Vascular responsiveness in renal and hindquarter vascular beds: effect of hypertension

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Abstract

Hypertension is defined as a chronic elevation in arterial pressure. Established hypertension is typically accompanied by an elevation in peripheral vascular resistance, a critical determinant of arterial pressure. The aim of this study was to characterise changes in the vascular reactivity of key vascular beds, the renal and hindquarter vasculature, in an experimental preparation of hypertension. This study also explored the differential vascular remodelling and functional alterations that may occur between and within vascular beds of rabbits with hypertension.

To characterise changes in vascular responsiveness that accompany hypertension, a moderate level of hypertension was induced via bilateral renal cellophane wrap in the rabbit. Sham-operated rabbits served as normotensive controls. Flow probes were chronically implanted around the lower abdominal aorta and the left renal artery to enable measurement of hindquarter and renal blood flow, respectively. Haemodynamic responses to the graded intra-arterial infusion of constrictor and dilator drugs were measured in conscious hypertensive and normotensive rabbits with homeostatic reflexes intact or pharmacologically blocked. Wire myography was used to assess specific, local changes in the vascular reactivity of small arteries that may otherwise be lost in the assessment of integrated vascular bed reactivity in vivo. Emphasis was placed on characterising: i) the inherent reactivity of vascular smooth muscle to various stimuli; ii) endothelial function; and iii) the contribution of T-type voltage-operated calcium channels to vascular responses. The reactivity of an arterial segment was defined by the maximum active force (Emax) to applied constrictor agents or the maximum relaxation to vasodilator agents, as well as the sensitivity of the arteries (EC50) to these vasoactive stimuli.

Mean arterial pressure was 40 mmHg greater in wrap than in sham-operated normotensive rabbits. This study demonstrated that the structural vascular amplifier was present in the renal vasculature of conscious renal hypertensive rabbits and reaffirmed previous reports of its existence in the hindquarter vascular bed. Compared to constrictor dose-vascular conductance response curves constructed in normotensive rabbits, those in the hypertensive rabbit were characterised by a lower resting conductance (raised resistance), lower range and a more gradual slope. During maximal dilatation, renal and hindquarter vascular resistance were greater in the hypertensive rabbit. These haemodynamic patterns are consistent with a structurally-based amplification of vascular responsiveness caused by vessels with narrower lumens and an increased wall thickness to lumen ratio. In vitro characterisation of vascular reactivity in isolated vessels indicated that deep femoral resistance arteries and renal interlobar arteries have enhanced vasoconstrictor sensitivity (agonist-dependent) and normal endothelial function. Importantly, there was substantial heterogeneity in vascular responses to vasoactive stimuli between vascular beds, between species (rat and rabbit) and between sequential vessel segments within the renal vasculature.

In conclusion, there are interacting differential changes in structure and inherent vascular reactivity, across and within vascular beds, which contribute to the enhanced vascular responsiveness in rabbits with cellophane renal-wrap hypertension.
Declaration

The following declaration page, signed by the candidate:

This is to certify that:

i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,

ii. due acknowledgement has been made in the text to all other material used,

iii. the thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Makhala Michell Khammy

B.Sc. (Hons)

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Dedication

In loving memory of Thongdeth Khammy, the best dad one could hope for (1956-2003)

and

with deep gratitude to Professor Emeritus Paul Ivan Korner (1925-2012) for his never ending passion for the vascular amplifier and the original ideas for this PhD project.
Chapter 1

General introduction
Hypertension: a historical perspective

Hypertension is defined as a chronic elevation in arterial pressure and is a currently the leading risk factor for the global burden of disease (Lim et al., 2013). It was not until the 1900s however that high blood pressure was conclusively shown to be a cardiovascular risk factor (Kotchen, 2011). Hypertension, or rather its associated cardiovascular complications, is one of the oldest documented conditions affecting humans.

Ancient history: ‘Hard Pulse Disease’

Several ancient texts appear to have used the nuances of an individual’s arterial pulse to identify medical conditions. In an ancient Chinese text that describes the fundamental doctrines of Chinese medicine, it is written that:

‘one should observe whether the pulse is superficial or whether it is deep, whether it is regular or uneven; and then it becomes clear where the disease originates and it can be cured. Nothing surpasses examination of the pulse, for with it errors cannot be committed.’

In another excerpt, the author is likely to be describing the now known association between excess salt intake and high blood pressure:

‘If too much salt is used in food, the pulse hardens.’

The excerpts are taken from the English translation of Suwen, the first of two texts in the Huangdi Neijing otherwise known as The Yellow Emperor’s Classic of Internal Medicine (Veith, 1966). It is not the only text to place diagnostic importance on the ‘hard pulse’. The Pulse Classic from 280 AD is dedicated entirely to the rationale behind pulse-based diagnosis (Yang, 1997).

Historically, the management of ‘hard pulse disease’ or apoplexy involved decreasing the volume of blood in the patient, otherwise known as bloodletting. Bloodletting was advocated by numerous ancient physicians, though the methods employed may have differed. In addition to acupuncture, various ancient Chinese texts recommend bloodletting via venesection, in which blood is withdrawn from the large external veins (Ruskin, 1956). The Greek physicians Hippocrates (460-370 BC), Erasistratus (304-250 BC) and Galen (131-201 AD) also recommended bloodletting via venesection (Hippocrates & Adams, 1849; Esunge, 1991). Tablets from the Royal Library of Ashurbanipal in Nineveh (669-626 BC) used multiple methods of blood letting for the treatment of apoplexy including venesection, leeches and cupping (Esunge, 1991). Some ancient treatments for hypertension still persist to this modern day. Acupuncture may have synergistic effects with validated pharmacological interventions making it a suitable adjunct therapy for the treatment of hypertension (Flachskampf et al., 2007; Yin et al., 2007). Meanwhile, leeches are still commonly used in Russia. The former leader of the Soviet Union, Joseph Stalin, was infamously provided this treatment after he suffered a cerebral haemorrhage that was attributed to his hypertension. There is no doubt however, that our
knowledge of hypertension and its management has advanced considerably since the recognition of the ‘hard pulse disease’.

Modern History: Hypertension

The first step towards our modern understanding of high blood pressure could arguably be attributed to the identification of the circulatory system by the physician William Harvey in 1628. In his treatise, *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* (anatomical studies on the motion of the heart and blood in animals), Harvey proposed that blood was pumped by the heart around the body within a closed circulatory system in a unidirectional manner, the latter being facilitated by the valves in the veins and the heart (Harvey & Leake, 1958). It was not until later that techniques were developed to measure blood pressure. The earliest record of blood pressure measurement was in 1733 when Reverend Stephen Hales measured intra-arterial pressure in the horse using fine glass tubes (Hales, 1964).

The first description of the condition we now call hypertension can be attributed to the German clinician Samuel S. Schaarschmidt in a publication that spanned three volumes and that was published posthumously by his pupil Ernst Anton Nicolai in 1753 (Schaarschmidt & Nicolai, 1753). Schaarschmidt mentions a condition that involves the ‘spastic constriction of the vascular bed’ contributing to the ‘vehement agitation and motion of the blood’ and its close association with an ‘idiopathic’ haemorrhage that is distinct from its symptomatic counterpart and ‘traceable to no other preceding disease’ (Backer, 1944; Backer, 1953). Schaarschmidt’s and Nicolai’s conceptualisation of this condition have the same fundamental principles we now know about hypertension. It is well accepted that increased peripheral resistance contributes to a high blood pressure and that hypertension is associated with the development of cardiovascular disease. Perhaps the most incredible part is Schaarschmidt’s understanding of the nature of essential or ‘idiopathic’ hypertension.

The English physician Frederick Mahomed also demonstrated an incredible understanding of hypertension, differentiating essential idiopathic hypertension from secondary hypertension (O’Rourke, 1992):

‘thus, general disorder may cause high arterial pressure and this, in its turn, kidney changes; while on the other hand, kidney changes may be primary and acute and they may in their turn produce impurity of blood and this general pressure. But whether we read the tale backwards or forwards it is the same tale in the end…’

He also went on to describe the asymptomatic nature of high blood pressure, writing that:

‘these persons appear to pass on through life pretty much as others do and generally do not suffer from their high blood pressures, except in their petty ailments upon which it imprints itself... As age advances the enemy gains accession of strength...’
Mahomed and Schaarschmidt had a sound grasp of the fundamentals of hypertension well before the ideas became universally accepted: it is unfortunate that both remain widely uncredited for their contributions to this day. In between the time of Schaarschmidt and Mahomed was the physician Richard Bright. He published a case report of a patient who, judging by the text, was likely to have had hypertension and concomitant chronic nephritis and heart failure by today’s definition. Bright alluded to the idea that,

‘the enlarged state of the heart would seem to bespeak some cause of obstruction to the circulation through the system beyond what we discovered...’

In a subsequent tabular medical report Bright noted an intimate association between the diseased kidney and cardiac hypertrophy (Bright, 1836). Upon finding no local causes for the hypertrophy, Bright astutely postulated that renal affliction

“so affects the minute and capillary circulation as to render greater action necessary to force blood through the distant subdivisions of the vascular system”,

a concept synonymous to what we now refer to as a greater peripheral resistance in hypertension. By current classification, Bright was treating patients with secondary hypertension. Secondary hypertension is a form of hypertension in which the underlying cause of the elevated arterial pressure is known (in this case, chronic nephritis or Bright’s disease). It is now known however that high blood pressure often occurs in the absence of renal disease. Indeed, most individuals with hypertension have ‘essential hypertension’ where the underlying cause is unknown. In relation to Bright’s disease, Gull and Sutton (1872) had previously questioned the primary role of the kidney in the development of left ventricular hypertrophy and vascular changes noting that cardiovascular changes often preceded renal afflications. The diagnosis of asymptomatic hypertension however was only really possible following development of the mercury sphygmomanometer in 1896 by Scipione Riva-Rocci: the device enabled blood pressure to be routinely measured in the clinic (Booth, 1977). The recognition that high blood pressure was a risk factor that should be measured in the clinic was perhaps driven by the insurance industry which, by 1918, had started to require systolic and diastolic blood pressure measurements by auscultation for life insurance examinations (Kotchen, 2011).
1.2 Hypertension: current understanding

The underlying cause of the elevation in blood pressure may be known, as in secondary hypertension, or in most cases, unknown, as in primary hypertension (Carretero & Oparil, 2000; Mancia et al., 2013). Approximately 90-95% of hypertensive patients have primary hypertension while the remaining 5-10% have secondary hypertension (Chiong et al., 2008). In regards to primary hypertension, otherwise known as essential hypertension, there is still considerable debate over its aetiology and pathogenesis. Perhaps the current predicament could be best described with a quote from one of the great contributors to the field of hypertension:

“virtually any factor that might contribute to this multifaceted disorder of regulation apparently found not only its convincing support but also its refutation in this mountain of scientific accomplishment.”

Folkow (1982).

The only pieces of information that appear to have resounding support from the general scientific community is that i) primary hypertension is a multifactorial condition with a hereditary genetic component; ii) an inherited predisposition alone is generally insufficient to initiate and maintain an elevated blood pressure and iii) extrinsic factors such as environmental factors are often required to either initiate or sustain an elevation in blood pressure (Folkow, 1982).

On the other hand, secondary hypertension is used to describe a long-term increase in blood pressure that has an identifiable underlying cause (Chiong et al., 2008; Weber et al., 2014). It does not require a genetic component or reinforcing genetic factors and normally results from the machinations of an existing condition on components involved in the control of arterial pressure. For example, in renovascular hypertension, renal artery stenosis causes a decrease in renal blood flow and renal perfusion pressure (Derkx & Schalekamp, 1994). This elicits an endocrine-driven compensatory increase in systemic blood pressure that acts in a manner that corrects the decrease in renal perfusion pressure. Another common type of secondary hypertension is that resulting from primary hyperaldosteronism. In these patients the overproduction of aldosterone independently of excessive renin secretion leads to greater sodium and water retention, resulting in an elevated blood pressure (Stewart, 1999). There are a number of other identifiable causes of hypertension, including obstructive sleep apnoea, pheochromocytoma and pregnancy-induced hypertension amongst others (Onusko, 2003; Chiong et al., 2008).

Although the initiating mechanism in primary and secondary hypertension differs, the mechanisms responsible for the continued pressor effect may be shared. Structural adaptation of the cardiovascular system has been shown to propagate and reinforce elevations in blood pressure and is a common denominator amongst all forms of hypertension (Folkow, 1971; Jern, 1992; Heagerty et al., 1993; Schwartzkopff et al., 1993; Rizzoni et al., 1996; Schiffrin, 2004). As knowledge about
blood pressure control and the pathophysiological processes involved in hypertension increases, so too does our ability to diagnose and manage hypertension.
1.3 Classification, management and socio-economic burden

Essential hypertension was initially thought to arise from the inheritance of a Mendelian dominant gene. It was believed to be a qualitative deviation from the norm that manifested as a bimodal distribution in blood pressure (Platt, 1959). It is now accepted that blood pressure has a unimodal continuous frequency distribution and that hypertension is a quantitative deviation from the norm that is represented by the right tail end of the distribution curve (Pickering et al., 1959; Oldham et al., 1960). For this reason, setting a systolic and diastolic blood pressure cut-off value for the diagnosis of hypertension is somewhat arbitrary. It is especially difficult when we consider that blood pressure has a continuously graded association with cardiovascular risk (Collaboration, 2002). Regardless, blood pressure cut-off values are necessary in order to standardise the diagnosis and management of hypertension (Mancia et al., 2013).

There are several guidelines available from various committees on the diagnosis and management of hypertension. Table 1-1 summarises the most recent classifications recommended by various major committees for individuals over the age of 18 and less than 80. Across all major guidelines, the optimal blood pressure sits below 120 mmHg for systolic blood pressure (SBP) and 80 mmHg for diastolic blood pressure (DBP). Hypertension is generally classified as having a clinic blood pressure equal to or exceeding 140/90 mmHg on repeated measurements, although the target blood pressure is dependent on the patient group. In hypertensive patients with chronic kidney disease and proteinuria, the Canadian Hypertension Education Program set a lower target pressure of 130/80 mmHg (Hackam et al., 2013). In adults over 60 years of age with no diabetes or chronic kidney disease, the Eighth Joint National Committee (JNC8) recommended a target blood pressure of 150/90 mmHg (James et al., 2014). For most populations however, the target pressure is set at 140/90 mmHg.

The first line of treatment for hypertension uncomplicated by other cardiovascular risks is non-pharmacological based therapy such as lifestyle changes. Lifestyle changes may include regular aerobic exercise, abstinence from smoking, reduction in salt and alcohol consumption and also weight loss. Lifestyle changes have been shown to help decrease blood pressure and have the added benefit of also decreasing other cardiovascular risk factors (Ornish et al., 1990; Appel et al., 2003). The American Society of Hypertension and the International Society of Hypertension (ASH/ISH) recommend implementing lifestyle changes in individuals with blood pressures as low as 120/80 mmHg, a category termed prehypertension (Weber et al., 2014). The British Heart Foundation (BHF)(Williams et al., 2004) and the European Society of Hypertension and European Society of Cardiology (ESH/ESC)(Mancia et al., 2013) adopt a more lenient stance: individuals with a SBP between 120-129 mmHg and a DBP between 80-89 mmHg are considered to have a normal blood pressure. Lifestyle changes are recommended only in patients with a high-normal blood pressure and other risk factors. Pharmacological treatment is normally recommended in patients with stage 1 hypertension (SBP: 140-159, DBP: 90-99 mmHg) but only after lifestyle changes have been implemented and proven ineffective. More severe stages of hypertension are tackled with
immediate pharmacological intervention. A summary of the current pharmacological interventions used in the clinic is provided in Figure 1-1. Most patients will require a combination of antihypertensive drugs to achieve adequate blood pressure control.

Ultimately however, guidelines recommend that the management of hypertension depend on the individual’s global (or total) cardiovascular risk (Mancia et al., 2013). The majority of individuals with high blood pressure also have concomitant cardiovascular risk factors such as smoking, sex, age, diabetes and dyslipidaemia. These factors are also known to interact, resulting in the potentiation (rather than mere summation) of cardiovascular risk (D’Agostino et al., 2008). Various algorithms for the calculation of global cardiovascular risk and the management of hypertension have been proposed and the reader is directed to guidelines provided by the BHF, ESH/ESC and ASH/ISH for a more detailed discussion (Williams et al., 2004; Mancia et al., 2013; Weber et al., 2014).

Despite an abundance of antihypertensive agents that tackle various mechanisms of blood pressure control (Oparil & Schmieder, 2015), 10-20% of the hypertensive population remains resistant to treatment (Sander & Giles, 2011; Sim et al., 2013). Resistant hypertension refers to an uncontrolled blood pressure despite treatment with ≥ 3 antihypertensive agents of different classes at optimal doses (of which one is a non-potassium sparing diuretic) or where ≥ 4 antihypertensive agents are required to control blood pressure (Sander & Giles, 2011). Patients with uncontrolled blood pressure on ≥ 5 antihypertensive agents, known as refractory hypertension, accounts for approximately 0.5% of hypertensive patients (Calhoun et al., 2014). Two major interventional approaches have been proposed for the treatment of resistant hypertension: renal denervation and baroreflex activation therapy (Krum et al., 2014; Oparil & Schmieder, 2015). Renal denervation has been the subject of much discussion but its clinical efficacy has yet to be conclusively demonstrated (Bhatt et al., 2014; Epstein & De Marchena, 2015).
### Table 1-1. Classification of blood pressure levels recommended in recent guidelines developed for hypertension management

<table>
<thead>
<tr>
<th>Category</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>Optimal</td>
<td>Optimal</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Prehypertension</td>
</tr>
<tr>
<td>High-normal</td>
<td>High-normal</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 (mild)</td>
<td>Grade 1</td>
<td>Stage 1</td>
</tr>
<tr>
<td>Grade 2 (moderate)</td>
<td>Grade 2</td>
<td>Stage 2</td>
</tr>
<tr>
<td>Grade 3 (severe)</td>
<td>Grade 3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Committee</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHF</td>
<td>(Williams et al., 2004)</td>
</tr>
<tr>
<td>ESH/ESC</td>
<td>(Mancia et al., 2013)</td>
</tr>
<tr>
<td>ASH/ISH</td>
<td>(Weber et al., 2014)</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; BHF, British Heart Foundation; ESH/ESC, European Society of Hypertension and European Society of Cardiology; ASH/ISH, American Society of Hypertension and the International Society of Hypertension. Blood pressure levels are based on clinic blood pressure. If an individual’s systolic and diastolic blood pressures lie in different categories, the determining category is decided by the higher blood pressure value.
Figure 1-1. Summary of the major determinants that influence arterial pressure and the main classes of antihypertensive agents that affect these determinants

Blood pressure is the function of cardiac output (CO) and total peripheral resistance (TPR). Cardiac output and total peripheral resistance are determined by a number of factors. There are numerous classes of antihypertensive agents currently used in the clinic, all of which affect one or more determinants of arterial pressure. CCBs, calcium channel blockers; and ACE, angiotensin converting enzyme. Adapted from (Golan, 2005), Chapter 25.
1.4 Socio-economic burden

Currently, hypertension is one of the most common conditions afflicting humans. Approximately a third of the adult population in any community has hypertension, including communities in developed and developing countries. The number of total sufferers is predicted to reach 1.56 billion individuals by 2025 (Kearney et al., 2005). The socioeconomic burden of hypertension is substantial because it is a major risk factor for a range of cardiovascular complications such as stroke, coronary artery disease, heart failure and peripheral artery disease (Stamler et al., 1989; MacMahon et al., 1990; Kannel, 1996; Makin et al., 2001; Mancia et al., 2013; Rapsomaniki et al., 2014). It is also a cause (and complication) of chronic kidney disease (Levey et al., 2003; Levey et al., 2005). In the United States alone, the cost of cardiovascular disease for 2010 was estimated at $315.4 billion (Go et al., 2014).

Figure 1-2. Proportion of mortality causes in Australia.

1.5 Haemodynamic changes in hypertension

The change in haemodynamic pattern over the course of the development of hypertension has provided useful temporal information on the pathogenesis of the disease. The development of catheterisation techniques that enabled the direct measurement of cardiac output resulted in an influx of haemodynamic studies in hypertensive patients during the 20th century.

The initial developmental stage of hypertension, referred to as early hypertension in some studies, is often characterised by an elevated cardiac output and an unchanged or lower than normal total peripheral resistance. It was generally assumed that young adult patients with no cardiovascular complications and patients with borderline or labile hypertension (characterised by an occasional elevation of blood pressure) were in the early developmental stages of hypertension. Lund-Johansen (1967) and Sannerstedt (1966b) reported that young males with elevated blood pressure but no other cardiovascular diseases had significantly elevated cardiac output but similar total peripheral resistance at rest to normotensive age-matched controls. Elevated cardiac output has also been reported in the initial developmental stages of experimental models of secondary hypertension such as renal hypertension (Ledingham & Cohen, 1963), although not always (Olmsted & Page, 1965). Cardiac output was also raised in patients with borderline hypertension while total peripheral resistance was within normal limits (Finkelman et al., 1965) or decreased (Eich et al., 1962). In another study however, patients with labile hypertension had an elevated total peripheral resistance when corrected for cardiac output (Julius & Conway, 1968). These patients may be in a pre-hypertensive state and susceptible to developing established hypertension. In a longitudinal study of borderline hypertensive patients, the majority of the cohort had elevated TPR after 10 years with most having developed established hypertension after 15-18 years (Lund-Johansen, 1983).

Total peripheral resistance is almost always elevated in the advanced stages while cardiac output is either normal (Bolomey et al., 1949; Werkö & Lagerlöf, 1949; Lund-Johansen, 1967) or reduced (Sannerstedt, 1966; Frohlich et al., 1967). In a small clinical study that compared patients with essential hypertension with control patients with similar metabolic rate, heart rate, haematocrit distribution and cardiac output, systemic resistance was significantly elevated in hypertensive patients (Bolomey et al., 1949). The increase in resistance is shared across most vascular beds including the hepatic portal (Culbertson et al., 1951; Wilkins et al., 1952), cerebral (Kety et al., 1948) and renal vasculature (Bolomey et al., 1949). Males with severe hypertension who had developed hypertension-induced cardiovascular damage had lower cardiac output, presumably because the heart was no longer able to operate at maximum efficiency or capacity (Sannerstedt, 1966). Older males (40-49 years) with long-standing hypertension had a similar cardiac output index to normotensive individuals (Lund-Johansen, 1967). The haemodynamic pattern of males with hypertension aged 30-39 is heterogeneous, possibly representing a period of transition from an elevated cardiac output to a normal cardiac output but raised systemic resistance (Sannerstedt, 1966).
1.6 Hypertension: dysregulation of blood pressure control

The multifactorial nature of hypertension is perhaps a reflection of the complex nature of blood pressure control. Blood pressure is controlled by multiple interacting regulatory systems and is the product of cardiac output and total peripheral vascular resistance. Changes in the resistance vasculature are of particular interest because total peripheral resistance is commonly increased in patients with established hypertension, regardless of whether they have primary or secondary hypertension (Bolomey et al., 1949; Sannerstedt, 1966; Frohlich et al., 1967; Julius, 1988). Vascular resistance is determined by numerous factors including vessel geometry, the arrangement of vascular networks, properties of the blood (viscosity and flow), vascular function and the extravascular mechanical forces of surrounding tissue. These properties are all altered in hypertension and are both the cause and consequence of hypertension.

As it is a characteristic shared across essential and secondary forms of hypertension and occurs independently of the initial causative factor, targeting elevated total peripheral resistance may be a logical step for the treatment of hypertension. Various mechanisms have been proposed to explain the elevation in total peripheral resistance. These may be structural (structural-based vascular remodelling, rarefaction), functional (increased vascular smooth muscle sensitivity to vasoconstrictor agents, enhanced myogenic activity, elevated neurogenic and hormonal stimulation, endothelial dysfunction, rarefaction) or mechanical. The following section describes the contribution of each factor.

1.6.1 Structural determinants of blood pressure

The structure of the cardiovascular system is closely coupled to its function. The relationship is dynamic: the vasculature for example is known to undergo physiological adaptations in response to changes in tissue requirements and pressure load. In this regard, the giraffe vasculature is exemplary. In the giraffe, arterial pressure is approximately 100 mmHg in the neck and approaches 200 mmHg near the heart. Due to their height, the lower limbs of the giraffe are subject to high hydrostatic pressures of about 250-350 mmHg (Goetz & Budtz-Olsen, 1955; Van Gitters et al., 1966; Hargens et al., 1987; Østergaard et al., 2011; Petersen et al., 2013). Small resistance arteries in the leg of the giraffe have been shown to have a greater media thickness and also wall thickness to lumen ratio than those from the neck of the giraffe (Petersen et al., 2013). Both geometrical parameters were correlated with the distance of the vessel from the ground. These structural adaptations are thought to protect against pressure-induced vascular damage not only in the altered vessel itself but in distal capillaries.

The human vasculature also undergoes structural alterations. These alterations may be physiological or pathophysiological in origin and may have mal-adaptive consequences. The following section discusses the relationship between cardiovascular structure and haemodynamics with particular focus on the vascular system.
1.6.1.1 Vessel geometry

The physical properties of blood vessels have a large impact on local and systemic haemodynamics. If we consider a segment of a blood vessel, the flow of blood ($Q$) through this segment is dependent on both the pressure gradient that drives blood through the vessel ($\Delta P$) and the resistance that results from friction between the flowing blood and the intravascular wall ($R$). The relationship between these variables is described by Ohm’s law:

$$Q = \frac{\Delta P}{R} \tag{1}$$

where $\Delta P$ is the difference in pressure at the start ($P_1$) and end ($P_2$) of a vessel segment. If we assume blood to be an incompressible fluid that adopts smooth laminar flow within a blood vessel, then Poiseuille’s law can be applied to further our understanding of haemodynamics. Jean Léonard Marie Poiseuille was a French physiologist that dedicated much of his research to understanding the haemodynamics of the microcirculation. Through a series of elaborate experiments exploring the flow of various fluids through small glass capillaries, Poiseuille claimed that volumetric flow rate ($Q$) was dependent on pressure ($P$), the diameter ($D$) and length ($L$) of the pipe, and $K''$, which he used to describe the function of temperature and the type of liquid in question (Poiseuille & Herschel, 1940). The relationship between these variables is described as follows:

$$Q = \frac{K''PD^4}{L} \tag{2}$$

Poiseuille’s law (3) which has been extensively applied in the understanding of vascular resistance is actually a derivation of Poiseuille’s original equation (2) and written as follows:

$$Q = \frac{\pi D^4 P}{128\mu L} \tag{3}$$

Poiseuille’s law (3) is now also referred to as the Hagen-Poiseuille equation in late recognition of the contribution made by Gotthilf Heinrich Ludwig Hagen. It is thought to have been derived by Eduard Hagenbach from the Navier-Stokes equations describing the motion of fluid substances. It takes into account the dynamic viscosity ($\mu$) of fluids. The equation can be rearranged to calculate the pressure loss ($\Delta P$) as follows:

$$\Delta P = \frac{128\mu LQ}{\pi D^4} \tag{4}$$
This can be alternatively expressed using the internal radius \( r \) rather than the diameter \( D \):

\[
\Delta P = \frac{8\mu L}{\pi r^4}
\]  

\( (5) \)

Combining Ohm’s law (1) and the Hagen-Poiseuille equation (5) the following equation is obtained:

\[
R = \frac{8\mu L}{\pi r^4}
\]  

\( (6) \)

Vascular resistance is thus determined by the viscosity of blood, the length of the vessel and the internal radius of the vessel. This reveals an important relationship, while resistance is proportional to viscosity and vessel length; it is inversely proportional to the fourth power of the vessel radius:

\[
R \propto \frac{1}{r^4}
\]

Thus even small changes in the internal radius of a vessel can dramatically change resistance and in turn blood flow. Physiologically this means that small arteries and arterioles (R₁ precapillary resistance vessels), which have a greater wall thickness to lumen ratio than larger conduit arteries courtesy of a greater proportion of smooth muscle cells in the media layer, are high resistance vessels with a greater capacity to influence total peripheral resistance than vessels of a larger internal radius. Arterioles with diameters less than approximately 30 μm are thought to have the greatest influence over peripheral resistance (Pries et al., 1994). Capillaries, despite having the smallest internal radius, have little impact on systemic vascular resistance because their walls are composed of a single layer of endothelial cells.

1.6.1.1 Vascular structural adaptation

As mentioned, vascular structural adaptation is often a physiological response to changes in wall stress. The vascular wall is constantly exposed to mechanical stress and multiple regulatory responses are available to limit major deviations from the basal levels of stress. The vascular wall encounters stress from several axes. Blood provides a radial distending force that pushes the arterial wall outward (intravascular pressure, \( P_i \)) while extravascular tissue places compressional forces on the vessel (tissue pressure, \( P_t \)). Transmural pressure (\( \Delta P \)) is opposed by elastic components of the vascular wall that generate constricting forces. These distending and constricting forces are in balance at equilibrium and the resulting wall tension \( T \) can be derived using Laplace’s law (Figure 1-3). In addition to stretch forces, the vascular wall also encounters parallel haemodynamic forces from flow-induced shear stress. Transient changes in stretch and shear stress are normalised to basal levels via numerous functional regulatory mechanisms (see regulation of
vascular tone in section 1.6.2). However, if the vascular wall is subject to chronic elevations in stretch and stress, the vessel may undergo structural changes.

According to the LaPace law, wall tension is the product of the internal radius and intravascular pressure. Thus, an increase in intravascular pressure or an increase in internal radius (for example, due to vasodilatation), would place the vascular wall under greater stress, prompting hypertrophy of the vascular wall. Such structural remodelling is important; inward hypertrophy of vascular smooth muscle decreases lumen diameter (and increases the media to lumen ratio), resulting in a lower tension per unit layer of muscle. In this way, otherwise damaging levels of wall stress can be limited (Coffman, 2011). Unfortunately, structural remodelling that involves a decrease in the lumen diameter also increases vascular resistance and thus arterial pressure. Structural remodelling in the vasculature can thus be positive-feedback in nature: a minor sustained increase in arterial pressure may eventually procure a profound increase in pressure. An increase in pressure however, also activates a number of homeostatic reflexes that operate to blunt the pressure rise. The relationship between vascular structure and arterial pressure is thus a complex interplay between feedback and feed forward mechanisms.

\[
\Delta P = P_i - P_t
\]

Laplace's law:

\[
T = r_i \Delta P
\]

where

- \(\Delta P\) is transmural pressure
- \(P_i\) is intravascular pressure
- \(P_t\) is tissue pressure
- \(r_i\) is internal radius
- \(T\) is tension

Figure 1-3. Relationship between transmural pressure (\(\Delta P\)) and wall tension (T) as described by Laplace's law.
1.6.1.1.1 Morphological evidence of vascular remodelling

Structural changes in the vasculature have long been known to occur in established essential hypertension. In 1868, the physician George Johnson (1868) noted that patients presenting with chronic nephritis, a disease often accompanied by elevated blood pressure, had

“decided hypertrophy of the arterial walls in most tissues examined, not only in the kidneys, but also in the skin, the intestines, the muscles, and the pia matter.”

Indeed, many years later, we now know that the central cause underlying an elevation in vascular resistance is a decrease in lumen diameter. The major contributors to vascular resistance are the small arteries and arterioles. Alterations in the structure of these blood vessels can have a profound effect on vascular resistance by virtue of Poiseuille’s law.

The development of high resolution microscopes have allowed a closer examination of how vascular wall components alter in hypertension. Two forms of structural adaptation have now been defined: inward eutrophic remodelling and hypertrophic remodelling (Intengan & Schiffrin, 2000). This is diagrammatically represented in Figure 1-4. Both classes of structural remodelling result in a decreased lumen diameter and an increased media thickness to lumen ratio. However, inward eutrophic remodelling achieves this without changing the amount of wall material while inward hypertrophic remodelling involves hypertrophy of the media and subsequent encroachment of the lumen (Mulvany et al., 1996). The former involves rearrangement of the existing wall material around a smaller lumen and can be quantified using the remodelling index (Baumbach & Heistad, 1989; Heagerty et al., 1993). Vascular wall hypertrophy is quantified by the growth index (Heagerty et al., 1993). Although both eutrophic and hypertrophic remodelling contribute to structural changes in blood vessels in hypertension (Heagerty et al., 1993), the relative contribution depends on a number of factors, including but not limited to: the form of hypertension, disease progression, pathophysiology of the rise in blood pressure (and thus the model of experimental hypertension used), the vascular bed and branching order of the vessels studied.
**Inward eutrophic remodelling**

Rearrangement of existing wall material results in a smaller outer diameter and encroachment of the lumen. These vessels have:

- Decreased lumen diameter
- Increased wall thickness to lumen ratio

**Inward hypertrophic remodelling**

Media layer undergoes inward hypertrophy (outer diameter is unchanged). The media cross-sectional area is increased and these vessels have:

- Decreased lumen diameter
- Increased wall thickness to lumen ratio

**Figure 1-4 Schema showing the two classes of vascular structural remodelling that occurs in resistance vessels in hypertension.**

One form of remodelling may be favoured over the other depending on the factors present.
Hypertrophic remodelling

Hypertrophic remodelling has been reported in both essential and secondary hypertension, although the former is arguably associated with eutrophic remodelling (Mulvany, 1993). Resistance arteries isolated from gluteal subcutaneous biopsies and omental arteries of hypertensive patients exhibited a greater media thickness and media thickness to lumen ratio than those isolated from normotensive subjects (Aalkjaer et al., 1987; Rosei et al., 1995). A significantly smaller lumen diameter is not always reported in hypertensive patients. Similar internal diameters have been reported between arteries of normotensive and hypertensive patients (Aalkjaer et al., 1987; Rosei et al., 1995). Mesenteric resistance vessels isolated from spontaneously hypertensive rats had a thicker media layer, smaller lumen and a greater media thickness to lumen ratio than vessels isolated from control rats (Mulvany et al., 1978; Mulvany et al., 1980; Mulvany et al., 1985; Sharifi et al., 1998). These changes have been observed in 3 month old spontaneously hypertensive rats with lumen diameter being significantly smaller from an even younger age (6 weeks) (Mulvany et al., 1980). Media hypertrophy was only observed post any significant elevation in blood pressure, presumably when sufficient time under conditions of increased loading had passed for structural changes to occur (Mulvany et al., 1980). Vascular structural adaptations, at least in spontaneously hypertensive rats, may thus occur secondary to chronically elevated blood pressure and reinforce any further blood pressure rises through an elevated total peripheral resistance. Adams et al. (1989) reported that an elevated hindquarter vascular resistance (4 week old) preceded any blood pressure increases in the spontaneously hypertensive rat. It has been proposed that some individuals with essential hypertension may have a lower threshold for the autoregulatory structural remodelling defence response. That is, otherwise harmless pressure loads (i.e. transient increases in BP in response to stress) prematurely activate structural changes in the vasculature that would normally occur following sustained or large elevations in arterial pressure. Importantly, vascular remodelling is not uniform between vascular beds or between longitudinal segments within the same vascular bed. Hypertrophic remodelling tends to occur in the larger \( R_1 \) vessels (Furuyama, 1962; Schmid-Schönbein et al., 1986). Post-mortem histometrical analysis of renal and superior mesenteric arteries showed that medial hypertrophy was more pronounced in arteries with an internal radius exceeding 100 μm. It was non-existent in vessels with an internal diameter smaller than 10 μm (Furuyama, 1962).

Hypertrophic remodelling predominates over eutrophic remodelling in secondary forms of hypertension, particularly renal hypertension. Small resistance arteries isolated from patients with renovascular hypertension had a greater media thickness to lumen ratio than those from normotensive controls. These arteries exhibited both vascular (media) hypertrophy and remodelling (growth index, 53%; remodelling index, 70%) whereas arteries isolated from patients with essential hypertension or pheochromocytoma had a remodelling index of 93 to 94% (Rizzoni et al., 1996). Hypertrophic remodelling is also particularly prominent in severe models of renal hypertension. It has been reported in small coronary, mesenteric, femoral but not renal arteries of one-kidney, one clip renal hypertensive rats (Korsgaard & Mulvany, 1988; Deng & Schiffrin, 1991; Li et al., 1996). Mesenteric resistance arteries isolated from deoxycorticosterone acetate (DOCA)-salt hypertensive
rats (Deng & Schiffrin, 1992) and salt-treated Dahl-sensitive rats (d’Uscio et al., 1997) also present with characteristics typical of hypertrophic remodelling. It is important to note that structural adaptations are not uniform across different vascular beds in the same disease model. Whether or not this reflects a difference in the rate of structural remodelling across vascular beds is not known. However, particular arterial segments appear to undergo eutrophic rather than hypertrophic remodelling. The basilar artery from salt-treated Dahl-sensitive rats (d’Uscio et al., 1997) and small renal arteries in one-kidney, one clip renal hypertensive rats (Li et al., 1996) were both shown to exhibit characteristics of eutrophic remodelling.

**Eutrophic remodelling**

In essential hypertension and animal models of genetic hypertension, structural changes in resistance arteries are primarily due to the rearrangement of existing wall material. Subcutaneous resistance arteries isolated from hypertensive patients had a greater media thickness to lumen ratio in the absence of any change in media cross-sectional area or individual cell size compared to arteries of normotensive patients (Schiffrin et al., 1992; Korsgaard et al., 1993; Schiffrin et al., 1993; Falloon & Heagerty, 1994). In addition to hypertrophic remodelling, resistance arteries of spontaneously hypertensive rats also undergo eutrophic remodelling (Intengan et al., 1999c). There are some discrepancies between the morphology characteristics reported and those typical of eutrophic remodelling. Arteries isolated from hypertensive patients did not always have a smaller external diameter than those from normotensive subjects (Schiffrin et al., 1992). Furthermore, while Falloon and Heagerty (1994) reported a remodelling index of 65% (and a comparatively smaller growth index of 18%) in vessels of hypertensive patients, this was not accompanied by a smaller lumen.
1.6.1.1.2 Functional evidence of vascular remodelling

Theoretical considerations

At any given level of smooth muscle shortening, a vessel with a greater media thickness to lumen ratio would narrow to a greater extent and develop more resistance. Figure 1-5 shows a mathematical simulation of changes in resistance to smooth muscle shortening in hypothetical, structurally remodelled vessels. The resultant resistance-response curve has several distinguishing features from that obtained in a normal vessel (Folkow et al., 1970a):

i) higher resistance at maximal vasodilatation
ii) steeper resistance curve
iii) higher maximal response

Figure 1-5 demonstrates two important conclusions. Firstly, the features mentioned in the aforementioned hypothetical simulation are typical of those observed in resistance-response curves obtained in perfused hindquarters of the spontaneously hypertensive rat. Secondly, they do not support the idea that enhanced vascular responses are due to an enhanced sensitivity to vasoconstrictor agents. If in hypertension, vascular smooth muscle cells did indeed have greater sensitivity to vasoconstrictor agents, then one would expect the vessels to contract to a greater extent than vessels from normotensive subjects when exposed to the same concentration of a vasoconstrictor agent. In a concentration-response curve, this would manifest as:

i) a lower vasoconstrictor threshold but no change in resistance during maximal vasodilatation
ii) similar steepness
iii) an unchanged maximal response

compared to the curve produced in a normotensive vessel. The hypertensive resistance curve would lie parallel and to the left of the normotensive resistance curve (Figure 1-5) yet this is not the case.

Functional studies

The role of altered vascular structure in the elevation of systemic resistance in hypertension is also strongly supported by the finding that vascular resistance was elevated in the vascular beds of hypertensive patients and animals, even when these vascular beds were maximally dilated when vascular smooth muscle tone is presumably no longer an influence. In vivo measurement of blood flow via venous occlusion plethysmography showed that resistance is clearly elevated in maximally dilated vasculatures and vessels of hypertensive patients compared to normotensive subjects. This has been shown in the resistance vessels of the forearm (Folkow et al., 1958; Doyle et al., 1959; Conway, 1963), hand (Sivertsson & Olander, 1968; Sivertsson et al., 1976) and calf (Sivertsson & Hansson, 1976). Borderline hypertensive males and normotensive males with a familial disposition
for hypertension also have smaller forearm vasodilator capacity than their normotensive counterparts (Takeshita & Mark, 1980; Takeshita et al., 1982). The degree of resistance may also be positively related to the severity of hypertension with severe hypertensive patients having greater forearm resistance than mild hypertensive patients (Conway, 1963). With the exception of Doyle et al. (1959), resistance was derived from blood flow measurements made during reactive hyperaemia, a potent vasodilator stimulus wherein a brief arterial occlusion causes a transient elevation in blood flow. Exercise was often used as an additional vasodilator stimulus (Conway, 1963; Folkow, 1971; Sivertsson and Hansson, 1976; Sivertsson and Olander, 1968; Sivertsson et al., 1976), while some studies applied heat to minimise sympathetic vasoconstrictor fibre activity (Krog et al., 1960). Ischaemic exercise was also used in a study that measured blood flow through skeletal muscle via intramuscular injection of Xenon (Amery et al., 1969). Similar to studies using venous occlusion plethysmography, the calculated skeletal muscle vascular resistance after ischaemic exercise was higher in hypertensive patients than in normotensive controls. In conscious renal hypertensive (cellophane wrap) rabbits with autonomic block, hindquarter vascular resistance at maximal dilatation was significantly greater than that of normotensive rabbits regardless of the vasodilator used (acetylcholine, adenosine and serotonin) (Wright et al., 1987). In spontaneously hypertensive rats, vascular resistance was raised in perfused hindquarters (Adams et al., 1989), while total peripheral resistance was raised in the anaesthetised animal (Leenen et al., 1994).

Many of the haemodynamic patterns observed in hypertension can be explained by inward vascular remodelling of resistance vessels. Vascular reactivity can be assessed by infusing vasoconstrictor and vasodilator agents intra-arterially into a vascular bed. This allows the effect of vasoconstrictor and vasodilator agents to be assessed without significantly altering systemic blood pressure, limiting activation of central haemodynamic mechanisms. In numerous studies using hypertensive subjects or experimental hypertension, dose-response (resistance) curves generated in response to vasoconstrictor agents model over the resistance curve of hypothetically structurally-adapted resistance vessels. In hypertensive and normotensive subjects, Sivertsson and Olander (1968) measured blood flow during intra-arterial infusion of noradrenaline into the hand. The threshold dose of noradrenaline was similar between both groups indicating that both groups had similar sensitivity to noradrenaline. Moreover, the dose-response curve was steeper in hypertensive subjects than their normotensive controls. A similar dose-response curve (unchanged vasoconstrictor threshold and steeper curve compared to control curve) was observed when increasing doses of noradrenaline or angiotensin II were infused into the forearm of patients with hypertension (Egan et al., 1988). The maximum vascular forearm resistance was also greater in hypertensive than normotensive subjects regardless of the vasoconstrictor agent. As vascular smooth muscle is presumably maximally contracted when the maximal resistance response is recorded, the elevated resistance in hypertensive subjects may be attributable to a structural decrease in lumen diameter. Similar results have been reported in animal preparations of hypertension. Graded noradrenaline infusion into the hindquarter vascular bed revealed similar noradrenaline thresholds but greater maximal contraction and a steeper dose-response curve in SHR than matched normotensive control rats (Folkow et al., 1970a). In perinephritic hypertensive rabbits, vascular resistance responses to the vasoconstrictor
agonists methoxamine, noradrenaline and angiotensin II were all similarly enhanced despite similar ED$_{50}$, dose required to produce 50% of the maximum response and indicative of sensitivity (Wright et al., 1987; Wright & Angus, 1999). Many studies also show that patients with hypertension have greater rises in blood pressure to chemical and reflex stimuli than individuals with normal blood pressure (Hines & Brown, 1933; Zbrozyna & Krebbel, 1985), although not always (Pickering & Kissin, 1936; Alam & Smirk, 1939; Russek & Zohman, 1945).
The pattern of resistance changes in response to smooth muscle shortening in the hypertensive rat closely models theoretical expectations from mathematical simulations using hypothetical vascular remodelled resistance vessels. The left panel shows the average resistance response curves for SHR and the normotensive controls in response to graded infusions of noradrenaline. The red dotted line is the resistance curve one would expect if smooth muscle sensitivity to vasoconstrictor agents were enhanced (i.e., a parallel leftward shift). The right panel shows mathematically-simulated resistance response curves to incremental increases in smooth muscle shortening in two hypothetical vessels, H and N, where H has a thicker media layer (30%).

Modified from Folkow et al. (1970a).
Cellular mechanisms underlying vascular remodelling in hypertension

The cellular mechanisms underlying vascular remodelling are numerous and include: aberrated growth, differentiation and migration of vascular smooth muscle cells, abnormal production of extracellular matrix proteins, inflammation and fibrosis. The mechanisms are only briefly discussed here and readers are directed to comprehensive reviews (Intengan & Schiffrin, 2000).

Hypertrophy of the vascular wall has been attributed to two processes: i) hypertrophy, wherein vascular smooth muscle cells are enlarged (Korsgaard & Mulvany, 1988); and ii) hyperplasia, wherein there is an increased number of vascular smooth muscle cells (Mulvany et al., 1978; Lee, 1985; Mulvany et al., 1985). In some cases, both an increase in cell size and number are implicated (Rizzoni et al., 1996). Hypertrophy of non-contractile elements has also been reported in mesenteric resistance arteries of the spontaneously hypertensive rat (Lee et al., 1983) and arteries of hypertensive patients (Tobian & Binion, 1952; Egan et al., 1988). Renal vessels collected post-mortem from hypertensive patients had greater sodium and water content in the medial and intimal layers than those from normotensive patients (Tobian & Binion, 1952). It is not clear what drives these hypertrophic processes, although angiotensin II has been shown to stimulate growth through a number of signalling pathways (Touyz, 2005).

Apoptosis, or programmed cell death, is a process intimately associated with growth (Thompson, 1995). It is thus not surprising that apoptosis can modulate vascular remodelling (Hamet et al., 1996; Intengan & Schiffrin, 2001). It is uncertain whether apoptosis is a primary process in remodelling or whether it is a secondary growth compensatory mechanism. The incidence of apoptosis was lower in small arteries of young spontaneously hypertensive rats compared to normotensive controls and this proceeded any vascular remodelling (Dickhout & Lee, 1999). This differential incidence of apoptosis may continue into adulthood (Diez et al., 1997) and may contribute to the greater media volume observed in spontaneously hypertensive rats. In other instances, apoptosis may be increased in the vasculature of hypertensive animals (Hamet et al., 1995). In eutrophic remodelling, growth of the inner smooth muscle layers in combination with an increase in apoptosis localised to the outer layers may contribute to the remodelling of the media layer around a smaller lumen without altering the amount of existing media (Intengan & Schiffrin, 2000). Increased apoptosis may also lead to rarefaction of the vasculature in hypertension (see section 1.6.1.2).

Inflammation plays a critical role in the pathophysiology of hypertension and in vascular remodelling. In hypertension vascular inflammation involves chemotactic chemokine production, oxidative stress, vascular smooth muscle cell proliferation, aberrant extracellular matrix production, increased expression of adhesion molecules and the activation, recruitment and infiltration of inflammatory cells into the sub-endothelial space (Touyz, 2005; Virdis et al., 2014). Inflammation has also been implicated in initiating fibrosis. The restructuring of the extracellular matrix scaffold that occurs with vascular fibrosis can contribute to vascular remodelling. Vascular fibrosis involves the accumulation of structural extracellular matrix components such as collagen and elastin, and adhesion proteins
such as fibronectin and laminin (Intengan & Schiffrin, 2001). Increased synthesis of collagen has been reported in the vascular wall of small mesenteric and cerebral arteries of spontaneously hypertensive rats (Sharifi et al., 1998; Intengan et al., 1999c) and subcutaneous resistance arteries isolated from patients with essential hypertension (Intengan et al., 1999a).
1.6.1.1.4 Reversing structural remodelling

Small artery remodelling may also contribute to other complications of hypertension. Aberrant changes in the structure of small resistance arteries are now known to be an independent predictor of cardiovascular events in hypertension. The media thickness to lumen ratio of small resistance arteries appears to have prognostic value for cardiovascular events in hypertensive patients with medium or high cardiovascular risk and patients with uncomplicated essential hypertension (Rizzoni et al., 2003; De Ciuceis et al., 2007; Mathiassen et al., 2007). The prognostic value of small artery remodelling may stem from its association with target (end) organ damage. Structural changes in small resistance arteries may compromise tissue perfusion to major organs and are thought to precede clinical manifestations of target organ damage (Park & Schiffrin, 2001). Reversing structural alterations in the vasculature may thus procure other benefits besides a decrease in total peripheral resistance and arterial pressure. Most studies in hypertensive patients that assessed structural remodelling recruited untreated patients. In a rare long-term study, Sivertsson & Hansson (1976), reported that the difference in vascular resistance between hypertensive patients and control subjects was markedly attenuated following 5 years of antihypertensive treatment. Another small study demonstrated that subcutaneous resistance vessels isolated from patients who had been on antihypertensive therapy for a year, displayed morphological and functional abnormalities despite comparable blood pressure to normotensive controls. Structurally, the media thickness to lumen ratio was still elevated. Functionally, the vessels were unable to sustain a contraction and also had lower Ca\(^{2+}\) sensitivity and elevated catecholamine uptake compared to vessels isolated from normotensive controls (Aalkjaer et al., 1989). Reversing structural alterations in the vasculature procures other benefits besides a decrease in total peripheral resistance and arterial pressure. Structural changes in resistance arteries disturbs the blood supply to major organs, contributing to end organ damage (Park & Schiffrin, 2001). Indeed, vascular remodelling is an independent predictor of cardiovascular events in hypertension (Rizzoni et al., 2003; Mathiassen et al., 2007).

1.6.1.2 Arrangement of the vascular network

The human vascular system comprises components arranged both in-series and in-parallel. The major vascular networks (coronary, brachial, gastrointestinal, hepatic, renal, hindquarter) and their associated major distributing arteries are arranged in parallel, the exception being the gastrointestinal and hepatic vascular networks that lie in-series with each other. Each peripheral vascular bed is composed of a branching network of arterial and venous vessels arranged in-series and in-parallel. The main distributing artery gives rise to branches of small arteries. These then branch and narrow into arterioles which in turn branch into capillaries. The capillaries in this capillary network then converge to form venules which converge further to form veins. An arteriolar vascular network may be described as a bifurcating tree with multiple orders of branching (A\(_1\), A\(_2\), A\(_3\), A\(_4\)...)(Chen et al., 1981; Zweifach et al., 1981; Roy & Mayrovitz, 1982; Meininger et al., 1984) although it is rarely so simple or symmetrical (Engelson et al., 1985; Engelson et al., 1986). The vasculature in the skeletal muscle is arranged in an arcading manner, with the A\(_2\) level arterioles...
interconnecting with similarly sized arterioles rather than dichotomising. These interconnected arterioles then give off lateral branches called transverse arterioles that branch into the capillary network (Zweifach et al., 1981). The level of resistance proffered by precapillary resistance vessels depends on their branching echelon (Roy & Mayrovitz, 1982; Meininger et al., 1984). The larger precapillary vessels (denoted R1 and comprising the small arteries and large arterioles, A1-A2) are the largest contributors to systemic vascular resistance. Smaller precapillary vessels (denoted R2 and comprising the smaller higher order arterioles, A3, A4) are primarily responsible for regulating blood flow through the capillaries at a rate optimal for gas and material exchange. Studies of the pressure distribution of a vascular bed during hypertension have shown that the largest contributors to an increased vascular resistance are large arterioles and in particular small arteries proximal to the microcirculation (Joyner et al., 1981; Meininger et al., 1984; Prewitt et al., 1984; Imig & Anderson, 1991). Thus, structural and functional changes in these vessels carry the most impact in elevating arterial pressure.

There is evidence that rarefaction of the vascular bed contributes to the elevated total peripheral resistance in hypertension. There are two (O'Rourke, 1992) types of rarefaction: (i) functional rarefaction, which describes a decreased number of perfused arterioles in the vascular network without a decrease in the number of physical arterioles, and (ii) structural (anatomical) rarefaction, where there is a decrease in the number of arterioles physically present. Both result in a lower number of parallel pathways available for the flow of blood, leading to an elevation in vascular resistance. Rarefaction has been shown in the skeletal muscle (Hutchins & Darnell, 1974; Bohlen et al., 1977; Chen et al., 1981; Prewitt et al., 1982b) and mesenteric vasculature of SHR (Henrich et al., 1978). In these studies, arterioles of the SHR had a similar or larger lumen diameter than arterioles of comparable order in normotensive controls. Rarefaction has also been demonstrated in multiple branching orders of pial vessels in the cerebral cortex of deoxycorticosterone acetate (DOCA)-saline rats, renal hypertensive rats and SHR (Sokolova et al., 1985). In the cerebral cortex, rarefaction was accompanied by a decrease in internal luminal diameter of the pial arteries, particularly the larger arterioles (Sokolova et al., 1985). Rarefaction of the capillary network has also been shown. In addition to a decreased density of A3 and A4 terminal arterioles, Chen et al. (1981) showed a decreased capillary density in SHR cremaster muscle compared to WKY controls under conditions of innervation, denervation and sodium nitroprusside-mediated maximum vasodilatation. In human essential hypertension, decreased microcirculatory density has been reported in the arteriolar networks supplying the bulbar conjunctiva (Harper et al., 1978), as well as the capillary bed networks supplying the skin (Antonios et al., 1999b), forearm (Prasad et al., 1995), quadriceps and pectoralis major muscles (Henrich et al., 1988). Post-mortem analysis of patients with chronic hypertension also revealed decreased vascularity in the mesenteric vascular bed (Short & Thomson, 1959).

Rarefaction of the arteriolar vascular network in hypertension may be a long-term autoregulatory response that serves to protect the tissue from the passive increase in blood flow brought about by the elevation in systemic pressure. During the early stages of hypertension in SHR, rarefaction of the
arteriole network is primarily due to the functional non-perfusion of vessels. The occlusion is temporary as demonstrated by an unchanged density in maximally dilated vascular beds despite an apparent reduction when assessing innervated vascular beds. As the condition progresses, these chronically constricted vessels degenerate, resulting in anatomical (structural) rarefaction where there is a true physical decrease in the number of arterioles in the vascular network (Prewitt et al., 1982b). Similarly, rarefaction reported in the skin capillary beds of patients with essential hypertension is due to both anatomical and functional rarefaction, but more so the former (Serné et al., 2001). In any case, a decrease in the number of arterioles decreases parallel conductance passages leading to an elevated resistance that helps limit large and potentially damaging increases in blood flow volume through the capillaries.

The capillary bed also undergoes rarefaction. Capillaries consist of a single layer of endothelial cells and are thus incapable of altering their vascular tone. Thus, capillary beds may cope with an elevated perfusion pressure by increasing resistance by decreasing capillary density (Prewitt et al., 1982b). In human essential hypertension, capillary rarefaction is likely to be anatomical rather than functional (Prasad et al., 1995) and like in SHR (Prewitt et al., 1982b) may begin to occur in the early stages of hypertension (Noon et al., 1997; Antonios et al., 1999a).

The contribution of arteriolar rarefaction to the increase in regional and systemic vascular resistance is debated. Mathematical modelling suggests that rarefaction may account for 20-40% of the total increase in total peripheral resistance (Greene et al., 1989; Korner & Angus, 1992). An earlier study however, demonstrated that its contribution to the overall elevation in total peripheral resistance in hypertension may be comparatively little. Hallbäck et al. (1976) reported on the likely haemodynamic consequence of a decreased number of resistance vessels by simulating the decrease through graded microplugging. Microspheres were introduced into the isolated hindquarters of SHR until sufficient vessels had been blocked as to increase baseline resistance at maximal vessel dilatation by 50%. Resistance curves generated during rarefaction simulation were different in many respects to dose-resistance response curves constructed in the SHR or renal hypertensive animal.

1.6.2 Functional determinants of vascular tone

Blood vessels normally exhibit some level of vascular tone under basal conditions because there is always some degree of vascular smooth muscle contraction. The degree of vascular tone is dependent on the balance between vasoconstrictor and vasodilator factors and these factors may be intrinsic or extrinsic to the vasculature. The intrinsic properties of blood vessels (e.g. myogenic response and endothelial factors) as well as those of the surrounding tissue (e.g. metabolic activity) are particularly critical for the local regulation of blood flow, ensuring the metabolic requirements of the surrounding tissue are met. Local regulatory mechanisms enable local autoregulation, the inherent capacity of an organ to maintain optimal blood flow regardless of fluctuations in perfusion pressure. Local autoregulation has been reported in a number of vascular beds, including the cerebral (Harder, 1984), skeletal muscle (Meiningher et al., 1987), coronary (Kuo et al., 1988) and
renal vascular bed (Gilmore et al., 1980). Extrinsic factors are often neurohumoral in origin. The renin-angiotensin-aldosterone system and the autonomic nervous system are imperative to the regulation of vascular tone and total peripheral resistance. Numerous other humoral factors also play a role, including vasopressin, atrial natriuretic peptide and endothelin. This section further explores the main functional mechanisms critical to the regulation of vascular tone, with particular emphasis on regulatory processes altered in hypertension.

1.6.2.1 Smooth muscle sensitivity to vasoconstrictor agents

Enhanced smooth muscle sensitivity to specific vasoconstrictor agonists has been reported in hypertension. In forearm resistance vessels, hypertensive patients were more sensitive to noradrenaline than control subjects (Doyle et al., 1959). In conscious SHR, enhanced vascular resistance responses were only observed at low and moderate doses of methoxamine whilst phentolamine elicited smaller total peripheral resistance responses in SHR than the WKY controls (Leenen et al., 1994). With respect to pressor-response curves, SHR were more sensitive to angiotensin II than WKY controls but only at 16 weeks of age and not before (Toal & Leenen, 1985). In contrast, sensitivity to noradrenaline was similar between SHR and WKY controls regardless of age (Toal & Leenen, 1985). Perinephritic hypertensive rabbits (Hamilton & Reid, 1983) reportedly had enhanced pressor sensitivity to the α1-adrenoceptor agonists noradrenaline and phenylephrine, but not angiotensin II or the α2-adrenoceptor agonist guanabenz. However, when using only pressor responsiveness to draw conclusions about vascular smooth muscle sensitivity, hypertrophy or vasomotor activity, the results from whole-animal studies are rather equivocal, partly because mean arterial pressure is influenced by both vascular and cardiac parameters. If studies were performed without autonomic blockade then in the whole animal, as in Hamilton and Reid (1983), cardiovascular reflexes (which are altered in hypertension) remain intact, potentially confounding results.

There are also results from multiple in vitro studies indicative of increased smooth muscle sensitivity to vasoconstrictor agents. Isolated perfused mesenteric arteries of genetically hypertensive rats had greater reactivity to 5-hydroxytryptamine (5-HT) than normotensive Wistar rats. Although the 5-HT dose-response curves in the arteries of hypertensive rats had a steeper slope and enhanced maximal pressures typical of vessels with an enhanced wall to lumen ratio, the 5-HT threshold was also lower, indicating an increase in 5-HT sensitivity (Haeusler & Finch, 1972). McGregor and Smirk (1970) note that although the responses to noradrenaline, angiotensin II and 5-HT were all enhanced in perfused mesenteric arteries of genetically hypertensive rats, 5-HT responses were markedly more enhanced than either noradrenaline and angiotensin II. The disproportionate enhancement contrasts against that observed in conscious hypertensive rabbits (Wright et al., 1987; Wright & Angus, 1999) and suggests that the hyper-responsiveness to 5-HT is non-structurally mediated. In isolated mesenteric artery ring preparations noradrenaline sensitivity was enhanced in 6-, 12-, 20- and 24-week old SHR to a greater extent than age-matched WKY rats, but only following cocaine-mediated blockade of neuronal uptake (Mulvany et al., 1980). Acute adrenergic denervation using the neurotoxin 6-hydroxydopamine increased noradrenaline sensitivity in mesenteric arteries
isolated from 5 month old SHR more than those from WKY (Whall et al., 1980). Both studies suggest that noradrenaline sensitivity may be enhanced in the SHR but masked by a corresponding increase in neuronal uptake. Notably, the increased noradrenaline sensitivity preceded the rise in blood pressure in SHRs (Mulvany et al., 1980). Contrastingly, in isolated aortic strip preparations, where the wall to lumen ratio has no haemodynamic consequence, vascular responses to noradrenaline (Hallbäck et al., 1971) were not enhanced in the SHR relative to normotensive controls. Of course, results from one vascular bed are not always representative for the entire resistance vasculature and certainly even more care is required when extrapolating results to the in vivo setting.

Elucidating the contribution of enhanced vascular smooth muscle vasoconstrictor sensitivity to vascular hyper-reactivity is difficult because not only does it seem to be dependent on the vascular region but also on the progress of the disease state. Enhanced 5-HT responses were reported in isolated perfused mesenteric arteries of genetically hypertensive rats but not the hindquarter vasculature, while the renal vasculature tended to have smaller 5-HT responses compared to control rats (Haeusler & Finch, 1972). Moreover, as the disease state progresses, sensitivity to a particular vasoconstrictor or vasodilator agonist may increase or decrease. Although isolated perfused hindquarter preparations of SHR were initially much more sensitive to methoxamine than weight-matched WKY rats, this difference gradually diminished between 9- and 14-weeks of age such that 14-week old SHR and their controls shared similar sensitivity to methoxamine (Adams et al., 1989).

