116±4.9*	100	176±22.0*	216±16.5*†
Capacitance Change (mL/kg/min)	0	2.2±0.6*	0	2.2±0.7*	5.1±2.2

Mean Aortic Pressure (P_{Ao}), Heart Rate (HR), Cardiac Output (CO), Stroke Volume (SV), Left-Ventricular End-Diastolic Pressure (P_{LVED}), Left-Ventricular End-Diastolic Area ($Area_{LVED}$).

*P < 0.05 (interventions vs. baseline), †P < 0.05 (SNP+BAT vs. SNP).

Table 3 – Aortic Stiffness and PWV of BAT during the control state, SNP, and BAT during SNP administration (n=5)

	Baseline	BAT (Dose 1)	Baseline	SNP	SNP + BAT (Dose 1)
Proximal Stiffness (mmHg/mm)	24.2±1.4	26.2±1.5*	23.9±1.3	26.7±1.6*	30.3±1.3*†
Abd. Stiffness (mmHg/mm) (n=3)	205±45	167±41*	203±35	165±33*	110±31*†
Proximal PWV (m/s)	4.1±0.2	3.9±0.2	4.2±0.1	4.0±0.2	3.8±0.2*†
Abdominal PWV (m/s)	7.1±1.5	5.2±0.9*	6.9±1.2	5.5±1.0*	4.1±0.6*†
Total PWV (m/s)	5.1±0.2	4.4±0.2*	5.4±0.2	4.5±0.2*	3.8±0.1*†

*P < 0.05 (interventions vs. baseline), †P < 0.05 (SNP+BAT vs. SNP).

6.1.3 Effects of Angiotensin II with BAT

The effects of Ang II are listed in Table 4 and shown in the right-hand column of Figure 3. Ang II increased mean P_{Ao} by 40.4 ± 3.5 mmHg and decreased conductance by 37.7 ± 5.2%. Abdominal blood volume decreased at a rate of 1.4 ± 0.5 mL/kg/min. $Area_{LVED}$ increased by 11.8 ± 2.3% and P_{LVED}, by 3.5 ± 1.0 mmHg, but SV decreased by 26.6 ± 8.1%. CO was

unchanged. There was a trend for HR to increase (18.2 ± 2.5%, P = 0.06). The effects of Ang II on aortic stiffness and PWV are listed in Table 5. Ang II had no consistent effect on proximal aortic stiffness, while abdominal aortic stiffness increased 52.9 ± 6.0%. All measures of PWV increased with Ang II; proximal aortic PWV by 17.0 ± 2.7%, abdominal aortic PWV by 43.6 ± 3.5%, and total PWV by 27.0 ± 2.7%.

During Ang II infusion, BAT (Dose 1) reduced mean P_{Ao} by 22.2 ± 4.9 mmHg, but did not change arterial conductance. The BAT-induced decrease in mean P_{Ao} was equal to that during the control state (i.e., 22 mmHg). HR decreased by 12.1 ± 1.7%. BAT reversed the decrease in abdominal blood volume and increased it at a rate of 0.58 ± 0.96 mL/kg/min. BAT decreased $Area_{LVED}$ by 6.0 ± 1.3% and P_{LVED} by 1.5 ± 0.4 mmHg. CO decreased by 9.5 ± 3.0%. No dose of BAT had a significant effect on either proximal or abdominal aortic stiffness. BAT Dose 1 decreased all measures of PWV; proximal aortic PWV by 8.0 ± 2.1%, abdominal aortic PWV by 15.4 ± 3.6%, and total PWV by 9.9 ± 2.2%.

BAT at Dose 3 during Ang II infusion reversed the P_{Ao} effects of Ang II, decreasing mean P_{Ao} 38.2 ± 7.7 mmHg, while HR, CO, $Area_{LVED}$, and P_{LVED} did not change significantly with BAT at Dose 2 or Dose 3. Dose 3 of BAT increased conductance from 62.3 ± 5.2% to 80.2 ± 3.3% of baseline and the rate of increase of abdominal blood volume to 1.8 ± 0.9 mL/kg/min. With respect to aortic stiffness and PWV, only total PWV changed significantly with BAT Doses 2 or 3, decreasing to 106.2 ± 6.4% of baseline with BAT Dose 3.

Table 4 – Results of BAT during Ang II administration; Doses 1, 2, and 3

	Baseline	Ang II	Ang II + BAT (Dose 1)	Ang II + BAT (Dose 2)	Ang II + BAT (Dose 3)
P_{Ao} (mmHg)	82.9±3.9	123.2±4.5*	101.0±5.9*†	92.9±7.7†‡	85.0±9.3†‡§
HR (bpm)	68.3±4.0	80.7±6.4	70.9±4.4†	71.1±4.6	68.7±4.6
SV (mL)	16.9±2.7	12.4±1.8*	12.5±1.9*	12.3±1.4*	11.2±1.1*
CO (mL/min)	1171±238	978±143	885±150*†	880±137*†	762±70*†
P_{LVED} (mmHg)	8.5±0.6	12.0±0.6*	10.5±0.4†	9.8±0.5†	10.0±0.4†
$Area_{LVED}$ (mm^2)	1217±112	1360±141*	1275±144†	1248±157†	1286±191†
Conductance, % control	100	62.3±5.2*	64.7±3.6*	73.1±3.6*†	80.2±3.3*†‡§
Capacitance Change (mL/kg/min)	0	-1.4±0.5*	0.58±0.96†	1.7±0.9†	1.8±0.9†‡

Abbreviations as in Table 2 – Results of BAT during the control state, SNP, and BAT during SNP administration. *P < 0.05 (interventions vs. baseline), †P < 0.05 (BAT vs. Ang II), ‡P < 0.05 (Doses 2 and 3 vs. Dose 1), §P < 0.05 (Dose 3 vs. Dose 2).

Table 5 – Aortic Stiffness and PWV of BAT during the control state, Ang II, and BAT during Ang II administration

	Baseline	Ang II	Ang II + BAT (Dose 1)	Ang II + BAT (Dose 2)	Ang II + BAT (Dose 3)
Prox. Stiffness (mmHg/mm)	23.6±1.1	29.6±3.8	26.5±0.8*	26.9±2.5	26.3±2.7
Abd. Stiff. (mmHg/mm) (n=3)	136±15	207±22*	177±12*	137±6	168±13
Proximal PWV (m/s)	3.9±0.1	4.5±0.1*	4.2±0.1*†	4.0±0.2†	4.0±0.2†
Abdominal PWV (m/s)	6.1±0.7	8.7±0.8*	7.3±0.6*†	7.0±0.5†	6.9±0.6*†
Total PWV (m/s)	4.8±0.1	6.1±0.1*	5.5±0.2*†	5.3±0.3†	5.2±0.3†‡§

*P < 0.05 (interventions vs. baseline), †P < 0.05 (BAT vs. Ang II), ‡P < 0.05 (Doses 2 and 3 vs. Dose 1), §P < 0.05 (Dose 3 vs. Dose 2)

