ALTERATION IN VASCULAR FUNCTION DUE TO HIGH INTRAVASCULAR PRESSURE:

Arterial stiffness and the role of beta-adrenoceptors

by Camille Leblanc [©]

A thesis submitted to the School of Graduate Studies in partial fulfilment of the

requirements for the degree of

Master of Science in Medicine

Cardiovascular and Renal Sciences

Division of Biomedical Sciences

Faculty of Medicine

Memorial University of Newfoundland

May 2018

St. John's, Newfoundland

Abstract

Background: Hypertension-induced arterial stiffness is associated with high risks of morbidity and mortality. The sympathetic nervous system plays an important role in the control of cardiovascular function through beta-adrenoceptors (β-AR). The involvement of β-AR in the control of arterial stiffness in a state of hypertension and normotension was examined. **Methods:** Pulse wave velocity (PWV) was assessed within a narrow range of blood pressures in isoflurane-anaesthetized 13-14-week-old male spontaneously hypertensive (SH) and Wistar-Kyoto (WKY) rats. **Results:** Baseline PWV was higher in SH (9.2±0.9 m/s) compared to WKY rats (6.7±0.4 m/s). The stimulation of β_2- but not β_3-AR reduced PWV in SH rats despite comparable reductions in blood pressure. Sodium nitroprusside infusions initiated a dose-dependent reduction of PWV in SH rats.

Conclusion: The evidence suggests that a reduction in central vascular tone may play a key role in decreasing elevated PWV independently from reduction in blood pressure in a state of hypertension.

Key words: arterial stiffness, high intravascular pressure, pulse wave velocity, beta-adrenoceptors, vascular tone

Acknowledgements

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Symbols

ANOVA: Analysis of variance

ATP: Adenosine triphosphate

α-AR: Alpha-adrenoceptor

β-AR: Beta-adrenoceptor

cAMP: Cyclic adenosine monophosphate

CMDV: Corrected mean density value

cDBP: Central diastolic blood pressure

cPP: Central pulse pressure

cSBP: Central systolic blood pressure

DAPI: 4' 6-diamidino-2-phenylindole

ECM: Extracellular matrix

E_{inc}: Elastic modulus

eNOS: Endothelial nitric oxide synthase

ET-1: Endothelin-1

Gs: Stimulatory G protein

HR: Heart rate

IF: Immunofluorescence

ISH: Isolated systolic hypertension

MAP: Mean arterial pressure

MBDV: Mean background density value

MDV: Mean density value

MMP: Matrix metalloproteinase

NGS: Normal goat serum

NE: Norepinephrine

NO: Nitric oxide

NOS: Nitric oxide synthase

PBS: Phosphate-buffered saline

pDBP: Peripheral diastolic blood pressure

PKA: Protein Kinase A

pPP: Peripheral pulse pressure

pSBP: Peripheral systolic blood pressure

PWV: Pulse wave velocity

RAAS: Renin-angiotensin-aldosterone system

SDS: Sodium dodecyl sulfate

SH: Spontaneously hypertensive

SNP: Sodium nitroprusside

SNS: Sympathetic nervous system

TBS: Tris-buffered saline

VSMC: Vascular smooth muscle cell

WKY: Wistar-Kyoto rat

1. Introduction

1.1 Circulatory system

Blood vessels deliver oxygen and nutrients to organs and tissues in the body, as well as enable the removal of metabolic waste and CO_2 from cells. Systemic arteries carry oxygenated blood coming from the heart and circulate it throughout the body, while systemic veins return deoxygenated blood to the heart. The blood can then be pumped to the pulmonary vasculature where it will be oxygenated. In the pulmonary vasculature, the arteries carry deoxygenated blood to the lungs where it can be oxygenated, and the veins return oxygenated blood to the heart, from the pulmonary vasculature. The systemic artery with the largest diameter, the aorta, originates from the left cardiac ventricle. It then branches into small arteries, and subsequently into arterioles. Arterioles branch into capillaries, which allow the exchange of oxygen and nutrients with tissues. Small arteries such as arterioles are often referred to as resistance vessels because they have the greatest sectioned area, along with the greatest fall in blood pressure compared to other arteries, which suggests that they are the segment of the circulation with the greatest vascular resistance. Resistance vessels alter the lumen diameter by vasoconstriction or vasodilation, which increases and decreases vascular resistance, respectively (Marieb and Hoehn, 2013).

Blood vessels are composed of three layers; the tunica intima, the tunica media, and the tunica externa (tunica adventitia) (Figure 1). The tunica intima is the inner lining of blood vessels, and is composed of a single layer of endothelial cells and the underlying

1

subendothelial space that play many important roles in the vasculature, including the secretion of dilatory factors such as nitric oxide (NO). Capillaries are composed of endothelial cells, and are unique blood vessels, as they lack the tunica media and externa. Capillaries are known as the major exchange site for oxygen, nutrients, and waste. The tunica media is composed chiefly of elastin, an elastic fiber, as well as vascular smooth muscle cells (VSMCs). Two essential functions of VSMCs include contraction and dilation. The associated alteration in vascular tone leads to an increased or decreased lumen diameter, which subsequently alters resistance to blood flow as well as blood pressure. Arteries are mainly composed of VSMCs, and also have a high elastin content, which increases vascular elasticity. Elastin can be found in the tunica externa and the tunica media, as well as the border between the intima and media (internal elastic lamina) and between the tunica media and externa (external elastic lamina). However, it is predominantly present in the tunica media, between VSMCs (Marieb and Hoehn, 2013). Finally, the outer lining, the tunica externa, is composed of connective tissue, which serves to reinforce the tissue and connect it to neighboring tissues (Marieb and Hoehn, 2013). The extracellular matrix (ECM) of this layer of tissue is composed of collagen (mostly type I and III) (Shekhonin et al., 1987), which is a rigid fiber, as well as nerve fibers and lymphatic vessels (Marieb and Hoehn, 2013).
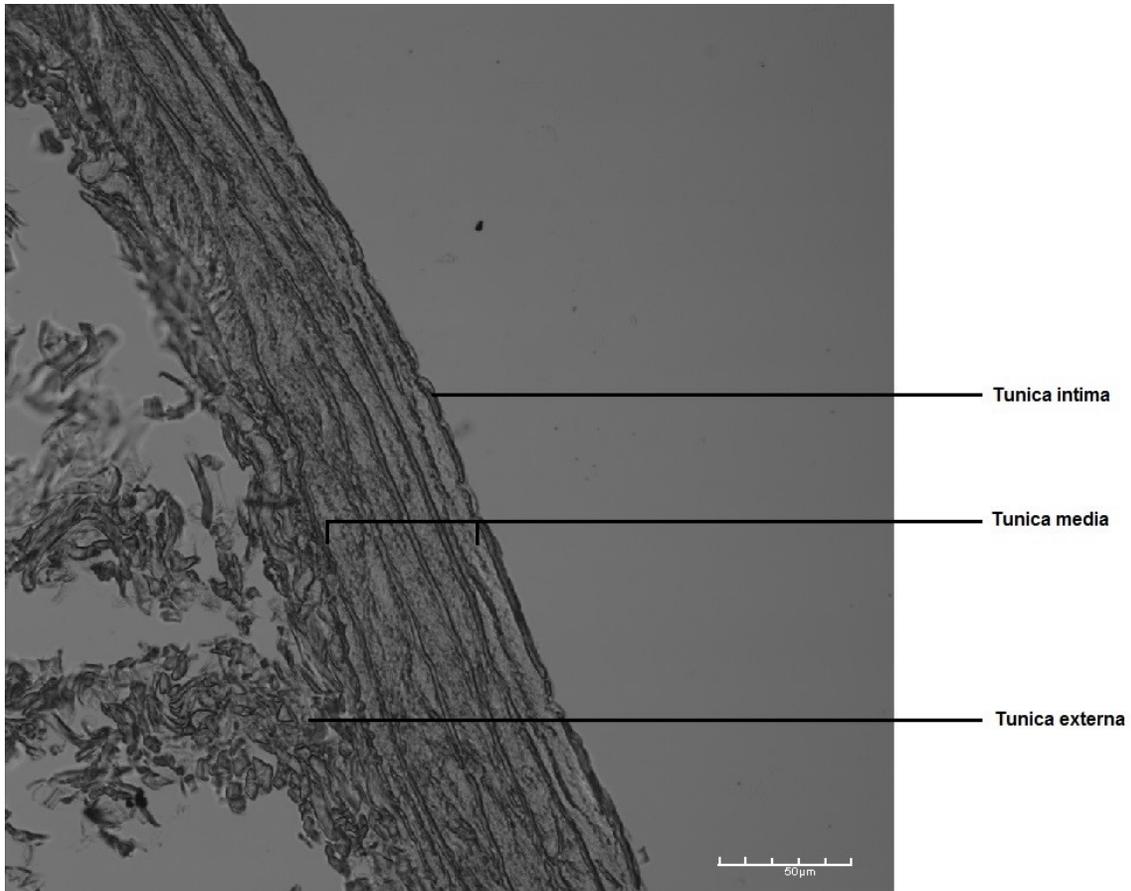
Figure 1. Schematic of rat abdominal aorta using transmitted light imaging, with a 20X objective and 1.9X magnification. The image shows the three layers of the arterial wall; the tunica intima, tunica media and the tunica externa.

1.2 Hypertension

Hypertension is defined as chronic high intravascular arterial pressure. Hypertension has traditionally been diagnosed when blood pressure reaches ≥140/90 mmHg (Chobanian et al., 2003). Blood pressure classifications can be found in Table 1. However, more complicated criteria have been developed, such as taking multiple blood pressure readings in the same clinical visit (among other measures for diagnosis) (Leung et al., 2017). Hypertension is the most common ailment in clinical settings (Chobanian et al., 2003). Essential (or primary) hypertension is a subset of hypertension that is diagnosed when the hypertension has no obvious cause. Secondary hypertension, which is less common, occurs due to other ailments such as renal occlusive disease or hyperthyroidism (Marieb and Hoehn, 2013). Hypertension is one of the leading causes of stroke, heart failure, renal failure, and myocardial infarction, which can contribute to a higher risk of mortality (James et al., 2014). Chronic high systolic pressure and reduced diastolic blood pressure often occur in subjects with increased central vascular stiffness (Mackenzie et al., 2002). High systolic pressure increases cardiac metabolism and therefore requires more blood flow to the heart, while reduced diastolic pressure (produced by a reduction in vascular resistance) results in decreased perfusion pressure in the coronary arteries. Eventually, this reduces the delivery of oxygen to the myocardium, which may result in heart failure (Mackenzie et al., 2002).

Table 1. Hypertension classification according to Chobanian and colleagues (2003)

Blood pressure classification		Systolic pressure (mmHg)	Diastolic pressure (mmHg)
Normal		<120	<80
Pre-hypertension		120-139	80-89
Hypertension	Stage 1	≥140	≥ 90
	Stage 2	≥ 160	≥ 100

1.2.1 The spontaneously hypertensive (SH) rat – a widely used animal model of hypertension

The spontaneously hypertensive (SH) rat was developed by selective breeding to study the pathophysiology of hypertension (Okamoto and Aoki, 1963). Male SH rats develop hypertension with a systolic pressure of approximately 154 ± 5 mmHg by 13 to 14 weeks of age, independently from environmental stimuli (data from the current investigation). The systolic blood pressure of the normotensive Wistar-Kyoto (WKY) rat is approximately 106 ± 4 mmHg at 13 to 14 weeks of age (data from the current investigation). Transplantation of both kidneys of an SH rat to their normotensive counterpart, the Wistar-Kyoto (WKY) rat, has been shown to induce hypertension in the WKY rat. The transplantation of both kidneys from a WKY to an SH rat, on the other hand, resulted in normal blood pressure in the SH rat. Therefore, it has been suggested that hypertension in the SH rat originates from the kidneys (Rettig, 1993). The SH rat is a suitable model for experiments pertaining to high intravascular pressure, since the pathophysiology is very similar to that of humans (Pinterova et al., 2011). For example, left ventricular hypertrophy is one of the complications of chronic hypertension in SH

rats (Institute of Laboratory Animal Resources, 1976). Arterial stiffness is another common complication of hypertension reported in SH rats (Sehgel et al., 2013), as well as in humans (Zieman et al., 2005). The manifestation of increased arterial stiffness can also occur independently from high blood pressure.

1.3 Arterial Stiffness

Arterial stiffness can be defined as a reduction in the elasticity of arteries. Increased aortic stiffness is often associated with hypertension, and increased rates of cardiovascular morbidity and mortality (Zieman et al., 2005). In normal physiological conditions, the aorta serves as a conduit vessel and a pressure buffer; since central arteries have high elastin content, they distend when blood pressures increase during systole, and recoil during diastole. This is known as the Windkessel effect, which allows dampening of the pulse pressure, as well as continuous blood flow and perfusion of smaller blood vessels and capillaries (Belz, 1995). When arteries become stiffer, their ability to distend is diminished, as well as their ability to dampen pulse pressure, which contributes to larger pulse pressures in arterial stiffness (Belz, 1995). There is increasing evidence that the assessment of arterial stiffness can predict cardiovascular risk (Zieman et al., 2005; Boutouyrie et al., 2014).

1.3.1 Assessment of arterial stiffness

The mechanical and dynamic properties of blood vessels are often described in various ways. Viscoelasticity describes the time-dependent mechanical properties of blood vessels; once they are subjected to a deformation, there is a delay to reach the appropriate shape relative to the deformation (visco-), and to return to its original shape once the stress is removed (-elasticity). Pressure, diameter, and wall thickness can be

measured to quantify these dynamic changes in vivo (Milnor, 1989) and contribute to defining the mechanical properties of arteries, by using indices of stiffness such as pulse pressure and pulse wave velocity (PWV).

Pulse pressure is the difference between systolic and diastolic pressure. As age increases, systolic blood pressure increases (Franklin et al., 1997), and diastolic blood pressure tends to decrease (Mackenzie et al., 2002). This creates a widening of the pulse pressure with increasing age. Pulse pressure seems to be a predictor of cardiovascular health and disease. It has been suggested that pulse pressure is a better predictor of coronary heart disease than blood pressure alone in patients >50 years of age (Franklin et al., 1999). As well, pulse pressure predicts the risk of stroke and mortality (Domanski et al., 1999). There is an increase in pulse pressure in smaller arteries partly due to increasing vascular resistance. Peripheral pulse pressure is also increased due to wave reflection; the closer you get to a peripheral reflection site, the less the delay between the original and reflected waves, and the more their summation affects the pulse amplitude. Brachial measurement of pulse pressure reflects arterial stiffness in the peripheral vasculature as opposed to the central vasculature (Pauca et al., 1992). If pulse pressure is to be used for measurement of arterial stiffness for cardiovascular disease risk assessment, it is best to use central aortic blood pressure measurements as opposed to peripheral blood pressure, since it has been reported to be a better predictor of cardiovascular events (McEniery et al., 2014). In a circulation with compliant arteries, pulse pressure tends to be lower than one with increased stiffness, because of the Windkessel effect and because the reflected pressure wave tends to return to the aortic root during diastole, as opposed to systole. Therefore, the impact of wave reflection on

the pulse wave is lower in compliant arteries (Mackenzie et al., 2002). PWV, the rate at

which the pulse wave travels in a given direction, is one of the most important factors

contributing to the timing of wave reflection.

PWV is also a measure of arterial stiffness that can predict cardiovascular risk in a

state of hypertension (Mackenzie et al., 2002). This measure of stiffness can be calculated

by dividing the distance (d) between two pressure points by pulse transit time (Δt), the

time it takes for the pulse to travel this distance;

$$PWV = d/\Delta t$$

This technique reflects the stiffness of the aorta, since the pulse travels faster in stiffer

arteries (Mackenzie et al., 2002). The arterial pressure wave is composed of the forward

pressure created by systole and the reflected pressure wave. Thus, the foot-to-foot method

is often used to avoid the influence of wave reflection. With this technique, the time

between the foot of the central diastolic pressure wave and the foot of the peripheral

diastolic pressure wave is used to assess pulse transit time (Mackenzie et al., 2002).

Vascular stiffness, and therefore PWV, has been reported to be dependent on heart rate as

well as blood pressure. Pulse transit time should therefore be measured at isobaric blood

pressures and similar heart rates (Butlin et al., 2015; Tan et al., 2012). In clinical practice,

determination of PWV has been used in the assessment of arterial stiffness, and seems to

be considered the "gold standard" for evaluating vascular elasticity (Laurent et al., 2006).

Although PWV is the most common method of assessing arterial stiffness in the

literature, there are other indices of stiffness that can be measured, such as the elastic

modulus (E_{inc}), the stiffness index and the augmentation index. Essentially, the elastic

modulus gives an indication of the elasticity of arteries. It can be defined as the ratio of

the force exerted on the blood vessel relative to its deformation, otherwise known as the stress to strain ratio. This parameter is defined as incremental, since it is obtained from measurements of small changes in blood pressure and vessel diameter (Milnor, 1989). The Moens-Korteweg equation can be used to calculate the elastic modulus in vivo, using PWV;

$$PWV = \sqrt{\frac{E_{inc} \times h}{2r\rho}}$$

where E_{inc} = elastic modulus, h = wall thickness, r = lumen radius and ρ = blood density (Marque et al., 2001). However, this equation assumes a homogenous vascular wall, as well as homogeneous and non-viscous fluid, which is not the case for blood vessels studied in vivo (Shahmirzadi et al, 2012). The stiffness index (β) is a measure of the mechanical properties of blood vessels, which relates arterial diameter to blood pressure (Milnor, 1989). In experimental settings, this index can be obtained by measuring central systolic (P_s) and diastolic (P_d) pressures, and systolic (D_s) and diastolic (D_d) lumen diameters;

$$\beta = \frac{P_s/P_d}{(D_s - D_d)/D_d}$$

The augmentation index is also used as a metric of arterial stiffness. It assesses the alteration in the central pressure wave attributed to changes in wave reflection. It is often defined as the measurement of the augmentation of central blood pressure by the reflected pulse wave (Milnor, 1989).

Arterial stiffness can be evaluated by assessing a vessel segment (such as a section of the aortic wall), or by assessing a larger section of the vasculature (such as the entire aorta, assessed by PWV). The limitation of assessing a simple vessel segment, as is the

case with the stiffness index, is that it does not accurately reflect the entirety of the central vasculature. For example, the increased elasticity in the thoracic aorta compared to the abdominal aorta (Wolinsky et al., 1969) could disturb the accuracy of these metrics for the assessment of arterial stiffness. Furthermore, many investigations do not seem to address the potential effect of blood pressure levels on PWV (McEniery et al., 2003; Vuurmans et al., 2003; Wallace et al., 2007; Mahmud and Feely, 2008; Fok et al., 2012). This is a major limitation in the current literature, since PWV has been reported to be dependent on blood pressure levels; lower blood pressures are associated with lower PWV, and higher blood pressures are associated with higher PWV (Tan et al., 2012). Correcting for blood pressure (e.g. by measuring PWV at isobaric blood pressures) is of great importance to avoid confounding the results, especially when assessing the effect of blood pressure lowering drugs on PWV.

1.3.2 Pathophysiological changes in arterial stiffness

The mechanical and hemodynamic properties of blood vessels are fundamental factors influencing vascular compliance. For example, McEniery and colleagues (2005) have observed a positive correlation between increased aortic stiffness and isolated systolic hypertension (ISH). Hypertension and acute vascular distention are among the main factors that can contribute to an increase in arterial stiffness. Acute distention of blood vessels (e.g. by elevated blood pressure) leads to increased stiffness and PWV. Any treatment that could ultimately reduce blood pressure, such as the administration of sodium nitroprusside (SNP; a NO donor/vasodilator), could alter stiffness and PWV due to simple blood pressure alteration. Therefore, measurements of stiffness and PWV must

be controlled for blood pressure, especially when comparing stiffness in hypertensive and normotensive individuals.