It is possible however, that increased vascular smooth muscle cell sensitivity to vasoconstrictor agents, in combination with a greater wall to lumen ratio, may explain hyper-reactivity to vasoconstrictor agents in hypertension. The relative contribution of the two factors may depend on the vascular bed being analysed, the specific vasoconstrictor agent as well as the state of disease progression. Increased noradrenaline sensitivity may be involved in the transition from a high cardiac output but normal TPR haemodynamic pattern in labile, early/mild hypertension to a normal cardiac output but raised TPR commonly seen in established hypertension.

### 1.6.2.2 The myogenic response

The myogenic response is the intrinsic ability of smooth muscle to respond to changes in intraluminal pressure or smooth muscle tension (Bayliss, 1902) and occurs independently of neural, humoral and metabolic factors (Folkow, 1949; Folkow, 1953; Folkow, 1964). When an increase in intraluminal pressure causes passive distension of an arteriole, the vessel constricts in response to the increase in smooth muscle tension. At the molecular level, an increase in pressure causes membrane depolarisation of vascular smooth muscle cells, potentially through the activation of transient receptor potential channels (Welsh et al., 2002; Earley et al., 2004). Membrane depolarisation then activates L-type voltage-operated calcium channels and the subsequent influx of Ca$^{2+}$ leads to the increase in intracellular Ca$^{2+}$ necessary for smooth muscle contraction (Davis & Hill, 1999). The myogenic response is thus an effective method of maintaining an optimum level of vascular tone and thus resistance. The magnitude of the myogenic response increases as vessel size decreases, although only down until the 2nd and 3rd order arterioles (Davis, 1993). Higher orders of
arteriolar branching have comparatively lower myogenic responsiveness (Davis, 1993). It is thought that by maintaining vascular resistance upstream, smaller downstream arterioles and capillaries are protected from gross changes in perfusion pressure that could otherwise damage the vascular integrity. Maintaining vascular resistance upstream also means that tissue perfusion can be maintained regardless of changes in perfusion pressure. Although the myogenic response is a mechanism intrinsic to the vascular smooth muscle and operates independently of neural, humoral and metabolic factors, it is modulated by said factors. Indeed, as the myogenic response is positive-feedback in nature, these factors may be required to limit the myogenic response and curtail large increases or decreases in transmural pressure. There are suggestions however that the myogenic response is self-limiting at high perfusion pressures (Davis, 1993).

### 1.6.2.3 Metabolic factors

The blood flow to tissue depends on the metabolic requirements of the tissue in question. A local feedback mechanism exists to ensure that equilibrium is maintained between the supply and demand of oxygen. Changes in local metabolic factors – such as the concentration of tissue metabolites and ions and the partial pressure of oxygen ($P_{O_2}$) and carbon dioxide ($P_{CO_2}$) – affect vascular smooth muscle tone through various signal transduction mechanisms. For example the tissue metabolite adenosine is a potent vasodilator agonist, particularly in the coronary vasculature (Drury & Szent-Györgyi, 1929; Wilson et al., 1990). As tissue metabolism increases, adenosine formation increases in response to the characteristic decrease in $P_{O_2}$, increase in $P_{CO_2}$ and decrease in pH in the interstitium. Other tissue metabolites and ions such as carbon dioxide, lactic acid (and thus H$^+$) and potassium ions also facilitate vasodilatation. As vasodilatation increases blood flow and returns interstitial $P_{O_2}$ back to adequate levels, less vasodilator factors are released and vascular tone increases.

### 1.6.2.4 Autocrine and paracrine factors

The endothelium plays an essential role in vascular homeostasis and is the source of multiple autocrine and paracrine factors that regulate vascular tone. As the intima is the innermost layer of a vessel, endothelial cells are directly exposed to haemodynamic forces and circulating mediators. The endothelium is thus in a prime location to both sense and respond to haemodynamic, humoral and local stimuli (Epstein et al., 1994; Topper & Gimbrone Jr, 1999). The endothelium can actively regulate smooth muscle function (Furchgott, 1983) and may influence structural changes in the vasculature (Epstein et al., 1994). Alongside the myogenic constriction inherent to vascular smooth muscle cells, the tonic release of vasodilator factors from the endothelium determines the basal vascular tone. Perhaps a good indication of the endothelium’s importance in vascular function is that endothelial dysfunction is often a hallmark of vascular diseases and a risk factor for cardiovascular diseases (Perticone et al., 2001; Endemann & Schiffrin, 2004; Brunner et al., 2005). Endothelial dysfunction is often a manifestation of hypertension. Impaired endothelial-dependent relaxation has been observed in subcutaneous resistance vessels isolated from patients with essential hypertension (Park et al., 2001; Park & Schiffrin, 2001). Impaired endothelial function has
also been reported in the forearm (Panza et al., 1990) and brachial artery (Park et al., 2001) of essential hypertensive patients. Endothelial dysfunction may also precede the condition (Taddei et al., 1996).

The endothelium regulates smooth muscle reactivity through the release of vasoconstrictor and vasodilator mediators (Furchgott & Vanhoutte, 1989). Although endothelial derived substances include nitric oxide, prostacyclin (prostaglandin I₂) and the endothelins, this section places particular emphasis on the vasodilator nitric oxide and the vasoconstrictor peptide endothelin-1, both of which have been extensively studied in the pathogenesis of hypertension.

**Nitric oxide**

Nitric oxide is an endothelium-derived substance that can rapidly diffuse into vascular smooth muscle cells to cause vasodilatation: possible only because of its small molecular size and lipophilic nature (Arnold et al., 1977; Ignarro et al., 1986). In the vascular smooth muscle cell, the molecule stimulates the production of cyclic GMP by activating soluble guanylate cyclase. The subsequent elevation in cyclic GMP causes smooth muscle relaxation (Ignarro, 1989). It is also a free radical – its half-life is measured in seconds (Angus & Cocks, 1987) – and is thus a suitable modulator of local vascular tone. The production of nitric oxide from intracellular L-arginine via endothelial nitric oxide synthase (Palmer et al., 1988; Palmer & Moncada, 1989; Rees et al., 1990) can occur in response to the activation of specific endothelial receptors or in response to the shear stress that results when blood flows over the endothelial layer. Basal vascular tone is predominantly dependent on flow-mediated nitric oxide production because even physiological levels of stress can stimulate nitric oxide production (Rubanyi et al., 1986; Kuchan & Frangos, 1994). Nitric oxide production also occurs following the activation of certain endothelial receptors. Receptor-mediated smooth muscle vasodilatation is generally transient (flow-mediated vasodilatation is more sustained) as it requires an increase in intracellular Ca²⁺ to facilitate formation of the Ca²⁺/calmodulin complex necessary for activation of endothelial nitric oxide synthase (Busse & Mülsch, 1990; Förstermann et al., 1991). In addition to modulating smooth muscle tone, the molecule also has a number of other physiological effects: it inhibits platelet aggregation and adhesion to the endothelium (Radomski et al., 1987; Radomski et al., 1990) and possesses anti-proliferative (Cornwell et al., 1994; Dzau et al., 2002) and anti-inflammatory properties (MacMicking et al., 1997). Given these protective properties and its ability to elicit vasodilatation, it is not surprising that decreased nitric oxide bioavailability has been implicated in the pathogenesis of hypertension. Endothelial dysfunction is thought to contribute to some extent to the rise in total peripheral resistance in hypertension and may be a consequence of diminished sensitivity to nitric oxide, lower nitric oxide bioavailability or enhanced release of endothelium-derived constricting factors (De Vriese et al., 2000).

**Endothelin-1**

Endothelin-1 is part of a class of potent vasoconstrictor peptides produced primarily in endothelial cells called endothelins (Yanagisawa et al., 1988). There are three known isoforms in humans (endothelin-1, 2 and 3) all of which are structurally and pharmacologically distinct from each other
(Inoue et al., 1989). Of these, endothelin-1 is thought to be an important paracrine mediator of vascular tone (Wagner et al., 1992). The peptide is able to mediate both vasoconstriction and vasodilatation. The former occurs via activation of either of the endothelin receptors localised to vascular smooth muscle: endothelin-A (ET\textsubscript{A}) and endothelin-B (ET\textsubscript{B}) (Wood et al., 1990; Sakamoto et al., 1991; Sumner et al., 1992; Seo et al., 1994; Haynes et al., 1995). The latter occurs through the release of nitric oxide and prostacyclin following activation of ET\textsubscript{B} receptors located on endothelial cells (De Nucci et al., 1988).

The endothelin system is important in the control of salt and water homeostasis and aberrations in this system have been reported in hypertension, particularly salt-sensitive hypertension (Boesen, 2015). ET\textsubscript{A}-receptor antagonism or dual blockade of the ET\textsubscript{A} and ET\textsubscript{B} receptor has been shown to lower blood pressure and reverse vascular hypertrophy in a number of experimental preparations of hypertension including angiotensin II infusion-induced hypertension (Alexander et al., 2001; Boesen et al., 2010), Dahl Salt-sensitive rats (Barton et al., 1998) and DOCA-salt sensitive but not renovascular hypertensive rats (Li et al., 1994; Li et al., 1996; Alcock et al., 1998). The role of the endothelin system in essential hypertension is unclear. Selective pharmacological blockade of the ET\textsubscript{A} receptor with intravenously infused BQ-123 lowered blood pressure in a genetic model of hypertension in the rat (Nishikibe et al., 1993). The non-selective ET\textsubscript{A/B} receptor antagonist TAK-044 decreased systemic blood pressure and peripheral vascular resistance in normotensive subjects (Haynes et al., 1996). In patients with essential hypertension, intra-arterial infusion of the selective ET\textsubscript{A} receptor antagonist BQ-123 increased forearm vascular blood flow. This elevation was enhanced when administered in combination with the selective ET\textsubscript{B} receptor antagonist BQ-788. Administration of BQ-788 alone however caused a relatively transient vasodilatation in hypertensive patients despite decreasing forearm vascular blood flow in normotensive patients. Similarly, in patients with chronic renal failure and elevated blood pressure, the selective ET\textsubscript{A} receptor antagonist BQ-123 decreased renal vascular resistance and systemic blood pressure while the selective ET\textsubscript{B} receptor antagonist BQ-788 did not (Goddard et al., 2004). Combination therapy decreased blood pressure without affecting total renal vascular resistance while the ET\textsubscript{B} receptor antagonist alone elevated blood pressure and caused systemic and renal vasoconstriction. Treatment with the orally active mixed ET\textsubscript{A} and ET\textsubscript{B} receptor antagonist over a period of 4 weeks significantly lowered the blood pressure of patients with essential hypertension (Krum et al., 1998).

1.6.2.5 Neurohumoral factors

Unlike the R\textsubscript{2} precapillary resistance vessels where vascular tone is modulated primarily by myogenic tone, the larger R\textsubscript{1} precapillary resistance vessels are extensively modulated by neurohumoral factors. Neural regulation of vascular tone primarily involves the sympathetic branch of the autonomic nervous system. The parasympathetic nervous system and sensory neurones also contribute (Hill et al., 2001), albeit probably less so, to the neural regulation of vascular responses.
Neural regulation

Perivascular sympathetic nerves are a major influence on vascular reactivity and tone. Activation of sympathetic nerve fibres running along the adventitia of blood vessels stimulates the release of neurotransmitters from nerve fibre varicosities that then alter vascular smooth muscle activity. Numerous neurotransmitters of sympathetic perivascular nerves have been identified including: noradrenaline, adenosine tri-phosphate and neuropeptide Y (Burnstock, 1976). Neurotransmitters may be released alone or with other neurotransmitters in a phenomenon described as cotransmission, where a single nerve is able to produce, store and release more than one transmitter (Burnstock, 1976). The relative proportion of each neurotransmitter depends on the vascular bed (Burnstock & Ralevic, 1994) and parameters of the stimulation (Kennedy et al., 1986; Todorov et al., 1999). This contributes to the heterogeneity reported in the vasomotor responses to neural factors between and within vascular beds. Further differences arise from the action of neuromodulating substances that are co-localised and co-released with neurotransmitters (Burnstock, 1976; Lundberg et al., 1982; Ekblad et al., 1984) and the activation of parasympathetic and sensory neurons. Synaptic transmission is modulated via various other mechanisms. In noradrenergic transmission, α₂-adrenoceptor activation feedbacks negatively to dampen noradrenaline release while neuronal uptake limits the concentration of noradrenaline in the junction.

There is evidence that essential hypertension is initiated but not necessarily maintained by an overactive sympathetic nervous system. The mechanisms driving this over-activation may include enhanced central sympathetic outflow and anomalous noradrenaline neuronal uptake. Direct measurement of multiunit postganglionic sympathetic activity via clinical microneurography has shown higher nerve firing rates in sympathetic fibres to the peripheral skeletal muscle vasculature of hypertensive (Schlaich et al., 2004) and borderline hypertensive patients (Anderson et al., 1989). The rate of sympathetic nerve firing can also be indirectly assessed by measuring noradrenaline spillover. The rate of noradrenaline release into plasma is thought to be proportional to the rate of sympathetic nerve firing (Esler et al., 1990). As clinical microneurography is limited to the nerve fibres of skin and skeletal muscle, measuring organ-specific noradrenaline spillover rate via radiotracer techniques offers important information on regional sympathetic activity (Esler et al., 1984b). In normal healthy humans, sympathetic nerve fibres supplying the lung are responsible for close to a third of total noradrenaline spillover. The kidneys and skeletal muscle are thought to be the next two major sites of noradrenaline release, contributing approximately 17-25% each to the total noradrenaline release into plasma. Comparatively, sympathetic nerves of the heart, liver, gastrointestinal tract and skin each contribute less than 10% to total noradrenaline release into plasma (Esler et al., 1984b; Esler et al., 1984c). One of the hallmarks of essential hypertension in young patients is elevated mean total noradrenaline spillover (Esler et al., 1984a; Esler et al., 1986; Esler et al., 1991; Schlaich et al., 2004). Renal and cardiac noradrenaline spillover are also elevated (Esler et al., 1984a; Esler et al., 1986; Esler et al., 1991; Schlaich et al., 2004). Curiously, the increase in cardiorenal sympathetic tone is only able to account for approximately half of the increase in mean total noradrenaline spillover (Esler et al., 1986). Elevated spillover has been
attributed to increased sympathetic nerve firing rates and blunted neuronal noradrenaline reuptake, particularly cardiac neuronal noradrenaline reuptake (Schlaich et al., 2004). Plasma catecholamine levels have also been used as an index of sympathetic activity. One of the major limitations however, is that plasma concentration also depends on the rate of removal of the catecholamine from plasma and thus, does not directly correspond to catecholamine release or sympathetic tone (Esler, 1993). Nonetheless, it remains useful as a rough indication of sympathetic activity. As is found with noradrenaline spillover studies, elevated plasma noradrenaline is more commonly reported in younger rather than in older hypertensive patients (Goldstein, 1983; Esler et al., 1986) and plasma noradrenaline levels are normal in established hypertension (Julius, 1988).

Sympathetic overactivity may be more important in creating the initial pressure load that causes structural vascular adaptation. In patients with labile hypertension (elevated cardiac output, normal TPR), concomitant administration of the non-selective β-antagonist propranolol and muscarinic antagonist atropine decreased cardiac output to levels comparable to normotensive subjects. Propranolol by itself failed to completely decrease cardiac output to normal levels. The results suggest that sympathetic and parasympathetic activity are increased in labile hypertension (Julius et al., 1971). In experimental hypertension, immunosympathectomy-mediated ablation of the peripheral sympathetic nervous system of neonatal SHR and genetically hypertensive New Zealand rats protected against the development of hypertension (Clark, 1971; Folkow et al., 1972; Lee et al., 1987). However, although blood pressure was comparable to that of normotensive control rats, it was still higher than the blood pressure of immunosympathectomised normotensive control rats. This suggests that other non-neurogenic mechanisms are present to maintain the elevated blood pressure (Folkow et al., 1972). In the absence of a large elevation in pressure load, vascular walls of immunosympathectomised hypertensive rats were still hypertrophied. The wall composition however, was altered. The increased wall thickness was attributed to hypertrophy of adventitia in the tunica externa rather than smooth muscle cell hyperplasia (Lee et al., 1987).

**Renin-angiotensin system**

The renin-angiotensin system plays an important role in salt and water homeostasis and blood pressure regulation. In addition to the systemic renin-angiotensin system, individual tissues may possess their own local renin-angiotensin system (Paul et al., 2006). The kidney, for example, has a local intrarenal renin-angiotensin II system that operates independently from its systemic counterpart (Giani et al., 2015).

Like the sympathetic nervous system, aberrant activation of the renin-angiotensin system can contribute to hypertension development. The renin angiotensin system comprises two main opposing regulatory components. The peptide angiotensin II exerts its actions via two receptors: AT₁ and AT₂. The majority of the hypertensinogenic effects associated with activation of the renin-angiotensin system are mediated via the AT₁ receptor, including: vasoconstriction (Finnerty, 1962), proliferation of smooth muscle cells (Geisterfer et al., 1988; Daemen et al., 1991), aldosterone synthesis and thus salt and water retention. The peptide may elicit vasoconstriction directly, through the activation
of angiotensin II receptors (de Gasparo et al., 2000), and indirectly, by stimulating the production of other vasoconstrictor agents or augmenting sympathetic activity (Reid, 1992). Direct vasoconstriction is mediated by the angiotensin II receptor subtype 1 (AT₁) localised to vascular smooth muscle cells (de Gasparo et al., 2000). Angiotensin II also stimulates the production of other vasoconstrictor factors including endothelin-1 (Emori et al., 1991; Dohi et al., 1992).

The counter regulatory component of the renin-angiotensin system is mediated by angiotensin (1-7), a cleavage product of angiotensin II and angiotensin I (Ferrario et al., 2005; Paul et al., 2006). Angiotensin (1-7) counter regulates the actions of angiotensin II (Ferrario et al., 1997) by facilitating a multitude of vasodilator mechanisms (Li et al., 1997b; Li et al., 1997a) and anti-proliferative effects (Tallant et al., 1999). The two components of the renin-angiotensin system are not independent of each other. Nor are they limited to purely vasoconstrictor or vasodilator responses. Angiotensin II, for example, predominantly elicits vasoconstriction but is also capable of mediating dilatation via vasodilator prostaglandins (Aiken & Vane, 1973; Toda & Miyazaki, 1981; Schuijt et al., 2001) and the nitric oxide-bradykinin-cyclic guanylate cyclase AMP pathway (Siragy & Carey, 1997). The interaction between the two arms of the renin-angiotensin system is bidirectional and complex: it is the balance of the two arms that dictates whether vasoconstriction or vasodilatation predominates (Ferrario et al., 2005).

### 1.6.2.6 Voltage-operated calcium channels

Calcium (Ca²⁺) is a ubiquitous intracellular signalling molecule that facilitates a myriad of cellular functions including vasoconstriction and vasodilatation (Berridge et al., 2003). Ca²⁺ involved in signalling may be sourced from the extracellular environment or internal stores. In general, membrane depolarisation-mediated contractions are reliant on the influx of extracellular calcium through voltage-operated calcium channels whereas agonist-mediated responses tend to utilise calcium released from intracellular stores.

In vascular smooth muscle, the balance between calcium influx into the cell and extrusion out of the cell is a major determinant of vascular tone and thus resistance and blood pressure (Nelson et al., 1990). At physiological transmural pressures, resistance arteries and arterioles exist in a state of partial constriction. As discussed in section 1.6.2.2, this is due to an intrinsic myogenic contractile response of vascular smooth muscle cells. An elevation in pressure causes membrane depolarisation and activation of voltage-operated calcium channels, specifically the L-type voltage-operated calcium channels. Once opened, L-type channels mediate the intracellular influx required for the maintenance of persistent vascular tone. The importance of this process in regulating smooth muscle tone is highlighted by the clinical use of calcium channel blockers. Calcium channel blockers are a major class of antihypertensive treatment and their effectiveness lies largely in their ability to decrease vascular resistance (Weber et al., 2014).
1.7 The renal vasculature and arterial pressure regulation

The kidneys play such an integral role in the regulation of arterial pressure that for years aberrations in the kidney were considered the prime causes behind hypertension. Richard Bright is noted for his insight into the diseased kidney, having identified what is now termed acute or chronic nephritis, inflammation of nephrons in the kidneys (Bright, 1836). A less renowned but no less astounding observation was made in a short tabular medical report where he noted an intimate association between the diseased kidney and cardiac hypertrophy. Upon finding no local causes for the hypertrophy, Bright astutely postulated that renal affliction

"so affects the minute and capillary circulation as to render greater action necessary to force blood through the distant subdivisions of the vascular system",

a concept synonymous to what we now refer to as a greater peripheral resistance in hypertension. He would be among the first of many to implicate the kidney in hypertension and blood pressure control.

1.7.1 Vascular architecture

The basic anatomical arrangement of the renal vasculature is common across multiple mammalian species (Moffat & Fourman, 1963; Kriz, 1981; Pallone et al., 1990). The main renal artery generally branches into 5 segmental arteries just prior to the hilum of the kidney. The segmental arteries then branch into a total of 6-10 interlobar arteries that ascend within the renal pelvis to run between the renal pyramids. These arteries penetrate the renal parenchyma and branch to form arcuate arteries that travel along an arc near the corticomedullary boundary. Interlobular arteries branch off from arcuate arteries at right angles to ascend through the cortex towards the renal capsule, eventually terminating as end arteries or superficial glomeruli afferent arterioles. Afferent arterioles also branch off the side at multiple sites along the interlobular arteries to supply juxtamedullary glomeruli (juxtamedullary afferent arterioles). The glomeruli capillaries then reunite to form efferent arterioles. While efferent arterioles of juxtamedullary glomeruli form capillaries that supply the inner zone of the medulla, efferent arterioles of cortical glomeruli branch into peritubular capillaries that form a capillary plexus around tubules in the cortex.

1.7.2 The cortical and medullary microcirculation

The renal vasculature is composed of two distinct microcirculations that are differentially regulated: the cortical and medullary circulations. Medullary blood flow is primarily dependent on flow through the efferent arterioles of juxtamedullary glomeruli while cortical blood flow is dependent on flow through cortical glomeruli. The two microcirculations have vastly different pharmacodynamic profiles (Table 1-2). These differential responses may provide a means of independently regulating medullary and cortical blood flow. Given that different regions of the kidney have varying degrees of influence over blood pressure, characterisation of the differential responses to various factors is of considerable importance.
In contrast to cortical blood flow, medullary blood flow appears to be relatively insensitive to the effects of vasoconstrictor agonists. Noradrenaline, endothelin-1 and angiotensin II have little effect on medullary blood flow despite the presence of receptors for all three agonists in the medulla (Yukimura et al., 1996; Miyata et al., 1999). In a number of studies, intravenous noradrenaline infusion decreased cortical but not medullary blood flow in anaesthetised rabbits and rats and conscious rabbits (Parekh & Zou, 1996; Correia et al., 2000; Evans et al., 2000a; Oliver et al., 2002). Renal sympathetic nerve stimulation, which releases noradrenaline amongst other neurotransmitters, has also been shown to influence medullary blood flow to a lesser extent than cortical blood flow (Leonard et al., 2001; Guild et al., 2002; Eppe et al., 2004). Although activation of α2-adrenoceptor-mediated nitric oxide production has been shown to oppose α1-adrenoceptor-mediated vasoconstrictor effects of noradrenaline in the medulla (Zou & Cowley, 2000), this dilatory mechanism is not specific to the medullary circulation and thus unlikely to account for the relative insensitivity of the medulla circulation to renal nerve stimulation (Eppe et al., 2004).

As with noradrenaline, intrarenal artery or intravenous endothelin-1 infusion decreased total renal blood flow and cortical blood flow but not medullary blood flow in anaesthetised or conscious rabbits (Evans et al., 2000a; Evans et al., 2000b; Weekes et al., 2000; Rajapakse et al., 2002). Similarly, angiotensin II has been shown to either increase or not change medullary blood flow despite decreasing cortical blood flow. Intravenous angiotensin II infusion did not alter medullary blood flow in anaesthetised rats (Parekh & Zou, 1996), conscious rabbits (Evans et al., 2000a) or anaesthetised rabbits in which renal arterial pressure had been fixed at 65 mmHg (Evans et al., 2000b). Direct intrarenal artery angiotensin II infusion into anaesthetised rabbits elicited a biphasic response characterised by an initial decrease that was followed by an increase in medullary blood flow (Rajapakse et al., 2002). The only vasoconstrictor agonist that appears to consistently lower medullary blood flow is vasopressin. Intravenous infusion of the selective V1 receptor agonist [Phe2, Ile3, Orn8]-vasopressin decreased medullary blood flow without affecting cortical or total renal blood flow (Nakanishi et al., 1995; Franchini & Cowley Jr, 1996; Parekh & Zou, 1996; Evans et al., 2000a; Evans et al., 2000b). Nakanishi et al. (1995) further examined regions of the medulla and found that vasopressin decreased inner medullary blood flow (-35%) to a greater extent than outer medullary blood flow (-15%) in anaesthetised renal-denervated rats.

The lack of response to vasoconstrictor agents has been attributed to the difference in vascular geometry of cortical and juxtaglomerular arterioles as well as the presence of local counter-regulatory dilator mechanisms in the medullary circulation (Evans et al., 2004). Briefly, juxtaglomerular arterioles have a larger internal diameter than the arterioles of cortical glomeruli. Given that vascular resistance is inversely proportional to the fourth power of the internal radius, a given level of smooth muscle shortening caused by a given concentration of constrictor agonist, would cause a greater resistance increase in the cortical than the medullary microcirculation. Furthermore, juxtamedullary glomeruli have a unique geometrical structure that dampens resistance changes: efferent arterioles of juxtamedullary glomeruli have a thicker muscular wall and a larger internal diameter than afferent arterioles (Trueta & Barclay, 1947; Denton et al., 1992).
The medullary circulation is generally more susceptible to vasodilator factors than the cortical circulation. Nitric oxide (Mattson et al., 1992; Mattson et al., 1997; Zou & Cowley, 1997) and prostaglandins such as prostacyclin and PGE2 (Roman & Lianos, 1990; Agmon & Brezis, 1992; Oliver et al., 2002) are an important determinant of basal medullary tone. The concentration of interstitial nitric oxide is higher in the medulla than the cortex and accordingly, an enhanced nitric oxide synthase activity has been reported in the medulla (Mattson & Higgins, 1996; Zou & Cowley, 1997; Cowley et al., 2003). Although the medulla and cortical circulations have comparable sensitivity to nitric oxide (Kalyan et al., 2002), the medulla circulation appears to be more effective at producing and releasing nitric oxide once stimulated (Rajapakse et al., 2002; Cowley et al., 2003). Like noradrenaline, endothelin-1 and angiotensin II may elicit nitric oxide-mediated vasodilation via activation of the AT1 and ETB receptor, respectively (Gurbanov et al., 1996; Mattson et al., 1997; Zou et al., 1998; Hercule & Oyekan, 2000; Dickhout et al., 2002; Rajapakse et al., 2002). Renal prostaglandins have also been shown to dilate medullary vessels in response to vasoconstriction elicited by certain agonists (Parekh & Zou, 1996). In this manner, the vasoconstrictor effects of these factors are dampened by opposing vasodilator effects.

The regulation of medullary blood flow is particularly interesting given that the medullary microcirculation has been implicated in the long-term control of arterial pressure. The chronic infusion of vasoconstrictor agents into the medullary interstitium at doses that affect medullary blood flow but not cortical blood flow has been shown to cause hypertension (Cowley Jr, 1997). Additionally, the chronic infusion of vasodilator agonists at doses that preferentially altered medullary blood flow in the spontaneously hypertensive rat blunted the degree of hypertension (Cowley Jr, 1997).

1.7.3 The renal vasculature and the pressure-natriuresis mechanism

The kidney is integral to the long-term control of arterial pressure, in part due to the pressure-natriuresis mechanism (Guyton et al., 1972; Guyton, 1991). This mechanism involves modulation of sodium and water excretion, and thus extracellular fluid volume, in response to deviations in arterial pressure from its set point. For example, elevation of mean arterial pressure (and thus renal arterial pressure) above its set point prompts an increase in sodium and water excretion, thus lowering extracellular fluid volume, blood volume and arterial pressure (Cowley Jr, 1992; Cowley Jr & Roman, 1996; Cowley Jr, 1997). Conversely, lowering of arterial pressure below its set point (and thus renal arterial pressure) prompts the pressure-natriuresis mechanism to preserve sodium and water, thereby increasing blood volume and arterial pressure. The level of sodium and water reabsorption is believed to correlate with changes in renal interstitial hydrostatic pressure (Mattson, 2003; Evans et al., 2005). Thus, the pressure-natriuresis mechanism is dependent firstly, on the ability to detect deviations from the set arterial pressure, and secondly, on the ability to communicate this pressure change to the renal interstitium. The latter is facilitated by the relatively poor autoregulatory capacity of certain parts of the medullary circulation (namely the vasa recta capillaries)(Mattson et al., 1993): a change in renal arterial pressure proportionally alters the pressure in the medullary circulation (Mattson, 2003). The change in medullary perfusion pressure, specifically through the vasa recta
capillaries, then proportionally alters renal interstitial hydrostatic pressure (Cowley Jr, 1997; Mattson, 2003). Thus, the renal arterial pressure and renal interstitial hydrostatic pressure share an almost linear relationship (Komolova & Adams, 2010).

1.7.4 The kidney and hypertension

The importance of the kidney in the development and maintenance of high blood pressure is clearly demonstrated in a number of renal transplant studies in experimental preparations of hypertension and patients.

Renal homografts between rats with opposing genetic predispositions to hypertension caused a chronic reversal of the hypertensive phenotype (Bianchi et al., 1974; Dahl et al., 1974). Bilaterally nephrectomised Milan Hypertensive rats transplanted with kidneys from normotensive controls achieved and maintained normal blood pressure regardless of age (Bianchi et al., 1973; Bianchi et al., 1974; Fox & Bianchi, 1976). Conversely, following bilateral nephrectomy, Milan Normotensive rats that received a renal homograft from Milan Hypertensive rats developed hypertension. Similar results have been reported regardless of the strain of primary hypertension. Post-transplantation, hypertension of similar severity to the donor strain was observed when borderline hypertensive rats received a kidney from a spontaneously hypertensive rat, whilst the transplantation of a WKY kidney normalised blood pressure (Kawabe et al., 1978).

Besides animal models of essential hypertension, a similar result has been reported in humans. Patients with essential hypertension and subsequent kidney failure that had received kidney transplants from normotensive donors had normal blood pressure when followed up 4.5 years later (Curtis et al., 1983). In an observational prospective study of 85 transplanted patients with 8 years follow up, recipients without a history of familial hypertension were ten-fold more likely to require antihypertensive treatment if the donor was hypertensive than normotensive. However in recipients with familial hypertension the requirement for antihypertensive therapy was not influenced by the donor’s blood pressure status. Familial hypertension appears to blunt the elevated blood pressure induced by a hypertensive donor kidney (Guidi et al., 1996). The results from these studies are conveniently explained by an intrinsic genetic renal fault and perhaps this is why the Dahl dictum ‘hypertension follows the kidney’ gained such support. It is now known that things are not so simple, certainly the renal genetic profile influences hypertension development but it likely does so alongside other factors.

Firstly these series of cross-transplantation studies do not discount extrarenal influences. Dahl S rats with a Dahl Salt Resistant (Dahl R) kidney are protected from hypertension only if they are on a low salt diet. If dietary salt intake is increased (0.4% to 8% NaCl) these rats develop hypertension of similar severity to that observed in Dahl S kidney recipients (Morgan et al., 1990). The protective property of the transplanted normotensive Dahl R kidney was insufficient in the presence of overwhelming dietary salt intake suggesting an extrarenal component in hypertension development.
There are also renal components of a non-genetic nature that contribute to hypertension development. In a series of patient case studies, post-transplantation hypertension was attributed to a variety of mechanisms including renal transplant artery stenosis (Zeier, 1998). As renal homografts used whole kidneys, pathophysiological alterations in the renal vasculature would also have been exchanged during transplantation. Given the importance of the renal vasculature in arterial pressure regulation, the exchange of narrow vessels with healthy vessels or vice-versa could well explain the data obtained from cross-transplantations in animal models of essential hypertension.

1.7.5 The renal vasculature and hypertension

There have been many studies exploring the pathophysiology, pharmacology and functional characteristics of the renal vasculature in hypertension. In an animal model of primary hypertension SHR are known to have greater renal vascular resistance than their normotensive WKY controls (Arendshorst & Beierwaltes, 1979) due to smaller arteriolar lumen diameters in the SHR (Gattone li et al., 1983; Smeda et al., 1988; Skov et al., 1992; Notoya et al., 1996). Although many agree on the presence of narrowed renal arteries in the kidney, the cause of this narrowing is still debated. In fixed SHR isolated arteries, microscopy was used to analyse arterial wall thickness and the cross-sectional area of the different layers of the arterial wall. The cross-sectional area of the adventitia and intima were comparable between hypertensive and normotensive rats whilst the media appeared to be hypertrophied in hypertensive rats. Smeda et al. (1988) thus attributed renal arterial vessel narrowing to the hypertrophic media and its encroachment of the lumen. Narrowed afferent renal arterioles have also been observed using a vascular cast technique where resin is perfused into the kidneys to generate a literal cast of the renal vasculature (Skov et al., 1992; Notoya et al., 1996). However in contrast to Smeda et al. (1988) morphometric analysis revealed smaller cross-sectional area of the media in SHR compared to normotensive WKY (Skov et al., 1992; Notoya et al., 1996). Others have reported smaller arteriole lumen diameters in SHR in the absence of any gross structural changes. They instead linked this phenomenon to changes in vascular reactivity and hypoplasticity (Gattone li et al., 1983). One of the earliest reports of hypertrophied renal arteries was in subjects with chronic nephritis or Bright’s disease (Johnson, 1846). In contrast to other hypertrophied arteries, hypertrophied renal arteries appeared to have a thickened longitudinal layer of smooth muscle in addition to the characteristically thickened outer circular layer (Johnson, 1868).
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MES, Multicolored English Strain; NZW, New Zealand White; MW, Munich-Wistar; W, Wistar rats; WKY, Wistar-Kyoto rats; MAP, mean arterial pressure; RAP, renal arterial pressure; CBF, cortical blood flow; MBF, medullary blood flow; RBF, Total renal blood flow; ↓, decrease; ↑ increase; ↑†, transient increase; ↓†, biphasic and ←, no change.
1.8 Experimental models of hypertension

Numerous models of experimental hypertension have been developed to accommodate the growing interest in the development, progression and pathogenesis of hypertension. The first experimental model of persistent hypertension was developed by means of renal ischaemia when Goldblatt used clamps to constrict the main renal arteries of dogs. The resultant renal ischaemia caused a moderate elevation in systolic blood pressure without impairing renal function, reflecting a type of secondary hypertension associated with benign nephrosclerosis in humans (Goldblatt et al., 1934). Since then, numerous experimental models of hypertension have been developed.

1.8.1 Spontaneously hypertensive rat

The spontaneously hypertensive rat (SHR) is the most commonly used experimental model of essential hypertension (Pinto et al., 1998). The strain was first developed by selective inbreeding between Wistar rats exhibiting spontaneous and persistently elevated blood pressure (Okamoto & Aoki, 1963). The SHR model is useful because it shares multiple characteristics with human essential hypertension including: a genetic aetiology that is polygenic rather than Mendelian in origin; a neurogenic component during the developmental stage of hypertension; the potential to be influenced by environmental factors; elevated total peripheral resistance; and enhanced vascular reactivity (Trippodo & Frohlich, 1981).

SHR display a haemodynamic pattern reminiscent of human essential hypertension. Elevated total peripheral resistance is a basis in both conscious and anaesthetised adult SHR, a common haemodynamic aberration observed in patients with established hypertension. This is often accompanied by a normal cardiac output compared to age-matched normotensive controls (Pfeffer & Frohlich, 1973b; Pfeffer & Frohlich, 1973a; Albrecht, 1974; Tadepalli et al., 1974; Iriuchijima et al., 1975; Ferrone et al., 1979; Smith & Hutchins, 1979). The regional vascular beds responsible for the elevation in total peripheral resistance are debated. Using radioactive microspheres, Tobia et al. (1974) demonstrated similar per cent distribution of cardiac output to the major vascular beds between SHR and normotensive controls, implying a uniform elevation in regional vascular resistance. Others report that elevations in regional vascular resistance vary across vascular beds, implying non-uniformity in the individual contribution of each vascular bed to the overall increase in systemic resistance. The splanchnic and renal vasculature are suggested to be the greatest contributors (Albrecht, 1974; Ferrone et al., 1979). It is more difficult to find equivalences between young SHR and young hypertensive or borderline labile hypertensive patients. As previously mentioned, patients with early hypertension often report with elevated cardiac output (hyperkinetic circulation), but comparable total peripheral resistance compared to normotensive subjects. As the disease progresses, total peripheral resistance and mean arterial pressure elevates and cardiac output normalises (Borst & Borst-de Geus, 1963; Coleman et al., 1971). This total body autoregulatory response has been corroborated in conscious unrestrained male SHR (Smith & Hutchins, 1979), but many other studies report an absence of the antecedent hyperkinetic circulation required to initiate the autoregulation in the first place. It is important to note also that from ages 30-60 days,
SHR undergo haemodynamic changes comparable to age-matched normotensive controls and these are likely to reflect the altering tissue requirements of growing animals (Albrecht, 1974).

### 1.8.2 Page hypertension

Page hypertension is a model of hypertension that is induced by wrapping the kidneys in cellophane (Page, 1939a). The resultant fibrocollagenous hull that develops in reaction to the cellophane encapsulates and compresses the renal parenchyma causing renal hypo perfusion, ischaemia, activation of the renin-angiotensin system and interstitial nephritis (Haydar et al., 2003). The development of hypertension in this model may be due to activation of the renin-angiotensin system and the release of pressor substances such as angiotensin II (Denton & Anderson, 1985), though in dogs with cellophane renal wrap, hypertension developed in the absence of systemic increases in angiotensin II (Hart et al., 2001). Recent studies suggest an important role for renal interstitial inflammation and reactive oxygen species generation (Vanegas et al., 2005), processes that may facilitate sodium retention and increased volume load via an increase in intrarenal angiotensin II (Rodríguez-Iturbe et al., 2004). In the clinic, the model resembles post-traumatic renal hypertension (Glenn & Harvard, 1960; Grant Jr et al., 1971; Maling et al., 1976; von Knorring et al., 1981; Watts & Hoffbrand, 1987; Monstrey et al., 1989).

The procedure was first demonstrated by Page (1939a) to cause moderate and persistent hypertension in dogs. In unilateral cellophane renal-wrapped animals, the contralateral kidney remained unaffected and removal of the wrapped kidney post establishment of hypertension normalised mean arterial pressure. Wrapping both kidneys (bilateral wrapping) tended to enhance the severity as well as stabilise the hypertension though not always (Page, 1939b). In dogs, mean arterial pressure gradually increased from 130 mmHg two weeks post-operative, peaking at approximately 2 months before plateauing at a pressure slightly lower than the peak at 200 mmHg (Page, 1939b). Like in essential hypertension, the development of renal hypertension involved an initial increase in cardiac output before an elevated TPR became the prominent pressor mechanism (Ferrario et al., 1970).

Cellophane wrapping of the kidneys has since been performed in rabbits and the pathogenesis of hypertension appears to progress in a similar chronological manner to that of cats and dogs (Page, 1939b; Campbell et al., 1973; Denton et al., 1983; Kline et al., 1986; Wright et al., 1987). Similar to in canine, bilateral cellophane renal-wrap in rabbits and rats caused a gradual increase in mean arterial pressure over time. Mean arterial pressure was unchanged in renal wrap rabbits 10 days post-renal wrap. However after 28 days mean arterial pressure was raised by 42 mmHg and increased by only an additional 10 mmHg over 28 more days (52 mmHg from baseline of 79 mmHg, 56 days post-renal wrap) suggesting a plateau in mean arterial pressure (Denton et al., 1983). In a separate study, Denton et al. (1983) found that Multicolored English strain rabbits with bilateral cellophane renal-wrap had higher arterial pressures (+33 mmHg) than sham rabbits 4 weeks post renal-wrap. At 5 weeks post renal-wrap, mean arterial pressure was approximately 45 mmHg greater in the wrap than the sham rabbits (Wright et al., 1987). In the male Sprague-Dawley rat, renal wrap
increased mean arterial pressure from 120 mmHg to approximately 157 mmHg over a course of 7 weeks (Vanegas et al., 2005). The gradual increase in blood pressure is a reflection of the underlying pathogenesis. Perinephritis and compression of kidney parenchyma have both been implicated in Page hypertension.

Cellophane elicits a profound tissue reaction termed perinephritis. Two to five days post-renal wrap, the cellophane elicits a rapid inflammatory response from tissue surrounding the kidney. Fibrous and collagenous deposits, granulation tissue and rapidly proliferating connective tissue are seen 7 days post-operative and eventually form the characteristic fibrocollagenous hull that surrounds the kidney. The hull continues to develop and thicken significantly over the next 2-3 weeks and remains even when the cellophane has become fragmented and embedded within the omentum 2-3 months later. Two months post-cellophane wrapping, subacute, chronic productive and constrictive perinephritis continues to occur. The cellophane is believed to act as a chemotactic stimulant for polymorphonuclear cells which may explain the accumulation of polymorphonuclear leukocytes in the interstitial tissue and tubular lumen of dogs (Graef & Page, 1940). Similarly rats with bilateral cellophane renal-wrap have extensive tubulointerstitial damage and accompanying lymphocyte and macrophage infiltration. In contrast, glomeruli are left undamaged with very little accumulation of immune cells. Some mesangial expansion may occur (Graef & Page, 1940; Vanegas et al., 2005).

From 2 months, compression of the renal parenchyma leads to focal cortical tubular atrophy and fibrosis of cortical tissue. The extent of the fibrosis appears to vary between animals. Scarring reminiscent of ischaemia and infarction has also been observed after 2 months and may result from marked compression of the renal tissue (Graef & Page, 1940). Rats with bilateral cellophane wrapping of the kidneys that received the immunosuppressive anti-inflammatory drug mycophenolate mofetil (MMF), accumulated less inflammatory cells in the renal tubulointerstitial region than untreated rats and did not develop high blood pressure, indicative of the major role perinephritis has in this preparation of renal hypertension. Moreover, blood pressure remained at baseline levels despite formation of the typical fibrocollagenous hull suggesting that compression of the kidney parenchyma by the hull is alone insufficient to elevate blood pressure (Vanegas et al., 2005). This does not completely exclude renal tissue compression as a contributing factor to the elevation in mean arterial pressure in this model.

The hull is responsible for compression of the parenchyma. The concept was postulated by Page whom found that an incision in the hull led to protrusion of the kidney from the incision (Page, 1939a). Measurement of deep renal vein wedge pressure, an index of renal interstitial hydrostatic pressure (Hinshaw, 1964), provided quantitative evidence. Renal interstitial hydrostatic pressure was more than double in the bilaterally wrapped rabbits than normotensive rabbits 4 weeks post-operative (18.3 ± 2.0 vs. 8.4 ± 1.1 mmHg) (Denton & Anderson, 1989). Renal hydrostatic pressure measured by direct intrarenal fine-needle manometry was higher in the bilaterally wrapped rat than sham-operated rats (22.3 ± 5.2 vs. 7.6 ± 4.0 cm H₂O)(Vanegas et al., 2005). The pocket located between the capsule and kidney parenchyma is also pressurised (30 mmHg) so the higher renal pressure is unlikely to be a mere reflection of its positive linear relationship with elevated arterial
pressure (Hinshaw, 1964). Removal of the hull (decapsulation) decreased blood pressure (Campbell et al., 1973) but the magnitude of the decrease was variable (Page, 1939b).

Compression of the renal tissue may initiate another pressor mechanism that, once initiated, operates independently of whether compression persists. For example, compression of the kidneys is postulated to stimulate the release of pressor substances. These may act in concert with the external mechanical compression to increase renal vascular resistance and arterial pressure. Angiotensin II is one such pressor. Chronic infusion of the angiotensin converting enzyme inhibitor enalapril (5 μg/kg/h via osmotic mini-pump) into renal-wrapped rabbits blunted the elevation in mean arterial pressure (25 ± 4 mmHg) compared to untreated renal-wrapped rabbits (48 ± 5 mmHg). The smaller increase in mean arterial pressure was accompanied by a smaller decrease in renal blood flow and smaller increase in renal vascular resistance (Denton & Anderson, 1985). Given these data some are inclined to implicate activation of the renin-angiotensin system in the pathogenesis of Page hypertension.

Activation of the renin-angiotensin system in the pathogenesis of hypertension is contentious, partly because the complexity of the renin-angiotensin system makes it difficult to accurately measure and correctly interpret changes in components of the renin-angiotensin system. There are several theories as to how the renin-angiotensin system is involved in hypertension: i) angiotensin-mediated vasoconstriction of resistance vessels to increase arterial and so total peripheral resistance; ii) angiotensin mediated constriction of renal arteries; and iii) angiotensin-mediated release of aldosterone which promotes sodium and water retention. Plasma renin activity, a measure of angiotensinogen conversion to angiotensin I, has been reported to increase (Lewis & Lee, 1971; Denton & Anderson, 1985), decrease (Campbell et al., 1973), or remain unchanged following bilateral cellophane wrapping in rabbits (Denton et al., 1983). In dogs with bilateral cellophane wrapped kidneys, plasma renin activity was comparable or lower relative to normotensive controls (Mogil et al., 1969; Hart et al., 2001). Provided the autoregulatory homeostatic mechanisms of the renin-angiotensin system are intact, hypertension should logically be accompanied by a lower than normal plasma renin activity as a high renal perfusion pressure would be detected by the juxtaglomerular apparatus to negatively feedback on renin release. Studies that report an unchanged or increase in plasma renin activity suggest an aberrant increase in renin secretion and/or enzyme activity. In several studies the increase may be a result of the laparotomy itself which can cause a transient increase in plasma renin activity (McKenzie et al., 1967). However, plasma renin activity is chronically elevated at 4, 6 and 12 weeks (Lewis & Lee, 1971; Denton & Anderson, 1985). This cannot be attributed to the non-specific effect of surgery and is indicative of over activity of the enzyme renin. Despite the increase in plasma renin activity, a corresponding increase in the vasoressor agonist angiotensin II is not always reported. Plasma levels of angiotensin II in renal-wrapped rats 7 weeks post-operative was similar to levels in normotensive rats (Vanegas et al., 2005) and remain unchanged throughout the 12-week experimental period in cellophane-wrapped dogs (Hart et al., 2001). It may be that the conversion of angiotensin I to angiotensin II by
angiotensin converting enzyme is not similarly raised but rather unchanged or decreased in compensation.
1.9 Measuring cardiovascular parameters \textit{in vivo}

Hypertension is a systemic disorder with pathophysiological changes beyond that of a persistent elevation in blood pressure. Blood pressure itself is a constantly fluctuating parameter that both influences and is influenced by other variables in an integrative cardiovascular system. Thus, in order to assess vascular responsiveness and its role in the pathophysiology of hypertension, it is imperative that vascular responsiveness is studied \textit{in vivo} where the cardiovascular system is intact. Assessment of vascular responsiveness \textit{in vivo} requires the measurement of not only blood pressure but blood flow. The following section discusses the choices of methods measuring vascular responsiveness in experimental animals. Note that the first criterion for \textit{in vivo} measurement of vascular responsiveness is that the technique be suitable for use in conscious animals. Systemic anaesthesia is known to profoundly alter cardiovascular function and integrative cardiovascular responses so this study analyses cardiovascular parameters in conscious animals to obtain a true reflection of pathophysiological changes in hypertension.

1.9.1 Blood pressure

Significant technological advances now enable the continuous direct measurement of blood pressure in conscious animals via radiotelemetry and indwelling catheters connected to external transducers. Radiotelemetry involves implantation of a pressure-sensitive catheter into either the lower abdominal aorta, femoral artery or the thoracic aorta via the left carotid artery (Carlson & Wyss, 2000; Mills et al., 2000; Butz & Davisson, 2001; Vecchione et al., 2002; Kramer & Kinter, 2003). In order to choose an appropriate location for placement of a catheter, one must consider the physical size of the animal as the catheter must not occlude or significantly impede blood flow. The accompanying transmitter body is also placed in the animal’s body, for example mid-scapular or the inner flank (Butz & Davisson, 2001). The result is a chronically instrumented animal from which chronic, continuous blood pressure data are automatically collected. Alternatively, heparinised catheters may be inserted into an artery and the distal end connected to a calibrated external pressure transducer. The signal is then amplified and recorded.

Both techniques have similar attributes and applications and have been validated in conscious animals of various species including rats (Brockway et al., 1991), mice (Van Vliet et al., 2003), dogs (Truett et al., 1996) and non-human primates (Schnell & Wood, 1993; Reinhart et al., 2002). Radiotelemetry and indwelling catheters connected to external transducers provide several advantages over indirect methods of blood pressure measurement. Continuous data acquisition provides information not only on the acute regulation of blood pressure but also its long-term regulation. These techniques also allow blood pressure variability to be discerned, an attribute especially useful in nocturnal animals with diurnal variations and in examining the relationship between blood pressure and locomotor activity. Additionally, unlike indirect methods which measure blood pressure from a small sample of cardiac cycles and thus cannot be assumed to truly represent the average blood pressure, continuous direct measurements result in smaller variations in estimates of the mean and thus provide a better indication of the mean (Van Vliet et al., 2003; Kurtz...
et al., 2005). Real-time continuous data acquisition of blood pressure also enables quantification of
blood pressure changes however minute or transient, in relation to other measured variables (i.e.
regional vascular blood flow, sympathetic activity, target organ damage) or various interventions (i.e.
Drug infusions).

Each has advantages over the other and is briefly discussed in the following section (for a more
comprehensive comparison of indirect and direct methods as well as methodology considerations,
the author refers the reader to a review by Kurtz et al. (2005)). Radiotelemetry allows blood pressure
measurement in completely unrestrained animals, further minimising restraint stress that may
otherwise be imposed on an animal. Indwelling catheters are comparatively inexpensive and remain
the most accurate technique for direct blood pressure measurement as the system can be
recalibrated at will and with ease. Employing a swivel-tether system with indwelling catheters also
allows the simultaneous measurement of other parameters alongside blood pressure, for example
blood flow measurement via chronically implanted ultrasonic Doppler flow probes. Further still, blood
vessels can be catheterised for the acute or chronic infusion of fluids or drugs. Thus, this method
can facilitate an elaborate set-up capable of simultaneously measuring multiple cardiovascular
parameters in response to the systemic or local infusion of a desired pharmacological agent.

Pressure-response curves however do not allow proper interpretation of vascular responsiveness.
Firstly, blood pressure is not solely dependent on vascular responsiveness but also cardiac function.
Furthermore, in a conscious animal with intact cardiovascular reflexes, any changes in systemic
blood pressure to a given dose of a pharmacological agent could potentially be dampened by
homeostatic mechanisms. Secondly, because systemic blood pressure can only be lowered or
elevated to a certain degree before it becomes detrimental to the conscious animal; full
dose-pressor response curves cannot generally be generated. Thus, sensitivity, which is defined by the
dose required to elicit 50% of the maximum response, cannot be ascertained. Thirdly, even if a
change in systemic blood pressure is presumed to be due to alterations in vascular responsiveness,
measuring a systemic change provides no information on which vascular beds are involved. It is thus
more useful to measure regional blood flow concurrently with systemic pressure, from which regional
vascular resistance can then be derived.

1.9.2 Regional vascular resistance

The regional blood flow in vascular beds of conscious unrestrained animals can be measured
continuously and instantaneously with little error using Doppler ultrasound flowmeter systems (Gill,
1985). The system is commercially available and involves the implantation of the ultrasonic flow
probe around the vessel of interest. These flow probes may be implanted at multiple sites
throughout the vasculature for simultaneous measurement of regional vascular blood flow (Haywood
et al., 1981) and are applicable for both acute (Hartley & Cole, 1974) and chronic use (Vatner et al.,
1970). The technique has been validated and utilised in a variety of species including the rat
(Haywood et al., 1981), dog (Hartley et al., 1978), sheep and rabbit. Transit-time ultrasonic
perivascular flow probes can also be utilised to measure regional blood flow in conscious animals (Craig Hartman et al., 1994; Bednarik & May, 1995).
1.10 Aims of thesis

Systemically, the renal vasculature is critical in the short-term and long-term control of arterial pressure. Locally, the renal vasculature is integral to maintaining renal haemodynamics and perfusion pressure at levels optimal for glomerular filtration. It is known that elevated total peripheral resistance is a common component in established essential hypertension and secondary forms of hypertension like renovascular hypertension. The elevation in total peripheral resistance has been attributed to both structural and functional changes in peripheral vascular beds, though structural remodelling of the vasculature is thought to be particularly important in this aspect. Functional changes in the vasculature may include alterations in vascular smooth muscle sensitivity to vasoconstrictor agents and voltage-operated calcium channel-mediated calcium signalling. The latter is particularly interesting given that recent studies have shown the clinical benefit of voltage-operated calcium channel blockers with both T- and L-type activity over traditionally L-type selective calcium channel blockers in hypertensive patients with renal dysfunction. Thus the main aims of this project were:

- To examine the physiological role of T-type voltage-operated calcium channels in renal vascular reactivity.
- To assess differential pharmacological and functional characteristics of different regions of the renal vasculature in a preparation of hypertension.
- To examine how renal haemodynamics are changed in a conscious animal preparation of hypertension and to determine whether the changes are due to structurally-based vascular amplification, functional changes in the renal vasculature, or a combination of both.
Chapter 2

Pharmacological characterisation of rat renal arteries
2.1 Introduction

Vasomotor responses to stimuli are often heterogeneous when comparing vessels within the same vascular bed and between different vascular beds (Hill et al., 2001). Vessels from different regions of the renal vasculature (Figure 2.1) are also likely to be heterogeneous in their vasomotor responses. Studies of the pharmacological regulation of renal vascular resistance have focused primarily on the regulation of pre- and postglomerular arterioles and vasa recta. This is not surprising given that arterioles are major effectors in the homeostatic maintenance of renal perfusion and thus fluid balance and long-term arterial pressure control.

Pre and post-glomerular arterioles are differentially regulated. In the rabbit, afferent arteriolar vascular tone is predominantly modulated by the myogenic response intrinsic to vascular smooth muscle and the macula densa-mediated tubuloglomerular feedback response (Edwards, 1983; Ito et al., 1992; Ito et al., 1993; Ito & Ren, 1993; Juncos et al., 1995). The myogenic response is particularly important in proximal upstream segments of the afferent arteriole while the terminal ends are modulated primarily by the macula densa-mediated tubuloglomerular feedback response. The two mechanisms however have been shown to interact dynamically in the rat afferent arteriole (Walker et al., 2000). In contrast, rabbit renal efferent arteriolar vascular tone is regulated predominantly by vasoactive hormones released from upstream glomeruli (Edwards, 1983). Angiotensin II for example has been shown to constrict cannulated efferent arterioles, but not afferent arterioles or interlobular arteries of the rabbit renal vasculature (Edwards, 1983). In isolated perfused afferent arterioles, angiotensin II elicited constriction in both afferent and efferent arterioles. However, efferent arterioles were more sensitive (lower EC50) and constricted to a greater extent (greater change in luminal diameter)(Ito et al., 1993). Nitric oxide and various prostaglandins have been implicated alongside angiotensin II in the regulation of rabbit efferent vascular tone (Arima et al., 1994; Arima et al., 1996).

Comparatively little is known about the small arteries of the renal vasculature, the no man’s land of the renal vasculature (Hollenberg et al., 1969). Interlobar and arcuate arteries are a part of both the medullary and cortical circulation and are generally considered of lesser importance in the homeostatic maintenance of renal perfusion pressure and thus knowledge is limited by the paucity of studies. It is of interest however to pharmacologically characterise these arteries. Arcuate arteries form part of the renal resistance circuit alongside interlobular arteries, afferent and efferent arterioles and descending vasa recta. Although interlobar arteries are generally not considered to be part of the renal resistance circuit, they appear to be more prone to pathological changes than their smaller arteriolar counterparts (Moritz & Oldt, 1937; Hollenberg et al., 1969). In essential hypertensive patients, Hollenberg et al. (1969) reported an association between arteriography-determined vascular abnormalities in distal interlobar and arcuate arteries and clinical indices of severity and complications of hypertension. Whilst the abnormal haemodynamic consequences of pathological vascular abnormalities in the small arteries may not really alter glomerular perfusion pressure due to the autoregulatory capabilities of pre- and post-glomerular arterioles, the burden on arterioles from upstream haemodynamic disturbances is still undesirable. In this sense,
understanding the regulation of vascular tone in the upstream interlobar and arcuate arteries under physiological settings may be important. Vasoactive mediators previously implicated in both physiological and pathological conditions were of particular interest in this study and include angiotensin II, endothelin-1, noradrenaline and nitric oxide.

Angiotensin II is the main effector of the renin-angiotensin system (RAS) and exhibits both endocrine and paracrine properties (Paul et al., 2006). The peptide has numerous interrelated physiological roles, including, but not limited to, regulation of the systemic arterial pressure, sodium-fluid homeostasis, modulation of the sympathetic nervous system and vascular development and growth (Kobori et al., 2007). Angiotensin II has also been implicated in the pathological adaptation of the vasculature in hypertension (Schiffrin et al., 2000). In the vasculature, angiotensin II has a predominantly vasoconstrictor action. Angiotensin II has been shown to elevate systemic resistance in numerous mammalian species including humans (Finnerty, 1962), monkeys (Forsyth et al., 1971), dogs and, rabbits (Wright et al., 1987). Angiotensin II has also been shown to increase vascular resistance in many vascular beds, including but not limited to, the coronary circulation of dogs (Fowler & Holmes, 1964), hindquarter vasculature of rabbits (Wright et al., 1987) as well as the cutaneous, hepatic and mesenteric vasculature of the Rhesus monkey (Forsyth et al., 1971). Angiotensin II is also a potent vasoconstrictor of the renal vasculature (Finnerty, 1962; Heyndrickx et al., 1976; Alberola et al., 1994; Evans et al., 2000a), though it has been shown to differentially affect cortical and medullary blood flow (Evans et al., 2000a).

Endothelin-1 is one of three isoforms of endothelins, a class of potent vasoconstrictor peptides (Yanagisawa et al., 1988; Inoue et al., 1989). Unlike angiotensin, endothelin-1 normally acts as a paracrine or autocrine factor. Endothelin-1 has been implicated in the pathogenesis of both pulmonary and essential hypertension (Giaid et al., 1993; Iglarz & Schiffrin, 2003). The integrated vascular response to endothelin-1 is complex. The endogenous endothelins act at two G-protein coupled receptors: ET<sub>A</sub> and ET<sub>B</sub> (Davenport, 2002). ET<sub>A</sub> located on vascular smooth muscle facilitates vasoconstriction and cellular proliferation (Komuro et al., 1988; Yanagisawa et al., 1988). ET<sub>B</sub>-mediated responses are location-dependent. Those localised to vascular smooth muscle facilitate vasoconstriction (Seo et al., 1994). Comparatively, those located on endothelial cells mediate vasodilatation (D'Oreléans-Juste et al., 2002). Similarly to angiotensin II, vascular responses to endothelin-1 are heterogeneous between vascular beds (Wright & Fozard, 1988; Cocks et al., 1989). Given this, it seems prudent to also assess endothelin-1 responses in isolated vessels of the renal vasculature.

The renal vasculature also has extensive sympathetic innervation. Adrenergic innervation is found along almost all vessels with vascular smooth muscle, including the extrarenal, interlobar, arcuate and interlobular arteries and the afferent and efferent glomerular arterioles (Ljungqvist & Wågermark, 1970; Doležel et al., 1976; Barajas et al., 1992). As such, a portion of this chapter pharmacologically characterises the role of sympathetic transmission in the regulation of renal vascular tone. The role of neuronal uptake and pre-synaptic α<sub>2</sub>-adrenoceptor-mediated autoinhibitory feedback in modulating this sympathetic transmission is also estimated.
As mentioned, there is significant heterogeneity in the responsiveness of different vascular beds to vasoactive factors (Forsyth et al., 1971; Heyndrickx et al., 1976; Peach, 1977; Hill et al., 2001). Sequential segments within a vascular bed may also show a disparity in responses: the varying responsiveness of pre- and post-glomerular arterioles to angiotensin II is an apt example of this phenomenon (Edwards, 1983). Thus, in addition to assessing renal vascular responses to important vasoactive compounds (angiotensin II, endothelin-1, noradrenaline and nitric oxide), this study looked for differences in anatomically distinct regions of the vascular bed. To achieve this, large branches of the main renal artery were isolated alongside interlobar arteries (Figure 2-1).
Figure 2-1. Illustration showing the renal vasculature in a kidney that has been hemi-dissected.