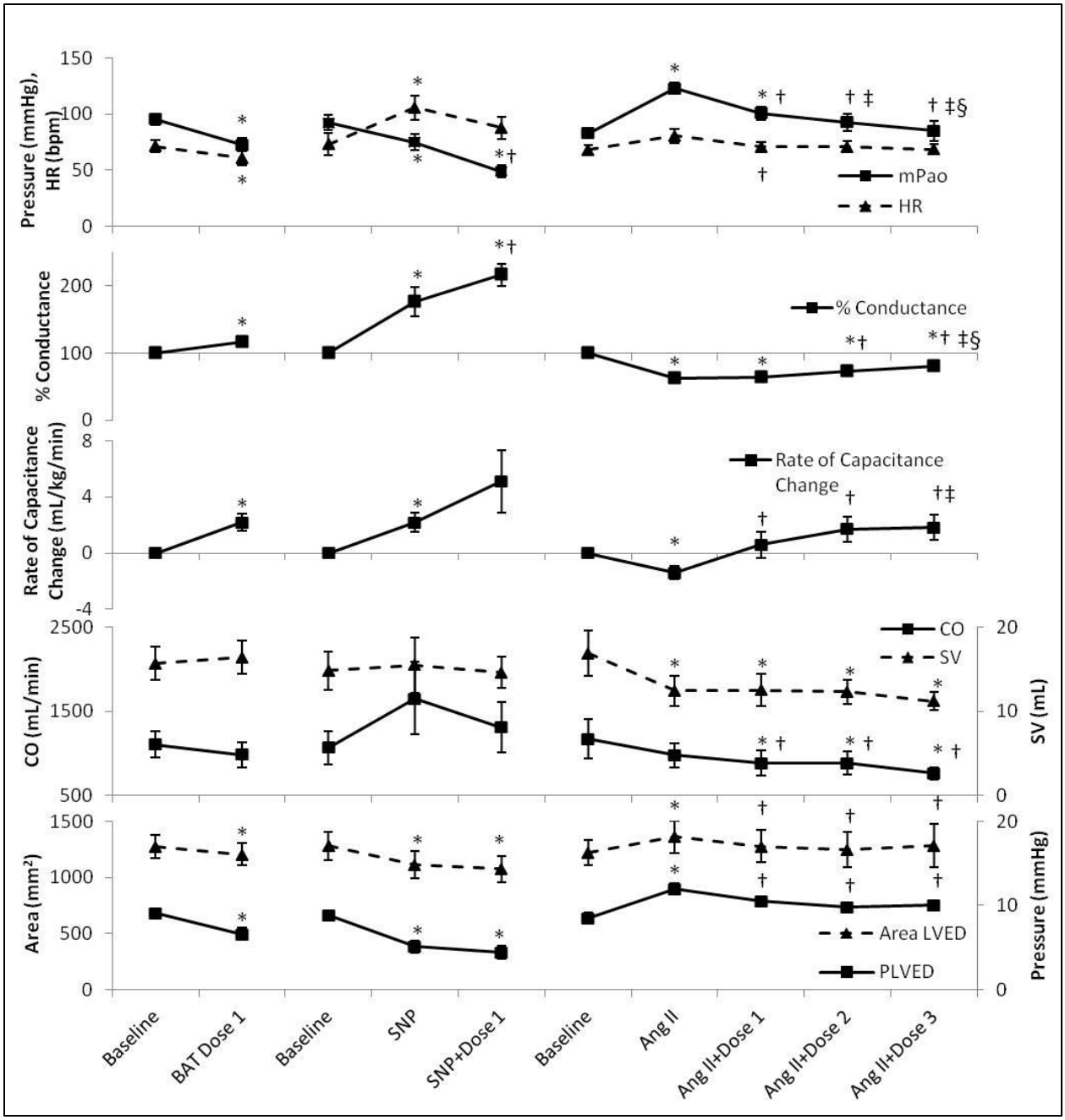

Figure 3 - Results BAT during the control state, SNP, and Ang II administration.

Mean Aortic Pressure (P_{Ao}), Heart Rate (HR), Cardiac Output (CO), Stroke Volume (SV), Left-Ventricular End-Diastolic Pressure (LVEDP), Left-Ventricular End-Diastolic Area (LVED Area).

*P < 0.05 (interventions vs. baseline), †P < 0.05 (BAT vs. SNP or Ang II), ‡P < 0.05 (Doses 2 and 3 vs. Dose 1), §P < 0.05 (Dose 3 vs. Dose 2).

7 Discussion

[Portions of this discussion have been adapted for publication, and therefore have been improved upon and received contributions from the co-authors; Dr. Dimitrios Georgakopoulos, Dr. Israel Belenkie, and Dr. John Tyberg.]

As has been previously demonstrated,[81] BAT achieved a substantial, sustained reduction in mean arterial blood pressure. This was observed in all cases, including the application of BAT during the pharmacological challenges of SNP and Ang II. There was no difference in the pressure drop between the device acting independently versus acting in vasculature that had been dilated by SNP or constricted by Ang II. BAT caused large increases in venous capacitance and arterial conductance, also observed in the presence of SNP and Ang II. The effect of BAT on venous capacitance was additive during SNP infusion and was not attenuated by Ang II. Furthermore, BAT was able to reverse the effects of Ang II on venous capacitance, a potentially important effect of this technology for clinical application.[85] BAT also increased arterial conductance, though to a lesser extent than SNP. During Ang II administration, arterial conductance increased with increasing doses of BAT, though not to baseline values while mean aortic pressure did return to baseline.

7.1 Hemodynamic effects of BAT

BAT profoundly affects 3 of the 4 determinants of CO:[36] LV afterload, preload, and heart rate. The fourth, contractility, has been shown to be unaffected.[1] BAT increases both arterial conductance and venous capacitance, thus tending to decrease both LV afterload and preload. Decreasing afterload increases CO, and decreasing preload decreases CO. These opposite effects tend to cancel each other but, under the conditions in our study, BAT always decreased CO suggesting that decreased preload was dominant. Mean arterial pressure is equal to CO divided by systemic conductance (the reciprocal of resistance) and, because the increase in conductance was greater than the decrease in CO, mean arterial pressure always decreased. Normally, a decrease in arterial pressure produces an increase in heart rate (via the baroreceptor mechanism) but because of its effect on vagal outflow, BAT always decreased

heart rate. Since the decrease in heart rate was similar to the decrease in CO, SV did not change.

7.2 BAT with Vasoactive Agents

BAT decreased blood pressure equally when applied alone or when applied during SNP infusion. It was also equally effective when applied during Ang II infusion. The decrease in pressure due to BAT (Dose 1) alone and with Ang II was associated with decreased LV preload, which implies that the mechanism by which arterial pressure is reduced is at least in part related to this effect, in addition to the decrease in afterload due to the increase in conductance.

The ability of SNP to increase abdominal blood volume has been previously demonstrated,[44;54] and it could therefore be reasonably presumed that BAT's ability to decrease blood pressure would be diminished when applied to a vasculature already partially-dilated by SNP. This, however, is not the case, as can be observed from the results with SNP infusion. While the chosen rate of SNP infusion was sufficient to lower mean P_{Ao} 17.4 ± 0.7 mmHg, application of BAT Dose 1 at this reduced pressure was able to achieve a further decrease of 26.0 ± 2.1 mmHg. This decrease is comparable to the decrease achieved by BAT on its own of 22.5 ± 1.5 mmHg.

The ability of BAT to decrease blood pressure in the presence of Ang II is consistent with a previous report by Lohmeier et al.[6] In an investigation of BAT with chronic Ang II-induced hypertension, they showed that BAT decreased blood pressure by an amount equal to that under control conditions. Similarly, in our study, the effect of BAT (Dose 1) was not attenuated during the Ang II infusion. However, the key finding of Lohmeier et al. was that with no adjustment to the BAT dose and with continued infusion of Ang II, the effectiveness of BAT was attenuated by 80% after one week. In our acute study, the Ang II-induced pressure increase was completely reversed in a dose-dependent manner with BAT.