The architecture of blood vessels makes important contributions to their mechanical properties within the circulatory system, to the delivery of blood to organs and tissues in the body, and is intimately tied to the elasticity of the vessels involved (Milnor, 1989). According to the current literature, the most important pathophysiological alterations that occur in arterial stiffness affect the tunica media, the ECM and the endothelium (Zieman et al., 2005).

Similarities between atherosclerosis and arterial stiffness has led to the idea that atherosclerosis may contribute to reduced arterial compliance. Atherosclerosis can be defined as a cardiovascular disease in which there is accumulation of fatty deposits on arterial walls, which can lead to blockage of blood flow. For example, atherosclerosis has similar mechanisms to arterial stiffness that alter ECM proteins, such as elastin fracture and collagen accumulation. This ultimately leads to an alteration of the mechanical properties of arteries (Palombo and Kozakova, 2016).

The tunica media of blood vessels is composed of VSMCs circumferentially arranged around the lumen (Rhodes and Simons, 2007; Todd et al., 1983). Larger arteries such as the aorta have a thicker layer of VSMCs than smaller arteries (Marieb and Hoehn, 2013). Previous work has focused on the involvement of the endothelium and the ECM in vascular stiffness (Dao et al., 2005; Wang et al., 2003). The role of VSMCs in arterial stiffness is a topic that is increasingly investigated. For example, Sehgel and colleagues (2013) confirmed increased stiffness in aortic VSMCs of SH rats compared to WKY rats. This was achieved by atomic force microscopy, which consists of applying pressure to a

11

surface (in this case, VSMCs) and measuring the resistance to stress, also known as the elastic modulus. Reports from other investigations have indicated increased vascular wall thickness in SH rats compared to WKY rats in the thoracic aorta (Marque et al., 1999; Van Gorp et al., 1995). In hypertension, increased VSMC mass as well as an increase in DNA content in VSMCs (polyploidy) has also been identified in the aorta (Werstiuk and Lee, 2000). Brandts and colleagues (2013) investigated the relationship between aortic wall thickness (measured with magnetic resonance imaging) and central arterial stiffness (assessed by PWV) in hypertensive and normotensive patients. Aortic wall thickness was found to be significantly higher in hypertensive compared to normotensive patients, and aortic PWV had a strong positive correlation with aortic wall thickness. Thus, the increased arterial wall thickness observed in hypertensive subjects has been thought to contribute to arterial stiffness in hypertension. The mechanism of involvement of VSMCs in the alteration of arterial stiffness is not well understood. However, the involvement of the ECM in vascular compliance has been well established (Toda et al., 1980; Basalyga et al., 2004; Dao et al., 2005).

The ECM of the tunica media is largely composed of elastin fibers, which provide the elastic properties of the blood vessel (Shekhonin et al., 1987). The ECM of the tunica externa, on the other hand, contains mostly collagen (type I and III) (Shekhonin et al., 1987) as well as nerve fibers (Marieb and Hoehn, 2013). In normal physiological conditions, elastin is most involved in the elasticity of the arterial wall (Lehmann, 1999), and is also the protein with the highest content in large arteries, such as the aorta (Jacob, 2003). Collagen fibers, on the other hand, are mostly located in the tunica externa

(Marieb and Hoehn, 2013), contribute to the viscoelastic behavior of the vascular wall, and maintain the integrity of the arterial wall (Lehmann, 1999).

In hypertension, arteries are distended due to increased intra-arterial pressures. The elastin fibers become acutely distended, and the pressure is transferred to rigid collagen fibers. Therefore, arterial stiffness in hypertension is increased partly due to the shift in pressure load from the distended elastic fibers to the rigid collagen fibers (Nichols and O'Rourke, 2005). Increasing age is another factor that can aggravate arterial stiffness (McEniery et al., 2005). With increasing age, the concentration of elastin relative to other arterial wall components decreases and is accompanied by an increase in collagen content. In addition, age-related arterial wall changes, such as the gradual fragmentation of elastin, may also contribute to decreased compliance (Toda et al., 1980). Degradation of elastin fibers can occur as a result of matrix metalloproteinase (MMP) activity such as MMP-2, which can lead to degradation of ECM proteins. Wang and colleagues (2003) have observed age-related increases in MMP-2 activity, and have located MMP-2 in the aorta near disintegrated elastin fibers. In addition, the binding of calcium ions to elastin fibers calcifies the arterial wall and leads to elastin fragmentation in the media, which increases arterial stiffness (Basalyga et al., 2004). Rodrigues and colleagues (2010) have also shown that MMPs can cleave β_2-AR in SH rats, which could minimize the function of β_2-AR agonists in SH rats. In addition, this may account for the increased vascular tone in SH compared to WKY rats (Fitzpatrick and Szentivanyi, 1980). Calcium levels in arterial walls progressively rise with increasing age, accelerating the process of arterial stiffening (Reaven and Sacks, 2004). However, the most common hypothesis for the degradation of elastin with increasing age is that chronic exposure to systolic pressure

within the vascular wall exacerbates the fragmentation of elastin over time (Dao et al., 2005).

The role of the endothelium in the alteration of arterial stiffness has been studied extensively. The involvement of endothelial cells in altering arterial stiffness has been explained by studies investigating the relationships between endothelial dysfunction and PWV, as well as the involvement of NO, an endothelium-derived vasodilator, and endothelin-1 (ET-1), a potent vasoconstrictor released by the endothelium.

Endothelial dysfunction and arterial stiffness can appear prior to hypertension. In fact, vascular stiffness has been suggested to initiate endothelial cell "contraction", which contributes to increased endothelial stiffness (Huveneers et al., 2015). Wallace and colleagues (2007) measured PWV (not corrected for blood pressure) in patients with ISH, and determined endothelial function by flow-mediated dilation. They reported higher PWV and lower flow-mediated dilation in ISH patients compared to controls. Flow-mediated dilation is the ability of large arteries to adjust to low blood flow by increasing vascular tone, which is largely mediated by NO release (Joannides et al., 1995). Endothelium dysfunction occurs when the ability of the endothelium to alter vascular tone is impeded. This evidence suggests that there may be a relationship between increased aortic stiffness and endothelial dysfunction.

NO and ET-1 also contribute to the alteration in vascular stiffness in the central arteries (Wallace et al., 2007). In addition, Kinlay and colleagues (2001) investigated the effect of NO on arterial stiffness. Arterial stiffness was assessed using the Moens-Korteweg equation for PWV, while E_{inc} was calculated as 75% of the slope of the stress-strain curves ($E_{inc} = 0.75$ x stress/strain). The brachial arteries of seven patients were

14

infused with L-NMMA (nitric oxide synthase inhibitor), which initiated an increase in PWV. The administration of nitroglycerin (a NO donor), however, was associated with reduced PWV. This evidence suggests that NO release has an inverse relationship with arterial stiffness in humans. Stiffening of endothelial cells can also result in reduced NO production, leading to impairment of VSMC relaxation (Huveneers et al., 2015). The association between increased NO levels and the reduction of arterial stiffness has been mostly assessed by PWV, since other means of assessing arterial stiffness (such as the stiffness index and Moens-Korteweg's elastic modulus) are dependent on arterial diameter.

An effect of ET-1 on arterial stiffness has been confirmed by Marano and colleagues (1999), who reported an increase in arterial compliance in carotid arteries of both SH and WKY rats when incubated with ET-1 antagonists in vitro. In addition, Vuurmans and colleagues (2003) have observed higher PWV, central systolic blood pressure, as well as increased pulse pressure with elevated ET-1 levels in patients with renal failure and/or heart failure. Though PWV was not corrected for blood pressure, other investigations have found a relationship between ET-1 and arterial stiffness. It has been suggested that ET-1 contributes to increased arterial stiffness by inhibiting collagen degradation in hypertension (Ergul et al., 2006), which may be attributed to ET-1 mediated smooth muscle cell contraction. McEniery and colleagues (2003) investigated the local effects of ET-1 on PWV (not corrected for blood pressure) in anesthetized sheep. The infusion of ET-1 was accompanied by an increase in PWV, while the infusion of an ET-1 antagonist was accompanied by a reduction in PWV. Therefore, ET-1 does seem to have a direct effect on arterial stiffness at the local level.

1.4 Autonomic nervous system activity and control of arterial blood pressure

The autonomic nervous system is composed of the sympathetic nervous system (SNS) and the parasympathetic nervous system. The SNS innervates vascular smooth muscle, and is involved in the regulation of vasomotor tone and blood pressure, and can also affect heart rate. The parasympathetic nervous system is involved in the regulation of heart rate (Guyenet, 2006). These two systems work together as a component of a feedback mechanism termed the baroreflex, which mediates the short-term regulation of blood pressure.

1.4.1 Sympathetic nervous system

The SNS is often called the fight-or-flight system, and it mediates a number of unconscious cardiovascular responses, such as stress-induced increases in heart rate and blood pressure. The SNS is composed of preganglionic and postganglionic neurons. Preganglionic neurons originate from the thoracic or lumbar regions of the spinal cord (Marieb and Hoehn, 2013). The axons of preganglionic neurons in the SNS synapse with postganglionic neurons. Postganglionic axons, on the other hand, extend to the organ on which it has an effect (Marieb and Hoehn, 2013). They are predominantly adrenergic, which means that they mostly secrete noradrenaline (Guyton and Hall, 2011). Negative feedback mechanisms mediated by autonomic nerves and hormones can serve to regulate arterial pressure through sympathetic nerves (Guyenet, 2006) that innervate VSMCs. These sympathetic nerves release norepinephrine, which can stimulate adrenoceptors, although it is not the most important regulator of the renin-angiotensin-aldosterone system (RAAS) (Marieb and Hoehn, 2013). The SNS influences blood pressure by increasing heart rate, vascular tone, as well as the force of cardiac contraction, which

leads to an increase in stroke volume and cardiac output (Guyenet, 2006). Furthermore, the SNS can initiate venous constriction, which leads to an increase in venous return to the heart. Venous constriction can contribute to increasing stroke volume (Charkoudian and Rabbitts, 2009). SNS activity can also stimulate the RAAS, which also contributes to the regulation of blood pressure and blood volume. The RAAS regulates renin release, which subsequently controls angiotensin II and aldosterone levels. Aldosterone causes renal tubules to retain sodium and water, while angiotensin II causes vasoconstriction. Therefore, the RAAS contributes to maintaining normal blood pressures through the release or inhibition of renin (Marieb and Hoehn, 2013).

1.4.2 Baroreflex

The baroreceptor reflex also contributes to the rapid control of blood pressure, which is mediated by parasympathetic and sympathetic nerves. This reflex relies on baroreceptors located in the carotid sinuses and in the aortic arch, which are activated by increases in blood pressure. However, if blood pressure decreased suddenly, baroreceptors are also capable of decreasing their activity in proportion to the fall in blood pressure. With a fall in blood pressure, there is a decrease in the frequency of baroreceptor impulses sent to the medulla of the brain, leading to increased activity of the vasomotor center. The frequency of action potentials directed to the heart and blood vessels by the SNS from the vasomotor center would increase in this case, leading to an increase in heart rate and vasoconstriction of the peripheral vasculature, which results in an increase in blood pressure. On the other hand, an increase in arterial blood pressure stretches the mechanoreceptors in the arterial wall, inhibiting the vasomotor center, which leads to a reduction in heart rate and vascular tone. This allows the blood pressure to

lower, and ultimately return towards normal (Guyton and Hall, 2011).

1.4.3 Adrenoceptors

Adrenoceptors are G protein-coupled receptors (Werstiuk and Lee, 2000) located on the cell membranes of sympathetic nerve endings, endothelial cells and/or VSMCs (Bulbring and Tomita 1987). They were first discovered in cardiac muscle by Ahlquist, (1948) and were subsequently classified in one of two groups, the alpha-adrenoceptors (α-AR) or beta-adrenoceptors (β-AR). These adrenoceptors were later discovered to have subtypes of their own; among the α-AR subtype, are the α_1- and α_2-AR. In the cardiovascular system, they are mostly located in VSMC membranes and their nerve terminals (Bulbring and Tomita 1987). Their activation leads to smooth muscle contraction, and inhibition of norepinephrine release from adrenergic terminals (Marieb and Hoehn, 2013). There are three β-AR subtypes (β_1-, β_2-, and β_3-AR), which are involved in accelerating heart rate (chronotropism; β_1-AR), increasing the strength of contraction of the heart (inotropism; β_1-AR), as well as vasodilation (β_2-, and β_3-AR) (Cohen et al., 1999; Ablad et al., 1974; Dessy et al., 2004).

1.4.4 Beta-adrenoceptors

Studies using selective agonists and antagonists allowed the sub-classification of beta-adrenoceptors into β_1- and β_2-AR (Lands et al., 1967). β_3-ARs, on the other hand, were initially identified in adipocytes, (Emorine et al., 1989) and subsequently in cardiac tissue (Cohen et al., 1999). Another β-AR, which was first termed putative β_4-AR, was discovered in 1989 (Kaumann, 1989). However, this receptor is now termed low-affinity β_1-AR, and is possibly a distinctive conformational state of the typical β_1-AR (Granneman, 2001). β_1- and β_2-AR are usually referred to as the typical β-ARs, and any

other receptors, such as β_3-AR and the low affinity β_1-AR (previously named the putative

β_4-AR) are referred to as atypical β-ARs (Shafiei and Mahmoudian, 1999).

The release of adrenaline from the adrenal medulla or noradrenaline from autonomic

nerves can stimulate β-AR activity (Dessy et al., 2004). β_1-ARs are usually found in heart

tissue, where their activation exerts positive inotropic and chronotropic effects (Ablad et

al., 1974). β_2-AR is the predominant adrenoceptor found in VSMCs (Brodde and Michel,

1992; Takata and Kato, 1996). However, β_1-AR and an atypical β-AR resembling the low

affinity β_1-AR have also been identified in VSMCs (Brawley et al., 2000). β_2-AR has

been identified throughout the cardiovascular system, such as in the rat thoracic aorta

(Brawley et al., 2000), carotid arteries (Chiba and Tsukuda, 2001), and heart tissue

(Morisco et al., 2001). In the vasculature, β_2-ARs are predominantly involved in

adrenergic-mediated peripheral vasodilation, although the activation of β_1-AR also

reduces vascular tone. Relaxation induced by isoprenaline (non-selective β_1- and β_2-AR

agonist) was not attenuated by NO release inhibition or 'disruption' of the endothelium,

indicating that the relaxation was not endothelial-dependent, and presumably had a direct

effect upon VSMCs (Chruscinski et al., 2001). β_3-ARs are located in the cell membranes

of cardiomyocytes (Cohen et al., 1999), adipose tissue (Ferrer-Lorente, 2005), and the

endothelium (Trochu et al., 1999). β_3-ARs are involved in contractile effects in the heart

(Cohen et al., 1999), and have been shown to mediate direct VSMC relaxation in the rat

abdominal aorta (Matsushita et al., 2006). β_3-AR stimulation with a selective agonist,

BRL37344, also caused relaxation in denuded internal mammary arteries (Shafiei and

Mahmoudian, 1999). The rat thoracic aorta also seems to relax in response to activation

of atypical receptors, such as β_4-AR (low affinity β_1-AR) (Brawley et al., 2000). β-ARs

can be found throughout the vascular system, and are intimately involved in the control of vascular tone and heart rate.

Structure and Pathway

β-ARs are G-coupled receptors composed of seven transmembrane helices of amino acids. Their glycosylation sites are at the amino terminus (Lefkowitz, 2007), where norepinephrine or epinephrine may bind (Marieb and Hoehn, 2013). β-agonists activate a stimulatory G protein (Gs), which stimulates adenylyl cyclase. Adenylyl cyclase converts ATP to cyclic adenosine monophosphate (cAMP). This increase in cAMP results in the activation of protein kinase A (PKA), which leads to phosphorylation of proteins. This results in a decrease in intracellular Ca^{2+} levels, leading to relaxation (Figure 2). (Werstiuk and Lee, 2000)

These receptors can be stimulated by selective or non-selective β-agonists. For example, selective beta-agonists such as terbutaline, a selective β_2-agonist, (Johansson, 1995) and BRL37344, a selective β_3-agonist (Candelore et al., 1999), target specific beta-adrenoceptors. Others, such as isoprenaline, a non-selective β_1- and β_2-agonist, (Gao et al., 2010) are recognized as non-selective since they target more than one subtype of β-AR.

1.4.5 Beta-adrenoceptors and hypertension

In essential hypertension, SNS activity is increased, and baroreflex mechanisms seem to be attenuated (Lucini et al., 1994). In addition, impaired β-AR mediated relaxation has also been identified in the thoracic aorta of SH rats (Cheng and Shibata, 1981). Ageing has also been linked to a decrease in β-AR–Gs–adenylyl cyclase coupling, downregulation of β-AR, modifications in G-protein levels (Werstiuk and Lee, 2000),

and lower β-AR activity (Fleisch et al., 1970). In addition, Bray and colleagues (2000) reported a significant relationship between blood pressure and polymorphisms in the β_2-AR gene. For example, they reported increased frequency of Gly16 and Glu27 alleles in the β_2-AR gene of hypertensive compared to normotensive subjects. This evidence supports the idea that β_2-AR may have an important role in hypertension. Mallem and colleagues (2004) have identified an up-regulation of β_3-ARs in the endothelial layer of the aorta of SH rats by immunohistochemistry, which did not accompany an increase in β_3-AR vasodilatory response. The current literature suggests that β-AR expression and activity is modified in pathophysiological conditions such as hypertension. It has been suggested that β-AR blockade is associated with an impairment of relaxations of the conduit blood vessels and this could significantly contribute to increased vascular stiffness (Klapholz, 2009; Dudenbostel and Glasser, 2012). However, the role of β-AR in the alteration of arterial stiffness is still not established.
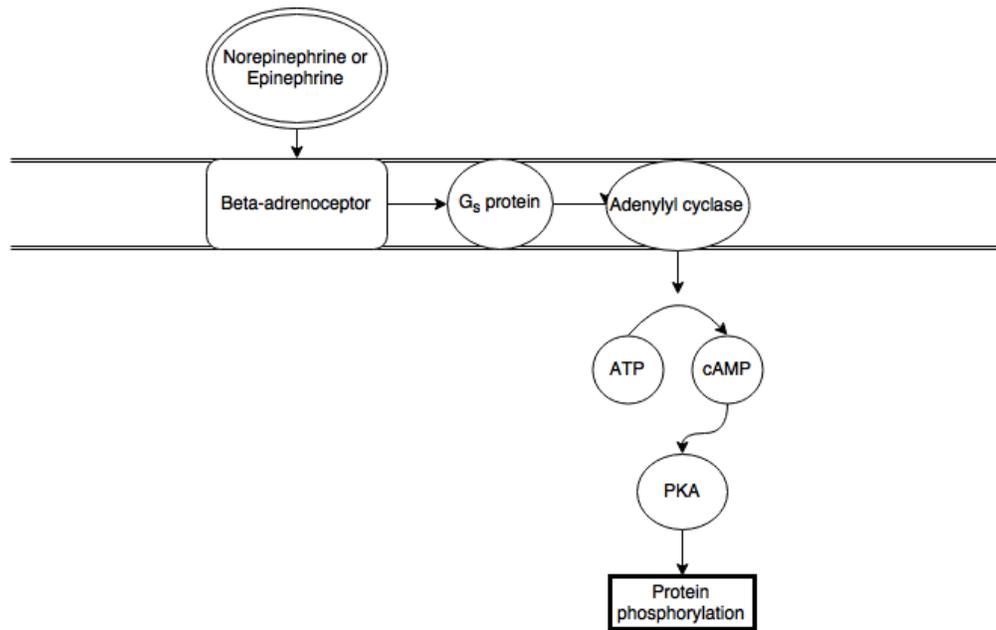
Figure 2. Typical beta-adrenoceptor pathway. Norepinephrine or epinephrine binds to the β-adrenoceptor, activate Gs, which activates adenylyl cyclase. Adenylyl cyclase then converts ATP to cAMP, which will activate a protein kinase (PKA), which leads to the phosphorylation of proteins. Adapted from (Santulli and Iaccarino, 2013), used under Creative Commons Attribution License.