Extrarenal arteries were isolated from the anterior and posterior branches (not shown) of the main renal artery. Interlobar arteries were isolated from the outer medulla prior to their branching into arcuate arteries. Figure modified from Human Physiology: An Integrated Approach, 2014, Pearson Education Inc.
2.2 Methods

2.2.1 Experimental animals

This study was approved by the University of Melbourne Animal Ethics Committee and performed in accordance with the Australian code for the care and use of animals for scientific purposes (8th edition, 2013, National Health and Medical Research Council, Canberra). Male Sprague-Dawley rats (Biomedical Science Animal Facility, University of Melbourne, Victoria, Australia) were housed in high-top cages under constant climatic conditions in groups of 2-4 (22°C, 12 h light/dark cycle) and provided with water and food ad-libitum.

2.2.2 Tissue collection

Male Sprague-Dawley rats (300-350 g) were anaesthetised by spontaneous inhalation of isoflurane (5% with O₂/room air; Veterinary Companies of Australia Pty. Ltd, Kings Park, NSW, Australia) and killed by rapid removal of the heart. The mesentery and kidneys were removed and promptly placed in cold Krebs’ physiological salt solution (PSS, composition in mmol/L: NaCl 119; KCl 4.7; KH₂PO₄ 1.18; MgSO₄.7H₂O 1.17; NaHCO₃ 25; CaCl₂ 2.5; EDTA 0.03; glucose 5.5) saturated with carbogen (O₂ 95%; CO₂ 5%) at pH 7.4.

2.2.3 Renal arteries

The basic anatomical arrangement of the renal vasculature is similar across multiple species (Kriz, 1981; Pallone et al., 1990). A comprehensive analysis of the microvasculature organisation of the rat kidney is found in Moffat and Fourman (1963). Segments of the anterior and posterior branches of the main renal artery were isolated. These arteries were isolated before they entered the hilum and for convenience are termed extrarenal arteries throughout the chapter. Kidneys were then hemi-dissected and the papilla and calyces removed to expose the interlobar arteries running along the renal columns between the renal pyramids (Figure 2-1). Proximal segments of the interlobar arteries located in the outer medulla were dissected under a stereoscopic microscope. Arteries were mounted on stainless steel wires (diameter 40 μm) connected to a force transducer in isolated chambers of an isometric wire myograph (620M; Danish Myo Technology; Aarhus, Denmark). Each chamber contained PSS that was bubbled with carbogen and thermoregulated at 37°C. Vessels were allowed to equilibrate un-stretched for 30 min.

2.2.4 Mesenteric arteries

A small segment (2 mm) of the 3rd and 1st order branches were isolated from the mesenteric vasculature and cleaned of connecting tissue under a stereoscopic microscope. Arteries were mounted in the same manner as isolated renal arteries.

2.2.5 Normalisation of arteries

The smooth muscle in vessels is arranged in a circumferential manner and contracts with the greatest force when stretched to its ideal length. The optimal passive distending force for the
development of maximum active force can be determined by generating a passive length-tension curve for each vessel (Angus & Wright, 2000). The process is explained below and in Figure 2.2.

Following equilibration, the wire attached to the micrometer was brought up to touch the wire on the transducer arm. The wires were then moved slightly apart. At this point the wall force \( f \) is zero \( (f = 0) \). The wires were then moved apart every minute to passively distend the vessel in steps. At each step \( (i) \), the distance between the two wires \( (f_i) \) is indicated by the micrometer reading and is recorded alongside the resulting circumferential wall force \( (F_i) \). Provided the length of the vessel is known, wall tension \( (T) \), which is the circumferential wall force \( (F) \) per unit length \( (l) \) can be determined via:

\[
T = \frac{\Delta F}{l}
\]  

(1)

where \( l \) is the unit length of artery and taken as \( 2 \times \) vessel segment length \( (g) \) as the artery is in fact two sheets stretched flat on the wires. The length of the internal circumference of the vessel \( (L) \) at each step \( (i) \)th can be determined using the following:

\[
L = (\pi + 2)d + 2f
\]  

(2)

where \( d \) is the diameter of the wires and \( f \) is the distance between the two wires. Once wall tension and internal circumference have been determined for each step \( (T_i \) and \( L_i \) respectively), the values can be fitted by the equation:

\[
T_i = A expBL_i
\]  

(3)

The result is a passive length-tension curve specific to the mounted vessel. The internal circumference of the vessel when distended by a transmural pressure of 100 mmHg but under no active tone \( (L_{100}) \) was then determined. To achieve this, an isobar straight line \( T \) for a transmural pressure \( (P) \) of 100 mmHg \((13.3 \text{ kPa})\) was generated using a rearrangement of the Laplace relationship:

\[
T = \frac{PL}{2\pi}
\]  

(4)

A computer iterative fitting technique was used to determine the intersection of the passive length-tension curve and the isobar to give the \( L_{100} \). Note that step-stretches were only continued until the transmural pressure exceeded the desired value (in this case 100 mmHg) to avoid overstretching.
the vessel. The internal diameter (D) for a transmural pressure of 100 mmHg (D_{100}) was then easily derived using the known relationship between the circumference and diameter of a circle:

\[ L = 2\pi r = \pi D \] (5)

Previous experiments in rat isolated vessels have shown that maximum active tension is generated when vessels are stretched to an internal circumference for 0.9L_{100} (Mulvany & Halpern, 1977). Vessels were thus partially relaxed to an internal circumference of 0.9L_{100} by adjusting the micrometer. The isometric force at this setting is the resting passive force (\(f_r\)); the artery is under no active tone and is maximally dilated. The aforementioned calculations and curve fits can now be easily performed by a DMT normalisation module that can be added on to LabChart acquisition software.
Figure 2.2. Standard set up and normalisation of vessels in the wire myograph.

A. DMT myograph 620. B. Top-down view of vessel mounted in 6 ml myograph chamber and a close-up top-down view of a mounted vessel. C. Schematic of a trace showing the normalisation procedure. Vessels first equilibrate unstretched for 30 min (1). The wire attached to the micrometer is brought up to touch the wire on the transducer side, causing a negative deflection (2). The wires are moved slightly apart. At this point, there is zero wall force \( f = 0 \). The wires are then moved apart every min (3) and the wall force \( F_i \) and micrometer reading \( f_i \) recorded in order to generate a passive length-tension curve (3i). A computer iterative fitting technique then determines the micrometer setting required to set an internal circumference equivalent to a transmural pressure of 100 mmHg \( (L_{100} \text{ or } IC_{100}) \). The vessel is relaxed slightly to an internal circumference equivalent to 0.9\( L_{100} \) (4) and remains at this resting passive force \( f_r \) (6i). Contraction is elicited with KPSS and noradrenaline (10 \( \mu \)M, 2 min) to ascertain vessel viability (5) before it is washed to relax to baseline with PSS. The vessel is then exposed to KPSS (2 min) to provide a reference maximal contraction (6). The vessel is allowed to equilibrate for 15 min prior to any protocol (7). The change in force \( \Delta f \) is the force generated to stimuli \( f_s \) – \( f_r \) (6i).
2.2.6 Establishing viability of the preparation

Following normalisation via passive stretch equivalent to 0.9$L_{100}$, vessels were equilibrated for 15 min. To establish the viability of the preparation, vessels were maximally contracted with a combination of potassium depolarising solution (KPSS; PSS with an equimolar substitution of KCl for NaCl) and noradrenaline (10 μM). Exposure lasted 2 min before vessels were washed with PSS to the resting passive force. Tissues were equilibrated for a further 15 min and then exposed for 2 min to KPSS to provide a reference maximal contraction (Figure 2-2C).

2.2.7 Agonist-induced vasoconstriction

Cumulative concentration-response curves were generated to noradrenaline (0.001 – 3.0 μM), methoxamine (0.01 – 10 μM), endothelin-1 (0.01 – 100 nM), angiotensin II (1 – 100 nM) and the thromboxane A2-mimetic U46619 (1 – 30 nM). Drugs were added in cumulative half-log10 increments allowing for responses to reach a plateau between additions. For experiments assessing the effect of angiotensin II, vessels were pre-treated with the NOS inhibitor L-nitro-arginine methyl ester (L-NAME; 100 μM) and the cyclooxygenase inhibitor indomethacin (3 μM) for 30 min. They were then pre-contracted to approximately 10% of the KPSS-induced maximum contractile response with arginine vasopressin (AVP).

2.2.8 Reuptake, post-ganglionic α2-adrenoceptor stimulation and β-adrenoceptors

To further characterise the processes involved in noradrenaline-mediated vasoconstriction in renal arteries, this study looked at the role of neuronal uptake, α2-adrenoceptors and β-adrenoceptors. Neuronal reuptake is the primary process for terminating the effect of endogenous noradrenaline at sympathetic neuroeffector junctions. To test the role of neuronal reuptake, noradrenaline concentration-response curves were generated firstly in the absence and then in the presence of the neuronal uptake inhibitor desipramine (0.1 μM, 30 min)(Angus et al., 1992b). Curves were generated in the same vessel so that each preparation served as its own control. To ascertain the role of α2-adrenoceptors and β-adrenoceptors on noradrenaline-mediated contraction, curves were generated in the absence and presence of yohimbine (0.1 μM)(Faber, 1988) and propranolol (3 μM), respectively. To ensure noradrenaline responses were reproducible, 2 noradrenaline concentration-response curves were generated 30 min apart in the absence of any antagonist (time control).

2.2.9 Electrical field stimulation

Following a 20 min equilibration period, tissues received square wave electrical field stimulation via platinum electrodes connected to a low-output-resistance stimulator (Grass SD9/S88, Quincy, MA, USA). The tissue then received 3 stimulation trains (3 s at 6.25, 12.5 and 25 Hz, 30 V) each 1 min apart. Arteries were then treated with the competitive and selective α1-adrenoceptor antagonist prazosin (0.1 μM, 5 min) followed by the irreversible non-selective α-adrenoceptor antagonist benextramine (3 μM, 5 min). Baths were repeatedly washed every 5 min for 30 min. As the prazosin is continually washed out, this prazosin-benextramine treatment leaves α2-adrenoceptors irreversibly blocked without affecting α1-adrenoceptors (Angus et al., 1988). Arteries were then re-stimulated.
with the same parameters. Following 20 min of equilibration, vessels were exposed to exogenous noradrenaline (10 μM) for 2 min and restimulated with the same parameters. To ensure the responses observed were not due to direct stimulation of the muscle, arteries received stimulation trains (3 s at 25 Hz, 30 V) each 1 min apart in the presence of the sodium channel blocker tetrodotoxin (0.1 μM). As tetrodotoxin inhibits voltage-gated sodium channels in neuronal cell membranes, it inhibits action potentials. Vessel contraction in the presence of tetrodotoxin suggests direct smooth muscle stimulation. In such cases, data were excluded.

### 2.2.10 Drugs

Drugs and suppliers were as follows: angiotensin II amide (Tocris Bioscience, Bristol, United Kingdom); arginine vasopressin (AusPep, Parkville, Victoria, Australia); benextramine (Sigma Aldrich, St Louis, Missouri, USA); endothelin-1 (AusPep); indomethacin (Sigma); L-nitro-arginine-methyl-ester (Sigma); methoxamine hydrochloride (Sigma); noradrenaline bitartrate (Sigma); prazosin hydrochloride (Sigma); propranolol hydrochloride (Sigma); tetrodotoxin (Sapphire Bioscience, Sydney, NSW, Australia); yohimbine hydrochloride (Sigma) and U46619 (1,5,5-hydroxy-11,9-(epoxymethano)prosta-5Z,13E-dienoic acid, Sigma). All drugs used for in vitro assessment were prepared using MilliQ water and stored as stock solutions at 4 °C or −20 °C until required.

### 2.2.11 Data and statistical analyses

The isometric contractile response and resting passive tension of smooth muscle are dependent on the volume of smooth muscle cells (of which vessel length is a surrogate). Due to the anatomical structure of the renal vasculature, isolating small arteries of the same length was technically difficult. Knowledge of the length of the vessel segment and the internal diameter at a set pressure allows determination of wall tension. Wall tension \( T \) is the circumferential wall force per unit length \( l \) in mN/mm and takes into account the length of each vessel segment. The equation is as follows:

\[
T = \frac{F}{l},
\]  

(6)

Active wall tension \( \Delta T \) is the tension pulled in response to isometric activation and is calculated as the difference in wall tension when the vessel is exposed to activating \( T_{active} \) and to non-stimulatory solutions \( T_{relaxed} \):

\[
\Delta T = T_{active} - T_{relaxed}
\]  

(7)

Alternatively, it can be calculated as follows:

\[
\Delta T = \frac{\Delta F}{l},
\]  

(8)

Where \( \Delta F \) is the force pulled in response to isometric activation and calculated as the difference in wall force when the vessel is exposed to activating and non-stimulatory solution.
The effective active pressure (mN/mm$^2$) is the estimated active transmural pressure that would have been obtained upon isometric activation had the *in situ* vessel been at the same derived internal circumference and under the same induced wall tension *in vivo*. The effective active pressure is the tension per internal circumference as determined by the Laplace relationship and calculated as follows:

$$\Delta P = \frac{2\pi \Delta T}{L}$$  (9)

where $L$ is $0.9L_{100}$. As 1 mN/mm$^2$ = 1 kPa and 1 mmHg = 0.1333 kPa, the active pressure units of mN/mm$^2$ were converted to mmHg using the conversion rate of 1 mN/mm$^2$ = 7.501 mmHg. The effective active pressure is useful because the derivation also takes into account the diameter of the vessel. This is especially important since the study is comparing vessels from different branching levels of the renal vasculature.

Data are expressed as change in effective active pressure (mmHg) ± 1 standard error of the mean (SEM) of $n$ experiments. Data are also expressed as mean change in force (mN) ± 1 SEM of $n$ experiments in some instances. Sigmoidal concentration–contraction response curves for each individual experiment were fitted using Prism 5 (GraphPad Software, San Diego, CA, USA). Student’s unpaired t-test was used to compare respective $pEC_{50}$ (negative log of the concentration of agonist required to elicit half-maximal response; $EC_{50}$) and $E_{max}$ (maximum response) of agonist concentration-response curves between groups unless otherwise stated. Student’s paired t-test was used to ascertain the effect of desipramine, propranolol or yohimbine on noradrenaline concentration-response curves within the same tissues. For responses to nerve stimulation, only the third stimulation peak of each stimulation frequency is presented. Curves were compared with repeated measures ANOVA with Greenhouse-Geisser correction for correlation (Ludbrook, 1994) using Prism 6 (GraphPad Software). Two sided $p \leq 0.05$ was considered statistically significant.
2.3 Results

2.3.1 Isolated renal and mesenteric arteries: general characteristics

The general characteristics of isolated renal and mesenteric arteries are summarised in Table 2-1. Proximal interlobar arteries in the outer medulla of the kidney had internal diameters of 330 ± 10 μm (n = 71 individual arteries). Branches off the main extrarenal artery were substantially larger at an average internal diameter of 617 ± 14 μm (n = 70, p < 0.0001). Extrarenal arteries contracted to a greater force (15.62 ± 0.66 mN) than interlobar arteries (8.81 ± 0.44 mN, p < 0.0001) when maximally activated with KPSS and this difference remained when wall tension was compared (4.51 ± 0.23 vs. 2.32 ± 0.11 mN/mm, p < 0.0001). However, normalisation of responses to internal diameter by calculating effective active pressure abolished this differentiation. Extrarenal and interlobar arteries reached similar maximal effective pressures (105 ± 4 vs. 105 ± 5 mmHg, respectively, p = 0.909). The results may imply that the media thickness of both arterial branches were similar.

Third order and 1st order mesenteric arteries had internal diameters of 300 ± 15 μm (n = 7) and 477 ± 26 μm (n=7) respectively. Third order mesenteric arteries had comparable internal diameters to proximal interlobar arteries (p = 0.344). Proximal interlobar arteries contracted to a similar force (8.81 ± 0.44 vs. 10.90 ±0.70 mN, p = 0.145) and wall tension (2.32 ± 0.11 vs. 2.76 ± 0.16, p = 0.229) as third order mesenteric arteries. However, when responses were normalised to internal diameters, third order mesenteric arteries pulled greater effective active pressure than interlobar arteries (138 ± 7 vs. 105 ± 4 respectively, p = 0.017). In contrast, although extrarenal arteries are substantially larger than 1st order mesenteric arteries (p = 0.002), both contract to a comparable maximal force (15.62 ± 0.57 vs. 16.83 ± 1.21 mN, respectively, p = 0.575), wall tension (4.51 ± 0.23 vs. 4.21 ± 0.73 mN respectively, p = 0.676) and effective maximal pressure (105 ± 5 vs. 135 ± 12 mmHg, respectively, p = 0.065).

2.3.2 Agonist-induced vasoconstriction

Noradrenaline, methoxamine, endothelin-1 and U46619 all caused concentration-dependent vasoconstriction in interlobar and extrarenal arteries. Of the vasoconstrictor agents assessed, only responses to α-adrenoceptors were appreciably different between interlobar and extrarenal arteries.

Interlobar arteries were significantly more sensitive to both noradrenaline (2.6-fold, n = 13; p = 0.001, Student’s paired t-test; Figure 2-3A, B) and methoxamine (2.4-fold, n = 5-6, p < 0.05, Student’s unpaired t-test; Figure 2-3C, D) than extrarenal arteries. The reason for this regional difference is explored in section 2.3.3. The difference in sensitivity is particularly clear when responses are expressed as effective active pressure (Figure 2-3B, D). As effective active pressure normalises for the internal circumference of vessels, the derived parameter allows clearer comparisons of pharmacological responses in arteries of vascular branches significantly different in diameter. For example, maximal active wall tension (circumferential wall force per unit length, mN/mm) elicited by noradrenaline was greater in extrarenal than interlobar arteries (Emax 4.81 ±
0.56 vs. 2.41 ± 0.30 mN/mm; \( p = 0.001 \), Student’s paired t-test; Figure 2-3A), presumably due to their larger internal circumference. Expressing noradrenaline responses in effective active pressure however, revealed that noradrenaline elicited similar wall force per unit area of smooth muscle (mN/mm²) in interlobar (135 ± 17 mmHg) and extrarenal arteries (128 ± 14 mmHg; \( p = 0.565 \), Student’s paired t-test; Figure 2-3B).

Unlike noradrenaline, methoxamine elicited similar maximal wall tension (Emax 3.29 ±0.33 vs. 4.09 ± 0.63 mN/mm) in interlobar and extrarenal arteries. Maximal effective active pressure was also comparable between the two arteries (Emax 109 ± 6 vs. 99 ± 15 mmHg). Although interlobar arteries tended to reach a greater maximal effective pressure than extrarenal arteries in response to both endothelin-1 (Emax 144 ± 18 vs. 109 ± 8 mmHg, \( p = 0.115 \)) and U46619 (Emax 131 ± 16 vs. 113 ± 12 mmHg, \( p = 0.382 \)), this was not statistically significant. Endothelin-1 sensitivity was comparable between interlobar (\( pEC_{50} \) 8.28 ± 0.02) and extrarenal arteries (\( pEC_{50} \) 8.18 ± 0.07, \( n = 5 \); Figure 2-4A). Similarly, there was no regional difference in U46619 sensitivity with a \( pEC_{50} \) of approximately 7.4 obtained in both arteries (\( n = 6 \); Figure 2-4B).

In contrast to the other vasoconstrictor drugs, angiotensin II was a poor vasoconstrictor agent in both interlobar and extrarenal arteries (Figure 2-4C). Treatment of vessels with indomethacin and L-NAME did not significantly alter basal tone. Although the level of arginine vasopressin-mediated tone applied to vessels varied considerably (5 to 30 mmHg), the level of pre-tone did not appear to affect the magnitude of the angiotensin II response. Notably, the response to angiotensin II was inconsistent even with pre-treatment. Four interlobar vessels (of \( n = 7 \)) and three extrarenal vessel (of \( n = 6 \)) had very poor responses (change in \( \Delta P < 5 \) mmHg; Figure 2-5) and were excluded from the group data. It is noted that of all the vessels tested, both responsive and non-responsive to angiotensin II, were viable vessels. There appeared to be no correlation between maximum effective active pressure reached with KPSS and the quality of response to angiotensin II (Figure 2-5, right panels).

### 2.3.3 Reuptake, pre-synaptic \( \alpha_2 \)-adrenoceptors and the \( \beta_2 \)-adrenoceptors

The re-uptake inhibitor desipramine (0.1 μM) did not affect the noradrenaline response in interlobar arteries (\( n = 5 \), Figure 2-6B). Similarly, desipramine did not alter the maximum contractile response or the potency of noradrenaline in extrarenal arteries (\( n = 6 \)). The antagonist did however lower the noradrenaline vasoconstrictor threshold from approximately 10 nM to 3 nM.

To determine whether \( \alpha_2 \)-adrenoceptors were involved in shaping noradrenaline-mediated responses in renal arteries, experiments were performed in the presence of the selective \( \alpha_2 \)-adrenoceptor antagonist yohimbine (1 μM, Figure 2-6C). Yohimbine caused a parallel rightward shift of the noradrenaline concentration-response curve in extrarenal (\( n = 6 \)) but not interlobar arteries (\( n = 5 \)). Potency decreased by a factor of 2 following yohimbine treatment (\( pEC_{50} \) =7.06 ± 0.15 to 6.73 ± 0.12; \( p = 0.001 \), Student’s paired t-test), whereas the maximum contractile response was similar pre- and post-treatment (96 ± 8 vs. 95 ± 7 mmHg, respectively). In contrast, yohimbine did not affect
the noradrenaline response in interlobar arteries. Similar potencies ($pEC_{50} = 7.27 \pm 0.13$ vs. $7.16 \pm 0.21$) and maximum contractions ($115 \pm 11$ vs. $121 \pm 9$ mmHg) were recorded pre- and post-treatment.

The non-selective $\beta$-adrenoceptor inhibitor propranolol (0.1 μM) did not affect noradrenaline-mediated contractile responses in interlobar or extrarenal arteries ($n = 5$, Figure 2-6D). Similar $pEC_{50}$s were recorded in interlobar arteries in the absence ($7.07 \pm 0.14$) and presence ($7.22 \pm 0.06$) of propranolol. Maximum contractile responses were also unchanged ($166 \pm 34$ vs. $160 \pm 34$ mmHg). In extrarenal arteries, propranolol had little effect on $pEC_{50}$ ($6.58 \pm 0.17$ vs. $6.76 \pm 0.13$) and the maximum response ($187 \pm 18$ vs. $193 \pm 19$ mmHg).

In untreated interlobar and extrarenal arteries, noradrenaline concentration-response curves were reproducible (Figure 2-6A). This suggests minimal time-dependent changes in vessel contractility and noradrenaline sensitivity. Thus, changes to the noradrenaline response in the presence of yohimbine can be attributed to inhibition of $\alpha_2$-adrenoceptors.

### 2.3.4 Electrical field stimulation

The force responses of rat interlobar and extrarenal arteries to electrical field stimulation are shown in Figure 2-7(Top panel). The response of extrarenal arteries ($n = 9$) to nerve stimulation was significantly greater than that of interlobar arteries ($n = 7$; $p = 0.032$, two-way ANOVA repeated measures). Electrical field stimulation (3 s at 25 Hz, 30 V) caused a contraction of $10 \pm 4$ mmHg ($n = 7$) in interlobar arteries and $31 \pm 7$ mmHg ($n = 9$) in extrarenal arteries. Irreversible block of $\alpha_2$-adrenoceptors potentiated the force response to nerve stimulation (12.5 and 25 Hz) in both interlobar and extrarenal arteries ($p < 0.05$, two-way ANOVA repeated measures). When these interlobar and extrarenal vessels were then treated with desipramine, no further potentiation was observed except for the response to 6.25 Hz in extrarenal arteries ($p < 0.05$, two-way ANOVA repeated measures).

The small response to nerve stimulation observed in the interlobar arteries appears to be region-specific. Mesenteric arteries of comparable internal diameter ($n = 7$) also had greater responses ($p = 0.023$, two-way ANOVA repeated measures; Figure 2-7, middle panel). In contrast, extrarenal arteries displayed similar responses to mesenteric arteries isolated from comparable branching levels ($n = 7$, $p = 0.055$; Figure 2-7, bottom panel). As observed in the renal vessels, irreversible block of $\alpha_2$-adrenoceptors also potentiated responses to nerve stimulation in mesenteric arteries, but only at the higher frequencies (12.5 Hz for 1st order, 12.5 Hz and 25 Hz for 3rd order mesenteric arteries). Further treatment with desipramine did not affect responses. Contractile responses were tetrodotoxin-insensitive and thus likely due to neural activation and not direct smooth muscle stimulation.
Table 2.1. General characteristics of renal and mesenteric arteries isolated from the rat kidney

<table>
<thead>
<tr>
<th></th>
<th>Renal</th>
<th>Mesenteric</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interlobar</td>
<td>Extrarenal</td>
<td>3rd order</td>
<td>1st order</td>
</tr>
<tr>
<td>n</td>
<td>71</td>
<td>70</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Internal diameter (µm)</td>
<td>330 ± 10</td>
<td>617 ± 14 ***</td>
<td>300 ± 15</td>
<td>477 ± 26 *** ††</td>
</tr>
<tr>
<td>Δ Force (mN)</td>
<td>8.81 ± 0.44</td>
<td>15.62 ± 0.66 ***</td>
<td>10.9 ± 0.70</td>
<td>16.83 ± 1.21 **</td>
</tr>
<tr>
<td>Active tension (mN/mm)</td>
<td>2.32 ± 0.11</td>
<td>4.51 ± 0.23 ***</td>
<td>2.76 ± 0.11</td>
<td>4.21 ± 0.30 **</td>
</tr>
<tr>
<td>Maximal effective pressure (mmHg)</td>
<td>105 ± 4</td>
<td>105 ± 5</td>
<td>138 ± 7 †</td>
<td>135 ± 12</td>
</tr>
</tbody>
</table>

Internal diameter was estimated for transmural pressure of 100 mmHg (L100). Δ Force is the increase in force (mN) to KPSS (124 mM) and active tension is the change in force per mm length of the isolated artery. Maximal active pressure (mmHg) is the estimated pressure that the artery would have reached in vivo in response to KPSS (124 mM) at the estimated internal diameter. Data are expressed as mean ± SEM. n refers to the pooled number of each vessel type from the total number of rats used. In general, 2 extrarenal and 2 interlobar arteries were isolated from each rat. ***p < 0.001, 1st order mesentery or extrarenal artery compared to 3rd order and interlobar arteries respectively. †p < 0.05, 3rd order mesenteric artery compared to interlobar artery; ††p < 0.01, 1st order mesenteric artery compared to extrarenal artery, Student’s unpaired t-test.
Figure 2-3. Irrespective of their smaller diameter, isolated interlobar arteries (●) exhibited greater sensitivity to α-adrenoceptors than extrarenal arteries (○)

Panels on the left are responses to noradrenaline (A) and methoxamine (C) expressed as the change in force (mN). Panels on the right are responses to noradrenaline (B) and methoxamine (D) expressed as the calculated effective active pressure ($\Delta P = \frac{2\Delta T}{D}$) that the artery would have reached in vivo at the estimated internal diameter ($L_{100}$). Effective active pressure normalises for internal circumference so that any divergence in responses cannot be attributed to a difference in their internal diameters. *$p < 0.05$, $\rho EC_{50}$ of extrarenal vs. interlobar artery, Student’s unpaired t-test for comparison of methoxamine curves and Student’s paired t-test for comparison of noradrenaline responses, where $\rho EC_{50}$ is the negative log of the concentration of constrictor agent required to elicit a half-maximal response. †$p < 0.05$, $E_{max}$ of extrarenal vs. interlobar artery, Student’s unpaired t-test for comparison of methoxamine curves and Student’s paired t-test for comparison of noradrenaline responses, where $E_{max}$ is the maximum contractile response of the artery to the constrictor agent. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the $EC_{50}$ ± 1 SEM. $n =$ number of arteries from separate rats.
Figure 2-4. Estimated effective pressure in response to various vasoconstrictor agents in rat isolated interlobar (●) and extrarenal (●) arteries

Vascular responses to endothelin-1 (A), U46619 (B) and angiotensin II (C) were comparable between interlobar and extrarenal arteries. Prior to assessing angiotensin II-mediated responses, vessels were pretreated with L-NAME (100 μM) and indomethacin (3 μM) for 30 min. A small amount of tone was then applied with arginine vasopressin (Treatment). Vertical bars are ± 1 SEM; where no error bar is visible the SEM is within the symbol. Horizontal error bars represent the EC50 ± 1 SEM. n, number of arteries from separate rats.
Figure 2-5. Angiotensin II-mediated concentration-response curves in isolated interlobar (top panel, n = 7) and extrarenal arteries (bottom panel, n = 4) from different rats

Responses to angiotensin II were inconsistent and poor in both interlobar and extrarenal arteries. All vessels were treated with a combination of L-NAME (100 μM) and indomethacin (3 μM) and pre-constricted to a low level of tone with arginine vasopressin. Curves 1, 5, 6 and 7 from the top panel and curves 2, 4 and 6 from the bottom panel had comparatively poor responses to angiotensin II (change in ΔP < 5 mmHg) and were excluded from the group data. The panels on the right compare the effective active pressure obtained when vessels were maximally contracted with KPSS (x-axis) to the sum of the maximum effective active pressure achieved by angiotensin II and AVP tone (closed circles). Open circles are the changes in maximum effective pressure elicited by angiotensin II alone (i.e. minus the effect of AVP tone).
Figure 2-6. The role of reuptake, post-synaptic modulation and β-adrenoceptors on noradrenaline-mediated changes in effective active pressure in rat isolated interlobar and extrarenal arteries.

Noradrenaline concentration-response curves in the absence (interlobar ●, extrarenal ○, solid lines) and presence (interlobar ○, extrarenal ●, dotted lines) of A. vehicle; B. desipramine (0.1 μM); C. yohimbine (0.1 μM); or D. propranolol (3 μM). Only yohimbine and propranolol significantly altered noradrenaline-mediated responses and only in extrarenal, not interlobar arteries. **p < 0.01, pEC₅₀ pre- vs post-treatment with antagonist (paired Student’s t-test), where pEC₅₀ is the negative log₁₀ of the noradrenaline concentration required to elicit 50% of the maximum response. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the EC₅₀ ± 1 SEM. n = number of arteries from separate rats.
Table 2-2. The effect of desipramine, propranolol and yohimbine on noradrenaline potency in rat isolated renal interlobar and extrarenal arteries (effective pressure graphs).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Interlobar</th>
<th>Extrarenal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC₅₀</td>
<td>n</td>
</tr>
<tr>
<td>Time control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>6.86 ± 0.16</td>
<td>5</td>
</tr>
<tr>
<td>post</td>
<td>6.96 ± 0.07</td>
<td>5</td>
</tr>
<tr>
<td>Desipramine (0.1 µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>7.53 ± 0.07</td>
<td>5</td>
</tr>
<tr>
<td>post</td>
<td>7.50 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td>Propranolol (0.1 µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>7.07 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td>post</td>
<td>7.22 ± 0.06</td>
<td>5</td>
</tr>
<tr>
<td>Yohimbine (0.1 µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>7.35 ± 0.13</td>
<td>5</td>
</tr>
<tr>
<td>post</td>
<td>7.08 ± 0.20</td>
<td>5</td>
</tr>
</tbody>
</table>

**p < 0.01, pEC₅₀ of noradrenaline curve post-treatment vs. pre-treatment where pEC₅₀ is the negative log₁₀ of the concentration of agonist required to elicit half-maximal response (paired Student’s t-test). n = number of arteries from separate rats.
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Extrarenal
Interlobar

pre-synaptic
\(\alpha_2\)-adrenoceptor inhibition

pre-synaptic
\(\alpha_2\)-adrenoceptor inhibition + neuronal uptake inhibition

\(\Delta P\) (mmHg)

Stimulation frequency (Hz)
Figure 2-7. Estimation of the role of pre-synaptic α2-adrenoceptor mediated autoinhibitory feedback and neuronal uptake in renal arteries: a comparison to mesenteric arteries of similar branching echelons

Contractile responses to electrical field stimulation (3 sec at 6.25, 12.5 and 25 Hz, 30 V) in rat isolated interlobar (●, n = 7), extrarenal (●, n = 8), 1st order (●, n = 6) and 3rd order (●, n = 6) mesenteric arteries. Data are presented in three different panels for clarity. Top panel: Comparison of responses in interlobar and extrarenal arteries. Middle panel: Comparison of responses in interlobar and 3rd order mesenteric arteries. Bottom panel: Comparison of responses in extrarenal and 1st order mesenteric arteries. Three stimulus-response lines were generated in each vessel. Left panel: Responses in the absence of any antagonists and thus intact modulatory mechanisms. Centre panel: Responses following irreversible inhibition of α2-adrenoceptor-mediated autoinhibitory feedback (prazosin-benextramine pre-treatment, refer to methods). Right panel: Responses following inhibition of neuronal uptake (desipramine, 0.1 μM, 30 min) and autoinhibitory feedback (prazosin-benextramine pre-treatment). Response was calculated as increase in effective active pressure (ΔP, mmHg). *p < 0.05 compared to control response to the same stimulation frequency; "p < 0.05 compared to response after pre-synaptic α2-adrenoceptor inhibition at the same stimulation frequency, two-way ANOVA repeated measures with Tukey’s multiple comparisons. *p < 0.05, for comparisons of control frequency-response curves obtained in vessels from different regions; comparisons are indicated by lines, two-way ANOVA repeated measures. Vertical bars are ± 1 SEM. n = number of arteries from separate rats.
2.4 Discussion

There have been many studies assessing the pharmacological profile of isolated renal arterioles. The pharmacological characteristics of the main renal artery have also been assessed in multiple species, though not nearly as much as the renal arterioles. There is scarce information however, on small renal arteries such as interlobar arteries, despite their potentially greater propensity for pathological changes than small arterioles (Moritz & Oldt, 1937; Hollenberg et al., 1969).

2.4.1 Heterogeneity within segments of the renal vascular bed

This study demonstrated both similarities and differences in the vascular reactivity of renal arteries of differing regions of the renal vasculature. U46619, endothelin-1 and angiotensin II elicited similar responses regardless of the branching level of the vessel. Furthermore, although the response to angiotensin II is noticeably blunted compared to arteries from other vascular beds (see 2.4.3 for discussion); endothelin-1 potency appears to be conserved across multiple species. Endothelin-1 was similarly potent between the isolated rat extrarenal (pEC₅₀ 8.18) and interlobar arteries (pEC₅₀ 8.28) and comparable potency has been reported in the isolated main renal artery of humans (pEC₅₀ 8.39) (Maguire et al., 1994), Wistar rats (pEC₅₀ 8.5) (Devadason & Henry, 1997) and canines (pEC₅₀ 8.7) (Cocks et al., 1989).

While there were no regional differences in the response to endothelin-1, angiotensin II or the thromboxane A₂ mimetic U46619, there were several discrepancies in regards to adrenoceptor-mediated responses. Interlobar arteries were more sensitive than extrarenal arteries to both noradrenaline (2.6-fold) and methoxamine (2.4-fold). There are numerous potential reasons underlying this difference. Noradrenaline is known to mediate vasoconstriction in most vascular beds via post-junctional α₁-adrenoceptors and the renal vasculature is no exception (Schmitz et al., 1981; Docherty, 1998). A greater α₁-adrenoceptor density in interlobar arteries compared with extrarenal arteries may be reasonably expected to lead to greater vasoconstrictor sensitivity. The response to noradrenaline is modulated by various mechanisms including activation of other pre- and post-junctional adrenoceptors. This study used various pharmacological antagonists to examine the role of such modulating mechanisms in interlobar and extrarenal arteries.

Pre-treatment with the α₂-adrenoceptor antagonist yohimbine (10⁻⁷M) did not alter vasoconstrictor responses to exogenous noradrenaline in interlobar arteries, suggesting minimal α₂-adrenoceptor-mediated modulation of responses. Similar results have been reported in isolated interlobar arteries of male Wistar rats (Chen et al., 1997). The negative feedback mechanism is unlikely to play a role in this scenario given that the vasoconstriction is to exogenous noradrenaline and not endogenously-released noradrenaline from the nerve terminal. However, yohimbine (10⁻⁷M, 10⁻⁸ M) has been shown to cause dose-dependent inhibition of the noradrenaline concentration-response curve in isolated interlobar arteries of dogs (Toda et al., 1984). In contrast, yohimbine pre-treatment of extrarenal arteries decreased noradrenaline potency from 7.06 ± 0.15 to 6.73 ± 0.12. The reason for this is unclear. It may be that in extrarenal arteries, noradrenaline is causing vasoconstriction via
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post-junctional α2-adrenoceptors in addition to α1-adrenoceptors. Activation of post-junctional α2-adrenoceptors (α2A, α2B) on vascular smooth muscle can induce arterial vasoconstriction (Docherty, 1998) and this is thought to occur in rat tail arteries (Medgett et al., 1984) as well as renal and mesenteric but not coronary arteries of dogs (Toda et al., 1984). Given that yohimbine is only marginally selective for α2C-adrenoceptors (MacDonald et al., 1997; Lalchandani et al., 2002) over α2A (4-fold) and α2B (15-fold), inhibition of post-junctional α2-adrenoceptors could well explain why yohimbine caused a parallel rightward shift of the noradrenaline concentration-response curves.

The role of β-adrenoceptors in noradrenaline-mediated vasoconstriction was also assessed. The enhanced sensitivity to noradrenaline in extrarenal arteries could be explained in part by a larger β2-adrenoceptor presence on the vascular smooth muscle. The non-selective β-blocker propranolol caused a parallel leftward shift of the noradrenaline concentration-response curves in extrarenal, but not interlobar arteries. Propranolol may have potentiated vasoconstriction by inhibiting β2-mediated vasodilatation, a process that can otherwise functionally antagonise vasoconstriction (Graves & Poston, 1993). The renal vasculature is thought to express both β1- and β2-adrenoceptors (Taira et al., 1977), although compared to other vascular beds, there is little evidence in the literature to support the presence of β-adrenoceptor-mediated vasodilatation in isolated vessels of the renal vasculature (Boonen et al., 1990; Heesen & Mey, 1990).

2.4.2 Nerve-mediated responses

It is well established that the sympathetic nervous system plays an important role in controlling vascular tone. Neural control of vascular tone is actioned through a variety of mediators including the neurotransmitter noradrenaline and the co-transmitters neuropeptide Y and adenosine triphosphate (Lundberg & Tatemoto, 1982). In small renal arteries of the rat, neural-mediated force responses are predominantly mediated though noradrenaline-mediated activation of the α1A-adrenoreceptor with minor contribution from neuropeptide Y (Chen et al., 1997). In rat mesenteric arteries, the force response to nerve stimulation is mediated predominantly via the α-adrenoceptors, with minimal contribution from adenosine triphosphate receptors (Angus et al., 1988). The renal vasculature is also known to have extensive adrenergic innervation (Barajas et al., 1992).

To further characterise the role of sympathetic activation in the renal vasculature, electrical field stimulation was utilised to activate neurally-released transmitter-mediated force responses in isolated vessels. Nerve stimulation elicited frequency-dependent contractile responses in both extrarenal and interlobar arteries. However, despite extensive adrenergic innervation of interlobar arteries (Barajas et al., 1992), nerve stimulation elicited rather small contractile responses relative to that elicited in 3rd order mesenteric arteries of comparable internal diameters and those elicited in extrarenal arteries (Figure 2-7). The difference between arteries cannot be explained by their size as the responses are expressed as effective active pressure (where responses have been normalised for internal diameter). It is possible that at interlobar arteries, neurotransmitter release from sympathetic varicosities is subjected to a greater degree of modulation than at synapses found at
extrarenal and mesenteric arteries. This study assessed the contribution of two major modulators of neurotransmitter release from adrenergic nerves: autoinhibitory feedback and neuronal uptake.

Autoinhibitory feedback at the neuroeffector junction is thought to be mediated by pre-junctional α2-adrenoceptors (Starke et al., 1989). There are numerous subtypes of the α2-adrenoceptor (α2A, 2B, 2C), but the α2A (α2D in rats) and α2C subtype are thought to be the primary subtypes that mediate pre-synaptic inhibition of transmitter release (Hein et al., 1999). In this study, inhibition of pre-synaptic α2-adrenoceptors showed a marked enhancement of responses in interlobar, extrarenal, 1st and 3rd order mesenteric arteries, particularly at higher frequencies (Figure 2-7). Such potentiation has been previously reported in rat isolated mesenteric resistance arteries (Angus et al., 1988; Angus et al., 1990). Indiscriminate blockade of pre and post-synaptic α2-adrenoceptors with yohimbine has also been shown to potentiate responses to transmural stimulation of sympathetic fibres in dog isolated renal interlobar arteries (Toda et al., 1984). Notably, autoinhibitory feedback did not alter the difference in magnitude of nerve-stimulated responses observed with interlobar and extrarenal arteries. Thus the smaller responses observed in interlobar arteries cannot be attributed to a greater auto-inhibitory feedback presence.

In contrast, neuronal uptake does not appear to play a major role in regulating neurotransmitter release in neuroeffector junctions at renal arteries. At a concentration previously shown to potentiate responses to nerve stimulation and noradrenaline via neuronal reuptake inhibition in rat atria (Lew & Angus, 1983), desipramine caused no major enhancement of nerve-stimulated responses on top of that observed with pre-synaptic α2-adrenoceptor inhibition in either interlobar or extrarenal arteries. Desipramine also had no major effect on the maximal contraction or sensitivity to exogenous noradrenaline in interlobar or extrarenal arteries (Figure 2-6). This is in contrast to data presented in the isolated perfused renal artery of the rat where desipramine caused non-competitive inhibition of noradrenaline mediated contractions (Hrdina & Ling, 1970). The disparity may be explained by the non-specific effects of desipramine. Desipramine is able to inhibit the α1-adrenoceptor with a reported pA2 of approximately 7 and has been shown to decrease the potency of methoxamine and catecholamines to various degrees in multiple vascular beds (McCulloch & Story, 1972; Angus et al., 1992b). This is not always the case, and the lack of desipramine-mediated inhibition of exogenous noradrenaline responses in our study also suggests a lack of inhibitory activity at α1-adrenoceptors at 0.1 μM. Notably, Hrdina and Ling (1970) used higher concentrations than that used here (5x10^{-7} – 10^{-6}M). It is possible that in the renal artery, desipramine begins to exert α1-adrenoceptor inhibitory activity at concentrations above 5x10^{-7}M. At concentrations selective for neuronal uptake, Angus et al. (1990) showed that desipramine had no significant effect on nerve-stimulated responses in rat isolated mesenteric arteries. From the data, it appears that the major modulatory mechanism at neuroeffector junctions in the renal vasculature is autoinhibitory feedback. Neuronal uptake is negligible except at lower stimulation frequencies when there is not yet sufficient neurotransmitter available to activate pre-synaptic autoinhibitory feedback.
2.4.3 Angiotensin II-mediated vascular responses

Angiotensin II has a multitude of physiological roles, several of which contribute to its potent vasopressor effect. The peptide stimulates vascular smooth muscle contraction, enhances myocardial contractility and augments sympathetic nervous system activity (Kobori et al., 2007). Despite its potent vasoconstrictor effects in vivo however (Champion et al., 1998), this study observed a poor contractile response to angiotensin II (10^{-11} - 10^{-7}M) in rat isolated extrarenal and interlobar arteries.

Most of the physiological effects of angiotensin II are mediated via two G-protein coupled receptors, AT_1 (subtypes AT_{1A} and AT_{1B}) and AT_2 (Chiu et al., 1989; Whitebread et al., 1989; Bumpus et al., 1991). Although vasoconstriction is mediated primarily via the AT_{1A} receptor subtype localised in smooth muscle cells (Champion et al., 1998; de Gasparo et al., 2000), both the AT_1 and AT_2 receptor have been found in the vasculature. AT_1 appears to be preferentially expressed in vascular smooth muscle cells while AT_2 receptors have a larger presence in endothelial cells.

The poor contractile response to angiotensin II may be due to its ability to facilitate vasodilatation in addition to vasoconstriction. AT_2 receptor stimulation has previously been shown to counteract physiological roles mediated by the AT_1 receptor, such as vasoconstriction (Siragy & Carey, 1997; Dimitropoulou et al., 2001; Batenburg et al., 2004). The AT_2 receptor is able to elicit vasodilatation via both endothelium-dependent and independent signalling mechanisms. Angiotensin II can cause vasodilatation by stimulating nitric oxide production and release from the endothelium. In renal and coronary resistance vessels, AT_2 receptor-mediated vasodilatation was proposed to occur via a bradykinin-nitric oxide-cGMP cascade (Siragy & Carey, 1997; Siragy et al., 1999). Flow-mediated vasodilatation via shear-stress-induced nitric oxide release may also be dependent on AT_2 receptor activation (Endlich et al., 1999; Matrougui et al., 1999). This is not always the case however. In the anaesthetised rat, neither AT receptor-mediated nitric oxide release nor shear stress-mediated nitric oxide release was observed following angiotensin II infusion (Schuijt et al., 2001). Additionally, whilst the tonic release of nitric oxide and epoxyeicosatrienoic acids both dampen angiotensin II mediated vasoconstriction, only the latter appears to be coupled to the AT_2 receptor (Kohagura et al., 2000).

Angiotensin II also stimulates the production and release of vasodilator prostanoids, particularly in the renal vasculature. In canines, angiotensin II infusion was accompanied by enhanced prostaglandin E_2 production in the renal but not hindquarter vasculature (Aiken & Vane, 1973). Toda and Miyazaki (1981) demonstrated that angiotensin II caused vasodilatation of perfused renal and coronary arteries through angiotensin II-mediated prostaglandin I_2 release. Vasodilator prostanoids have also been shown to dampen angiotensin II-mediated vasoconstriction to a greater extent than nitric oxide (Schuijt et al., 2001). Both endothelium-dependent and independent release of prostanoids has been reported. In rat cerebral arteries, angiotensin II elicited vasodilatation required both an intact functional endothelium and cyclooxygenase enzymes (Haberl et al., 1990). Prostaglandin E_2 and I_2 production may also occur following angiotensin II activation of AT_1 or AT_2 receptors in porcine aortic vascular smooth muscle cells (Jaiswal et al., 1993).
Endothelium-independent vasodilatation may also occur via the recruitment of Ca\(^{2+}\)-activated potassium channels following AT\(_2\) receptor activation (Dimitropoulou et al., 2001). This has been reported in rat mesenteric resistance vessels where there is a high expression of AT\(_2\) receptor mRNA (Dimitropoulou et al., 2001). Angiotensin II may also be functionally opposing vasoconstriction by facilitating vasodilatation via the AT\(_1\) receptor. An endothelial-dependent AT\(_1\) receptor vasodilatation characterised by enhanced production and release of nitric oxide was reported in the rat carotid artery (Boulanger et al., 1995), rabbit afferent artery (Ito et al., 1993) and interlobar and arcuate arteries of the mouse (Thorup et al., 1998).

Thus, to ensure angiotensin-mediated vasoconstriction was not being masked by its concomitant vasodilator properties, angiotensin II concentration-response curves were also generated in arteries pre-treated with the non-selective cyclooxygenase inhibitor indomethacin and the nitric oxide synthase inhibitor L-NAME. Although this regimen revealed contractions in some arteries, approximately half still failed to contract to angiotensin II. Of those that did contract to angiotensin II, the response was poor.

Alternatively, the lack of response to angiotensin II may be due to relatively low expression of AT\(_{1A}\) receptors in the renal vasculature. AT\(_{1A}\) mRNA expression in the renal vasculature is limited to afferent arterioles, vasa recta (Gasc et al., 1994) and arcuate arteries (Terada et al., 1993; Miyata et al., 1999). Little or no AT\(_{1A}\) mRNA has been detected in the vascular smooth muscle of other renal arteries (Gasc et al., 1994). Accordingly, in vitro receptor autoradiography has shown that AT\(_1\) receptors are primarily localised to glomeruli and areas of the outer medulla associated with vasa recta bundles (Mendelsohn et al., 1986; Sechi et al., 1992).

Poor angiotensin II mediated vasoconstriction in renal arteries highlights the importance of renal arterioles in regulating renal haemodynamics. The afferent arteriole in particular appears to be the main site for regulating renal haemodynamics. Although the renal vasculature is under neural and humoral regulation, the afferent arteriole also has an intrinsic capacity to modulate its vascular resistance. These intrinsic mechanisms occur independently of neural (Shipley, 1951) and hormonal mechanisms and are thus autoregulatory. Importantly, autoregulatory mechanisms take precedence over extrinsic influences enabling blood flow to remain relatively constant over a range of systemic arterial pressures (Shipley, 1951; Arendshorst & Gottschalk, 1975).

The physiological relevance for the relatively low vasoconstrictor capacity of angiotensin II has not been determined. It is postulated that poor angiotensin-mediated vasoconstriction of renal arteries may be a means to prevent excessive vasoconstriction of the renal vasculature. The potential for excessive vasoconstriction may occur for example, following activation of the local renal renin-angiotensin system. Locally expressed renin-angiotensin systems have been demonstrated in numerous tissues including the kidney (Kobori et al., 2007), the heart and the vasculature (Oliver & Sciaccia, 1984) amongst others (Paul et al., 2006). These local systems may act independently or in
a dynamic and cooperative (or antagonistic) manner with the systemic renin-angiotensin system (Paul et al., 2006).

Figure 2-8. Schema of the proposed pathways through which angiotensin II can elicit vasoconstriction and vasodilatation

Angiotensin II is known to cause vasoconstriction primarily through the AT₁ receptor. It is now thought to also cause vasodilatation predominantly through activation of the AT₂R. A number of pathways have been implicated in angiotensin II-mediated vasodilatation: these pathways are likely to interact.
2.4.4 Limitations and considerations

The isolated artery preparation provides useful information on the reactivity of individual arterial branches without the influence of extravascular neurohumoral variables that often confound studies in the whole intact kidney. However, as mentioned, vasomotor responses to stimuli are often heterogeneous when comparing vessels within the same vascular bed and between different vascular beds (Hill et al., 2001). Nonetheless, the renal vasculature appears to share attributes observed in the mesenteric resistance vasculature. It is curious however that the response to nerve stimulation and angiotensin II are quite poor in interlobar arteries. The reasons have not yet been determined.
Pharmacological characterisation of rat renal arteries
Chapter 3

Role of T-type-voltage-operated calcium channels in rat renal vascular reactivity
3.1 Introduction

Many classes of antihypertensive drugs decrease arterial pressure by lowering peripheral vascular resistance, specifically by decreasing peripheral vascular tone. Most calcium channel blockers (CCBs) used to treat hypertension were developed with the intent to selectively inhibit vascular-specific Ca.1.2b L-type voltage-operated calcium channels (VOCC), the primary VOCC responsible for vascular smooth muscle contraction and thus vascular tone. Despite the effectiveness of CCBs and other anti-hypertensive therapies, approximately 30% of patients remain resistant to current pharmacological therapy. The mechanisms underlying resistant hypertension remain unknown leading researchers to re-explore mechanisms involved in the regulation of vascular tone.

The L-type calcium channel is only one of five different types of VOCC (L-, T-, N-, P-/Q-, and R-type) and at least another three of these (T, P-/Q-) are suspected to have some role in regulating the vascular tone of small vessels. Structurally, voltage-operated calcium channels are formed from a complex of 4 subunits (α1, α2δ, β1-4, and γ) with the pore-forming α1-subunit conferring a 1000-fold selectivity for Ca2+ over Na2+ (Tsien et al., 1987). The remaining three auxiliary subunits (α2δ, β1-4, and γ) are not necessary for the channel to function but appear to modulate channel kinetics, voltage-gating properties and expression levels. Voltage-operated calcium channels are currently classified by the responsible gene encoding the pore-forming α1-subunit (Ertel et al., 2000). There are ten genes known to encode the α1-subunit (α1S, C, D, F, A, B, E, G, H, I) from three subfamilies: Ca.1 (L-), Ca.2 (P-/Q-), N- and R-) and Ca.3 (T-). Besides the L-type calcium channel, the T-type calcium channel is of particular interest. Not only is it extensively expressed in multiple vasculatures, but CCBs with antagonistic activity at the T-type channel have been shown to provide additional renoprotective benefits in hypertensive patients.

3.1.1 Biophysical properties of T-type channels

Numerous studies have characterised the biophysical properties of the T-type channel in the hopes of elucidating its physiological function. Unlike other VOCCs, T-type channels are uniquely low voltage-activated, requiring only small depolarisations of the plasma membrane. In contrast, L-/P-/Q- and N-type channels are high voltage-activated while R-type channels are activated by intermediate voltages. T-type channels also possess other properties that enable it to be differentiated from high voltage-activated channels. Several electrophysiological properties have been reported across a spectrum of tissues:

a) Low voltage-activated
b) Facilitate transient inward current due to fast inactivation
c) Overlapping voltage range for steady state activation and inactivation
d) Deactivate slowly upon repolarisation culminating in a slowly deactivating tail current
e) Have slow activation and inactivation kinetics near threshold voltages that accelerate with progressively stronger depolarisations, generating a classical criss-crossing pattern when current recordings generated from a current-voltage (I-V) protocol are superimposed
Role of T-type calcium channels in rat renal vascular tone

f) A small unitary conductance under conditions of saturating Ba\(^{2+}\) concentrations (Carbone & Lux, 1984; Armstrong & Matteson, 1985)

g) Similar unitary conductance between Ba\(^{2+}\) and Ca\(^{2+}\) (Ba\(^{2+}\) is more permeant than Ca\(^{2+}\) through high voltage-operated calcium channels (Nilius et al., 1985) and

h) Relatively dihydropyridine insensitive.

Although fast inactivation (b), an overlapping voltage range for steady-state activation and inactivation curves (c) and dihydropyridine insensitivity (h) are not properties exclusive to T-type channels, the remainder remain insofar, uniquely T-.

The biophysical properties reported for T-type channels are heterogeneous. This is explained in part by the different conditions used in electrophysiological studies and the existence of different isoforms of the T-type channel. In mammals there are three known genes encoding the \(\alpha_1\)-subunit of T-type channels: CACNA1G encodes for Ca\(^{v}\)3.1 (\(\alpha_{1G}\))(Perez-Reyes et al., 1998), CACNA1H encodes for Ca\(^{v}\)3.2 (\(\alpha_{1H}\))(Cribbs et al., 1998) and CACNA1I encodes for Ca\(^{v}\)3.3 (\(\alpha_{1I}\))(Lee et al., 1999a). Though these subtypes share biophysical properties, they also possess marked differences. For example, Lee et al. (1999a) reported that the steady-state inactivation curve of Ca\(^{v}\)3.3 currents sat at more positive potentials than Ca\(^{v}\)3.1 and Ca\(^{v}\)3.2. Notably, the voltage-dependence of steady-state inactivation was similar between all channels when a physiological concentration of Ca\(^{2+}\) was used as the charge carrier (Klöckner et al., 1999). Lee et al. (1999a) had used a high concentration of Barium as the charge carrier. The differences between these two studies (and many others) highlight the difficulty in synthesising data from electrophysiological studies that use different assay conditions. The charge carrier and the concentration of the charge carrier are known to alter voltage dependence (Kaku et al., 2003; Kuo et al., 2011), a fact highlighted in (Table 3-1). The cell system used will also affect the biophysical characteristics (Chemin et al., 2001b; Kuo et al., 2011). The diversity in the reported biophysical properties of T-channel subtypes is also likely to be due to the existence of numerous splice variants (Perez-Reyes & Lory, 2006).

3.1.2 Pharmacological inhibition of T-type channels

Information about the T-type channel has come from studies using T- and L-type calcium channel pharmacological inhibitors. Problematically, these inhibitors lack sufficient selectivity and data obtained with these inhibitors should be interpreted with caution. The following section summarises what is known about inhibitors currently used to inhibit T-type calcium channels.

Nickel

At low micromolar concentrations, NiCl\(_2\) is a selective antagonist of the Ca\(^{v}\)3.2. The half maximal inhibitory concentration for cloned human Ca\(^{v}\)3.2 ranges from 6 \(\mu\)M when expressed in Xenopus oocytes (Lee et al., 1999a) to 7-13 \(\mu\)M in HEK293 cells (Lee et al., 1999a; Williams et al., 1999). NiCl\(_2\)-mediated block of Ca\(^{v}\)3.2 current is relatively voltage-independent and is only minimally blunted when current is evoked by more positive test potentials (Mlinar & Enyeart, 1993; Lee et al., 1999b). Nickel-mediated block of the other T-type channel isoforms appears to be species-dependent. Comparison of cloned human \(\alpha\)-subunits showed that Ca\(^{v}\)3.1 was more sensitive to NiCl\(_2\).
than Ca,3.3 (Monteil et al., 2000b). In rats however the order of sensitivity is reversed with Ca,3.3 subunits more sensitive to nickel than Ca,3.1 (Lee et al., 1999b). Regardless, much higher concentrations (15 to 30-fold) are required to block Ca,3.1 and Ca,3.3 compared to Ca,3.2 and these concentrations are also likely to inhibit HVA channels (Lee et al., 1999b). Its subtype-selectivity may explain the variation seen in the ability of nickel to block native T-currents in different tissues. The metal is most effective in tissues thought to have relatively high Ca,3.2 expression (Perchenet et al., 2000). In cardiomyocytes, nickel (40 μM) significantly inhibited T-current without affecting L-type current (Hagiwara et al., 1988; Zhou & Lipsius, 1994). In vascular smooth muscle cells from rat aorta the reported IC₅₀ was 10 μM (Akaike et al., 1989). In contrast, much higher concentrations of nickel were generally required to block native T-current in neuronal cells (30-800 μM)(Huang, 1989; Kaneda & Akaike, 1989; Kaneda et al., 1990; Fraser & MacVicar, 1991; Takahashi & Akaike, 1991; Ye & Akaike, 1993).

Mibefradil

For years, mibefradil (Ro 40-5967) was considered a selective T-type channel inhibitor (McDonough & Bean, 1998; Klugbauer et al., 1999). Mibefradil was originally developed by Roche in 1986 in response to several issues that limited the therapeutic utility of hypertensive drugs (poor bioavailability, short half-life, negative inotropy, excessive peripheral vasodilatation and reflex tachycardia)(Clozel et al., 1997). Although mibefradil was chemically modified from verapamil, the tetralol derivative is structurally distinct from all 3 major classes of antihypertensive drugs (Osterrieder & Holck, 1989). Mibefradil was found to inhibit Ca²⁺ current mediated by native T-type voltage-operated calcium channels in vascular smooth muscle cells (Mishra & Hermsmeyer, 1994), neurons (Viana et al., 1997; McDonough & Bean, 1998; Todorovic & Lingle, 1998), human medullary thyroid carcinoma cells (Mehrke et al., 1994), HEK293 cells (Cribbs et al., 1998; Martin et al., 2000) and Xenopus oocytes expressing cloned subtypes of T-type channels (Ca,3.1, Ca,3.2, Ca,3.3)(Martin et al., 2000).

The concentration of mibefradil required to block native T-current depends on the particular preparation. The reported lowest IC₅₀ was 130 nM in vascular smooth muscle (Clozel et al., 1997). The concentration required to inhibit current in guinea pig left atria was 100-fold greater (IC₅₀ = 14 μM)(Osterrieder & Holck, 1989). The heterogeneity in IC₅₀ values can be attributed to the presence of different channel isoforms in different tissues and the drug's preference for particular subtypes. The order of sensitivity to mibefradil is Ca,3.1 > Ca,3.2 > Ca,3.3 (Klugbauer et al., 1999; Martin et al., 2000), accounting for its greater vascular-to-cardiac selectivity. Mibefradil has even greater affinity for α₂δ and α₁δ in physiological calcium (2 mM CaCl₂) than in 10 mM BaCl₂. Mibefradil is thought to compete for its binding site with the permeant species: Ba²⁺ is believed to be a more effective competitor for the channel than Ca²⁺ (Martin et al., 2000).

Although mibefradil has a reported IC₅₀ of 100-300 nM for T-type channels in vascular smooth muscle (Mishra & Hermsmeyer, 1994; Martin et al., 2000; Hansen et al., 2001; Morita et al., 2002), at best, mibefradil is selective for T-type over L-type channels over a narrow range (Osterrieder & Holck,
1989; Mehrke et al., 1994; Mishra & Hermsmeyer, 1994; Martin et al., 2000). In vascular smooth muscle cells cultured from neonatal rats mibefradil is 10-30 fold more selective for T-type channels (Osterrieder & Holck, 1989; Mishra & Hermsmeyer, 1994). In HEK293 cells expressing cloned T-type calcium channels and the L-type Ca,1.2 isoform, mibefradil had a 10- to 15-fold preference for T-type over L-type channels (Martin et al., 2000). In rabbit sinus node cells, mibefradil was almost equally effective at blocking T- and L-type Ca2+ current (Protas & Robinson, 2000). Mibefradil itself does not antagonise L-type channels. Instead, the membrane permeable molecule has a methoxyacetyl side chain that is hydrolysed by intracellular enzymes resulting in the metabolite des-methoxycetyl (Wiltshire et al., 1992; Wu et al., 2000). This metabolite is able to inhibit the L-type current in cells derived from pancreatic β-cells (Wu et al., 2000). The active metabolite may act via the intracellular domain (Wu et al., 2000) and thus at a site distinct from that of dihydropyridines which bind to the extracellular domain of α1-subunits. The clinical effectiveness of mibefradil as an antihypertensive therapy, originally attributed to inhibition of T-type VOCC, was concluded by 2006 to be mediated by the Ca,1.2 L-type VOCC as mibefradil failed to elicit the same decrease in mean arterial blood pressure in Ca,1.2SMAKO knockout mice as in control mice.