7.3 Capacitance and Conductance

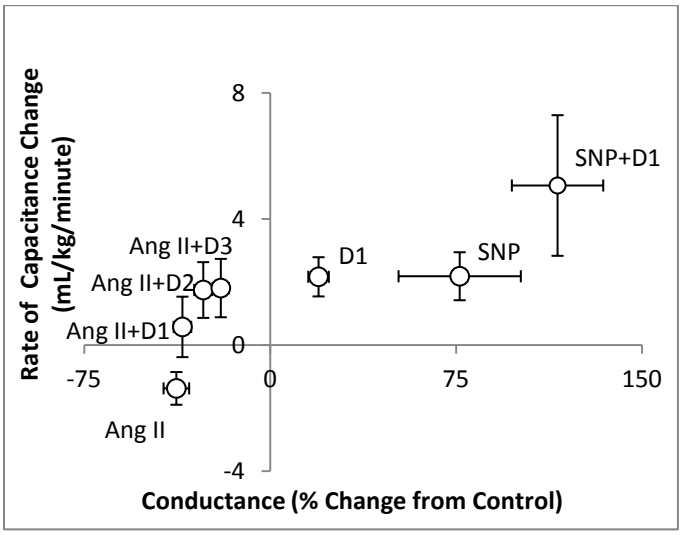

Figure 4 - Rate of capacitance change vs. % conductance for all interventions.

Baseline is represented at the axes intersection 0,0. Drug interventions are indicated as Ang II and SNP, while D1, D2, and D3 represent stimulating currents, Dose 1, Dose 2, and Dose 3. Ang II+D2 and Ang II+D3 data points are partially superimposed.

A large capacitive effect was observed during all activations of the device at Dose 1. Vasoactive agents can affect arterial and venous tone to varying degrees. Changing arterial/ arteriolar caliber is typically associated with changes to systemic vascular resistance (i.e., the reciprocal of conductance), with a generally ignored effect on vascular capacitance.[39] Changes to venous or venular caliber are associated with changes in vascular capacitance, with a trivial effect on total systemic vascular resistance. Thus, to compare venous and arterial effects quantitatively we have developed plots of (venous) capacitance versus (arterial) conductance.[38;59-61]

Under the conditions of our study (anesthetized, ventilated animals), while capacitance changes resulting from our chosen doses of SNP and BAT were similar, SNP had a much greater effect on arterial conductance. This is indicative of its greater capability as an arterial dilator in addition to its comparable effect as a a venous dilator. While BAT is associated with decreased

preload (i.e., P_{LVED} and $Area_{LVED}$) with Dose 1 alone and with Ang II, preload did not change significantly with Dose 1 during SNP infusion. In this case, BAT's ability to increase arterial conductance and reduce heart rate are responsible for the decrease in blood pressure.

The comparative effects of Ang II on capacitance and conductance are also interesting (see Figure 4). Ang II decreased both conductance and capacitance, yet only the capacitive effects (mobilizing blood to the central circulation) were fully reversed with BAT. Although mean arterial pressure returned to pre-Ang II levels with the highest dose of BAT (Dose 3), arterial conductance was not fully restored, returning to only 80% of baseline. With no changes in preload observed between Dose 1 and Doses 2 and 3, the increases in conductance with higher BAT doses were likely playing a larger role in the reduction of mean arterial pressure.

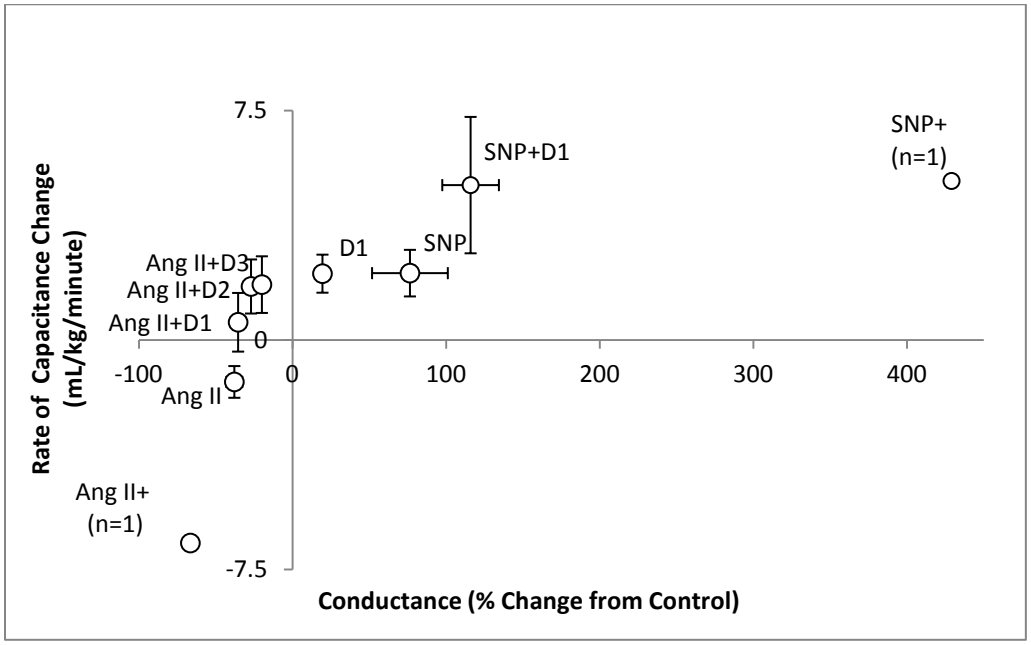

Figure 5 – Capacitance vs. conductance with augmented SNP and Ang II doses.

Figure 4 repeated with points added representing augmented doses of SNP and Ang II (n=1 for both), denoted by '+' symbol.

In Figure 5, two points have been added to the capacitance vs. conductance plot. These points each represent a single application of an augmented dose of Ang II and SNP. These values were

obtained early in the study when drug doses were being established. While the n of 1 limits the conclusions that can be drawn from these data points, they are helpful in placing the existing data in context.

7.4 Aortic Stiffness and Pulse Wave Velocity

Results from measurement of aortic stiffness and pulse wave velocity are generally supportive of the conclusions made thus far in the discussion of hemodynamic measurements and venous capacitance. Changes to stiffness and PWV typically mirror changes in mean P_{ao}. This is consistent with expected results, with higher pressure and greater stretch of the artery resulting in a more collagen engagement, and therefore greater stiffness.[25]

The exceptions to the trend of stiffness changing proportionally to P_{ao} are the observations for proximal stiffness. Changes to proximal aortic stiffness cannot be predicted based on changes to mean P_{ao} alone. The direction of change also depends on initial mean P_{ao}. See Figure 6 for a typical example of an aortic pressure-diameter relationship over a range of pressures. When pressure decreases below physiological values, the proximal aortic stiffness, as determine by the slope of the pressure-diameter loop, begins to increase. The mechanism for this increase is not entirely clear, with possible contributions from a sympathetic reflex response, as well as limited elastin engagement.[86;87] For this reason it cannot be simply stated that BAT, or any intervention, decreases or increases proximal aortic stiffness. When BAT was applied alone and during SNP, it increased proximal aortic stiffness. When applied during Ang II infusion, BAT decreased proximal aortic stiffness. The difference between the two cases is the baseline pressure. Results of abdominal aortic stiffness, where stiffness is much greater due to increased collagen content,[88] are more predictable, with the limited data supporting a passive dependency of aortic stiffness on blood pressure.

