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for Sec. Research Graduate School Committee

THE RENAL SYMPATHETIC NERVES: IMPLICATIONS FOR VASCULAR REMODELLING IN THE SHR KIDNEY.

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Addendum

Errata

Page 103, lines 2 and 3: "are not consistent with these previous studies." to "are not consistent with these previous studies (Rudd *et al.*, 1986; Bello-Reuss *et al.*, 1975; Bello-Reuss *et al.*, 1977)."

Page 114, line 7: "hypertrophy and/or remodelling and therefore the development of hypertrophy" to "hypertrophy and/or remodelling and therefore the development of hypertension."

Addendum

Methodological point: In all experiments rats were obtained from the Baker Medical Research Institute. Both the spontaneously hypertensive and Wistar-Kyoto rats were derived from the original Okamoto strain.

Chapter 3, methodological point: Although stereology was performed on both SHR and WKY rats, due to the time and cost required, it was not considered necessary to also perform the vascular casting on sham-operated and unilaterally denervated WKY rats.

Chapter 6, page 102, last line: Should be added. "It has previously been demonstrated that acute renal denervation does lead to diuresis and natriuresis in young SHR, irrespective of changes in renal haemodynamics (Rudd *et al.*, 1986)."

Chapter 7, page 110: Should be added prior to *Section 7.2.2.* "Whilst information gained from cultured cells about a potential trophic action of catecholamines is important, extrapolation of such information to whole organs and animal should be made with caution. Analysis of the role of the renal sympathetic nerves *in vivo*, is complicated by an array of factors that influence cellular homeostasis and function, but is important in understanding the physiological involvement in the development of hypertension."

Additional reference

Rudd, M. A., Grippo R. S. and Arendshorst W. J. (1986). Acute renal denervation produces a diuresis and natriuresis in young SHR but not WKY rats. *American Journal of Physiology* 251: F655-61.

Table of contents

Declaration

I hereby declare that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, nor material which has been accepted for the award of any other degree or diploma at Monash or any other university or tertiary institution, except where due acknowledgment is made in the text of the thesis. I also declare this thesis is less than 100,000 words in length exclusive of tables, bibliographies, appendices and footnotes.

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Publications

Published, peer reviewed journal articles

Malpas S.C., Shweta A., Head G.A., Anderson W.P. (1996). Functional response to graded increases in renal nerve activity during hypoxia in conscious rabbits. *American Journal of Physiology*, 271: R1489-99.

Evans R.G., Shweta A., Malpas S.C., Fitzgerald S.M. (1997). Renal effects of rilmenidine in volume loaded anaesthetized dogs. *Clinical & Experimental Pharmacology & Physiology*, 24: 64-7.

Evans R.G., Stevenson K.M., Malpas S.C., Fitzgerald S.M., Shweta A., Tomoda F., Anderson W.P. (1997). Chronic renal blood flow measurement in dogs by transit-time ultrasound flowmetry. *Journal of Pharmacology and Toxicological Methods*, 38: 33-9.

Shweta A., Malpas S.C., Anderson W.P., Evans R.G. (1999). Effects of naloxone on the haemodynamic and renal functional responses to plasma volume expansion in conscious rabbits. *Pflügers Archiv - European Journal of Physiology*, 439: 150-7.

Denton K.M., Lamden M., Shweta A., Alcorn D., Anderson W.P. (2001). Chronic angiotensin converting enzyme inhibition enhances renal vascular responsiveness to acetylcholine in anaesthetized rabbits. *Journal of Hypertension*, 19: 1497-503.

Denton K.M., Shweta A., Anderson W.P. (in press). Pre- and post-glomerular resistance responses to different levels of sympathetic activation by hypoxia. *Journal of the American Society of Nephrology*.

Publications arising from this thesis

Shweta A., Denton K.M., Lambert G.W., Anderson W.P. (in preparation). Chronic unilateral renal denervation causes renal vasodilatation in the spontaneously hypertensive rat. To be submitted to *Hypertension*.

Shweta A., Kett M.M., Denton K.M., Bertram J.F., Anderson W.P. (in preparation). Stereological analysis of the pre- and post-glomerular renal vasculature of the spontaneously hypertensive rat – effect of unilateral renal denervation. To be submitted to *Hypertension*.

Abstracts

S.C. Malpas, A. Shweta, G.A. Head, W.P. Anderson. Renal functional response to graded sympathetic activation with hypoxia in conscious rabbits. *Proceedings of the High Blood Pressure Research Council of Australia* (Melbourne, 1995).

S.C. Malpas, A. Shweta, G.A. Head, W.P. Anderson. Renal functional response to graded sympathetic activation with hypoxia in conscious rabbits. *Proceedings of the International Society of Hypertension* (Glasgow, 1996).

R.G. Evans, A. Shweta, S.C. Malpas, S.M. Fitzgerald, W.P. Anderson. Renal effects of rilmenidine in pentobarbitone anaesthetized volume loaded dogs. *Proceedings of the Australian Physiological and Pharmacological Society* (Perth, 1996).

A. Shweta, S.C. Malpas, W.P. Anderson, R.E. Evans. Role of endogenous opioids in the systemic haemodynamic and renal responses to plasma volume expansion in conscious rabbits. *Proceedings of the High Blood Pressure Research Council of Australia* (Fremantle, 1997) – **Oral presentation.**

A. Shweta, S.C. Malpas, W.P. Anderson, R.E. Evans. The role of endogenous opioids in the systemic and renal response to plasma volume expansion in conscious rabbits. *Cardiovascular Function in Health and Disease* (Sydney, 1998) – **Poster presentation.**

A. Shweta, K.M. Denton, W.P. Anderson. The effects of chronic unilateral renal denervation on the renal vasculature of spontaneously hypertensive rats. *Proceedings of the High Blood Pressure Research Council of Australia* (Melbourne, 1999) – **Poster presentation.**

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A. Shweta, K.M. Denton, W.P. Anderson. The effects of chronic unilateral renal denervation on the renal vasculature of spontaneously hypertensive rats (SHR). *Monash Postgraduate Symposium* (Monash University, 2000) – **Poster presentation.**

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A. Shweta, M.M. Kett, K.M. Denton, J.F. Bertram, W.P. Anderson. The role of the renal nerves in renal vascular hypertrophy of SHR. *IUPS satellite "Cardio-Renal Control in Health and Disease"* (Queenstown, NZ, 2001). – **Oral presentation.**

K.M. Denton, A. Shweta, W.P. Anderson. Graded reflex increases in renal sympathetic activity cause differential pre- and post-glomerular vascular resistance responses in anaesthetised rabbits. *IUPS* (Christchurch, NZ, 2001).