Mibefradil also directly inhibits voltage-operated K+ channels in rabbit coronary arterial smooth muscle cells with a Kd of 1.08 μM (Hong et al., 2012). These channels are found in vascular smooth muscle cells and activate in response to membrane depolarisation (Wilde & Lee, 1989; Volk et al., 1991; Smirnov & Aaronson, 1992). For this reason they may limit the magnitude of membrane depolarisation (Nelson & Quayle, 1995; Brayden, 1996). They also help set the steady-state membrane potential (Nelson & Quayle, 1995; Brayden, 1996). The functional consequence of mibefradil-mediated inhibition of Kv current has not been assessed but the Kv selective inhibitor 4-AP is known to cause membrane depolarisation and vasoconstriction. The development of the more selective T-type channel inhibitors NNC 55-0396 (Li et al., 2005) and TTA-A2 (Uebele et al., 2009) may greatly improve our understanding of T-type VOCC channels in the vasculature.

**NNC 55-0396**

NNC 55-0396 is considered a relatively more selective inhibitor of T-type channels than mibefradil and other classical antagonists. Its relative selectivity for T-type channels can be attributed to its structure. NNC 55-0396 was developed from its analogue, mibefradil. The molecule has a cyclopropanecarboxylate side chain rather than the problematic methylacetate in mibefradil (Huang et al., 2004). Consequently, unlike mibefradil, NNC 55-0396 is not metabolised into the L-type inhibitor des-methoxycetyl (Li et al., 2005). This may explain why the compound is able to inhibit current through recombinant Ca,3.1 channels in HEK293 cells (IC_{50} = 7 μM) without affecting native high voltage-activated current (Li et al., 2005). However, although Li et al. (2005) showed that 100 μM of NNC 55-0396 did not affect high voltage-activated currents in insulin-secreting beta derived cells, more recent studies suggest that NNC 55-0396 is non-selective between 1-10 μM (Kuo et al., 2010; Björling et al., 2013). NNC 55-0396-mediated inhibition is both voltage- and frequency-dependent. Antagonism is less effective in more hyperpolarised membranes while the rate of
inhibition of T-type Ca\textsuperscript{2+} current is enhanced with increased stimulation frequency (Huang et al., 2004).

**New generation dihydropyridines**

Several non-classical dihydropyridines have been shown to cause dual blockade of T- and L-type calcium channels. Efonidipine inhibits T- and L-type calcium channels but not P/Q-, R- or N-type calcium channels (Furukawa et al., 2004). Whilst the S(+) isomer doesn’t discriminate between these two channels, the R(-) efonidipine isomer selectively blocks T-type calcium channels (Furukawa et al., 2004). Of the three T-type calcium channel subtypes, efonidipine is able to block Ca\textsubscript{3.1} and Ca\textsubscript{3.2} channels but not Ca\textsubscript{3.1} channels (Furukawa et al., 2009). In contrast, both nimodipine and nivaldipine inhibit Ca\textsubscript{3.2} and Ca\textsubscript{3.3} but not Ca\textsubscript{3.1} current (Furukawa et al., 2009).

### 3.1.3 T-type channels and vascular function

Under basal conditions, resistance vessels such as the small arteries and arterioles normally exhibit some degree of vascular tone because they are partially constricted. Vascular smooth muscle contraction necessitates an increase in intracellular calcium. The rise in intracellular calcium may occur via electromechanical coupling or pharmacomechanical coupling (Somlyo & Somlyo, 1968). The latter is dependent primarily on the release of calcium from intracellular stores. The former involves the depolarisation of vascular smooth muscle cells, activation of voltage-operated calcium channels and the subsequent influx of extracellular calcium into the cell. The L-type channel has long been considered the primary voltage-operated calcium channel involved in mediating calcium influx. There is now evidence to suggest that the T-type channel may also have a role in the maintenance and management of vascular tone.

Until recently, the T-type VOCC has largely been deemed inconsequential to the regulation of vascular tone because certain biophysical properties are difficult to reconcile with what is required to maintain vascular tone. Firstly, the membrane potential of vascular smooth muscle cells spans -30 to -45 mV in pressurised vessels (Loutzenhiser et al., 1997; Knot & Nelson, 1998; Welsh & Segal, 1998). This is outside the voltage range in which the T-type channel can maintain the persistent calcium influx necessary for vascular tone: a property termed the window current. The window current, which is described as the range of voltages over which the steady-state activation and inactivation curves overlap, was determined by Hirano et al. (1989) to sit between -65 and -45 mV for T-type channels. Secondly, the magnitude of the window current mediated through T-type channels is small, particularly when compared to that of L-type channels. Although the window current for the Ca\textsubscript{3.3} channel (-70 to -40 mV) lies close to the resting membrane potential of smooth muscle cells, courtesy of a steady-state activation curve that sits at more depolarised potentials than other cloned T-type channels (Lee et al., 1999a), Ca\textsubscript{3.3} are not found in the vasculature.

Alternative splicing of genes encoding the \(\alpha_1\)-subunit of T-type channels have been shown to confer distinct biophysical and pharmacological properties to different isoforms (Mittman et al., 1999;
Monteil et al., 2000a; Emerick et al., 2006; Perez-Reyes, 2006). It has been suggested that splice variants of Ca,3.1 and Ca,3.2 may have biophysical properties that are more conducive to the regulation of vascular tone and reactivity. Specifically, their window current may be shifted to more depolarised potentials, resulting in a greater overlap with the range of membrane potentials reported under physiological conditions and thus a greater voltage range for sustained calcium influx (Monteil et al., 2000a; Kuo et al., 2011).

3.1.3.1 Expression in the vasculature

T-type channels have been identified in multiple vascular beds, including cerebral, mesenteric, subcutaneous and renal vascular beds. In the rat mesenteric arteries and arterioles, gene expression analysis revealed mRNA for both T- (Ca,3.1 and Ca,3.2) and L-type (Ca,1.2) channels (Gustafsson et al., 2001; Braunstein et al., 2009), though the latter are not always found in smaller terminal arterioles (Gustafsson et al., 2001). Accordingly, immunolocalisation indicated the presence of Ca,3.1 and Ca,3.2 protein in both mesenteric arteries and arterioles. In the cerebral circulation, immunohistochemistry identified T- (Ca,3.1 and Ca,3.2) and L-type (Ca,1.2) in the smooth muscle cells and endothelial cells of the basilar, middle and posterior cerebral artery as well as in the smaller lateral branches (Navarro-Gonzalez et al., 2009; Kuo et al., 2010; El-Rahman et al., 2013).

T-type channels are particularly abundant in the renal vasculature. In rat preglomerular arterioles, smooth muscle cells may either co-express or exclusively express either L- or T-type calcium channels (Gordienko et al., 1994). Human interlobar and arcuate and mouse intrarenal vessels express Ca,1.2, Ca,2.1a and Ca,3.1 and Ca,3.2 mRNA. In humans, the relative abundance of these channels were typically in the order Ca,1.2 > Ca,3.2 > Ca,2.1 and Ca,3.1, though at times Ca,3.2 were dominant (Hansen et al., 2011). Immunohistochemistry demonstrated that Ca,3.1 protein was found throughout the human and localised to smooth muscle cells (Hansen et al., 2011). Unfortunately there is not yet an antibody for the human Ca,3.2 isoform. Studies in rats have found mRNA expression of both T-type isoforms (Ca,3.1 and Ca,3.2) and the L-type Ca,1.2 in intrarenal vessels, including preglomerular afferent arterioles, outer medullary vasa recta vessels and juxtamedullary but not cortical efferent arterioles (Hansen et al., 2001). This expression pattern is also reflected at the protein level. Immunofluorescence microscopy in the rat kidney showed Ca,3.2 protein expression in arterioles, capillaries and glomeruli (Hansen et al., 2001) but not the cortical efferent arterioles (Poulsen et al., 2011). This appears to be species-specific as rat cortical efferent arterioles do not express Ca,3.1 while mouse and human cortical efferent arterioles do (Hansen et al., 2011; Poulsen et al., 2011). Notably, the expression of Ca,3.1 is generally confined to the media layer of vascular smooth muscle whilst Ca,3.2 is predominantly observed in endothelial cells (Braunstein et al., 2009; Hansen et al., 2011). This location-specific expression compliments the information we have about the differential function of the Ca,3.1 and Ca,3.2 channels: Ca,3.1 is thought to facilitate vasoconstriction whereas Ca,3.2 has been linked to vasodilatation (Hayashi et al., 2005; Jensen & Holstein-Rathlou, 2009; Poulsen et al., 2011)(Figure 3-1).
3.1.3.2 Vascular contraction

Ca.3.1 has been implicated in the vasoconstriction of renal, cerebral and mesenteric arterioles. T-type calcium channels are thought to contribute to Ca\(^{2+}\) entry required during local electromechanical coupling (Hansen et al., 2000; Hansen et al., 2001; Braunstein et al., 2009).

Resting vascular tone

As previously mentioned, T-type channels may facilitate the persistent influx of calcium required to maintain vascular tone via its window current. The role of T-type channels in the renal vasculature is particularly interesting because it may have therapeutic implications in the treatment of hypertension with concomitant renal nephropathy. Different calcium channel blockers used in the treatment of hypertension with chronic kidney disease elicit varying degrees of renal protection. The L/T-type calcium channel inhibitor efonidipine decreased proteinuria, a clinical parameter for renal damage and a risk factor of cardiovascular events, more effectively than the L-type selective amlodipine. The difference in renal protection may be explained by the divergent action of calcium channel inhibitors in the afferent and efferent arteriole. This divergent action is likely to be due to the different profile of VOCC in the pre- and post-glomerular arteries.

In the renal vasculature, afferent arteriolar tone and efferent arteriolar tone are predominantly mediated by L- and T-type channels, respectively. T-type channels are unlikely to be involved in regulating afferent arteriolar tone since neither nickel nor mibefradil did not modify the basal diameter of preglomerular afferent arterioles (2001) when used at a concentration considered T-type selective. This is supported by the observation that L-type selective inhibitors appear to act preferentially in the preglomerular circulation. Intrarenal infusion of nicardipine (Abe et al., 1983), nifedipine (Heller & Horacek, 1990; Honda et al., 2001) and verapamil (Heller & Horacek, 1990) in anaesthetised dogs increased glomerular filtration rate and filtration fraction, an indicator of glomerular capillary pressure. Increases in glomerular capillary pressure can occur with preglomerular arteriolar dilation provided that the postglomerular efferent arteriolar diameter is unaltered or is decreased. The preferential dilatation of canine afferent arterioles was visualised using intravital pencil-lens charge-coupled device camera video microscopy (Honda et al., 2001). (Heller & Horacek, 1990), reported a decrease in afferent arteriolar resistance but no change in efferent arteriolar resistance, once again suggesting preferential dilatation of afferent arterioles. A similar response to verapamil was observed in the anaesthetised rat (Ichikawa et al., 1979). Although the decrease in afferent arteriolar resistance could be a passive consequence of the decrease in mean arterial pressure in the study by Honda et al. (2001) and Ichikawa et al. (1979), the doses of nifedipine and verapamil administered by Heller and Horacek (1990) did not affect systemic pressure: dilatation of afferent arterioles is likely to be a direct effect of the selective L-type channel inhibitors. The unresponsiveness of the efferent arteriole to selective L-type channel inhibitors suggests a greater role for non L-type VOCC in controlling efferent arteriolar tone. Dihydropyridines that cause dual block of T- and L-type channels, such as manidipine and efonidipine (Furukawa et al., 2009), have been shown to increase renal blood flow without
significantly affecting glomerular filtration fraction in healthy humans (Allison et al., 1985), mild hypertensive patients (Takabatake et al., 1993) and spontaneously hypertensive rats (Yokoyama et al., 1992). This suggests that the dual blockers are able to dilate both efferent and afferent arterioles, possibly by inhibiting T- and L-type channels, respectively.

**Myogenic response**

T-type channels are thought to facilitate the myogenic response to low intravascular pressures. In cerebral arteries, T-type channels contributed to the myogenic response elicited at low intraluminal pressures (El-Rahman et al., 2013). In perfused mesenteric arteries isolated from Ca,3.1− mice, small increases in intraluminal pressure (40-80 mmHg) failed to elicit the constriction responses observed in arteries of wild-type mice (Björling et al., 2013). In contrast, the myogenic response to large increases in intraluminal pressure (100-120 mmHg) was unaffected suggesting that T-type channels are not necessary for the genesis of the myogenic response activated during high intraluminal pressures. These studies complement the data obtained in mice with smooth muscle-specific deletion of Ca,1.2 channels. In tibialis arteries isolated from these mice, the myogenic response is inhibited at intraluminal pressures between 50 and 90 mmHg but preserved at lower pressures (Moosmang et al., 2006), possibly through the recruitment of T-type channels.

**Depolarisation-mediated vasoconstriction**

The mesenteric arterial vasculature is thought to recruit both L- and T-type channels to facilitate Ca²⁺ entry; however T-type channels are proposed to progressively dominate towards the peripheral and terminal branches of arterioles (Braunstein et al., 2009) where L-, P-/Q-, N- and R-type VOCC mRNA are minimally expressed or not at all (Gustafsson et al., 2001; Jensen et al., 2004). The dominance of L-type channels in larger resistance vessels may explain why mesenteric arteries isolated from Ca,3.1 knockout mice had normal contractile responses to potassium (Hansen et al., 2011): these vessels do not recruit T-type channels during depolarisation mediated vasoconstriction in the first place. Thus, the finding by Hansen et al. (2011) does not preclude the involvement of T-type channels in mediating depolarisation-mediated vasoconstriction in smaller resistance vessels. Pursuant to this, the T-type selective inhibitors mibefradil, NNC 55-0396 and (R)-efonidipine caused concentration-dependent inhibition of potassium-mediated Ca²⁺ influx and inhibited responses to a greater extent than nifedipine. Potassium-mediated Ca²⁺ influx in rat mesenteric arterioles was insensitive to the selective L-type channels inhibitor nifedipine at concentrations that typically cause 50% inhibition of the response (IC₅₀) (Braunstein et al., 2009). Mibefradil and NNC 55-0396 caused almost complete inhibition at higher doses (10 μM) (Jensen et al., 2004; Braunstein et al., 2009). Similarly, intravital videomicroscopy of mesenteric arterioles in anaesthetised rats showed that vasoconstriction induced by local current stimulation was abolished by mibefradil and nickel, but not by high concentrations of nifedipine or nimodipine (Gustafsson et al., 2001). In mouse efferent arterioles, vasoconstriction induced by potassium (100 mM) was inhibited by nickel (1 mM) and mibefradil (1 nM) (Poulsen et al., 2011). Note that in the studies by Gustafsson et al. (2001) and Braunstein et al. (2009), complete inhibition of Ca²⁺ responses and vasoconstriction was only obtained at concentrations now believed to inhibit both L- and T-type VOCC (Jensen & Holstein-
At concentrations considered selective for T-type channels, mibefradil (100 nM) has been shown to inhibit approximately 30% of Ca\(^{2+}\) entry mediated by T-type channels in the rat terminal mesenteric arteriole (Jensen et al., 2004).

**Agonist-mediated vasoconstriction**

Recent studies using Ca\(_{3.1}\) knockout mice have demonstrated the importance of the channel in agonist-mediated vascular responses (Hansen et al., 2011; Hansen, 2014). Compared to wild-type mice, mesenteric arteries isolated from Ca\(_{3.1}\) knockout mice have decreased sensitivity to the α\(_1\)-adrenoceptor agonist phenylephrine (Hansen et al., 2011). Phenylephrine was also less efficacious in these vessels despite an unchanged maximum response to potassium-mediated depolarisation. In contrast, a later study showed that the maximum response to noradrenaline was enhanced in the mesenteric arteries of Ca\(_{3.1}\) knockout mice (Björling et al., 2013). The reason for this difference is unknown: the role of T-type channels appears to vary depending on the agonist. In mouse cortical efferent arterioles, the response to the thromboxane A2 analogue U46619 but not angiotensin II was partially inhibited by mibefradil at a concentration that is selective for the T-type channel (Poulsen et al., 2011). It could be that different agonists preferentially recruit different subtypes or splice variants.

The magnitude of the contribution of T-type channels also depends on the vascular bed and the different regions of the vascular bed, perhaps a consequence of the variable distribution of T-type channels. While a blunted response to phenylephrine was observed in mesenteric arteries deficient in Ca\(_{3.1}\), segmental intrarenal arteries examined were more sensitive and reached a greater maximum contraction than arteries of wild-type mice (Hansen et al., 2011). The renal vasculature is a prime example of the varying contribution of T-type channels to agonist-mediated vascular tone across different regions. The distribution profile of VOCC in the renal microvasculature (afferent arteriole, predominantly L-; efferent arteriole, predominantly T-) may explain why classical dihydropyridines selective for the L-type channel only inhibited agonist-mediated responses in the afferent arteriole while dual L/T-type channel blockers indiscriminately inhibited agonist-mediated responses (Carmines & Navar, 1989; Hayashi et al., 1996; Saruta et al., 1996).

### 3.1.3.3 Vascular dilatation

T-type channels have been shown to mediate vasodilatation through two mechanisms: an endothelial-dependent mechanism involving the Ca\(_{3.1}\) channel and a smooth muscle-dependent mechanism involving the Ca\(_{3.2}\) channel (Figure 3-1). The Ca\(_{3.1}\) channel localised on endothelial cells is thought to mediate the vasodilation that occurs secondarily to depolarisation-mediated vasoconstriction. Mesenteric arteries isolated from Ca\(_{3.1}\) knockout mice exhibit a diminished secondary dilatation (Svenningsen et al., 2014). The dilatation is thought to be endothelial dependent, requiring the activation of eNOS and the subsequent production of nitric oxide (Figure 3-1; IIb). This pathway is facilitated by the co-localisation of Ca\(_{3.1}\) channels and eNOS in endothelial cells, an observation reported in mice mesenteric arteries. Accordingly, in mice deficient in Ca\(_{3.1}\) channels, nitric oxide and cGMP production was attenuated (Svenningsen et al., 2014).
Ca,3.2 knockout mice exhibit impaired coronary vasorelaxation responses and constitutively constricted arteries yet normal contractile function (Chen et al., 2003). It was speculated that the Ca,3.2 channel localised to smooth muscle cells mediates relaxation through a pathway that involves large conductance calcium sensitive potassium channels (BKCa). In coronary arteries deficient in Ca,3.2 that were treated with a BKCa channel opener, relaxation responses were normalised to that observed in vessels of wild-type mice (Chen et al., 2003). It is now known that the Cav3.2 channel is linked to BKCa through the ryanodine receptor. Calcium influx through the Ca,3.2 channel was shown to activate ryanodine receptors, leading to the localised release of calcium from intracellular stores. The subsequent rise intracellular calcium activated BKCa channels, leading to hyperpolarisation and thus smooth muscle relaxation (Harraz et al., 2014).

3.1.3.4 Developmental role

Due to their electrophysiological properties T-type channels may continue to regulate Ca²⁺ movement at low voltages even when cells are unstimulated (Cohen et al., 1988; Crunelli et al., 2005). The ability to continuously regulate Ca²⁺ homeostasis enables T-type channels to contribute to cell differentiation and proliferation, including that of vascular smooth muscle cells (Gordienko et al., 1994; Kuga et al., 1996). Their expression is not surprisingly dependent on processes such as cell proliferation, differentiation and hypertrophy and they may be expressed exclusively in a cell, with other high-voltage activated currents, or not at all (Akaike et al., 1989; Kuga et al., 1990; Richard et al., 1992; Gordienko et al., 1994). Low-voltage activated current was reported in cultured but not freshly isolated coronary myocytes from human (Quignard et al., 1997) and rabbit vessels (Matsuda et al., 1990). Proliferating smooth muscle cells are suggested to have a greater expression of T-type current than differentiated cells (Akaike et al., 1989; Richard et al., 1992). In primary cultures of rat aortic myocytes, expression of low-voltage activated currents coincided with the period of time where cell proliferation was maximum (Richard et al., 1992). Approximately 90% of cells in the synthesis phase showed T-type current compared to the 37% of cells in the G1 phase (Kuga et al., 1996). In growing non-confluent cells, (2008) found expression of T-type channel mRNA. No mRNA expression was observed in cytostatic confluent cells.

3.1.4 Study aims

To conclude, the understanding of the physiological and pathophysiological roles of T-type channels is in part hindered by a lack of selective T-type calcium channel inhibitors and confounded by the erroneous use of calcium channels presumed to be T-type selective. The aim of this study was to characterise, functionally, the role of T- and L-type voltage-operated calcium channels in two processes that mediate vascular contraction and dilatation: electromechanical coupling and pharmacomechanical coupling. Electromechanical coupling is essential in membrane depolarisation-mediated vasoconstriction and traditionally associated with activation of L-type voltage-operated calcium channels whilst pharmacomechanical coupling has particular importance in agonist-mediated vascular responses. This study also aimed to define the selectivity profile of pharmacological inhibitors of the T-type calcium channel.
Figure 3-1. The role of T-type channels (Ca\textsubscript{3.1} and Ca\textsubscript{3.2}) in vasoconstriction and vasodilatation of resistance vessels.

In response to depolarisation of the smooth muscle cell, L-type voltage-operated calcium channels open to enable Ca\textsuperscript{2+} influx (I). An increase in this second messenger facilitates smooth muscle contraction. Of the three subtypes of T-type calcium channels, only Ca\textsubscript{3.1} and Ca\textsubscript{3.2} are thought to be present in the vasculature. Cav3.1 has been linked to both vasoconstriction and endothelia-dependent vasodilatation (IIa, b) while Ca\textsubscript{3.2} has been linked to endothelial-independent vasodilatation (III).
Table 3-1. The effect of charge carrier on the biophysical properties of T-type VOCCs stably transfected into HEK-293 cells.

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<th>Role of T-type calcium channels in rat renal vascular tone</th>
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<th>Activation kinetics</th>
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<td>$V_{50}$ (mV)</td>
<td>Slope ($k$)</td>
<td>$V_{h}$ (mV)</td>
<td>Slope ($k$)</td>
<td>$\tau_{act}$ (ms)</td>
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<td>$Ca_{3.1} (\alpha_{1G})$ (rat)</td>
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<td>-72</td>
<td>-4.8</td>
<td>15</td>
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<td></td>
<td>-46</td>
<td>4.1</td>
<td>-73</td>
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<td>30</td>
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<tr>
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<td>$Ca_{3.3} (\alpha_{1I})$ (rat)</td>
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$V_{max}$, $V_{50}$ and $V_{h}$, midpoint of voltage dependence of activation or inactivation, respectively (mV); $k$, slope factor of the curve; $\tau_{act}$ and $\tau_{inact}$, time constants of activation and inactivation (ms), respectively.
Figure 3-2. IC$_{50}$ estimates of CCBs for native T- and L-type channels and cloned human/rat T- (Ca$_{3.1-3.3}$) and L- (Ca$_{1.2}$) type channels expressed in Xenopus oocytes or HEK-293 cells

IC$_{50}$, the concentration required to elicit 50% of the maximum response is indicated by circles: closed circles and open circles indicate human and rat channels respectively.

References: 1, Clozel et al. (1997); 2, Mishra and Hermsmeyer (1994); 3, Monteil et al. (2000a); 4, Monteil et al. (2000b); 5, Akaike et al. (1989); 6, Lee et al. (1999b); 7, Diaz et al. (2005); 8, Williams et al. (1999); 9, Cribbs et al. (1998); 10, Martin et al. (2000); 11, Mehrke et al. (1994); 12, Huang et al. (2004); 13, Furukawa et al. (2004); 14, Hong et al. (2012); 15, Furukawa et al. (2009); and 16, Bernatchez et al. (2001).
3.2 Methods

Rat isolated interlobar arteries were isolated as per the methodology in Chapter 2 (section 2.2).

3.2.1 Characterising the contribution of T and L-type VOCC to renal vascular reactivity

The functional involvement of T- and L-type VOCC in vascular responses to membrane depolarisation- and agonist-mediated responses were assessed in rat isolated interlobar arteries.

Membrane depolarisation-induced contraction

To test the contribution of T- and L-type VOCC to membrane depolarisation-induced contraction this study assessed the effect of various calcium channel inhibitors on potassium-induced membrane depolarisation (Table 3-2).

Following recovery from the KPSS reference contraction, interlobar vessels were equilibrated with either a calcium channel antagonist ± L-NAME (100 μM) or vehicle (PSS). Tissues were then exposed to graded, non-cumulative increases in depolarising potassium solution (10, 20, 40, 62 mM; composition in mmol/l: PSS with an equimolar substitution of KCl at 10, 20, 40 or 60 mmol/l for NaCl). Each exposure lasted two min and tissues were rapidly washed to their baseline thereafter. Arteries were re-equilibrated with their respective antagonist for a further 15 min prior to each subsequent exposure to depolarising potassium solution (Figure 3.3B).

Agonist-induced contraction

To test the contribution of the T- and L-type VOCC to receptor-operated vascular contraction, the effects of calcium channel antagonists on the contractile response to the non-selective adrenocceptor agonist noradrenaline and the potent vasoconstrictor peptide endothelin-1 were assessed. Both vasoconstrictor agents were chosen for their physiological and pathophysiological relevance. Noradrenaline is an endogenous neurotransmitter released from post-ganglionic neurons of the sympathetic nervous system to alter vascular tone. Endothelin-1 is an endogenous peptide implicated in various renal pathologies (Li et al., 1996; Brooks, 1997), pulmonary (MacLean, 1998; Humbert et al., 2004), essential and experimental models of hypertension (Cardillo et al., 1999; Intengan et al., 1999b).

Following recovery from the KPSS reference contraction, interlobar and extrarenal vessels were equilibrated for 30 min with either NNC 55-0396 (100 or 300 nM) or felodipine (1 or 3 nM). Cumulative concentration-response curves were generated to noradrenaline (0.001 - 1 μM) or endothelin-1 (0.1 - 100 nM).

3.2.2 Characterising the selectivity profile of NNC 55-0396 and mibefradil

To further functionally characterise the selectivity profile of NNC 55-0396 and mibefradil, this study also assessed whether the compounds could inhibit vasoconstrictor responses mediated by L-type
VOCC. This study examined the effects of NNC 55-0396 on vasoconstriction elicited by the selective L-type VOCC agonist BAY K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluromethylphenyl)-pyridine-5-carboxylate). BAY K 8644 is an analogue of nifedipine that binds to the same site as dihydropyridines to enhance Ca\(^{2+}\) influx (Schramm et al., 1983; Schramm et al., 1985). To determine the appropriate concentration of BAY K 8644 that would elicit a sub-maximal sustained constriction against which mibefradil- and NNC 55-0396-mediated relaxation could be suitably assessed, concentration-response curves were generated to BAY K 8644 in rat isolated interlobar arteries.

Following recovery from the KPSS reference contraction, concentration-response curves were generated to BAY K 8644 (1–100 nM) to determine an appropriate concentration that would produce a stable contraction equivalent to approximately 80% of the maximum contraction response elicited by KPSS. The responses to BAY K 8644 were generally poor (see section 3.3.3) in vessels under resting tension levels. To enhance this contraction, BAY K 8644 responses were also generated in interlobar arteries pre-constricted with either 10 or 20 mM potassium depolarising solution. BAY K 8644 was found to cause a moderate contraction at 10 nM and was chosen as the most appropriate concentration for all subsequent experiments.

To determine whether NNC 55-0396 and mibefradil were able to inhibit responses mediated by L-type VOCC, vessels were contracted with BAY K 8644 (10 nM) under three different conditions as follows: (i) under resting tension; (ii) pre-constricted with 10 mM potassium depolarising solution and (iii) pre-constricted with 20 mM potassium depolarising solution. Responses to BAY K 8644 were allowed to plateau before addition of either NNC 55-0396 (100 nM) or mibefradil (100 nM). As felodipine is a dihydropyridine known to selectively inhibit L-type VOCC, felodipine (1 nM) was used as a positive control. Parallel time controls were also performed. Responses to the calcium channel antagonists were recorded at 5 and 10 min and then every 10 min thereafter until no further relaxation was observed.

3.2.3 Drugs

The drugs used in this study were obtained from the following suppliers: BAY K 8644 (Bayer, Leverkusen, Germany); ET-1 (GenScript, Piscataway, NJ, USA); felodipine (Hässle, Mölndal, Sweden); mibefradil hydrochloride (Roche, Australia); nicardipine (Bayer); nickel chloride (BDH Merck, Poole, England); nifedipine (Sigma Aldrich, St Louis, Missouri, USA); nisoldipine (Bayer); NNC 55-0396 (Tocris Bioscience, Bristol, UK); noradrenaline bitartrate (Sigma); and Nω-nitro-L-arginine methyl ester (Sigma). ET-1 was prepared as 10\(^{-4}\) M stock solutions in 10% dimethylformamide (DMF). Nifedipine and felodipine were prepared as 10\(^{-2}\) M and 10\(^{-3}\) M stock solutions respectively in dimethyl sulfoxide (DMSO). All other drugs were prepared using milliQ water. Drugs were stored at -20°C until required and diluted to required concentrations with milliQ water. The dihydropyridines were protected from light for the duration of the experiments.
3.2.4 Statistical analysis

Data are expressed as mean (% KPSS contraction) ± 1 SEM of n experiments. In rat isolated interlobar arteries sigmoidal concentration–contraction response curves were fitted using Prism 5 (GraphPad Software, San Diego, CA, USA) for each individual experiment. One-way ANOVA with Dunnett’s post-test for multiple comparisons was used to compare the effect of different calcium channel antagonists or the same calcium channel antagonist at varying concentrations on the EC$_{50}$ and Emax of concentration-response curves. In all cases, two-sided $p \leq 0.05$ was taken as statistically significant.

Alternate method of data analysis

In order to ascertain the contribution of T-type currents and L-type currents to membrane depolarisation-mediated contraction, this method of analysis exploited the fact that T-type currents activate and inactivate at lower membrane potentials than L-type currents. Lower concentrations of potassium chloride (10 and 20 mM) have been shown to depolarise the membrane potential ($E_m$) to approximately -55 mV and -40 mV, respectively (Figure 3-3A) (Fujiwara & Angus, 1996). These voltages fall within the activation range of T- (-65 to -40 mV), but not L-type VOCC (-30 to 0 mV) (Hirano et al., 1989). Thus 10 and 20 mM potassium depolarising solution are proposed to preferentially activate the low-voltage activated T-type VOCC (Figure 3-3B). The assay makes two important assumptions. Firstly that the ability of potassium depolarising solution and KCl to depolarise the membrane does not differ and that the membrane potential is depolarised to a similar magnitude with both solutions. Secondly, that the physiological resting membrane potential is similar between the two vascular beds (mesenteric artery vs. interlobar artery). A step-wise representation of the rationale behind this alternate method of analysis is presented in Figure 3-3.
Table 3-2. List of pre-treatments used to assess the contribution of T- and L-type VOCC to contractions elicited by graded, non-cumulative increases in depolarising potassium solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Proposed mechanism of action*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (PSS)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMSO vehicle (0.003%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.3, 1.0, 3.0 nM</td>
<td>Selective L-type VOCC inhibitor</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>30, 100, 300 nM</td>
<td>Selective T-type VOCC inhibitor</td>
</tr>
<tr>
<td>NNC 55-0396</td>
<td>30, 100, 300 nM</td>
<td>Selective T-type VOCC inhibitor</td>
</tr>
<tr>
<td>NNC 55-0396 + Felodipine</td>
<td>100 nM</td>
<td></td>
</tr>
<tr>
<td>NNC 55-0396 + Felodipine</td>
<td>300 nM</td>
<td></td>
</tr>
<tr>
<td>NiCl₂</td>
<td>300 μM</td>
<td>Selective Ca,v,3.2 inhibitor</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>100 nM</td>
<td>Selective L-type VOCC inhibitor</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>100 nM</td>
<td>Selective L-type VOCC inhibitor</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>30, 100, 300 nM</td>
<td>Selective L-type VOCC inhibitor</td>
</tr>
<tr>
<td>L-NAME</td>
<td>100 μM</td>
<td>Nitric oxide synthase inhibitor</td>
</tr>
<tr>
<td>L-NAME + NNC 55-0396</td>
<td>100 μM + 30 nM</td>
<td></td>
</tr>
<tr>
<td>L-NAME + NNC 55-0396</td>
<td>100 μM + 100 nM</td>
<td></td>
</tr>
</tbody>
</table>

*proposed mechanism of action at the concentration used in this study
Figure 3-3. Step-wise representation of the rationale behind the second method of analysis used to assess the functional role of voltage-operated calcium channels in the vasculature.

A. Chart tracing showing simultaneous recordings of membrane potential (Em) and tension in a rat isolated mesenteric artery in response to increasing concentrations of KCl in the presence of prazosin (100 nM). Figure is modified from Fujiwara and Angus (1996). Note that 10 and 20 mM KCl depolarised the membrane potential to approximately -55 mV and -40 mV respectively, voltages that lie within the activation range of the T-type channel. B. Interlobar vessels were exposed to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K^+). Concentrations of 10 and 20 mM K^+ are proposed to preferentially activate the low-voltage activated T-type VOCC whilst 40 and 62 mM K^+ would activate both T- and L-type VOCC. C. The L-type-mediated component of the contraction can then be derived by subtracting the 20 mM K^+-mediated from the 40 mM K^+-mediated response (T- and L-component).
3.3 Results

3.3.1 Characterising the contribution of T and L-type VOCC to renal vascular reactivity

3.3.1.1 Membrane depolarisation-induced contraction

Equimolar solutions containing 10, 20, 40 and 62 mM K⁺ elicited membrane depolarisation-induced contractions of 3.7 ± 1.8, 40.7 ± 5.6, 84.1 ± 2.6 and 99.7 ± 2.6% of the KPSS-induced maximum contraction, respectively. An EC₅₀ of 23.4 ± 1.4 mM and maximum contractile response of 103 ± 3 %KPSS was obtained when sigmoidal concentration-contraction response curves were fitted (n = 6).

Selective L-type VOCC inhibitors

Felodipine caused concentration-dependent rightward shifts of the potassium concentration response curve and depressed the maximum response. Significant rightward shift was observed with felodipine (n = 4) at all concentrations tested (EC₅₀ 30.4 ± 0.9, 31.6 ± 1.6, 38.9 ± 0.7 mM at 0.3, 1.0 and 3.0 nM respectively, p < 0.0001 vs. control). The maximum response was depressed only at 1.0 and 3.0 nM of felodipine (Emax 85 ± 2 and 82 ± 3 %KPSS, respectively, p < 0.0001 vs. control)(Figure 3-4A and Table 3-3). Felodipine (1 and 3 nM) caused a significant decrease in the maximum response. The changes cannot be attributed to the vehicle DMSO (0.003%) as it did not alter the EC₅₀ or the maximum response of the potassium concentration-response curve (Figure 3-4A and Table 3-3). The selective L-type VOCC inhibitors nifedipine and nicardipine also caused a rightward shift (EC₅₀ 42.5 ± 0.4 and 44.9 ± 1.9 mM, respectively, p < 0.0001 vs. control, Student’s unpaired t-test) and depression of the maximum response of the potassium concentration-response curve (Emax 39 ± 3 and 77 ± 5 %KPSS respectively, p < 0.0001 vs. control, Student’s unpaired t-test).

Selective T-type VOCC inhibitors

The T-type selective antagonists NNC 55-0396 and mibefradil also caused concentration-dependent rightward shifts of the potassium concentration response curve and depression of the maximum response (Figure 3-4B, C and Table 3-3). Significant rightward shift of the control curve was observed at 100 nM and 300 nM of mibefradil (EC₅₀ 31.1 ± 1.3 mM, n = 4 and 41.4 ± 0.8 mM, n = 4, respectively, p < 0.0001 vs. control). A depression of the maximum response was also observed at 100 and 300 nM (Emax 85 ± 5 and 63 ± 4 %KPSS, respectively, p < 0.0001). NNC 55-0396 (100 nM, n = 6) also caused a rightward shift of the potassium concentration-response curve (EC₅₀ 29.8 ± 1.8 mM, p = 0.0002) and decreased the maximum response (Emax 89 ± 3 %KPSS, p < 0.0001) to a comparable magnitude as that observed with mibefradil (100 nM). Responses were completely abolished with 10 μM of NNC 55-0396 (n = 4). In contrast, the selective Ca₃.2 inhibitor, NiCl₂ (10 μM, n = 7), did not affect the potassium concentration-response curve with an EC₅₀ (24.3 ± 2.9 mM) and maximum response (103 ± 4 %KPSS) comparable to that of the control curve. The concentration used was specifically chosen to block Ca₃.2 (IC₅₀ = 10 μM) but not Ca₃.1 or Ca₃.3. (IC₅₀ > 200 μM)(Lee et al., 1999b). When NiCl₂ was used at a concentration likely to affect all T-type channel subtypes (300 μM, n = 7), the potency of potassium-mediated responses was unaltered.
although a decrease in the maximum response was observed (Emax 85 ± 3 %KPSS, p = 0.002 vs. control).

**Combined L- and T-type VOCC inhibition**

Treating vessels with a combination of NNC 55-0396 (100 nM) and felodipine (1 nM) caused a further rightward shift (EC₅₀ 36.8 ± 0.9 mM) in the potassium concentration-response curve than either antagonist alone without further decreasing the maximum response (Emax 85 ± 2 %KPSS, n = 5). Combining higher concentrations (n = 5) however, elicited a greater rightward shift (EC₅₀ 42.0 ± 0.7 mM) and a further depression of maximum (Emax 54 ± 4 %KPSS) than either NNC 55-0396 (300 nM) or felodipine (3 nM) alone.

**Acute NO inhibition**

Treatment of vessels with L-NAME (100 μM, n = 6) did not alter responses to potassium-induced membrane depolarisation-mediated vasoconstriction or resting tension. However, L-NAME appeared to amplify the inhibitory effect of NNC 55-0396 (Figure 3-5). Co-treatment with L-NAME (100 μM) and NNC 55-0396 (100 nM) caused a further rightward shift (EC₅₀ 35.1 ± 1.6 mM, n = 5) than NNC 55-0396 alone (EC₅₀ 29.8 ± 1.8 mM).

**Other considerations**

In order to take into the account the possibility that different concentrations of potassium depolarising solution (and thus membrane depolarisation) recruit a different profile of voltage-operated calcium channels, it was also important to assess the effect of calcium channel inhibitors at each potassium concentration. To facilitate this analysis, data previously represented in non-linear regression curve fits are now re-represented graphically as per Figure 3-6 – Figure 3-8. The response to 10 mM K⁺ (3.7 ± 1.8 %KPSS contraction) was too small a signal to comfortably assess the effect of any calcium channel inhibitor (Figure 3-6A). The exception is perhaps NiCl₂ (300 μM) which tended to potentiate rather than inhibit the response to 10 mM K⁺, although this did not reach significance. 20 mM K⁺ elicited a larger contractile response (40.7 ± 5.6 % KPSS contraction) wherein the effect of antagonists could be assessed. Almost complete inhibition was obtained with NNC 55-0396 (300 nM, 2.5 ± 0.6 % KPSS contraction, p < 0.05 vs. control), mibefradil (300 nM, 6.0 ± 0.7 %KPSS contraction, p < 0.05 vs. control), felodipine (3 nM, 3.4 ± 1.9 %KPSS contraction, p < 0.05 vs. DMSO vehicle control) and nisoldipine at 30 nM (1.8 ± 0.5 %KPSS contraction, p < 0.05 vs. DMSO vehicle control) and 100 nM (1.1 ± 0.6 %KPSS contraction, p < 0.05 vs. DMSO vehicle control). At higher concentrations of potassium depolarising solution (40 and 62 mM K⁺, Figure 3-6C, D), several calcium channel antagonists caused significant inhibition of the contractile response at lower concentrations than that required to inhibit responses to 20 mM K⁺, including NNC 55-0396, mibefradil and felodipine. NiCl₂ (300 μM) also caused moderate inhibition of responses to 40 and 62 mM K⁺ (p < 0.05 vs. control). All statistical analyses were performed using one-way ANOVA with Dunnett’s post-test for multiple comparisons.
Combining NNC 55-0396 and felodipine resulted in more effective inhibition of membrane depolarisation-mediated vasoconstriction responses than either antagonist alone (p < 0.0001, one-way ANOVA with Bonferroni’s post-test for multiple comparisons), particularly at higher concentrations of potassium depolarising solution (40 and 62 mM K⁺, Figure 3-7). The presence of L-NAME (100 μM) potentiated (p < 0.0001, one-way ANOVA with Bonferroni’s post-test for multiple comparisons) the inhibitory capacity of NNC 55-0396 (300 nM) on 40 mM K⁺-mediated contractile responses, but had little effect on NNC 55-0396-mediated inhibition at other concentrations of potassium depolarising solution (Figure 3-8).

3.3.2 Receptor-operated vascular contraction

At concentrations that caused a significant rightward shift in the potassium concentration-response curve, felodipine (1 and 3 nM, n = 4) did not alter noradrenaline potency in interlobar arteries (Figure 3-9A and Table 3-4). A leftward shift in the noradrenaline concentration-response curve was observed with felodipine in extrarenal arteries, but only at 3 nM. In contrast NNC 55-0396 (100 nM) decreased noradrenaline potency by ~ 4-fold in interlobar (pEC50, 7.30 ± 0.11 to 6.70 ± 0.05, n = 4, p < 0.01) and extrarenal arteries (pEC50, 7.09 ± 0.17 to 6.47 ± 0.1, n = 6, p < 0.05) without affecting Emax (Figure 3-9B and Table 3-4). Higher concentrations of NNC 55-0396 had no further effect on noradrenaline potency, suggesting maximal inhibition was achieved at 100 nM. At the concentrations tested, neither felodipine nor NNC 55-0396 significantly altered the maximum contractile response. This is in contrast to the ability of NNC 55-0396 to inhibit potassium-mediated membrane depolarisation-induced contraction where inhibition was concentration-dependent and responses could be completely abolished with sufficiently high concentrations (Figure 3-4B). That NNC 55-0396 can abolish potassium-induced contractions (even those elicited with high potassium concentrations and thus strong depolarisations) indicates non-selective activity at the L-type VOCC. However if one considers that many L-type VOCC selective antagonists have failed to inhibit noradrenaline-mediated vasoconstriction, then this study has shown that NNC 55-0396 is acting through T-type VOCC to inhibit noradrenaline mediated contraction at concentrations up to 100 nM. At what concentration NNC 55-0396 loses its selectivity for the T-type VOCC is unknown and will be addressed in section 3.3.3. When vessels were pre-treated with a combination of NNC 55-0396 (100 nM) and felodipine (1 nM) to assess the effect of simultaneous blockade of T- and L-type VOCC respectively on noradrenaline-mediated contraction, the response was similar to that observed in the presence of NNC 55-0396 (100 nM) alone (Figure 3-9).

NNC 55-0396-mediated inhibition of receptor-operated vascular contraction appears to be constrictor-selective. The drug does not alter endothelin-1 potency in interlobar or extrarenal arteries (Figure 3-10; Table 3-5). It does however decrease the maximal response induced by endothelin-1 but only at 300 nM and only in interlobar arteries (Emax, 122 ± 3 to 86 ± 5 %KPSS contraction, n = 5, p < 0.001 vs. control). Felodipine (3 nM) also decreased the maximal response induced by endothelin-1 in interlobar arteries (Emax, 122 ± 3 to 104 ± 4 %KPSS, n = 4, p < 0.001 vs. control) though the magnitude of inhibition was comparatively lower. Pre-treatment with a combination of NNC 55-0396 (100 nM) and felodipine (1 nM) decreased maximal endothelin-1 contraction in
extrarenal (Emax, 101 ± 2 to 87 ± 2 %KPSS, n = 4, p < 0.05 vs. control, Figure 3-11) but not interlobar arteries. Co-treatment with higher concentrations of NNC 55-0396 (300 nM) and felodipine (3 nM) however, did decrease maximal endothelin-1 contraction in interlobar arteries (Emax, 122 ± 3 to 74 ± 5 %KPSS, n = 5, p < 0.001 vs. control).

3.3.3 Characterising the selectivity profile of NNC 55-0396

Determining an optimum concentration of BAY K 8644

BAY K 8644 caused a concentration-dependent vasoconstriction (1 – 100 nM). However, under basal resting conditions the response to BAY K 8644 fell into two levels (Figure 3-11A). Half of the tested arteries (n = 3 of 6) responded well, reaching a maximum of 47 ± 1 %KPSS with a pEC50 of 8.19 ± 0.09. The other half responded poorly, reaching a maximum of 15 ± 3 %KPSS with a pEC50 of 7.66 ± 0.12. It has been suggested that the drug enhances Ca2+ influx by modifying channel opening time or rate of channel opening and thus requires that the L-type VOCC be in its active state (Schramm et al., 1985). This requirement may explain why under basal conditions, BAY K 8644 can cause vasoconstriction in some vascular beds (Mikkelsen et al., 1985; Salaices et al., 1985), but little to no vasoconstriction in other vascular beds that may be more quiescent (Schramm et al., 1983; Salaices et al., 1985; Uski & Andersson, 1985; Asano et al., 1986; Hansen et al., 2001). There are few reports where BAY K 8644 alone is able to cause significant vasoconstriction. In rat thoracic aorta BAY K 8644 induced concentration-dependent vasoconstriction (pEC50 = 6.66), with 10^{-6} M causing a contraction similar in magnitude to that of a maximally activated artery (Mikkelsen et al., 1985). Concentration-dependent vasoconstriction was also observed in middle cerebral arteries (Salaices et al., 1985). However, direct opening of the channel was insufficient to reach the maximal contractile capacity of the artery. BAY K 8644 (10^{-6} M) was able to elicit approximately only 50% of the maximum contraction with higher doses causing relaxation (Salaices et al., 1985). Middle cerebral arteries were also comparatively less sensitive (pEC50 = 8.5) to the drug than thoracic aorta. Most studies however observe little to no vasoconstriction. This includes afferent arterioles (Hansen et al., 2001), rabbit mesenteric artery (Kanmura et al., 1984) and aortic strips (Schramm et al., 1983), rat mesenteric, femoral (Asano et al., 1986) and tail (Su et al., 1984; Asano et al., 1986) arteries and feline basilar (Uski & Andersson, 1985) and femoral arteries (Salaices et al., 1985). In such quiescent arteries, partial depolarisation with potassium solution (10 to 15 mM) was usually sufficient to enhance BAY K 8644-mediated concentration-dependent vasoconstriction. Partial depolarisation activates a greater proportion of L-type VOCC whose activity BAY K 8644 is then able to modulate. Consequently this study also generated concentration-response curves to BAY K 8644 in arteries pre-constricted with 10 or 20 mM K+ depolarising solution to pre-activate L-type VOCC. In arteries pre-constricted with 10 mM K+, BAY K 8644 caused a maximum contraction of 59 ± 7 %KPSS contraction with a pEC50 of 8.37 ± 0.15 (Figure 3-11B). In arteries pre-constricted with 20 mM K+, a greater maximal contraction was achieved (75 ± 3 %KPSS contraction) with similar potency (pEC50, 8.09 ± 0.10, Figure 3-11C). Thus in arteries pre-contracted with 20 mM K+ depolarising solution, BAY K 8644 (10 nM) was found to produce a stable contraction (approximately
75-80% of its KPSS-induced maximum contractile response) suitable for testing the effect of NNC 55-0396 and other established calcium channel inhibitors.

**Does NNC 55-0396 have inhibitory activity at the L-type VOCC?**

BAY K 8644-mediated pre-contraction in vessels pre-constricted with 20 mM potassium was similar between groups (approximately 75-80%) \( p = 0.336 \), one-way ANOVA with Bonferroni post-test for multiple comparisons). At a concentration that caused significant inhibition of potassium-mediated responses (Figure 3-4B), NNC 55-0396 (100 nM) caused a small relaxation response, with a maximum decrease in tone of 23 ± 6% KPSS contraction \( p < 0.05 \) compared to that of time control: 0.8 ± 2.3 %KPSS contraction). At the same concentration (100 nM), mibefradil elicited a slightly greater relaxation response than NNC 55-0396. The decrease in tone was 29 ± 6 % KPSS contraction \( p < 0.05 \) compared to that of time control. In contrast, felodipine (1 nM), caused very little relaxation of BAY K 8644 mediated pre-constriction. At 30 min, tone had decreased by only 8 ± 4% KPSS contraction. Of note, the relaxation response often took in excess of 25 min to plateau regardless of the inhibitor being assessed (Figure 3-12).

3.3.3.1 **T-/L- selectivity ratio**

To determine the T-/L- selectivity ratio, an assay was developed to dissect apart the contribution of T- and L-type VOCC to membrane depolarisation-mediated vasoconstriction. As 20 mM K⁺ is presumed to result predominately from T-type VOCC activation, the concentration of calcium channel inhibitor required to inhibit 50% of the response was taken as the inhibitor’s IC\(_{50}\) at the T-type VOCC. The calculated IC\(_{50}\)s are as follows: mibefradil (54 nM), NNC 55-0396 (55 nM), felodipine (1.6 nM) and nisoldipine (3 nM). It is noted that although L-type selective inhibitors felodipine and nisoldipine appear to inhibit T-type channels at low nanomolar concentrations, this may be due to an inhibitory effect of the DMSO vehicle.

The arithmetically-derived L-type component of membrane depolarisation-mediated vasoconstriction was taken as the response to 40 mM K⁺ minus the response to 20 mM K⁺ (within tissue) and is shown in (Figure 3-13). The data suggest that mibefradil does not inhibit the L-type component for all the concentrations tested. Although mibefradil did tend to inhibit the response at 300 nM, this inhibition was not significant \( p = 0.070 \) vs. control, one-way ANOVA with Dunnett’s post-test for multiple comparisons). Similarly, the L-type component was not affected by any concentration of NNC 55-0396 \( p = 0.151 \) vs. control) or felodipine \( p = 0.476 \) vs. DMSO vehicle control, one-way ANOVA with Dunnett’s post-test for multiple comparisons). In contrast nisoldipine caused concentration-dependent inhibition of the L-type VOCC-mediated component of membrane depolarisation-mediated vasoconstriction. Full inhibition was observed at 30 nM enabling determination of its IC\(_{50}\) at L-type VOCC in a functional preparation (IC\(_{50}\) ~12). Thus the T-/L- selectivity ratio could only be appropriately determined for nisoldipine (ratio of 4).
Figure 3-4. Effect of various calcium channel inhibitors on membrane depolarisation-mediated vasoconstriction in rat isolated renal interlobar arteries

Contractile responses of rat isolated renal interlobar arteries exposed for 2 min to graded, non-cumulative and sequential increases in depolarising potassium solution (10, 20, 40 and 62 mM) in the absence (control, PSS, ●, n = 6) and presence of increasing concentrations of A. felodipine at 0.3 nM (○, n = 4), 1 nM (●, n = 4), 3 nM (●, n = 4) and vehicle (0.003% DMSO, ◊ n = 4); B. NNC 55-0396 at 30 nM (●, n = 7), 100 nM (●, n = 6), 300 nM (●, n = 5) and 10000 nM (◊, n = 1); C. mibefradil at 30 nM (●, n = 4), 100 nM (●, n = 4) and 300 nM (●, n = 4); and D. NiCl₂ at (10 µM, ●, n = 4) and (300 µM, ●, n = 7).

Vertical bars are ± 1 SEM; where no error bar is visible the SEM is within the symbol. Horizontal error bars represent the EC⁵₀ ± 1 SEM. *p < 0.05, EC⁵₀ compared to control group; † p < 0.05, maximum response compared to control group (one-way ANOVA with Dunnett’s post-test for multiple comparisons).

n, number of arteries from separate rats.
Figure 3-5. Role of nitric oxide in membrane depolarisation-mediated contractile responses and L- and T-type VOCC-mediated responses

Contractile responses of rat isolated renal interlobar arteries exposed for 2 min to graded, non-cumulative and sequential increases in depolarising potassium solution (10, 20, 40 and 62 mM) in the absence (control, PSS, ●, n = 6) and presence of nitric oxide synthase inhibitor L-NAME (100 μM, ○, n = 6). Separate arteries were cotreated with L-NAME (100 μM) and either 30 nM NNC 55-0396 (○, n = 5) or 100 nM NNC 55-0396 (○, n = 4). Solid lines and closed symbols indicate vessels treated with NNC 55-0396 only (30nM, ●, n = 7; 100nM, ●, n = 5) and are re-shown for comparison. Vertical bars are ± 1 SEM; where no error bar is visible the SEM is within the symbol. Horizontal error bars represent the EC\textsubscript{50} ± 1 SEM. *p < 0.05, EC\textsubscript{50} compared to that of vessels treated with respective concentration of NNC 55-0396 (unpaired t-test). n, number of arteries from separate rats.
Table 3-3. The effect of various calcium channel antagonists on the EC$_{50}$ and Emax of K$^+$-mediated membrane depolarisation-induced contraction in rat isolated interlobar arteries.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC$_{50}$ (mM)</th>
<th>Emax</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.4 ± 1.4</td>
<td>103 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>DMSO (0.003%)</td>
<td>28.5 ± 2.4</td>
<td>103 ± 2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Felodipine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 nM</td>
<td>30.4 ± 0.9 **</td>
<td>104 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>1.0 nM</td>
<td>31.6 ± 1.6 **</td>
<td>85 ± 2 ***</td>
<td>4</td>
</tr>
<tr>
<td>3.0 nM</td>
<td>38.9 ± 0.7 ***</td>
<td>82 ± 3 ***</td>
<td>4</td>
</tr>
<tr>
<td><strong>Mibefradil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 nM</td>
<td>27.0 ± 2.5</td>
<td>96 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>100 nM</td>
<td>31.1 ± 1.3 *</td>
<td>85 ± 5 **</td>
<td>4</td>
</tr>
<tr>
<td>300 nM</td>
<td>41.4 ± 0.8 ***</td>
<td>63 ± 4 ***</td>
<td>4</td>
</tr>
<tr>
<td><strong>NNC 55-0396</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 nM</td>
<td>27.4 ± 1.5</td>
<td>100 ± 1</td>
<td>7</td>
</tr>
<tr>
<td>100 nM</td>
<td>29.8 ± 1.8 *</td>
<td>89 ± 3 ***</td>
<td>6</td>
</tr>
<tr>
<td>+ felodipine (1 nM)</td>
<td>37.0 ± 0.6 ***</td>
<td>85 ± 2 ***</td>
<td>5</td>
</tr>
<tr>
<td>300 nM</td>
<td>37.9 ± 1.1 ***</td>
<td>77 ± 1 ***</td>
<td>5</td>
</tr>
<tr>
<td>+ felodipine (3 nM)</td>
<td>42.0 ± 0.7 ***</td>
<td>54 ± 4 ***</td>
<td>5</td>
</tr>
<tr>
<td><strong>NiCl$_2$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM</td>
<td>24.3 ± 2.9</td>
<td>103 ± 4</td>
<td>7</td>
</tr>
<tr>
<td>300 μM</td>
<td>22.8 ± 2.9</td>
<td>85 ± 3 ***</td>
<td>7</td>
</tr>
<tr>
<td><strong>Nicardipine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td>42.5 ± 0.4 ***</td>
<td>39 ± 3 ***</td>
<td></td>
</tr>
<tr>
<td><strong>Nifedipine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td>44.9 ± 1.9 ***</td>
<td>77 ± 5 ***</td>
<td>4</td>
</tr>
<tr>
<td><strong>L-NAME</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 μM</td>
<td>23.2 ± 2.1</td>
<td>105 ± 4</td>
<td>6</td>
</tr>
<tr>
<td>+ NNC 55-0396 (30 nM)</td>
<td>27.0 ± 1.3 *</td>
<td>93 ± 1</td>
<td>4</td>
</tr>
<tr>
<td>+ NNC 55-0396 (100 nM)</td>
<td>35.1 ± 1.6 **</td>
<td>80 ± 5</td>
<td>5</td>
</tr>
</tbody>
</table>

EC$_{50}$, concentration required to achieve half-maximal response in mM; Emax the maximum contractile effect of the potassium-mediated response expressed as % KPSS-induced maximum contraction; DMSO, dimethyl sulfoxide; L-NAME, L-Nitro-Arginine methyl ester. Calcium channel antagonists were incubated for 30 min prior to noradrenaline contraction. *p < 0.05, **p < 0.01, ***p < 0.001, compared to control, one-way ANOVA with Dunnett’s post-test for multiple comparisons. n, number of arteries from separate rats.
Role of T-type calcium channels in rat renal vascular tone

A. 10 mM K^+

B. 20 mM K^+

C. 40 mM K^+

D. 62 mM K^+

% KPSS

control | NNC 55-0396 (nM) | mibebradil (nM) | nickel (µM) | DMSO | felodipine (nM) | nisoldipine (nM)
Figure 3-6. Calcium channel inhibitors caused concentration-dependent inhibition of potassium-mediated responses

Interlobar vessels isolated from male SD rats were exposed for 2 min to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K+). These responses were performed in the absence (control, n = 6) or presence of either NNC 55-0396 (30, 100 or 300 nM, n = 5-7), mibebradil (30, 100 or 300 nM, n = 4), nickel (10, 300 μM, n = 4-7), felodipine (0.3, 1.0 or 3.0 nM, n = 4), nisoldipine (30, 100, 300 nM, n = 4) or DMSO vehicle (0.0003%, n = 4). Each treatment was equilibrated for 30 min (see section 3.2.1 for detailed methods). *p < 0.05 response compared to control or respective DMSO vehicle control with one-way ANOVA and Dunnett’s post-test for multiple comparison. n, number of arteries from separate rats.
Interlobar vessels isolated from male SD rats were exposed for 2 min to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K⁺). Potassium depolarising solution-mediated responses were performed in PSS and in the presence of two combination pre-treatments: NNC 55-0396 (100 nM) with felodipine (1 nM) and NNC 55-0396 (300 nM) with felodipine (3 nM). The effect of each antagonist alone is also shown for comparison purposes. Each treatment was equilibrated for 30 min (see section 3.2.1 for detailed methods). *p < 0.05 response vs. control response unless otherwise indicated, one-way ANOVA with Bonferroni post-test for multiple comparisons. n = number of arteries from separate rats.
Interlobar vessels isolated from male SD rats were exposed for 2 min to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K⁺). Potassium depolarising solution-mediated responses were performed in PSS and in the presence of either L-NAME (100 μM), L-NAME (100 μM) + NNC 55-0396 (30 nM) or L-NAME (100 μM) + NNC 55-0396 (100 nM). The effect of each NNC 55-0396 concentration alone is also shown for comparison purposes. Each treatment was equilibrated for 30 min (see section 3.2.1 for detailed methods). *p < 0.05 response vs. control response unless otherwise indicated, one-way ANOVA with Bonferroni’s post-test for multiple comparisons. n = number of arteries from separate rats.
Figure 3-9. Selective T- and L-type channel inhibitors decrease noradrenaline potency without altering efficacy in isolated interlobar and extrarenal arteries

Concentration-dependent responses to noradrenaline in the absence (control, PSS, •, n = 6) and presence of felodipine (1 nM: ○, n = 5) and NNC 55-0396 (100 nM: ▲, n = 4). The effect of co-treatment with felodipine (1 nM) and NNC 55-0396 (100 nM) was also assessed (□, n = 4). The effect of high concentrations of felodipine (3 nM: ●, n = 5) and NNC 55-0396 (300 nM: ▲, 300 nM, n = 5) were assessed separately and in combination (■, n = 5). Vertical bars are ±1 SEM; where no error bar is visible the SEM is within the symbol. Horizontal error bars represent the EC_{50} ±1 SEM. *p < 0.05, EC_{50} compared to control (one-way ANOVA with Dunnett’s post-test for multiple comparison). n, number of arteries from separate rats. For clarity of curves, individual * symbols are not shown.
### Table 3-4. The effect of various calcium channel antagonists on the $pEC_{50}$ and $Emax$ of noradrenaline in rat isolated interlobar and extrarenal arteries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$pEC_{50}$</th>
<th>$Emax$ (%KPSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noradrenaline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.30 ± 0.11</td>
<td>116 ± 5</td>
</tr>
<tr>
<td>NNC 55-0396</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td>6.70 ± 0.05**</td>
<td>114 ± 6</td>
</tr>
<tr>
<td>300 nM</td>
<td>6.67 ± 0.07***</td>
<td>118 ± 5</td>
</tr>
<tr>
<td><strong>Felodipine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 nM</td>
<td>7.19 ± 0.10</td>
<td>125 ± 5</td>
</tr>
<tr>
<td>3 nM</td>
<td>6.99 ± 0.15</td>
<td>123 ± 2</td>
</tr>
<tr>
<td>NNC 55-0396 (100 nM) + felodipine (1 nM)</td>
<td>6.90 ± 0.10*</td>
<td>118 ± 2</td>
</tr>
<tr>
<td>NNC 55-0396 (300 nM) + felodipine (3 nM)</td>
<td>6.48 ± 0.05***</td>
<td>109 ± 7</td>
</tr>
</tbody>
</table>

$pEC_{50}$, negative log_{10} of the concentration required to achieve a half-maximal response; $Emax$ the maximum contractile effect of noradrenaline expressed as % KPSS-induced maximum contraction. Calcium channel antagonists were incubated for 30 min prior to noradrenaline contraction. *$p < 0.05$, ***$p < 0.001$ compared to control (one-way ANOVA with Dunnett’s post-test for multiple comparison). $n$, number of arteries from separate rats.
Figure 3-10. Differential effect of L- and T-type channel inhibition on endothelin-1 mediated vasoconstriction in the interlobar and extrarenal arteries.