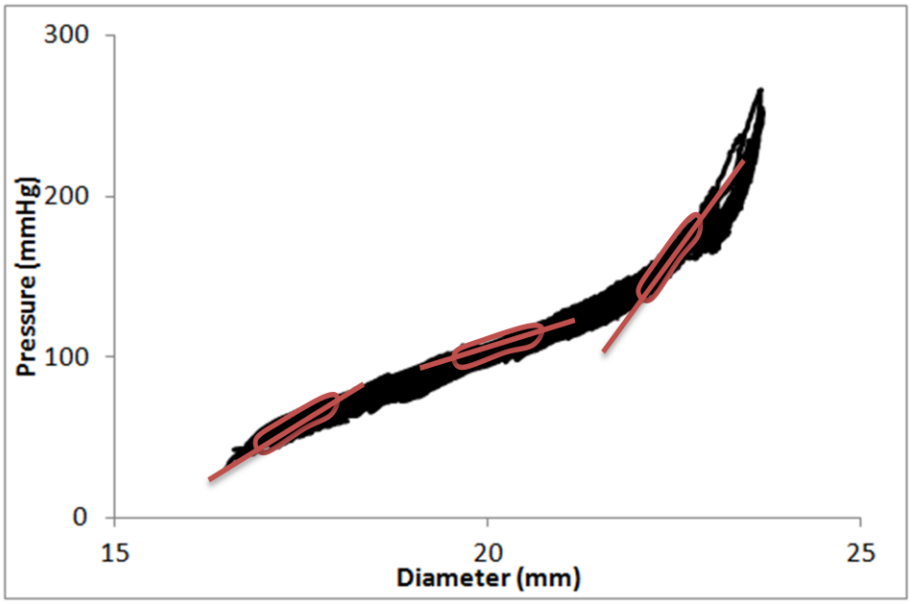

Figure 6 – Typical ascending aorta pressure-diameter loops over a broad pressure range due to Ang II infusion. Red lines show approximate loops and their slopes at different pulse pressures.

Proximal PWV measurements provide a more consistent measure of arterial stiffness than the proximal pressure-diameter loop stiffness for our experimental setup. This is because stiffness is measured at a single location (ascending aorta), while proximal PWV is measured as the temporal difference between ascending aorta and diaphragmatic aorta pressure waveforms for a known distance, and therefore represents an average stiffness for the proximal aorta. For both cases, BAT with SNP and BAT with Ang II, PWV appears to move in the direction of the pressure effects of the intervention, though results of BAT with SNP are not statistically significant.

Abdominal and total PWV values tend to agree with our conclusions stated previously about the overall hemodynamic effects of BAT. BAT has demonstrated the ability to decrease blood pressure through a relatively large venous effect, increasing abdominal blood volume and decreasing preload (P_{LVED} and $Area_{LVED}$). This is in addition to BAT's arterial effect, increasing conductance. While the importance of the venous vs. arterial effects were demonstrated when BAT was applied with SNP and Ang II (i.e. when BAT was applied with SNP, preload did not

decrease significantly, and we placed a greater importance on the conductance changes) PWV and stiffness do not depend on such a distinction. If BAT is not changing stiffness directly, then PWV and stiffness should only depend on arterial pressure/stretch, regardless of the means by which this pressure change is achieved.

During Ang II infusion, BAT Dose 3 was able to reverse the Ang II pressure and venous effects, while restoring conductance to only 80% of baseline. Only abdominal PWV shows a statistically significant higher value at BAT Dose 3 from baseline. Other values tend to be higher, but not statistically. While this may be interpreted as some small evidence to suggest that Ang II may have an aortic/arterial stiffening effect that is not entirely reversed by BAT, variability in the data prevents such a conclusion being made. Additional experiments, with a greater sensitivity in stiffness and PWV measurements would be required to make such conclusions.

7.5 IVC Occlusion vs. BAT

A study by Ferguson et al.[86] found that infusion of SNP produced no significant alteration to the pressure-diameter relationship, as compared to performing an IVC occlusion. When lowering pressure with SNP, the shifted pressure-diameter curve was the same as that achieved by IVC occlusion. It is therefore not surprising that no significant difference for stiffness or PWV appeared between BAT and an IVC occlusion. These results support our observations with pharmacological interventions, in addition to the literature,[29;30] that acute changes to aortic stiffness through pharmacological or mechanical means are unlikely.

While we have recorded and reported PWV values due to the experimental consistency of the measurement as well as the correlation to PWV use in the clinical setting,[84] it is likely best to view PWV as being dependent on stiffness. This dependence is described by the Moens-Korteweg equation, where elastic modulus (stiffness) is the variable of interest in determining PWV.[18]

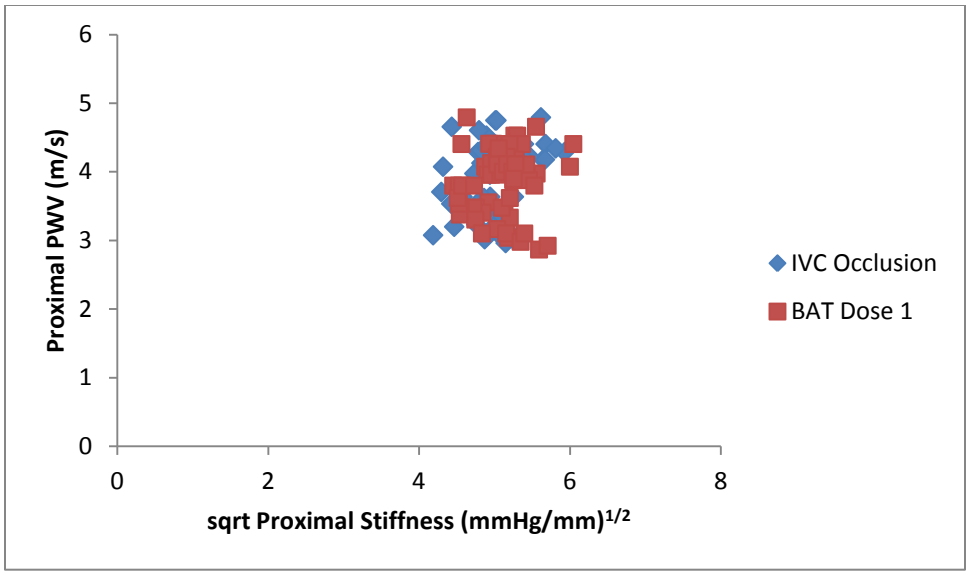

Figure 7 - Proximal PWV vs. square root of proximal aortic stiffness (n=6).