K.M. Denton, M. Lamden, A. Shweta, D. Alcorn, W.P. Anderson. Chronic ACE inhibition enhances renal vascular responsiveness to acetylcholine in anaesthetised rabbits. (Submitted to the *American Heart Association Meeting*, 2001).

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A. Shweta, K.M. Denton, G.W. Lambert, W.P. Anderson. Micropuncture analysis of the renal haemodynamics following chronic unilateral denervation in SHR kidneys. (Submitted to the *High Blood Pressure Research Council of Australia*, 2001).

A. Shweta, K.M. Denton, G.W. Lambert, W.P. Anderson. Pre- and post-glomerular conductance and glomerular capillary pressure in chronic unilaterally denervated SHR kidneys. (Submitted to the *International Society of Hypertension*, 2002).

Summary

This thesis examined the hypothesis that the renal sympathetic nerves are responsible for producing or initiating the hypertrophy/remodelling of the pre-glomerular vasculature in the spontaneously hypertensive rat (SHR). There are a number of possible mechanisms by which the renal nerves could lead to the development of hypertrophy/remodelling of the renal vasculature, and they could be direct or indirect. The SHR demonstrates an increase in renal sympathetic nerve activity and there is also evidence of hyperinnervation of the vasculature. Therefore one possibility is that the release of noradrenaline, which may act as a growth promoting factor, could lead to hypertrophy of the pre-glomerular vasculature. In-order to interpret the results without the confounding effects of a reduction in blood pressure (bilateral renal denervation delays the onset of hypertension in this strain – *Chapter 3*), unilateral renal denervation was used throughout this thesis at a period when hypertension is rapidly developing in this strain.

In *Chapter 4*, four weeks following unilateral renal denervation (UD_x) the lumen diameters of the interlobular arteries and afferent and efferent arterioles were examined using the vascular casting technique. The main finding of this chapter was that there was no significant affect of UD_x on the lumen dimensions of the pre-glomerular vasculature. However, there was a significant reduction in calculated relative resistance of the efferent arteriole.

Chapter 5 examined the wall and lumen dimensions of the proximal interlobular and arcuate arteries following UD_x in SHR and Wistar-Kyoto rats, using unbiased stereological techniques. UD_x produced no significant alterations in the wall of either the proximal interlobular or arcuate arteries. The lumen diameter of the arcuate artery was also not significantly different in the unilaterally denervated kidneys compared to the sham operated kidneys, but the proximal interlobular arteries of the unilaterally denervated SHR kidneys were significantly narrower. The results of *Chapters 4 & 5* therefore, demonstrate no apparent effect on the pre-glomerular vasculature, apart from

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the narrowing of the proximal interlobular artery. The finding of a reduced calculated relative resistance of the efferent arteriole (*Chapter 4*) and narrowing of the proximal interlobular artery (*Chapter 5*), was unexpected, and *Chapter 6* examined the functional consequences of such changes.

The specific aim of *Chapter 6* was to examine the functional consequences of the above mentioned findings in renal vascular structure following unilateral renal denervation in SHR. Micropuncture was used to estimate glomerular capillary pressure in unilaterally denervated and sham operated SHR kidneys, whole kidney function was also measured. Four weeks following UD_x there was a significantly greater total renal vascular conductance and a reduced estimated glomerular capillary pressure compared to the sham operated kidneys. This observation was consistent with the findings of the previous two chapters indicating a predominantly post-glomerular effect.

The findings of this thesis therefore, provide strong evidence against there being a role for the renal sympathetic nerves in the hypertrophy of the pre-glomerular vasculature in the SHR. However, the findings do suggest the involvement of the renal sympathetic nerves in the structural integrity of the efferent arteriole.

Symbols and abbreviations

Symbols

α	alpha
β	beta
π	pi (equals 3.142)
π_a	arterial colloid oncotic pressure
π_e	efferent oncotic pressure
π_{gc}	glomerular oncotic pressure
ΔP	hydrostatic pressure difference
Σ	sum of
η	viscosity
*	times

Abbreviations

A

AA	afferent arteriole
A-D	analog to digital
ACEi	angiotensin converting enzyme inhibitor
AI	angiotensin I
ANOVA	analysis of variance
ARC	arcuate artery

B

bFGF	basic fibroblast growth factor
BSA	bovine serum albumin
BD_x	bilaterally denervated

C

^{14}C	radioactive labelled isotope of carbon
°C	degree(s) Celsius
C_a	arterial protein concentration

D

Dahl-S	Dahl salt-sensitive rats
DBP	diastolic blood pressure
dH_2O	distilled water
DNA	deoxyribonucleic acid
DOCA	desoxycorticosterone acetate

E

EA	efferent arteriole
ERBF	effective renal blood flow
ERPF	effective renal plasma flow

F

F_1	first generation offspring
FE_{Na}^+	fractional sodium excretion
FF	filtration fraction

G

g	gram(s)
G	glomerulus
GFR	glomerular filtration rate
GH	genetically hypertensive rat

H

[^{3}H]	tritiated/radioactive isotope of hydrogen
h	hour(s)
HR	heart rate

I

ID	inner diameter
ILA	interlobular artery
i.p.	intraperitoneally
i.v.	intravenously

J

K

K_f	glomerular ultrafiltration coefficient
kg	kilogram(s)
kid	kidney
KOH	potassium hydroxide

L

l	length
LH	Lyon hypertensive rat
LK	left kidney
LL	Lyon low blood pressure rat
LN	Lyon normotensive rat
lum	lumen

M

M	molar
MAP	mean arterial pressure
µm	micrometre(s)
mg	milligram(s)
MHS	Milan hypertensive strain
min	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mmHg	millimetres of mercury
mRNA	messenger ribonucleic acid

N

n	number
NA	noradrenaline
Na^+	sodium ion
NaCl	sodium chloride
ng	nanogram(s)
NGF	nerve growth factor

O

OD	outer diameter

P

PAH	para-aminohippuric acid
PBS	phosphate buffered saline
PDGF	platelet derived growth factor
P_{gc}	glomerular capillary pressure
PO_4	phosphate
PRA	plasma renin activity
P_t	proximal tubule pressure