Concentration-dependent responses to endothelin-1 in the absence (control, PSS, ●, n = 6) and presence of felodipine (1 nM: ○, n = 4;) and NNC 55-0396 (100 nM: △, n = 4). The effect of co-treatment with felodipine (1 nM) and NNC 55-0396 (100 nM) was also assessed (□, n = 5). The effect of high concentrations of felodipine (3 nM: ●, n = 4) and NNC 55-0396 (300 nM: ▲, 300 nM, n = 4) were assessed separately and in combination (■, n = 4). Vertical bars are ± 1 SEM; where no error bar is visible the SEM is within the symbol. Horizontal error bars represent the EC_{50} ± 1 SEM. *p < 0.05, EC_{50} compared to control (one-way ANOVA with Dunnett’s post-test for multiple comparison). †p < 0.05, Emax compared to control (one-way ANOVA with Dunnett’s post-test for multiple comparison). n, number of arteries from separate rats.
Table 3-5. The effect of various calcium channel antagonists on the \( pEC_{50} \) and Emax of endothelin-1 in rat isolated interlobar and extrarenal arteries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pEC(_{50})</th>
<th>Emax (%KPSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interlobar</td>
<td>Extrarenal</td>
</tr>
<tr>
<td>Control</td>
<td>8.31 ± 0.05</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>8.12 ± 0.03</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>101 ± 2</td>
<td>9</td>
</tr>
<tr>
<td>NNC 55-0396</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nM</td>
<td>8.19 ± 0.06</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>96 ± 6</td>
<td>5</td>
</tr>
<tr>
<td>300 nM</td>
<td>8.15 ± 0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>98 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>Felodipine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 nM</td>
<td>8.16 ± 0.03</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>95 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>3 nM</td>
<td>8.21 ± 0.04</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>91 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>NNC 55-0396 (100 nM) + felodipine (1 nM)</td>
<td>8.11 ± 0.04</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>85 ± 3**</td>
<td>4</td>
</tr>
<tr>
<td>NNC 55-0396 (300 nM) + felodipine (3 nM)</td>
<td>8.05 ± 0.15*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>77 ± 4***</td>
<td>5</td>
</tr>
</tbody>
</table>

\( pEC_{50} \), negative \( \log_{10} \) of the concentration required to elicit a half-maximal response; Emax the maximum contractile effect of noradrenaline expressed as % KPSS-induced maximum contraction. Calcium channel antagonists were incubated for 30 min prior to noradrenaline contraction. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \) compared to control (one-way ANOVA with Dunnett’s post-test for multiple comparison). \( n \), number of arteries from separate rats.
Figure 3.11. Individual concentration-response curves to the selective L-type VOCC agonist BAY K 8644 following the application of varying degrees of vascular tone

Responses to BAY K 8644 were inconsistent when applied to vessels under resting tension (A). Applying a small amount of tone with either 10 mM K⁺ (B) or 20 mM K⁺ consistently enabled BAY K 8644 to elicit a concentration-dependent contractile response. Curves represent responses in arteries isolated from different rats. The mean curve is superimposed and indicated by open circles and dotted lines (○) with SEM of n = 6.
Figure 3-12. L-type calcium channel agonist BAY K 8644 is unable to sustain contraction in the presence of T-type selective calcium channel inhibitors

The ability of NNC 55-0396 (○, 100 nM, n = 5), felodipine (●, 1 nM, n = 4) or mibefradil (●, 100 nM, n = 4) to inhibit BAY K 8644-mediated constriction (10 nM) was assessed in arteries pre-constricted with 20 mM K⁺ solution (tone). BAY K 8644-mediated constriction was allowed to plateau before testing the effect of a calcium channel inhibitor. Responses to the calcium channel antagonists were recorded at 0, 5 and 10 min and then every 10 min thereafter until no further relaxation was observed. Vertical bars are ±1 SEM; where no error bar is visible the SEM is within the symbol. †p < 0.05, ΔRmax (%KPSS contraction) compared to time control (one-way ANOVA with Dunnett’s post-test for multiple comparison). n, number of arteries from separate rats.
Role of T-type calcium channels in rat renal vascular tone

Figure 3.13. Ability of various calcium channel antagonists to inhibit the L-type component of membrane depolarisation-mediated vasoconstriction of rat isolated interlobar arteries

Interlobar vessels isolated from male SD rats were exposed for 2 min to graded, non-cumulative, sequential increases in depolarising potassium solution (10, 20, 40, 62 mM K⁺). Potassium depolarising solution-mediated responses were performed in PSS (control) or vehicle control (DMSO 0.0003 %) and in the presence of increasing concentrations of A. mibefradil; B. NNC 55-0396; C. felodipine; and D. nisoldipine. The proposed L-type component of membrane depolarisation-mediated vasoconstriction was taken as the response to 40 mM K⁺ minus the response to 20 mM K⁺ (within tissue) and has been superimposed onto each graph (○). The half maximal inhibitory concentrations (IC₅₀) of each inhibitor for the T- and L-type components were then determined from the corrected curves allowing the estimation of T- and L-type VOCC selectivity (refer to section 3.2.4 and Figure 3-3 for more details). Vertical bars are ±1 SEM.
3.4 Discussion

In the present study, *in vitro* pharmacological characterisation was used to show that T-type voltage-operated calcium channels have functional importance in small renal arteries (interlobar). Both T- and L-type voltage-operated calcium channels may be required to elicit maximal contraction in vascular responses elicited by depolarisation-activated Ca$^{2+}$ influx. T-type channels may also play a role in agonist-mediated vasoconstrictor responses, though they do not appear to be necessary for the contraction to occur *per se*.

In interlobar arteries, depolarisation-mediated contractions elicited with potassium were sensitive to the L-type selective voltage-operated calcium channel inhibitors, nifedipine, felodipine and nisoldipine. Both nifedipine and felodipine have little inhibitory action at all three subtypes of the T-type channels (Cav3.2, Cav3.2 and Cav3.1) when expressed in Xenopus oocytes. Nisoldipine has been shown to abolish native L-type current at 30 nM without impacting current mediated by T-type calcium channels a current that was subsequently successfully abrogated with mibefradil (1 μM) (Mishra & Hermsmeyer, 1994). Not surprisingly, the data in this study corroborates the essential role L-type voltage-operated calcium channels play in the electromechanical coupling required during depolarisation-mediated vasoconstriction. Mibefradil also inhibited the response to depolarisation-mediated contractions suggesting a role for T-type channels alongside L-type channels in small renal arteries of the rat. Mibefradil decreased both the potency and efficacy of potassium-mediated contractions at a concentration (100 nM) considered selective for the T-type calcium channel (Figure 3-4.C). Further inhibition was achieved at 300 nM. Higher concentrations were not tested as the compound is reportedly non-selective at concentrations greater than 10$^{-6}$M, a concentration that is unfortunately commonly employed when studying T-type calcium channels (Hong et al., 2012).

Mibefradil has been shown to inhibit voltage-dependent potassium channels (Hong et al., 2012), chloride channels in macrovascular endothelial cells (Nilius et al., 1997) and L-type voltage channels through one of its hydrolysed metabolites (Wu et al., 2000) when utilised at micromolar concentrations. Even then, the compound retains a 10-fold selectivity for T-type calcium channels over L-type calcium channels (Bezprozvanny & Tsien, 1995).

NNC 55-0396 also decreased the potency and efficacy of potassium-mediated contraction, further reinforcing a functional role for T-type calcium channels in electromechanical coupling and vasoconstriction in small renal arteries (Figure 3-4.B). NNC 55-0396 is a structural analogue of mibefradil with greater selectivity for T- over L-type calcium channels than mibefradil (Huang et al., 2004). As per mibefradil, NNC 55-0396 caused partial inhibition of potassium-mediated vasoconstriction at concentrations ranging of 100-300 nM. Problematically, the selectivity of this inhibitor for T-type calcium channels has recently been disputed. NNC 55-0396 inhibited nifedipine-insensitive high-voltage activated current with an IC$_{50}$ of 960 nM (Björling et al., 2013). The identities of the high-voltage activated calcium channels suspected to facilitate nifedipine-insensitive current are the P- and Q-type voltage-operated calcium channels. P- and Q-type calcium channels are splice variants of the Ca$^{2+}$ gene (Ca$^{2+}$,1a and Ca$^{2+}$,1b respectively) and are now known to be expressed in vascular smooth muscle cells of renal resistance vessels. Notably, both P- and Q-type voltage-
operated calcium channels have been suggested to be of functional importance in depolarisation-mediated constriction of small arteries (interlobar and arcuate) of the human renal vasculature (Hansen et al., 2011), rat renal afferent arterioles and vasa recta (Hansen et al., 2000). Note that in the rat, P- and Q-type currents have not been explicitly demonstrated in the vascular smooth muscle of all small renal arteries, only in preglomerular afferent arterioles (Andreasen et al., 2006). Further studies are required to assess the contribution of P- and Q-type current to potassium-mediated vasoconstriction in rat small renal arteries. At the moment, these currents can only be discerned from other high-voltage activated currents pharmacologically through their sensitivity to ω-agatoxin (Mintz et al., 1992).

As different concentrations of potassium-depolarising solution (and thus different degrees of membrane depolarisation) may recruit a different profile of voltage-operated calcium channels, we found it prudent to analyse the effect of each calcium channel inhibitor at each potassium concentration (10, 20, 40 and 62 mM K⁺). At 20 mM, potassium has been shown to cause small depolarisations to a membrane potential of -40 mV (Fujiwara & Angus, 1996). This is within the activation range of T- but not L-type calcium channels (Hirano et al., 1989). Yet, the data showed that both T- and L-type selective calcium channel inhibitors were able to completely abolish the contractile response to potassium (Figure 3-6B). This may suggest that a) the L-type calcium channel inhibitors utilised are not as selective as previously thought at the concentrations employed or b) potassium 20 mM does not exclusively recruit T-type channels to elicit vasoconstriction. Given the abundance of studies demonstrating the L-type selectivity of felodipine, nifedipine and nisoldipine, the most recent being a study by Furukawa et al. (2009), option b seems a more likely scenario. At 40 mM and 62 mM, potassium has been shown to cause large depolarisations to membrane potentials of about -25 mV. These depolarisations are presumed to activate high voltage-operated calcium channels (Hirano et al., 1989; Fujiwara & Angus, 1996) and are large enough to potentially inactivate T-type calcium channels. The ability of mibefradil and NNC 55-0396 to partially inhibit responses to 40 and 62 mM potassium indicates that a), T-type channels are activated and functionally contribute to contractions elicited by high potassium or b), mibefradil and NNC 55-0396 are non-selective, inhibiting other high voltage-activated calcium channels from 0.1 μM upwards.

Notably, the selective T-type channel inhibitor nickel did not affect the magnitude of the contractile response to potassium. The concentration utilised in the study was within the range of the estimated IC₅₀ values for Ca,3.2 (Figure 3-2), a channel predominantly linked to smooth muscle-dependent vasodilatation (Hansen, 2014). Using the same concentration employed in this study (10 μM), Poulsen et al. (2011) showed that nickel prolonged the duration of potassium-mediated constriction in mouse cortical efferent arterioles by inhibiting the secondary vasodilatation response that typically follows depolarisation elicited by high potassium solution. In the presence of nickel, complete closure of the efferent arteriole lasted approximately 47 seconds compared to 16 seconds in the absence of the metal ion. The present study did not quantitate the duration of the constriction elicited by potassium. However, it was clear from simple observation that potassium-mediated contraction was not prolonged with nickel as the general shape of the potassium-mediated
contractile response was preserved (rapid contraction followed almost immediately by a decrease in tone). This suggests that in the larger intrarenal arteries like the interlobar arteries, the Ca\textsubscript{v}3.2 channel is unlikely to be mediating the secondary relaxation response, at least in the rat kidney. When applied to interlobar arteries at a higher concentration (300 μM), nickel caused partial inhibition of the contractile response to 40 and 62 mM of potassium. At this concentration, nickel has inhibitory properties at all three T-type channels (Figure 3-2) and may be impeding vascular contraction by blocking Ca\textsubscript{v}3.1-mediated calcium influx into smooth muscle cells.

Ca\textsubscript{v}3.2 has been implicated in the local longitudinal conduction of dilatation following the initial depolarisation-mediated constriction (Figueroa et al., 2007). Propagation of the electrical signal along endothelial cells may activate Ca\textsubscript{v}3.2 channels, leading to an increase in intracellular calcium. This activates eNOS and calcium-activated potassium channels, leading to nitric oxide-mediated and hyperpolarisation-mediated smooth muscle relaxation, respectively. Ca\textsubscript{v}3.1 channels located on endothelial cells are also thought to mediate vasodilatation via a nitric oxide pathway (Figure 3-1). Recent studies suggest that the function of T-type channels is intimately linked with changes in nitric oxide availability. In the present study, L-NAME-mediated inhibition of nitric oxide synthesis did not significantly affect resting tension nor potassium-mediated vasoconstriction (Figure 3-5 and Figure 3-8), indicative of little to no basal nitric oxide release. The combination of acute nitric oxide inhibition and T-type channel inhibition caused a greater rightward shift of the potassium-response curve than the inhibition of T-type channels alone (Figure 3-5). The reason for this is unknown. Acute inhibition of nitric oxide production has been shown to enhance the contribution of T-type channels to vascular tone, particularly in larger arterioles. This may occur via the recruitment of cytoplasmic Ca\textsubscript{v}3.1 to the cell membrane or increased Ca\textsubscript{v}3.1 protein expression. This was observed in the cerebral vasculature in vitro and in cremaster muscle arterioles in vivo (Howitt et al., 2013). The enhanced inhibitory capacity of NNC 55-0396 in the presence of acute nitric oxide inhibition, may point to the recruitment of T-type channels to the cell membrane in smooth muscle cells. This may lead to a greater contribution of T-type current to the calcium influx required for membrane depolarisation-mediated contraction. If this is the case however, it is unusual that L-NAME alone failed to enhance the constrictor response to potassium.

The contribution of T- and L-type calcium channels to agonist-mediated vasoconstriction was region-dependent. T-type calcium channels may contribute to the pharmacomechanical coupling mechanism that occurs during agonist-mediated vasoconstriction in both large and small renal arteries while L-type calcium channels are limited to large renal arteries. Felodipine (3 nM) caused a parallel leftward shift of the noradrenaline response-curve in extrarenal but not interlobar arteries (Figure 3-9A). NNC 55-0396 (100 nM) caused a parallel leftward shift (4-fold) of the noradrenaline concentration-response curve in both interlobar and extrarenal arteries. A higher concentration of NNC 55-0396 had no added inhibitory effect, suggesting that the functional contribution of T-type channels to noradrenaline-mediated vasoconstriction was completely inhibited by 100 nM. The data suggests that although T-type calcium channels may contribute to the increase in intracellular calcium, its activation was not required for the contraction to occur per se. The results can be
aligned with the current paradigm surrounding pharmacomechanical coupling mechanisms. Pharmacomechanical coupling can occur independently of changes in membrane potential and thus the increase in intracellular Ca\(^{2+}\) required for contraction does not necessitate the activation of voltage-operated calcium channels and the subsequent influx of extracellular Ca\(^{2+}\). The increase in intracellular calcium is instead thought to be due to the release of calcium from intracellular stores via store-operated calcium channels in addition to calcium influx via receptor-operated calcium channels (Somlyo & Somlyo, 1968). Although pharmacomechanical coupling can occur independently of electromechanical coupling, the two processes are likely to interact in a synergistic manner (Nelson et al., 1990; Orallo, 1996). Indeed transmembrane influx of Ca\(^{2+}\) can occur secondarily to Ca\(^{2+}\) release from intracellular stores via multiple mechanisms (Nelson et al., 1988). This study speculates that in small renal arteries of the rat, T-type calcium channels are involved in the transmembrane Ca\(^{2+}\) influx. This may explain why T-type channel inhibition resulted in an attenuated sensitivity to noradrenaline. The exact mechanism through which T-type calcium channels are recruited during agonist-mediated vascular contraction is unknown. T-type channel inhibition has also been shown to reverse angiotensin II-mediated vasoconstriction of efferent arterioles. Two pathways common between these two agonists is protein kinase C and inositol-1,4,5-triphosphate (IP\(_3\))-mediated intracellular Ca\(^{2+}\) release, and so they may be a good starting point to assess the intracellular signalling mechanism of T-type calcium channels.

Endothelin-1 has also been shown to mediate vasoconstriction through pharmacomechanical coupling. The peptide mobilises intracellular calcium through the activation of the inositol-1,4,5-triphosphate (IP\(_3\)) pathway (Marsden et al., 1989). The rise in intracellular calcium may in turn activate chloride channels. The resultant chloride efflux causes membrane depolarisation which can then activate voltage-operated calcium channels to maintain elevated intracellular calcium levels (Iijima et al., 1991). This study showed that, unlike with noradrenaline constrictor responses, endothelin-1-mediated vasoconstriction in small renal arteries (interlobar) required both T- and L-type calcium channels to achieve maximal efficacy (Figure 3-10). In contrast, Gordienko et al. (1994) proposed that L- but not T-type channels are involved in the endothelin-1 mediated rise in intracellular calcium. Gordienko et al. (1994) showed that in smooth muscle cells isolated from interlobar and arcuate arteries, endothelin-1 enhanced current carried by L-type channels but not current facilitated by low-voltage activated channels. The reason for this difference is unknown. Compared to the interlobar arteries, the maximum contraction elicited by endothelin-1 was only decreased when arteries were co-treated with both NNC 55-0396 (100 nM) and felodipine (1 nM)(Figure 3-10). It is possible that larger renal arteries may be able to recruit voltage-operated calcium channels of the type not being inhibited, to compensate, enabling the artery to elicit maximal responses to endothelin-1 when treated with only one type of calcium channel inhibitor. Co-treatment with greater concentrations of both inhibitors failed to cause any further depression of the endothelin-1-mediated contraction. Thus although T- and L-type channels may contribute to endothelin-1 mediated constriction, they are not required for the contraction to occur.
3.4.1 Limitations

Studies about T-type calcium channels are limited by the lack of clearly selective T-type calcium channel inhibitors. In order to determine whether NNC 55-0396 has inhibitory activity at the L-type voltage-operated calcium channel, the ability of the compound to reverse L-type mediated contraction was assessed. NNC 55-0396 (100 nM), mibebradil (100 nM) but not felodipine (1 nM) partially relaxed contractile responses to BAY K 9644 (Figure 3-12). The relaxation must be due to the added compound, as contractile responses maintain a stable tone over time. Although this may indicate that NNC 55-0396 and mibebradil have inhibitory activity at the L-type voltage calcium channel, interpretation of the results is complicated by the need to pre-constrict vessels with a low potassium concentration (20 mM). BAY K 8644-mediated contractions could only be achieved in arteries pre-constricted to a small level of tone with potassium (20 mM). If it is presumed that 20 mM potassium preferentially activates T-type calcium channels, then the relaxation response to NNC 55-0396 and mibebradil of BAY K 8644-mediated contractions could arguably be attributed to its action at T-type channels rather than L-type channels. Indeed, the magnitude of the decrease in tone elicited by mibebradil and NNC 55-0396 is comparable to the amount of tone elicited by 20 mM of potassium.

3.4.2 Conclusion

In vascular smooth muscle cells isolated from rat preglomerular (Andreasen et al., 2006), interlobar and arcuate arteries (Gordienko et al., 1994), cells may express either T-type, L-type or both calcium channels. The inability of BAY K 8644 to consistently elicit contraction in this preparation and other renal vessels (Su et al., 1984; Hansen et al., 2001) reinforces the idea that activation of only L-type calcium channels may be insufficient to cause contraction. Although the concentrations of mibebradil and NNC 55-0396 utilised in this study are below their IC$_{50}$ at high voltage-activated channels, without accompanying electrophysiological experiments it is difficult to say for certain whether the compounds ability to inhibit depolarisation-mediated responses was due to the inhibition of T-type channels, non L-type high voltage-activated channels (P-/Q-type channels) or both. In cells expressing multiple types of voltage-operated calcium channels, the combined effort of all channels may be necessary to achieve a sustained and high enough calcium influx for contraction. Consequently, in vessel segments, the cooperative action of all voltage-operated calcium channels may be required to elicit a maximal vasoconstrictor response to depolarisation-mediated contractions. It is also clear that T-type calcium channels play a functional role in agonist-mediated vasoconstriction, though they are not a pre-requisite. The relative contribution of T-type channels to agonist-mediated contraction is both agonist-dependent and region-dependent. Even vessels isolated from the same vascular bed but different segments may differentially recruit voltage-operated calcium channels.
Chapter 4

The structural vascular amplifier in the renal and hindquarter vascular beds of conscious hypertensive rabbits
4.1 Introduction

A chronic elevation in arterial pressure is accompanied by structural changes in resistance arteries irrespective of the type of hypertension. The structural adaptation of resistance arteries, particularly the R1 resistance arteries with internal diameters between 50 and 200 μm, is thought to enhance vascular responsiveness and resistance and thus contribute to the maintenance of hypertension. The augmented vascular responsiveness is thought to be a product of an increased wall thickness to lumen ratio in the resistance vessels (Folkow et al., 1958). The increased wall thickness to lumen ratio may be a result of hypertrophic or inward eutrophic remodelling of the blood vessels. The former is characterised by smooth muscle hypertrophy and subsequent encroachment of the blood vessels. The latter involves the rearrangement of existing wall material around a smaller lumen (Baumbach & Heistad, 1989; Heagerty et al., 1993; Mulvany et al., 1996). It is not uncommon for structural adaptations to involve both hypertrophic and eutrophic remodelling (Heagerty et al., 1993), although one form may predominate depending on the form of hypertension.

Subcutaneous resistance arteries of patients with renovascular hypertension exhibit both vascular growth and eutrophic remodelling (Rizzoni et al., 1996). Both forms of remodelling have also been reported in animal preparations of secondary hypertension such as renovascular hypertension (Korsgaard & Mulvany, 1988; Deng & Schiffrin, 1991; Li et al., 1996) and deoxycorticosterone acetate-salt hypertension (Deng & Schiffrin, 1992). There is also substantial heterogeneity with vascular remodelling among patients with the same form of hypertension. This is true even when comparing vessels of patients that have been isolated from the same anatomical location (Intengan et al., 1999a). Regardless, both forms of structural adaptation result in a smaller internal radius (ri) and an increase in the wall thickness to lumen ratio (w:ri). This may be accompanied by a decrease in wall distensibility (Intengan & Schiffrin, 2000).

Structural vascular remodelling has functional haemodynamic consequences and these were first discussed at length by Björn Folkow (Folkow et al., 1958). It was surmised that encroachment of the lumen by hypertrophied media would lead to a structural decrease in the lumen diameter that would persist even when vascular smooth muscle was completely relaxed (Folkow, 1971). Functionally, this would translate into a raised flow resistance, even when vessels are maximally dilated. The increased wall to lumen ratio also augments changes in resistance. As discussed in section 1.5.1.1.1, mathematical modelling of an increased wall to lumen ratio demonstrated that in the absence of changes in vascular smooth muscle activity, a given level of smooth muscle shortening (i.e. from a given level of constrictor stimulus), would cause a more pronounced narrowing of the vessel lumen and thus an amplified resistance change than what would normally occur in a normal vessel (Folkow, 1971). The narrowing has since been shown to be relatively uniform over the range of vasomotor tone (Korner & Angus, 1992). Theoretical dose-resistance response curves of structurally remodelled arteries were characterised by a higher resting resistance at maximal dilatation, a steeper slope and a higher maximal response, compared to dose-resistance response curves modelled in ‘normal’ vessels (Folkow, 1971). Correspondingly, a given level of dilator stimulus would cause a more marked widening of the vessel lumen and thus a greater fall in resistance. As
resistance is inversely proportional to the fourth power of the internal radius \( R \propto 1/r^4 \), small decreases in the internal radius of a vessel can elicit substantially augmented resting resistance and resistance changes. Thus, a decreased internal radius and increased wall thickness to lumen ratio can confer an enhanced vascular responsiveness that does not necessitate an increase in vascular smooth muscle activity. This forms the basis of the vascular structural amplifier hypothesis.

The existence of the vascular structural amplifier has been the subject of much debate (Wright et al., 1987; Folkow, 1995; Izzard et al., 1999; Wright & Angus, 1999; Folkow, 2000; Korner et al., 2000; Izzard et al., 2002b; Izzard et al., 2002a; Lee & Bund, 2002; Wright et al., 2002; Bund & Lee, 2003; Izzard et al., 2003). In a number of comprehensive studies, Folkow presented sound evidence for the structural vascular amplifier in the spontaneously hypertensive rat. In the hindquarter vascular bed, Folkow et al. (1970a) measured perfusion pressure in response to graded infusions of noradrenaline. As flow was fixed in this preparation, resistance was proportional to pressure. The perfused hindquarter of the spontaneously hypertensive rat exhibited greater flow resistance at maximum dilatation, elicited a greater maximum resistance response and had a steeper dose-resistance response curve than the perfused hindquarter of the normotensive control rat. The vasoconstrictor threshold, which is sometimes used as an index of sensitivity, was comparable between the spontaneously hypertensive rat and the normotensive control rat. Thus, the experimental data from this study displayed all the hallmarks of the structural vascular amplifier. The coronary vasculature of the SHR has also been shown to exhibit similar structural adaptations (Noresson et al., 1977). These structural adaptations are not confined to specific vascular beds. They have also been shown to occur throughout the systemic vasculature (Folkow et al., 1970b). Under conditions of maximal dilatation, the systemic resistance was greater in spontaneously hypertensive rats than in normotensive control rats (Folkow et al., 1970b). The amplifier property has since been demonstrated in other preparations and vascular beds, including the hindquarter and systemic vasculature of conscious rabbits with renovascular hypertension (Wright et al., 1987; Wright et al., 2002; Korner et al., 2010) and the forearm vasculature of patients with mild essential hypertension (Egan et al., 1988).

A raised minimum flow resistance during maximum dilatation has been demonstrated in a number of isolated vascular beds of patients with essential hypertension, renovascular hypertension and hypertension that accompanies aldosteronism (Folkow et al., 1958; Doyle et al., 1959; Conway, 1963; Sivertsson & Olander, 1968; Sivertsson & Hansson, 1976). In mild hypertensive patients, graded regional infusions of noradrenaline and angiotensin II into the forearm generated a response pattern characterised by an increased minimum flow resistance, unaltered threshold sensitivity, a steeper slope and a greater maximum response (Egan et al., 1988). The same pattern was reported following the graded infusion of noradrenaline into the hands of patients with essential and renal vascular hypertension (Sivertsson & Olander, 1968).

A series of experiments have also confirmed the presence of the vascular amplifier in the conscious rabbit with renovascular (cellophane wrap) hypertension (Wright et al., 1987; Wright & Angus, 1999;
The structural vascular amplifier in the renal bed of hypertensive rabbits

Wright et al., 2002). The resting hindquarter vascular resistance and total peripheral resistance is greater in the conscious renovascular hypertensive rabbit than normotensive rabbits, even during autonomic and neurohumoral block (West et al., 1975; Angus et al., 1976; Wright et al., 1987; Wright & Angus, 1999). Vascular responsiveness is enhanced in the renovascular hypertensive rabbit. In the hindquarter of conscious renovascular hypertensive rabbits and their normotensive controls, Wright et al. (1987) generated dose-vascular response curves to a number of vasoconstrictor (dose-conductance response curves) and vasodilator (dose-resistance response curves) drugs, enabling the comparison of parameters such as slope, maximum response, $ED_{50}$ and range. In comparison to sham normotensive rabbits, renovascular hypertensive rabbits exhibited enhanced vascular responsiveness to all the vasoconstrictor agents assessed: the resting conductance, range and slope of the dose-vascular conductance curves were all approximately half of the values measured in normotensive rabbits. The uniformity of responses across different agonists is in accordance with the non-specific nature of the amplifier hypothesis. The vascular amplifier is a property of the adapted vascular structure, which would presumably affect the responses to all stimuli equally. This is further supported by the similar response patterns of each vasodilator agent: the resting conductance, range and slope of the dose-vascular conductance curves of adenosine, acetylcholine and serotonin were all approximately half of the values measured in normotensive rabbits. Importantly hindquarter sensitivity ($ED_{50}$) was comparable between hypertensive and normotensive rabbits for all vasoconstrictor and vasodilator agents. In line with observations in various vascular beds, vasoconstrictor- and vasodilator-mediated changes in total peripheral resistance were also amplified in the conscious hypertensive rabbit in the absence of any changes in sensitivity (Wright & Angus, 1999). A generalised increase in response to constrictor agents is not always observed. In the hypertensive rabbit (unilateral renal cellophane wrap with contralateral kidney removed), an enhanced pressor response to noradrenaline and the specific $\alpha_1$-adrenoceptor agonist phenylephrine, but not angiotensin II, was observed (Hamilton & Reid, 1983).

Dose-mean arterial pressure response curves in the hypertensive rabbit were leftward shifted with no change in slope (Hamilton & Reid, 1983), suggesting an increased sensitivity. The use of dose-mean arterial pressure curves however, are not necessarily a good correlate of vascular resistance in the conscious animal with integrated systems. Firstly, blood pressure is a factor of both cardiac output and vascular function. Secondly, in the conscious animal, haemodynamic changes will be influenced by a number of homeostatic regulatory mechanisms.

To address these issues, this study used close intra-arterial infusion and areflexic preparations. In the Wright et al. (1987) and Wright and Angus (1999) studies, intra-arterial infusion into the lower abdominal aorta and left atrium, respectively, enabled the construction of dose-vascular conductance response curves before sufficient venous circulation of the agonist caused limiting changes in systemic blood pressure. Areflexic preparations are necessary for the assessment of local vascular responses because cardiovascular reflexes can profoundly influence the cardiovascular and haemodynamic responses to stimuli. The baroreflex is critical for limiting short-term blood pressure fluctuations and thus helps maintain a stable mean arterial pressure at a particular set point for a short time frame (Guyton, 1991). An increase in mean arterial pressure and therefore transmural
pressure, causes deformation of the vessel wall and activation of stretch-sensitive baroreceptors located on the carotid sinus and aortic arch (James, 1971). An increased firing rate of afferent baroreceptor pathways to the medullary cardiovascular control centre stimulates efferent neural pathways to elicit bradycardia and vasodilatation, rapidly counteracting the elevation in mean arterial pressure. Conversely, a decrease in mean arterial pressure results in decreased firing rates. The resultant tachycardia and vasoconstriction increases mean arterial pressure, diminishing deviations from the set point (Head et al., 2002)(Figure 4-1). Non-arterial baroreceptors localised to the heart and pulmonary circulation also contribute to the afferent drive (Korner, 1989). Thus, any attempt to assess local vascular responses in an intact conscious animal necessitates the use of areflexic preparations. This is especially pertinent when comparing local vascular responses between normotensive and hypertensive animals. Cardiovascular reflexes are aberrant in hypertension (Gribbin et al., 1971; Korner, 1989; Head, 1995; Grassi et al., 1998) and can thus confound data obtained in conscious systemic preparations. Vagal baroreflex control of heart rate is diminished in experimental models of hypertension and in patients with established hypertension (Korner et al., 1974; West & Korner, 1974; Ricksten & Thoren, 1981; Head & Adams, 1988; Kumagai et al., 1990), while the baroreceptor threshold is set to a higher pressure in both essential and secondary forms of hypertension (McCubbin et al., 1956). Wright et al. (1987) have showed evidence of the vascular amplifier in the hindquarter and systemic resistance vasculature of the conscious renovascular hypertensive rabbit even during pharmacologically-mediated autonomic block.

The vascular amplifier has also been demonstrated in the hindquarter vasculature of the hypertensive rabbit under neurohumoral blockade. In addition to neurally-mediated cardiovascular reflexes, haemodynamic changes in the conscious animal are also buffered by humoral factors released in response to changes in arterial pressure and tissue perfusion pressure. In addition to the autonomic nervous system, the renin-angiotensin system and arginine vasopressin are also important in maintaining arterial pressure. All three mechanisms are known to interact with each other under physiological and pathophysiological conditions (Folkow, 1982; Zimmerman et al., 1984; Johnston, 1985). The regulatory mechanisms activated in response to a decrease in mean arterial pressure are summarised in Figure 4-1. If a particular mechanism becomes inactive or blunted for whatever reason, other regulatory mechanisms may become more active to compensate. For example, acute pharmacological blockade of the autonomic nervous system in the Long-Evans rat caused a profound decrease in blood pressure that saw a preferential elevation in plasma vasopressin but not plasma renin or angiotensin II (Hiwatari et al., 1985). Plasma renin and angiotensin II increased following concomitant blockade of the autonomic nervous system and vasopressin receptors. To ensure that the vascular responses observed can be attributed to structural adaptation of the vasculature and not aberrations of the autonomic or humoral systems, this study also assessed haemodynamic changes in rabbits with autonomic blockade and neurohumoral blockade.
Figure 4-1. Compensatory homeostatic mechanisms involved in regulating blood pressure

This schematic summarises the main effector pathways of the neural and humoral systems that are activated following a fall in blood pressure. These pathways work in concert to raise arterial pressure back to its set value.
In reality, vascular responsiveness depends on the interaction between vascular geometry, transmural pressure, mechanical properties of the wall and functional determinants of vascular tone (Folkow & Karlström, 1984; Folkow, 1990; Wright et al., 2002). Changes in vascular geometry per se, account for only a small fraction of the elevation in vascular reactivity and resistance in hypertension. Rather, vascular responsiveness is enhanced because adaptations in vessel geometry amplify functionally-driven changes in resistance. In this way, an enhanced vascular reactivity does not necessitate an increase in vascular smooth muscle activity.

It has been suggested that in hypertension, mechanical properties of the resistance arteries undergo changes that may contribute to a raised vascular resistance. Wall distensibility and compliance are used to describe the elastic behaviour of a blood vessel. They both, for example, describe the ability of a vessel to minimise alterations in transmural pressure. The elastic behaviour of a blood vessel depends on the collective elastic moduli of the structural components of the wall, the three main elastic components of a vessel being the vascular smooth muscle, elastin and the less distensible (stiffer) collagen fibres (Bank et al., 1996). It also depends on vessel geometry and transmural pressure. Thus it is plausible that the adaptive structural changes in the vasculature of hypertensive patients and animals would be accompanied by changes in wall distensibility and compliance (Folkow & Karlström, 1984). Increased wall stiffness has been demonstrated in small second order cerebral arteries of the stroke-prone spontaneously hypertensive rat (Hajdu & Baumbach, 1994) and carotid arteries of rats with established renovascular hypertension (Zanchi et al., 1997). Other studies however, have reported decreased elastic moduli in resistance arteries of hypertensive patients (Intengan et al., 1999a). In comparison to normotensive controls, a lower incremental elastic modulus under isobaric conditions was reported in mesenteric small arteries of spontaneously hypertensive rats perfused at physiological intravascular pressures (Laurant et al., 1997). It has been proposed that wall stiffness may be initially decreased before increasing in response to collagen deposition and various processes involving adhesive and extracellular matrix determinants (Intengan & Schiffrin, 2000). However, differences in elastic properties of resistance arteries are not always observed between hypertensive and normotensive individuals and experimental preparations. Some studies report similar mechanical properties between hypertensive and normotensive arteries (Thybo et al., 1996; Intengan & Schiffrin, 1998; Park et al., 2000).

Functional changes in the vasculature have long been associated with the elevated peripheral resistance in hypertension. There are a number of functional determinants of vascular tone, including: (i) circumferential stress from transmural blood pressure and activation of the intrinsic myogenic component; (ii) shear stress from blood flow; (iii) metabolic factors; (iv) neurohumoral factors; and (v) locally-released vasoactive stimuli. Each functional factor is altered in its own right in hypertension: (i) an increase in mean arterial pressure is accompanied by an almost proportional increase in myogenic activity; (ii) endothelial function and the production, release and delivery of nitric oxide may be aberrant; (iii) metabolic activity; (iv) increased stimulation of the renin-angiotensin system, sympathetic nervous system and adrenal system (depending on the experimental model of hypertension); and (v) an imbalance in the production of vasoactive and
vasodilator stimuli such as prostanoids. A more detailed coverage of these functional factors and aberrations in hypertension has been discussed in section 1.4.

Another factor that can influence vascular reactivity is the architecture of the vasculature. In hypertension, vascular beds may undergo rarefaction which describes a decrease in the number of perfused arterioles. Rarefaction may be structural, in which there is a decrease in the anatomical number of arterioles or it may be functional, where blood flow is occluded (i.e. via intense vasoconstriction) but there is no change in the anatomical number of arterioles. Structural (anatomical) rarefaction of the arteriolar (pre-capillary) vascular network is thought to contribute to the greater resting vascular resistance in hypertension (Prewitt et al., 1982b; Korner & Angus, 1992). A more detailed discussion on its contribution to an elevated total peripheral resistance in hypertension is covered in section 1.6.1.2.

It should be reiterated that vascular reactivity is dependent on the interaction of all the aforementioned factors. Wright et al. (2002) have attempted to estimate the relative contribution of the vascular amplifier, rarefaction and functional determinants of vascular tone to the raised total peripheral resistance in conscious renovascular hypertensive rabbits. They suggested that the interaction between structural changes and functional factors and their combined influence on total peripheral resistance can be represented by the following equation:

$$\Delta TPR_{H-N} = \text{amplifier} \times \sum (\Delta \text{functional factors}) + \text{rarefaction}$$

where $\Delta TPR_{H-N}$ is the difference in resting total peripheral resistance between hypertensive (H) and normotensive (N) groups and ‘amplifier’ is the hypertensive:normotensive ratio of resistance slopes generated from dose-response curves (Wright et al., 2002).

Although the structural amplifier has been reported in the hindquarter and systemic vasculature of the conscious renovascular hypertensive rabbit, it is unknown if this resistance amplifier also extends to the renal vasculature. A raised renal vascular resistance is consistently reported in hypertension. Renal vascular resistance is elevated in renovascular hypertensive rats (Ferrone et al., 1979), pre-hypertensive SHR (Dilley et al., 1984; Harrap & Doyle, 1986) and SHR with established hypertension (Ferrone et al., 1979; Fink & Brody, 1979; Berecek et al., 1980), even under conditions of supra-maximal and maximal vasodilatation (Fink & Brody, 1979; Göthberg et al., 1979; Berecek et al., 1980). It has also been shown in the conscious rabbit with bilateral cellophane wrap hypertension (Bolt & Saxena, 1983; Denton et al., 1983). The only exception is a study by Folkow et al. (1971) in which the perfused kidney of spontaneously hypertensive rats had a lower resting renal resistance then the normotensive control. In humans, Doppler ultrasonography has shown that the renal resistance index, which depends on a composite of vascular properties including intrarenal resistance, pulse pressure and arterial compliance (Tublin et al., 2003), is elevated in patients with essential hypertension (Veglio et al., 1995; Galesic et al., 2000). Renal vascular resistance is also elevated in normotensive individuals with a familial history of hypertension (van Hooft et al., 1991),
although not always (Blackshear et al., 1987). The former suggests that changes in renal haemodynamics occur in the early stages of hypertension. Given that their blood pressures were normal, the observed changes in renal haemodynamics were unlikely to be due to structural changes that have arisen from an increased pressure load. This does not preclude the involvement of structurally-driven increases in renal vascular resistance in established hypertension.

In established hypertension, the raised renal vascular resistance has been attributed to structural changes in the vasculature as well as changes in functional processes involved in regulating the vascular tone of small arteries and arterioles, the principal repositories of renal vascular resistance (Arendshorst & Beierwaltes, 1979; Skov & Mulvany, 2004). A number of studies have identified structural changes in the renal vasculature. Morphometric analysis of perfusion-fixed renal vessels of the adult SHR (21 weeks old) revealed that, with the exception of preglomerular arterioles, renal arteries with an internal diameter exceeding 70 μm (main renal artery, interlobar arteries, arcuate arteries and some interlobular arteries) all exhibited characteristics of hypertrophic remodelling: greater media cross-sectional area and a greater media to lumen ratio compared to control vessels (Smeda et al., 1988). (Kett et al., 1995) also reported hypertrophic remodelling in arcuate and interlobular arteries of the SHR (10-week-old). Preglomerular afferent arterioles of SHR (6, 9, 10, 12 and 20 week old) had smaller lumens than their normotensive controls, but were not associated with an encroachment of the lumen by hypertrophied media (Gattone Li et al., 1983; Smeda et al., 1988; Kimura et al., 1991; Skov et al., 1992; Notoya et al., 1996). A greater wall thickness to lumen ratio is also noticeably absent in morphological studies of renal afferent arterioles (Gattone Li et al., 1983; Smeda et al., 1988; Skov et al., 1992; Notoya et al., 1996). The narrowing of arterioles is still likely to be structurally-driven since morphological analysis was often performed during maximal vasodilatation and thus in the absence of functional contributions (Skov et al., 1992). However, these structural changes are unlikely to be adaptive changes in response to elevated pressure load. Renal arteriolar narrowing has been observed in the absence of substantial increases in blood pressure, including in young SHR (pre-hypertensive, 6 week old). Efferent arterioles of SHR do not present with a narrowed lumen: they may have lumens that are comparable to that of the normotensive controls (Notoya et al., 1996) or conversely, an enlarged lumen (Kimura et al., 1991).

Much of this information however has been drawn from the SHR. There is comparatively little information on the structure of human renal arteries in hypertension (for obvious ethical reasons), unless one examines the post-mortem examination notes of Bright (1836), Johnson (1868) and Gull and Sutton (1872). There is also a scarcity of information on the vascular structural adaptations that occur in secondary forms of hypertension.

Although renal vascular resistance is generally elevated in hypertension and vascular remodelling has been reported in some studies, it is not known if the structural vascular amplifier applies in the renal vasculature of hypertensive patients and experimental models of hypertension. In the renal vasculature, graded noradrenaline infusion generated steeper dose-response curves in the spontaneously hypertensive rat than the normotensive control rat (Folkow et al., 1971). An increased maximal pressor response was also observed, suggesting an increase in the wall thickness to lumen
ratio. However, in contrast to other regional vascular beds, vascular resistance in the renal vasculature during maximum dilatation was lower in the spontaneously hypertensive rat than the normotensive control rat suggestive of larger internal diameters in the hypertensive animal. The renal vasculature is unique – possessing a remarkable autoregulatory capacity – and may differentially alter compared to other peripheral vascular beds. In contrast to resistance arteries of other peripheral vascular beds which undergo adaptive structural changes in response to an increased pressure load from a raised systemic pressure, the structural changes in small renal arteries may occur independently of pressure load. Despite presenting with morphological properties characteristic of hypertrophic remodelling, antihypertensive treatments that lowered systemic blood pressure failed to normalise the structural changes (Kett et al., 1995; Anderson et al., 1997), despite normalising non-renal arteries (Lee et al., 1991).

This study thus builds on previous studies by Angus, Korner and Wright by assessing the role of the vascular structural amplifier in the renal vasculature of the conscious renovascular hypertensive rabbit. To assess local vascular responses, vasodilator and vasoconstrictor agents were assessed in sham normotensive and cellophane wrap hypertensive rabbits with effectors intact and during autonomic and neurohumoral block.
4.2 Methods

This study was approved by the University of Melbourne Animal Ethics Committee and performed in accordance with the *Australian code for the care and use of animals for scientific purposes* (8th edition, 2013, National Health and Medical Research Council, Canberra). New Zealand White (NZW) rabbits (2.4–2.8 kg) of either sex were used (Nanowie, Small Animal Production Unit, Bellbrae, Victoria, Australia). Rabbits were housed in pairs in floor pens in the Biomedical Science Animal Facility (BSAF) under constant climatic conditions (21°C, 12 h light/dark cycle) and provided with water and food ad-libitum. Diet comprised normal chow supplemented with fresh vegetables and fruit. Rabbits were allowed to acclimatise for 7 days in their floor pens before the first procedure. Each rabbit underwent three surgical procedures on day 0, 21 and 33 prior to the first experiment on day 35 (Figure 4.2).

4.2.1 Surgical procedures

General procedures

All i.v. drug administration was via a 24G intravenous cannula inserted into the marginal ear vein. Rabbits were anaesthetised with i.v. propofol (10–20 mg/kg; Diprivan, AstraZeneca, North Ryde, New South Wales, Australia). Following endotracheal intubation (3.0 uncuffed endotracheal tube), anaesthesia was maintained with 2% isoflurane (Baxter Healthcare, NSW, Australia) supplemented with medical O₂ and room air using an isoflurane anaesthetic vaporiser (Penlon Sigma Delta; Penlon Limited, Abingdon, UK). Body temperature was monitored with a rectal temperature and maintained at 38°C with a homeothermic heating blanket (Harvard Apparatus, Massachusetts, USA). Before each surgery, rabbits were administered the antibiotic enrofloxacin (10 mg/kg s.c. via 30G needle; Ilium Enrotril, Troy Laboratories, NSW, Australia) and the analgesic agent buprenorphine hydrochloride (0.05 mg/kg i.v.; Temgesic, Reckitt Benckiser, Berkshire, UK). Warm sterile 0.9% saline (10 ml slow bolus i.v.; Baxter Healthcare) was administered to prevent dehydration.

Surgical procedure 1 (Day 0)

Following anaesthesia, bilateral cellophane wrap was performed by accessing the kidneys via flank incisions (left and right retroperitoneal). Kidneys were carefully mobilised from surrounding tissue and each kidney wrapped in sterile cellophane (12 x 12 cm). The cellophane was held in place by loosely tied silk (0 braided silk; Ethicon, New Jersey, USA) around the hilum of the kidney. In the sham group the kidneys remained undisturbed. A perivascular ultrasonic flow probe (sized for vessel of 2 mm OD, MC2PSB Precision S flow probe with back exit; Transonic Systems Inc. Ithaca, USA) was implanted around the left renal artery to measure renal blood flow. The connector of the Transonic flow probe was tunnelled subcutaneously so that it lay under the skin along the rabbit’s back before being buried at the nape of the neck.
Surgical procedure 2 (Day 21)

Following general anaesthesia, via a midline laparotomy, rabbits were implanted with a perivascular ultrasonic flow probe (sized for vessel of 3 mm OD, MC3PSB Precision S flow probe with side exit; Transonic Systems Inc) around the lower abdominal aorta above the bifurcation for the measurement of hindquarter blood flow. The connector of the Transonic flow probe was tunneled subcutaneously before being buried at the nape of the neck alongside the connector for renal flow.

Surgical procedure 3 (Day 35)

Following general anaesthesia, the left carotid artery was exposed by making a small incision (1-2 cm in length) over the left carotid artery, midway between the jaw and sternum. Ropivacaine (approximately 200-300 μl bolus s.c. via 30 gauge needle; Naropin, Astra, NSW, Australia), a long acting local anaesthetic, was administered prior to incision. A clear polyvinyl catheter (ID, 1.2 mm; OD, 1.7mm) was inserted via the left carotid artery and threaded down until it was positioned in the thoracic aorta for the subsequent infusion of vasoactive drugs. The catheter was filled with a predetermined volume of heparin (1000 units/ml) to prevent clotting and secured in position with a Dacron patch and cyanoacrylate glue (Vetbond, 3M, North Ryde, NSW, Australia). Flow was unobstructed and the artery remained patent. The tip was heat-sealed before being tunneled subcutaneously to join the flow probe connectors at the nape of the neck. The catheter and the flow probes were exteriorised and protected from damage by a custom-made rabbit jacket that did not inhibit free movement.

Post-operative care

Post-surgery rabbits were placed in a heated recovery box and closely monitored for several hours before being returned to their home floor pens. Enrofloxacin was re-administered daily for three days post-surgery except for after the third surgical procedure when it was administered only 24 h post-surgery. Rabbits were monitored daily until stable weight gain was achieved and then weekly thereafter. Animals displaying strong discomfort or poor recovery were anaesthetised with propofol (10-20 mg/kg i.v.) and euthanased with saturated KCl (approximately 5 ml i.v.).

Sutures

For the abdominal and flank incisions, the muscle, subcutaneous and subcuticular layers were closed with a locked blanket stitch (Mersilene 3/0 braided polyester fibre; Ethicon, US), running stitch (Prolene 5/0 monofilament, Ethicon, US) and subcuticular stitch (Ethilon 3/0 monofilament nylon; Ethicon, US), respectively. The incision at the nape of the neck was closed with a single blanket stitch (Ethilon 3/0 monofilament nylon; Ethicon).

4.2.2 Measurement of haemodynamic and cardiovascular parameters

During each experimental protocol rabbits were housed in a polycarbonate box (Nalgene; Nalge, Rochester, NY, U.S.A). Lim et al. (2012) have shown that mean arterial pressure and sympathetic
activity recorded in rabbits restrained in a polycarbonate box do not significantly differ from measurements recorded in unrestrained rabbits in their home pen. All minor surgical procedures on experimental days were performed under local analgesia with a 50/50% v/v mix of 1% w/v ropivacaine (Naropin, Astra) and 1% w/v lignocaine (Xylocaine, Astra). The central ear artery was cannulated and the catheter connected to a pressure transducer (Argon Medical Devices, Athens, Greece) for measurement of pulsatile blood pressure. The transducer was connected to a PowerLab 8SP (AD Instruments Pty Ltd, Bella Vista, NSW, Australia) via a bridge amplifier (AD Instruments) for data collection. Mean arterial pressure measured via the central ear artery is typically 5-10 mmHg lower than carotid mean arterial pressure (Edwards et al., 1959). The marginal ear vein was cannulated (24G Surflo, Terumo Inc.) and connected to an infusion pump (Terufusion Syringe Pump, Terumo Corporation, Japan) for antagonist administration (if applicable). Flowprobe connectors and the intra-aortic catheter were retrieved from their compartment in the rabbit jacket. Flowprobe connectors were connected to a flowmeter (TS420 Transit Time Perivascular Flowmeter, Transonic Systems Inc.) for measurement of renal (RQ; ml/min) and hindquarter (HQ; ml/min) blood flow. Voltage signal output was filtered at 40 Hz when acquiring phasic flow and 1 Hz for average flow. The intra-aortic catheter was connected to an infusion pump (Adelab Scientific, SA, Australia) for infusion of agonist drugs. Heart rate (HR; beats/min), hindquarter vascular conductance (HVC; ml/min/mmHg) and renal vascular conductance (RVC; ml/min/mmHg) were continuously computed by Chart v 5.5.6 (AD Instruments). Following the experimental procedure, the intra-aortic catheter was re-filled with heparin (1000 units/ml; Pfizer, NY, USA), re-sealed and returned to its position within the jacket together with the flow probe leads after washing with 70% ethanol and sterile saline. The minor wound over the central ear artery was closed with a single blanket stitch (Prolene 5/0 monofilament polypropylene; Ethicon).

4.2.3 Experimental protocols

Each rabbit underwent three experimental protocols with 2-4 recovery days separating each protocol. They were performed in the following order: (i) P1, effectors intact; (ii) P2, ganglionic block; and (iii) P3, neurohumoral block. Following experimental preparations a recovery period of thirty min was allowed for cardiovascular and haemodynamic parameters to stabilise.

Study day 1 – Effectors intact

In order to assess vascular reactivity and determine whether vascular structural amplification occurs in the renal vasculature, the responses to various vasoconstrictor and vasodilator agents were assessed. The agonists were also chosen to allow comparison with earlier work in this preparation from our group (Wright et al., 1987; Wright & Angus, 1999; Korner et al., 2010). Dose-response curves were generated to angiotensin II (0.001 – 1.0 μg/kg/min i.a.), adenosine (10 – 1000 μg/kg/min i.a.), noradrenaline (0.1 – 10 μg/kg/min i.a.), acetylcholine (1 – 100 μg/kg/min i.a.) and methoxamine (1 – 1000 μg/kg/min i.a.) in the order listed. Each agonist dose was infused i.a. (variable rate) until haemodynamic responses plateaued. Methoxamine was administered last due to the longer duration required for parameters to return to baseline. Doses of agonists have been
previously optimised (Wright et al., 1987). At least 50 min separated the infusion of each different agonist to allow all haemodynamic parameters to return to baseline.

**Study day 2 – Ganglionic block**

Pharmacological inhibition of ganglia was induced with a slow bolus of mecamylamine (4 mg/kg i.v.). To prevent blood pressure from decreasing precipitously following mecamylamine administration, plasma volume was expanded by administering a warm 10% polygeline/electrolyte solution (10 ml i.v. slow bolus; Haemaccel, Hoechst Australia Pty Ltd, Victoria, Australia). Ganglionic block was maintained by constant i.v. infusion throughout the experimental protocol (mecamylamine, 2.5 mg/kg/h at 10 ml/h i.v.) (Wright et al., 1987). Cardiovascular parameters were allowed to stabilise for 40 min before administration of agonist drugs. The maximum doses of adenosine and acetylcholine were limited to 300 and 10 μg/kg/min i.a., respectively, to prevent excessive hypotension.

**Study day 3 – Neurohumoral block**

Neurohumoral block was achieved with i.v. administration of: (i) vasopressin V₁ receptor antagonist, des-Gly-[Phe1,D-Tyr(Et)2,Lys6,Arg8]-vasopressin (1 μg/kg bolus and 0.1 μg/kg/h infusion); (ii) angiotensin-converting enzyme inhibitor enalaprilat (1 mg/kg bolus and 1.5 mg/kg/h infusion); and (iii) mecamylamine (4 mg/kg slow bolus and 2.5 mg/kg/h infusion) at 10 ml/h (Terufusion Syringe Pump, Terumo Corporation, Japan). As performed on study day 2, the bolus i.v. injection of mecamylamine was followed by a slow bolus of a warm 10% polygeline/electrolyte solution (10 ml i.v. slow bolus) to prevent precipitous decreases in blood pressure. The dose regimen of enalaprilat and vasopressin V₁ receptor antagonism required to maintain inhibition of angiotensin-converting enzyme and vasopressin-mediated pressor effects, respectively, has previously been optimised and validated by Whorlow et al. (1998) and Wright et al. (2002). The combination of these antagonist regimens and autonomic blockade has been used to successfully elicit and maintain neurohumoral blockade in rabbits over several hours (Wright et al., 2002).

### 4.2.4 Using the nasopharyngeal reflex as a test for autonomic and neurohumoral blockade

Prior to the infusion of agonists, successful autonomic blockade during study day 1 and 2 was confirmed by the absence of the nasopharyngeal reflex typically activated upon exposure to cigarette smoke (Allen, 1928; White & McRitchie, 1973). Inhalation of noxious stimuli such as cigarette smoke activates trigeminal afferent nerves located in the nasopharynx. Subsequent enhancement of vagal and sympathetic activity culminates in apnoea, a reflex that acts to minimise inspiration of noxious stimuli. The nasopharyngeal reflex is thus characterised by marked bradycardia due to increased vagal activity and elevated peripheral resistance due to enhanced sympathetic-mediated vasoconstriction (White & McRitchie, 1973). Both serve to conserve circulating blood oxygen during apnoea. In this study, rabbits were briefly exposed to cigarette smoke pre- and post-induction of ganglionic block. A simple smoke-transfer apparatus was utilised to extract and administer cigarette smoke. The apparatus consisted of a 25 ml plastic syringe connected via a three way tap to a short
length of flexible rubber tubing and another longer piece of tubing. Cigarette smoke from a freshly lit cigarette (Marlboro; Phillip Morris Ltd., Melbourne, Australia) was extracted into the syringe via the short length of tubing. The smoke was then blown towards the nares of the rabbit via the longer piece of tubing for 5-7 s to facilitate active inhalation of smoke. The nasopharyngeal reflex was transient and cardiovascular parameters quickly reverted to baseline values with no long-term impact on cardiovascular parameters. The effect of cigarette smoke on mean arterial pressure, heart rate, hindquarter and renal vascular conductance was recorded at the point of peak response change. The effect of smoke is within animal and was analysed using a Student’s paired t-test.

4.2.5 Post-mortem

Rabbits were killed 2-4 days post the third study day. Rabbits were heparinised (5000 USP), anaesthetised with propofol (10-20 mg/kg i.v.) and killed with 4 ml of saturated KCl. Both kidneys were removed and their wet-weight recorded before prompt placement in cold Krebs’ physiological salt solution (PSS) (composition in mmol/l: NaCl 119; KCl 4.7; KH2PO4 1.18; MgSO4.7H2O 1.17; NaHCO3 25; CaCl2 2.5; EDTA 0.03; glucose 5.5) saturated with carbogen (O2 95%; CO2 5%) at pH 7.4 for in vitro assessment of the renal vasculature (see Chapter 5). The hindlegs were also removed and placed on ice for the assessment of vascular reactivity in hindquarter resistance vessels (see Chapter 5).

4.2.6 Drugs

Drugs and suppliers were as follows: acetylcholine bromide (Sigma, St. Louis, MO, USA); adenosine (Sigma), angiotensin II amide; arginine vasopressin (AusPep, Parkville, Victoria, Australia); enalaprilat (gift from Merck, Rahway, New Jersey, USA); mecamylamine (Sigma); methoxamine hydrochloride (Sigma); noradrenaline bitartrate (Sigma); vasopressin V1 receptor antagonist des-Gly-[Phe1,D-Tyr(Et)2,Lys6,Arg8]-vasopressin (Bachem, Bubendorf, Switzerland). All drugs used for in vivo assessment were prepared using sterile 0.9% sodium chloride solution. Angiotensin II was stored as a stock solution at -20°C until required. All other drugs were made fresh daily.

4.2.7 Statistical analysis

**Analysis of haemodynamic circulating variables**

The haemodynamic variables assessed were mean arterial pressure (MAP), heart rate (HR), hindquarter flow (HQ), hindquarter vascular conductance (HVC), renal flow (RQ) and renal vascular conductance (RVC). Haemodynamic variables at rest were compared between groups (hypertensive vs. normotensive) using Student’s unpaired t-test. Within each group, circulatory variables before and after ganglionic block or neurohumoral block were compared using a Student’s paired t-test. Within each group, haemodynamic variables in rabbits with intact effectors and in rabbits during ganglionic and neurohumoral block were compared via one-way ANOVA with Dunnett’s post-test for multiple comparisons; the response with effectors intact was taken as the control response. Within animal, the effect of smoke on each variable was tested using Student’s paired t-test. The ability of
ganglionic or neurohumoral block to affect the response of variables to smoke were also assessed using Student’s paired t-test. Ideally, rabbits would have undergone all three protocols enabling the use of repeated measures ANOVA for more powerful statistical comparisons. Unfortunately, not all haemodynamic parameters were measured for all three protocols. Two sham rabbits and one hypertensive rabbit had an abnormally low resting mean arterial pressure on study day 3. Although they otherwise appeared healthy, the low arterial pressure meant that they were excluded from receiving neurohumoral block. In another two sham and two hypertensive rabbits, renal flow signal was low and unusable. There were also instances where the rabbits had chewed through their flow probe leads between experimental days.

Analysis of dose-response curves

The response to each dose was taken at the point where a plateau in renal and hindquarter vascular conductance had been reached. The corresponding values for mean arterial pressure, heart rate and flow were also measured. Prior to the infusion of each agonist, all parameters had to be stable for a period of at least ten minutes. To demonstrate that each parameter was stable prior to the infusion of each agonist, dose-response curves show readings taken at t=10, 5 and 0 min before the first dose of agonist was administered. Values taken at t=0 min were considered the resting haemodynamic response (and differed from the baseline haemodynamic variables recorded at the start of each day 30 min post experimental set-up).