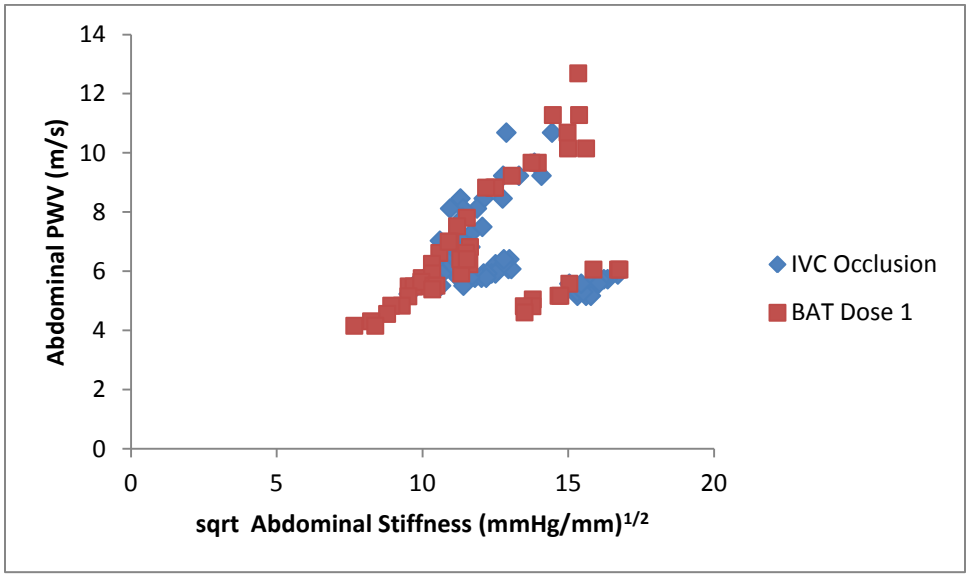

Figure 8 - Abdominal PWV vs. square root of abdominal aortic stiffness (n=4).

To demonstrate the dependency of PWV on stiffness, Figure 7 and Figure 8 show PWV for IVC occlusion and transient BAT plotted against the square root of stiffness. Proximal PWV shows no correlation with proximal aortic stiffness. This is due to our experimental methodology.

Proximal PWV is representative of the average aortic stiffness over the measurement area (ascending aorta to diaphragm), while proximal aortic stiffness is a representation of a single location (ascending aorta). The relationship is better seen in the abdomen. Figure 8 shows a linear relationship between abdominal PWV and the square root of abdominal aortic stiffness. The limited correlation between experiments is not surprising, and is due to the previously described challenges associated with sonometric crystal implantation in the abdomen.

The relationship between aortic stiffness and pressure, which is likely to be our independent variable in acute cases, can be seen in Figure 9. Aortic stiffness increases with increased pressure, and likely represents the increase in collagen engagement, due to the stretch of the artery.

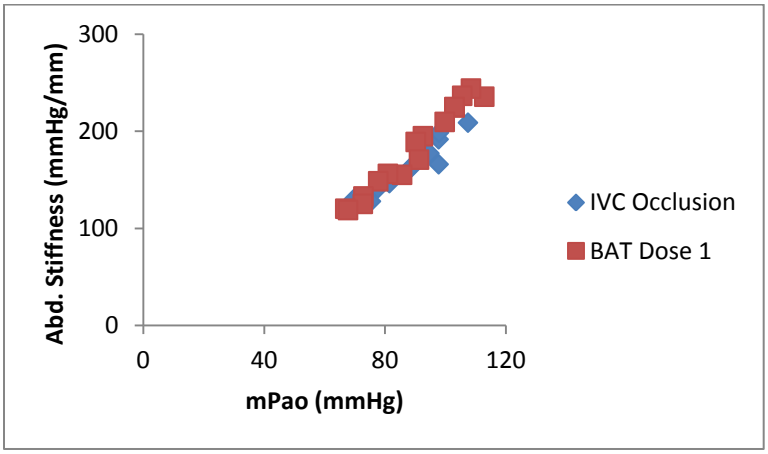

Figure 9 – Typical example of transient abdominal aortic stiffness vs. mean arterial pressure for BAT and IVC occlusion.

Figure 10 helps better illustrate the lack of a relationship in our data between proximal aortic stiffness and proximal PWV. At the aortic root, at lower pressures, stiffness increases with a pressure decrease, whereas the PWV averaged across the thoracic aorta decreases with decreasing pressure (Figure 11), and likely represents decreasing average thoracic aortic stiffness.

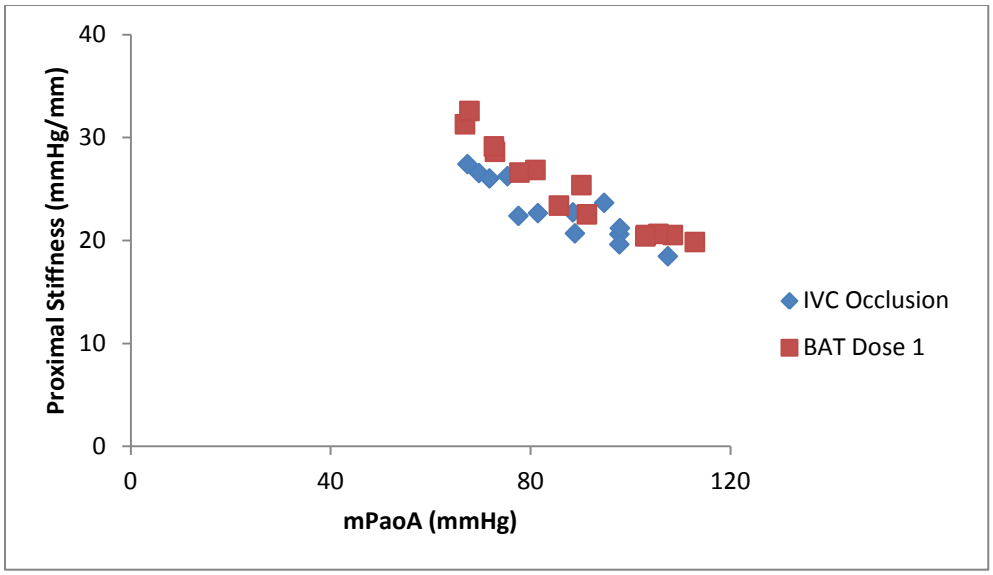

Figure 10 – Typical example of proximal aortic stiffness vs. mean aortic pressure for BAT and IVC occlusion.

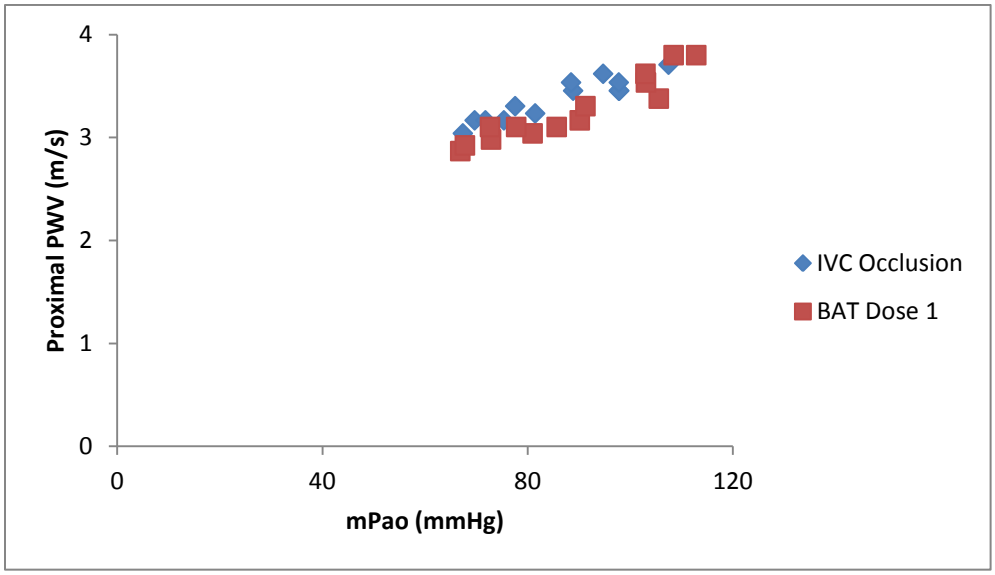

Figure 11 - Typical example of proximal aortic PWV vs. mean aortic pressure for BAT and IVC occlusion.