Q

R

r	radius
R	relative resistance
RBF	renal blood flow
RK	right kidney
RPF	renal plasma flow
rpm	revolutions per minute
RSNA	renal sympathetic nerve activity
RVLM	rostral ventrolateral medulla
RVR	renal vascular resistance

S

SBH	Sabra hypertensive strain
SBN	Sabra normotensive strain
SBP	systolic blood pressure
SD	standard deviation
sec	second(s)
sem	standard error of the mean
SEM	scanning electron microscope/microscopy

SFP	stop-flow pressure
SHR	spontaneously hypertensive rat
SHR_{BDx}	bilaterally denervated SHR
SHR_{Dx}	unilaterally denervated SHR
SHR_S	sham operated SHR
SHR-SP	stroke prone spontaneously hypertensive rat
SNBF	single nephron blood flow
SNGFR	single nephron glomerular filtration rate
SNS	sympathetic nervous system

T

TGF	tubuloglomerular feedback

U

UFR	urine flow rate
$U_{Na}{}^{+}V$	urinary sodium excretion
UD_x	unilaterally denervated

V

V	volume
VSMC	vascular smooth muscle cell(s)
V_V	volume density

W

WKY	Wistar-Kyoto rats
WKY_{Dx}	unilaterally denervated WKY
WKY_S	sham operated WKY
W:L	wall to lumen ratio

XYZ

CHAPTER 1

CHAPTER 1

Review of literature

1.1 Hypertension

High blood pressure (hypertension) is a major factor in the pathogenesis of coronary heart disease, stroke and renal failure (Chalmers *et al.*, 1999). The World Health Organisation has defined hypertension as a systolic blood pressure of 140 mmHg or greater and/or a diastolic blood pressure of 90 mmHg or greater in subjects who are not receiving antihypertensive medication (Chalmers *et al.*, 1999).

The prevalence of hypertension in the Australian population is high, with approximately 1 in 5 of the adult population having high blood pressure (Heart Foundation of Australia, 1999). With cardiovascular diseases accounting for 41% of deaths by major diseases (including cancers; Heart Foundation of Australia, 1999). It is therefore, of major importance to understand the underlying causes of hypertension, in terms of both the burden of the cost to the public health system (Chalmers *et al.*, 1999) and in targeting treatment more effectively to the individual in-order to improve quality of life.

In this case prevention is better than cure, but first we must understand the pathophysiology of the condition.

1.2 Essential hypertension

In 90% of hypertensive patients the cause of the increase in arterial pressure is unknown, and they are said to have essential or primary hypertension (Ganong, 1993). Secondary hypertension accounts for the remainder of patients, with hypertension developing as a consequence of an underlying condition such as renal artery stenosis, coarctation of the aorta, diabetes or Cushing's syndrome (Ganong, 1993; Laragh and Brenner, 1995). Within a population the prevalence of hypertension depends on age,

sex, the blood pressure distribution in the population and the number of blood pressure measurements used to define the hypertension (Epstein, 1983). Patients with hypertension have widely differing cardiovascular characteristics, and so it can be said that essential hypertension is not a homogeneous disorder (Abboud, 1982), although generally patients show an increase in total peripheral resistance and cardiac output (Epstein, 1983; Mongeau, 1991). It has been postulated, since the 1930s (Page, 1939), that hypertension is multi-factorial in that it has a number of causative components, with both genetic and/or environmental factors being of major importance.

There is a wide body of evidence highlighting the importance of the kidneys (Kawabe *et al.*, 1979; Curtis *et al.*, 1983; Rettig *et al.*, 1989; Rettig *et al.*, 1990; Rettig and Unger, 1991; Kopf *et al.*, 1993; Rettig *et al.*, 1993) and sympathetic nervous system (Abboud, 1982; Esler, 1995; Laragh and Brenner, 1995) in the development of essential hypertension. The kidney has long been known to play a pivotal role in long term blood pressure regulation (Guyton and Hall, 2000), and so it would be common sense to think that any change in the kidneys may indeed effect blood pressure.

The best model to study human essential hypertension is man, and while many studies have been performed in humans, more complicated questions often involve the need for more invasive procedures. One of the most widely used animal models of essential hypertension is the spontaneously hypertensive rat (SHR), which will be covered in detail in *Section 1.3.1.1 The spontaneously hypertensive rat.* The SHR also provides us with the option of studying the genetic involvement in the development of hypertension. Animal models have been extensively used, therefore, to study the cause of hypertension, as not all questions can be answered ethically in human subjects.

1.3 Animal models of hypertension

1.3.1 Genetic models of hypertension

There are a number of animal models of hypertension, some of which demonstrate similarities to human essential hypertension. The following sections will review the animal models, which demonstrate a genetic predisposition to the development of hypertension.

1.3.1.1 The spontaneously hypertensive rat

The Japanese strain of SHR has been the most widely studied animal model of essential hypertension (Okamoto, 1969; Yamori, 1994), and this is the strain of choice

for my studies. The development of the SHR began with Kozo Okamoto in 1959 (Okamoto, 1969). Male Wistar-Kyoto (WKY) rats with mild hypertension were mated with females with relatively high blood pressure. The blood pressure of the offspring was continually monitored in-order to select for those with hypertension. Okamoto succeeded in producing a colony of rats, via selective brother-sister inbreeding, that always developed hypertension, and in 1963 they were reported as being spontaneously hypertensive (Yamori, 1994). SHR are currently the best animal model we have of human essential hypertension, because they not only develop hypertension spontaneously, but also have other hypertension related complications without additional inducements, such as salt loading which induces hypertension in the Dahl rat strain. Such hypertension related complications include cardiac failure, cerebral vascular lesions and renal failure (Okamoto, 1969).

SHR colonies were distributed worldwide well before they had become fully inbred, therefore, there are phenotypic and genotypic differences among SHR strains around the world (De Jong and Ganten, 1994). Also controversy exists over the best control when using SHR. The most commonly used control is the WKY rat, which is genetically the closest relative to the SHR, however, again this strain was distributed prior to the SHR becoming fully inbred, and thus they remain genetically heterogeneous.

SHR develop hypertension early and gradually and share some similarities to essential hypertension in humans. High blood pressure can develop as early as 4-weeks of age in SHR (Adams *et al.*, 1989; Korner *et al.*, 1993; Rizzoni *et al.*, 1995), and is well established by the age of 10-weeks (see *Figure 1.1*; Dickhout and Lee, 1998). SHR and WKY rats display similar levels of blood pressure up to the age of 4-weeks, this has enabled investigation into the mechanisms involved in the initiation of hypertension. The most rapid period of blood pressure development in the SHR is between the ages of 6- to 10-weeks (Okamoto, 1969). Although the time frame of development of hypertension is not identical to humans, the early onset of hypertension in SHR allows us to study the phase prior to established hypertension.