Statistical comparisons between the vascular response of hypertensive and normotensive rabbits were performed using dose-vascular conductance curves for constrictor agents and dose-vascular resistance curves for dilator agents. The advantage of this is that (i) vascular conductance falls to a clear minimum of zero with high doses of constrictor drugs (whereas resistance would continue to rise with a poorly-defined maximum plateau) and (ii) vascular resistance falls to a clearer minimum plateau with dilator drugs (whereas conductance would continue to rise to a poorly-defined maximum). This enabled more rigorous comparisons of the dose-vascular response curves between hypertensive and normotensive rabbits. Given that resistance and conductance are reciprocals of each other, they both measure the same vascular properties and can be used interchangeably. The dose-absolute response curves were characterised based on (i) the range, defined as the resting response at t=0 min to the maximum response observed, (ii) the ED50, defined as the dose required to elicit 50% of the maximum response and (iii) the average slope of the dose-vascular response curve. To determine the ED50, sigmoidal logistic dose-response curves for each individual experiment were fitted using Prism 5 using absolute values (GraphPad Software). The average slope was determined from the quasi-linear part of the curve. Each curve was fitted with a linear regression over the dose range that elicited the majority of the response changes: this was determined by inspection and the same dose range was used throughout for each particular rabbit group and agonist. The dose ranges used for constrictor dose-vascular response curves are indicated in Figure 4-13. The dose ranges used for acetylcholine and adenosine dose-vascular resistance curves are indicated in Figure 4-14 and Figure 4-16, respectively.
The parameters of the dose-vascular response curves (resting vascular resistance or conductance, range, slope and ED$_{50}$ and plateau response) were compared between groups (hypertensive vs. normotensive rabbits) using Student’s unpaired t-test. In all cases, two-sided $p \leq 0.05$ was taken as statistically significant. To determine whether there were agonist dose-dependent changes in a particular haemodynamic variable within group, one-way ANOVA with repeated measures was used with Dunnett’s post-test for the comparison of the response at each dose back to the resting value (t=0 min).

Delta responses was defined as the change in response from resting value at t=0 min. Sigmoidal logistic dose-resistance and conductance curves were also generated for each individual experiment using delta values in the same manner as described for absolute values. Comparisons of peak response changes between hypertensive and normotensive groups were performed using Student’s unpaired t-test.
Figure 4-2. Timeline of surgical procedures and experimental protocols for each rabbit.

Rabbits were allowed to acclimatise in floor pens in the animal facility for one week. All rabbits received a flow probe around the left renal artery at day 0 (S1). Half the cohort also underwent bilateral cellophane wrap during the same surgery to induce hypertension (wrap hypertensive) while the other half did not (sham normotensive). On day 21, all rabbits were implanted with a flow probe around the lower abdominal aorta (S2). On day 33 (S3), an intra-thoracic catheter was inserted via the left carotid artery to allow close arterial infusion of drugs. P1, P2 and P3 indicate experimental days with effectors intact, ganglionic blockade or neurohumoral blockade, respectively. Rabbits were euthanased on day 41.
4.3 Results

4.3.1 General characteristics

There were a total of 13 sham normotensive rabbits (female, $n = 7$; male, $n = 6$) and 9 wrap hypertensive rabbits (female, $n = 6$; male, $n = 3$) that completed the entire study. The starting body weight of rabbits allocated to the sham normotensive (2.53 ± 0.07 kg) and wrap group (2.5 ± 0.05 kg) were comparable ($p > 0.05$, Student’s unpaired t-test; Table 4-1 and Figure 4-3). Rabbits in the wrap group gained little or no weight in the first 7 post-operative days whereas sham-operated rabbits tended to gain a small amount of weight. In the following 5 weeks, wrap and sham rabbits gained weight at comparable rates. There was no significant difference in body weight between sham and wrap rabbits throughout the study period ($p = 0.62$, two-way ANOVA, repeated measures, Table 4-1). Induction of hypertension had no appreciative impact on kidney wet weight (weight post-removal of the fibrocollagenous hull). The mean left kidney weight was 8.70 ± 0.59 and 9.35 ± 0.88 g for sham and wrap rabbits, respectively ($p = 0.53$, Student’s unpaired t-test). Mean right kidney weight was 9.41 ± 0.39 and 9.55 ± 0.51 g for sham and wrap rabbits, respectively ($p = 0.83$, Student’s unpaired t-test).
Table 4-1. Body weight (kg) of sham and wrap hypertensive rabbits over the study period of 6 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0( ^a )</td>
<td>7</td>
<td>14</td>
<td>21( ^b )</td>
<td>28</td>
<td>33( ^c )</td>
<td>35 (P1)</td>
</tr>
<tr>
<td>Sham</td>
<td>13</td>
<td>2.53 ± 0.07</td>
<td>2.58 ± 0.08</td>
<td>2.74 ± 0.08</td>
<td>2.82 ± 0.10</td>
<td>2.91 ± 0.09</td>
<td>2.97 ± 0.09</td>
<td>2.86 ± 0.09</td>
</tr>
<tr>
<td>Wrap</td>
<td>9</td>
<td>2.50 ± 0.05</td>
<td>2.44 ± 0.06</td>
<td>2.60 ± 0.06</td>
<td>2.75 ± 0.05</td>
<td>2.82 ± 0.04</td>
<td>2.92 ± 0.06</td>
<td>2.87 ± 0.04</td>
</tr>
</tbody>
</table>

Surgery days are indicated by superscripts: On day 0 \(^a\) rabbits had via retroperitoneal flank incisions, bilateral renal cellophane wrap or sham operation and were implanted with a flow probe around the left renal artery. On day 21 \(^b\) rabbits were implanted with a flow probe around the lower abdominal aorta via a midline laparotomy. On day 33 \(^c\), an intra-thoracic aortic catheter was implanted via the left carotid artery to allow close arterial drug infusion. Protocol 1 (P1), protocol 2 (P2) and protocol 3 (P3) were performed on days 35, 37 and 39, respectively. Rabbits were euthanased on day 41 \(^d\). Data are mean body weight (kg) ± SEM. \( n \) = number of rabbits.
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Figure 4-3. Body weight of sham-operated and cellophane-wrapped rabbits over the study period of 6 weeks

Surgery days (S) and experimental protocols (P) are indicated by arrows: on day 0 (S1), rabbits were implanted with a flow probe around the left renal artery via a retroperitoneal flank incision. Rabbits allocated into the wrap group also had bilateral renal cellophane-wrap surgery. On day 21 (S2), rabbits were implanted via a midline laparotomy a flow probe around the lower abdominal aorta. On day 33 (S3), an intra-thoracic catheter was inserted via the left carotid artery to allow close arterial infusion of drugs. Arrows accompanied by P1, P2 or P3 indicate experimental days with effectors intact, ganglionic blockade or neurohumoral blockade, respectively. Rabbits were euthanased on day 41 (E). Error bars are ± 1 SEM. n, number of rabbits.
4.3.2 Baseline cardiovascular and haemodynamic variables

Five weeks post-operatively, wrap hypertensive rabbits with intact effectors had a mean arterial pressure that was 39 mmHg greater than sham normotensive rabbits (MAP, 120 ± 4 vs. 80 ± 2 mmHg, hypertensive vs. normotensive; p < 0.0001, Student’s unpaired t-test; Table 4-2) and a comparable heart rate (216 ± 13 vs. 221 ± 6 beats/min, wrap vs. sham, p = 0.701, Student’s unpaired t-test). Baseline hindquarter blood flow was significantly lower (p = 0.027) in hypertensive rabbits (47.3 ± 3.6 ml/min) than normotensive rabbits (58.8 ± 3.2 ml/min). Correspondingly baseline hindquarter vascular conductance was significantly lower in hypertensive rabbits (0.400 ± 0.034 vs. 0.738 ± 0.037 ml/min/mmHg, wrap and sham, respectively, p = 0.0002, Student’s unpaired t-test). Renal blood flow tended to be lower in hypertensive (30.5 ± 7.5 ml/min) than normotensive rabbits (43.6 ± 3.4 ml/min) but this difference was not statistically significant (p = 0.110). Resting renal vascular conductance however was substantially lower in the hypertensive rabbit than its normotensive counterpart (0.260 ± 0.068 vs. 0.488 ± 0.058 ml/min/mmHg, wrap and sham, respectively, p = 0.020, Student’s unpaired t-test). In terms of vascular resistance, this meant that resistance was approximately 2-fold greater in both the hindquarter and renal vasculature of hypertensive rabbits when compared to their normotensive sham controls. The hypertensive to normotensive ratio (H:N) of vascular resistance was calculated as 1.85 and 2.13 in the hindquarter and renal vascular bed, respectively.

4.3.3 Effects of autonomic and neurohumoral blockade on responses

The experimental protocols performed in this study were also performed during autonomic and neurohumoral block. Areflexic preparations are necessary to assess local vascular responses without the confounding effects of cardiovascular reflexes and opposing actions mediated by local factors.

Using the nasopharyngeal reflex as a test for autonomic and neurohumoral blockade

The responses to cigarette smoke exposure in sham and wrap rabbits pre-ganglionic block (day P2) or pre-neurohumoral block (day P3) were characteristic of the nasopharyngeal reflex. A typical trace of this response is shown in Figure 4-6. In sham rabbits, pre-ganglionic block (Protocol 2, P2, Figure 4-4) and pre-neurohumoral block (Protocol 3, P3, Figure 4-5), there was a small but significant increase in mean arterial pressure (ΔMAP: P2, 9 ± 2 mmHg, p = 0.001; P3, 10 ± 1 mmHg, p < 0.0001), profound bradycardia (ΔHR: P2, -113 ± 15 beats/min, p < 0.0001; P3, -93 ± 9 beats/min, p < 0.0001) and decreased hindquarter vascular conductance (ΔHVC: P2, -0.492 ± 0.052 ml/min/mmHg, p < 0.0001; P3, -0.478 ± 0.067 ml/min/mmHg, p = 0.001) in response to smoke. Renal vascular conductance decreased on both days (Figure 4-4D and Figure 4-5D), but this was only statistically significant on Protocol Day 3 (ΔRVC: P3, -0.096 ± 0.033 ml/min/mmHg, p = 0.033). As shown in Figure 4-4 and Figure 4-5, the cardiovascular and haemodynamic pattern of the nasopharyngeal reflex in wrap rabbits with effectors intact differed in some aspects to sham rabbits. Although cigarette smoke inhalation elicited marked bradycardia (ΔHR: P2, -87 ± 9 beats/min, p < 0.0001; P3, -102 ± 17 beats/min, p = 0.001) and peripheral vasoconstriction in the hindquarter
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vasculature ($\Delta$HVC: P2, $-0.227 \pm 0.032$ ml/min/mmHg, $p < 0.0001$; P3, $-0.234 \pm 0.040$ ml/min/mmHg, $p = 0.001$), mean arterial pressure and renal vascular resistance were unchanged.

Following the induction of either ganglionic block or neurohumoral block, the haemodynamic changes in response to cigarette smoke were significantly blunted in both sham and wrap groups, indicative of a successful blocking regimen in these rabbits. During autonomic and neurohumoral blockade, mean arterial pressure (Figure 4-4A and Figure 4-5A) and heart rate (Figure 4-4B and Figure 4-5B) remained relatively stable upon exposure to smoke. Although a decrease in heart rate was observed upon exposure to smoke in sham rabbits during neurohumoral block, the magnitude of the decrease was substantially smaller than when effectors were intact ($\Delta$HR $-93 \pm 9$ vs. $-14 \pm 4$ beats/min, pre- and post-neurohumoral block, respectively, $p = 0.0002$). The magnitude of smoke-induced peripheral vasoconstriction was also significantly decreased during ganglionic and neurohumoral block, though it was not completely absent (Figure 4-4C and Figure 4-5C).

Baseline variables across all three study days

The baseline mean arterial pressures of sham rabbits during study days P1, P2 and P3 decreased progressively in small increments (MAP, $80 \pm 2$, $74 \pm 2$ and $70 \pm 3$ mmHg, respectively). Only the blood pressures between study day P1 and P3 were statistically significant ($p < 0.01$, one-way ANOVA with Bonferroni post-test for multiple comparisons). A similar pattern was observed with the baseline mean arterial pressures of wrap rabbits during study days P1, P2 and P3 (MAP, $119 \pm 3$, $109 \pm 5$, $106 \pm 5$ mmHg, respectively), although these were not statistically significant. Baseline heart rate, hindquarter vascular conductance and renal vascular conductance were stable across all three study days for both groups.

Effect of ganglionic blockade on baseline variables

In both sham and wrap rabbits, administration of the ganglion blocking drug mecamylamine caused a decrease in mean arterial pressure (Table 4-3). Ganglionic block also caused a profound increase in heart rate, an expected response given the dominance of parasympathetic (vagal) influence on heart rate in the rabbit. Ganglionic blockade resulted in greater increases in hindquarter vascular conductance (or greater decreases in resistance) in the hypertensive than the normotensive rabbits. Hindquarter vascular conductance increased by $0.107 \pm 0.054$ and $0.200 \pm 0.059$ ml/min/mmHg in normotensive and hypertensive rabbits, respectively. Only the latter was statistically significant ($p = 0.010$). Comparatively, renal vascular conductance was not affected by ganglionic blockade in either sham ($\Delta$RVC, $0.020 \pm 0.045$ ml/min/mmHg; $p = 0.672$) or wrap rabbits ($\Delta$RVC, $0.001 \pm 0.041$ ml/min/mmHg; $p = 0.989$).

Effect of neurohumoral blockade on baseline variables

Neurohumoral blockade caused a similar cardiovascular pattern to ganglionic blockade. Induction of neurohumoral blockade lowered mean arterial pressure ($p < 0.0001$) and elevated heart rate, although the tachycardia was only statistically significant in sham ($\Delta$HR, $55 \pm 10$ beats/min; $p < 0.0001$) not wrap rabbits ($\Delta$HR, $34 \pm 16$ beats/min; $p = 0.074$). Hindquarter vascular conductance
also increased in both sham ($\Delta$HVC, 0.251 ± 0.086 ml/min/mmHg; $p = 0.023$) and wrap groups ($\Delta$HVC, 0.250 ± 0.025 ml/min/mmHg; $p < 0.0001$).

Neurohumoral block caused greater decreases in mean arterial pressure of wrap rabbits than ganglionic block alone, with mean arterial pressure of wrap rabbits falling by 31 ± 4 mmHg during neurohumoral block but only by 15 ± 3 mmHg during ganglionic block ($p < 0.0001$, Table 4-3). In line with this, neurohumoral block also elicited greater increases in renal vascular conductance than autonomic block alone. Renal vascular conductance in sham rabbits was elevated during neurohumoral block ($\Delta$RVC, 0.202 ± 0.026 ml/min/mmHg; $p = 0.0002$), an effect not observed with ganglionic block. Renal vascular conductance also appeared to be elevated in wrap rabbits during neurohumoral blockade, though the effect was not statistically significant ($\Delta$RVC, 0.080 ± 0.042; $p = 0.112$). This may suggest that endogenous angiotensin II and/or vasopressin rather than neural inputs set the resting renal vascular tone in the normotensive rabbit.

It has been reported that the resting circulatory variables of hypertensive rabbits alter to a greater magnitude following autonomic block than those of sham rabbits. This study demonstrated that the neurohumoral or ganglion block-induced changes in resting circulatory variables were comparable between sham and wrap rabbits. The only exception being that neurohumoral blockade elicited a greater decrease in mean arterial pressure in wrap than sham rabbits ($\Delta$MAP, -31 ± 4 vs. -17 ± 2 mmHg, wrap and sham, respectively, $p < 0.01$). Mean arterial pressure of hypertensive rabbits was still significantly higher than in normotensive rabbits following ganglionic ($p < 0.0001$) or neurohumoral blockade ($p < 0.0001$, Student’s unpaired t-test). Hindquarter vascular resistance was also greater than corresponding values of sham rabbits during ganglionic ($p = 0.030$) and neurohumoral block ($p = 0.005$), although the differences were slightly dampened (H:N hindquarter vascular resistance ratios of 1.85, 1.42 and 1.56 during effectors intact, ganglionic and neurohumoral block, respectively). Unlike in the hindquarter vascular bed, the raised renal vascular resistance in hypertensive rabbits was largely unaffected by either blocking regimen. Similar H:N ratios were determined during effectors intact, ganglionic block and neurohumoral block (H:N ratio of renal vascular resistance was 2.13, 1.90 and 1.65, respectively).
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<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>n</th>
<th>Wrap</th>
<th>n</th>
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<td></td>
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</tr>
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<td>255 ± 12</td>
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<tr>
<td>Hindquarter flow (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>58.8 ± 3.2</td>
<td>12</td>
<td>47.3 ± 3.6 *</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>53.6 ± 4.9</td>
<td>10</td>
<td>58.6 ± 5.2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>NHB</td>
<td>58.2 ± 7.7</td>
<td>9</td>
<td>52.2 ± 4.6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Hindquarter vascular conductance (ml/min/mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>0.738 ± 0.037</td>
<td>12</td>
<td>0.400 ± 0.034***</td>
<td>9</td>
<td>0.54 (1.85)</td>
</tr>
<tr>
<td>GB</td>
<td>0.869 ± 0.079</td>
<td>10</td>
<td>0.642 ± 0.075**, #</td>
<td>9</td>
<td>0.71 (1.42)</td>
</tr>
<tr>
<td>NHB</td>
<td>1.094 ± 0.108**</td>
<td>9</td>
<td>0.714 ± 0.034**, #</td>
<td>9</td>
<td>0.65 (1.53)</td>
</tr>
<tr>
<td>Renal flow (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>43.6 ± 3.4</td>
<td>9</td>
<td>30.5 ± 7.5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>33.8 ± 3.1</td>
<td>7</td>
<td>28.3 ± 2.1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>NHB</td>
<td>36.1 ± 3.3</td>
<td>7</td>
<td>28.8 ± 2.9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Renal vascular conductance (ml/min/mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>0.532 ± 0.041</td>
<td>9</td>
<td>0.250 ± 0.060**</td>
<td>7</td>
<td>0.47 (2.13)</td>
</tr>
<tr>
<td>GB</td>
<td>0.565 ± 0.049</td>
<td>7</td>
<td>0.298 ± 0.017***</td>
<td>7</td>
<td>0.53 (1.90)</td>
</tr>
<tr>
<td>NHB</td>
<td>0.657 ± 0.047</td>
<td>7</td>
<td>0.395 ± 0.054**</td>
<td>7</td>
<td>0.60 (1.65)</td>
</tr>
</tbody>
</table>

Intact, effectors intact; GB, ganglionic block (mecamylamine); NHB, neurohumoral block via concomitant vasopressin V1 antagonism, angiotensin-converting enzyme inhibition and ganglionic block (see Methods for details); H:N, wrap : sham ratio; VC, vascular conductance and VR, vascular resistance (reciprocal of vascular conductance). Parameters were measured in rabbits with effectors intact (Day 35), during ganglionic block (Day 37) and during neurohumoral block (Day 39). Measurements were taken after a 30 min stabilisation in rabbits with effectors intact and after a 40 min stabilisation period in rabbits with block. Values are mean ± SEM. *p < 0.05, ***p < 0.001 vs. corresponding mean value in sham group, Student's unpaired t-test. ##p < 0.01, ###p < 0.001, compared with corresponding effectors intact values within group, one-way ANOVA with Dunnett's post-test for multiple comparisons. n, number of rabbits.
Table 4-3. Acute effects of ganglionic blockade and neurohumoral blockade on haemodynamic variables in normotensive (sham) and renal cellophane-wrapped (wrap) conscious rabbits

<table>
<thead>
<tr>
<th>Variable</th>
<th>GB</th>
<th>GB</th>
<th>NHB</th>
<th>NHB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td>Sham</td>
<td>Wrap</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>n</td>
<td>12</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Pre-block</td>
<td>75 ± 2</td>
<td>109 ± 5</td>
<td>70 ± 3</td>
<td>106 ± 5</td>
</tr>
<tr>
<td>Post-block</td>
<td>62 ± 1***</td>
<td>94 ± 6***</td>
<td>53 ± 2***</td>
<td>76 ± 7***</td>
</tr>
<tr>
<td>Δ</td>
<td>-13 ± 2</td>
<td>-15 ± 3</td>
<td>-17 ± 2</td>
<td>-31 ± 4##2, #3***</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>n</td>
<td>12</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Pre-block</td>
<td>206 ± 4</td>
<td>207 ± 4</td>
<td>204 ± 7</td>
<td>207 ± 9</td>
</tr>
<tr>
<td>Post-block</td>
<td>248 ± 5***</td>
<td>255 ± 12**</td>
<td>259 ± 13***</td>
<td>241 ± 16</td>
</tr>
<tr>
<td>Δ</td>
<td>42 ± 4</td>
<td>47 ± 11</td>
<td>55 ± 10</td>
<td>34 ± 16</td>
</tr>
<tr>
<td>Hindquarter flow (ml/min)</td>
<td>n</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Pre-block</td>
<td>56.4 ± 3.5</td>
<td>47.5 ± 2.2</td>
<td>59.5 ± 5.3</td>
<td>48.7 ± 2.3</td>
</tr>
<tr>
<td>Post-block</td>
<td>53.6 ± 4.9</td>
<td>58.5 ± 5.2*</td>
<td>58.2 ± 6.9</td>
<td>53.2 ± 5.1</td>
</tr>
<tr>
<td>Δ</td>
<td>-2.9 ± 2.7</td>
<td>10.9 ± 4.1#1</td>
<td>-1.3 ± 3.4</td>
<td>4.5 ± 3.8</td>
</tr>
<tr>
<td>Hindquarter vascular conductance (ml/min/mmHg)</td>
<td>n</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Pre-block</td>
<td>0.762 ± 0.036</td>
<td>0.443 ± 0.025</td>
<td>0.859 ± 0.067</td>
<td>0.449 ± 0.020</td>
</tr>
<tr>
<td>Post-block</td>
<td>0.869 ± 0.079</td>
<td>0.642 ± 0.075**</td>
<td>1.137 ± 0.112*</td>
<td>0.699 ± 0.035***</td>
</tr>
<tr>
<td>Δ</td>
<td>0.107 ± 0.054</td>
<td>0.200 ± 0.059</td>
<td>0.251 ± 0.086</td>
<td>0.250 ± 0.025</td>
</tr>
<tr>
<td>Renal flow (ml/min)</td>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Pre-block</td>
<td>40.7 ± 6.0</td>
<td>32.4 ± 3.7</td>
<td>33.1 ± 4.4</td>
<td>32.4 ± 3.3</td>
</tr>
<tr>
<td>Post-block</td>
<td>33.8 ± 3.1</td>
<td>28.3 ± 2.1</td>
<td>36.1 ± 3.3</td>
<td>28.8 ± 2.9</td>
</tr>
<tr>
<td>Δ</td>
<td>-6.8 ± 5.2</td>
<td>-4.1 ± 5.2</td>
<td>3.1 ± 2.9</td>
<td>-3.6 ± 4.0</td>
</tr>
<tr>
<td>Renal vascular conductance (ml/min/mmHg)</td>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Pre-block</td>
<td>0.532 ± 0.072</td>
<td>0.297 ± 0.044</td>
<td>0.455 ± 0.051</td>
<td>0.309 ± 0.042</td>
</tr>
<tr>
<td>Post-block</td>
<td>0.552 ± 0.049</td>
<td>0.298 ± 0.017</td>
<td>0.657 ± 0.047***</td>
<td>0.395 ± 0.054</td>
</tr>
<tr>
<td>Δ</td>
<td>0.020 ± 0.045</td>
<td>0.001 ± 0.041</td>
<td>0.202 ± 0.026##1</td>
<td>0.086 ± 0.036</td>
</tr>
</tbody>
</table>

GB, ganglionic block (mecamylamine); NHB, neurohumoral block via concomitant vasopressin V₁ antagonism, angiotensin-converting enzyme inhibition and ganglionic block (see Methods for details). Pre-block values were recorded after a stabilisation period (30 min) following set-up. Post-block values were recorded 40 min into the GB or NHB protocol when parameters were stable. *p < 0.05, **p < 0.01, ***p < 0.001, pre-block vs. post-block within treatment (GB or NHB) and within animal, Student’s paired t-test. #p < 0.05, ##p < 0.05, ###p < 0.01, comparisons indicated by superscript numbers: 1, compared with corresponding sham group value during GB; 2, compared with corresponding wrap group value during GB; and 3, compared with corresponding sham group value during NHB, Student’s unpaired t-test. n, number of rabbits.
Figure 4-4. Haemodynamic variables in response to the smoke-induced nasopharyngeal reflex before and after ganglionic block in normotensive and hypertensive conscious rabbits

The effects of ganglionic block on smoke-induced changes in A. mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter vascular conductance (HVC); and D. Renal vascular conductance (RVC). Haemodynamic variables are shown at baseline and in response to smoke before (pre-treatment, black) and 40 min post ganglionic blockade (red) in sham (left panel within each graph) and wrap rabbits (right panel within each graph). The exact p-value is given where comparisons were significant (unless the p value fell below 0.0001); p-values in red indicate a significant smoke-induced response in rabbits prior to ganglionic blockade (pre) and p-values in black indicate a significant smoke-induced response in rabbits during ganglionic blockade (post). All comparisons were within animal and made using Student’s paired t-test. Vertical lines indicate comparisons between the smoke-induced response changes prior to ganglionic blockade (pre) and during ganglionic blockade (post). Vertical bars are ± 1 SEM; where no error bar is visible the SEM is within the symbol. n, number of rabbits.
Figure 4-5 Haemodynamic variables in response to the smoke-induced nasopharyngeal reflex before and after neurohumoral block in normotensive and hypertensive conscious rabbits

The effects of neurohumoral block on smoke-induced changes in A. mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter vascular conductance (HVC); and D. Renal vascular conductance (RVC). Haemodynamic variables are shown at baseline and in response to smoke before (pre-treatment, black) and 40 min post neurohumoral blockade (red) in sham (left panel within each graph) and wrap rabbits (right panel within each graph). The exact p-value is given where comparisons were significant (unless the p value fell below 0.0001); p-values in blue indicate a significant smoke-induced response in rabbits prior to neurohumoral blockade (pre) and p-values in black indicate a significant smoke-induced response in rabbits during neurohumoral blockade (post). All comparisons were within animal using Student’s paired t-test. Vertical lines indicate comparisons between the smoke-induced response changes prior to neurohumoral blockade (pre) and during neurohumoral blockade (post). Vertical bars are ± 1 SEM; where no error bar is visible the SEM is within the symbol. n, number of rabbits.
The structural vascular amplifier in the renal bed of hypertensive rabbits

Figure 4-6. Typical trace showing haemodynamic changes associated with the smoke-induced nasopharyngeal reflex (left panel) and inhibition of these changes during ganglionic block (right panel)

The trace is taken from a normotensive rabbit on day P2. The left panel was taken prior to mecamylamine infusion and the right taken 40 min post mecamylamine infusion. Note the profound bradycardia, slight pressor effect and decrease in hindquarter vascular conductance (HVC) in response to cigarette smoke (indicated by dotted vertical line). The decrease in renal vascular conductance (RVC) in this trace was not observed in all rabbits. Abbreviations: MAP, mean arterial pressure; HR, heart rate; MHQ, mean hindquarter flow and MRQ, mean renal flow.
4.3.4 Infusion of vasoconstrictor drugs

In sham and wrap rabbits, angiotensin II (Figure 4-7-4.8), methoxamine (Figure 4-9-4.10) and noradrenaline (Figure 4-11.4.12) all elicited dose-dependent constrictor responses typified by graded decreases in hindquarter and renal vascular conductance and increases in mean arterial pressure.

4.3.4.1 Pressor responses

Noradrenaline

Although in this study noradrenaline and methoxamine were administered via close intra-arterial infusion, both agonists still increased mean arterial pressure at the two highest doses (Figure 4-9A and Figure 4-11A). This may be due to recirculation of the drug or alternatively due to the direct effects of intra-arterial infusion on local vascular conductance in vascular regions downstream of the thoracic aorta. Hypertensive rabbits had enhanced pressor responses to both noradrenaline and methoxamine. These enhanced pressor responses manifested as a parallel leftward shift of the linear portion of the log dose-absolute mean arterial pressure response curves (Figure 4-9A and Figure 4-11A). In rabbits with effectors intact, noradrenaline tended to elicit a greater increase in mean arterial pressure in the hypertensive than normotensive rabbits (ΔMAP, 41 ± 4 vs. 55 ± 3 mmHg, respectively); however, this was not statistically significant. Thus, the greater peak absolute mean arterial pressure in the hypertensive rabbits (MAP, 172 ± 5 vs. 119 ± 5 mmHg, hypertensive and normotensive, respectively; ΔMAP = 53 mmHg) appeared to diminish during ganglionic block (MAP, 187 ± 9 vs. 140 ± 5 mmHg, respectively; ΔMAP = 47 mmHg; p < 0.001) and neurohumoral block (MAP, 181 ± 6 vs. 147 ± 6 mmHg, respectively; ΔMAP = 35 mmHg; p < 0.01, one-way ANOVA with Bonferroni post-test for multiple comparisons). This was despite peak changes in mean arterial pressure being comparable between normotensive and hypertensive rabbits during ganglion (ΔMAP, 80 ± 5 vs. 96 ± 7 mmHg, respectively) and neurohumoral block (ΔMAP, 93 ± 5 vs. 103 ± 4 mmHg, respectively; Figure 4-12A).

Methoxamine

A similar pressor response pattern was observed with the α1-adrenoceptor agonist methoxamine. In rabbits with intact effectors, methoxamine elicited a greater peak absolute pressure in hypertensive than normotensive rabbits (MAP, 155 ± 5 vs. 110 ± 4 mmHg, respectively; p < 0.01). However, while the enhanced peak pressor response was still evident during autonomic block (MAP, 156 ± 6 vs. 118 ± 6 mmHg; p < 0.01), it was not during neurohumoral block (MAP, 140 ± 8 vs. 125 ± 7 mmHg, ΔMAP = 15 mmHg). There was a tendency for methoxamine to cause greater changes in mean arterial pressure in hypertensive rabbits with intact effectors but this was not statistically significant (ΔMAP, 34 ± 3 vs. 48 ± 5 mmHg, normotensive and hypertensive, respectively). The peak change in mean arterial pressure was also comparable between normotensive and hypertensive rabbits during
autonomic block (ΔMAP, 60 ± 5 vs. 66 ± 6 mmHg) and neurohumoral block (ΔMAP, 71 ± 7 vs. 64 ± 8 mmHg).

Angiotensin II

Compared to noradrenaline and methoxamine, angiotensin II elicited profound elevations in mean arterial pressure. The dose range of angiotensin II was greater than that of methoxamine and noradrenaline and this may have prolonged the time taken to perform the dose-response curve and thus the time available for the drug to recirculate and impact on systemic pressure. Angiotensin II elicited a greater maximal absolute mean arterial pressure in hypertensive than normotensive rabbits (Emax, 192 ± 3 vs. 155 ± 4 mmHg; p < 0.001, one-way ANOVA with Bonferroni post-test for multiple comparisons). This difference remained during ganglionic (Emax, 183 ± 5 vs. 148 ± 5 mmHg, hypertensive and normotensive, respectively; p < 0.001) and neurohumoral block (Emax, 178 ± 6 vs. 155 ± 6 mmHg; p < 0.05, one-way ANOVA with Bonferroni post-test for multiple comparisons). Peak changes in mean arterial pressure between normotensive and hypertensive rabbits were not different when effectors were intact (ΔMAP, 73 ± 3 vs. 73 ± 5 mmHg, respectively) or during ganglionic block (ΔMAP, 85 ± 5 vs. 92 ± 4 mmHg) and neurohumoral block (ΔMAP, 104 ± 5 vs. 104 ± 8 mmHg). As with noradrenaline and methoxamine, angiotensin II-mediated peak changes in mean arterial pressure were not different between normotensive and hypertensive rabbits, the greater absolute mean arterial pressure in hypertensive rabbits may simply be a consequence of their elevated baseline mean arterial pressure. In the sham normotensive rabbit, angiotensin II caused greater peak changes in mean arterial pressure during ganglionic and neurohumoral block (ΔMAP, 85 ± 5 mmHg and 104 ± 5 mmHg, respectively) than when effectors were intact (ΔMAP, 73 ± 3 mmHg; Figure 4-8A). Thus, despite the drop in baseline mean arterial pressure with the blocking regimens, the maximum absolute mean arterial pressure elicited by the highest dose of angiotensin II (Emax, 192 ± 3 mmHg), was not significantly altered following ganglionic (Emax 183 ± 5 mmHg) or neurohumoral blockade (Emax, 178 ±6 mmHg; Figure 4-7A). A similar pattern was observed for hypertensive rabbits (Figure 4-7A and Figure 4-8).

The enhanced absolute pressor response observed with each constrictor drug in the hypertensive rabbit was retained during ganglionic and neurohumoral block. Despite starting at a lower resting pressure, rabbits with autonomic or neurohumoral block still managed to reach the same maximum absolute mean arterial pressure achieved when effectors were intact. Thus, dose-pressor response curves generated in the presence of ganglionic or neurohumoral block all had noticeably steeper curves, presumably because there were no longer any cardiovascular reflex mechanisms to buffer the increase in mean arterial pressure. It appears however that the slope of the dose-pressor response curves were comparable between normotensive and hypertensive rabbits, even during ganglionic and neurohumoral block. Though this may argue against structurally-based augmentation of pressor responses – which would have seen a steeper slope for hypertensive rabbits – there are a number of complications with using dose-pressure response curves to assess local vascular responses, as discussed in the introduction. Thus this study assessed the vascular conductance.
4.3.4.2 Heart rate

In rabbits with intact effectors, the bradycardia observed during the higher doses of methoxamine and noradrenaline infusion coincided with substantial changes in arterial pressure (Figure 4-9B and Figure 4-11B). The magnitude of the bradycardia was attenuated by ganglionic block and thus likely to be a reflex bradycardia activated as part of the baroreceptor reflex to dampen the increased pressure. The reflex bradycardia observed with noradrenaline was slightly more pronounced in the normotensive than hypertensive rabbits (ΔHR, -63 ± 11 vs. -42 ± 14 beats/min, respectively; Figure 4-12B), though this was not statistically significant. The bradycardia was completely abolished by autonomic and neurohumoral block in both normotensive and hypertensive rabbits. Methoxamine also elicited reflex bradycardia, reaching a similar minimum heart rate of 158 ± 6 and 170 ± 12 beats/min, for normotensive and hypertensive rabbits, respectively (Figure 4-9B). The peak decrease in heart rate in normotensive and hypertensive rabbits with intact effectors was -35 ± 7 beats/min and -53 ± 7 beats/min, respectively (Figure 4-10B). Unlike with noradrenaline, ganglionic and neurohumoral block only partially attenuated the bradycardia.

In contrast to noradrenaline and methoxamine, angiotensin II caused a small increase in heart rate in the hypertensive rabbit with intact effectors, with a maximum heart rate change of +20 ± 10 beats/min at the highest dose of angiotensin II (p < 0.0001, one-way ANOVA with Bonferroni post-test for multiple comparisons). Heart rate remained relatively stable during angiotensin II intra-arterial infusion in the normotensive rabbit with intact effectors. Ganglionic and neurohumoral block in the normotensive rabbit revealed a substantial tachycardia: peak heart rate changes were +47 ± 5 and +49 ± 10 beats/min for ganglionic and neurohumoral block, respectively (p < 0.001). The tachycardia in hypertensive rabbits also tended to be more pronounced during ganglionic (ΔHR, 35 ± 9 beats/min) and neurohumoral (ΔHR, 35 ± 7 beats/min) block than with effectors intact, though this was not statistically significant (p>0.05).
4.3.4.3 Hindquarter and renal vascular conductance

Dose-absolute hindquarter and renal vascular conductance response curves were derived for angiotensin II, methoxamine and noradrenaline as explained in Methods. The resting hindquarter vascular conductance (measured at 0 min prior to each agonist infusion) of normotensive rabbits was similar \((p = 0.702, \text{one way ANOVA, Tukey post-test for multiple comparisons})\) for angiotensin II \((0.760 \pm 0.036 \text{ ml/min/mmHg})\), noradrenaline \((0.842 \pm 0.064 \text{ ml/min/mmHg})\), methoxamine \((0.742 \pm 0.040 \text{ ml/min/mmHg})\), adenosine \((0.788 \pm 0.063 \text{ ml/min/mmHg})\) and acetylcholine \((0.808 \pm 0.053 \text{ ml/min/mmHg})\), reflecting the stability of the preparation over the course of an entire experiment. In comparison, resting hindquarter vascular conductance values were approximately halved in the hypertensive rabbit (Table 4-5) and remained significantly lower during ganglionic (Table 4-7) and neurohumoral block (Table 4-9). In accordance with the elevation in MAP, all constrictor agents elicited vasoconstriction in the renal and hindquarter vasculature of hypertensive and normotensive rabbits. Although close intra-arterial infusion of agonists was employed (to enable the generation of complete dose-response curves before sufficient venous recirculation of the agonist influenced systemic pressure), the elevation in systemic pressure at high doses of constrictor agents may be partly due to recirculated drug.

Hindquarter vasculature

In the hypertensive rabbit, the slope and range of dose-hindquarter vascular conductance curves were approximately 40-70% of the corresponding values in the sham rabbit. With effectors intact, constrictor dose-hindquarter vascular conductance curves generated in hypertensive rabbits had significantly smaller ranges than those in normotensive rabbits (Table 4-5). The H:N range ratios were 0.47 \((p < 0.0001)\), 0.61 \((p = 0.0004)\) and 0.41 \((p = 0.0003)\) for angiotensin II, methoxamine and noradrenaline, respectively. The ranges of constrictor dose-response curves in hypertensive rabbits remained significantly lower during ganglionic (Table 4-7) and neurohumoral block (Table 4-9). Dose-hindquarter vascular conductance curves generated to angiotensin II and noradrenaline in hypertensive rabbits also had slopes that were less steep than those of normotensive rabbits. The H:N slope ratios of angiotensin II and noradrenaline were determined to be 0.43 \((p = 0.007)\) and 0.47 \((p = 0.039)\), respectively. These were largely unaltered by ganglionic and neurohumoral block. Although the range of methoxamine dose-hindquarter vascular conductance curves was not significantly different between hypertensive and normotensive rabbits with effectors intact (H:N slope ratio, 0.68; \(p = 0.108\)), slopes were noticeably less steep in hypertensive rabbits (Figure 4-13, left panels) during ganglionic \((p = 0.022); H:N \text{ slope ratio, 0.60; Table 4-7}) and neurohumoral block \((p = 0.056; H:N \text{ slope ratio, 0.61; Table 4-9}).

The sensitivity of the hindquarter vasculature to constrictor agents was largely unaltered with hypertension. The hindquarter vasculature of hypertensive rabbits tended to be more sensitive to angiotensin II \((ED_{50}, 0.144 \pm 0.035 \mu g/kg/min \text{ i.a.})\) than that of normotensive rabbits \((ED_{50}, 0.252 \pm 0.035 \mu g/kg/min \text{ i.a.})\), though this was not statistically significant \((p = 0.054; \text{Table 4-5})\). Angiotensin II sensitivity was comparable during ganglionic \((ED_{50}, 0.153 \pm 0.025 \text{ vs. 0.211 \pm 0.022} \mu g/kg/min\)
The structural vascular amplifier in the renal bed of hypertensive rabbits

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i.a., wrap and sham rabbits respectively; \( p = 0.102 \) (Table 4-7) and neurohumoral block (ED\(_{50}\), 0.184 ± 0.039 vs. 0.194 ± 0.034 μg/kg/min i.a., wrap and sham rabbits respectively; \( p = 0.853 \) (Table 4-9). The hindquarter vasculature of normotensive rabbits and hypertensive had similar sensitivity to noradrenaline, as indicated by comparable ED\(_{50}\) values (2.78 ± 0.56 vs. 3.67 ± 0.60 μg/kg/min i.a., respectively; \( p = 0.293 \) (Table 4-5). Noradrenaline sensitivity of the hindquarter vasculature remained comparable between normotensive and hypertensive rabbits during ganglionic (2.61 ± 0.42 vs. 2.97 ± 0.34 μg/kg/min i.a., respectively; \( p = 0.507 \) (Table 4-7) and neurohumoral block (2.96 ± 0.33 vs. 3.19 ± 0.65 μg/kg/min i.a., respectively; \( p = 0.768 \) (Table 4-9). With effectors intact, there was a small but statistically significant difference in methoxamine sensitivity between the hindquarter vasculature of normotensive and hypertensive rabbits. The hindquarter vasculature of hypertensive rabbits were slightly less sensitive to methoxamine than their normotensive counterparts (ED\(_{50}\), 37.3 ± 2.4 vs. 26.2 ± 3.2 μg/kg/min i.a., respectively; \( p = 0.025 \). This difference was not observed during ganglionic (ED\(_{50}\), 38.6 ± 3.0 vs. 34.5 ± 1.7 μg/kg/min i.a., respectively; \( p = 0.259 \) nor neurohumoral block (ED\(_{50}\), 41.4 ± 1.6 vs. 36.2 ± 1.8 μg/kg/min i.a., respectively; \( p = 0.053 \).

Renal vasculature

As observed in the hindquarter vasculature, resting vascular conductance, range and slope values of dose-renal vascular response curves were significantly lower in the hypertensive than normotensive rabbits. Although, resting renal vascular conductance was greater in normotensive than hypertensive rabbits, dose-renal vascular conductance curves converged to a similar minimum conductance at the highest dose of constrictor agonist (Figure 4-13, right panels). A common bottom plateau was particularly apparent for angiotensin II and noradrenaline dose-vascular response curves. Thus, with effectors intact, dose-renal vascular conductance curves in hypertensive rabbits had smaller ranges as well as slopes that were less steep than those in normotensive rabbits (Table 4-5). The H:N range ratio was 0.46, 0.41 and 0.38 for angiotensin II, methoxamine and noradrenaline, respectively. Neither ganglionic (Table 4-8) nor neurohumoral block (Table 4-10) significantly altered these ratios for angiotensin II and methoxamine. The H:N range ratio for noradrenaline was slightly elevated during ganglionic (0.59) and neurohumoral (0.52) block when compared with effectors intact (0.38). However, the range was still considerably lower in hypertensive than normotensive rabbits during ganglionic (0.283 ± 0.032 vs. 0.483 ± 0.042 ml/min/mmHg, hypertensive and normotensive, respectively; \( p = 0.003 \)) and neurohumoral (0.318 ± 0.043 vs. 0.609 ± 0.047 ml/min/mmHg, hypertensive and normotensive, respectively; \( p = 0.002 \) block.

In rabbits with intact effectors, the H:N slope ratio for renal vascular conductance was 0.59, 0.52 and 0.42 for angiotensin II, methoxamine and noradrenaline, respectively. While ganglionic block resulted in a lower H:N slope ratio for noradrenaline dose-renal vascular conductance curves (0.78),
The structural vascular amplifier in the renal bed of hypertensive rabbits

Neurohumoral block significantly alter the difference in slopes (H:N slope ratio, 0.47). Regardless, the slopes of dose-renal vascular conductance curves generated in hypertensive rabbits were consistently less steep than those in normotensive rabbits, irrespective of ganglionic and neuroeffector status.

Unlike the hindquarter vasculature in which vasoconstrictor sensitivity was largely unaltered in the hypertensive rabbit, the renal vasculature did exhibit changes in vasoconstrictor sensitivity. The ED_{50} values in the hindquarter vasculature for angiotensin II and methoxamine were comparable between normotensive and hypertensive rabbits with intact effectors. Ganglionic and neurohumoral block revealed that angiotensin II sensitivity was approximately 2-fold greater in the renal vasculature of sham than wrap rabbits. The ED_{50} for angiotensin II in the renal vasculature of sham and wrap rabbits were as follows: during effectors intact, 0.037 ± 0.009 vs. 0.036 ± 0.005 μg/kg/min i.a.; during ganglionic block, 0.020 ± 0.002 vs. 0.040 ± 0.001 μg/kg/min i.a., p = 0.036; during neurohumoral block, 0.018 ± 0.002 vs. 0.042 ± 0.009 μg/kg/min i.a., p = 0.013. The ED_{50} values in the renal vasculature for noradrenaline were higher in wrap than sham rabbits with intact effectors (ED_{50}, 2.19 ± 0.033 vs. 1.21 ± 0.030 μg/kg/min i.a., respectively; p = 0.048; Table 4-6). The ED_{50}s remained higher in the renal vasculature of wrap than sham rabbits during ganglionic block (ED_{50}, 1.47 ± 0.180 vs. 0.60 ± 0.09 μg/kg/min i.a.; p = 0.001; Table 4-8), but not neurohumoral block (ED_{50}, 0.97 ± 0.11 vs. 1.24 ± 0.31 μg/kg/min i.a.; Table 4-10).

4.3.4.4 Regional haemodynamics

Notably, the hindquarter but not the renal vascular bed exhibits passive changes in flow in relation to the changes in mean arterial pressure. That renal blood flow appears to be maintained at a constant flow during low doses of agonist infusion (i.a.) may be due to the autoregulatory ability of the renal vasculature to control renal flow and renal perfusion pressure. There were also notable differences in vasoconstrictor sensitivity between the two vascular beds. The renal vascular beds of both sham and wrap rabbits were significantly more sensitive than the hindquarter vasculature to angiotensin II and noradrenaline (lower ED_{50}s; Table 4-4). In contrast, the renal vasculature of the sham rabbits with intact effectors (but not wrap rabbits) was less sensitive to methoxamine than the hindquarter vasculature (ED_{50}, 40.9 ± 2.1 vs. 25.7 ± 3.2 μg/kg/min i.a., renal and hindquarter vasculature, respectively; p = 0.002). This difference was abolished with ganglionic and neurohumoral block.
Figure 4-7. Relationship between angiotensin II dose (i.a.) and haemodynamic variables in normotensive and hypertensive rabbits

A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line, $n = 8-14$) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line, $n = 6-9$) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Responses 10, 5 and 0 min prior to intra-arterial infusion of angiotensin II demonstrate the stability of the preparation. Values are mean ± SEM. $n =$ number of rabbits.
Figure 4-8. Changes (Δ) in haemodynamic variables from the resting value during angiotensin II infusion (i.a.) in normotensive and hypertensive rabbits

Changes (Δ) in A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line, n = 8-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line, n = 6-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Dose-response curves show responses to intra-arterial infusion of angiotensin II. Values are mean ± SEM. n = number of rabbits.
Figure 4-9. Relationship between methoxamine dose (i.a.) and haemodynamic variables in normotensive and hypertensive rabbits

A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line, \( n = 7-14 \)) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line, \( n = 5-9 \)) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Responses 10, 5 and 0 min prior to intra-arterial infusion of angiotensin II demonstrate the stability of the preparation. Values are mean ± SEM. \( n = \) number of rabbits.
Figure 4-10. Changes (Δ) in haemodynamic variables from the resting value during methoxamine infusion (i.a.) in normotensive and hypertensive rabbits

Changes (Δ) in A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line, n = 7-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line, n = 5-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Dose-response curves show responses to intra-arterial infusion of methoxamine. Values are mean ± SEM. n = number of rabbits.
Figure 4-11. Relationship between noradrenaline dose (i.a.) and haemodynamic variables in normotensive and hypertensive rabbits

A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line, n = 7-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line, n = 5-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Responses 10, 5 and 0 min prior to intra-arterial infusion of noradrenaline demonstrates the stability of the preparation. Values are mean ± SEM. n = number of rabbits.
Figure 4.12. Changes (Δ) in haemodynamic variables from the resting value during acetylcholine infusion (i.a.) in normotensive and hypertensive rabbits

Changes (Δ) in A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line, n = 7-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line, n = 5-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Dose-response curves show responses to intra-arterial infusion of noradrenaline. Values are mean ± SEM. n = number of rabbits.
Figure 4.13. Hindquarter vascular conductance (HVC, left panels) and renal vascular conductance (RVC, right panels) in response to the vasoconstrictor agonists.

Angiotensin II (A, B; sham, n = 8-11; wrap, n = 6-8), methoxamine (C, D; sham, n = 7-11; wrap, n = 5-9) and noradrenaline (E, F; sham, n = 7-9; wrap, n = 5-8) were infused intra-arterially. The average sigmoidal dose-response fits of values within each group are shown. The slopes were determined from the quasi-linear portion of the curves, the dose range of which was decided by inspection. In panel F, the dose-range over which slope was ascertained differed between wrap (30-100 µg/kg/min i.a.) and sham rabbits (10-100 µg/kg/min i.a.). Values are mean ± SEM. n = number of rabbits.
Table 4-4. Comparison of the sensitivity (ED<sub>50</sub> values) to various agonists in the hindquarter and renal vascular bed of sham and wrap rabbits

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Protocol</th>
<th>Hindquarter</th>
<th>Renal</th>
<th>Hindquarter</th>
<th>Renal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>EI</td>
<td>0.252 ± 0.035</td>
<td>0.037 ± 0.009 ***</td>
<td>0.144 ± 0.035</td>
<td>0.036 ± 0.005 *</td>
</tr>
<tr>
<td>(μg/kg/min i.a.)</td>
<td>GB</td>
<td>0.211 ± 0.022</td>
<td>0.020 ± 0.002 ***</td>
<td>0.153 ± 0.025</td>
<td>0.040 ± 0.009 **</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>0.194 ± 0.034</td>
<td>0.018 ± 0.002 ***</td>
<td>0.184 ± 0.039</td>
<td>0.042 ± 0.009 **</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>EI</td>
<td>26.2 ± 3.2</td>
<td>40.9 ± 2.1 **</td>
<td>37.3 ± 3.3</td>
<td>35.7 ± 5.6</td>
</tr>
<tr>
<td>(μg/kg/min i.a.)</td>
<td>GB</td>
<td>34.5 ± 1.7 *</td>
<td>32.0 ± 2.6</td>
<td>38.6 ± 3.0</td>
<td>37.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>36.2 ± 1.8 **</td>
<td>33.9 ± 1.2</td>
<td>41.4 ± 1.6</td>
<td>36.3 ± 9.6</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>EI</td>
<td>2.78 ± 0.56</td>
<td>1.21 ± 0.30 *</td>
<td>3.67 ± 0.47</td>
<td>2.19 ± 0.33 **</td>
</tr>
<tr>
<td>(μg/kg/min i.a.)</td>
<td>GB</td>
<td>2.61 ± 0.42</td>
<td>0.60 ± 0.09 ***</td>
<td>2.97 ± 0.34</td>
<td>1.47 ± 0.18 ***</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>2.96 ± 0.33</td>
<td>1.24 ± 0.31 **</td>
<td>3.19 ± 0.65</td>
<td>0.97 ± 0.11 *</td>
</tr>
<tr>
<td>Adenosine</td>
<td>EI</td>
<td>-</td>
<td>76 ± 21</td>
<td>-</td>
<td>121 ± 40</td>
</tr>
<tr>
<td>(μg/kg/min i.a.)</td>
<td>GB</td>
<td>-</td>
<td>96 ± 47</td>
<td>-</td>
<td>125 ± 44</td>
</tr>
<tr>
<td></td>
<td>NHB</td>
<td>-</td>
<td>79 ± 22</td>
<td>-</td>
<td>79 ± 24</td>
</tr>
</tbody>
</table>

Values are ED<sub>50</sub> (mean ± 1 SEM), the dose of agonist required to elicit 50% of the maximal response.*p < 0.05, **p < 0.01, ***p < 0.001 compared to the corresponding ED<sub>50</sub> value obtained in the hindquarter vasculature during the same blocking protocol, Student’s unpaired t-test. #p < 0.05, ##p < 0.01, compared to the sensitivity of the vascular bed to a particular agonist when effectors are intact, one-way ANOVA with Bonferroni post-test for multiple comparisons. For statistical comparisons of ED<sub>50</sub> values between hypertensive and normotensive groups, refer to tables summarising the parameters of agonist-hindquarter and -renal vascular conductance curves (Table 4-5 – Table 4-10).
## Table 4-5. Comparison of the range, slope and sensitivity to vasoconstrictor drugs in the hindquarter vascular bed of sham and wrap rabbits with intact effectors

<table>
<thead>
<tr>
<th>Agonist-HVC response curve parameters</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td>H:N</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.760 ± 0.036</td>
<td>0.377 ± 0.018***</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>-0.566 ± 0.044</td>
<td>-0.268 ± 0.013 ***</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.346 ± 0.050</td>
<td>-0.149 ± 0.026 **</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt; (μg/kg/min i.a.)</td>
<td>0.252 ± 0.035</td>
<td>0.144 ± 0.035</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Methoxamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.742 ± 0.040</td>
<td>0.474 ± 0.047 ***</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.481 ± 0.046</td>
<td>0.293 ± 0.037 ***</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.413 ± 0.059</td>
<td>-0.281 ± 0.043</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt; (μg/kg/min i.a.)</td>
<td>26.2 ± 3.2</td>
<td>37.3 ± 2.4 *</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.842 ± 0.064</td>
<td>0.450 ± 0.051 ***</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.499 ± 0.056</td>
<td>0.203 ± 0.032 ***</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.355 ± 0.073</td>
<td>-0.166 ± 0.031 *</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt; (μg/kg/min i.a.)</td>
<td>2.78 ± 0.56</td>
<td>3.67 ± 0.60</td>
<td>1.32</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. HVC, hindquarter vascular conductance and H:N, hypertensive:normotensive ratio. *p < 0.05, **p < 0.01, ***p < 0.001 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits.
### Table 4-6. Comparison of the range, slope and sensitivity to vasoconstrictor drugs in the renal vascular bed of sham and wrap rabbits with intact effectors

<table>
<thead>
<tr>
<th>Agonist-RVC response curve parameters</th>
<th>Sham</th>
<th>Wrap</th>
<th>H:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>n = 9</td>
<td>n = 6</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.537 ± 0.043</td>
<td>0.290 ± 0.072 **</td>
<td>0.54</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.462 ± 0.030</td>
<td>0.214 ± 0.045 **</td>
<td>0.46</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.210 ± 0.021</td>
<td>-0.123 ± 0.023 *</td>
<td>0.59</td>
</tr>
<tr>
<td>ED(_{50}) (μg/kg/min i.a.)</td>
<td>0.037 ± 0.009</td>
<td>0.036 ± 0.005</td>
<td>0.97</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>n = 8</td>
<td>n = 5</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.569 ± 0.024</td>
<td>0.337 ± 0.075 **</td>
<td>0.59</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.412 ± 0.045</td>
<td>0.170 ± 0.036 **</td>
<td>0.41</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.425 ± 0.084</td>
<td>-0.223 ± 0.065 *</td>
<td>0.52</td>
</tr>
<tr>
<td>ED(_{50}) (μg/kg/min i.a.)</td>
<td>40.9 ± 2.1</td>
<td>35.7 ± 5.6</td>
<td>0.87</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>n = 8</td>
<td>n = 6</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.533 ± 0.039</td>
<td>0.269 ± 0.054 **</td>
<td>0.50</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.421 ± 0.031</td>
<td>0.160 ± 0.026****</td>
<td>0.38</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.218 ± 0.039</td>
<td>-0.091 ± 0.013 *</td>
<td>0.42</td>
</tr>
<tr>
<td>ED(_{50}) (μg/kg/min i.a.)</td>
<td>1.21 ± 0.30</td>
<td>2.19 ± 0.33 *</td>
<td>1.81</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. RVC, renal vascular conductance and H:N, hypertensive:normotensive ratio. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits.
Table 4-7. Comparison of the range, slope and sensitivity to vasoconstrictor drugs in the hindquarter vascular bed of sham and wrap rabbits during ganglionic block

<table>
<thead>
<tr>
<th>Agonist-HVC response curve parameters</th>
<th>Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td>H:N</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>n = 11</td>
<td>n = 8</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.863 ± 0.066</td>
<td>0.609 ± 0.072 *</td>
<td>0.71</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.602 ± 0.061</td>
<td>0.371 ± 0.051 *</td>
<td>0.62</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.406 ± 0.048</td>
<td>-0.215 ± 0.055 *</td>
<td>0.53</td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td>0.211 ± 0.022</td>
<td>0.153 ± 0.025</td>
<td>0.73</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>n = 9</td>
<td>n = 9</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.955 ± 0.078</td>
<td>0.609 ± 0.051 **</td>
<td>0.64</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.631 ± 0.068</td>
<td>0.357 ± 0.048 **</td>
<td>0.57</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.615 ± 0.082</td>
<td>-0.371 ± 0.049 *</td>
<td>0.60</td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td>34.5 ± 1.7</td>
<td>38.6 ± 3.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>n = 6</td>
<td>n = 8</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>1.11 ± 0.056</td>
<td>0.632 ± 0.062 ***</td>
<td>0.57</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.477 ± 0.061</td>
<td>0.331 ± 0.032 *</td>
<td>0.69</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.509 ± 0.097</td>
<td>-0.280 ± 0.044 *</td>
<td>0.55</td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td>2.61 ± 0.42</td>
<td>2.97 ± 0.34</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. HVC, hindquarter vascular conductance and H:N, hypertensive:normotensive ratio. *p < 0.05, **p < 0.01, ***p < 0.001 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits.
### Table 4-8. Comparison of the range, slope and sensitivity to vasoconstrictor drugs in the renal vascular bed of sham and wrap rabbits during ganglionic block

<table>
<thead>
<tr>
<th>Agonist-RVC response curve parameters</th>
<th>Group</th>
<th>Sham</th>
<th>Wrap</th>
<th>H:N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n = 9</td>
<td>n = 7</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.537 ± 0.043</td>
<td>0.310 ± 0.017 ***</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.492 ± 0.041</td>
<td>0.243 ± 0.020 ***</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.251 ± 0.031</td>
<td>-0.132 ± 0.016 **</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>ED_{50} (μg/kg/min i.a.)</td>
<td>0.020 ± 0.002</td>
<td>0.040 ± 0.009 *</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Methoxamine</td>
<td></td>
<td>n = 8</td>
<td>n = 6</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.679 ± 0.039</td>
<td>0.360 ± 0.030****</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.587 ± 0.041</td>
<td>0.223 ± 0.032****</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.468 ± 0.035</td>
<td>-0.294 ± 0.054 *</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>ED_{50} (μg/kg/min i.a.)</td>
<td>32.0 ± 2.6</td>
<td>37.1 ± 1.5</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td></td>
<td>n = 9</td>
<td>n = 6</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>0.573 ± 0.032</td>
<td>0.354 ± 0.028 ***</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>0.483 ± 0.042</td>
<td>0.283 ± 0.032 **</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.225 ± 0.014</td>
<td>-0.164 ± 0.017 *</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>ED_{50} (μg/kg/min i.a.)</td>
<td>0.60 ± 0.09</td>
<td>1.47 ± 0.18 ***</td>
<td>2.45</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. RVC, renal vascular conductance and H:N, hypertensive: normotensive ratio. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. corresponding value in sham rabbit group. Student’s unpaired t-test. n = number of rabbits.
Table 4-9. Comparison of the range, slope and sensitivity to vasoconstrictor drugs in the hindquarter vascular bed of sham and wrap rabbits during neurohumoral block

<table>
<thead>
<tr>
<th>Agonist-HVC response curve parameters</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td>H:N</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>n = 9</td>
<td>1.091 ± 0.101</td>
<td>0.642 ± 0.029 **</td>
<td>0.59</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>n = 7</td>
<td>0.773 ± 0.076</td>
<td>0.440 ± 0.028 **</td>
<td>0.57</td>
</tr>
<tr>
<td>Average slope</td>
<td></td>
<td>-0.488 ± 0.058</td>
<td>-0.275 ± 0.042 *</td>
<td>0.56</td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td></td>
<td>0.194 ± 0.034</td>
<td>0.184 ± 0.039</td>
<td>0.94</td>
</tr>
<tr>
<td>Methoxamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>n = 8</td>
<td>1.035 ± 0.089</td>
<td>0.748 ± 0.049 **</td>
<td>0.72</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>n = 9</td>
<td>0.764 ± 0.081</td>
<td>0.417 ± 0.048 **</td>
<td>0.66</td>
</tr>
<tr>
<td>Average slope</td>
<td></td>
<td>-0.688 ± 0.080</td>
<td>-0.420 ± 0.095</td>
<td>0.61</td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td></td>
<td>36.2 ± 1.8</td>
<td>41.4 ± 1.6</td>
<td>1.11</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td>n = 5</td>
<td>1.060 ± 0.103</td>
<td>0.703 ± 0.039 **</td>
<td>0.66</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td>n = 8</td>
<td>0.564 ± 0.075</td>
<td>0.319 ± 0.054 *</td>
<td>0.57</td>
</tr>
<tr>
<td>Average slope</td>
<td></td>
<td>-0.519 ± 0.052</td>
<td>-0.296 ± 0.026 **</td>
<td>0.57</td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td></td>
<td>2.96 ± 0.33</td>
<td>3.19 ± 0.65</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. HVC, hindquarter vascular conductance and H:N, hypertensive:normotensive ratio. *p < 0.05, **p < 0.01 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits.
Table 4-10. Comparison of the range, slope and sensitivity to vasoconstrictor drugs in the renal vascular bed of sham and wrap rabbits during neurohumoral block

<table>
<thead>
<tr>
<th>Agonist-RVC response curve parameters</th>
<th>Group</th>
<th>Sham</th>
<th>Hypertensive</th>
<th>H:N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n = 8</td>
<td>n = 7</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td>0.660 ± 0.031</td>
<td>0.408 ± 0.055***</td>
<td>0.62</td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td></td>
<td>0.606 ± 0.029</td>
<td>0.341 ± 0.055**</td>
<td>0.56</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td></td>
<td>-0.342 ± 0.031</td>
<td>-0.212 ± 0.037*</td>
<td>0.62</td>
</tr>
<tr>
<td>Average slope</td>
<td></td>
<td>0.018 ± 0.002</td>
<td>0.042 ± 0.009*</td>
<td>2.33</td>
</tr>
<tr>
<td>ED\text{50 (μg/kg/min i.a.)}</td>
<td></td>
<td>33.9 ± 1.2</td>
<td>36.3 ± 9.6</td>
<td>1.07</td>
</tr>
<tr>
<td>Methoxamine</td>
<td></td>
<td>n = 7</td>
<td>n = 5</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td></td>
<td>0.776 ± 0.053</td>
<td>0.420 ± 0.057**</td>
<td>0.54</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td></td>
<td>0.648 ± 0.060</td>
<td>0.201 ± 0.043***</td>
<td>0.31</td>
</tr>
<tr>
<td>Average slope</td>
<td></td>
<td>-0.618 ± 0.031</td>
<td>-0.181 ± 0.047****</td>
<td>0.29</td>
</tr>
<tr>
<td>ED\text{50 (μg/kg/min i.a.)}</td>
<td></td>
<td>1.24 ± 0.31</td>
<td>0.97 ± 0.11</td>
<td>0.78</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td></td>
<td>n = 9</td>
<td>n = 5</td>
<td></td>
</tr>
<tr>
<td>Resting (ml/min/mmHg)</td>
<td></td>
<td>0.691 ± 0.055</td>
<td>0.416 ± 0.049**</td>
<td>0.60</td>
</tr>
<tr>
<td>Range (Δ ml/min/mmHg)</td>
<td></td>
<td>0.609 ± 0.047</td>
<td>0.318 ± 0.043**</td>
<td>0.52</td>
</tr>
<tr>
<td>Average slope</td>
<td></td>
<td>-0.387 ± 0.024</td>
<td>-0.181 ± 0.028**</td>
<td>0.47</td>
</tr>
<tr>
<td>ED\text{50 (μg/kg/min i.a.)}</td>
<td></td>
<td>1.24 ± 0.31</td>
<td>0.97 ± 0.11</td>
<td>0.78</td>
</tr>
</tbody>
</table>

RVC, renal vascular conductance and H:N, hypertensive:normotensive ratio. Values are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits.
4.3.5 Infusion of dilator drugs

4.3.5.1 Pressor, heart rate and blood flow responses

Both adenosine and acetylcholine caused dose-dependent decreases in mean arterial pressure. In rabbits with intact effectors, the maximum decrease in pressure induced by acetylcholine tended to be greater in wrap (ΔMAP, -48 ± 5 mmHg) than sham (ΔMAP, -34 ± 3 mmHg) rabbits, though this was not statistically significant (p = 0.083). Adenosine elicited a significantly larger decrease in mean arterial pressure in wrap (ΔMAP, -35 ± 3 mmHg) than sham (ΔMAP, -18 ± 3 mmHg; p < 0.0001) rabbits. Both dilator agents also elicited a reflex tachycardia in response to the decrease in pressure. Acetylcholine elicited a comparable peak increase in heart rate of 44 ± 11 and 52 ± 9 beats/min in wrap and sham rabbits, respectively. The magnitude of the tachycardia during adenosine infusion was also similar between both rabbit groups (ΔHR, 61 ± 11 vs. 65 ± 10 beats/min). Reflex tachycardia was not observed in either wrap or sham rabbits during ganglionic or neurohumoral block in response to adenosine (Figure 4-16B and Figure 4-17B) or acetylcholine (Figure 4-14B and Figure 4-15B). In all rabbit groups, hindquarter flow increased and renal blood flow remained largely unaltered despite falls in mean arterial pressure (Figure 4-14 and Figure 4-15). In hypertensive rabbits with intact effectors, hindquarter blood flow increased from a mean resting value of 51.2 to 71.7 ml/min while renal blood flow remained between 27-31 ml/min across the entire dose range of acetylcholine.