1.4.6 Beta-adrenoceptor blockade and hypertension

β-blockers are a class of drugs used for lowering high blood pressure. These competitive antagonists essentially prevent norepinephrine and noradrenaline from stimulating β-ARs (Haeusler, 1990; Girard et al., 1995). They may contribute to lowering blood pressure by decreasing heart rate, which can lead to a decrease in cardiac output. In addition, treatment with β-blockers inhibits β_1-AR-mediated renin secretion, which decreases angiotensin II and aldosterone levels (Laragh, 2001). With decreased levels of aldosterone, renal tubules cannot retain as much sodium and water, which could ultimately lead to lowering of blood volume and blood pressures.

The first generation of β-blockers includes non-selective drugs such as propranolol. The second generation of β-blockers is β_1-selective, also known as cardioselective, since their primary action is reduction in heart rate. Finally, the third generation of β-blockers is those who have additional vasodilatory effects in addition to simple beta-blockade, such as nebivolol (Pederson and Cockroft, 2006). Although non-selective β-blocker hypertension treatments also block β_2-AR, the vasoconstriction is minimal due to these additional therapeutic effects. Propranolol was among the first β-blockers, and was used for the treatment of angina (Quirke, 2006). Angina is a cardiovascular disorder associated with chest pain, often caused by insufficient blood flow to the heart due to coronary artery spasms or blockage. Scientists later modified the structure of various beta-blockers to add further cardiovascular benefits including β_1-AR selectivity, intrinsic sympathomimetic activity (partial agonists), α-AR blockade, and vasodilation (NO donors) (Frishman, 1981).

Some β-blockers selectively target β_1-ARs, such as metoprolol and atenolol (Frishman, 1984). According to Del Colle and colleagues (2007), both β_1- selective and non-selective β-blockers have a similar effect in a state of hypertension. β-blockers usually lower blood pressure by lowering heart rate by blocking the activity of β_1-ARs, however other β-blockers have also been developed with secondary mechanisms to reduce blood pressure (Frishman, 1984). For example, some β-blockers have intrinsic sympathomimetic activity; they can act as β-blockers, and act as competitive antagonists of norepinephrine and epinephrine to oppose the effects of catecholamines (Jaillon et al., 1990). For example, partial agonists like pindolol also stimulate β-receptors, but are only partially effective in comparison to typical agonists (Frishman, 1984). These β-blockers cause vasodilation and a reduction in heart rate, which decreases peripheral vascular resistance and blood pressure (in small doses). Other β-blockers also block α-AR and/or β-AR, such as labetalol and carvediol (Frishman, 1984). These α-blocking properties cause vasodilation, which reduces peripheral vascular resistance. Other β-blockers are NO donors, such as nebivolol, and stimulate NO production through activation of endothelial nitric oxide synthase (eNOS), thus increasing vasodilation (Maffei et al., 2006).

1.5 Rationale

Currently, there is very little known of the nature and role of the adrenergic-mediated responses as a contributor to arterial stiffness. The effects of β-AR stimulation on arterial stiffness have received little attention. Therefore, this project was aimed at systemically determining how activating β-AR affects vascular stiffness in a state of high compared to normal intravascular arterial pressure.

The SNS plays a pivotal role in the control of vascular function through the activation of adrenoceptors (Guyenet, 2006). Accordingly, beta-adrenergic blockers are commonly and widely used in the treatment of a host of cardiovascular disorders. It has been suggested that these drugs do not lower central aortic systolic and pulse pressure but seem to only lower arterial blood pressure at the distal circulation (Klapholz, 2009; Williams et al., 2006). Thus, it has been implied that the lack of vascular relaxation may be responsible for increased arterial stiffness (Dudenbostel and Glasser, 2012). Essentially, the use of β-blockers has been suggested to be associated with an impediment in relaxation of the conduit blood vessels and may significantly contribute to increased vascular stiffness, consequently increasing the risk of morbidity and mortality. Nevertheless, little is known of the effects of β-AR on vascular stiffness, which will be the main topic addressed in this thesis.

In clinical practice, determination of PWV has been used in the assessment of arterial stiffness, and is considered the "gold standard" for evaluating vascular elasticity (Laurent et al., 2006). The measure of aortic stiffness is pressure-dependent due to the structural properties of the arterial wall (Shadwick, 1999). Therefore, we chose to use PWV as our measure of vascular stiffness, and made our measurements of PWV over a similar range of pressures (85-95 mmHg with venous occlusion) for consistency throughout all groups. We used the foot-to-foot approach to measure pulse transit time, since this approach has been suggested to be valid for assessing pulse transit time in rodents (Mitchell et al., 1997). To obtain adequate transit time for our measurements, we measured the pulse transit time from the aortic arch to the bifurcation of the femoral arteries. Due to the greater elasticity of the thoracic aorta in comparison to the abdominal aorta, PWV

measured in this investigation is mostly from the thoracic aorta, which produces a slower PWV than that of the abdominal aorta.

In the current investigation, the SH rat was used as a model of hypertension due to the similar pathophysiology in humans (Pinterova et al., 2011), and WKY were used as normotensive controls for comparison with SH rats. β-AR agonists were infused in SH and WKY rats to determine the effect of β-AR stimulation on PWV (isoprenaline, β_1- and β_2-AR; terbutaline, β_2-AR; BRL37344, β_3-AR). Isoprenaline is amongst the most potent β-AR agonists (Apperley et al., 1976). Terbutaline and BRL37344 were used in this study due to their selectivity for β_2- and β_3-AR, respectively (O'Donnell and Wanstall, 1976; Vulliemoz et al., 1975; Lofdahl et al., 1983; Trochu et al., 1999; Brawley, 2000). Beta-blockers such as NO donors or those with sympathomimetic activity have a multitude of effects in addition to beta-blockade. We therefore decided to use beta-agonists to better understand how beta-adrenoceptor activity influences arterial stiffness, without needing to address the confounding effects that come with the use of beta-blockers. Further studies using beta-blockers will be useful in assessing the therapeutic value of beta-blocker therapy in high systemic arterial pressure due to increased vascular stiffness. In addition, sodium nitroprusside (vasodilator) was infused in SH and WKY rats to determine the effect of potent and non-selective relaxation of VSMC on PWV. Furthermore, there is currently a lack of literature on β-AR expression and activity in the abdominal aorta. Thus, we decided to determine the expression of the different subtypes of β-AR using immunofluorescence analysis.

1.6 Objectives

The objectives of this investigation were to define the role of different subtypes of beta-adrenoceptors in the control of arterial stiffness, as assessed by PWV. The present investigation will:

1. Investigate the effects of sub-types of β-AR (β_1, β_2 and/or β_3) on PWV, using β-AR agonists (isoprenaline, terbutaline, BRL37344).

2. Determine whether a state of high intravascular pressure alters the contributions of β-AR in reducing PWV in SH rats.

3. Determine the expression of β-AR in the abdominal aorta.

4. Determine whether vasodilation (with SNP), independent from β-AR stimulation, reduces PWV in a state of hypertension and normotension.

1.7 Hypothesis

Two main hypotheses were addressed in the current investigation. First, the activation of β-AR leads to a reduction in PWV, an index of arterial stiffness. Secondly, the reduction in arterial stiffness is due to relaxation of the large central arteries such as the aorta, as opposed to a reduction in blood pressure alone.

2. Materials and Methods

All procedures on animals were carried out in accordance with the guidelines of the Canadian Council on Animal Care, with the approval of the Institutional Animal Care Committee of Memorial University of Newfoundland. All animals were kept in a temperature-controlled environment ($22 \pm 2°C$) on a 12h-12h light-dark cycle, and had access to normal tap water and standard chow ad libitum. Animals were purchased from Charles River Laboratories International, Inc. (USA).

2.1 Surgical preparation of animals

WKY and SH rats were anesthetized (induction 5% isoflurane in 100% oxygen, maintenance 1.5-1.75% in 100% oxygen), and were injected with buprenorphine (0.01 mg/kg s.c.). Core body temperature was maintained at $37 \pm 1°C$ using a heating lamp and monitored with a rectal thermometer. The left femoral and carotid arteries were isolated and catheterized (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm connected to I.D. 0.28 mm, O.D. 0.61 mm). The catheters were advanced approximately 2 cm. The left femoral vein was also isolated and catheterized (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm). All catheters were filled with heparinized normal saline (25 iu/ml). Animals were stabilized for 45-60 min, and continuous central (aortic arch) and peripheral (left femoral artery) blood pressure, as well as heart rate was recorded by AcqKnowledge (3.9.1.6) software (Biopac Systems Canada Inc.) with a pressure transducer (P23XL; Spectramed Statham). The signals were then amplified (DA 100A; Biopac Systems Inc.), where the amplifier was connected to a universal interface module (UIM 100; Biopac Systems Inc.), and to an acquisition unit (MP100; Biopac Systems Inc.). The analog

output signal was then converted to a digital signal (USB1W; Biopac Systems Inc.), and displayed in AcqKnowledge (3.9.1.6).

2.2 Experimental protocol

Male WKY and SH rats of 13-14 weeks of age were assigned to one of the following five treatment subgroups: normal saline (time control), sodium nitroprusside (vasodilator), isoprenaline (β_1- and β_2-AR agonist), terbutaline (β_2-AR agonist), and BRL37344 (β_3-AR agonist).

After the stabilization period, baseline blood pressure and heart rate were recorded for 20 min before three cumulative doses (isoprenaline: 0.001, 0.003, & 0.01 μg/kg/min; terbutaline, 1, 3, & 10 μg/kg/min; BRL37344, 1, 3, & 10 μg/kg/min; and sodium nitroprusside, 1, 3, & 10 μg/kg/min) were continuously infused for 20 min. A pilot study was conducted to identify drug doses that would ensure adequate reduction in blood pressure with terbutaline, BRL37344 and SNP. The pilot study was also used to determine doses of isoprenaline that could modestly decrease blood pressure, while avoiding an increase in heart rate (which could affect PWV independently from β-AR stimulation). Equivalent volumes of normal saline were infused for parallel time-control groups (infusion rates: 0.002, 0.006, & 0.02 ml/min). Central aortic and peripheral blood pressures as well as heart rate were continuously monitored throughout the experiments.

Following the completion of each experiment, animals were sacrificed by anesthetic overdose, KCl injection and thoracotomy. A piece of silk suture was then placed between the tips of the carotid and femoral catheters (post-mortem), to measure the distance (d) (in mm) between the two points where blood pressure was captured. A section of the abdominal aorta below the kidney and above the bifurcation of femoral arteries of four

saline-treated animals (WKY, n=4; SH, n=4) was excised and placed in 10% neutral buffered formalin (pH=7.4). Each blood vessel segment was embedded in paraffin for immunofluorescence investigations. The heart of each animal was also excised, and the right ventricle and left ventricle plus septum were separated and weighed.

2.3 Immunofluorescence

Immunofluorescence was used to identify β_1-, β_2-, and β_3-adrenoceptors in the rat abdominal aorta. Sections from saline groups (WKY: n = 4, SH: n = 4) were stained and imaged for immunofluorescence analysis. Immunofluorescence protocols were similar to those of Daneshtalab and colleagues (2010). Dilutions of antibodies were adjusted to ensure selective staining of tissues.

Paraffin embedded tissues were cut in 6 μm sections, deparaffinized in xylene, and rehydrated with decreasing concentrations of ethanol. 3% Hydrogen peroxide was then added to the tissues for 15 min to block endogenous peroxidase. Heated citrate buffer (pH 6.0) [1.92 g/l] (10 min) was used as antigen retrieval for β_1-AR sections, and 1% sodium dodecyl sulfate (SDS) (3 min) was used as antigen retrieval for β_2- and β_3-AR sections. All samples were placed in a humidifier with 10% normal goat serum (NGS) with 0.1% triton x in phosphate-buffered saline (PBS) to block for 1h-2h. Sections were then incubated overnight at 4 °C with primary rabbit polyclonal β_1-, β_2-, or β_3-AR antibodies (β_1-AR; 1:200, β_2-AR: 1:150, β_3-AR: 1:50) in 2% NGS in PBS. Each section was washed in phosphate-buffered saline (PBS) after every step for 3-5 min on a shaker.

The following day, tissues were washed with tris-buffered saline (TBS) four times for 10 min on a shaker. Secondary antibodies were prepared in the dark, then applied to the sections in a humidifier and left for 30 min. Secondary antibodies consisted of Cy5

goat anti-rabbit IgG (β_1- and β_2-AR; 1:500, β_3-AR; 1:600) and 4' 6-diamidino-2-

phenylindole (DAPI; 1:1000) in 2% NGS in TBS. Slides were once again washed with

TBS four times for 10 min on a shaker, and covered with 50% glycerol in PBS to be

cover-slipped and sealed with clear nail polish.

Sections from the abdominal aorta of SH and WKY rats (n=4) were imaged with

confocal microscopy at 20x optical magnification and 1.9x digital magnification, and

analyzed with Fluoview software (Olympus Fluoview, FV100). Laser parameters

(intensity, offset and transmitted light) were the same within each staining group (β_1-, β_2-

and β_3-AR).

Images were then analyzed with ImageJ software (National Institutes of Health,

ImageJ1.40). First, the images were calibrated and gray scaled. Afterwards, background

integrated density and mean integrated density were measured. The corrected mean

density value (CMDV) was used, where CMDV=MDV – MBDV; MDV is mean density

value, and MBDV is mean background density value. The mean of three background

value measurements was used for MBDV. The mean of CMDV in WKY (n=4) and in SH

(n=4) was used for comparison of β-AR density between groups.

2.4 Data measurements and calculations:

Arterial stiffness was determined by measuring PWV (Figure 3). Since PWV is

pressure-dependent and needs to be measured in a pressure-normalized state, the

following method was used to calculate PWV: prior to drug infusions, pressure was

applied to the inferior vena cava (by abdominal compression) to temporarily reduce

venous return, with the objective of capturing a diastolic pressure of 85-95 mmHg for

PWV measurements in all animals.

A
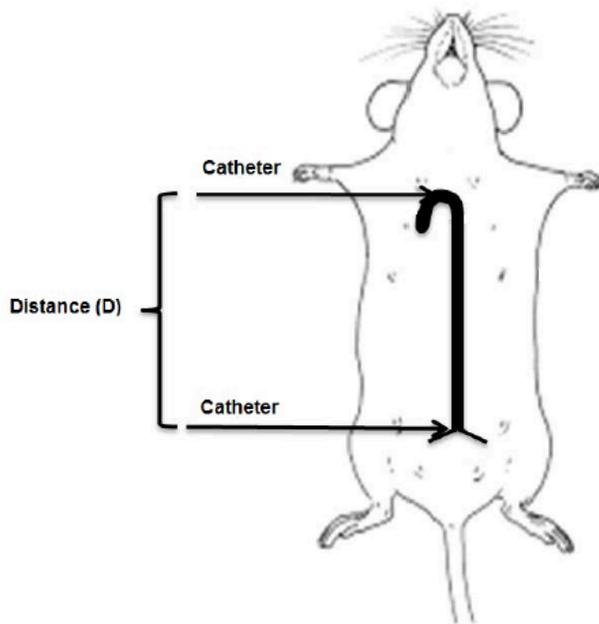
B
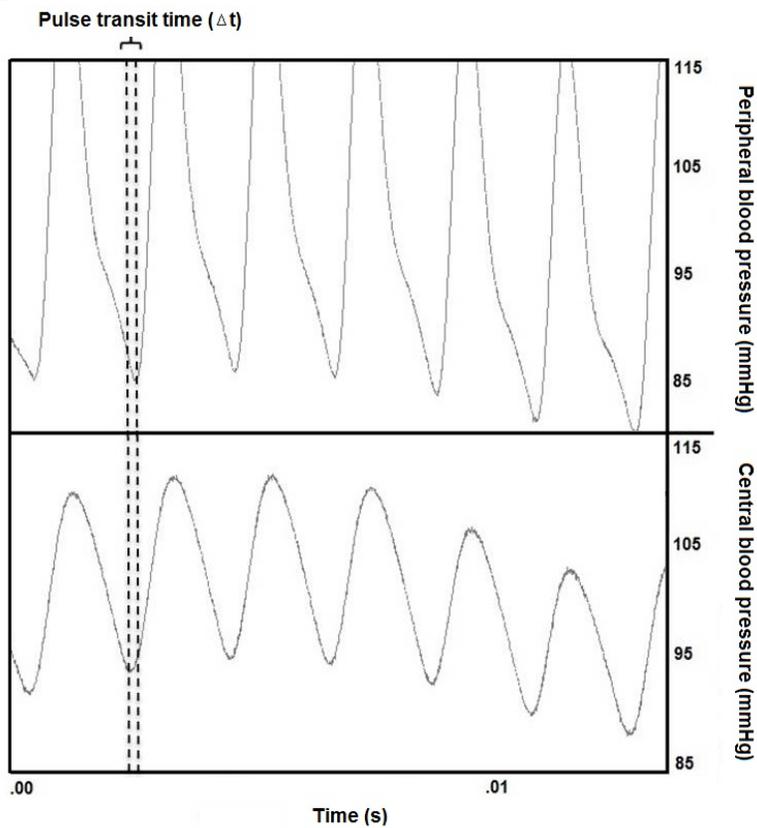
 Figure 3. Pulse wave velocity (PWV) was calculated by dividing the distance between two pressure points (**A**) by the pulse transit time (**B**). Pulse transit time is the time it takes for the pulse to travel along the vasculature.

Pressure was then released, and blood pressure was allowed to stabilize (Butlin et al., 2015). PWV was measured at baseline (post blood-flow occlusion) and 10-20 min after the start of each dose. PWV was calculated by dividing the distance (d) between the tip of the carotid artery catheter and the tip of the femoral artery catheter (measured prior to the excision of the abdominal aorta) by pulse transit time (Δt), the time it takes for the pulse to travel this distance;

$$PWV = d/\Delta t$$

2.5 Statistical analyses

Baseline values were compared with two-way analysis of variance (ANOVA) to determine if there were differences between group means. Drug treatment values were compared to saline time-control values with one-way ANOVA (Tukey test as needed) to determine significance between means. Hemodynamic values in all drug infusion groups were compared to their respective saline-infused time-control. ANOVA was used to compare the CMGV of the different subtypes of β-AR. A $p<0.05$ was the criterion for statistical significance for all comparisons. All statistical analyses were done using Sigma Plot 12.2.0.45. In total, 132 experiments have been conducted, 69 of which were included in the final analysis. After extraction of the heart, 11 WKY rats were excluded from the final analysis due to heart defects such as three ventricles. 3 SH and 23 WKY rats were also excluded due to abnormal resting heart rate and/or blood pressure at baseline. The remaining 26 experiments were excluded due to missing data, experimental or measurement errors.

2.6 Chemicals

All drugs were dissolved in 0.9% saline. Isoflurane was purchased from CDMV (Halifax, Canada), buprenorphine from Schering Plough Ltd (UK), heparin from SoloPak Laboratories Inc. (USA), isoprenaline from Winthrop Laboratories (USA), terbutaline and SNP from Sigma Aldrich (USA), and BRL37344 from Tocris Bioscience (UK). β_1-AR antibodies were purchased from Abcam Inc. (Toronto, ON), β_2- and β_3-AR antibodies from Santa Cruz (USA), Cy5 goat anti-rabbit IgG and normal goat serum from Jackson ImmunoResearch Laboratories Inc. (USA), DAPI, 10x PBS, tris base and triton X-100 from Fisher Scientific Inc. (USA), and citric acid from Sigma Aldrich (USA).

3. Results

There were no significant differences in body weight between SH and WKY rats
(282 ± 4g vs. 290 ± 4g). The ratio of the left ventricle plus septum to right ventricle of SH
rats (4.0 ± 0.1) was statistically greater than that in WKY (3.3 ± 0.1), which is an
indication of left ventricular hypertrophy likely due to systemic hypertension.