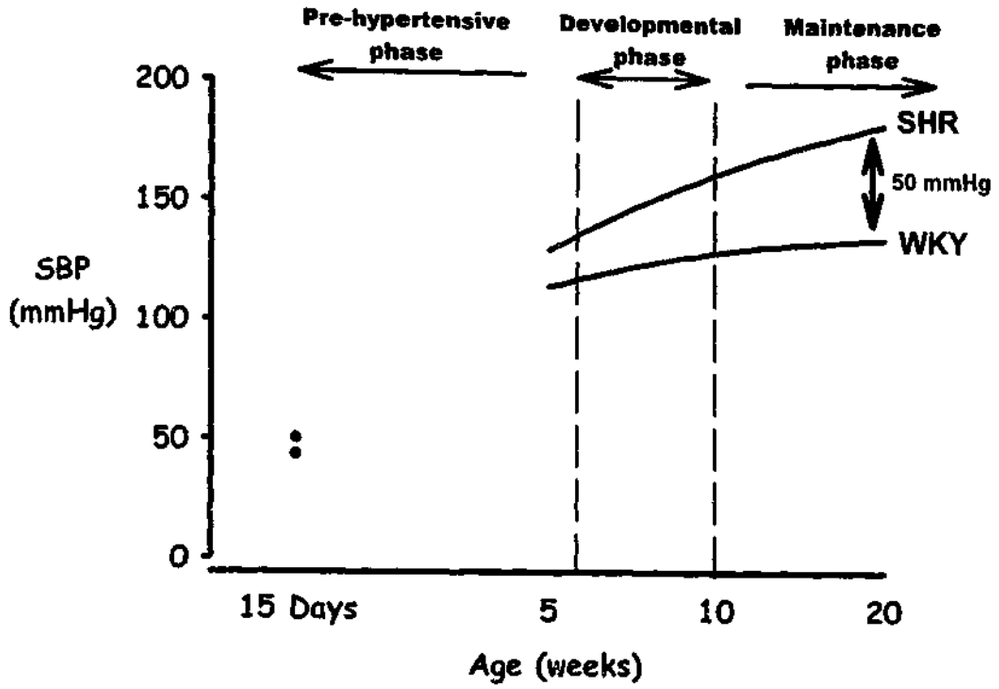

Figure 1.1 A schematic diagram demonstrating the systolic blood pressure (SBP) development in SHR and WKY rats, from birth to 20-weeks of age. Modified from (Okamoto, 1969; Gray, 1984).

1.3.2.2 Other rat models of genetic hypertension

There are many strains of genetically hypertensive rats. The New Zealand strain of genetically hypertensive (GH) rat was the first breakthrough in developing an appropriate model of essential hypertension (Smirk and Hall, 1958). The ability to develop such a model supported the genetic involvement in the development of hypertension, since hypertension was passed on from parent to offspring. Other models of genetic hypertension quickly followed, including; the Milan hypertensive strain (MHS; Horie *et al.*, 1986), Lyon hypertensive strain (De Jong and Ganten, 1994), Dahl-salt sensitive strain (Friedman, 1988), the DOCA-salt hypertensive strain (De Jong and Ganten, 1994) and the SHR (Okamoto, 1969; Yamori, 1994). However, many of these genetic models of hypertension are far from ideal in that hypertension does not develop in a similar way to human essential hypertension. The MHS, for example, develops very mild hypertension over the first 2 months after birth and shows no further augmentation of hypertension during the process of aging, which is quite different to the situation in human essential hypertension (Horie *et al.*, 1986). The GH rat has been shown to have a greater blood pressure from as early as 2 days of age (Jones and Dowd, 1970). The difference in blood pressure between the GH rat and the normotensive

strain increases up to 10-weeks of age and then stabilises at adult levels (Horie *et al.*, 1986).

The Lyon strains of hypertensive (LH), normotensive (LN) and low blood pressure (LL) rats were derived from the Sprague-Dawley strain (De Jong and Ganten, 1994). As early as 5-weeks of age LH rats have a higher systolic blood pressure when compared to age-matched LN and LL rats (De Jong and Ganten, 1994). Bilateral renal denervation does not alter the development of hypertension (Boussairi *et al.*, 1991) and therefore an overactive sympathetic nervous system is not thought to be of importance in this form of hypertension (De Jong and Ganten, 1994).

The Dahl rats were selectively bred for susceptibility or resistance to the hypertensive effect of high-salt diets (8% NaCl), and also originated from the Sprague-Dawley strain (De Jong and Ganten, 1994). If these rats are placed on a high-salt diet from the time of weaning, the blood pressure rise is rapid (De Jong and Ganten, 1994). On the other hand, if these rats are not given high-salt until 3 months of age then the blood pressure rise is less rapid (De Jong and Ganten, 1994). These rats provide an interesting model for the interaction of an environmental factor (salt) with genotype. The Sabra rat strain is a randomly-bred albino rat that is maintained at the Hebrew University in Jerusalem, however there are no written records however regarding its origin (De Jong and Ganten, 1994). Two sub-strains were selected according to their sensitivity to DOCA-salt. The Sabra hypertensive strain (SBH) develops hypertension when treated with DOCA-salt, while the Sabra normotensive strain (SBN) does not (De Jong and Ganten, 1994).

However of all these genetic models of hypertension none have been studied over the years as extensively as the SHR model.

1.4 The sympathetic nervous system in hypertension

The sympathetic nervous system (SNS), in particular the sympathetic innervation of the kidneys has been proposed as a candidate for initiating or causing the development of hypertension. Increased plasma noradrenaline levels, indicating an increase in efferent sympathetic nerve activity (Esler, 1995; Esler and Kaye, 1998), have been shown not only in SHR (Donohue *et al.*, 1988; Korner *et al.*, 1993; Yoshida *et al.*, 1995) but also in human essential hypertension (Esler, 1995). Many studies have also demonstrated an increase in peripheral sympathetic nerve activity to the heart and blood vessels of SHR compared to normotensive controls, particularly in the first 4-8

weeks of life (Judy *et al.*, 1976; Thorén and Ricksten, 1979; Lundin *et al.*, 1984; Smith *et al.*, 1984; Janssen *et al.*, 1987; Thorén, 1987; Gattone *et al.*, 1990; Wyss *et al.*, 1992).