4.3.5.2 Hindquarter and renal vascular conductance

Hindquarter and renal vascular responses to dilator agents were expressed as resistance (see Methods, section 4.2.7). Within each group (wrap or sham), adenosine and acetylcholine elicited different vascular responses.

Acetylcholine

This study was unable to construct sigmoidal logistic dose-absolute vascular conductance or resistance curves to the dilator acetylcholine in both the hindquarter and renal vascular beds. The construction of dose-absolute vascular response curves was difficult for various reasons. Despite the use of close intra-arterial infusion to minimise changes in systemic pressure, high doses of acetylcholine elicited substantial falls in pressure that necessitated the cessation of agonist infusion, particularly during ganglionic and neurohumoral block. Thus, many dose-vascular resistance curves did not have a clear lower plateau, resulting in poorly fitted sigmoidal logistic curves. There were also instances where acetylcholine caused little to no change in hindquarter vascular conductance despite causing a profound decrease in mean arterial pressure (n = 3). As sigmoidal logistic dose-response vascular conductance curves could not be generated, standard pharmacological analysis could not be utilised to assess the ED50 in wrap and sham rabbit groups. Regardless, it is clear that dose-hindquarter (and renal) vascular resistance response curves generated during acetylcholine infusion (i.a.) differ between wrap and sham rabbits (Figure 4-14 and Figure 4-15).
In the hindquarter vasculature of hypertensive rabbits with intact effectors, the range (resting resistance at t=0 min to minimum resistance recorded) was more than double that observed in the normotensive rabbits (H:N ratio, 2.2; \( p = 0.002 \)). The range remained significantly greater during ganglionic (H:N ratio, 1.7; \( p = 0.029 \)) but not neurohumoral block (H:N ratio, 1.4; \( p = 0.181 \)). In general, the slopes of the quasi-linear part of the dose-hindquarter vascular resistance curve tended to be steeper in hypertensive than normotensive rabbits (Table 4-11). The average slope was significantly steeper in the hypertensive than normotensive rabbits during neurohumoral block (H:N ratio, 1.8; \( p = 0.018 \)), but not quite during ganglionic block (H:N ratio, 1.8; \( p = 0.077 \)) or with effectors intact (H:N ratio, 1.5; \( p = 0.099 \)). Resting hindquarter vascular resistance was also significantly greater in the hypertensive (2.34 ± 0.25 ml/min/mmHg) than the normotensive group (1.35 ± 0.10 ml/min/mmHg; \( p = 0.001 \)). Resistance remained elevated during ganglionic (\( p = 0.0027 \)) and neurohumoral block (\( p = 0.001 \)). With effectors intact, the H:N resting vascular resistance ratio was calculated as 1.7, the reciprocal of which (0.58) is comparable to the H:N ratios of resting vascular conductance determined for the constrictor agonists (0.50 –0.64).

**Adenosine**

There was considerable variability in vascular responses between individual animals within each group. In sham rabbits with intact effectors, adenosine caused little to no change in hindquarter vascular conductance in 5 of 11 rabbits. There was a clear dose-dependent decrease in hindquarter vascular conductance in 4 rabbits (Figure 4-18) where one would have expected an increase. Another two rabbits appeared to have biphasic responses where conductance decreased at lower doses of adenosine before increasing with higher doses (Figure 4-19). Hindquarter vascular conductance also decreased or remained largely unaltered in response to adenosine i.a. infusion in wrap rabbits.

The mean vascular responses to the i.a. infusion of adenosine were expressed as vascular resistance and are shown in Figure 4-16. Although there was a dose-dependent decrease in hindquarter flow in both hypertensive (\( p < 0.0001 \)) and normotensive (\( p = 0.001 \)) rabbits with intact effectors, adenosine only caused dose-dependent increases in hindquarter vascular resistance in the former (\( p = 0.039 \)) and not the latter (\( p = 0.199 \), one way ANOVA with repeated measures). When autonomic ganglia and homeostatic neurohumoral mechanisms were blocked, adenosine failed to elicit any notable changes in hindquarter vascular resistance in both hypertensive and normotensive rabbits. The observed decrease in hindquarter flow was likely to be passive and due to the fall in mean arterial pressure (Figure 4-16).

In contrast to the hindquarter vasculature, renal blood flow increased in normotensive rabbits (\( p = 0.007 \)). There was also a trend for renal blood flow to increase in hypertensive rabbits but this did not reach statistical significance (\( p = 0.089 \), one way ANOVA with repeated measures). When changes in systemic pressure were taken into account, the graded infusion of adenosine elicited a dose-dependent decrease in renal vascular resistance in both hypertensive (\( p = 0.008 \)) and normotensive rabbits (\( p < 0.0001 \), although in some rabbits adenosine had minimal impact on
renal vascular resistance. This enabled the generation of sigmoidal logistic dose-absolute vascular resistance curves and estimations of the ED$_{50}$. ED$_{50}$ values were similar between normotensive and hypertensive rabbits across all three protocol days (Table 4-13). Of note, the standard errors of the means were substantial, and thus beg the question as to whether the calculated ED$_{50}$s are a true representation of the vascular responses observed.

The dose-renal vascular resistance response curves differed between sham and wrap rabbits. The slopes of adenosine dose-renal vascular resistance response curves tended to be steeper in hypertensive (-0.96 ± 0.20) than normotensive rabbits (-0.40 ± 0.07; $p = 0.018$), resulting in a H:N slope ratio of 2.4. However, this was only observed with effectors intact. Slopes were not significantly different between hypertensive and normotensive rabbits during ganglionic (-0.70 ± 0.14 vs. -0.42 ± 0.08; $p = 0.111$) or neurohumoral block (-0.60 ± 0.07 vs. -0.40 ± 0.06; $p = 0.055$). This is probably due to the fact that while slopes tended ($p>0.05$) to become less steep in hypertensive rabbits when autonomic ganglia and neurohumoral effectors were blocked, the slopes reported for normotensive rabbits remained unaltered ($p>0.05$, one-way ANOVA with Tukey post-test for multiple comparisons). Consequently, H:N slope ratios were smaller during ganglionic (1.7) and neurohumoral block (1.5) than in rabbits with intact effectors (2.4). Additionally, with intact effectors, the ranges of the dose-renal vascular resistance response curves were greater in hypertensive than normotensive rabbits ($p = 0.020$; H:N range ratio, 2.6). As observed with slope, this difference was blunted during ganglionic (H:N range ratio, 1.3) and neurohumoral block (H:N range ratio, 1.3).
The structural vascular amplifier in the renal bed of hypertensive rabbits
Figure 4-14. Relationship between acetylcholine dose (i.a.) and haemodynamic variables in normotensive and hypertensive rabbits

A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line; \( n = 8-14 \)) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line; \( n = 5-9 \)) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Values 10, 5 and 0 min prior to intra-arterial infusion of acetylcholine demonstrate the stability of the preparation. The shaded region represents the dose range from which average slopes of dose-vascular conductance curves were determined. Values are mean ± SEM. \( n \) = number of rabbits.
The structural vascular amplifier in the renal bed of hypertensive rabbits

![Diagram showing changes in hemodynamic variables during acetylcholine infusion](image)

Figure 4.15. Changes (Δ) in haemodynamic variables from the resting value during acetylcholine infusion (i.a.) in normotensive and hypertensive rabbits

Changes (Δ) in A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line; n = 8-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line; n = 5-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Values are mean ± SEM. n = number of rabbits.
Table 4.11. Parameters of acetylcholine dose-vascular resistance curves generated in the hindquarter vasculature of hypertensive and normotensive rabbits with intact effectors and during ganglionic and neurohumoral block

<table>
<thead>
<tr>
<th>ACh-hindquarter vascular resistance response curve parameters</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
</tr>
<tr>
<td>Effectors intact</td>
<td>n = 9</td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.35 ± 0.10</td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.58 ± 0.06</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.57 ± 0.08</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;(μg/kg/min i.a.)</td>
<td>-</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>n = 10</td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.08 ± 0.10</td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.35 ± 0.05</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;(μg/kg/min i.a.)</td>
<td>-</td>
</tr>
<tr>
<td>Neurohumoral block</td>
<td>n = 7</td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>0.92 ± 0.07</td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.28 ± 0.05</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;(μg/kg/min i.a.)</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. H:N, hypertensive to normotensive ratio. *p < 0.05, **p < 0.01 vs. corresponding value in sham rabbit group. Student’s unpaired t-test. n = number of rabbits. ED<sub>50</sub> values could not be determined (see results, section 4.3.5).
The structural vascular amplifier in the renal bed of hypertensive rabbits

Table 4-12. Parameters of acetylcholine dose-vascular resistance curves generated in the renal vasculature of hypertensive and normotensive rabbits with intact effectors and during ganglionic and neurohumoral block

<table>
<thead>
<tr>
<th>ACh-renal vascular resistance response curve parameters</th>
<th>Group</th>
<th>Sham</th>
<th>Hypertensive</th>
<th>H:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectors intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.90 ± 0.17</td>
<td>4.72 ± 1.03 *</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.75 ± 0.11</td>
<td>1.81 ± 0.70</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.67 ± 0.07</td>
<td>-1.65 ± 0.77</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>ED$_{50}$ (µg/kg/min i.a.)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganglionic block</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 6</td>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.76 ± 0.19</td>
<td>2.87 ± 0.25 **</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.50 ± 0.06</td>
<td>1.09 ± 0.22 **</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.43 ± 0.05</td>
<td>-0.79 ± 0.14 **</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>ED$_{50}$ (µg/kg/min i.a.)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurohumoral block</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 7</td>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.68 ± 0.25</td>
<td>2.32 ± 0.26</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.38 ± 0.11</td>
<td>0.55 ± 0.06</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.29 ± 0.06</td>
<td>-0.41 ± 0.16</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>ED$_{50}$ (µg/kg/min i.a.)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. H:N, hypertensive to normotensive ratio. *p < 0.05, **p < 0.01 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits. ED$_{50}$ values could not be determined (see results, section 4.3.5).
Figure 4.16. Relationship between adenosine dose (i.a.) and haemodynamic variables in normotensive and hypertensive rabbits

A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line; n = 8-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line; n = 6-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Values 10, 5 and 0 min prior to intra-arterial infusion of adenosine demonstrate the stability of the preparation. The shaded region represents the dose range from which average slopes of dose-vascular conductance curves were determined. Values are mean ± SEM. n = number of rabbits.
Figure 4-17. Changes (Δ) in haemodynamic variables from the resting value during acetylcholine infusion (i.a.) in normotensive and hypertensive rabbits

Changes (Δ) in A. Mean arterial pressure (MAP); B. Heart rate (HR); C. Hindquarter flow (HQ); D. Renal flow (RQ); E. Hindquarter vascular conductance (HVC); and F. Renal vascular conductance (RVC) in normotensive (sham, filled circle, solid line; n = 6-14) and cellophane-wrapped hypertensive rabbits (wrap, open circle, dotted line; n = 5-9) with effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) are shown. Values are mean ± SEM. n = number of rabbits.
Table 4-13. Parameters of adenosine dose-renal vascular resistance after ganglionic block, neurohumoral block or with effectors intact.

<table>
<thead>
<tr>
<th>Adenosine-renal vascular resistance response curve parameters</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Hypertensive</td>
<td>H:N</td>
<td></td>
</tr>
<tr>
<td>Effectors intact</td>
<td>n = 6</td>
<td>n = 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.93 ± 0.07</td>
<td>4.63 ± 0.95 *</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.87 ± 0.08</td>
<td>2.24 ± 0.53 *</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Average slope</td>
<td>-0.40 ± 0.07</td>
<td>-0.96 ± 0.20 *</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td>69 ± 23</td>
<td>121 ± 40</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Ganglionic block</td>
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<td>n = 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (mmHg/ml/min)</td>
<td>1.94 ± 0.05</td>
<td>3.45 ± 0.23 ****</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Range (Δ mmHg/ml/min)</td>
<td>0.88 ± 0.09</td>
<td>1.14 ± 0.15</td>
<td>1.3</td>
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</tr>
<tr>
<td>Average slope</td>
<td>-0.42 ± 0.08</td>
<td>-0.70 ± 0.14</td>
<td>1.7</td>
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<tr>
<td>ED₅₀ (μg/kg/min i.a.)</td>
<td>96 ± 47</td>
<td>125 ± 44</td>
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</tr>
<tr>
<td>Neurohumoral block</td>
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<td>Resting (mmHg/ml/min)</td>
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<td>2.56 ± 0.19 **</td>
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<td>Range (Δ mmHg/ml/min)</td>
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<td>ED₅₀ (μg/kg/min i.a.)</td>
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<td>79 ± 24</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± 1 SEM. H:N, hypertensive to normotensive ratio. *p < 0.05, **p < 0.01, ****p < 0.0001 vs. corresponding value in sham rabbit group, Student’s unpaired t-test. n = number of rabbits.
Phasic BP, phasic blood pressure (mmHg); Mean BP, mean arterial blood pressure (mmHg); Hindquarter Q, phasic hindquarter flow (ml/min); MHQ, mean hindquarter flow (ml/min) and HQ c, mean hindquarter vascular conductance; conductance = MHQ/Mean BP (ml/min/mmHg). Adenosine i.a. infusion rates are as indicated. Abrupt movements by the conscious rabbit were marked by the letter ‘m’. Renal flow is not shown because the renal flow probe was not operational in this particular rabbit.
Figure 4-19. Record of biphasic response to intra-arterial administration of adenosine in a normotensive rabbit with intact effectors

Phasic BP, phasic blood pressure (mmHg); Mean BP, mean arterial blood pressure (mmHg); Hindquarter Q, phasic hindquarter flow (ml/min); MHQ, mean hindquarter flow (ml/min); HQ c, mean hindquarter vascular conductance; conductance = MHQ/Mean BP (ml/min/mmHg); Renal Q, phasic renal flow (ml/min); MRQ, mean renal flow (ml/min); RQ c, mean renal vascular conductance; conductance = MRQ/Mean BP (ml/min/mmHg). Adenosine i.a. infusion rates are as indicated. Abrupt movements by the conscious rabbit were marked by the letter ‘m’ at markers no. 27 and 31. The yellow rectangle highlights the substantial decrease in hindquarter vascular conductance that occurred during adenosine infusion (Δ ~ -0.5 ml/min/mmHg).
4.4 Discussion

It has previously been shown that changes in vascular resistance in the systemic vasculature and the hindquarter vasculature are augmented in conscious rabbits with renovascular hypertension. It is unknown if the magnitude of the structural vascular amplifier is similar across different vascular beds. This study found that vascular resistance and reactivity is elevated in the renal vasculature of the conscious hypertensive rabbit and that the vascular structural amplifier plays a role in this elevation.

Elevated resting vascular resistance

As was previously determined (Wright et al., 1987), hindquarter vascular resistance was greater in renovascular hypertensive rabbits than normotensive rabbits. The resting H:N ratio for vascular resistance in the hindquarter vasculature of rabbits with intact effectors was 1.85, slightly lower than previous reports of 2.16 (Korner et al., 2010). When effectors were inhibited, vascular tone decreased in both rabbit groups. However, pharmacological blockade of autonomic ganglia caused a more pronounced increase in hindquarter vascular conductance (or decrease in resistance) in hypertensive than normotensive rabbits. This was also reported in experimental renal hypertension and in patients with primary or renal hypertension (Overbeck et al., 1971; Korner et al., 1973; Tarazi & Dustan, 1973). It may allude to a role for increased sympathetic vasoconstriction in the elevation of peripheral resistance in hypertension or be an example of the vascular amplifier. Although neurohumoral block increased hindquarter vascular conductance in normotensive and hypertensive rabbits, an observation consistent with total peripheral conductance (Wright et al., 2002), the magnitude of the increase in hypertensive rabbits was similar to that elicited by ganglionic block alone. This may indicate that angiotensin II and vasopressin V1-receptor activation play a relatively minor role in setting hindquarter vascular tone. Of note, resistance remained greater in the hindquarter vasculature of hypertensive than normotensive rabbits during ganglionic and neurohumoral block (H:N, 1.42 and 1.53, respectively). This implies that a portion of the raised hindquarter vascular resistance was not due to an enhanced neural activation or humoral activity. That hindquarter vascular resistance remained significantly higher in hypertensive than normotensive rabbits at maximal dilatation (achieved with the highest acetylcholine dose) suggests that the residual resistance in hypertensive rabbits was due to a structurally-driven increase in resistance.

Vascular resistance was also raised in the renal vasculature of conscious hypertensive rabbits. A raised resting renal vascular resistance in the conscious hypertensive rabbit has previously been reported in rabbits with bilateral cellophane nephritis using the radioactive microsphere method (Bolt & Saxena, 1983) and para-aminohippurate clearance (Denton et al., 1983). With intact effectors, resting renal vascular resistance was more than 2-fold greater in the hypertensive rabbit. Bolt and Saxena (1983) reported a similar fold-difference. In contrast, in rabbits that had arterial pressures comparable to those in the present study, Denton et al. (1983) reported a resting renal
vascular resistance that was almost 5-fold greater in hypertensive than normotensive rabbits. This was accompanied by a marked decrease in renal blood flow which was not observed in this study.

Contrary to the hindquarter vasculature, neural innervation may have little input in setting the resting renal vascular tone in hypertensive rabbits. Ganglionic block had no significant effect on renal vascular resistance in either rabbit group. Accordingly, resting renal vascular resistance remained approximately 2-fold greater in the hypertensive than normotensive rabbits during ganglionic block (H:N ratio 1.90). It should be raised that in some rabbits, the absence of marked renal vasoconstriction that typically occurs during the nasopharyngeal response (Evans et al., 2000a) suggests partial denervation of the kidney, perhaps due to placement of the flow probe around the left renal artery. Neurohumoral block, which encompasses ganglionic block and the inhibition of angiotensin II production and vasopressin V$_1$-receptors, increased renal vascular conductance in normotensive rabbits only. Resting renal vascular resistance remained greater in hypertensive rabbits during neurohumoral block, albeit to a lesser degree (H:N ratio 1.65). The data suggest that while angiotensin II and vasopressin, may contribute to the resting renal vascular tone in normotensive rabbits, they are not so important in the hypertensive renal vasculature. As observed in the hindquarter vasculature, vascular resistance remained greater in the renal vasculature of hypertensive than normotensive rabbits at maximal dilatation (achieved with the highest dose of acetylcholine or adenosine). Provided that maximum dilatation was truly achieved at the highest doses of dilator agents, then the raised resting renal vascular resistance must be due to structural rather than functional changes in the vasculature.

Structural rarefaction of the arteriolar network or structural remodeling that involves a decrease in the internal radius of resistance vessels, could both contribute to the elevated renal and hindquarter vascular resistance in the hypertensive rabbit. The relative contribution of these structural changes was not assessed. Theoretically, a decrease in the internal radius of R$_1$ arteries would be more effective at raising vascular resistance than the anatomical loss of arterioles. The internal radius has a fourth power relationship with resistance ($R \propto 1/r_i^4$) so that even minute decreases in the internal radius can substantially affect resistance. In contrast, rarefaction of arterioles increases vascular resistance by decreasing the number of parallel conductance channels ($1/R_{total} = 1/R_1 + 1/R_2 + ... + 1/R_n$). In renal hypertension, both structural remodeling and rarefaction have been reported in skeletal muscle vascular beds (Prewitt et al., 1984; Wright et al., 1987; Lombard et al., 1989; Hansen-Smith et al., 1990). Theoretical simulations suggest that rarefaction may contribute anywhere between 20-40% of the total change in vascular resistance (Greene et al., 1989; Korner & Angus, 1992). The two processes are not mutually exclusive. Indeed, vascular remodeling of larger upstream R$_1$ resistance vessels may facilitate functional rarefaction by amplifying resistance changes. The majority of information about microvascular rarefaction of renal vessels in hypertension has been obtained in preparations of renovascular hypertension induced by renal artery stenosis (Chade, 2013). Direct assessment of arteriolar density in the renal vascular bed is required to determine if structural rarefaction occurs in the cellophane wrap model of hypertension and if so, to what extent does it contribute to the raised resting vascular resistance.
Bolt and Saxena (1983) have previously shown that in the perinephritic hypertensive rabbit the increase in renal vascular resistance exceeds the increase in total peripheral resistance, though this was only assessed in rabbits with intact effectors. The present study showed that the H:N ratios for resting vascular resistance were greater in the renal than hindquarter vasculature, even under conditions of maximum dilatation and in rabbits in which the autonomic nervous system and the neurohumoral axis had been blocked. It is speculated that in this preparation, the renal vasculature is more susceptible to structural changes that drive an increased vascular resistance (i.e. decreased internal radius and/or anatomical rarefaction) than the hindquarter vasculature. This greater susceptibility may stem from its autoregulatory capabilities. The renal microcirculation is normally protected from increases in systemic pressure because the preglomerular vasculature undergoes a proportionate level of vasoconstriction in response to the increase in pressure. In this manner, a relatively constant glomerular perfusion pressure is maintained across a wide range of systemic pressures (Navar, 1978). In this study, renal flow tended to be lower in hypertensive (30.5 ± 7.5 ml/min) than normotensive (43.6 ± 3.4 ml/min) rabbits but this was not statistically significant. This may reflect the autoregulatory ability of the renal vasculature to maintain a relatively constant renal flow despite marked changes in arterial pressure. Renal autoregulation has been shown to be preserved in uncomplicated hypertension: the lower and upper pressure limit of autoregulation is elevated and so the microcirculation remains protected in the face of chronically elevated systemic pressure (Iversen et al., 1986; Iversen et al., 1987; Bidani & Griffin, 2002). Thus, while the hindquarter and preglomerular renal vasculature are faced with similar enhanced pressure loads in hypertension, the preglomerular renal vasculature is also persistently constricted as part of its autoregulatory duties. Persistent vasoconstriction is thought to facilitate not only anatomical rarefaction (Prewitt et al., 1982b), but inward vascular remodeling (Intengan & Schiffrin, 2001), both of which can raise resting resistance under conditions of maximum dilatation. It is important to note that the kidney is also being compressed by the fibrocollagenous hull that develops and encases the organ during perinephritic hypertension (Graef & Page, 1940). The pressure between the capsule and the kidney reaches approximately 30 mmHg after 4 weeks (Brace et al., 1974). External compression of the renal parenchyma leads to rarefaction and a gradual decrease in glomeruli number (Korner, 1982), contributing to the elevation in renal vascular resistance.

### 4.4.1 The structural vascular amplifier

The structural vascular amplifier describes an enhanced change in vascular resistance to a given constrictor or dilator stimulus that is consequential to structural changes in resistance arteries. The vascular amplifier can be defined as the hypertensive : normotensive ratio of vascular resistance slopes determined during the infusion of stimuli (Korner & Angus, 1992). Although it was possible to construct dose-vascular conductance curves to constrictor agents from resting to maximum constriction, it was difficult to construct vascular resistance curves to dilator agents due to the constraints of hypotension in the conscious rabbit. Thus, estimations of slope were obtained from regression lines that were constructed from the quasi-linear portions of the logistic curves.
This study confirmed previous reports that constrictor dose-vascular conductance response curves generated in the hindquarter vasculature of hypertensive rabbits have a resting resistance, average slope and range that is approximately half that in normotensive rabbits (Wright et al., 1987). That is, a given level of stimulus elicited a smaller change in hindquarter vascular conductance in the hypertensive than the normotensive rabbit. This phenomenon has been termed the conductance attenuator and is synonymous with the term resistance amplifier when responses are alternatively expressed in resistance rather than conductance units (Wright & Angus, 1999).

Taking the inverse of conductance to give resistance, this study found that the estimated slope was greater in the hindquarter vasculature of hypertensive than normotensive rabbits. The estimates of slope differences between hypertensive and normotensive rabbits were comparable for angiotensin II and noradrenaline (H:N ratio, 2.3 and 2.1, respectively). The difference in slopes between hypertensive and normotensive rabbits was lower for methoxamine (H:N ratio, 1.5). The reason for this difference is unknown. Wright et al., (1987) have previously reported that the differences in slope and range between hypertensive and normotensive rabbits were uniform across all constrictor agonists. This observation aligns with structurally-driven enhancement of vascular reactivity: a structural change in vascular geometry would affect the responses of all stimuli indiscriminately.

The magnitude of the difference in hindquarter vascular resistance slopes between hypertensive and normotensive rabbits was slightly less during autonomic and neurohumoral block. There was also a corresponding decrease in the hypertensive:normotensive ratio for range and resting vascular resistance. Notably, these observations only applied to angiotensin II and noradrenaline dose-hindquarter vascular resistance curves. In contrast, Wright et al. (2002) reported that, for all agonists, neurohumoral block had little effect on the hypertensive : normotensive slope ratio.

Enhanced vasoconstrictor sensitivity has been reported in various experimental preparations of hypertension (Molvany et al., 1980; Amann et al., 1981; Hamilton & Reid, 1983). In agreement with a similar study by Wright et al. (1987), this study found no evidence of an enhanced sensitivity to vasoconstrictors in the hindquarter vasculature of cellophane wrap hypertensive rabbits. The sensitivity values of the hindquarter vascular bed to angiotensin II and noradrenaline were comparable between rabbit groups, irrespective of whether effectors were intact or not. Hindquarter vascular beds of hypertensive rabbits were actually less sensitive to methoxamine than that of normotensive rabbits. However, although the difference in sensitivity was statistically significant, the magnitude of the decreased sensitivity was small and unlikely to have major physiological implications. When considered together, a raised resting vascular resistance, decreased range, steeper slope and relatively unaltered vasoconstrictor sensitivity provides evidence of the structural vascular amplifier in the hindquarter vasculature.
Vascular responses to constrictor agents were differentially altered in the renal and hindquarter vasculature of hypertensive rabbits. In contrast to the hindquarter vasculature, the renal vasculature of hypertensive rabbits had altered sensitivity to vasoconstrictor agonists. Interestingly, autonomic and neurohumoral block revealed a 2-fold decrease in sensitivity (greater ED$_{50}$) to angiotensin II. The renal vasculature of hypertensive rabbits also had a 2-fold decrease in sensitivity to noradrenaline but not methoxamine, despite both agonists mediating vasoconstriction through the $\alpha_1$-adrenoceptor. The lower sensitivity to noradrenaline disappeared with neurohumoral block. The reason for the decreased sensitivity is unknown: renal vascular vasoconstrictor sensitivity is generally enhanced or unaltered in established hypertension (Collis & Vanhoutte, 1977; Berecek et al., 1980; Smeda et al., 1988). The decrease in sensitivity may serve to counterbalance the enhanced renal vascular resistance. However, it is arguable whether a 2-fold decrease in vasoconstrictor sensitivity would have any significant physiological repercussions.

As in the hindquarter vasculature, the ranges of the constrictor dose-RVC curves were consistently lower in hypertensive than normotensive rabbits irrespective of the constrictor agent or blocking protocol. When expressed as resistance, the values for range in the hypertensive rabbit with effectors intact were more than double those in corresponding normotensive controls. Importantly, the estimated average slopes of dose-renal vascular conductance curves constructed in hypertensive rabbits were approximately half the values obtained in normotensive rabbits (H:N slope ratios, 0.59, 0.52, and 0.42 for angiotensin II, methoxamine and noradrenaline, respectively). The relative magnitude of the differences in range and slope between hypertensive and normotensive rabbits varied with ganglionic and neurohumoral block. The reason for this is unclear: ganglionic and neurohumoral block did not affect renal vascular responses in any discernible pattern. Regardless, slope values remained considerably lower in the hypertensive rabbit. The data suggest that the renal vasculature is also a conductance attenuator (or resistance amplifier) in rabbits with cellophane wrap hypertension.

Responses to dilator agents

As the structural vascular amplifier is conferred by structural changes in the vasculature, it was expected that the vascular responses of dilator drugs would be enhanced to a similar magnitude as those observed across all constrictor agents. This has been demonstrated in the hindquarter and peripheral vasculature of the hypertensive rabbit (Wright et al., 1987; Wright & Angus, 1999). In the present study, adenosine dose-renal vascular resistance curves constructed in hypertensive rabbits had slopes and ranges that were approximately 2.5-fold greater than those in normotensive rabbits, while ED$_{50}$ values were unchanged. These differences between wrap and sham rabbits were not present during ganglionic and neurohumoral block. Although dose-renal vascular resistance curves constructed in response to acetylcholine also appeared to have a greater slope and range in hypertensive rabbits, this was not statistically significant except during ganglionic block. For both dilator agents, the renal vascular responses between wrap and sham rabbits during neurohumoral
block were comparable: the only notable difference was the elevation in resting renal resistance in hypertensive rabbits. The data suggest that the resistance amplifier property of the renal vasculature is largely due to activity of humoral factors, perhaps angiotensin II or vasopressin V₁ receptor agonists. The data are in contrast to those obtained with the infusion of constrictor agents. It is unclear why such a difference exists. It is noted however that the analysis of dilator vascular responses was somewhat hindered due to the substantial variability between animals (in part due to small sample numbers).

Unfortunately, the effect of hypertension on hindquarter vascular responses to dilator drugs could not be rigorously ascertained in this study because the vascular responses to these drugs were inconsistent. The hypertensive : normotensive range ratio for acetylcholine dose-hindquarter vascular resistance curves was 2.2 in rabbits with effectors intact. The magnitude of this difference decreased with ganglionic block and was no longer significant during neurohumoral block. In contrast, slopes were significantly steeper in the hindquarter vasculature of hypertensive than normotensive rabbits, but only during neurohumoral block. Adenosine on the other hand, failed to elevate hindquarter vascular conductance in normotensive and hypertensive rabbits. Adenosine is known to directly cause potent vasodilatation in most vascular beds (Granger & Norris, 1980; Sollevi & Fredholm, 1981; Lagerkranser et al., 1984; Van Wylen et al., 1989; Wilson et al., 1990; Sakai et al., 1998) via the activation of A₂ receptors. It has been shown to elicit marked elevations in hindquarter vascular conductance (or decrease in resistance) in animal preparations similar to those used in this study (Wright et al., 1987). In addition to vasodilatation, adenosine has also been shown to elicit vasoconstriction. The ability of adenosine to elicit both vasoconstriction and vasodilatation may result in very little net effect and thus negligible changes in hindquarter vascular conductance.

However, although adenosine has been shown to elicit vasoconstriction, this has been limited to the hepatic, renal and pulmonary vasculature. In the pulmonary and renal vasculature, adenosine elicits vasoconstriction through the activation of A₁ receptors. In the hepatic vasculature, the decrease in hepatic blood flow reported in response to the systemic infusion of adenosine is unlikely to be the result of a direct vasoconstrictor effect (Lagerkranser et al., 1984; Fredholm & Sollevi, 1986). The direct infusion of adenosine into the hepatic artery has been shown to increase hepatic blood flow (Scholtholt et al., 1967). The vasoconstriction reported with systemic adenosine infusion may be an autoregulatory response to maintain hepatic perfusion pressure in the face of decreasing systemic pressure. This is unlikely to explain the lack of adenosine-mediated vasodilatation in the hindquarter vasculature of these rabbits: in comparison to the renal, hepatic, cerebral and coronary microcirculation, the hindquarter circulation has limited local autoregulatory capacity. It is unusual that despite sharing almost identical experimental protocols, Wright et al. (1987) observed a marked elevation in hindquarter vascular conductance in response to adenosine infusion but this study did not. The only major point of technical difference between this study and that of Wright et al. is the location of the implanted catheter used to administer agonists. This study administered agonists into the thoracic aorta rather than the lower abdominal aorta, a site more distant to the hindquarter vasculature. It may be that the extra distance that must be covered by adenosine before it reaches
the hindquarter vasculature allowed sufficient time for the activation of homeostatic mechanisms (i.e. vasoconstriction) working to restore the fall in arterial pressure presumably elicited by dilatation of the splanchnic circulation. However, adenosine also failed to increase hindquarter vascular conductance during ganglionic and neurohumoral block when the homeostatic mechanisms were no longer present to confound local vascular responses. Furthermore, if the activation of systemic homeostatic mechanisms was indeed the issue, then it is unusual that a similar blunting of responses was not observed with acetylcholine or any of the vasoconstrictor agents.

In a small number of rabbits adenosine failed to elicit major changes in renal vascular conductance. The capacity of adenosine to activate opposing vascular responses may explain why, in this study, adenosine failed to elicit major changes in renal vascular conductance in a small number of rabbits. Unlike the hindquarter vasculature, adenosine-mediated vasoconstriction has been reported in the renal vasculature and is likely to occur through the A1 adenosine receptor (Murray & Churchill, 1984). Single intravascular adenosine injections into the renal artery elicited vasoconstriction in normotensive patients (Marraccini et al., 1996) and anaesthetised animals (Hashimoto & Kumakura, 1965; Scott et al., 1965; Spielman & Osswald, 1978). As mentioned, the direct vasoconstriction effect of adenosine is likely to be via activation of the A1 adenosine receptor. Adenosine-mediated renal vasoconstriction is inhibited by the selective A1 adenosine receptor antagonist DPCPX (Dietrich et al., 1991). Furthermore, the selective A1 adenosine receptor agonist N6-cyclohexyl adenosine caused constriction in preglomerular arterioles in the split hydrenephrotic kidney of anaesthetised rats (Holz & Steinhausen, 1987; Dietrich et al., 1991). The continuous infusion of adenosine into the anaesthetised animal elicited a transient decrease in renal blood flow that then returned to values at or above baseline values (Tagawa & Vander, 1970; Osswald et al., 1978; Spielman & Thompson, 1982; Hall et al., 1985; Okumura et al., 1992). A net elevation in renal blood flow (or conductance) has also been observed in normotensive subjects (Edlund et al., 1994), patients with essential hypertension (Smits et al., 1991; Wierema et al., 2005) and anaesthetised animals (Hansen et al., 2005).

The propensity of adenosine to facilitate vasodilatation or vasoconstriction is dependent on the region of the renal vasculature and the distribution of adenosine receptor subtypes in these regions. In the cortical microcirculation of anaesthetised dogs and rats, adenosine decreased blood flow (Macias et al., 1983; Agmon et al., 1993), possibly by constricting preglomerular arterioles via activation of A1 adenosine receptors (Hansen & Schnermann, 2003; Hansen et al., 2005). In the medullary microcirculation, intrarenal infusion of adenosine elicited sustained vasodilatation (Macias et al., 1983). Renal interstitial medullary infusion of physiological levels of adenosine elicited dose-dependent vasodilatation in the medullary circulation (Zou et al., 1999). As discussed in the introduction, the cortical and medullary microcirculations are structurally and functionally distinct. This is likely to explain the marked regional heterogeneity in vascular responses observed with not only adenosine, but a number of vasoconstrictor agents (Parekh et al., 1996; Evans et al., 2000a; Evans et al., 2000b)(see section 1.7.2; Table 1-2)Thus the global renal response to adenosine infusion will depend on the balance of regional responses. Alternatively, the autoregulatory capacity
of the renal vasculature to maintain renal perfusion pressure and blood flow may explain why adenosine and acetylcholine failed to markedly alter renal vascular conductance in some rabbits.
<table>
<thead>
<tr>
<th>Baseline vascular conductance</th>
<th>Hindquarter vascular bed</th>
<th>Renal vascular bed</th>
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</thead>
<tbody>
<tr>
<td>Effectors intact</td>
<td>Ganglionic block</td>
<td>Neurohumoral block</td>
</tr>
<tr>
<td>0.54 (1.9)</td>
<td>0.71 (1.4)</td>
<td>0.65 (1.5)</td>
</tr>
<tr>
<td>0.47 (2.1)</td>
<td>0.53 (1.9)</td>
<td>0.60 (1.7)</td>
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**Constrictor agents**

<table>
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<th>Angiotensin II</th>
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<tr>
<td>0.57, ns</td>
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<td>↔</td>
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<tr>
<td>Methoxamine</td>
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<td>0.64 (1.6)</td>
<td>0.64 (1.6)</td>
<td>0.72 (1.4)</td>
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<td>Range</td>
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<tr>
<td>0.61 (1.6)</td>
<td>0.57 (1.8)</td>
<td>0.66 (1.5)</td>
</tr>
<tr>
<td>Slope</td>
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<tr>
<td>0.68 (1.5), ns</td>
<td>0.60 (1.7)</td>
<td>0.61 (1.6), ns</td>
</tr>
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<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
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</tr>
<tr>
<td>1.42</td>
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<tr>
<td>0.53 (1.9)</td>
<td>0.57 (1.8)</td>
<td>0.66 (1.5)</td>
</tr>
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<td>Range</td>
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<td>0.41 (2.4)</td>
<td>0.69 (1.4)</td>
<td>0.57 (1.8)</td>
</tr>
<tr>
<td>Slope</td>
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<tr>
<td>0.47 (2.1)</td>
<td>0.55 (1.8)</td>
<td>0.57 (1.8)</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↔</td>
<td>↔</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Summary of the hypertensive : normotensive conductance ratios for parameters of constrictor dose-vascular conductance response curves including resting vascular conductance (ml/min/mmHg), range (Δml/min/mmHg), slope and ED<sub>50</sub> (µg/kg/min i.a.). ↔, no change; and ns, non-significant but a trend was observed. Numbers in parenthesis are the resistance ratios (calculated as 1/conductance ratio).
Table 4.15. Summary of the changes in vascular responses that occur with hypertension in the hindquarter and renal vascular bed

<table>
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<th>Dilator agents</th>
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<th>Renal vascular bed</th>
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</thead>
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<td>Effectors intact</td>
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</tr>
<tr>
<td>Acetylcholine</td>
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</tr>
<tr>
<td>Resting vascular resistance</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Range</td>
<td>2.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Slope</td>
<td>1.5, ns</td>
<td>1.8, ns</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting vascular resistance</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slope</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Summary of the hypertensive: normotensive resistance ratios for parameters of dilator dose-vascular resistance response curves including resting vascular resistance (mmHg/ml/min), range (ΔmmHg/ml/min), slope and ED<sub>50</sub> (µg/kg/min i.a.). ↔, no change; -, not determined and ns, non-significant but a trend was observed.
4.4.2 Limitations

Although the potentially confounding effect of extrinsic mechanisms such as sympathetic activity was limited in this study by inhibiting autonomic ganglia, angiotensin II production and V1-receptor activation, the role of intrinsic functional counter-regulatory mechanisms in influencing the vascular responses to constrictor and dilator agents cannot be excluded. In addition to local autoregulatory systems, this study did not factor in the differential regulation of regional renal blood flow, having measured only total renal blood flow.

The renal vasculature is autoregulatory and comprises two circulations (medullary and cortical) that are differentially regulated. Although the medulla receives approximately 10% of total renal blood flow (Pallone et al., 1990), medullary blood flow is closely regulated: numerous studies report smaller changes in medullary blood flow than cortical or total renal blood flow in response to vasoactive agents (Nobes et al., 1991; Evans et al., 2000a; Evans et al., 2000b), the exception being V1-receptor agonists where the opposite is reported (Evans et al., 1998b). In some studies, medullary blood flow was unaltered by vasoactive agents (see section 1.7.2; Table 1-2). This apparent protection against vasoconstriction has been attributed to a number of intrinsic mechanisms including the anatomical arrangement of the medullary circulation, the vascular geometry of afferent and efferent juxtamedullary arterioles (see section 1.7.2) and local dilatory mechanisms (Cowley et al., 2003). Basal nitric oxide production is of greater importance in the medulla than the cortical circulation, indicated by the observation that nitric oxide synthase inhibition preferentially decreased medullary blood flow (Mattson et al., 1997; Rajapakse et al., 2002; Cowley et al., 2003). Furthermore, both acetylcholine and bradykinin, vasodilator agents that stimulate nitric oxide release, elicited greater increases in medullary blood flow than cortical blood flow. Since application of exogenous nitric oxide donors elicited similar increases in blood flow in both circulations, it was proposed that the medullary circulation was not necessarily more sensitive to nitric oxide, rather it had a greater capacity to produce and release nitric oxide once stimulated (Kalyan et al., 2002; Rajapakse et al., 2002). Vasodilator prostaglandins have also been implicated in the control of medullary blood flow (Roman & Lianos, 1990; Mattson & Roman, 1991).

Thus, in this study, local renal vascular responses to constrictor and dilator agents may have been confounded by the autoregulatory nature of the renal vasculature, particularly of the medullary circulation. Furthermore, given the importance of the medullary circulation in pressure diuresis/natriuresis and thus long-term arterial pressure control, it is of interest to determine whether the cortical and medullary circulation undergo different degrees of structural adaptation.

4.4.3 Other points of discussion

As expected, substantial increases in pressure induced by noradrenaline and methoxamine were accompanied by a reflex bradycardia that was abolished with autonomic block. Despite noradrenaline eliciting greater changes in pressure than methoxamine, both agonists mediate a reflex bradycardia of similar magnitude. This may be due to dampening of the reflex bradycardia to
noradrenaline via the activation of cardiac $\beta_1$-adrenoceptors (positive chronotropic effect) and in $\beta_2$-adrenoceptors in vascular smooth muscle, particularly in the skeletal muscle (vasodilatation).

Interestingly, reflex bradycardia was not observed with angiotensin II in normotensive or hypertensive rabbits despite the drug eliciting greater absolute pressor responses than noradrenaline and methoxamine. Instead, heart rate was unchanged during angiotensin II infusion in the normotensive rabbit while tachycardia was observed in the hypertensive rabbit with intact effectors. It has previously been shown that slow intravenous infusion of angiotensin II can accelerate the heart whilst bolus administration causes a reflex bradycardia followed by a steady tachycardia (Nishith et al., 1962; Lee et al., 1980; Guo & Abboud, 1984). In the same perinephritic model of renovascular hypertension in the rabbit, angiotensin II caused bradycardia (Wright et al., 2002). The reason for the difference in responses is unknown. Angiotensin II has been shown to act centrally to inhibit vagal efferent activity and thus the reflex bradycardia that typically accompanies elevations in arterial pressure (Lumbers et al., 1979; Lee et al., 1980). This may explain why normotensive rabbits with intact effectors had a stable heart rate during angiotensin II infusion. This does not explain however, the profound tachycardia that was unmasked following ganglionic and neurohumoral block. Angiotensin II is likely to be increasing heart rate via other means. Angiotensin II has been shown to act at sympathetic nerve terminals to augment noradrenaline release and consequently $\beta$-adrenoceptor-mediated positive chronotropy and inotropy (Ziogas et al., 1984). Angiotensin II can also act independently of tissue noradrenaline release and $\beta$-adrenoceptor activation by directly stimulating the sino-atrial node (Kobayashi et al., 1978; Cross et al., 1981; Nakashima et al., 1982). This has also been demonstrated in the rabbit isolated atrial preparation (Rioux et al., 1976). During ganglionic and neurohumoral block, the enhanced positive chronotropic effect could be due to the absence of the opposing reflex bradycardia that accompanies elevations in arterial pressure.

4.4.4 Summary

The data suggest that in chronic renovascular hypertension in rabbits: (i) the renal vasculature contributes to the enhanced peripheral vascular resistance alongside the hindquarter vasculature (and may do so to a greater extent); (ii) unlike in the hindquarter vascular bed, a significant change in neural activity does not appear to be the cause of the raised resting vascular resistance in the renal vascular bed; (iii) circulating humoral factors such as angiotensin II and vasopressin $V_1$ agonists influences resting hindquarter but not renal vascular resistance; and (iv) the raised resting resistance under maximum dilatation points to structural changes in the vasculature (rarefaction and/or decrease in lumen diameter). This study also confirmed previous reports of the structural vascular amplifier in the hindquarter vascular bed of perinephritic hypertensive rabbits and presents evidence of the structural vascular amplifier in the renal vasculature. These conclusions were based on the following observations: (i) the range and estimated slopes of constrictor dose-vascular conductance curves were significantly lower in both the hindquarter and renal vasculature of hypertensive rabbits compared to normotensive rabbits; (ii) vascular resistance was still greater in the hindquarter and renal vascular beds of hypertensive rabbits under conditions of maximal or near-maximal dilatation; and (iii) vasoconstrictor sensitivity was not enhanced in either vascular bed.
The augmentation of vascular resistance changes in the absence of changes in smooth muscle activity can be explained by vascular remodelling of the renal and hindquarter resistance arteries, specifically that which involves an increase in the wall thickness to lumen ratio. It should be noted however, that the magnitude of the resistance amplifier (or conductance attenuator) was not uniform across all constrictor and dilator agents within a vascular bed. The reason for this is unknown. In the renal vasculature, the lack of uniformity may be linked to the existence of distinct microcirculations that are differentially regulated and potentially differentially altered structurally.
Chapter 5

In vitro characterisation of regional vascular reactivity: effect of hypertension
5.1 Introduction

Elevated total peripheral resistance and enhanced vascular reactivity in hypertension is attributed predominantly to a structurally-driven decrease in lumen diameter and increase in wall to lumen ratio in resistance vessels (Folkow et al., 1958; Folkow et al., 1970b; Mulvany, 2012), as well as rarefaction of peripheral vascular beds (Chen et al., 1981; Prewitt et al., 1982a; Henrich et al., 1988; Greene et al., 1989; Prasad et al., 1995; Antonios et al., 1999b). Functional changes in the resistance vasculature have also been suggested to contribute to vascular hyper-reactivity. These changes include enhanced intrinsic myogenic activity, enhanced vascular smooth muscle sensitivity to endogenous vasoconstrictor substances and endothelial dysfunction. The results supporting functionally-driven increases in total peripheral resistance are ambiguous. This is particularly true for the renal vasculature which may be differentially altered by hypertension compared to other peripheral vascular beds. In hypertension, deviations in renal vascular tone and renal vascular reactivity are of particular interest because of the implications for renovascular resistance and accordingly renal perfusion pressure and arterial pressure. The experiments described in this chapter assess the local functional changes in resistance arteries of the renal and hindquarter vasculature of hypertensive and normotensive rabbits to complement the information obtained on structural changes in whole integrated vascular beds in vivo (see Chapter 4).

Enhanced renal vascular reactivity in hypertension may be due to a combination of structural and functional changes in the renal resistance vasculature. Increased smooth muscle sensitivity to constrictor agents would cause a parallel leftward shift of the agonist-dose response curve and no change in the maximum response. In contrast, encroachment of the vessel lumen by hypertrophied media (increased media to lumen ratio) would cause an asymmetrical leftward shift of the dose response curve (steeper slope) and a greater maximum response. ED$_{50}$ may also be lower in the absence of altered sensitivity (for reasons discussed in Chapter 1) but the decrease would be similar for all constrictor agents. In the isolated perfused kidney of 4-6-month-old SHR, enhanced vascular responses to noradrenaline, serotonin and angiotensin II were characterised by a greater maximal response but a lower ED$_{20}$ (dose required to elicit 20% of maximum response) and no change in slope compared to normotensive control rats (Collis & Vanhoutte, 1977). The magnitude of the decrease in ED$_{20}$ was not uniform but rather, agonist-specific. The data suggests that both structural and functional changes may contribute to the enhanced vascular reactivity in SHR. Similarly, dose-response curves generated in the renal vasculature of stroke-prone SHR to noradrenaline, serotonin, angiotensin II and vasopressin were characteristic of vessels that had both a greater wall to lumen ratio and enhanced vascular smooth muscle sensitivity (Berecek et al., 1980). Enhanced vascular responses were present from an early age (1-month-old) and increased with age (4s and 6-months-old). In contrast, Collis et al. (1980) reported a lack of increased smooth muscle sensitivity to noradrenaline and angiotensin II in young SHR (6-week-old). Although responses to renal nerve stimulation were enhanced in the young SHR, this was attributed to enhanced noradrenaline release rather than an increased sensitivity to the agonist itself. Furthermore, while Chatziantoniou et al. (1990) reported an enhanced vascular response to angiotensin II in young SHR (6-week-old), this was linked to the diminished presence of vasodilator prostaglandins in the SHR. Fink and Brody
Abnormalities in endothelial function occur secondary to the development of high blood pressure but may contribute to disease progression by exacerbating or maintaining the elevation in peripheral vascular resistance (Boulanger, 1999). The endothelium releases numerous smooth muscle contracting and relaxing factors and is integral in maintaining vascular homeostasis (Furchgott & Vanhoutte, 1989). Impaired endothelial function may disrupt the balance between vasodilator and vasoconstrictor agents such that vasoconstriction is favoured. Impaired endothelial function, often indicated by an impaired relaxation response to the endothelial-dependent vasodilator acetylcholine, has been reported in patients with essential hypertension (Linder et al., 1990; Panza et al., 1990; Taddei et al., 1993; Iiyama et al., 1996), although not always (Cockcroft et al., 1994). Endothelial dysfunction has also been reported in secondary forms of hypertension. Impaired relaxation to acetylcholine has been observed in the forearm vasculature and isolated subcutaneous arteries of renovascular and primary aldosteronism hypertensive patients (Taddei et al., 1993; Rizzoni et al., 1996). In experimental preparations of secondary hypertension, a decreased vasodilator response to acetylcholine has been detected in the mesenteric resistance arteries and aortae of one-kidney, one-clip renovascular hypertensive rats, two-kidney, one-clip renovascular hypertensive rats, deoxycorticosterone hypertensive rats and in rats with coarctation-induced hypertension (Lockette et al., 1986; Tesfamariam & Halpern, 1988; Lüscher et al., 1990; Van de Voorde et al., 1992).

Data on the renal vasculature of experimental models of hypertension are relatively sparse. Perfusion studies in whole vascular beds have provided important information on the sensitivity of vasoconstrictor agents but it is well known that different regions of the vasculature respond differently to the same stimuli (Hill et al., 2001). The renal vascular bed is no exception (Chapter 2). The contribution of various regions of the renal vasculature to elevated vascular resistance is thus likely to be different.

The present study explored the in vitro pharmacodynamics of isolated segments of small arteries taken from two sites in the rabbit kidney: the larger interlobar arteries (≈600 µm i.d.) and the arcuate arteries (≈250 µm i.d.) that lie along the corticomedullary border. For direct comparison we selected a small branch of the deep femoral artery (≈250 µm i.d.). The aim was to evaluate the full range (Emax) and sensitivity (EC50) of the three arterial segments under normalised conditions of passive tension. Of great interest was the comparison of Emax and EC50 values between arteries from wrap and sham rabbits. This study found dramatic differences in the pharmacodynamics of the three vessels highlighting that there is no general effect of Page hypertension. Rather, the study points to specific, local changes in pharmacodynamics that may be lost in the integration of the full vascular bed reactivity in vivo.
### Table 5.1. Summary of renal vascular whole kidney responses to various vasoconstrictor agents and the characteristics of their concentration-response curves

<table>
<thead>
<tr>
<th>Model</th>
<th>Preparation</th>
<th>RU</th>
<th>Age (mth)</th>
<th>Stimulant</th>
<th>Th</th>
<th>Shift</th>
<th>Slope</th>
<th>Emax</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male and female SHR</td>
<td>Isolated perfused kidney</td>
<td>na</td>
<td>4-6</td>
<td>Angiotensin II</td>
<td>↑</td>
<td>←</td>
<td>x</td>
<td>↑</td>
<td>(Collis &amp; Vanhoutte, 1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Barium chloride</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>↑</td>
<td>←</td>
<td>x</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acetylcholine</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Male SHR</td>
<td>In situ autoperfused kidney</td>
<td>↑</td>
<td>3-4</td>
<td>Angiotensin II</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>(Fink &amp; Brody, 1979)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Angiotensin II</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td>(Berecek et al., 1980)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vasopressin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Angiotensin II</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>(Collis et al., 1980)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Barium chloride</td>
<td>x</td>
<td>x</td>
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<td>x</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>SPSHR</td>
<td>Isolated perfused kidney</td>
<td>↑</td>
<td>1, 2, 4</td>
<td>Angiotensin II</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>(Chatziantoniou et al., 1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vasopressin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Angiotensin II</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Barium chloride</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Male SHR</td>
<td>Isolated perfused kidney</td>
<td>↑</td>
<td>6-7 weeks</td>
<td>Angiotensin II</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>(Chatziantoniou et al., 1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Noradrenaline</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>↑</td>
<td>←</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>Intrarenal bolus injection into anaesthetised rat</td>
<td>↑</td>
<td>6 weeks</td>
<td>Angiotensin II</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rat; SPSHR, stroke-prone spontaneously hypertensive rat; RU, Resistance when vessel is maximally dilated; Th, threshold sensitivity to vasoconstrictor agent; Shift, direction of shift of the location of the concentration-response curve, i.e. EC\textsubscript{50} sensitivity (← indicates a leftward shift in the curve and an increase in sensitivity); Slope, slope of steep part of the vasoconstrictor-mediated concentration-response curve; Emax, maximal contractile response to vasoconstrictor agent. All concentration-response curve characteristics are in the hypertensive model vs. control. ↓, decrease; ↑, increase; ← leftward shift; x, no change; na, not assessed. *Authors expressed responses to vasoconstrictor agents as a fraction of the initial resistance value.
5.2 Methods

5.2.1 Experimental animals

This study used vessels isolated from the sham normotensive and cellophane-wrap hypertensive New Zealand White rabbits described in Chapter 4.

5.2.2 Tissue collection

Following the conclusion of in vivo experiments (Chapter 4), rabbits were euthanased 2-4 days post-experimental protocol 3. Rabbits were anaesthetised with propofol (10-20 mg/kg i.v.) and killed with 4 ml saturated KCl (i.v.). Kidneys were removed and their wet-weight recorded before prompt placement in cold Krebs physiological salt solution (PSS; composition in mmol/l: NaCl 119; KCl 4.7; KH₂PO₄ 1.18; MgSO₄.7H₂O 1.17; NaHCO₃ 25; CaCl₂ 2.5; EDTA 0.03; glucose 5.5) saturated with carbogen (O₂ 95%; CO₂ 5%) at pH 7.4. The rabbit hind limb was sectioned from the rabbit and placed on ice. The deep femoral artery of the left and right thigh was isolated and cleaned of connective tissue. Kidneys were hemi-dissected and ring segments of the distal interlobar (outer medulla to subcortical zone) and arcuate arteries (corticomedullary junction to cortex) were isolated and cleaned of connective tissue (Figure 5-1). Vessels were mounted on stainless steel wires (outer diameter, 40 μm) connected to a force transducer in isolated 6 ml chambers of an isometric wire myograph (620M; Danish Myo Technology; Aarhus, Denmark). Vessels were bubbled with carbogen and thermoregulated at 37° C.
Figure 5.1. Schema showing the location of interlobar and arcuate arteries in a hemi dissected kidney (left panel) and the deep femoral artery (no. 22) of the right thigh as viewed from the left side (right panel).

In vitro characterisation of regional vascular reactivity: effect of hypertension

5.2.3 Normalisation protocol

In most studies, arteries are distended under passive conditions of stretch in order to align actin-myosin fibres for optimum force development upon application of a constrictor agent. To determine the appropriate resting passive force, this study employed the passive force normalisation procedure (Angus & Wright, 2000). A passive length-tension curve was generated for each vessel to determine the internal circumference \( L \) of each vessel when distended to an equivalent transmural passive pressure of 80 mmHg \( (L_{80}) \), for sham vessels, or 110 mmHg \( (L_{110}) \), for wrap vessels. These transmural pressures were chosen because they approximate the physiological mean arterial pressure of these rabbits. From the internal circumference of \( L_{80} \) and \( L_{110} \), the internal diameter \( D \) was calculated \( (L = \pi D) \) for \( D_{80} \) or \( D_{110} \). To ensure the arteries were tested for active contraction on the upslope of the length-tension curve, the stretched \( L_{80} \) or \( L_{110} \) was relaxed to 0.9 \( L_{80} \) or 0.9 \( L_{110} \) by micrometer adjustment as standard procedure. The 0.9 \( D_{80} \) and 0.9 \( D_{110} \) were then directly calculated from the internal circumference of 0.9 \( L_{80} \) or 0.9 \( L_{110} \). For a general discussion on the necessity of normalisation and the calculations required, refer to section 2.2.5.

5.2.4 Experimental protocols

5.2.4.1 Effect of normalisation protocols

Generally, the transmural pressure chosen to determine the passive resting force of isolated small arteries via the wire myograph is 100 mmHg (Mulvany & Halpern, 1977). To determine whether the different transmural pressures of sham and wrap vessels significantly altered the maximal contractile capacity of the arteries, the contractile response to KPSS was assessed in both wrap and sham arteries, firstly when arteries were set to 0.9 \( L_{80} \) and subsequently when arteries were set to 0.9 \( L_{110} \) (Figure 5-2, Protocol 3).

5.2.4.2 Receptor-mediated vasoconstriction

Following recovery from the KPSS reference contraction, cumulative concentration-response curves were generated to either noradrenaline \( (0.001 \text{ – } 100 \mu M) \), methoxamine \( (0.01 \text{ – } 100 \mu M) \), endothelin-1 \( (0.1 \text{ – } 100 \text{nM}) \) or angiotensin II \( (0.1 \text{ – } 100 \text{nM}) \). Vessels assessing angiotensin II-mediated responses were pre-treated with the nitric oxide synthase inhibitor L-nitro-arginine methyl ester (L-NAME, 100 μM) and the cyclo-oxygenase inhibitor indomethacin \( (3 \mu M) \) for 30 minutes. Distal arcuate vessels were additionally pre-contracted with arginine vasopressin to approximately 10% of the maximum contractile response induced by KPSS. This preactivated the artery to a limited extent so that any small changes in tone to angiotensin II were visible. A schematic of these protocols is shown in Figure 5-2, Protocol 2A.
5.2.4.3 Receptor-mediated vasodilatation

Following recovery from the KPSS reference contraction, cumulative relaxation concentration-response curves were generated in vessels pre-constricted with methoxamine to approximately 80% of the maximum contractile response induced by KPSS. The vasodilator agents assessed included the endothelium-dependent acetylcholine and the endothelium-independent sodium nitroprusside (Figure 5-2, Protocol 2B). The response to adenosine, a compound that causes vasodilatation by an unknown mechanism, was also assessed.