3.1 Baseline hemodynamics

The baseline hemodynamic values are shown in Table 2. SH animals had
significantly higher systolic and diastolic (central and peripheral) blood pressures than
WKY rats. Also, the mean central and peripheral blood pressures of SH rats (132 ± 2
mmHg & 125 ± 2 mmHg) were significantly higher than those of WKY (90 ± 2 mmHg &
83 ± 2 mmHg) rats, respectively. Pulse pressures (central & peripheral) were significantly
greater in SH compared to WKY rats. In addition, central pulse pressure was significantly
different from peripheral pulse pressure in both WKY and SH animals. To compare PWV
at isobaric blood pressures, 71% of measurements were captured at a diastolic pressure of
85-95 mmHg (the remaining measurements were captured within 15% of this range).
PWV was significantly higher in SH rats compared to WKY at baseline. There was no
significant difference in heart rate between WKY and SH rats (Table 2).

3.2 Sodium nitroprusside

Sodium nitroprusside (vasodilator; 1, 3 & 10 µg/kg/min) was infused in SH and
WKY rats to determine the effect of potent and non-selective relaxation of VSMCs on

Table 2. Baseline values for central systolic blood pressure (cSBP; mmHg), central diastolic blood pressure (cDBP; mmHg), peripheral systolic blood pressure (pSBP; mmHg), peripheral diastolic blood pressure (pDBP), central pulse pressure (cPP; mmHg), peripheral pulse pressure (pPP; mmHg), heart rate (HR; beats/minute) and pulse wave velocity (PWV; m/s; measured at central diastolic pressures between 85-95 mmHg post-blood flow occlusion) of Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats in saline, isoprenaline, terbutaline, BRL37344, and sodium nitroprusside (SNP) groups. Each value is mean ± SEM. n = 6-8.

		Saline	**Isoprenaline**	**Terbutaline**	**BRL37344**	**SNP**
WKY	cSBP	103 ± 4	103 ± 3	110 ± 5	113 ± 3	100 ± 4
	cDBP	79 ± 4	82 ± 3	84 ± 4	89 ± 3	75 ± 3
	pSBP	107 ± 6	110 ± 4	119 ± 5	119 ± 6	104 ± 7
	pDBP	64 ± 4	67 ± 3	74 ± 4	76 ± 4	64 ± 3
	cPP	24 ± 2	22 ± 1	25 ± 3	25 ± 3	25 ± 3
	pPP	44 ± 3^a	43 ± 2^a	45 ± 2^a	43 ± 3^a	40 ± 4^a
	HR	317 ± 9	324 ± 8	335 ± 12	338 ± 7	313 ± 10
	PWV	6.5 ± 0.4	7.1 ± 0.3	7.4 ± 0.2	6.3 ± 0.6	6.0 ± 0.7
SH	cSBP	149 ± 5^b	165 ± 7^b	153 ± 6^b	155 ± 3^b	147 ± 5^b
	cDBP	117 ± 5^b	128 ± 6^b	119 ± 5^b	122 ± 3^b	115 ± 3^b
	pSBP	158 ± 5^b	177 ± 8^b	162 ± 7^b	161 ± 3^b	154 ± 5^b
	pDBP	100 ± 4^b	115 ± 7^b	106 ± 5^b	109 ± 4^b	100 ± 3^b
	cPP	32 ± 2^b	37 ± 2^b	34 ± 3^b	33 ± 2^b	32 ± 3^b
	pPP	58 ± 2^{ab}	62 ± 2^{ab}	56 ± 4^{ab}	52 ± 4^{ab}	54 ± 3^{ab}
	HR	338 ± 9	329 ± 11	339 ± 9	338 ± 7	342 ± 15
	PWV	10.3 ± 1.1^b	8.7 ± 0.7^b	9.6 ± 0.9^b	9.1 ± 0.9^b	8.3 ± 0.7^b

[a]Significantly different from cPP within strain; $p < 0.05$
[b]Significantly different from respective value in WKY; $p < 0.05$

PWV. The infusion of SNP significantly decreased PWV at all doses in SH rats, but not at any dose in WKY when compared to saline-infused time-controls (Figure 4). SNP infusions significantly decreased both central and peripheral pulse pressures at the two highest doses in SH rats, but not at any dose in WKY (Figure 5 and Figure 6). Administration of SNP also significantly decreased central systolic and diastolic blood pressures in SH rats at the highest two doses, but not at any dose in WKY rats (Figure 7 and Figure 8). SNP infusions did not alter heart rate in WKY or SH rats (Figure 9).

3.3 Terbutaline

Terbutaline (selective β_2-AR agonist; 1, 3, & 10 µg/kg/min) was infused in SH and WKY rats to determine the effect of β_2-AR stimulation on PWV. The infusion of terbutaline significantly decreased PWV at the two lowest doses in SH rats, but not at any dose in WKY rats (Figure 4). Administration of terbutaline at all doses significantly decreased central systolic and diastolic blood pressures in SH rats, and at the highest dose in WKY rats (Figure 7 and Figure 8). The infusion of terbutaline significantly increased heart rate in SH rats at the highest dose, but not at any dose in WKY rats (Figure 9). Terbutaline infusions did not alter central or peripheral pulse pressure in WKY or SH rats (Figure 5 and Figure 6).

3.4 BRL37344

BRL37344 (selective β_3-AR agonist; 1, 3, & 10 µg/kg/min) was infused in SH and WKY rats to determine the effect of β_3-AR stimulation on PWV. The infusion of BRL37344 significantly increased heart rate in hypertensive animals at the highest dose, but not in WKY rats (Figure 9). BRL37344 significantly decreased central systolic and

Figure 4. Pulse wave velocity (PWV) during infusions of equivalent volumes of saline (n=6; 0.002, 0.006, 0.02 ml/min) and increasing doses of isoprenaline (n=7; 0.001, 0.003, 0.01 µg/kg/min), terbutaline (n=7), BRL37344 (WKY n=6; SHR n=8) and sodium nitroprusside (SNP; WKY n=7; SHR n=8) (1, 3, 10 µg/kg/min) for 20 minutes each in (**A**) Wistar-Kyoto and (**B**) spontaneously hypertensive rats at 13-14 weeks of age. B, baseline; D1, dose 1; D2, dose 2; D3, dose 3. Values are mean ± SEM.

[a]Significantly different from respective value in saline-infused time-control group; $p < 0.05$
[b]Significantly different from respective value in WKY rats; $p < 0.05$
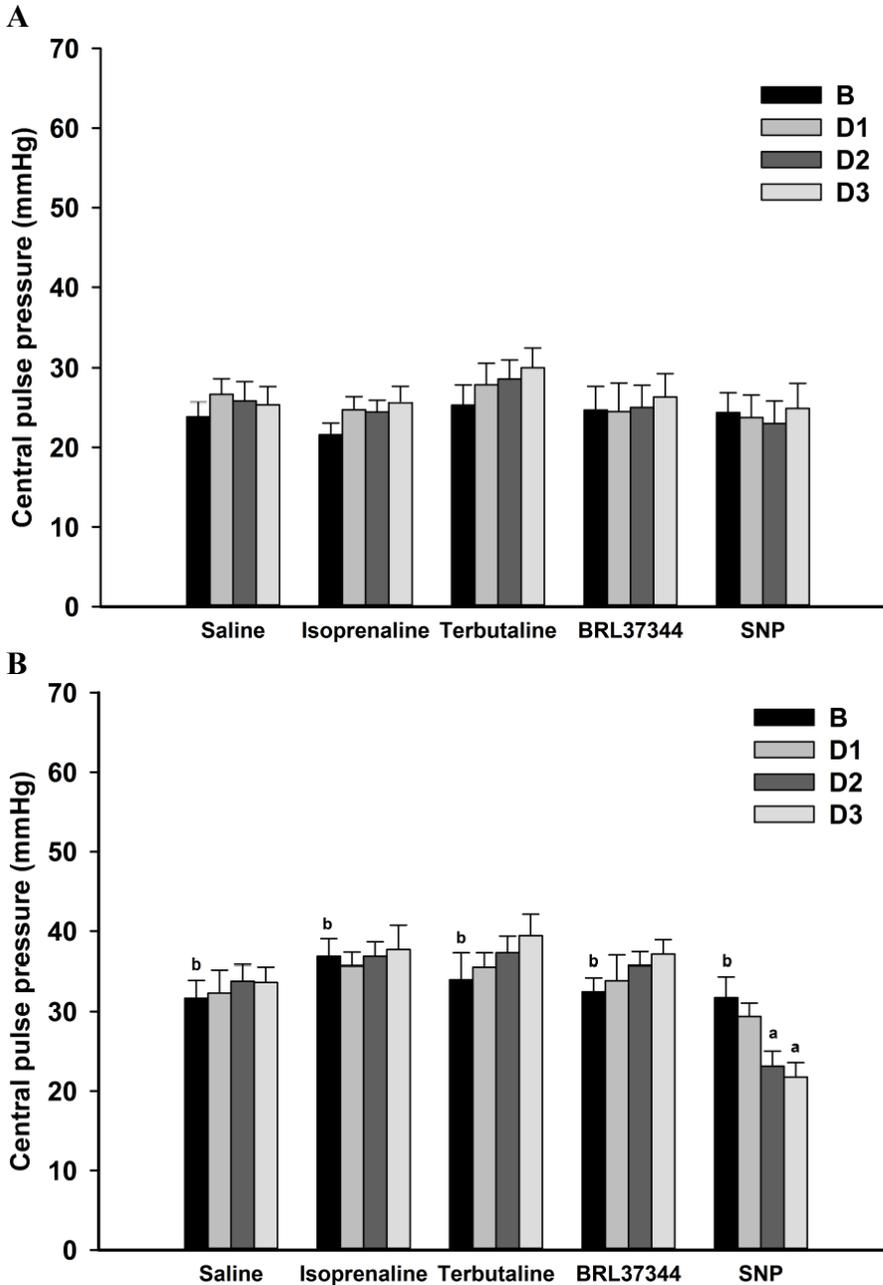
Figure 5. Central pulse pressure during infusions of equivalent volumes of saline (n=6; 0.002, 0.006, 0.02 ml/min) and increasing doses of isoprenaline (n=7; 0.001, 0.003, 0.01 μg/kg/min), terbutaline (n=7), BRL37344 (WKY n=6; SHR n=8) and sodium nitroprusside (SNP; WKY n=7; SHR n=8) (1, 3, 10 μg/kg/min) for 20 minutes each in (**A**) Wistar-Kyoto and (**B**) spontaneously hypertensive rats at 13-14 weeks of age. B, baseline; D1, dose 1; D2, dose 2; D3, dose 3. Values are mean ± SEM.

[a]Significantly different from respective value in saline-infused time-control group; $p < 0.05$
[b]Significantly different from respective value in WKY rats; $p < 0.05$
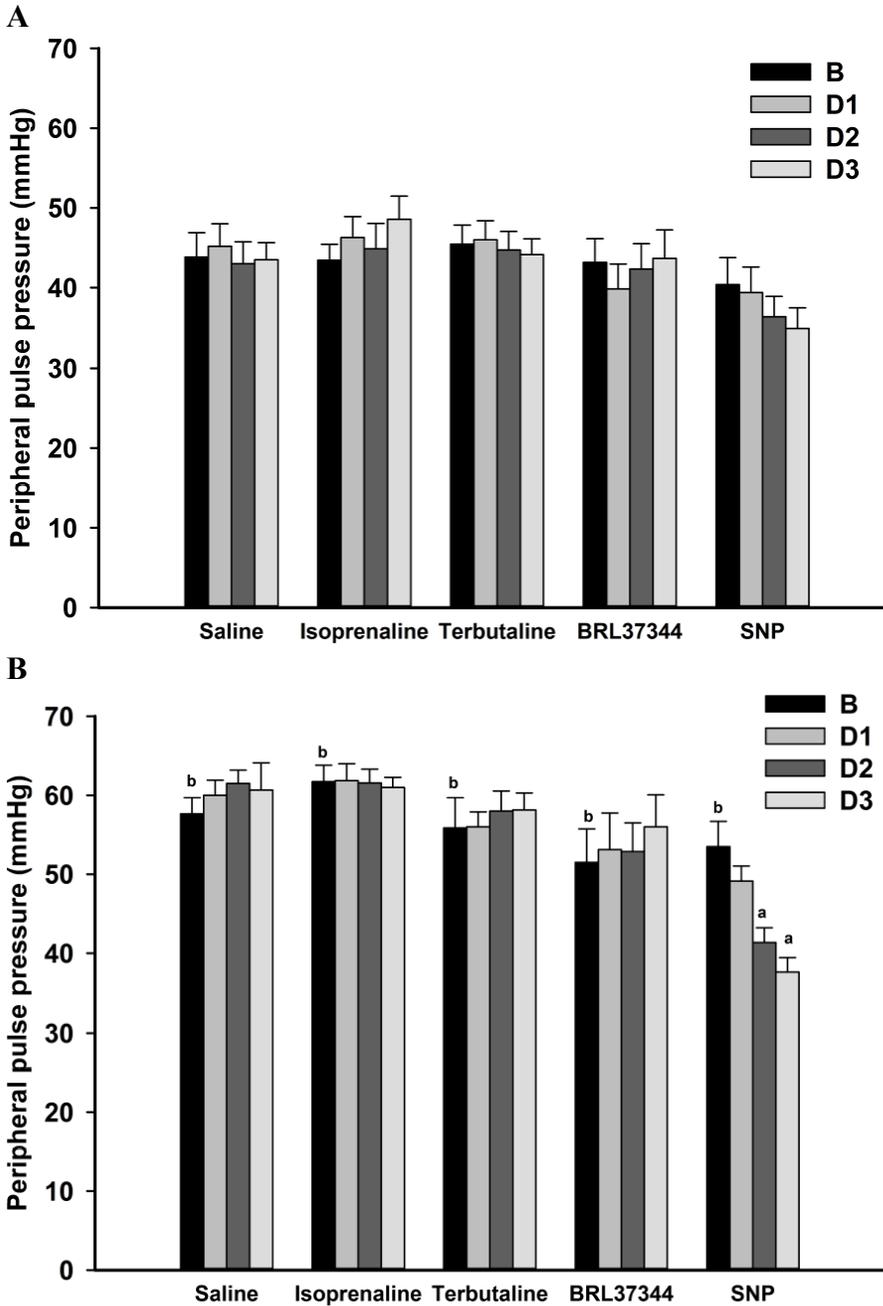
Figure 6. Peripheral pulse pressure during infusions of equivalent volumes of saline (n=6; 0.002, 0.006, 0.02 ml/min) and increasing doses of isoprenaline (n=7; 0.001, 0.003, 0.01 μg/kg/min), terbutaline (n=7), BRL37344 (WKY n=6; SHR n=8) and sodium nitroprusside (SNP; WKY n=7; SHR n=8) (1, 3, 10 μg/kg/min) for 20 minutes each in (**A**) Wistar-Kyoto and (**B**) spontaneously hypertensive rats at 13-14 weeks of age. B, baseline; D1, dose 1; D2, dose 2; D3, dose 3. Values are mean ± SEM.

[a]Significantly different from respective value in saline-infused time-control group; $p<0.05$
[b]Significantly different from respective value in WKY rats; $p<0.05$

Figure 7. Central systolic blood pressure during infusions of equivalent volumes of saline (n=6; 0.002, 0.006, 0.02 ml/min) and increasing doses of isoprenaline (n=7; 0.001, 0.003, 0.01 µg/kg/min), terbutaline (n=7), BRL37344 (WKY n=6; SHR n=8) and sodium nitroprusside (SNP; WKY n=7; SHR n=8) (1, 3, 10 µg/kg/min) for 20 minutes each in (**A**) Wistar-Kyoto and (**B**) spontaneously hypertensive rats at 13-14 weeks of age. B, baseline; D1, dose 1; D2, dose 2; D3, dose 3. Values are mean ± SEM.

[a]Significantly different from respective value in saline-infused time-control group; $p<0.05$
[b]Significantly different from respective value in WKY rats; $p<0.05$

41
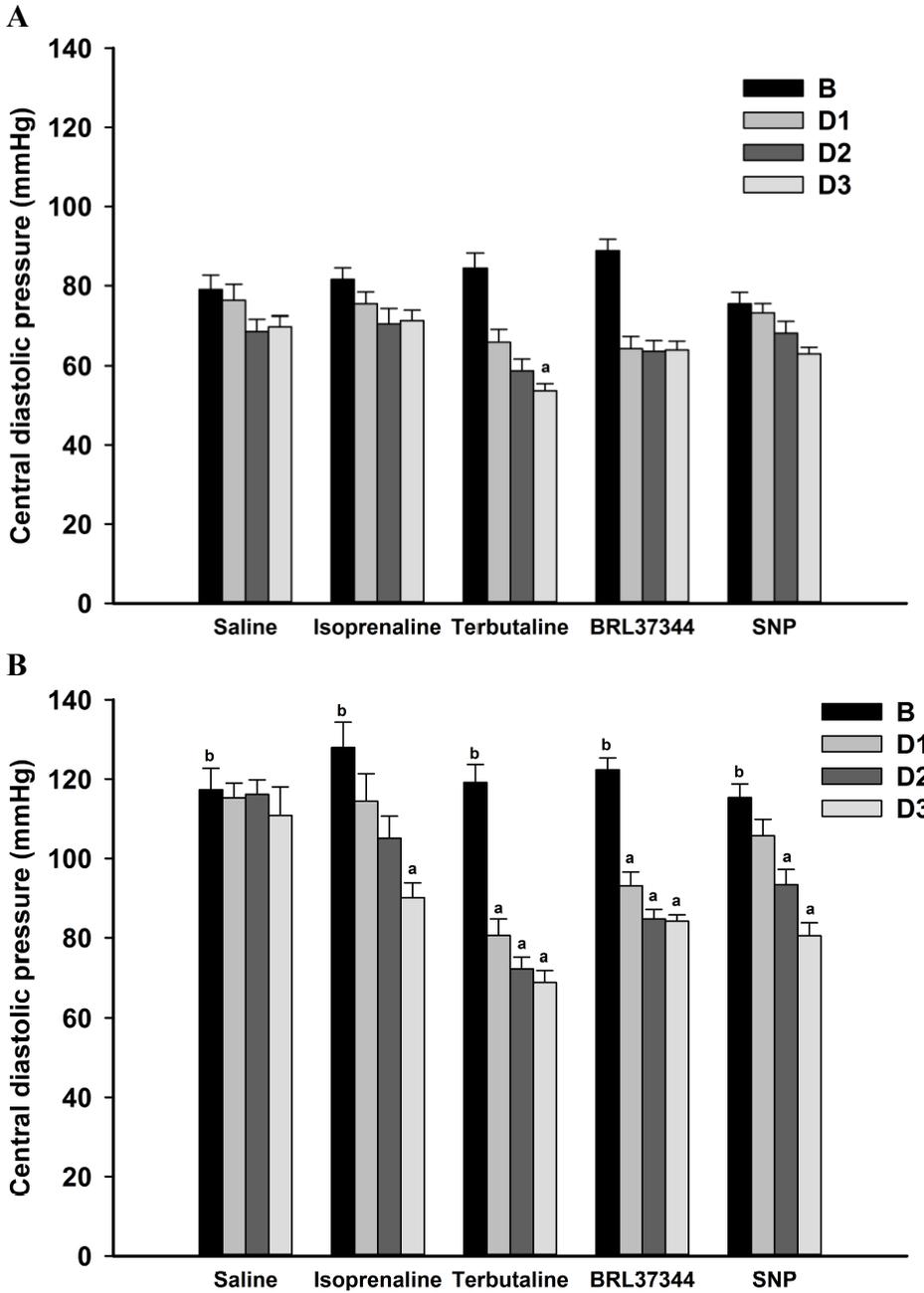
Figure 8. Central diastolic blood pressure during infusions of equivalent volumes of saline (n=6; 0.002, 0.006, 0.02 ml/min) and increasing doses of isoprenaline (n=7; 0.001, 0.003, 0.01 µg/kg/min), terbutaline (n=7), BRL37344 (WKY n=6; SHR n=8) and sodium nitroprusside (SNP; WKY n=7; SHR n=8) (1, 3, 10 µg/kg/min) for 20 minutes each in (**A**) Wistar-Kyoto and (**B**) spontaneously hypertensive rats at 13-14 weeks of age. B, baseline; D1, dose 1; D2, dose 2; D3, dose 3. Values are mean ± SEM.