Destruction of the SNS either by pharmacological or surgical means, early in the life of the SHR, markedly attenuates the increase in blood pressure, thus implicating the SNS in the development of hypertension (Korner *et al.*, 1993). Propranolol treatment, a β-adrenoceptor antagonist, during the pre-hypertensive stage in SHR, prevented the subsequent pressure rise and the development of structural changes of the resistance vessels (Weiss *et al.*, 1974). While a similar treatment at 8 months of age, when hypertension is well established, did not attenuate the pressure rise or any vascular changes (Weiss *et al.*, 1974). Similarly, treatment with α-adrenoceptor antagonists during the developmental stage of hypertension in the SHR, will attenuate the rise in arterial pressure, and either not affect or reduce ventricular weight (Takeda *et al.*, 1991; Young *et al.*, 1993; Smid *et al.*, 1995; McCarty and Lee, 1996). None of these studies examined the effect of α- or β-adrenoceptor blockade on the renal vasculature. The above mentioned treatments were also administered orally or intravenously and therefore would block adrenergic receptors systemically, no conclusion can be drawn therefore as to the main site of influence of the nerves in the development of hypertension in the SHR. It is my hypothesis therefore, that the renal sympathetic nerves produce hypertrophy and/or remodelling of the renal vasculature, commonly observed in the SHR kidney, thus leading to an increase in renal vascular resistance.

Treating newborn SHR and normotensive controls with nerve growth factor (NGF) antiserum, for the first five days of life, reduces blood pressure by 40% in SHR, levels which are equal to those seen in the normotensive controls (Folkow *et al.*, 1972). However, the blood pressure of the treated SHR remained 20-25% greater than that of the treated normotensive rats (Folkow *et al.*, 1972). Another group also treated newborn SHR for the first month of life with anti-NGF antibody plus guanethidine, and found that the development of hypertension could be prevented (Lee *et al.*, 1987; Lee *et al.*, 1991). Both these studies, and others, clearly show that the SNS is indeed important in the development of hypertension in the SHR (Folkow *et al.*, 1972; Lee *et al.*, 1987; Lee *et al.*, 1991; Korner *et al.*, 1993).

The presence of increased levels of noradrenaline synthesising enzymes in young SHR compared to WKY rats also implicates a role for the SNS (Gibbons, 1995). Young SHR also demonstrate an increase in NGF peptide and mRNA levels in blood vessels, kidneys and nerves (Donohue *et al.*, 1989; Ueyama *et al.*, 1992; Kapuscinski *et al.*, 1996). This increase in NGF levels correlates closely to the increase in arterial

pressure (Kapuscinski *et al.*, 1996). Similarly sympathetic overactivity has also been demonstrated in most young individuals with essential hypertension (Esler, 1995; Saruta and Kumagai, 1996).

The above mentioned evidence suggests that the SNS may play a role in the induction of essential hypertension in humans (Esler and Kaye, 1998) and SHR (Folkow *et al.*, 1972; Lee *et al.*, 1987; Lee *et al.*, 1991; Korner *et al.*, 1993). This could be through increased resting neural activity and/or trophically induced cardiovascular hypertrophy (Korner *et al.*, 1993). Within the kidney, the SNS stimulates renin release, promotes sodium and water reabsorption, and constricts the renal vasculature, thereby elevating blood pressure and inducing sodium retention (DiBona, 1982; Saruta and Kumagai, 1996). **Could the renal nerves be the cause of essential hypertension?**

1.4.1 The renal sympathetic nerves

The renal sympathetic innervation originates from the rostral ventrolateral medulla (RVLM) in the hindbrain, which receives input from the nucleus tractus solitarius and other bulbar and suprabulbar centres. Neurones project from the RVLM to the intermediolateral cell column from which pre-ganglionic neurones from L12 to T6 project to the coeliac ganglion, and post-ganglionic neurones then innervate the kidney (Dampney, 1994). Efferent sympathetic nerves have been demonstrated on the renal vascular tree, renin-containing juxtaglomerular cells and renal tubules (Dieterich, 1974; Barajas *et al.*, 1984; Luff *et al.*, 1991; Luff *et al.*, 1992). Evidence indicates that graded increases in renal sympathetic nerve activity produce frequency dependent increases in renal renin secretion, renal tubular sodium and water reabsorption and decreases in RBF and GFR (Moss *et al.*, 1992; DiBona, 1994). The functions of the renal nerves therefore, are multiple. The renal nerves are also involved in the release of vasoactive substances which have an important role in cardiovascular function, regulation of arterial pressure, and the development and/or maintenance of hypertension (for review see DiBona, 1982).

Noradrenaline, the primary neurotransmitter released from the renal sympathetic nerve terminals into the synaptic cleft, stimulates predominantly α_1-adrenoceptors found on the renal vasculature (DiBona and Kopp, 1997). The adrenal medulla releases adrenaline and together with noradrenaline, both circulating and that which overflows from the synaptic cleft, act on extra-junctional adrenoceptors on the renal vasculature (DiBona and Kopp, 1997). Interactions between the effects of neurotransmitters and other humoral factors on the renal vasculature may occur at the level of the pre-

junctional receptor with modulation of neurotransmitter release or the post-junctional receptor-signal transduction mechanisms with modulation of the vascular smooth muscle cell (VSMC) response (DiBona and Kopp, 1997).

The SNS has been shown to have a trophic influence on VSMCs (Erlinge *et al.*, 1993). For example, denervation of rabbit ear arteries, showed that the [^{3}H]-thymidine uptake (indicative of the amount of DNA present and thus proliferation) by the VSMC is reduced compared to control (Bevan, 1975). Chemical sympathectomy with 6-hydroxydopamine has also been shown to reduce the number of VSMC in rabbit aortic wall, and reduce the number of layers in rat mesenteric arteries (Laragh and Brenner, 1995). *In vitro* noradrenaline, adrenaline and isoproteronol stimulate proliferation of rat aortic VSMCs (Simpson *et al.*, 1982; Adams *et al.*, 1995; Laragh and Brenner, 1995).