7.6 Clinical Implications

Decreased vascular capacitance is a characteristic of hypertension, both experimentally[71] and clinically,[73] with reductions in venous blood volume and the mobilization of blood to the central circulation. The ability of BAT to increase venous capacitance, moving blood to the periphery, is likely an important contributor to the success of the therapy in hypertensive subjects.[81] The elevated level of circulating Ang II in hypertensive patients is a common therapeutic target via blockage of the renin-angiotensin system.[64] The ability of the device to lower blood pressure in the presence of Ang II is a promising result and consistent with the success of the device observed in hypertensive patients.[81]

BAT is seen as a potential therapy in heart failure.[65] Heart failure in animal models has indentified a decrease in vascular capacitance,[89] while limb plethysmography in heart failure patients has led to similar conclusions,[78] correlating impaired venous function to the severity of heart failure. Neurohumoral stimulation in severe acute decompensated heart failure is the body's attempt to improve cardiac output.[85] However, the associated decrease in venous capacitance, which would be expected to increase LV preload, is of limited value because of pericardial constraint to filling and may actually reduce LV preload by direct ventricular interaction.[90;91] Where there is a disruption of venous function in heart failure, the large capacitive effect of BAT may be beneficial by decreasing LV filling pressure and may also increase LV preload and output by direct ventricular interaction,[90;91] in addition to its effects on arterial conductance.

8 Conclusions

Acute electrical activation of the carotid baroreflex decreases mean aortic blood pressure and heart rate and increases arterial conductance. Arterial stiffness decreases with the BAT-associated pressure decrease. At the aortic root, the direction of the stiffness change depends on baseline pressure, though a decrease in thoracic PWV suggests a decrease in average thoracic aorta stiffness. Preload is reduced through decreased P_{LVED} and $Area_{LVED}$. A large increase in venous capacitance can be observed through changes in abdominal volume. Dilation of the vasculature by SNP does not attenuate BAT's effectiveness. With blood pressure

raised by infusion of Ang II the device continues to be effective, with dose-dependent blood pressure reduction associated with modulation of venous capacitance by baroreceptor activation. Modulation of venous capacitance may be an important effect of baroreceptor activation in hypertension and heart failure.

8.1 Future Directions

While the effectiveness of BAT in experimental[6] and clinical[81] hypertension has been demonstrated, the role and magnitude of venous capacitance change due to BAT in this disease state has not been quantified. This measure, including observation of changes to cardiac filling pressures would be a valuable addition to the literature.

Examination of venous capacitance and aortic conductance changes in a heart failure model would be especially valuable, given current investigations into the benefit of BAT in heart failure.[65] An acute animal model using coronary artery ligation or microsphere embolization would allow most of our experimental protocol to be reproduced for acute heart failure study. It would be hypothesized that the magnitude of change of venous capacitance would increase with increased sympathetic activity associated with heart failure.

Reference List

1. Georgakopoulos D, Wagner D, Cates AW, Irwin E, Lovett EG. Effects of electrical stimulation of the carotid sinus baroreflex using the Rheos device on ventricular-vascular coupling and myocardial efficiency assessed by pressure-volume relations in non-vagotomized anesthetized dogs. *Conf Proc IEEE Eng Med Biol Soc*. 2009;2009:2025-2029.

2. Berne RM, Levy MN. Cardiovascular Physiology. 2001. Mosby, St. Louis.

3. Martin PJ, Levy MN, Zieske H. Bilateral carotid sinus control of ventricular performance in the dog. *Circ Res*. 1969;24:321-337.

4. Masuda Y, Pace DG, Levy MN. The rate of arterial blood pressure change as a factor in the hysteresis of the baroreceptor reflex. *J Hypertens*. 1984;2:189-194.

5. Lohmeier TE, Dwyer TM, Irwin ED, Rossing MA, Kieval RS. Prolonged activation of the baroreflex abolishes obesity-induced hypertension. *Hypertension*. 2007;49:1307-1314.

6. Lohmeier TE, Dwyer TM, Hildebrandt DA, Irwin ED, Rossing MA, Serdar DJ, Kieval RS. Influence of prolonged baroreflex activation on arterial pressure in angiotensin hypertension. *Hypertension*. 2005;46:1194-1200.

7. Sopher SM, Smith ML, Eckberg DL, Fritsch JM, Dibner-Dunlap ME. Autonomic pathophysiology in heart failure: carotid baroreceptor-cardiac reflexes. *Am J Physiol*. 1990;259:H689-H696.

8. Ketch T, Biaggioni I, Robertson R, Robertson D. Four faces of baroreflex failure: hypertensive crisis, volatile hypertension, orthostatic tachycardia, and malignant vagotonia. *Circulation*. 2002;105:2518-2523.

9. Matthews GG. Introduction to Neuroscience. 2000. Blackwell Science, Inc..

10. Janiq W. The Integrative Action of the Autonomic Nervous System. 2006. Cambridge University Press.

11. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci*. 2006;7:335-346.

12. Borst C, Karemaker JM, Bouman LN, Dunning AJ, Schopman JG. Optimal frequency of carotid sinus nerve stimulation in treatment of angina pectoris. *Cardiovasc Res*. 1974;8:674-680.

13. Borst C, Karemaker JM, Dunning AJ, Bouman LN, Wagner J. Frequency limitation in the human baroreceptor reflex. *J Auton Nerv Syst*. 1983;9:381-397.

14. Milnor WR. Cardiovascular Physiology. 1990. Oxford University Press Inc..

15. Robertson D, Bioaggioni I, Burnstock G, Low P. Primer on the Autonomic Nervous System. 2012. Elsevier.

16. Pang CC. Autonomic control of the venous system in health and disease: effects of drugs. *Pharmacol Ther*. 2001;90:179-230.

17. Milan A, Tosello F, Fabbri A, Vairo A, Leone D, Chiarlo M, Covella M, Veglio F. Arterial stiffness: from physiology to clinical implications. *High Blood Press Cardiovasc Prev*. 2011;18:1-12.

18. Oates C. Cardiovascular Hemodynamics and Doppler Waveforms Explained. 2001. Greenwich Medical Media Ltd..

19. Stefanadis C, Stratos C, Vlachopoulos C, Marakas S, Boudoulas H, Kallikazaros I, Tsiamis E, Toutouzas K, Sioros L, Toutouzas P. Pressure-diameter relation of the human aorta. A new method of determination by the application of a special ultrasonic dimension catheter. *Circulation*. 1995;92:2210-2219.

20. Hallett JW, Mills JL, Earnshaw JL, Reekers JA, Rooke TW. Comprehensive Vascular and Endovascular Surgery. 2012. Mosby Elsevier, Philadephia, PA.