[a]Significantly different from respective value in saline-infused time-control group; $p<0.05$
[b]Significantly different from respective value in WKY rats; $p<0.05$

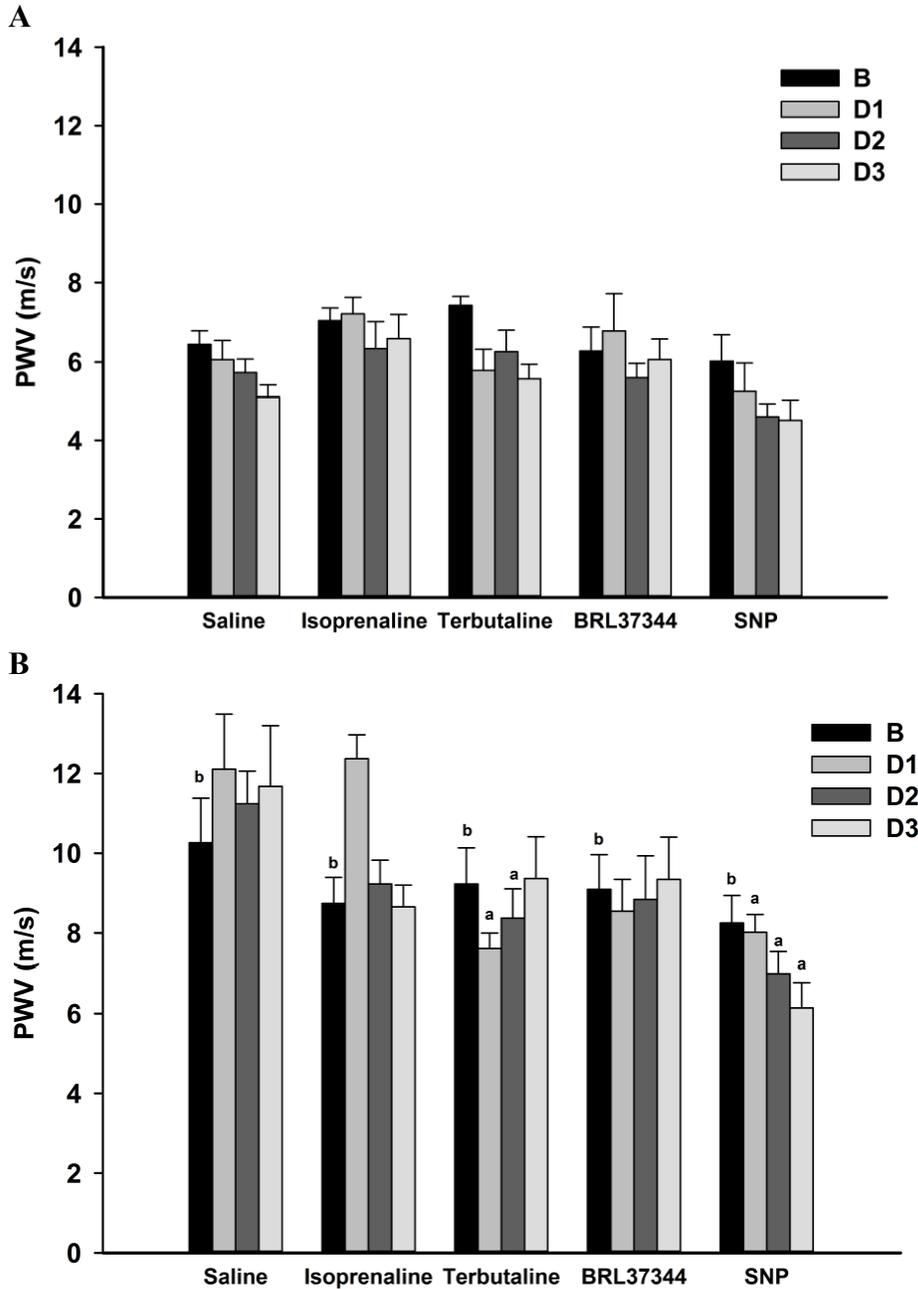**Figure 9.** Heart rate during infusions of equivalent volumes of saline (n=6; 0.002, 0.006, 0.02 ml/min) and increasing doses of isoprenaline (n=7; 0.001, 0.003, 0.01 μg/kg/min), terbutaline (n=7), BRL37344 (WKY n=6; SHR n=8) and sodium nitroprusside (SNP; WKY n=7; SHR n=8) (1, 3, 10 μg/kg/min) for 20 minutes each in (**A**) Wistar-Kyoto and (**B**) spontaneously hypertensive rats at 13-14 weeks of age. B, baseline; D1, dose 1; D2, dose 2; D3, dose 3. Values are mean ± SEM.

[a]Significantly different from respective value in saline-infused time-control group; $p<0.05$
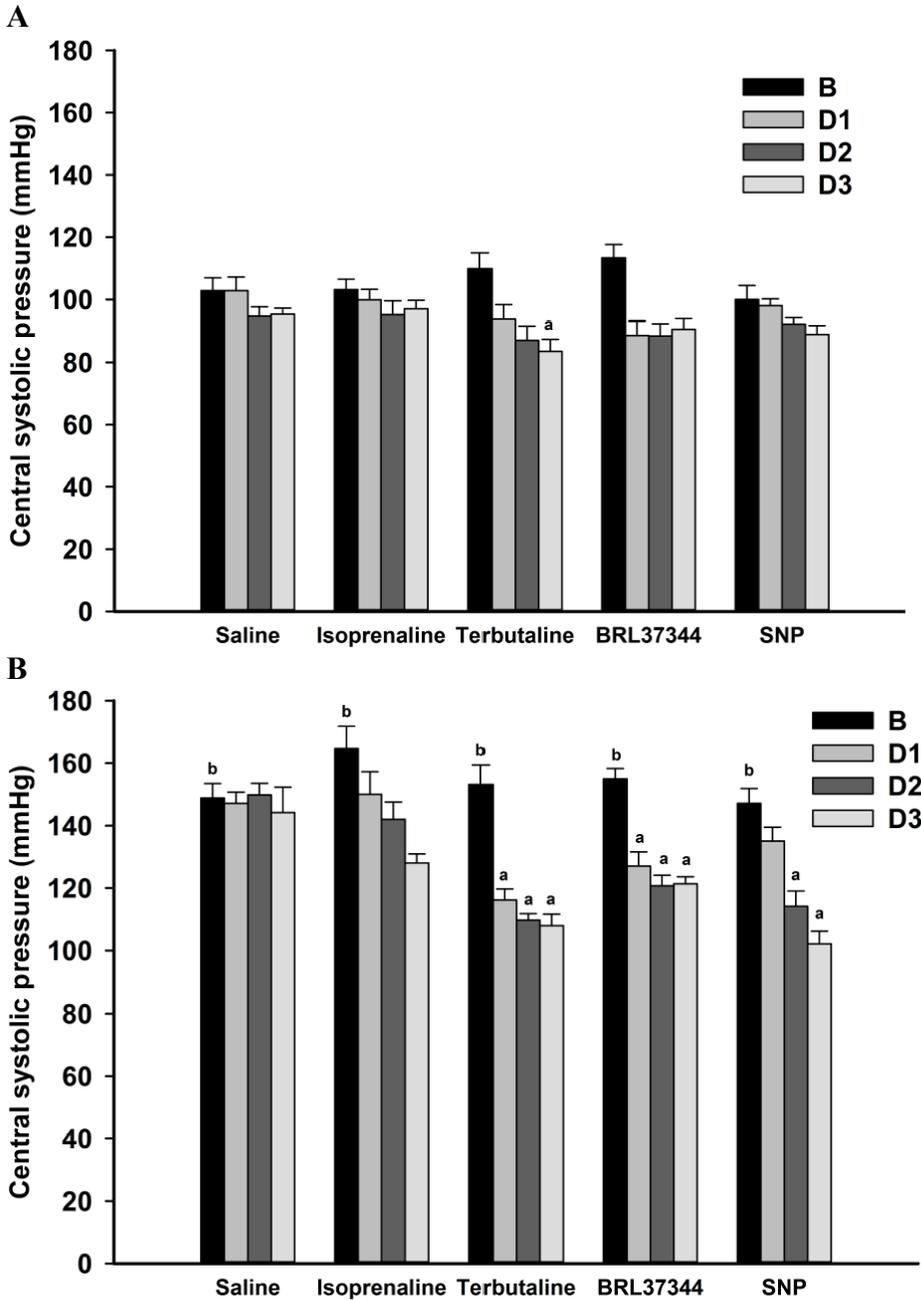[b]Significantly different from respective value in WKY rats; $p<0.05$

diastolic blood pressures at all doses in SH rats. However, it had no effect on central systolic and diastolic blood pressures of WKY rats (Figure 7 and Figure 8). The infusion of BRL37344 had no effects on PWV (Figure 4), as well as central and peripheral blood pressures in WKY and SH rats.

3.5 Isoprenaline

Isoprenaline (non-selective β_1- and β_2-AR agonist; 0.001, 0.003, & 0.01 µg/kg/min) was infused in SH and WKY rats to determine the effect of simultaneous β_1- and β_2-AR stimulation on PWV. The infusion of isoprenaline significantly decreased central diastolic blood pressure in SH rats at the highest dose, but not at any dose in WKY rats (Figure 8). The infusion of isoprenaline had no effect on PWV (Figure 4), as well as central and peripheral pulse pressure in WKY or SH rats (Figure 5 and Figure 6). Administration of isoprenaline did not significantly decrease central systolic pressure in WKY or SH rats (Figure 7). In addition, the administration of isoprenaline and SNP did not significantly affect heart rate in WKY or SH rats (Figure 9).

3.6 Presence of beta-adrenoceptors on the abdominal aorta

Corrected mean density values are shown in Table 3. Immunofluorescence revealed the expression of β_1-, β_2- and β_3-AR on endothelial cells, VSMCs and in the adventitial tissue of both WKY and SH rats (Figure 10, Figure 11, and Figure 12). Quantification of the density of β-AR indicated significantly higher expression of β_1-AR in the endothelium in comparison to VSMCs in both WKY and SH rats. However, there were no significant differences in the expression of β_2- and β_3-AR between the endothelium and the vascular smooth muscle. In addition, no significant differences in expression of β_1-, β_2-, or β_3-AR were found between WKY and SH rats.

Table 3. Corrected mean density values (arbitrary units) of β_1-, β_2- and β_3-adrenoceptors in vascular smooth muscle cells (VSMC) and the endothelium of the abdominal aorta of 13-14 week old Wistar-Kyoto (WKY; n=4) and spontaneously hypertensive (SH; n=4) rats. Each value is mean ± SEM.

		WKY	**SH**
VSMC	β_1-AR	11 ± 1	9 ± 2
	β_2-AR	21 ± 4	15 ± 2
	β_3-AR	21 ± 4	15 ± 2
Endothelium	β_1-AR	23 ± 3[a]	26 ± 4[a]
	β_2-AR	24 ± 4	16 ± 2
	β_3-AR	26 ± 4	18 ± 3

[a]Significantly different from respective value in VSMC within strain; $p < 0.05$
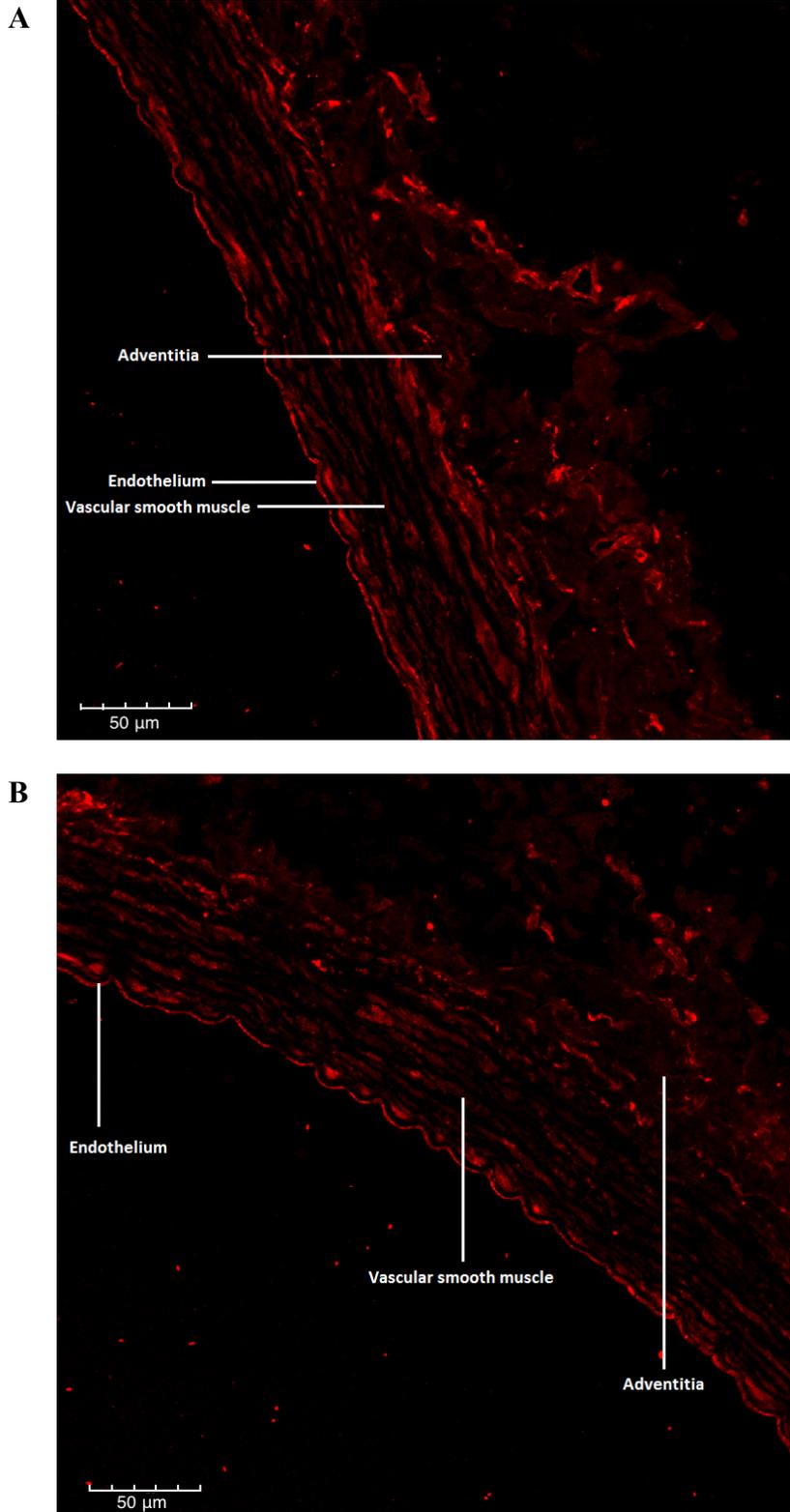
Figure 10. Typical immunofluorescence expression of β_1-adrenoceptor expression in abdominal aortas of WKY (**A**) and SH (**B**) rats.
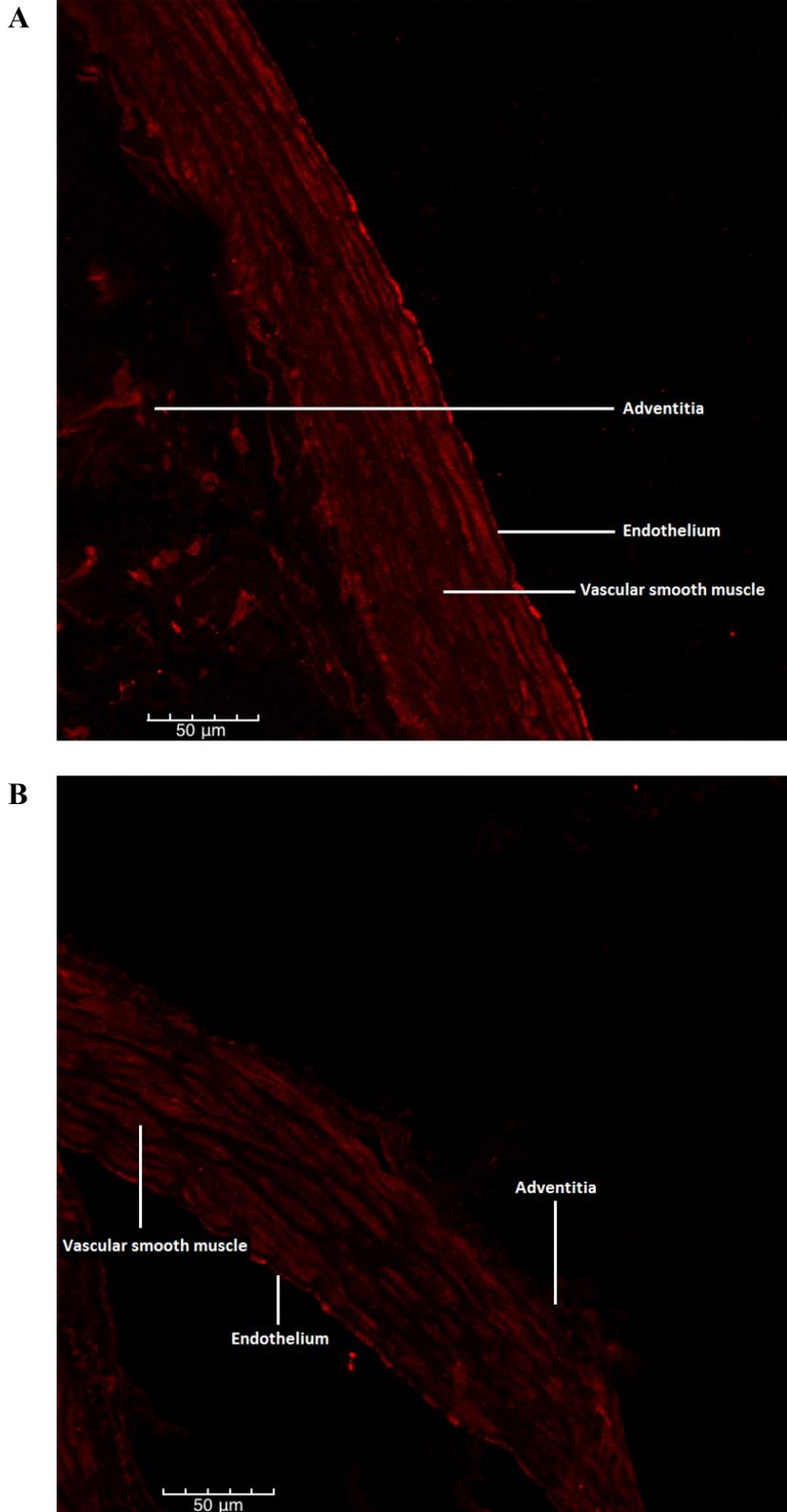
Figure 11. Typical immunofluorescence expression of β_2-adrenoceptor expression in abdominal aortas of WKY (**A**) and SH (**B**) rats.
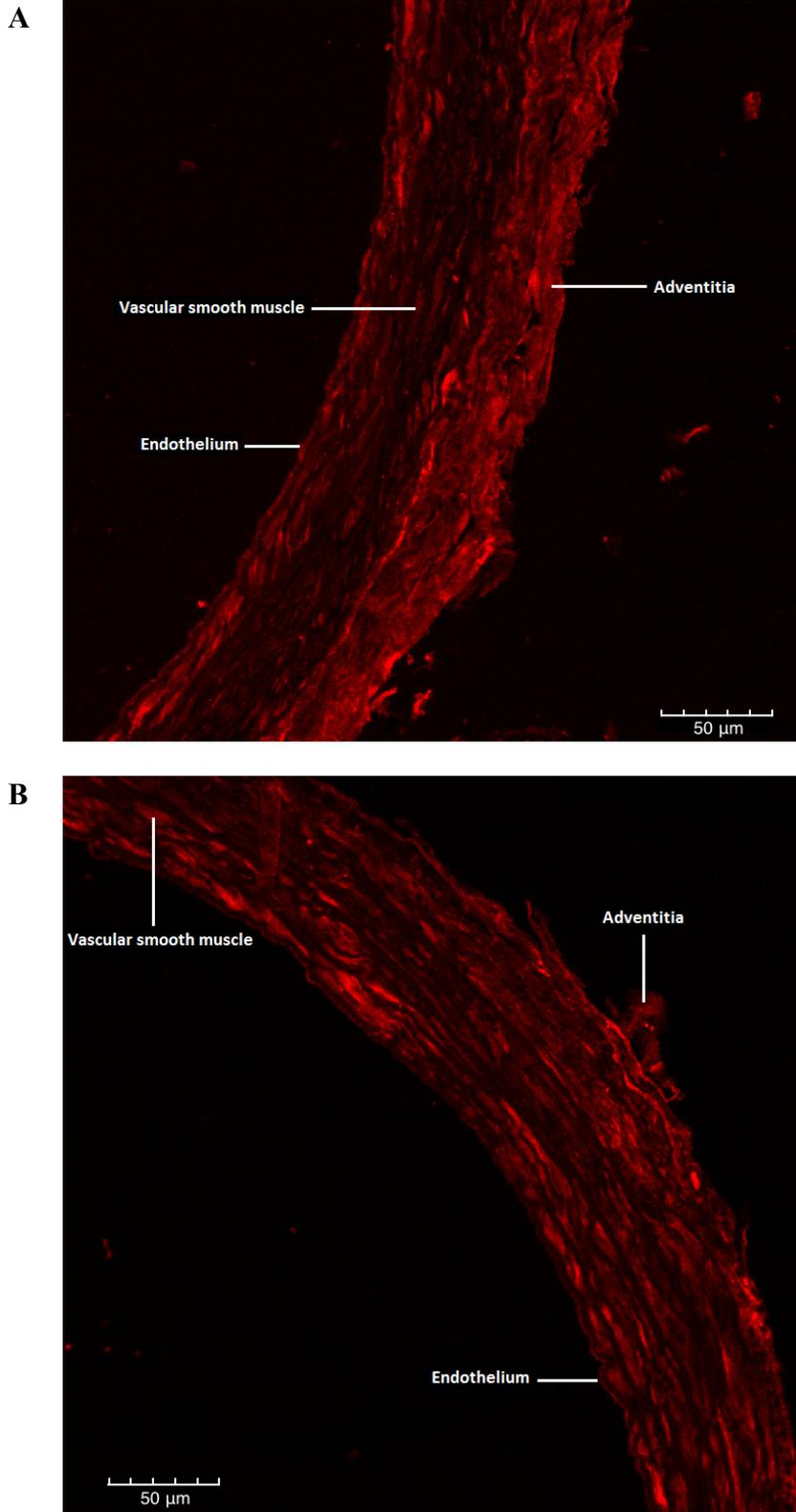
Figure 12. Typical immunofluorescence expression of β_3-adrenoceptor expression in abdominal aortas of WKY (**A**) and SH (**B**) rats.