5.2.4.4 Membrane depolarisation-mediated vasoconstriction

Following recovery from the KPSS reference contraction, tissues were exposed to graded, non-cumulative increases in depolarising potassium solution (10, 20, 40, 60 mM; composition in mmol/l: PSS with an equimolar substitution of KCl at 10, 20, 40 and 60 mmol/l for NaCl). Each exposure lasted two min and tissues were rapidly washed until they returned to their baseline. A 15 min period of equilibration separated each subsequent exposure to depolarising potassium solution (Figure 5-2, protocol 2C).

Contribution of T-type channels

To explore the contribution of T-type calcium channels to membrane-depolarisation induced contraction in the hypertensive rabbit, this study assessed the inhibitory effect of various calcium channel blockers on potassium-induced contraction in isolated renal arteries of hypertensive rabbits and their controls. Following recovery from the KPSS reference contraction, interlobar vessels were equilibrated with one of the following calcium channel inhibitors for 30 min: (i) NNC 55-0396 (100 nM); (ii) mibebradil (100 nM); and felodipine (1 nM). At the concentrations utilised, NNC 55-0396 and mibebradil are selective for T-type calcium channels while felodipine is selective for L-type calcium channels only. These concentrations have been shown to cause partial inhibition of potassium-mediated responses in rat interlobar arteries (Chapter 3). Tissues were then exposed to graded, non-cumulative increases in depolarising potassium solution (10, 20, 40, 60 mM; composition in mmol/l: PSS with an equimolar substitution of KCl at 10, 20, 40 or 60 mmol/l for NaCl). Each exposure lasted two min and tissues were rapidly washed to their baseline thereafter. Arteries were re-equilibrated with their respective antagonist for a further 15 min prior to each subsequent exposure to depolarising potassium solution (Figure 3-3B).

5.2.5 Drugs

Drugs and suppliers were as follows: acetylcholine bromide (Sigma, St. Louis, MO, USA); adenosine (Sigma), angiotensin II amide (AusPep, Parkville, Victoria, Australia); arginine vasopressin (AusPep); benextramine (Sigma); endothelin-1 (AusPep); indomethacin (prepared in 0.1 M Na₂CO₃; Sigma); L-nitro-arginine-methyl-ester (Sigma); methoxamine hydrochloride (Sigma); noradrenaline bitartrate (Sigma); prazosin HCl (Sigma); sodium nitroprusside (Sigma). All drugs used for in vitro assessment
were prepared using MilliQ water unless otherwise specified and stored as stock solutions at 4°C or −20°C until required.

5.2.6 Data analysis

The isometric contractile response and resting passive tension of smooth muscle is dependent on the length of a vessel. Due to the anatomical structure of the renal vasculature, isolating small arteries of the same length (g, µm) was technically difficult. Particular care was taken to record the length of each mounted vessel. In this study, the maximum contractile response to KPSS was expressed in three different ways: active force (mN); active tension (mN/mm); and effective active pressure (mmHg). Active force was calculated as the change in force when activated by a contractile agent (ΔF, mN). As described previously in section 2.2.11, knowledge of the length of the vessel segment allows determination of wall tension (T), the circumferential wall force per unit length (l) in mN/mm. Active tension can thus be calculated as follows:

\[ ΔT = \frac{ΔF}{l} \]

where l is the unit length of artery and taken as 2 x vessel segment length (g) as the artery is in fact two sheets stretched flat on the wires.

The effective active pressure (mN/mm²) is the estimated active transmural pressure that would have been obtained upon isometric activation had the in situ vessel been at the same derived internal circumference and under the same induced wall tension in vivo. The effective active pressure is the tension per internal circumference as determined by the Laplace relationship and calculated as follows:

\[ ΔP = \frac{2πΔT}{L} \]

where L is 0.9L₈₀ or 0.9L₁₁₀. As 1 mN/mm² = 1 kPa and 1 mmHg = 0.1333 kPa, the active pressure units of mN/mm² were converted to mmHg using the conversion rate of 1 mN/mm² = 7.501 mmHg. Data are expressed as the mean estimated effective pressure ± 1 SEM of n experiments. Sigmoidal concentration-response curves were fitted using Prism 5 (GraphPad Software, San Diego, CA, USA) for each individual experiment. Student’s unpaired t-test was used to evaluate the significance of differences between wrap hypertensive and sham normotensive rabbits. Student’s paired t-test was used when analysing the effect of normalisation pressure on various vascular parameters within (rather than between) vessels. In all cases, p ≤ 0.05 was taken as statistically significant.
In vitro characterisation of regional vascular reactivity: effect of hypertension

1. Normalisation protocol and assessment of vessel viability

![Diagram showing normalisation protocol and vessel viability](image)

2A. Cumulative concentration-response curve to vasoconstrictor agents

![Diagram showing vasoconstrictor agents](image)

2B. Cumulative concentration-response curve to vasodilator agents

![Diagram showing vasodilator agents](image)

2C. Role of VOCC in depolarisation-mediated contraction

![Diagram showing VOCC role](image)

3. Effect of different normalisation pressures on maximum contractile capacity

![Diagram showing normalisation pressures](image)

**Figure 5-2. Schema of protocols performed in isolated interlobar and arcuate arteries in the wire myograph.**

Vessels isolated from normotensive and hypertensive rabbits were normalised to transmural pressures of 0.9L80 and 0.9L110 respectively before one of the various protocols were performed (2A, B or C). Protocol 1 was not performed in vessels used to test the effect of different normalisation procedures on the maximum contractile capacity of the vessel (protocol 3).
In vitro characterisation of regional vascular reactivity: effect of hypertension

5.3 Results

5.3.1 General characteristics of sham normotensive and wrap hypertensive rabbits

The mean arterial pressure of hypertensive rabbits (118 ± 3 mmHg, n = 15) was significantly greater than that of normotensive rabbits (79 ± 2 mmHg, n = 23, p < 0.0001). Normotensive and hypertensive rabbits had comparable weights at the time of euthanasia (2.84 ± 0.07 kg vs. 3.01 ± 0.06 kg, respectively, p = 0.076).

5.3.2 General characteristics of isolated arteries

When normalised to their respective physiological pressures, arcuate and interlobar arteries isolated from sham and wrap hypertensive rabbits had comparable internal diameters (D; Table 5-2). Femoral arteries isolated from wrap hypertensive rabbits tended to have a larger internal diameter than those isolated from sham rabbits, but this was not statistically significant (p = 0.11).

5.3.3 Relationship between normalisation pressures and active tension

The relationship between the length (internal circumference)-tension and the passive and active tension of renal and femoral arteries isolated from wrap hypertensive and sham normotensive rabbits was assessed by stretching the blood vessels from an internal circumference equivalent to 0.9L₀.8₀ to 0.9L₁.₁₀.

Generally speaking, stretching arteries from 0.9L₀.₈₀ to 0.9L₀.₁₁₀ increased passive tension in arteries from both sham (+52 – 87%) and wrap arteries (+83 – 87%). However, this was only statistically significant for arcuate (+59%, p = 0.001) and deep femoral arteries (+52%, p = 0.042) from sham rabbits (Table 5-3) and interlobar (+83%, p = 0.033) and deep femoral arteries (+91%, p = 0.009) from wrap rabbits (Table 5-4). Correspondingly, with the exception of the deep femoral arteries from sham rabbits (p = 0.064), all vessels with a raised passive tension also had a greater internal diameter (0.9D).

The maximal active force, active tension and effective active pressure generated by arcuate and interlobar arteries in response to KPSS were comparable between the two levels of stretch regardless of whether they were isolated from sham (Table 5-3) or wrap rabbits (Table 5-4). Active tension developed in deep femoral arteries isolated from sham rabbits was also unaffected by the two levels of stretch. In contrast, deep femoral arteries isolated from wrap rabbits developed 15% greater maximal active tension at 0.9L₀.₁₁₀ compared to 0.9L₀.₈₀ (Table 5-3, p = 0.005), although this enhanced response was absent when maximal active tension was normalised for internal diameter (to generate the estimated effective active pressure, p = 0.126). The active pressure developed under KPSS was 26% greater in the wrap than sham femoral arteries at 0.9L₀.₈₀ and 30% greater at 0.9L₀.₁₁₀. In contrast, the active pressure developed in response to KPSS was similar between sham and wrap interlobar and arcuate arteries, irrespective of the chosen transmural pressure (0.9L₀.₈₀ or 0.9L₀.₁₁₀).
Table 5-2. General characteristics of arteries isolated from sham normotensive and wrap hypertensive rabbits

<table>
<thead>
<tr>
<th>Arteries</th>
<th>Arcuate</th>
<th>Interlobar</th>
<th>Deep femoral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td>Sham</td>
</tr>
<tr>
<td>n</td>
<td>24</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Normalisation pressure (mmHg)</td>
<td>80</td>
<td>110</td>
<td>80</td>
</tr>
<tr>
<td>Average length: g (mm)</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Internal diameter: D (µm)</td>
<td>272 ± 14</td>
<td>281 ± 19</td>
<td>615 ± 42</td>
</tr>
<tr>
<td>Internal diameter: 0.9D (µm)</td>
<td>245 ± 13</td>
<td>253 ± 17</td>
<td>554 ± 38</td>
</tr>
<tr>
<td>Passive tension (mN/mm)</td>
<td>0.43 ± 0.04</td>
<td>0.59 ± 0.03**</td>
<td>0.78 ± 0.07</td>
</tr>
<tr>
<td>Active force (mN)</td>
<td>7.02 ± 0.70</td>
<td>6.50 ± 0.76</td>
<td>20.28 ± 1.81</td>
</tr>
<tr>
<td>Active tension (mN/mm)</td>
<td>2.15 ± 0.18</td>
<td>2.27 ± 0.21</td>
<td>5.54 ± 0.42</td>
</tr>
<tr>
<td>Effective active pressure (mmHg)</td>
<td>128 ± 7</td>
<td>130 ± 8</td>
<td>153 ± 9</td>
</tr>
</tbody>
</table>

The internal diameter (D) was estimated at a transmural pressure of 80 mmHg ($D_{80}$) for sham normotensive arteries or 110 mmHg ($D_{110}$) for wrap hypertensive arteries, representing their respective physiological pressures. Passive tension is the isometric tension recorded at 0.9$L_{80}$ and 0.9$L_{110}$ for arteries isolated from sham and wrap rabbits, respectively. The maximum contractile response to KPSS was expressed in three different ways: active force (mN); active tension (mN/mm); and effective active pressure (mmHg), refer to Methods for details on calculations. Data are expressed as mean ± S.E.M. $n$ = number of rabbits, with 2-4 vessels isolated from each region from a single rabbit. *$p < 0.05$, **$p < 0.01$ compared to corresponding artery in sham rabbit, Student’s unpaired t-test.
Table 5-3. The effect of normalisation pressure on resting passive tension and maximum contractile capacity of arteries isolated from sham rabbits

<table>
<thead>
<tr>
<th></th>
<th>Arcuate</th>
<th>Interlobar</th>
<th>Deep femoral</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><strong>Average length (mm)</strong></td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td><strong>Normalisation pressure (mmHg)</strong></td>
<td>80</td>
<td>110</td>
<td>80</td>
</tr>
<tr>
<td>Internal circumference: L (µm)</td>
<td>894 ± 108</td>
<td>984 ± 112***</td>
<td>1533 ± 171</td>
</tr>
<tr>
<td>Internal diameter: D (µm)</td>
<td>285 ± 34</td>
<td>313 ± 36***</td>
<td>488 ± 55</td>
</tr>
<tr>
<td>Internal diameter at 0.9L: 0.9D (µm)</td>
<td>256 ± 31</td>
<td>282 ± 32***</td>
<td>439 ± 49</td>
</tr>
<tr>
<td>Passive tension (mN/mm)</td>
<td>0.44 ± 0.08</td>
<td>0.70 ± 0.09***</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>Active force (mN)</td>
<td>8.38 ± 2.41</td>
<td>8.68 ± 2.47</td>
<td>19.35 ± 2.20</td>
</tr>
<tr>
<td>Active tension (mN/mm)</td>
<td>2.67 ± 0.51</td>
<td>2.74 ± 0.51</td>
<td>5.44 ± 0.62</td>
</tr>
<tr>
<td>Effective active pressure (mmHg)</td>
<td>150 ± 12</td>
<td>141 ± 11</td>
<td>190 ± 22</td>
</tr>
</tbody>
</table>

The internal circumference length (L) and internal diameter (D) was estimated in vessels distended to a transmural pressure of 80 mmHg (L₈₀ and D₈₀). Vessels were partially relaxed to 0.9L₈₀ to determine passive resting tension and the maximum contractile response to KPSS. The latter was expressed in three different ways: active force (mN); active tension (mN/mm); and effective active pressure (mmHg), refer to Methods for details on calculations. The entire process was then repeated at a normalisation pressure of 110 mmHg. Data are expressed as mean ± S.E.M. n = number of vessels from separate rabbits. *p < 0.05, ***p < 0.001, compared to vessels stretched to L₈₀ or 0.9L₈₀. Student’s paired t-test.
Table 5-4. The effect of normalisation pressure on resting passive tension and maximum contractile capacity of arteries isolated from wrap rabbits

<table>
<thead>
<tr>
<th></th>
<th>Arcuate</th>
<th>Interlobar</th>
<th>Deep femoral</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Average length (mm)</td>
<td>1.1 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Normalisation pressure (mmHg)</td>
<td>80</td>
<td>110</td>
<td>80</td>
</tr>
<tr>
<td>Internal circumference: L (µm)</td>
<td>938 ± 133</td>
<td>1035 ± 159</td>
<td>1419 ± 69</td>
</tr>
<tr>
<td>Internal diameter: D (µm)</td>
<td>299 ± 42</td>
<td>330 ± 51</td>
<td>452 ± 22</td>
</tr>
<tr>
<td>Internal diameter at 0.9L: 0.9D (µm)</td>
<td>269 ± 38</td>
<td>297 ± 46</td>
<td>406 ± 20</td>
</tr>
<tr>
<td>Passive tension (mN/mm)</td>
<td>0.39 ± 0.03</td>
<td>0.73 ± 0.11</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>Active force (mN)</td>
<td>5.95 ± 0.08</td>
<td>5.85 ± 0.07</td>
<td>18.31 ± 1.38</td>
</tr>
<tr>
<td>Active tension (mN/mm)</td>
<td>2.74 ± 0.56</td>
<td>2.68 ± 0.47</td>
<td>5.29 ± 0.43</td>
</tr>
<tr>
<td>Effective active pressure (mmHg)</td>
<td>150 ± 9</td>
<td>135 ± 6</td>
<td>196 ± 17</td>
</tr>
</tbody>
</table>

The internal circumference length (L) and internal diameter (D) was estimated in vessels distended to a transmural pressure of 80 mmHg (L_{80} and D_{80}). Vessels were partially relaxed to 0.9L_{80} to determine passive resting tension and the maximum contractile response to KPSS. The latter was expressed in three different ways: active force (mN); active tension (mN/mm); and effective active pressure (mmHg), refer to Methods for details on calculations. The entire process was then repeated at a normalisation pressure of 110 mmHg. Data are expressed as mean ± S.E.M. n = number of vessels from separate rabbits. *p < 0.05, **p < 0.01, compared to vessels stretched to L_{80} or 0.9L_{80}, Student’s paired t-test.
5.3.4 Receptor-mediated vasoconstriction

Figure 5-3A shows the noradrenaline concentration-response curves of arteries isolated from sham and wrap hypertensive rabbits. In normotensive sham rabbits, the concentration-response curves to noradrenaline were similar between interlobar and arcuate arteries whilst femoral arteries were 11- to 14-fold less sensitive (p < 0.001). Arcuate, interlobar and femoral arteries all contracted to similar maximum effective active pressures (Emax, 162 ± 19, 180 ± 11 and 152 ± 19 mmHg, respectively). Interlobar and deep femoral arteries isolated from wrap hypertensive rabbits were 6-fold (p = 0.008) and 2-fold (p = 0.028) more sensitive than that of normotensive rabbits. In contrast, noradrenaline sensitivity in arcuate arteries was unchanged between sham and wrap rabbits. The maximum effective active pressure elicited by noradrenaline was unchanged with hypertension. Femoral arteries isolated from wrap rabbits tended to reach greater maximal effective active pressures (Emax, 208 ± 26 mmHg) than those from sham rabbits (Emax, 152 ± 19 mmHg) in response to noradrenaline, but this was not statistically significant (p = 0.106; Table 5-5).

In vessels isolated from sham rabbits, methoxamine concentration-response curves showed a similar pattern of regional sensitivity as that with noradrenaline (Figure 5-3B and Table 5-5). Femoral arteries were 8- to 9-fold less sensitive to methoxamine (pEC50, 5.13 ± 0.12, p < 0.0001) than arcuate (pEC50, 6.07 ± 0.09) and interlobar arteries (pEC50, 6.04 ± 0.09). Methoxamine was less potent than noradrenaline in interlobar (4-fold) and distal arcuate arteries (3-fold), but had similar potency in the femoral artery. As observed with noradrenaline, interlobar and deep femoral arteries isolated from wrap rabbits had greater sensitivity to methoxamine than arteries isolated from normotensive rabbits (4-fold, p = 0.001 and 4-fold, p = 0.009, respectively). Methoxamine also elicited a greater contraction in femoral arteries of hypertensive compared to normotensive rabbits (Emax, 216 ± 29 vs. 129 ± 21 mmHg, p = 0.034).

In contrast endothelin-1 sensitivity was comparable between the assessed arteries isolated from the sham rabbit. Furthermore, endothelin-1 sensitivity was not significantly altered in vessels isolated from hypertensive rabbits compared to normotensive rabbits. There was however, an increase in the maximum contractile response, though this was limited to interlobar arteries (Emax, 205 ± 21 vs. 153 ± 11 mmHg, wrap vs. sham, respectively; p = 0.031; Figure 5-4and Table 5-5).

The contractile response to the potent vasopressor agent angiotensin II was either non-existent or poor in arcuate arteries of normotensive rabbits even when any potential functional antagonism had been abolished with indomethacin and L-NAME pre-treatment (Figure 5-5). As in normotensive arcuate arteries, a response to angiotensin II was not always observed in arcuate arteries from wrap rabbits. In instances when a response was observed, the maximum elicited contraction was greater than that observed in normotensive arcuate arteries (Emax, 35 ± 24, vs. 3 ± 2 mmHg, respectively). In contrast, angiotensin II elicited profound vasoconstriction in interlobar (Emax, 95 ± 16 mmHg) and femoral arteries isolated from the sham rabbit (Emax, 225 ± 13 mmHg), particularly the latter. Hypertension did not significantly affect the maximum effective active pressure elicited by angiotensin II in interlobar arteries. Although there was a tendency for femoral arteries from wrap rabbits to contract to a greater
Emax than femoral arteries from sham rabbits, this was not statistically significant. Enhanced sensitivity to angiotensin II was only observed in interlobar arteries of hypertensive rabbits (3-fold, $p < 0.003$). The $pEC_{50}$ and Emax of each vasoconstrictor agonist in all preparations are summarised in Table 5-5.
Figure 5-3. Concentration-dependent increases in $\Delta P$ (mmHg) in interlobar, distal arcuate and 3rd order femoral arteries isolated from sham normotensive and wrap hypertensive rabbits in response to $\alpha$-adrenoceptor agonist drugs.

Maximal active pressure ($\Delta P$, mmHg) is the estimated pressure that the artery would have reached in vivo. Interlobar and femoral arteries from hypertensive rabbits were more sensitive to noradrenaline (A) and methoxamine (B) than those isolated from sham rabbits. Femoral arteries isolated from hypertensive rabbits contracted to a greater maximal response to methoxamine but not noradrenaline (not significant, $p = 0.108$). Vessels isolated from wrap rabbits are indicated by open shapes and dotted lines. Vessels isolated from sham rabbits are indicated by filled shapes and solid lines. *$p < 0.05$, **$p < 0.01$, $p_{EC50}$ compared to corresponding artery in sham rabbit group, Student’s unpaired t-test where $p_{EC50}$ is the negative log of the concentration of constrictor agent required to elicit a half-maximal response. †$p < 0.05$, $E_{max}$ compared to corresponding artery in sham group where $E_{max}$ is the maximum contractile response of the artery to constrictor agent. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the $EC_{50} ± 1$ SEM. $n =$ number of rabbits used in each experiment.
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Figure 5-4. Endothelin-1 responses were only enhanced with hypertension in renal interlobar arteries. Sensitivity to endothelin-1 was not altered in hypertension although the maximal contractile response was elevated in interlobar arteries. Maximal active pressure ($\Delta P$, mmHg) is the estimated pressure that the artery would have reached in vivo. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the EC$_{50}$ ± 1 SEM. $n$ = number of rabbits used in each experiment.

Figure 5-5. Angiotensin II-mediated increases in effective active pressure ($\Delta P$, mmHg) in interlobar, distal arcuate and 3rd order femoral arteries isolated from sham normotensive and wrap hypertensive rabbits. Angiotensin II responses were poor in distal arcuate vessels. Interlobar vessels from wrap rabbits had greater sensitivity to angiotensin II. All arteries were pre-treated with indomethacin (3 μM) and L-NAME (100 μM) for 30 min. Only arcuate vessels were pre-contracted to ~10% of the maximum response induced by KPSS using arginine vasopressin (Tone). Maximal active pressure ($\Delta P$, mmHg) is the estimated pressure that the artery would have reached in vivo. **p < 0.01, pEC$_{50}$ compared to corresponding artery in sham rabbit group, Student’s unpaired t-test, where pEC$_{50}$ is the negative log of the concentration of constrictor agent required to elicit a half-maximal response. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the EC$_{50}$ ± 1 SEM. $n$ = number of rabbits used in each experiment.
Table 5.5. Summary of vasoconstrictor sensitivity in the renal vasculature and femoral artery of wrap hypertensive and sham normotensive rabbits.

<table>
<thead>
<tr>
<th></th>
<th>Renal</th>
<th></th>
<th>Hindquarter deep femoral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>arcuate</td>
<td>distal interlobar</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>Wrap</td>
<td>Fold shift</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>( pEC_{50} )</td>
<td>6.66 ± 0.15</td>
<td>6.60 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Emax</td>
<td>162 ± 19</td>
<td>166 ± 25</td>
<td>-</td>
</tr>
<tr>
<td>Methoxamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>( pEC_{50} )</td>
<td>6.07 ± 0.09</td>
<td>6.02 ± 0.21</td>
<td>-</td>
</tr>
<tr>
<td>Emax</td>
<td>158 ± 22</td>
<td>187 ± 13</td>
<td>-</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>( pEC_{50} )</td>
<td>8.43 ± 0.22</td>
<td>8.55 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Emax</td>
<td>155 ± 12</td>
<td>150 ± 15</td>
<td>-</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>( pEC_{50} )</td>
<td>undetermined</td>
<td>8.31 ± 0.18</td>
<td>-</td>
</tr>
<tr>
<td>Emax</td>
<td>3 ± 2</td>
<td>35 ± 24</td>
<td>-</td>
</tr>
</tbody>
</table>

Arteries have been set to their physiological pressures (normotensive, 80 mm Hg and hypertensive, 110 mmHg). Data are expressed as mean ± SEM; fold shift, shift in \( pEC_{50} \) value from respective vehicle control where \( pEC_{50} \) is the negative log of the concentration of constrictor agent required to elicit a half-maximal response. \( n \) refers to the number of rabbits used in each experiment. *\( p < 0.05 \), **\( p < 0.01 \) vs corresponding artery in sham rabbit group unless accompanied by a number in which case, ^1 vs sham arcuate artery; ^2 vs sham interlobar artery, Student’s unpaired t-test.
5.3.5 Receptor-induced vasodilatation

For experiments assessing acetylcholine-mediated relaxation, methoxamine-mediated pre-constriction levels were similar across all vessels ($p > 0.05$, one-way ANOVA with Bonferroni’s post-test (Figure 5-6A). The endothelial-dependent vasodilator agent acetylcholine caused concentration-dependent relaxation in all vessel types assessed. Acetylcholine sensitivity was not significantly altered by hypertension in renal or femoral arteries and maximum relaxation was comparable between all groups.

Sodium nitroprusside also caused concentration-dependent relaxation in all vessel types (Figure 5-6B). In contrast to acetylcholine however, sodium nitroprusside was unable to completely reverse pre-constriction in distal arcuate arteries of either hypertensive or normotensive rabbits despite achieving almost complete relaxation in interlobar and femoral arteries. Residual tone ($\Delta P$) in the presence of the maximum concentration of sodium nitroprusside used in this study ($10^{-4.5}$ M), was $23 \pm 11$ mmHg and $51 \pm 29$ mmHg in the arcuate arteries of normotensive and hypertensive rabbits, respectively.

Adenosine caused little to no relaxation in renal arteries and in some instances elicited a small contractile response (Figure 5-7). Hypertension appeared to have no bearing on the effect of adenosine in femoral arteries. In contrast, adenosine was able to completely relax pre-constricted femoral arteries in a concentration-dependent manner. The sensitivity to adenosine was comparable between arteries isolated from hypertensive rabbits to those isolated from normotensive rabbits with $pEC_{50}$s of $6.36 \pm 0.10$ and $6.57 \pm 0.26$, respectively.
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Figure 5-6. Arteries isolated from hypertensive rabbits had similar relaxation capabilities as those isolated from normotensive rabbits.

A. Vasorelaxation responses to the endothelial-dependent vasodilator agonist acetylcholine; and B. endothelial-independent vasodilator agent sodium nitroprusside in interlobar, distal arcuate and deep femoral arteries isolated from normotensive and hypertensive rabbits. Vessels were pre-constricted with the α₁-selective adrenoceptor agonist methoxamine to 70-80% KPSS maximum response. Maximal active pressure (ΔP, mmHg) is the estimated pressure that the artery would have reached in vivo. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the EC_{50} ± 1 SEM. n = number of rabbits used in each experiment.
Figure 5-7. Adenosine-mediated vasodilatation was limited to isolated femoral arteries and was unimpaired with hypertension.

Vessels were pre-constricted with the α1-selective adrenoceptor agonist methoxamine to 70-80% KPSS maximum response. Maximal active pressure (ΔP, mmHg) is the estimated pressure that the artery would have reached in vivo. Vertical bars are ± 1 SEM; where no bar is visible the SEM is within the symbol. Horizontal bars represent the EC50 ± 1 SEM. n = number of rabbits used in each experiment.
5.3.6 Membrane depolarisation-induced vasoconstriction

Equimolar solutions containing 10, 15, 20, 40 and 62 mM K\(^+\) elicited concentration-dependent membrane depolarisation-induced contractions. Greater contractions were elicited with 20 (\(p = 0.022\)) and 40 mM K\(^+\) (\(p = 0.031\)) in wrap interlobar arteries than in sham interlobar arteries.

**Contribution of T-type calcium channels**

In all assessed arteries, felodipine (1 nM) did not alter membrane depolarisation-mediated constriction (Figure 5-9 – 5.11). NNC 55-0396 (100 nM) caused partial inhibition of the contraction elicited by 40 (change in \(\Delta P\), -43 mmHg; \(p < 0.05\); Figure 5-10) and 62 mM K\(^+\) (change in \(\Delta P\), -44 mmHg; \(p < 0.05\);Figure 5-11) in interlobar, but not arcuate, arteries of sham rabbits. The T-type selective calcium channel inhibitor caused similar inhibition of contractile responses in interlobar arteries of wrap rabbits. Mibefradil (100 nM) caused partial inhibition of the contractile response elicited by 62 mM K\(^+\) in sham interlobar arteries only (change in \(\Delta P\), -43 mmHg; \(p < 0.05\)). None of the calcium channel blockers inhibited the contractile response to 20 mM K\(^+\) (Figure 5-9) and the magnitude of the contractile response to 10 mM K\(^+\) was too small to reasonably assess the effect of an inhibitor (Figure 5-8).

![Figure 5-8. Membrane depolarisation-induced contraction in interlobar, arcuate and femoral arteries isolated from sham and wrap rabbits to segmental increases in potassium depolarising solution](image)

Interlobar arteries isolated from sham rabbits displayed augmented responses to 20 and 40 mM potassium depolarising solution. *\(p < 0.05\), compared to corresponding artery in sham rabbit group, Student’s unpaired t-test. Within each group, black lines represent the mean ± 1 SEM. Maximal active pressure (\(\Delta P\), mmHg) is the estimated pressure that the artery would have reached *in vivo*. Symbols represent individual artery responses. \(n =\) number of rabbits used in each experiment.
Figure 5-9. The effect of calcium channel blockers on the contractile response to potassium (20 mM) in renal arteries isolated from normotensive and hypertensive rabbits.

Arteries were depolarised and contracted by sequential incubation with K+ 10, 20, 40 and 62 mM in the absence and presence of L- and T-type calcium channel inhibitors: only the response to 20 mM K+ is shown here. Each treatment was equilibrated for 30 min (section 5.2.4.4). Within each group, black lines represent the mean ± 1 SEM and each symbol represents the response of an artery from a separate rabbit. Maximal active pressure (ΔP, mmHg) is the estimated pressure that the artery would have reached in vivo.
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Figure 5.10. The effect of calcium channel blockers on the contractile response to potassium (40 mM) in renal arteries isolated from normotensive and hypertensive rabbits.

Arteries were depolarised and contracted by sequential incubation with K⁺ 10, 20, 40 and 62 mM in the absence and presence of L- and T-type calcium channel inhibitors: only the response to 40 mM K⁺ is shown here. Each treatment was equilibrated for 30 min (section 5.2.4.4). *p < 0.05 vs. control response, one-way ANOVA with Dunnett post-test for multiple comparisons. Within each group, black lines represent the mean ± 1 SEM and each symbol represents the response of an artery from a separate rabbit. Maximal active pressure (ΔP, mmHg) is the estimated pressure that the artery would have reached in vivo.
Figure 5.11. The effect of calcium channel blockers on the contractile response to potassium (62 mM) in renal arteries isolated from normotensive and hypertensive rabbits.

Arteries were depolarised and contracted by sequential incubation with K+ 10, 20, 40 and 62 mM in the absence and presence of L- and T-type calcium channel inhibitors: only the response to 62 mM K+ is shown here. Each treatment was equilibrated for 30 min (see section 5.2.4.4). *p < 0.05 vs. control, one-way ANOVA with Dunnett post-test for multiple comparisons. Within each group, black lines represent the mean ± 1 SEM and each symbol represents the response of an artery from a separate rabbit. Maximal active pressure (ΔP, mmHg) is the estimated pressure that the artery would have reached in vivo.
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5.4 Discussion

The enhanced vascular resistance observed in the renal and hindquarter vasculature of renovascular hypertensive rabbits (Chapter 4) implied a greater than normal narrowing of resistance vessels in these vascular beds. Given that resistance is inversely proportional to the fourth power of the internal radius \( R \propto 1/r^4 \), even minor decreases in the internal radius of the vessel lumen can have profound effects on vascular resistance. This study found that vessels isolated from hypertensive rabbits had an enhanced vascular reactivity compared to those isolated from normotensive rabbits. The enhanced vascular reactivity appeared to region-dependent and was particularly prominent in the femoral resistance artery and renal interlobar artery. Enhanced narrowing of resistance vessels could be the result of structural alterations in the vasculature or enhanced vascular smooth muscle activity. The present investigation has found a role for both in the elevation of renal and hindquarter vascular resistance in the renovascular hypertensive rabbit.

The enhanced vascular smooth muscle activity in hypertensive vessels appeared to be region-dependent and agonist-specific. Femoral resistance arteries and interlobar arteries from hypertensive rabbits were significantly more sensitive to noradrenaline and methoxamine than their normotensive counterparts. Interlobar but not femoral arteries from hypertensive rabbits displayed enhanced sensitivity to angiotensin II while arcuate arteries were generally unaffected by hypertension. As discussed below, there are a number of functional changes in the vasculature that could contribute to this enhanced sensitivity.

5.4.1 \( \alpha \)-Adrenoceptor-mediated vasoconstriction

Femoral resistance arteries and distal interlobar but not arcuate resistance arteries isolated from hypertensive rabbits had enhanced sensitivity to noradrenaline and methoxamine. Enhanced noradrenaline sensitivity has also been reported in the isolated perfused kidney of the SHR (Collis & Vanhoutte, 1977; Berecek et al., 1980). These studies also reported an increase in the maximum response which, although not observed in the isolated renal interlobar and arcuate artery, was observed in the femoral artery. Other studies however, have reported an enhanced maximal contraction to noradrenaline and serotonin in the absence of any changes in sensitivity in resistance vessels isolated from hypertensive patients (Angus et al., 1992b) and SHR (Mulvany et al., 1980).

The enhanced vascular responsiveness to noradrenaline could be due to a number of reasons. Decreased neuronal uptake, decreased \( \beta_2 \)-adrenoceptor-mediated relaxation, increased membrane potential, greater \( \alpha \)-adrenoceptor expression, increased \( \alpha \)-adrenergic receptor affinity, increased efficacy and structural changes in the vasculature can all theoretically increase noradrenaline sensitivity. The effect of neuronal uptake blockade was not assessed in this study. Other studies however have shown that neuronal uptake is increased rather than decreased in spontaneously hypertensive rats. Cocaine-mediated inhibition of neuronal reuptake (Mulvany et al., 1980) or 6-hydroxydopamine-mediated destruction of noradrenergic neurons (Whall et al., 1980) enhanced sensitivity to noradrenaline in mesenteric resistance arteries of spontaneously hypertensive rats.
Neuronal uptake is reportedly greater in vessels of spontaneously hypertensive rats than Wistar-Kyoto controls (Whall et al., 1980). However, in subcutaneous resistance arteries isolated from hypertensive patients, treatment with the neuronal uptake blocker desipramine did not equalise noradrenaline sensitivity to that observed in vessels isolated from normotensive patients (Angus et al., 1992b). Of note, neither an enhanced neuronal uptake nor a smaller β2-adrenoceptor-mediated relaxation component could account for the enhanced sensitivity observed with the selective α1-adrenoceptor agonist methoxamine. In regards to receptor expression, greater expression of α1-adrenoceptors has not been reported in the renal vasculature of hypertensive preparations. An increased number of α2-adrenoceptors but not α1-adrenoceptors was reported in the kidney of stroke-prone spontaneously hypertensive rats (Graham et al., 1982). Alternatively, the intracellular signalling process following activation of α1-adrenoceptors may be more enhanced or more efficient. As methoxamine also elicits vasoconstriction through activation of the same receptor, it is conceivable that any changes in α1-adrenoceptor mediated intracellular signalling could explain an enhanced sensitivity to both noradrenaline and methoxamine. For example, agonist-mediated calcium permeability across the plasma membrane is increased in spontaneously hypertensive rats (Mulvany & Nyborg, 1980) and could explain the enhanced sensitivity in vessels of hypertensive rabbits. It is possible that the potassium (potential)-dependent calcium permeability of vascular smooth muscle is also enhanced in hypertension in interlobar arteries. Interlobar arteries isolated from the hypertensive rabbit contracted to a greater effective active pressure than its normotensive counterparts in response to 20 and 40 mM K+.

5.4.2 Endothelin-1-mediated vasoconstriction

It has been proposed that aberrations in the endothelin system contribute to the initiation and progression of hypertension, particularly in salt-dependent models of hypertension (Schiffrin, 2005). The endothelin receptor antagonist bosentan lowers blood pressure in patients with essential hypertension (Krum et al., 1998). Whether or not a pathogenic role exists for endothelin-1 in secondary forms of hypertension depends on the model of experimental hypertension used. Endothelin-1 has been reported to promote vascular hypertrophy. Chronic administration of an endothelin-1 receptor antagonist lowers blood pressure in deoxycorticosterone-acetate salt rats (Li et al., 1994), but not spontaneously hypertensive rats (Li & Schiffrin, 1995). Acute infusion of endothelin-specific antibodies caused a small decrease in arterial pressure of spontaneously hypertensive rats (Ohno et al., 1992). The pathogenic role of endothelin-1 in renovascular hypertension is unclear. Administration of the endothelin-1 receptor antagonist bosentan had no significant effect on the raised blood pressure or vascular hypertrophic remodelling in renovascular rats (Li et al., 1996), but lowered blood pressure of cellophane-wrapped renal hypertensive dogs (Donckier et al., 1995). The pathogenic role of endothelin-1 in renovascular hypertension does not appear to be correlated with circulating plasma levels (Donckier et al., 1995). This may suggest that the vascular response to endothelin-1, rather than the amount of endothelin-1, is augmented and contributing to the development and/or maintenance of high blood pressure in renal hypertension. Using the same model of renal hypertension as Donckier et al. (1995) but in the rabbit, this study demonstrated that in isolated femoral and renal resistance arteries, the potency and maximum
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contractile response to endothelin-1 was similar between hypertensive and normotensive rabbits. There was also no difference in endothelin-1 sensitivity between interlobar pulmonary arteries isolated from normotensive and hypertensive rabbits (Richard Hughes, MPhil, unpublished data). The data suggest that endothelin receptor signalling is unchanged in small arteries of the renal and hindquarter vasculature and larger arteries of the pulmonary vasculature.

5.4.3 Angiotensin II-mediated vascular responses

Angiotensin II has been implicated as an important mediator in the pathophysiology of renovascular hypertension. The peptide increases systemic pressure via two mechanisms: a fast pressor effect via systemic vasoconstriction and a slow pressor effect that encompasses numerous mechanisms (Brown et al., 1981; Lever, 1993). In experimental models of renovascular hypertension induced by unilateral renal artery stenosis, the resultant decrease in renal perfusion pressure prompts activation of the renin-angiotensin system, a compensatory process that elevates mean arterial pressure to restore renal perfusion pressure. This process may be auto-potentiating (Lever, 1993). Angiotensin II is now known to stimulate reactive oxygen species production and may contribute to chronic renovascular hypertension, not only by facilitating oxidative-stress mediated renal ischaemia and renal damage (Lerman et al., 2001), but by augmenting angiotensin II-mediated vasoconstriction (Romero & Reckelhoff, 1999). The present study however, employed a model of renovascular hypertension called Page hypertension where the elevation in arterial pressure is primarily attributed to interstitial nephritis (see section 1.11.2). It is unclear whether activation of the renin-angiotensin system contributes to the pathogenesis of the disease and if so, to what extent (Vanegas et al., 2005). This study assessed whether vascular smooth muscle had enhanced sensitivity to angiotensin II. Enhanced angiotensin II-mediated vasoconstriction was observed, but only in renal interlobar arteries. Arcuate arteries, which were unresponsive to angiotensin II if isolated from normotensive rabbits, occasionally, contracted in response to angiotensin II if isolated from hypertensive rabbits. Distal interlobar arteries from hypertensive rabbits were almost 3-fold more sensitive to angiotensin II than those isolated from normotensive rabbits. In contrast, contractile responses to angiotensin II in the femoral resistance arteries were comparable between hypertensive and normotensive rabbits. The enhanced response to angiotensin II in the renal vasculature is unlikely to be structurally-based. The maximal response elicited by angiotensin II in interlobar renal arteries was similar between normotensive and hypertensive vessels. This may point towards enhanced vascular smooth muscle sensitivity to angiotensin II, the mechanism for which is unknown.

5.4.4 Smooth muscle relaxation and endothelial function

In addition to enhanced smooth muscle activity to constrictor agents, another functional change in the hypertensive vasculature that could contribute to its enhanced vascular reactivity is endothelial dysfunction. Aberrant endothelial-dependent vasodilatation to acetylcholine has been reported in resistance arteries of stroke-prone spontaneously hypertensive rats (Tesfamariam & Halpern, 1988), two kidney-one clip renovascular hypertensive rats (Dohi et al., 1991), the forearm vasculature of patients with essential hypertension (Linder et al., 1990; Panza et al., 1990; Iiyama et al., 1996) and
renovascular hypertension (Higashi et al., 2002). The role of endothelial dysfunction in the cellophane-wrap model of renovascular hypertension has yet to be assessed in vitro. This study found no impairment of endothelium-dependent (acetylcholine) or endothelium-independent (sodium nitroprusside) vasodilatation in any of the arteries assessed. The relaxation response to the endogenous nucleoside adenosine was also comparable between hypertensive and normotensive rabbits across all arteries assessed. Similarly, the functional response to acetylcholine has been shown to be unaffected in isolated subcutaneous resistance arteries of hypertensive patients (Angus et al., 1992b; Thybo et al., 1996) and mesenteric arteries of spontaneously hypertensive rats (Angus et al., 1992a). Preservation of endothelial function suggests that any vascular amplification in these arteries is likely to be attributable to alterations in vascular smooth muscle activity and not the endothelium. This study did not, however, assess the role of endothelial derived hyperpolarising factor, an important vasodilator in resistance vessels (Garland et al., 1995; Brandes et al., 2000), or the role of endothelial-derived constrictor prostanoids. Constrictor prostanoids may be released in response to acetylcholine and this has been reported in the main renal artery of spontaneously hypertensive rats (Lüscher et al., 1988). However, in similar experimental set-ups, enhanced release of constrictor prostanoids typically manifests as a biphasic response. An initial rapid relaxation is followed by a secondary contraction (Lüscher et al., 1990), a phenomenon absent from this study.

5.4.5 Structural considerations

In hypertension, small resistance arteries are thought to undergo structural remodelling, leading to a narrower lumen and a greater media thickness to lumen ratio. The decreased lumen radius may result from inward media growth (hypertrophic) (Furuyama, 1962; Mulvany et al., 1978; Mulvany et al., 1980; Mulvany et al., 1985; Schmid-Schönbein et al., 1986; Korsgaard & Mulvany, 1988; Deng & Schiffrin, 1991; Li et al., 1996; d’Uscio et al., 1997) or the rearrangement of existing wall material as to encroach on the lumen (eutrophic remodelling) (Korsgaard et al., 1993; Schiffrin et al., 1993). The result of the decreased lumen radius means that at any given level of smooth muscle activation, the resistance change is amplified. This architectural change in the vascular geometry is able to confer an enhanced vascular responsiveness without necessitating a change in agonist sensitivity (refer to Chapter 1 for a detailed discussion).

The present investigation found that the enhanced vascular reactivity observed in hypertensive vessels was not accompanied by any significant differences in the lumen diameter from normotensive vessels. Similar lumen diameters between subcutaneous resistance vessels isolated from normotensive and hypertensive patients have previously been reported despite hypertensive vessels displaying eutrophic remodelling (Schiffrin et al., 1992; Falloon & Heagerty, 1994). It is possible that a smaller lumen in hypertensive vessels was masked because they were normalised to an internal circumference of 0.9L_{110} rather than 0.9L_{80}. That is, hypertensive vessels were set to an internal circumference that they would have had in vivo when maximally dilated and under a transmural pressure of 110 mmHg rather than 80 mmHg. Normalising the vessel to a higher transmural pressure (L_{110} from L_{80}) increased the internal diameter of interlobar and femoral, but not arcuate arteries isolated from hypertensive rabbits. Using the myograph to measure vascular
structure usually requires that both normotensive vessels and hypertensive vessels are stretched to an equivalent level (i.e. internal diameter where the vessel is subjected to a transmural pressure of 100 mmHg and maximally dilated, \(0.9L_{100}\)), though others have used \(0.8L_{100}\) (Mulvany et al., 1978). While this would have been ideal, this study found that when hypertensive and normotensive vessels were normalised to the same transmural pressure (either \(0.9L_{110}\) or \(0.9L_{80}\)), their internal diameters were not appreciably different. Although this suggests that vessels isolated from normotensive and hypertensive rabbits had similar lumen diameters when normalised, we did not directly measure the internal radius or wall thickness. Direct measurement of the media thickness and lumen diameter can be performed with a light microscope using a water immersion lens at x400 total magnification (Angus & Wright, 2000).

As smooth muscle content was not directly measured, we can only speculate on whether smooth muscle volume was increased in vessels isolated from hypertensive rabbits using functional data. The maximal effective active pressure induced by KPSS was significantly greater in wrap femoral arteries than sham femoral arteries (27%), regardless of the transmural pressure at which the vessels were normalised. The hypertensive to normotensive (H:N) ratios in vessels normalised to a transmural pressure of 80 and 110 mmHg were 1.27 (27% greater, \(p = 0.087\)) and 1.30, respectively (30% greater; \(p = 0.066\)). The responses to noradrenaline, methoxamine, endothelin-1 and angiotensin II all were on average greater in the femoral arteries of hypertensive rabbits although only the response to methoxamine demonstrated a statistically significant difference. Nonetheless, the Emax H:N ratios were calculated as 1.37, 1.66, 1.24 and 1.23 for noradrenaline, methoxamine, endothelin-1 and angiotensin II, respectively while the average Emax ratio approached 1.37. This may point to a greater smooth muscle volume in the femoral arteries of hypertensive rabbits. In contrast, the maximum contraction elicited by vasoconstrictor agents in renal arteries was generally comparable between hypertensive and normotensive rabbits. In arcuate resistance arteries, the average Emax H:N ratio was only 1.06. Indeed, vascular responses in the arcuate arteries were almost completely unaltered by hypertension. The exception was that wrap arcuate arteries occasionally had a contractile response to angiotensin II whereas sham arcuate arteries were completely unresponsive on all occasions. In contrast to both femoral and arcuate resistance arteries, the larger distal interlobar arteries of hypertensive rabbits elicited a greater contractile response to endothelin-1 only. The Emax induced by KPSS was similar between distal interlobar arteries of the hypertensive and normotensive rabbit suggesting that the enhanced maximal response observed to endothelin-1 is likely to be an agonist-specific response.

5.4.6 Regional-specific differences in vascular reactivity

This study also highlights the heterogeneity between different vascular beds and even between arterial segments of the same vascular bed. In conjunction with chapter 2, it also highlights species-specific differences and emphasises the caution that needs to be adopted when extrapolating results between species and vascular beds. These differences are also agonist-specific, with major discrepancies observed with \(\alpha\)-adrenoceptor agonists and the peptide angiotensin II.
The non-selective adrenoceptor agonist noradrenaline and the selective $\alpha_1$-adrenoceptor agonist methoxamine elicited similar responses in distal interlobar and arcuate arteries. Differences in sensitivity have previously been reported between other intrarenal arteries. Interlobar arteries isolated from New Zealand White rabbits were less sensitive to noradrenaline and the selective $\alpha_1$-adrenoceptor agonist phenylephrine than larger preceding 1st order renal artery branches. In rats however, renal interlobar arteries were more sensitive to noradrenaline and methoxamine than larger extrarenal arteries (see Chapter 2). Thus, although differences in sensitivity and maximal efficacy were not observed between interlobar and arcuate arteries, there are clearly differences between other renal vascular segments. These differences also appear to be species-specific. The potency of $\alpha$-adrenoceptor-mediated vasoconstrictor responses depended on the vascular bed. Renal arteries were significantly more sensitive to both noradrenaline (14-fold) and methoxamine (11-fold), than femoral resistance arteries isolated from the hindquarter vasculature.

Profound differences in maximal efficacy and sensitivity were reported for angiotensin II. In the renal vasculature, the peptide caused moderate constriction in distal interlobar but not arcuate resistance arteries. The difference is particularly remarkable given that distal interlobar arteries directly precede arcuate arteries; the reason for this is unknown. The distal interlobar artery of the rabbit is the first small renal artery that has shown a substantial response to angiotensin II. It is commonly reported that angiotensin II causes very little, if any, contractile response in renal arteries preceding pre-glomerular afferent arterioles (Strandhoy et al., 1972; Toda & Miyazaki, 1981; Viegas et al., 2012). Chapter 2 showed that angiotensin II elicited poor contractile responses in small interlobar arteries and larger extrarenal arteries of the rat renal vasculature. Such findings have lent support to the notion that angiotensin II modulates vascular resistance by acting predominately on pre- and post-glomerular arteries and peritubular capillaries (vasa recta) (Edwards, 1983). That rabbit distal interlobar arteries had a moderate contractile response to angiotensin II in this study may indicate a role for small renal arteries in angiotensin II-mediated regulation of renal vascular reactivity in the rabbit. Angiotensin II-mediated responses also varied between different vascular beds. Angiotensin II elicited a profound contraction in femoral resistance arteries from the hindquarter vasculature, recording a maximal efficacy almost 2.5-fold higher than that in interlobar arteries of the renal vasculature.

Endothelin-1 has been shown to cause potent vasoconstriction in most arterial beds. The rank order of potency for vasoconstriction is generally: coronary = pulmonary > cerebral > renal = femoral > mesenteric arteries. Consistent with this, the current study demonstrated that endothelin-1 was similarly potent in isolated small renal arteries and femoral arteries. In contradiction to the aforementioned rank order however, endothelin-1 was not significantly more potent in the pulmonary vasculature than the renal and femoral. In fact, isolated 2nd order pulmonary interlobar arteries isolated from New Zealand White rabbits had an endothelin-1 potency ($\text{pEC}_{50} 8.06 \pm 0.15$, Hughes, MPhil, unpublished data) comparable to that in small renal and femoral arteries.
Unlike the vasoconstrictor agents assessed, responses to the vasodilator agent acetylcholine and sodium nitroprusside were consistent across the hindquarter and renal vascular bed and between sequential intrarenal arteries. The major exception appeared to be adenosine. Although adenosine was able to completely reverse methoxamine-mediated pre-constriction in femoral arteries, the agent had very little effect in both the renal interlobar and arcuate arteries. This is a significant difference from the effect of adenosine obtained in vivo (Chapter 4) in which the intra-arterial infusion of adenosine increased renal vascular conductance (vasorelaxation), but had little effect on hindquarter vascular conductance. It is important to note that the isolated artery represents a small fraction of the vasculature and that there is marked heterogeneity in the vascular response to exogenous adenosine across different vascular beds and within different regions of a vascular bed, particularly the renal vascular bed (Vallon et al., 2006). The in vitro data may simply suggest that the interlobar and arcuate arteries are not important in mediating vascular responses to exogenous adenosine infusion in vivo.

5.4.7 Membrane depolarisation-mediated contraction and T-type VOCC

Following on from Chapter 3, this study showed that at concentrations selective for T-type calcium channels, NNC 55-0396 and mibefradil decreased the contractile response induced by potassium (40 and 62 mM) in interlobar arteries only. The data suggest that (i) T-type VOCC contribute to membrane depolarisation-mediated vasoconstriction in interlobar but not the smaller resistance arcuate arteries and (ii) T-type VOCC are recruited during membrane depolarisation-mediated contraction and are required to elicit a maximum contractile response in interlobar arteries. The former is an unusual finding: it has been suggested that T-type VOCC are more important in smaller arteries and arterioles than larger arteries (Jensen et al., 2004; Ball et al., 2009; Hansen et al., 2011). Notably, felodipine (1 nM) did not inhibit potassium-mediated contractile responses in either arcuate or interlobar arteries. This may support a greater role for T- over L-type VOCC in depolarisation-mediated constriction of rabbit renal interlobar arteries. Given that neither T- nor L-type selective calcium channel inhibitors blocked depolarisation-mediated contraction in arcuate arteries, calcium influx may have occurred instead via P- and Q-type VOCC. The P-type VOCC has been shown to contribute to potassium-induced calcium influx in renal vascular smooth muscle cells and to membrane depolarisation-mediated constriction of rabbit renal afferent arterioles (Hansen et al., 2000). In human intrarenal arteries, the P- and Q-type VOCC contributed to membrane depolarisation-mediated vasoconstriction alongside L-type VOCC (Hansen et al., 2011). It is possible that the lack of inhibitory effect observed with felodipine was because the concentration used was too low. Felodipine has a reported pK$_B$ of approximately 11 nM in rat ventricular myocytes (Zahradníková et al., 2007) and an IC$_{50}$ of approximately 3 nM in small mesenteric arteries contracted with 62 mM K$^+$ (Angus et al., 2000).

The inhibitory effect of NNC 55-0396 appeared to be comparable between sham and wrap interlobar arteries, which does not support a greater role for T-type calcium channels in hypertension, at least in renal interlobar arteries of the renal hypertensive rabbit.
5.4.8 Summary

Taken together, the data suggest that renal distal interlobar arteries and femoral resistance arteries have enhanced vascular reactivity in rabbit cellophane wrap hypertension. In the renal interlobar arteries, enhanced vascular responses are likely to be driven primarily by increased vascular smooth muscle sensitivity to specific vasoconstrictors, particularly angiotensin II and α₁-adrenoceptor agonists. As endothelial function was also preserved in all three arteries, enhanced vascular responses in the renal interlobar and femoral resistance arteries are likely to be predominantly due to enhanced vascular smooth muscle activity. The enhanced smooth muscle activity to vasoconstrictor agents observed in this study may be due to changes in receptor occupancy–response relations (due to changes in receptor number, second messengers or coupling) or changes in intracellular signalling (such as changes in sensitivity to calcium), amongst others. In addition to changes in vasoconstrictor sensitivity, the enhanced vascular reactivity in femoral arteries may also involve changes in vascular structure. From functional data, it is speculated that femoral arteries have increased smooth muscle content (but an unchanged lumen diameter) and that this contributes to the greater than normal narrowing of vessels in hypertension. Direct measurement of these morphological characteristics is required to confirm these speculations. Of note, renal arcuate resistance arteries appeared to be unaffected by hypertension. The reason for this is unknown and remains to be investigated.
Chapter 6

General summary
6.1 Main findings

The renal vascular bed is structurally and functionally unique and is capable of influencing both the acute and long-term control of arterial pressure. This study explored renal vascular reactivity, firstly under physiological conditions and subsequently in an experimental preparation of hypertension. The main focus areas were: (i) the in vitro pharmacological characterisation of renal vascular reactivity along different arterial segments; (ii) the role of T-type voltage-operated calcium channels in renal vascular responses; (iii) the relevance of the structural resistance amplifier in renal hypertension and (iv) characterisation of functional changes in the renal vasculature that could contribute to the enhanced renal vascular reactivity reported in hypertension.

Pharmacological characterisation of renal vascular reactivity

In addition to segmental heterogeneity in the renal vasculature, this study found substantial heterogeneity in vascular responses between vascular beds of the same species and between vessels of comparable anatomical location in different species. The heterogeneity was primarily observed with angiotensin II and α-adrenoceptor-mediated responses: the responses to endothelin-1, U46619 and the vasodilator agonists acetylcholine and sodium nitroprusside were relatively uniform. Of particular interest was the observation that angiotensin II elicited poor vasoconstriction in numerous renal arteries. The peptide caused little constriction in extrarenal and interlobar arteries isolated from the rat. Although angiotensin II elicited potent vasoconstriction in the interlobar arteries isolated from the rabbit – highlighting species-specific differences – arcuate arteries, which directly branch off from interlobar arteries, were largely unresponsive to the peptide. The lack of response to angiotensin II may be due to the relatively low expression of AT1 receptors in these larger vascular segments (Gascon et al., 1994), although AT1 receptors have been found in arcuate arteries of the rat (Terada et al., 1993; Miyata et al., 1999).

Role of T-type voltage-operated calcium channels in renal vascular tone

The T-type voltage-operated calcium channels (CaV3.1 and CaV3.2 subtypes) have been shown to be expressed in small arteries and arterioles of the cerebral, mesenteric and skeletal muscle vasculature, as well as throughout the renal vasculature (Gustafsson et al., 2001; Braunstein et al., 2009; Navarro-Gonzalez et al., 2009; Kuo et al., 2010; Hansen et al., 2011; Poulsen et al., 2011; El-Rahman et al., 2013). These channels have been suggested to contribute to the vascular tone of small arteries and arterioles in addition to L-type voltage-operated calcium channels and may have particular importance at lower intravascular pressures (El-Rahman et al., 2013). The understanding of the physiological and pathophysiological roles of T-type calcium channels is in part hindered by a lack of selective T-type calcium channel inhibitors and confounded by the use of calcium channel inhibitors at concentrations outside their proposed window of selectivity. The present study used pharmacological inhibitors of T- and L-type calcium channels within their respective selectivity windows to show that although T-type calcium channels are not required for depolarisation- and receptor-mediated vasoconstriction to occur per se, recruitment of both T- and L-type calcium channels may be required to elicit maximum contraction.
The structural vascular amplifier and functional changes in the vasculature of renal hypertensive rabbits

The elevation in total peripheral resistance that occurs in established hypertension is thought to be driven in part, by structural changes in the vasculature. This study provided evidence for the structural vascular amplifier in the renal vasculature of conscious renal hypertensive rabbits and confirmed previous reports of its existence in the hindquarter vasculature. In both vascular beds, constrictor dose-vascular conductance response curves constructed in the hypertensive rabbit were characterised by a lower resting conductance (raised resistance), a more gradual slope and a lower range compared to curves constructed in normotensive rabbits. At the highest dose of adenosine and acetylcholine, presumably when maximal dilatation (or close to) is achieved, both renal and hindquarter vascular resistance remained higher in the hypertensive rabbit. These haemodynamic patterns to the graded i.a. infusion of constrictor drugs suggest that the enhanced vascular reactivity characteristic of hypertension is due to structural or architectural changes in the vasculature, at least in the cellophane wrap preparation of experimental renal hypertension. This conclusion is reinforced by the observation that there were negligible alterations in vasoconstrictor sensitivity between the two rabbit groups.

In addition to assessing vascular reactivity in the integrated cardiovascular system, vascular reactivity was also assessed in vitro in a controlled environment to further dissect out functional differences between vessels isolated from normotensive and hypertensive preparations. As was assessed in the rat renal vasculature, this study also looked at segmental heterogeneity in the renal vasculature. Importantly, vascular responses were expressed as the effective active pressure (ΔP) for the in vitro assessment of vascular reactivity. The effective active pressure normalises for both the length and the circumference of the isolated vessel enabling the comparison of vessels with different internal diameters. Importantly, effective active pressure does not normalise for the smooth muscle content per circumference area. Thus, use of effective active pressure does not mask changes in vascular responses that may arise from alterations in smooth muscle content per unit circumference (for example due to smooth muscle hypertrophy or hyperplasia in hypertension). In this way, it is advantageous over expressing vascular responses as a percentage of the maximum contraction elicited by potassium physiological salt solution.

This study found that femoral resistance arteries and distal interlobar arteries of hypertensive rabbits have enhanced vascular reactivity compared to corresponding arteries in normotensive rabbits. The enhanced vascular reactivity was agonist-specific. In hypertensive rabbits, interlobar arteries were more sensitive to noradrenaline, methoxamine and angiotensin II while femoral arteries were more sensitive to α-adrenoceptor activation only. There was also a trend for these agonists, including endothelin-1, to be more efficacious in femoral and interlobar arteries. In contrast, arcuate arteries which are considered part of the renal resistance circuit, appeared to be protected from major alterations in vascular reactivity in hypertension. The reason is unclear but it once again highlights the segmental heterogeneity within the renal vasculature.
The enhanced vasoconstrictor sensitivity observed in femoral and interlobar arteries is in contrast to the relatively unaltered vasoconstrictor sensitivity observed in the hindquarter and renal vascular beds of hypertensive and normotensive rabbits. The increased smooth muscle sensitivity observed in vitro does not necessarily veto the importance of the structural vascular amplifier in the hindquarter and renal vascular bed. Indeed, as previously discussed, vascular reactivity is dependent on the interaction of structural and functional determinants of vascular tone (Wright et al., 2002):

$$\Delta \text{TPR}_{H-N} = \text{'amplifier'} \times \sum (\text{functional factors}) + \text{rarefaction}$$

Thus, vascular hyper responsiveness that arises from increased vasoconstrictor sensitivity will be further amplified by vascular remodelling. The relative contribution of each factor has not been determined but is likely to be difficult given the complex interactions between these factors.

### 6.2 Concluding remarks and future directions

As with other vascular beds, the architectural arrangement and structural properties of the renal vasculature are closely coupled to its function. This study found that the renal vasculature was not exempt from the ‘structural vascular amplifier’ (or ‘structural factor’) and that the enhanced vascular renal vascular reactivity was both agonist- and region-specific in hypertensive rabbits. Furthermore, the T-type calcium channel was shown to contribute to the magnitude of smooth muscle contraction in the renal vasculature but was not necessary for the contraction to occur. It would be interesting to assess whether the contribution of the T-type calcium channel to vascular tone and contraction is elevated in hypertension and whether it could be a potential therapeutic target. Recent studies suggest that T-type calcium channels are upregulated under conditions of oxidative stress and decreased nitric oxide bioavailability (Kuo et al., 2014), both of which have been implicated in the pathophysiology of hypertension. Finally, the study highlights the power of studying vascular responses in both a conscious animal with an integrated cardiovascular system and in isolated controlled in vitro conditions. Characterising segmental heterogeneity is particularly important in the renal vasculature as different vascular regions have been shown to have different functional roles. These regions may also differentially alter in hypertension. Given that the renal vasculature is comprised of two distinct circulations – the medullary and cortical circulation – it is of interest to assess regional haemodynamic changes that occur in renal hypertension to determine if the structural vascular amplifier is a property of the entire renal vascular bed or if it is region-specific.
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