21. Cox RH. Passive mechanics and connective tissue composition of canine arteries. *Am J Physiol*. 1978;234:H533-H541.

22. Silver FH, Christiansen DL. Biomaterials Science and Biocompatibility. 1999. Springer-Verlag, New York.

23. Wolinsky H, Glagov S. Structure Basis for the Static Mechanical Properties of the Aortic Media. *Circ Res*. 1964;14:400-413.

24. Roach MR, Burton AC. The reason for the shape of the distensibility curves of arteries. *Can J Biochem Physiol*. 1957;35:681-690.

25. Lanzer P, Topol EJ. Panvascular Medicine Integrated Clinical Management. 1999. Springer, Cleveland, OH.

26. Dobrin P, Canfield T. Identification of smooth muscle series elastic component in intact carotid artery. *Am J Physiol*. 1977;232:H122-H130.

27. Greenwald SE, Moore JE, Jr., Rachev A, Kane TP, Meister JJ. Experimental investigation of the distribution of residual strains in the artery wall. *J Biomech Eng*. 1997;119:438-444.

28. Wagenseil JE, Mecham RP. Vascular extracellular matrix and arterial mechanics. *Physiol Rev*. 2009;89:957-989.

29. Boutouyrie P, Lacolley P, Briet M, Regnault V, Stanton A, Laurent S, Mahmud A. Pharmacological modulation of arterial stiffness. *Drugs*. 2011;71:1689-1701.

30. Hayoz D, Rutschmann B, Perret F, Niederberger M, Tardy Y, Mooser V, Nussberger J, Waeber B, Brunner HR. Conduit artery compliance and distensibility are not necessarily reduced in hypertension. *Hypertension*. 1992;20:1-6.

31. Aars H. Diameter and elasticity of the ascending aorta during infusion of noradrenaline. *Acta Physiol Scand*. 1971;83:133-138.

32. Levy BI, Tedgui A. Biology of the Arterial Wall. 1999. Kluwer Academic Publishers, Norwell, MA.

33. Gerova M, Gero J, Dolezel S, Blazkova-Huzulakova I. Sympathetic control of canine abdominal aorta. *Circ Res*. 1973;33:149-159.

34. Cox RH, Bagshaw RJ. Carotid sinus control of pulsatile hemodynamics in halothane-anesthetized dogs. *Am J Physiol*. 1980;238:H294-H299.

35. Joannides R, Richard V, Moore N, Godin M, Thuillez C. Influence of sympathetic tone on mechanical properties of muscular arteries in humans. *Am J Physiol*. 1995;268:H794-H801.

36. Levy MN. The cardiac and vascular factors that determine systemic blood flow. *Circ Res*. 1979;44:739-747.

37. Luecke T, Roth H, Herrmann P, Joachim A, Weisser G, Pelosi P, Quintel M. Assessment of cardiac preload and left ventricular function under increasing levels of positive end-expiratory pressure. *Intensive Care Med*. 2004;30:119-126.

38. Tyberg JV. How changes in venous capacitance modulate cardiac output. *Pflugers Arch --Europ J Physiol*. 2002;445:10-17.

39. Wang SY, Scott-Douglas NW, Manyari DE, Tyberg JV. Arterial versus venous changes in vascular capacitance during nitroprusside infusion: a vascular modeling study. *Canad J Physiol Pharmacol*. 1999;77:131-137.

40. Rothe CF. Reflex controls of veins and vascular capacitance. *Physiol Rev*. 1983;63:1281-1341.

41. Gelman S. Venous function and central venous pressure: a physiologic story. *Anesthesiology*. 2008;108:735-748.

42. Hainsworth R. Vascular capacitance: its control and importance. *Rev Physiol Biochem Pharmacol*. 1986;105:101-173.

43. Brooksby GA, Donald DE. Measurement of changes in blood flow and blood volume in the splanchnic circulation. *J Appl Physiol*. 1971;31:930-933.

44. Wright CI, Sheldon RS, Tyberg JV. Measuring transient systemic conductance and capacitance responses using a modified Brooksby-Donald technique in anesthetized, autonomically intact rabbits. *Can J Cardiol*. 2004;20:1127-1134.

45. Noble BJ, Drinkhill MJ, Myers DS, Hainsworth R. Mechanisms responsible for changes in abdominal vascular volume during sympathetic nerve stimulation in anaesthetized dogs. *Exp Physiol*. 1997;82:925-934.

46. Brooksby GA, Donald DE. Release of blood from the splanchnic circulation in dogs. *Circ Res*. 1972;31:105-108.

47. Shoukas AA, Sagawa K. Control of total systemic vascular capacity by the carotid sinus baroreptor reflex. *Circ Res*. 1973;33:22-33.

48. Carneiro JJ, Donald DE. Blood reservoir function of dog spleen, liver, and intestine. *Am J Physiol Heart Circ Physiol*. 1977;232:H67-H72.

49. Carneiro JJ, Donald DE. Change in Liver Blood-Flow and Blood Content in Dogs During Direct and Reflex Alteration of Hepatic Sympathetic-Nerve Activity. *Circ Res*. 1977;40:150-158.

50. Fielding JWL, Hallissey MT. Upper Gastrointestinal Surgery. 2004. Springer-Verlag.

51. Rubin SA, Misbach G, Lekven J, Parmley WW, Tyberg JV. Resistance and volume changes caused by nitroprusside in the dog. *Am J Physiol*. 1979;237:H99-103.

52. Malm L. Responses of resistance and capacitance vessels in feline nasal mucosa to vasoactive agents. *Acta Otolaryngol*. 1974;78:90-97.

53. Hirakawa S, Itoh H, Kotoo Y, Abe C, Endo T, Takada N, Fuseno H. The role of alpha and beta adrenergic receptors in constriction and dilation of the systemic capacitance vessels: a study with measurements of the mean circulatory pressure in dogs. *Jpn Circ J*. 1984;48:620-632.

54. Smiseth OA, Manyari DE, Lima JA, Scott-Douglas NW, Kingma I, Smith ER, Tyberg JV. Modulation of vascular capacitance by angiotensin and nitroprusside: a mechanism of changes in pericardial pressure. *Circulation*. 1987;76:875-883.

55. Anderson SM, Rich GF, Roos C, Lee LP, Lee JS. Fluid restitution and blood volume redistribution in anesthetized rabbits in response to vasoactive drugs. *Circulation*. 1994;90:509-514.

56. Hsieh NK, Chang HR, Hu CT, Chen HI. Effects of nitric oxide donor and nitric oxide synthase inhibitor on the resistance, exchange and capacitance functions of the canine intestinal vasculature. *Vascul Pharmacol*. 2008;48:122-128.

57. Lee RW, Standaert S, Lancaster LD, Buckley D, Goldman S. Cardiac and peripheral circulatory responses to angiotension and vasopressin in dogs. *J Clin Invest*. 1988;82:413-419.

58. Osborn JW, Fink GD, Kuroki MT. Neural mechanisms of angiotensin II-salt hypertension: implications for therapies targeting neural control of the splanchnic circulation. *Curr Hypertens Rep*. 2011;13:221-228.

59. Semeniuk LM, Belenkie I, Tyberg JV. Acute effects of toborinone on vascular capacitance and conductance in experimental heart failure. *Circulation*. 1998;59:58-63.

60. Chihara E, Manyari DE, Isaac DL, Tyberg JV. Comparative effects of nitroglycerin on intestinal vascular capacitance and conductance. *Can J Cardiol*. 2002;18:165-174.