1.4.1.1 Renal sympathetic nerves in SHR

Precocious development of the sympathetic innervation in the SHR kidney has been previously demonstrated (Gattone *et al.*, 1990). Both newborn SHR and WKY rats demonstrated equivalent monoaminergic innervation of the cortex around the renal hilus associated with interlobular arteries and afferent arterioles and along the arcuate arteries (Gattone *et al.*, 1990). At one week of age the SHR demonstrate innervation of the entire circumference of the mid-region of the cortex, whereas in the WKY rats monoaminergic innervation is present only two-thirds of the way around the mid-region of the renal cortex (Gattone *et al.*, 1990). By 2-weeks of age the entire renal cortex of both SHR and WKY rats is innervated (Gattone *et al.*, 1990). This study suggests that cortical innervation develops much sooner in the SHR than in the WKY rat. The increase in renal sympathetic activity and early development of the renal sympathetic nerves in the SHR may well lead to increased renal vascular resistance, either directly through physiological vasoconstriction, or via structural changes caused by vessel wall hypertrophy therefore resulting in hypertension (Korner and Angus, 1992).

1.4.1.2 Bilateral renal denervation

Liard, in 1977, first demonstrated that bilateral renal denervation of 5-week old SHR delayed the onset of development of hypertension by 2-3 weeks (Liard, 1977) his results have also been verified by others (Kline *et al.*, 1978; Winternitz *et al.*, 1980). The above mentioned studies strengthen the argument for the importance of the renal sympathetic nerves in the development of essential hypertension, at least in the SHR (Liard, 1977; Kline *et al.*, 1980; Winternitz *et al.*, 1980; Norman Jr and Dzielak, 1982;

Saynavalammi *et al.*, 1982; Gattone *et al.*, 1984; DiBona and Jones, 1991; Greenberg and Osborn, 1994; Yoshida *et al.*, 1995). Furthermore, when bilateral renal denervation is performed at 18-weeks of age, a stage when hypertension has already developed, blood pressure does not decrease towards normotensive levels (Winternitz *et al.*, 1980; DiBona and Jones, 1991). Thereby suggesting that there is a critical period during the lifespan of the SHR when the renal sympathetic nerves contribute to the development of hypertension, but not in the maintenance of hypertension (Winternitz *et al.*, 1980; DiBona and Jones, 1991). There is also evidence to suggest that the renal sympathetic nerves may also be involved in the initiation of human essential hypertension, but not its maintenance (Esler *et al.*, 1988).

As mentioned above bilateral renal denervation delays the onset of hypertension in the SHR (Liard, 1977; Kline *et al.*, 1980; Winternitz *et al.*, 1980; Norman Jr and Dzielak, 1982; Saynavalammi *et al.*, 1982; Gattone *et al.*, 1984; DiBona and Jones, 1991; Greenberg and Osborn, 1994; Yoshida *et al.*, 1995), therefore making it difficult to ascertain what observations are due to withdrawal of renal sympathetic nerve activity and those due to the reduction in blood pressure. Throughout this thesis I have chosen to use the model of chronic unilateral renal denervation, in the SHR, which is known not to alter blood pressure (Tomoda *et al.*, 1997). Chronic unilateral renal denervation allows us to study the effects of withdrawal of the renal nerves independently of any changes in blood pressure. By studying the SHR at an early age, it is possible to deduce the importance of the renal sympathetic nerves in the renal vascular structural changes, commonly seen in this strain, and perhaps the hypertension.

1.4.1.3 Unilateral renal denervation

It must be kept in mind when performing treatments/experiments in one kidney, that there may also be changes occurring in the contralateral kidney, which may interfere with the overall response of the ipsilateral kidney. Previous studies have focussed on the effects of unilateral renal denervation and demonstrated a resultant diuresis and natriuresis, with the opposite occurring in the contralateral kidney, in the absence of any changes in systemic or renal haemodynamics (Colindres *et al.*, 1980; DiBona and Rios, 1980; Golin *et al.*, 1987). This response is termed reno-renal reflexes and defined as "responses occurring in one kidney as a result of interventions on the same (ipsilateral) or the opposite (contralateral) kidney that are mediated by neurohumoral mechanisms" (Kopp, 1993; DiBona and Kopp, 1997). Kopp and Smith (1989) have suggested that an impaired reno-renal reflex mechanism exists in the SHR

which contributes to the maintenance but not the development of hypertension in this strain.

Acute unilateral renal denervation

A common observation following acute surgical denervation is a persistent diuresis and natriuresis (Bencsath *et al.*, 1985). The increase in urinary sodium excretion has been attributed to an increase in glomerular filtration rate (GFR) and filtered sodium load and also a reduction in renal tubular sodium reabsorption (for review; DiBona, 1982). Micropuncture studies in rats, under hydropenic and volume expanded conditions, demonstrated that the diuresis and natriuresis observed following acute denervation occurs without changes in GFR and single nephron GFR (SNGFR; Bello-Reuss *et al.*, 1975; Bello-Reuss *et al.*, 1977). During hydropenia, the contralateral kidney showed no change in urine flow rate (UFR) or sodium excretion (Bello-Reuss *et al.*, 1975). However during volume expansion, the contralateral kidney showed a decrease in UFR and sodium excretion (Bello-Reuss *et al.*, 1977). When similar experiments were performed in conscious chronically instrumented animals, the observations made were not consistent with previous findings (for review; DiBona and Kopp, 1997). This discrepancy following acute renal denervation is thought to be due to an enhanced basal efferent renal sympathetic tone following anaesthesia (Szenasi *et al.*, 1982).

Denervation hypersensitivity has also been observed following acute renal denervation, initially a greater exposure of receptors to catecholamines occurs through loss of the re-uptake mechanism for catecholamines at efferent nerve endings (Moss *et al.*, 1992). In the kidney, hypersensitivity has also been demonstrated as both an increase in the number of α_1-adrenoceptors and an enhanced vasoconstrictor and tubular response to infused catecholamines compared to controls (Moss *et al.*, 1992).

Chronic unilateral renal denervation

Most studies that have performed chronic renal denervation have surgically denervated both the left and right kidneys. Very few have studied the effect of chronic unilateral renal denervation. Wilson *et al* (1979) have shown that chronic unilateral renal denervation does produce diuresis, natriuresis and kaliuresis without changes in GFR or renal plasma flow (RPF) in euvolaemic anaesthetised rats.