4. Discussion

The aim of this investigation was to define the role of different subtypes of beta-adrenoceptors in the control of arterial stiffness in the rat aorta, as assessed by PWV. The main findings of this investigation indicate that the stimulation of β_2-AR (but not β_1- or β_3-AR) reduced PWV in SH rats, but not in WKY rats. The administration of SNP also reduces PWV in SH rats, but not in WKY rats. Since PWV was measured within a small range of blood pressures in all of our subjects, the alterations in PWV observed are independent from its effects on blood pressure. Furthermore, immunofluorescence results suggested that all three subtypes of β-AR were expressed on endothelial cells, VSMC and in the adventitial tissue of the abdominal aorta in both WKY and SH rats. The data also revealed greater expression of β_1-AR in the endothelium in comparison to VSMC. However, there were no differences in the level of expression of β_1-, β_2- or β_3-AR between WKY and SH rats.

In the present study, SH animals had significantly higher central and peripheral (systolic and diastolic) blood pressures, pulse pressures and PWV than WKY rats at baseline. In addition, central pulse pressure was significantly different from peripheral pulse pressure in both WKY and SH rats. There was no significant difference in heart rate between WKY and SH rats.

4.1 VSMC contribution to arterial stiffness

In this investigation, the infusion of SNP (a NO donor) reduced PWV at all doses in SH rats, but not at any dose in WKY rats. Reduced PWV indicates increased vascular compliance, since the pulse travels quicker in stiffer arteries. These results provide clear

demonstration of the contribution of VSMC tone to arterial stiffness. The evidence seems to indicate that a reduction in arterial tone increases vascular compliance selectively in hypertension. As described further below, this is in agreement with studies that have altered vascular tone and have observed a change in arterial stiffness (Fitch et al., 2001; McVeigh et al., 2001; Wilkinson et al., 2002).

Fitch and colleagues (2001) investigated the role of NO in vascular stiffness. Aortic PWV was measured after the administration of L-NAME (NOS inhibitor) or phenylephrine in Sprague-Dawley rats. In normal physiological conditions, the activation of eNOS generates NO by converting L-arginine to L-citrulline (Sessa et al., 1990). The administration of L-NAME would therefore limit the production of NO and NO-mediated vascular relaxation. Blood pressure and PWV (controlled for blood pressure changes) were reported to have increased significantly with the injection of L-NAME. At each level of mean arterial pressure, L-NAME also produced a greater increase in PWV than phenylephrine (Fitch et al., 2001). Similarly, the infusion of L-NMMA in normotensive individuals was associated with a dose-dependent increase of the augmentation index, an alternate measure of arterial stiffness (Wilkinson et al., 2002). The augmentation index is an indicator of the enhancement of the central blood pressure wave due to the extent of wave reflection off resistance sites and PWV, which determines the timing of the summation of the forward and backward pulse waves. Thus, these findings are an indicator that an increase in vascular tone can increase arterial stiffness by altering wave reflection. This evidence, in conjunction with our finding that SNP infusion reduces PWV in SH rats, supports the concept that VSMC relaxation can reduce PWV independently from blood pressure levels, and that this may be achieved with a NO

donor. This suggests that endothelial NO may also be able to reduce PWV by reducing aortic tone. Thus, further investigations conducted ex vivo could help confirm the specific roles of endogenous NO compared to exogenous NO (e.g. NO donors) on relaxation and arterial stiffness. Further studies could also help identify distinctions in vascular responses to endothelial-dependent dilators compared to endothelial-independent dilators (such as SNP).

Other studies have also investigated the possibility that an alteration in vascular tone may have a linear relationship with PWV. Since direct blood pressure measurement in the aorta is considered invasive, many of these studies investigated arterial stiffness in other conduit vessels. It is therefore important to note that these studies need to be carefully scrutinized due to the differences in the mechanical properties of central arteries in comparison to peripheral arteries, such as differences in exposure to high systolic pressure or wave reflection (Bergel, 1960). The distinctions in the mechanical properties of peripheral and central arteries may have differing impacts on vascular compliance. Nonetheless, Kinlay and colleagues (2001) investigated arterial compliance in the brachial arteries of seven patients. An ultrasound catheter was used to measure vascular wall thickness, cross-sectional area and blood pressure. These measurements allowed for the calculation of circumferential wall stress, among other indices of stiffness. Stiffness was assessed after the administration of L-NMMA (to inhibit endothelium-derived NO), and after the administration of nitroglycerin (exogenous NO donor). L-NMMA infusion decreased compliance and PWV, while nitroglycerin increased compliance and PWV. Thus, endothelium-derived NO was reported to increase arterial compliance (Kinlay et al., 2001). As well, McEniery and colleagues (2003) examined the relationship between

ET-1 administration and PWV in the iliac artery of anesthetized sheep. The administration of ET-1 was accompanied by an increase in iliac PWV, while the infusion of an ET-1 antagonist was accompanied with a reduction in PWV. It is to be noted that this study had not controlled or corrected PWV for blood pressure. The incubation of carotid arteries with ET-1 antagonists (in vitro) also significantly increased vascular cross-sectional compliance (measured using a microscope connected to a video recorder) in both WKY and SH rats (Marano et al., 1999). In addition, high ET-1 levels in patients with renal failure and/or heart failure were associated with increased aortic PWV (not corrected for blood pressure), while the infusion of an ET-1 receptor blocker (VML-588) reduced PWV and the augmentation index (Vuurmans et al., 2003). Although the aforementioned investigations have examined other arteries, the evidence suggests that there is also a linear relationship between ET-1-mediated vascular tone and arterial stiffness.

Furthermore, a reduction in vascular tone seems to reduce arterial stiffness in most conduit arteries. For example, Steppan and colleagues (2016) investigated the relationship between PWV and other metrics for arterial stiffness. Isoflurane-anesthetized Fisher and SH rats were infused with phenylephrine to achieve a central systolic blood pressure of 200 mmHg. The administration of phenylephrine was then stopped, and aortic PWV was measured as the blood pressure returned to normal. A nonlinear relationship between PWV and blood pressure (systolic, diastolic and MAP) was observed. The assumption was that changes in aortic PWV were due to alterations in vascular tone. However, a limitation to this study is that PWV was not measured at isobaric blood pressures, which makes it challenging to differentiate between the effects of blood

pressure on PWV and the effects of phenylephrine (or vasoconstriction). In addition, Fok and colleagues (2012) assessed PWV (using the Moens-Korteweg equation) between the human brachial and radial arteries in response to changes in vascular tone induced by different signaling pathways. The relationship between PWV and vascular tone was examined with the infusion of phentolamine alone, or in conjunction with nitroglycerin or norepinephrine in the brachial artery. Pearson correlation indicated a significant negative relationship between percent change in PWV and percent change in radial artery diameter, supporting the view that vascular tone is a contributing factor to vascular compliance. It is therefore likely that an increase in vascular tone may account for an increase in PWV, and vice-versa.

In the current investigation, PWV was reduced in response to the administration of SNP or terbutaline in SH, but not in WKY rats. In this investigation, baseline blood pressures were lower in WKY than SH rats, and this could have contributed to the smaller reduction in blood pressures in WKY rats. It is also possible that WKY rats have reduced sensitivity to beta-agonists in comparison to SH rats, which could account for the lack of significant blood pressure reduction with the infusion of these drugs. Studies have shown that SH rats have increased vascular tone (Fitzpatrick and Szentivanyi, 1980), which may also account for the increased response to SNP in SH compared to WKY rats in the current investigation. The increased vascular tone in SH rats could also contribute to arterial stiffness by altering wave reflection and PWV (Shirwany and Zou, 2010). Greater VSMC stiffness in SH rats in comparison to WKY rats could also account for the lesser (non-significant) PWV reduction in WKY rats. A study by Sehgel and colleagues (2013) confirmed increased VSMC stiffness in SH rats in comparison to WKY rats by

using atomic force microscopy. It is also well known that SH rats have increased aortic wall thickness compared to WKY rats (Marque et al., 1999; Van Gorp et al., 1995), attributed to increased VSMC mass (Werstiuk and Lee, 2000), VSMC hyperploidy and polyploidy (Owens and Schwartz, 1982). It is therefore likely that the administration of SNP did not significantly decrease PWV in WKY rats due to the distinctions in the structure of blood vessels between SH and WKY rats. It is also probable that the ability to reduce PWV in compliant arteries is limited, since pulse transit time can only be reduced to a certain extent. In this investigation, β-AR agonists were also infused in WKY and SH rats to determine if the stimulation of β-AR has an impact on PWV.

4.2 β-AR contribution to arterial stiffness

It has been suggested that β-AR blockade may contribute to increased vascular stiffness due to impairment of relaxation of the conduit blood vessels, preventing the reduction of central aortic pressure (Klapholz, 2009; Dudenbostel and Glasser, 2012). However, evidence supporting this claim is scarce. In this investigation, the selective β_2-AR agonist terbutaline significantly decreased PWV in SH rats, but not in WKY rats. As discussed previously, the primary effect of β_2-AR stimulation involves adrenergic-mediated VSMC relaxation (Chruscinski et al., 2001). β_2-AR stimulation has been reported to cause vascular relaxation of the rat aorta (O'Donnell and Wanstall, 1985). In fact, Kelly and colleagues (1989) reported that dilevalol (beta-blocker with β_2-AR agonist properties) reduced PWV, wave reflection and the augmentation index in hypertensive patients (Kelly et al., 1989). β_2-AR stimulation likely reduced PWV by reducing blood pressure and central vascular tone.

In the current investigation, the highest dose of terbutaline administered to SH rats did not significantly decrease PWV compared to their saline-infused time-control. It is possible that the sensitivity to the β_2-agonist was reduced over time and with increasing doses, thereby decreasing the PWV effect seen at lower doses. It has been shown that beta-agonists tend to lose their selectivity at high concentrations (Baker, 2010). In addition, the administration of terbutaline significantly increased heart rate at the highest dose in SH rats, but not at any dose in WKY rats. The results of a study conducted by Tan and colleagues (2012) raise the possibility that the changes in heart rate may influence PWV results. In their study, they investigated the effects of heart rate on central PWV at various mean arterial pressures. Phenylephrine and SNP were infused to compare PWV at different blood pressures, and a bradycardic agent (zatebradine) was infused to reduce heart rate. Higher heart rates were found to be associated with an increase in PWV at all MAP greater than 80 mmHg, where the greatest change was 6.03±0.93% in the 110-130 mmHg range (Tan et al., 2012). It is not possible to compare our data to that of Tan and colleagues, since they simultaneously altered heart rate and blood pressures. Nonetheless, it has been suggested that an increase in PWV observed with higher heart rates could be attributed to the increased exposure of blood vessels to systolic blood pressure, which causes distension of blood vessels and increased wave reflection (Wilkinson et al., 2000; Giannattasio et al., 2003). The highest doses of terbutaline and BRL37344 used in the current study both increased heart rate in SH rats, with PWV at similar levels. Furthermore, terbutaline decreased PWV at the lowest doses in SH rats, but BRL37344 did not. It is possible that the increase in heart rate may have overshadowed the effect of the highest dose of terbutaline on PWV. It is also possible that BRL37344 did not

increase PWV at the highest dose for the reason that PWV was not altered at low doses and was already near its peak. Alternately, it is also possible that β_3-AR stimulation does not cause significant aortic relaxation, which could account for the reduction in PWV with terbutaline, but not BRL37344. Rodrigues and colleagues (2010) had shown that MMPs can cleave β_2-AR in SH rats, which could minimize the function of β_2-AR agonists in SH rats. However, this does not seem to be the case in the present study. The administration of terbutaline significantly decreased peripheral and central systolic blood pressures at all doses in SH rats, and at the highest dose in WKY rats.

The administration of BRL37344, a selective β_3-AR agonist, did not lower PWV in SH or WKY rats at any dose. However, BRL37344 infusion reduced central systolic and diastolic blood pressures in SH rats, and therefore mean central blood pressure, suggesting that it significantly reduced total peripheral resistance. It is possible that aortic vasodilation induced by BRL37344 was not sufficient to significantly reduce PWV, despite a reduction in blood pressure. It has been reported that BRL37344 produces a slow relaxation (5-8 min for peak response), (Oriowo, 1994) and has low efficacy and potency in VSMC of the thoracic aorta (Emorine et al., 1989; Brawley et al., 2000). Peripheral vasodilation has previously been observed in conscious rats infused with BRL37344 (Shen et al., 1996). However, Brawley and colleagues (2000) observed that the rat thoracic aorta dilation in response to BRL37344 was minimal in comparison to other atypical β-AR agonists. Thus, the stimulation of β_3-AR by BRL37344 could have initiated peripheral vascular relaxation, with minimal relaxation in the central arteries. β_3-ARs also require higher concentrations of catecholamine to be stimulated than those required for β_1- and β_2-AR stimulation (Emorine et al., 1989). Therefore, it is possible

that BRL37344 elicited a reduction in total peripheral resistance and blood pressure, but with little or no alteration in the vascular tone of the aorta itself, resulting in no change in aortic PWV. The differences in central relaxation between β_2- and β_3-AR stimulation could account for the fact that there was PWV reduction with terbutaline, but not BRL37344 in SH rats.

The effects of nebivolol (β_1-blocker with NO donor) and atenolol (selective β_1-blocker) on PWV (not corrected for blood pressure) have previously been investigated in hypertensive patients (Mahmud and Feely, 2008). Both treatments reduced aortic PWV (nebivolol, 11.5 ± 0.5 to 9.9 ± 0.5 m/s; atenolol, 11.1 ± 0.4 to 9.8 ± 0.4 m/s), however atenolol did not reduce wave reflection. A reduction in heart rate may explain the improved arterial distensibility associated with β_1-AR blockade. Mahmud and Feely (2008) also suggested that the vasodilation caused by nebivolol could have contributed to the reduction in wave reflection. This is in agreement with a previous study in which the alterations in PWV in response to treatment with β-blockers (nebivolol or atenolol) were investigated. PWV and peripheral pulse pressure were significantly reduced from baseline at 2 and 10 weeks in all (hypertensive) treatment groups. Since nebivolol also significantly reduced the augmentation index, it was reported to be more effective in increasing arterial compliance in comparison to atenolol (Koumaras et al., 2014). The augmentation index was likely reduced with nebivolol due to its NO donor properties, which causes relaxation in the peripheral vasculature. Peripheral vasodilation leads to a reduction in total peripheral resistance, which reduces wave reflection. According to the evidence from investigations that have assessed the effect of β-blockers on arterial stiffness, the most efficient β-blockers in increasing vascular compliance and reducing

wave reflection seem to be selective β_1-blockers (due to reduction in heart rate) and β-blockers with NO donor properties (due to vascular relaxation).

The infusion of isoprenaline (a β_1- and β_2-AR non-selective agonist) produced no significant change in PWV in WKY or SH rats despite a modest reduction in diastolic blood pressure in SH rats in the current study. It may be argued that the doses of isoprenaline administered in this investigation were too low to have an impact on PWV. The doses used in the present study were chosen (based on a pilot study) to be sufficient to reduce diastolic blood pressure, but low enough to avoid producing increases in heart rate. We avoided higher doses that increased heart rate since they would have had the potential to cause heart rate-mediated increase in arterial stiffness (Tan et al., 2012). Previous investigations have found that the minimal effective dose (determined by blood pressure, heart rate, among others) of isoprenaline was 0.003-0.01 µg/kg (Apperley et al., 1976), while others have observed increased chronotropic responses at 0.04 and 0.01 µg/kg/min in SH and WKY rats, respectively (Saragoca and Tarazi, 1981). Furthermore, isoprenaline infusions in the current investigation did cause a modest (insignificant) reduction in blood pressure, suggesting that it must have been close to the appropriate range of doses.

Ultimately, the evidence suggests that β_2-AR stimulation reduces arterial stiffness most likely due to the reduction in central vascular tone. In fact, β-AR agonists and antagonists can have a multitude of hemodynamic effects that can influence vascular stiffness. This topic will be covered in the following section.

4.3 Hemodynamics of the aorta

In this investigation, peripheral pulse pressure was found to be significantly higher than central pulse pressure in both WKY and SH rats. Pulse pressure increases as it travels through the vasculature due to increasing vascular resistance in smaller arteries (Pauca et al., 1992), which increases wave reflection. Therefore, the difference between central and peripheral pulse pressure confirms that wave reflection is present in rodents, which is a characteristic of hemodynamic properties of the cardiovascular system also present in humans (Kelly et al., 1989; Koumaras et al., 2014).