61. Isaac DL, Belenkie I, Manyari DE, Tyberg JV. Vascular and cardiac effects of amlodipine in acute heart failure in dogs. *Canad J Cardiol*. 1998;14:1375-1382.

62. Lemke T, Williams D, Roche V, Zito S. Foye's Principles of Medicinal Chemistry. 2013. Lippincott Williams & Wilkins, Baltimore, MD.

63. Schiffrin EL, Touyz RM. Multiple actions of angiotensin II in hypertension: benefits of AT1 receptor blockade. *J Am Coll Cardiol*. 2003;42:911-913.

64. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Struijker Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Kjeldsen SE, Erdine S, Narkiewicz K, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Cifkova R, Dominiczak A, Fagard R, Heagerty AM, Laurent S, Lindholm LH, Mancia G, Manolis A, Nilsson PM, Redon J, Schmieder RE, Struijker-Boudier HA, Viigimaa M, Filippatos G, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Kiowski W, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Viigimaa M, Waeber B, Williams B, Zamorano JL, The task force for the management of arterial hypertension of the European Society of Hypertension, The task force for the management of arterial hypertension of the European Society of Cardiology. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J*. 2007;28:1462-1536.

65. Georgakopoulos D, Little WC, Abraham WT, Weaver FA, Zile MR. Chronic baroreflex activation: a potential therapeutic approach to heart failure with preserved ejection fraction. *J Card Fail*. 2011;17:167-178.

66. Krames E, Peckham P, Rezai A. Neuromodulation. 2009. Elsevier.

67. Carlsten A, Folkow B, Grimby G, Hamberger CA, Thulesius O. Cardiovascular effects of direct stimulation of the carotid sinus nerve in man. *Acta Physiol Scand*. 1958;44:138-145.

68. Doumas M, Guo D, Papademetriou V. Carotid baroreceptor stimulation as a therapeutic target in hypertension and other cardiovascular conditions. *Expert Opin Ther Targets*. 2009;13:413-425.

69. Martin DS, Rodrigo MC, Appelt CW. Venous tone in the developmental stages of spontaneous hypertension. *Hypertension*. 1998;31:139-144.

70. Yamamoto J, Trippodo NC, MacPhee AA, Frohlich ED. Decreased total venous capacity in Goldblatt hypertensive rats. *Am J Physiol*. 1981;240:H487-H492.

71. Young DB, Murray RH, Bengis RG, Markov AK. Experimental angiotensin II hypertension. *Am J Physiol*. 1980;239:H391-H398.

72. Draaijer P, Kool MJ, Maessen JM, Van Bortel LM, de Leeuw PW, van Hooff JP, Leunissen KM. Vascular distensibility and compliance in salt-sensitive and salt-resistant borderline hypertension. *J Hypertens*. 1993;11:1199-1207.

73. Delaney EP, Young CN, Disabatino A, Stillabower ME, Farquhar WB. Limb venous tone and responsiveness in hypertensive humans. *J Appl Physiol*. 2008;105:894-901.

74. Widgren BR, Berglund G, Wikstrand J, Andersson OK. Reduced venous compliance in normotensive men with positive family histories of hypertension. *J Hypertens*. 1992;10:459-465.

75. Gay R, Wool S, Paquin M, Goldman S. Total vascular pressure-volume relationship in conscious rats with chronic heart failure. *Am J Physiol Heart Circ Physiol*. 1986;251:H483-H489.

76. Wang SY, Manyari DE, Scott-Douglas NW, Smiseth OA, Smith ER, Tyberg JV. Splanchnic venous pressure-volume relation during experimental acute ischemic heart failure: differential effects of hydralazine, enalaprilat, and nitroglycerin. *Circulation*. 1995;91:1205-1212.

77. Cotter G, Metra M, Milo-Cotter O, Dittrich HC, Gheorghiade M. Fluid overload in acute heart failure--re-distribution and other mechanisms beyond fluid accumulation. *Eur J Heart Fail*. 2008;10:165-169.

78. Ikenouchi H, Iizuka M, Sato H, Momomura S, Serizawa T, Sugimoto T. Forearm venous distensibility in relation to severity of symptoms and hemodynamic data in patients with congestive heart failure. *Jpn Heart J*. 1991;32:17-34.

79. Thomson HL, Morris-Thurgood J, Atherton J, McKenna WJ, Frenneaux MP. Reflex responses of venous capacitance vessels in patients with hypertrophic cardiomyopathy [see comments]. *Clin Sci (Colch)*. 1998;94:339-346.

80. Illig KA, Levy M, Sanchez L, Trachiotis GD, Shanley C, Irwin E, Pertile T, Kieval R, Cody R. An implantable carotid sinus stimulator for drug-resistant hypertension: surgical technique and short-term outcome from the multicenter phase II Rheos feasibility trial. *J Vasc Surg*. 2006;44:1213-1218.

81. Scheffers IJ, Kroon AA, Schmidli J, Jordan J, Tordoir JJ, Mohaupt MG, Luft FC, Haller H, Menne J, Engeli S, Ceral J, Eckert S, Erglis A, Narkiewicz K, Philipp T, de Leeuw PW. Novel baroreflex activation therapy in resistant hypertension: results of a European multi-center feasibility study. *J Am Coll Cardiol*. 2010;56:1254-1258.

82. Scott-Douglas NW, Traboulsi M, Smith ER, Tyberg JV. Experimental instrumentation and left ventricular pressure- strain relationship. *Am J Physiol Heart Circ Physiol*. 1991;261:H1693-H1697.

83. Belenkie I, Dani R, Smith ER, Tyberg JV. Effects of volume loading during experimental acute pulmonary embolism. *Circulation*. 1989;80:178-188.

84. Tomiyama H, Yamashina A. Non-invasive vascular function tests: their pathophysiological background and clinical application. *Circ J*. 2010;74:24-33.

85. Fallick C, Sobotka PA, Dunlap ME. Sympathetically mediated changes in capacitance: redistribution of the venous reservoir as a cause of decompensation. *Circ Heart Fail*. 2011;4:669-675.

86. Ferguson JJ, III, Momomura S, Sahagian P, Miller MJ, McKay RG. The use of nitroprusside to characterize aortic pressure-diameter relationships. *Tex Heart Inst J*. 1989;16:5-10.

87. Stefanadis C, Vlachopoulos C, Karayannacos P, Boudoulas H, Stratos C, Filippides T, Agapitos M, Toutouzas P. Effect of vasa vasorum flow on structure and function of the aorta in experimental animals. *Circulation*. 1995;91:2669-2678.

88. McDonald DA. Regional pulse-wave velocity in the arterial tree. *J Appl Physiol*. 1968;24:73-78.

89. Ogilvie RI, Zborowska-Sluis D. Effect of chronic rapid ventricular pacing on total vascular capacitance. *Circulation*. 1992;85:1524-1530.

90. Atherton JJ, Moore TD, Lele SS, Thomson HL, Galbraith AJ, Belenkie I, Tyberg JV, Frenneaux MP. Diastolic ventricular interaction in chronic heart failure. *Lancet*. 1997;349:1720-1724.

91. Moore TD, Frenneaux MP, Sas R, Atherton JJ, Morris-Thurgood JA, Smith ER, Tyberg JV, Belenkie I. Ventricular interaction and external constraint account for decreased stroke work during volume loading in CHF. *Am J Physiol Heart Circ Physiol*. 2001;281:H2385-H2391.