To date very few studies have concentrated on the effects of chronic unilateral renal denervation on renal haemodynamics and vasculature in the SHR. The most

relevant and recent study was conducted by Tomoda *et al* (1997), in which 6-week old SHR underwent surgical denervation of the left kidney. Four weeks post-surgery rats underwent bilateral renal function experiments in which renal haemodynamics were measured in both the left denervated kidney and right innervated kidney (Tomoda *et al.*, 1997). The authors were able to show an increase in both GFR and renal blood flow (RBF) in the left kidney, even though both kidneys were exposed to the same level of systemic arterial pressure (Tomoda *et al.*, 1997). This group went on further to study the renal vasculature using a maximally dilated isolated, colloid perfused kidney preparation (Tomoda *et al.*, 1997). The results showed that for any given perfusion pressure, the left denervated kidney filtered more than the sham operated kidney, but had similar flows (Tomoda *et al.*, 1997). The authors went on to suggest that this was indicative of an increase in the lumen diameter of the pre-glomerular vessels (afferent arteriole), and perhaps a reduction in efferent arteriole lumen diameter (Tomoda *et al.*, 1997). No other study has been able to examine the vascular changes in response to renal denervation without altering systemic pressure. The Tomoda *et al* (1997) study was suggestive of pre-glomerular vascular dilatation, but was unable to measure such changes directly.

Poiseuille's equation is often used to calculate hydraulic resistance of blood vessels. However this law applies to "tubes" with constant diameter and straight axis, but blood vessels in many beds do not have such properties. Poiseuille's law therefore, leads to an underestimation of vascular resistance (Iordache and Remuzzi, 1995). In the systemic circulation approximately two thirds of the resistance is in the small arterioles (Guyton and Hall, 2000). As Poiseuille's equation states, resistance is inversely proportional to the fourth power of the radius, therefore very small changes in lumen diameter of a vessel will lead to large alterations in vascular resistance (Guyton and Hall, 2000). Certainly it must be kept in mind that this method has limitations.

1.5 The kidney and hypertension

The kidney is highly ranked among the numerous factors, which could play a role in the pathogenesis of both essential and SHR hypertension (Rettig and Unger, 1991; Rettig *et al.*, 1993; Ritz and Fliser, 1993), the main reason being the observation that many experimental procedures which affect the kidney can produce hypertension readily (de Leeuw *et al.*, 1983). The kidney is also one of the main targets of hypertensive disease and abnormalities which have been observed in essential

hypertension, but it may be that these abnormalities not only contribute to the maintenance of hypertension but also the progression of the disease. In SHR there are alterations in renal vascular structure, renal haemodynamics and the responsiveness of the vasculature to certain stimuli. As will be covered later, renal transplantation studies also demonstrate that the kidney from a hypertensive subject is capable of producing hypertension in a normotensive subject.

1.5.1 The kidney and long-term blood pressure control

A characteristic of established essential hypertension is a 35% reduction in RBF, a more than two-fold increase in renal vascular resistance (RVR) with a 40% increase in filtration fraction (FF; Zanchetti and Mancia, 1997). If the kidneys were functioning normally in essential hypertension then it could be hypothesised that the high pressure would cause an increase in sodium excretion and this would lead to a reduction in arterial pressure toward normal levels, this does not occur however (Zanchetti and Mancia, 1997).

The kidney has long been known to play a central role in the long-term control of blood pressure (Guyton *et al.*, 1972). If arterial pressure rises or falls to levels different from the pressure level of the "equilibrium point" (see *Figure 1.2, point A*), the kidney-volume mechanism will be activated and pressure would be reduced towards the "equilibrium point" once more (Guytón *et al.*, 1983). This mechanism therefore is said to have an "infinite" capability in returning arterial pressure back to the equilibrium point, and is called the "infinite gain principle" (Guyton *et al.*, 1983). This mechanism has the capability of over-riding other pressure control mechanisms, for long-term pressure control (Guyton and Hall, 2000).

There exists two ways in which the long-term level of arterial pressure can be changed permanently. These are to alter either the intake level of sodium or to change the ability of the kidney to excrete sodium. The pressure natriuresis relationship in SHR and in the normal state is shown in *Figure 1.2*. As can be seen the urinary sodium output curve is shifted towards higher pressure levels in the SHR (*Figure 1.2*). In the SHR, hypertension is thought to be due to increased vascular resistance, and in particular increased afferent arteriolar resistance or increased renal artery resistance, and this could lead to a reduction in sodium excretion due to a reduction in sodium filtration. It has been demonstrated that SHR have an altered pressure diuresis-natriuresis mechanism prior to the development of hypertension, and this could be one reason why arterial pressure is not returned towards normal levels in this strain (Roman

and Cowley Jr., 1985; Roman, 1987). It is my hypothesis that the renal sympathetic nerves play an initial role in producing the increased renal vascular resistance by altering renal vascular structure (ie hypertrophy and/or remodelling of the renal vasculature), or possibly by direct physiological renal vasoconstriction.

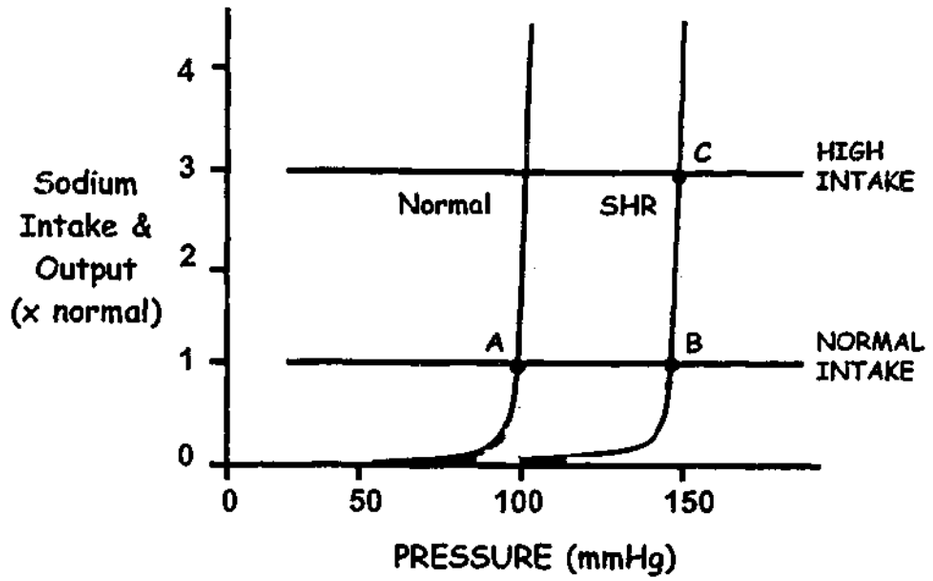

Figure 1.2 Schematic diagram demonstrating the effects of abnormal functional states of the kidney on arterial pressure. Points A-C denote the equilibration points caused by Goldblatt clamps on the renal arteries or SHR (B-C). Point A denoting the normal renal function curve. Modified from: (Guyton *et al.*, 1972).