SNP is well established to induce vascular relaxation in the isolated rat aorta (Bonaventura et al., 2008; Fok et al., 2012). In our current investigation, SNP infusion reduced both central and peripheral pulse pressure in SH rats. Central and peripheral (systolic and diastolic) blood pressures were also reduced with the administration of SNP, suggesting that there was peripheral vasodilation. Yet, we are unsure as to whether the reduction in central pulse pressure was due to vasodilation of the central and/or peripheral vasculature. Nonetheless, the administration of SNP reduced PWV in SH rats, but BRL37344 did not, despite comparable levels of blood pressure at each dose. Therefore, it is likely that central blood vessels are the main protagonists in reducing aortic PWV.

Drugs that reduce blood pressure such as the β-AR agonists and SNP used in this investigation may activate the baroreceptor reflex, leading to an activation of the SNS. The animals in this investigation were anesthetized with isoflurane, which is known to depress the baroreflex in normotensive and hypertensive rabbits (Bell, 1994), as well as in humans (Kotrly et al., 1984). SNP infusions did not increase heart rate despite large

59

reductions in blood pressure, suggesting that the baroreflex was indeed depressed, and unlikely to influence our results.

We expected that the effect of drugs on central arterial stiffness (PWV) might have been evident with changes in the central arterial pulse pressure. However, central arterial pulse pressure does not appear to be a very reliable metric of arterial elasticity since there does not seem to be a clear relationship between PWV reduction and central (or peripheral) pulse pressure in the current investigation. This could be due to the alteration of PWV, heart rate and vascular resistance, which affect wave reflection and augmentation (important contributors to pulse pressure). Further, a selective β-AR agonist may lose its selectivity at higher doses, and stimulate other subtypes of β-AR (Baker, 2010). Therefore, the increase in heart rate at the highest dose of terbutaline and BRL37344 could be attributed to the loss of selectivity. In the present study, we also investigated β-AR expression in the abdominal aorta of WKY and SH rats to determine whether β-AR density could account for differences in arterial stiffness and/or hemodynamic responses to β-AR stimulation.

4.4 Immunofluorescence

In the current investigation, β_3-AR expression in the abdominal aorta was investigated with immunofluorescence techniques. We have identified β_3-AR expression in the endothelium, VSMCs, and in the adventitial tissue of the abdominal aorta of both WKY and SH rats. β_3-AR was initially identified in adipocytes (Emorine et al., 1989; Ferrer-Lorente, 2005), and was subsequently identified in cardiomyocytes (Cohen et al., 1999). In addition, β_3-AR has been associated with NO-mediated vasodilation in human coronary microarteries (blood vessels that branch off from the coronary arteries) (Dessy

et al., 2004). Rautureau and colleagues (2002) also confirmed the presence of endothelial

β_3-AR in the rat thoracic aorta by immunohistochemistry techniques. The current

investigation has also revealed β_3-AR expression in VSMCs of the rat abdominal aorta.

It has been shown that stimulation of β_3-AR in the rat thoracic aorta causes

relaxation, which is greatly reduced by an eNOS inhibitor (Trochu et al., 1999). This

evidence suggests that β_3-AR-activity is largely endothelium-dependent. The infusion of

an NO donor such as SNP can initiate a strong relaxation of the entire vascular wall of the

aorta, since the vasa vasorum (network of vessels responsible for supplying blood to the

walls of the aorta) can distribute NO to the entire vascular wall. On the other hand, it is

possible that endothelial NO (e.g. released β_3-AR stimulation) is not capable of initiating

relaxation of the entire aortic vascular wall. Further studies are needed to elucidate this

idea.

Conversely, vascular relaxation triggered by the stimulation of β_1- and β_2-AR has

been linked to their presence in VSMCs. The evidence for β_1- and β_2-AR in the aortic

endothelium is sparse. However, endothelial β_1- and β_2-AR-mediated relaxation has been

reported in other blood vessels, but to a lesser extent than β_1- and β_2-AR located in

VSMC (Oriowo, 1995; Brawley et al., 2000). In this investigation, β_1- and β_2-AR were

found to be expressed in the endothelium and VSMCs of the abdominal aorta of both

WKY and SH rats. These findings concur with evidence of β_2-AR in VSMCs (Brodde

and Michel, 1992; Takata and Kato 1996), and those concluding that the role of vascular

β_1- and β_2-AR is relaxation (Chruscinski et al., 2001). Functional studies also suggested

greater relaxation to β_2-AR-stimulation than β_1-AR stimulation in the rat carotid artery

(Chiba and Tsukuda, 2001). Surprisingly, with quantification analysis, we found greater

expression of β_1-AR in the endothelium in comparison to VSMCs of both WKY and SH rats.

No differences in β_1-, β_2- or β_3-AR expression were found between WKY and SH rats, despite a previous report of β_3-AR upregulation in the endothelial layer of the thoracic aorta of SH rats with immunohistochemistry (Mallem et al., 2004). Differential levels of β-AR-mediated relaxation have previously been reported, in which the thoracic aorta showed greater relaxation compared to the abdominal aorta (Fleisch et al., 1970). Thus, it is possible that β_3-AR-mediated relaxation is more prominent in the thoracic aorta than in the abdominal aorta.

4.5 Limitations

This study has a number of limitations. Fluid-filled catheters were used rather than electronic pressure sensor devices that measure pressure at the tip of the catheters, which could have limited the accuracy of our assessment of pulse transit time and PWV. In addition, the present investigation was an acute study, and cannot predict the effect of chronic changes in β-AR activity on arterial stiffness.

As previously discussed, it is recognized that the measurement of parameters such as PWV are influenced by changes in heart rate and blood pressure, among other factors. Thus, the complex dynamic nature of blood vessels could limit the accuracy of arterial stiffness measurements. Hence, most studies assessing arterial stiffness are usually carried out using isolated blood vessels. However, the major disadvantage of in vitro investigations is that the true hemodynamic effects of blood flow on the vascular wall are absent, and values obtained under such circumstances could miss a host of physiological parameters that continuously influence arterial stiffness. Likewise, the lack of perfect

control in measuring PWV at isobaric blood pressures during drug infusions could contribute to an overestimation of the differences observed in PWV between WKY and SH rats.

4.6 Future studies

The current investigation seems to support the hypothesis that vasodilation of the central arteries (e.g. with SNP) can have an important role in the reduction of stiffness in a state of hypertension. The findings also suggest that β_2-AR stimulation may play a role in the alteration of vascular compliance, however it is not clear whether this effect would occur independently from aortic relaxation. Furthermore, we confirmed the expression of β_3-AR in the abdominal aorta, and increased expression of β_1-AR in the endothelium in comparison to VSMCs. These findings are important in understanding the mechanical properties of the central arteries in a state of high intravascular pressure.

Further studies are required to establish the impact of long-term vascular relaxation on vascular compliance. It would also be interesting to investigate the effect of chronic treatment with selective β-blockers compared to β-blockers with NO donor properties on arterial stiffness in a state of hypertension. It has been suggested that β-AR blockade may contribute to increased vascular stiffness due to impairment of relaxation (Klapholz, 2009; Dudenbostel and Glasser, 2012). However, evidence to support this claim is lacking, and the current literature on this subject suggests that selective β_1-blockers and those with NO donor properties may in fact reduce arterial stiffness. Relaxation of the large central arteries seems to play an important role in increasing vascular compliance. Hence, future studies are needed in order to investigate the effects of selective β-blockers on arterial stiffness to provide insight on effective treatment options for hypertension.

5. References

Ablad, B., Carlsson, B., Carlsson, E., Dahlof, C., Ek, L., & Hultberg, E. (1974). Cardiac effects of beta-adrenergic receptor antagonists. *Advances in Cardiology, 12*(0), 290-302.

Ahlquist, R. P. (1948). A study of the adrenotropic receptors. *The American Journal of Physiology, 153*(3), 586-600.

Apperley, G. H., Daly, M. J., & Levy, G. P. (1976). Selectivity of beta-adrenoceptor agonists and antagonists on bronchial, skeletal, vascular and cardiac muscle in the anaesthetized cat. *British Journal of Pharmacology*, *57*(2), 235–246.

Baker, J. G. (2010). The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors. *British Journal of Pharmacology, 160*(5), 1048-1061. doi:10.1111/j.1476-5381.2010.00754.x [doi]

Basalyga, D. M., Simionescu, D. T., Xiong, W., Baxter, B. T., Starcher, B. C., & Vyavahare, N. R. (2004). Elastin degradation and calcification in an abdominal aorta injury model: Role of matrix metalloproteinases. *Circulation, 110*(22), 3480-3487. doi:01.CIR.0000148367.08413.E9 [pii]

Bell, L. B. (1994). Baroreflex modulation by isoflurane anesthesia in normotensive and chronically hypertensive rabbits. *Advances in Pharmacology (San Diego, Calif.), 31*, 389-408.

Belz, G. G. (1995). Elastic properties and windkessel function of the human aorta. *Cardiovascular Drugs and Therapy / Sponsored by the International Society of Cardiovascular Pharmacotherapy, 9*(1), 73-83.

Bergel, D. H. (1961). The static elastic properties of the arterial wall. *The Journal of Physiology, 156*(3), 445-457.

Bonaventura, D., Lunardi, C. N., Rodrigues, G. J., Neto, M. A., & Bendhack, L. M. (2008). A novel mechanism of vascular relaxation induced by sodium nitroprusside in the isolated rat aorta. *Nitric Oxide : Biology and Chemistry, 18*(4), 287-295. doi:10.1016/j.niox.2008.02.004 [doi]

Boutouyrie, P., Fliser, D., Goldsmith, D., Covic, A., Wiecek, A., Ortiz, A., . . . London, G. M. (2014). Assessment of arterial stiffness for clinical and epidemiological studies: Methodological considerations for validation and entry into the european renal and cardiovascular medicine registry. *Nephrology, Dialysis, Transplantation : Official Publication of the* In vivo/in vitro comparison of rat abdominal aorta wall viscosity: influence of endothelial function *European Dialysis and Transplant Association - European Renal Association, 29*(2), 232-239.

Brandts, A., Westenberg, J. J., van Elderen, S. G., Kroft, L. J., Roes, S. D., Tamsma, J. T., . . . de Roos, A. (2013). Site-specific coupling between vascular wall thickness and function: An observational MRI study of vessel wall thickening and stiffening in hypertension. *Investigative Radiology, 48*(2), 86-91. doi:10.1097/RLI.0b013e31827f6410 [doi]

Brawley, L., Shaw, A. M., & MacDonald, A. (2000). Beta 1-, beta 2- and atypical beta-adrenoceptor-mediated relaxation in rat isolated aorta. *British Journal of Pharmacology, 129*(4), 637-644. doi:10.1038/sj.bjp.0703091 [doi]

Bray, M. S., Krushkal, J., Li, L., Ferrell, R., Kardia, S., Sing, C. F., . . . Boerwinkle, E. (2000). Positional genomic analysis identifies the beta(2)-adrenergic receptor gene as a susceptibility locus for human hypertension. *Circulation, 101*(25), 2877-2882.

Brodde, O. E., & Michel, M. C. (1992). Adrenergic receptors and their signal transduction mechanisms in hypertension. *Journal of Hypertension.Supplement : Official Journal of the International Society of Hypertension, 10*(7), S133-45.

Bulbring, E., & Tomita, T. (1987). Catecholamine action on smooth muscle. *Pharmacological Reviews, 39*(1), 49-96.

Butlin, M., Lindesay, G., Viegas, K. D., & Avolio, A. P. (2015). Pressure dependency of aortic pulse wave velocity in vivo is not affected by vasoactive substances that alter aortic wall tension ex vivo. *American Journal of Physiology.Heart and Circulatory Physiology, 308*(10), H1221-8. doi:10.1152/ajpheart.00536.2014 [doi]

Candelore, M. R., Deng, L., Tota, L., Guan, X. M., Amend, A., Liu, Y., . . . Weber, A. E. (1999). Potent and selective human beta(3)-adrenergic receptor antagonists. *The Journal of Pharmacology and Experimental Therapeutics, 290*(2), 649-655.

Charkoudian, N., & Rabbitts, J. A. (2009). Sympathetic neural mechanisms in human cardiovascular health and disease. *Mayo Clinic Proceedings, 84*(9), 822-830. doi:10.1016/S0025-6196(11)60492-8 [doi]

Cheng, J. B., & Shibata, S. (1981). Vascular relaxation in the spontaneously hypertensive rat. *Journal of Cardiovascular Pharmacology, 3*(5), 1126-1140.

Chiba, S., & Tsukada, M. (2001). Vascular responses to beta-adrenoceptor subtype-selective agonists with and without endothelium in rat common carotid arteries. *Journal of Autonomic Pharmacology, 21*(1), 7-13. doi:jap199 [pii]

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L.,Jr, . . . National High Blood Pressure Education Program Coordinating Committee. (2003). The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: The JNC 7 report. *Jama, 289*(19), 2560-2572. doi:10.1001/jama.289.19.2560 [doi]

Chruscinski, A., Brede, M. E., Meinel, L., Lohse, M. J., Kobilka, B. K., & Hein, L. (2001). Differential distribution of beta-adrenergic receptor subtypes in blood vessels of knockout mice lacking beta(1)- or beta(2)-adrenergic receptors. *Molecular Pharmacology, 60*(5), 955-962.

Cohen, M. L., Bloomquist, W., Kriauciunas, A., Shuker, A., & Calligaro, D. (1999). Aryl propanolamines: Comparison of activity at human beta3 receptors, rat beta3 receptors and rat atrial receptors mediating tachycardia. *British Journal of Pharmacology, 126*(4), 1018-1024. doi:10.1038/sj.bjp.0702364 [doi]

Daneshtalab, N., Dore, J. J., & Smeda, J. S. (2010). Troubleshooting tissue specificity and antibody selection: Procedures in immunohistochemical studies. *Journal of Pharmacological and Toxicological Methods, 61*(2), 127-135. doi:10.1016/j.vascn.2009.12.002 [doi]

Dao, H. H., Essalihi, R., Bouvet, C., & Moreau, P. (2005). Evolution and modulation of age-related medial elastocalcinosis: Impact on large artery stiffness and isolated systolic hypertension. *Cardiovascular Research, 66*(2), 307-317. doi:S0008-6363(05)00040-4 [pii]

Del Colle, S., Morello, F., Rabbia, F., Milan, A., Naso, D., Puglisi, E., . . . Veglio, F. (2007). Antihypertensive drugs and the sympathetic nervous system. *Journal of Cardiovascular Pharmacology, 50*(5), 487-496. doi:10.1097/FJC.0b013e318135446c [doi]

Dessy, C., Moniotte, S., Ghisdal, P., Havaux, X., Noirhomme, P., & Balligand, J. L. (2004). Endothelial beta3-adrenoceptors mediate vasorelaxation of human coronary microarteries through nitric oxide and endothelium-dependent hyperpolarization. *Circulation, 110*(8), 948-954. doi:10.1161/01.CIR.0000139331.85766.AF [doi]

Domanski, M. J., Davis, B. R., Pfeffer, M. A., Kastantin, M., & Mitchell, G. F. (1999). Isolated systolic hypertension : Prognostic information provided by pulse pressure. *Hypertension (Dallas, Tex.: 1979), 34*(3), 375-380.

Dudenbostel, T., & Glasser, S. P. (2012). Effects of antihypertensive drugs on arterial stiffness. *Cardiology in Review, 20*(5), 259-263. doi:10.1097/CRD.0b013e31825d0a44 [doi]

Emorine, L. J., Marullo, S., Briend-Sutren, M. M., Patey, G., Tate, K., Delavier-Klutchko, C., & Strosberg, A. D. (1989). Molecular characterization of the human beta 3-adrenergic receptor. *Science (New York, N.Y.), 245*(4922), 1118-1121.

Ferrer-Lorente, R., Cabot, C., Fernandez-Lopez, J. A., & Alemany, M. (2005). Combined effects of oleoyl-estrone and a beta3-adrenergic agonist (CL316,243) on lipid stores of diet-induced overweight male wistar rats. *Life Sciences, 77*(16), 2051-2058. doi:S0024-3205(05)00412-1 [pii]

66

Fitch, R. M., Vergona, R., Sullivan, M. E., & Wang, Y. X. (2001). Nitric oxide synthase inhibition increases aortic stiffness measured by pulse wave velocity in rats. *Cardiovascular Research, 51*(2), 351-358. doi:S0008636301002991 [pii]

Fitzpatrick, D. F., & Szentivanyi, A. (1980). The relationship between increased myogenic tone and hyporesponsiveness in vascular smooth muscle of spontaneously hypertensive rats. *Clinical and Experimental Hypertension, 2*(6), 1023-1037.

Fleisch, J. H., Maling, H. M., & Brodie, B. B. (1970). Beta-receptor activity in aorta; variations with age and species. *Circulation Research, 26*(2), 151-162.

Fok, H., Jiang, B., Clapp, B., & Chowienczyk, P. (2012). Regulation of vascular tone and pulse wave velocity in human muscular conduit arteries: Selective effects of nitric oxide donors to dilate muscular arteries relative to resistance vessels. *Hypertension (Dallas, Tex.: 1979), 60*(5), 1220-1225. doi:10.1161/HYPERTENSIONAHA.112.198788 [doi]

Franklin, S. S., Gustin, W.,4th, Wong, N. D., Larson, M. G., Weber, M. A., Kannel, W. B., & Levy, D. (1997). Hemodynamic patterns of age-related changes in blood pressure. the framingham heart study. *Circulation, 96*(1), 308-315.

Franklin, S. S., Khan, S. A., Wong, N. D., Larson, M. G., & Levy, D. (1999). Is pulse pressure useful in predicting risk for coronary heart disease? the framingham heart study. *Circulation, 100*(4), 354-360.

Frishman, W. (1981). Beta-adrenoceptor antagonists: New drugs and new indications. *New England Journal of Medicine, 305*(9), 505-506.

Frishman, W. (1984). *Clinical pharmacology of the beta-adrenoceptor blocking drugs* (2nd ed. ed.). Norwalk: Appleton-Century-Crofts.

Gao, F., de Beer, V. J., Hoekstra, M., Xiao, C., Duncker, D. J., & Merkus, D. (2010). Both beta1- and beta2-adrenoceptors contribute to feedforward coronary resistance vessel dilation during exercise. *American Journal of Physiology.Heart and Circulatory Physiology, 298*(3), H921-9. doi:10.1152/ajpheart.00135.2009 [doi]

Giannattasio, C., Vincenti, A., Failla, M., Capra, A., Ciro, A., De Ceglia, S., . . . Mancia, G. (2003). Effects of heart rate changes on arterial distensibility in humans. *Hypertension (Dallas, Tex.: 1979), 42*(3), 253-256. doi:10.1161/01.HYP.0000085199.33254.15 [doi]

Girard, A., Meilhac, B., Mounier-Vehier, C., & Elghozi, J. L. (1995). Effects of beta-adrenergic blockade on short-term variability of blood pressure and heart rate in essential hypertension. *Clinical and Experimental Hypertension (New York, N.Y.: 1993), 17*(1-2), 15-27.

Granneman, J. G. (2001). The putative beta4-adrenergic receptor is a novel state of the beta1-adrenergic receptor. *American Journal of Physiology.Endocrinology and Metabolism, 280*(2), E199-202.

Guyenet, P. G. (2006). The sympathetic control of blood pressure. *Nature Reviews.Neuroscience, 7*(5), 335-346. doi:nrn1902 [pii]

Guyton, A. C., & Hall, J. E. (2011). *Textbook of medical physiology* (10th ed. ed., pp. 709). Philadelphia: W.B. Saunders Company.

Haeusler, G. (1990). Pharmacology of beta-blockers: Classical aspects and recent developments. *Journal of Cardiovascular Pharmacology, 16 Suppl 5*, S1-9.

Huveneers, S., Daemen, M. J., & Hordijk, P. L. (2015). Between rho(k) and a hard place: The relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. *Circulation Research, 116*(5), 895-908. doi:10.1161/CIRCRESAHA.116.305720 [doi]

Institute of Laboratory Animal Resources. (1976). History of the spontaneously hypertensive rat. *Spontaneously hypertensive (SHR) rats: Guidelines for breeding, care, and use* (pp. G6-G7). Washington, D.C.: National Academies.

Jacob M.P. (2003) Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions. *Biomed. Pharmacother.* **57**:195–202.

Jaillon, P. (1990). Relevance of intrinsic sympathomimetic activity for beta blockers. *The American Journal of Cardiology, 66*(9), 21C-23C.

James, P. A., Oparil, S., Carter, B. L., Cushman, W. C., Dennison-Himmelfarb, C., Handler, J., . . . Ortiz, E. (2014). 2014 evidence-based guideline for the management of high blood pressure in adults: Report from the panel members appointed to the eighth joint national committee (JNC 8). *Jama, 311*(5), 507-520. doi:10.1001/jama.2013.284427 [doi]

Joannides, R., Haefeli, W. E., Linder, L., Richard, V., Bakkali, E. H., Thuillez, C., & Luscher, T. F. (1995). Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation, 91*(5), 1314-1319.

Johansson, L. H. (1995). Factors behind the functional beta 2-adrenoceptor selectivity of terbutaline. *Pharmacology & Toxicology, 77 Suppl 3*, 21-24.

Kaumann, A. J. (1989). Is there a third heart beta-adrenoceptor? *Trends in Pharmacological Sciences, 10*(8), 316-320. doi:0165-6147(89)90065-5 [pii]

Kelly, R., Daley, J., Avolio, A., & O'Rourke, M. (1989). Arterial dilation and reduced wave reflection. benefit of dilevalol in hypertension. *Hypertension (Dallas, Tex.: 1979), 14*(1), 14-21.

Kinlay, S., Creager, M. A., Fukumoto, M., Hikita, H., Fang, J. C., Selwyn, A. P., & Ganz, P. (2001). Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo. *Hypertension (Dallas, Tex.: 1979), 38*(5), 1049-1053.

Klapholz, M. (2009). Beta-blocker use for the stages of heart failure. *Mayo Clinic Proceedings, 84*(8), 718-729. doi:10.4065/84.8.718 [doi]

Kotrly, K. J., Ebert, T. J., Vucins, E., Igler, F. O., Barney, J. A., & Kampine, J. P. (1984). Baroreceptor reflex control of heart rate during isoflurane anesthesia in humans. *Anesthesiology, 60*(3), 173-179.

Koumaras, C., Tziomalos, K., Stavrinou, E., Katsiki, N., Athyros, V. G., Mikhailidis, D. P., & Karagiannis, A. (2014). Effects of renin-angiotensin-aldosterone system inhibitors and beta-blockers on markers of arterial stiffness. *Journal of the American Society of Hypertension : JASH, 8*(2), 74-82. doi:10.1016/j.jash.2013.09.001 [doi]

Lands, A. M., Luduena, F. P., & Buzzo, H. J. (1967). Differentiation of receptors responsive to isoproterenol. *Life Sciences, 6*(21), 2241-2249.

Laragh J. (2001) Laragh's lessons in pathophysiology and clinical pearls for treating hypertension. *American Journal of Hypertension.* (14) 296-304.

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., . . . European Network for Non-invasive Investigation of Large Arteries. (2006). Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *European Heart Journal, 27*(21), 2588-2605. doi:ehl254 [pii]

Lefkowitz, R. J. (2007). Seven transmembrane receptors: Something old, something new. *Acta Physiologica (Oxford, England), 190*(1), 9-19. doi:APS1693 [pii]

Lehmann, E. D. (1999). Terminology for the definition of arterial elastic properties. *Pathologie-Biologie, 47*(6), 656-664.

Leung, A. A., Daskalopoulou, S. S., Dasgupta, K., McBrien, K., Butalia, S., Zarnke, K. B., . . . Hypertension Canada. (2017). Hypertension canada's 2017 guidelines for diagnosis, risk assessment, prevention, and treatment of hypertension in adults. *The Canadian Journal of Cardiology, 33*(5), 557-576. doi:S0828-282X(17)30110-1 [pii]

Lindholm, L. H., Carlberg, B., & Samuelsson, O. (2005). Should beta blockers remain first choice in the treatment of primary hypertension? A meta-analysis. *Lancet (London, England), 366*(9496), 1545-1553. doi:S0140-6736(05)67573-3 [pii]

Lofdahl, C. G., Marlin, G. E., & Svedmyr, N. (1983). Pafenolol, a highly selective beta 1-adrenoceptor-antagonist, in asthmatic patients: Interaction with terbutaline. *Clinical Pharmacology and Therapeutics, 33*(1), 1-9. doi:0009-9236(83)90557-X [pii]

Lucini, D., Pagani, M., Mela, G. S., & Malliani, A. (1994). Sympathetic restraint of baroreflex control of heart period in normotensive and hypertensive subjects. *Clinical Science (London, England : 1979), 86*(5), 547-556.

Mackenzie, I. S., Wilkinson, I. B., & Cockcroft, J. R. (2002). Assessment of arterial stiffness in clinical practice. *QJM : Monthly Journal of the Association of Physicians, 95*(2), 67-74.

Maffei, A., Vecchione, C., Aretini, A., Poulet, R., Bettarini, U., Gentile, M. T., . . . Lembo, G. (2006). Characterization of nitric oxide release by nebivolol and its metabolites. *American Journal of Hypertension, 19*(6), 579-586. doi:S0895-7061(05)01198-2 [pii]

Mahmud, A., & Feely, J. (2008). Beta-blockers reduce aortic stiffness in hypertension but nebivolol, not atenolol, reduces wave reflection. *American Journal of Hypertension, 21*(6), 663-667. doi:10.1038/ajh.2008.156 [doi]

Mallem, M. Y., Toumaniantz, G., Serpillon, S., Gautier, F., Gogny, M., Desfontis, J. C., & Gauthier, C. (2004). Impairment of the low-affinity state beta1-adrenoceptor-induced relaxation in spontaneously hypertensive rats. *British Journal of Pharmacology, 143*(5), 599-605. doi:sj.bjp.0705990 [pii]

Marano, G., Grigioni, M., Palazzesi, S., & Ferrari, A. U. (1999). Endothelin and mechanical properties of the carotid artery in wistar-kyoto and spontaneously hypertensive rats. *Cardiovascular Research, 41*(3), 701-707. doi:S0008-6363(98)00240-5 [pii]

Marieb, E. N., & Hoehn, K. (2013). *Human anatomy & physiology* (9th ed. ed.). Glenview, IL: Pearson Education Inc.

Marque, V., Kieffer, P., Atkinson, J., & Lartaud-Idjouadiene, I. (1999). Elastic properties and composition of the aortic wall in old spontaneously hypertensive rats. *Hypertension (Dallas, Tex.: 1979), 34*(3), 415-422.

Marque, V., Van Essen, H., Struijker-Boudier, H. A., Atkinson, J., & Lartaud-Idjouadiene, I. (2001). Determination of aortic elastic modulus by pulse wave velocity and wall tracking in a rat model of aortic stiffness. *Journal of Vascular Research, 38*(6), 546-550.

Matsushita, M., Tanaka, Y., & Koike, K. (2006). Studies on the mechanisms underlying beta-adrenoceptor-mediated relaxation of rat abdominal aorta. *Journal of Smooth*

Muscle Research = Nihon Heikatsukin Gakkai Kikanshi, 42(6), 217-225. doi:JST.JSTAGE/jsmr/42.217 [pii]

McEniery, C. M., Cockcroft, J. R., Roman, M. J., Franklin, S. S., & Wilkinson, I. B. (2014). Central blood pressure: current evidence and clinical importance. *European Heart Journal*, *35*(26), 1719–1725.

McEniery, C. M., Qasem, A., Schmitt, M., Avolio, A. P., Cockcroft, J. R., & Wilkinson, I. B. (2003). Endothelin-1 regulates arterial pulse wave velocity in vivo. *Journal of the American College of Cardiology, 42*(11), 1975-1981. doi:S0735109703012427 [pii]

McEniery, C. M., Yasmin, Hall, I. R., Qasem, A., Wilkinson, I. B., Cockcroft, J. R., & ACCT Investigators. (2005). Normal vascular aging: Differential effects on wave reflection and aortic pulse wave velocity: The anglo-cardiff collaborative trial (ACCT). *Journal of the American College of Cardiology, 46*(9), 1753-1760. doi:S0735-1097(05)01781-X [pii]

McVeigh, G. E., Allen, P. B., Morgan, D. R., Hanratty, C. G., & Silke, B. (2001). Nitric oxide modulation of blood vessel tone identified by arterial waveform analysis. *Clinical Science (London, England : 1979), 100*(4), 387-393.

Milnor, W. R. (1989). Properties of the vascular wall. *Hemodynamics* (2nd ed.,). Baltimore: Williams & Wilkins.

Mitchell, G.F., Pfeffer, M.A., Finn, P.V., Pfeffer, J.M. (1997). Comparison of techniques for measuring pulse-wave velocity in the rat. *Journal of Applied Physiology* (82) 203–210.

Morisco, C., Zebrowski, D. C., Vatner, D. E., Vatner, S. F., & Sadoshima, J. (2001). Beta-adrenergic cardiac hypertrophy is mediated primarily by the beta(1)-subtype in the rat heart. *Journal of Molecular and Cellular Cardiology, 33*(3), 561-573. doi:10.1006/jmcc.2000.1332 [doi]

Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries: Theoretic, Experimental and Clinical Principles*. 5th ed. London, England: Edward Arnold; 2005.

O'Donnell, S. R., & Wanstall, J. C. (1976). The contribution of extraneuronal uptake to the trachea-blood vessel selectivity of beta-adrenoceptor stimulants in vitro in guinea-pigs. *British Journal of Pharmacology*, *57*(3), 369–373.

O'Donnell, S. R., & Wanstall, J. C. (1985). Responses to the beta 2-selective agonist procaterol of vascular and atrial preparations with different functional beta-adrenoceptor populations. *British Journal of Pharmacology, 84*(1), 227-235.

Okamoto, K., & Aoki, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japanese Circulation Journal, 27*, 282-293.

Oriowo, M. A. (1994). Atypical beta-adrenoceptors in the rat isolated common carotid artery. *British Journal of Pharmacology, 113*(3), 699-702.

Oriowo, M. A. (1995). Different atypical beta-adrenoceptors mediate isoprenaline-induced relaxation in vascular and non-vascular smooth muscles. *Life Sciences, 56*(15), PL269-75. doi:0024320595000763 [pii]

Owens, G. K., & Schwartz, S. M. (1982). Alterations in vascular smooth muscle mass in the spontaneously hypertensive rat. role of cellular hypertrophy, hyperploidy, and hyperplasia. *Circulation Research, 51*(3), 280-289.

Palombo, C., & Kozakova, M. (2016). Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications. *Vascular Pharmacology, 77*, 1-7. doi:10.1016/j.vph.2015.11.083 [doi]

Pauca, A. L., Wallenhaupt, S. L., Kon, N. D., & Tucker, W. Y. (1992). Does radial artery pressure accurately reflect aortic pressure? *Chest, 102*(4), 1193-1198. doi:S0012-3692(16)34305-7 [pii]

Pinterova, M., Kunes, J., & Zicha, J. (2011). Altered neural and vascular mechanisms in hypertension. *Physiological Research / Academia Scientiarum Bohemoslovaca, 60*(3), 381-402. doi:932189 [pii]

Quirke, V. (2006). Putting theory into practice: James black, receptor theory and the development of the beta-blockers at ICI, 1958-1978. *Medical History, 50*(1), 69-92.

Rautureau, Y., Toumaniantz, G., Serpillon, S., Jourdon, P., Trochu, J. N., & Gauthier, C. (2002). Beta 3-adrenoceptor in rat aorta: Molecular and biochemical characterization and signalling pathway.*British Journal of Pharmacology, 137*(2), 153-161. doi:10.1038/sj.bjp.0704867 [doi]

Reaven, P. D., Sacks, J., & Investigators for the Veterans Affairs Cooperative Study of Glycemic Control and Complications in Diabetes Mellitus Type 2. (2004). Reduced coronary artery and abdominal aortic calcification in hispanics with type 2 diabetes. *Diabetes Care, 27*(5), 1115-1120.

Rettig, R. (1993). Does the kidney play a role in the aetiology of primary hypertension? evidence from renal transplantation studies in rats and humans. *Journal of Human Hypertension, 7*(2), 177-180.

Rhodes, J. M., & Simons, M. (2007). The extracellular matrix and blood vessel formation: Not just a scaffold. *Journal of Cellular and Molecular Medicine, 11*(2), 176-205. doi:JCMM031 [pii]

Rodrigues, S. F., Tran, E. D., Fortes, Z. B., & Schmid-Schönbein, G. W. (2010). Matrix metalloproteinases cleave the β2-adrenergic receptor in spontaneously hypertensive rats. *American Journal of Physiology - Heart and Circulatory Physiology*, *299*(1), H25–H35.

Santulli, G., & Iaccarino, G. (2013). Pinpointing beta adrenergic receptor in ageing pathophysiology: Victim or executioner? evidence from crime scenes. *Immunity & Ageing : I & A, 10*(1), 10-4933-10-10. doi:10.1186/1742-4933-10-10 [doi]

Saragoca, M., & Tarazi, R. C. (1981). Impaired cardiac contractile response to isoproterenol in the spontaneously hypertensive rat. *Hypertension (Dallas, Tex.: 1979), 3*(3), 380-385.

Sehgel, N. L., Zhu, Y., Sun, Z., Trzeciakowski, J. P., Hong, Z., Hunter, W. C., . . . Vatner, S. F. (2013). Increased vascular smooth muscle cell stiffness: A novel mechanism for aortic stiffness in hypertension. *American Journal of Physiology.Heart and Circulatory Physiology, 305*(9), H1281-7. doi:10.1152/ajpheart.00232.2013 [doi]

Sessa, W. C., Hecker, M., Mitchell, J. A., & Vane, J. R. (1990). The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: L-glutamine inhibits the generation of L-arginine by cultured endothelial cells. *Proceedings of the National Academy of Sciences of the United States of America, 87*(21), 8607-8611.

Shadwick, R. E. (1999). Mechanical design in arteries. *The Journal of Experimental Biology, 202*(Pt 23), 3305-3313.

Shafiei, M., & Mahmoudian, M. (1999). Atypical beta-adrenoceptors of rat thoracic aorta. *General Pharmacology, 32*(5), 557-562. doi:S0306362398002833 [pii]

Shekhonin, B.V., Domogatsky, S.P., Idelson, G.L., Koteliansky, V.E., Rukosuev, V.S. (1987). Relative distribution of fibronectin and type I, III, IV, V collagens in normal and atherosclerotic intima of human arteries. *Atherosclerosis*. 67(1) 9-16.

Shen, Y. T., Cervoni, P., Claus, T., & Vatner, S. F. (1996). Differences in beta 3-adrenergic receptor cardiovascular regulation in conscious primates, rats and dogs. *The Journal of Pharmacology and Experimental Therapeutics, 278*(3), 1435-1443.

Shirwany, N. A., & Zou, M. (2010). Arterial stiffness: a brief review. *Acta Pharmacologica Sinica*, *31*(10), 1267–1276. http://doi.org/10.1038/aps.2010.123

Steppan, J., Sikka, G., Hori, D., Nyhan, D., Berkowitz, D. E., Gottschalk, A., & Barodka, V. (2016). Seeking a blood pressure-independent measure of vascular properties. *Hypertension Research : Official Journal of the Japanese Society of Hypertension, 39*(1), 27-38. doi:10.1038/hr.2015.109 [doi]

Takata, Y., & Kato, H. (1996). Adrenoceptors in SHR: Alterations in binding characteristics and intracellular signal transduction pathways. *Life Sciences, 58*(2), 91-106. doi:0024320595022139 [pii]

Tan, I., Butlin, M., Liu, Y. Y., Ng, K., & Avolio, A. P. (2012). Heart rate dependence of aortic pulse wave velocity at different arterial pressures in rats. *Hypertension, 60*(2), 528-533. doi:10.1161/HYPERTENSIONAHA.112.194225 [doi]

Toda, T., Tsuda, N., Nishimori, I., Leszczynski, D. E., & Kummerow, F. A. (1980). Morphometrical analysis of the aging process in human arteries and aorta. *Acta Anatomica, 106*(1), 35-44.

Todd, M. E., Laye, C. G., & Osborne, D. N. (1983). The dimensional characteristics of smooth muscle in rat blood vessels. A computer-assisted analysis. *Circulation Research, 53*(3), 319-331.

Trochu, J. N., Leblais, V., Rautureau, Y., Beverelli, F., Le Marec, H., Berdeaux, A., & Gauthier, C. (1999). Beta 3-adrenoceptor stimulation induces vasorelaxation mediated essentially by endothelium-derived nitric oxide in rat thoracic aorta. *British Journal of Pharmacology, 128*(1), 69-76. doi:10.1038/sj.bjp.0702797 [doi]

Van Gorp, A. W., van Ingen Schenau, D. S., Hoeks, A. P., Struijker Boudier, H. A., Reneman, R. S., & De Mey, J. G. (1995). Aortic wall properties in normotensive and hypertensive rats of various ages in vivo. *Hypertension (Dallas, Tex.: 1979), 26*(2), 363-368.

Vulliemoz, Y., Verosky, M., & Triner, L. (1975). Effect of albuterol and terbutaline, synthetic beta adrenergic stimulants, on the cyclic 3',5'-adenosine monophosphate system in smooth muscle.*The Journal of Pharmacology and Experimental Therapeutics, 195*(3), 549-556.

Vuurmans, T. J., Boer, P., & Koomans, H. A. (2003). Effects of endothelin-1 and endothelin-1 receptor blockade on cardiac output, aortic pressure, and pulse wave velocity in humans. *Hypertension (Dallas, Tex.: 1979), 41*(6), 1253-1258. doi:10.1161/01.HYP.0000072982.70666.E8 [doi]

Wallace, S. M., Yasmin, McEniery, C. M., Maki-Petaja, K. M., Booth, A. D., Cockcroft, J. R., & Wilkinson, I. B. (2007). Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. *Hypertension (Dallas, Tex.: 1979), 50*(1), 228-233. doi:HYPERTENSIONAHA.107.089391 [pii]

Wang, M., Takagi, G., Asai, K., Resuello, R. G., Natividad, F. F., Vatner, D. E., . . . Lakatta, E. G. (2003). Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates. *Hypertension (Dallas, Tex.: 1979), 41*(6), 1308-1316. doi:10.1161/01.HYP.0000073843.56046.45 [doi]

Werstiuk, E. S., & Lee, R. M. (2000). Vascular beta-adrenoceptor function in hypertension and in ageing. *Canadian Journal of Physiology and Pharmacology, 78*(6), 433-452.

Wilkinson, I. B., MacCallum, H., Flint, L., Cockcroft, J. R., Newby, D. E., & Webb, D. J. (2000). The influence of heart rate on augmentation index and central arterial pressure in humans. *The Journal of Physiology, 525 Pt 1*, 263-270. doi:PHY_0449 [pii]

Wilkinson, I. B., MacCallum, H., Cockcroft, J. R., & Webb, D. J. (2002). Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. *British Journal of Clinical Pharmacology, 53*(2), 189-192. doi:1528a.doc [pii]

Williams, B., Lacy, P. S., Thom, S. M., Cruickshank, K., Stanton, A., Collier, D., . . . CAFE Steering Committee and Writing Committee. (2006). Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: Principal results of the conduit artery function evaluation (CAFE) study. *Circulation, 113*(9), 1213-1225. doi:CIRCULATIONAHA.105.595496 [pii]

Wolinsky, H., & Glagov, S. (1969). Comparison of abdominal and thoracic aortic medial structure in mammals. deviation of man from the usual pattern. *Circulation Research, 25*(6), 677-686.

Zieman, S. J., Melenovsky, V., & Kass, D. A. (2005). Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arteriosclerosis, Thrombosis, and Vascular Biology, 25*(5), 932-943. doi:01.ATV.0000160548.78317.29 [pii]