1.5.2 Specific renal hypertension

The SHR is not the only experimental model that appears to display a renal basis to the development of hypertension, I have reviewed the literature on some of these in the following sections.

1.5.2.1 Renal artery stenosis/Goldblatt hypertension

Renal artery stenosis/Goldblatt hypertension was first described in 1934 (Goldblatt *et al.*, 1934). The aim was to produce an experimental form of hypertension similar to that seen in the population, in which intra-renal arteriolar sclerosis was so frequently observed (Goldblatt *et al.*, 1934). By the early 1950s it was evident that the main renal artery was the site of arteriosclerosis similar to that seen in other peripheral vascular beds (Korner and Angus, 1992). The changes observed included hypertrophy/remodelling of the vasculature thereby increasing wall to lumen ratio and

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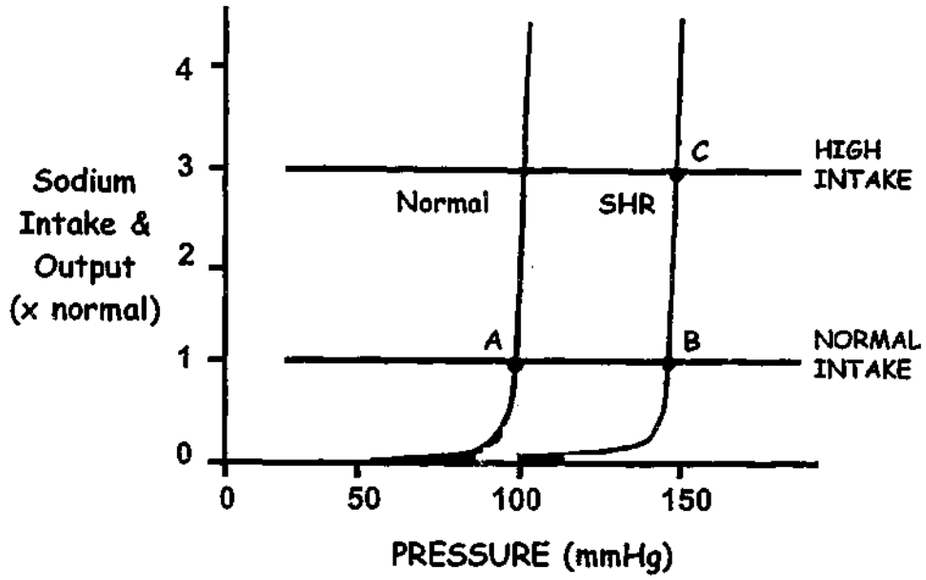

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thus RVR (Folkow, 1982; Heagerty and Izzard, 1995). The degree of hypertension is directly related to the degree of renal artery constriction via the Goldblatt clamps (Guyton and Hall, 2000). A number of studies have demonstrated an initial increase in plasma levels of renin and angiotensin II in chronic hypertension and in severe stenosis of one renal artery (Bianchi *et al.*, 1970; Woods *et al.*, 1986; Anderson *et al.*, 1987). Anderson *et al* (1987), have demonstrated an initial reduction in GFR following renal artery stenosis (one kidney model) to approximately 49% over the first 24 h, this value is restored to normal levels by 2-weeks (Anderson *et al.*, 1987). Two weeks following renal artery stenosis, plasma renin levels were also back to pre-stenosis levels (Anderson *et al.*, 1987). It has also been demonstrated that the rise in blood pressure in this model restores GFR and RBF to normal, but RVR remains increased (Anderson *et al.*, 1990).

1.5.2.2 Renal wrap/Page hypertension

Renal wrap or Page hypertension, first described in 1939 by Page and colleagues (Page, 1939), involves wrapping the kidneys in cellophane in-order to induce thickening of the renal capsule. This thickening eventually encases the kidney in a firm fibrous capsule approximately 3-4 mm in thickness (Page, 1939). The thickened capsule produces compression of the kidney leading to increased resistance to blood flow in a manner similar to that seen in renal artery stenosis. Bilateral renal wrap has been shown to produce 50% reductions in GFR and RBF over the first 28 days and a 42 mmHg rise in arterial pressure (Denton *et al.*, 1983). The authors later went on to show that angiotensin II plays a role in the developing hypertension as enalapril treatment attenuated the degree of renal wrap hypertension and reduction in RBF (Denton and Anderson, 1985).

1.5.3 Renal haemodynamics in the SHR

During the developmental phase of hypertension at 4-6 weeks of age, SHR exhibit a significantly increased RVR compared to age-matched WKY rats (Dilley *et al.*, 1984; Rettig *et al.*, 1990; Uyehara and Gellai, 1993). Micropuncture studies have shown an increase in both afferent and efferent arteriolar resistances in SHR compared to WKY rats at this age (Dilley *et al.*, 1984). It is believed that this increase in total renal resistance is primarily due to the greater increase in pre-glomerular resistance (Arendshorst and Beierwaltes, 1979b; Azar *et al.*, 1979; Hsu *et al.*, 1982; Dilley *et al.*,

1984). Along with the increase in RVR, SHR had reduced GFR and SNGFR, RBF, single nephron blood flow (SNBF) and RPF (Dilley *et al.*, 1984; Uyehara and Gellai, 1993). Although Dilley *et al.* (1984) measured similar glomerular capillary pressures in SHR and WKY rats at 6-weeks of age, the significant reduction in SNGFR can be explained by the reductions in glomerular ultrafiltration coefficient and single nephron plasma flow (Dilley *et al.*, 1984). SHR also demonstrate sodium and water retention compared to age-matched WKY rats (Dilley *et al.*, 1984).

Once hypertension has developed in SHR, and reached the maintenance phase, RVR is still significantly greater than in age-matched WKY rats (Arendshorst and Beierwaltes, 1979b). The increase in RVR is primarily due to the greater difference in mean arterial pressure, but similar RBF. GFR on the other hand is equal in SHR and WKY rats during this maintenance phase (Arendshorst and Beierwaltes, 1979b). Hydrostatic pressures along the renal vasculature were similar between SHR and WKY rats at 12-weeks of age (Arendshorst and Beierwaltes, 1979b). Afferent arteriolar resistance is significantly increased in SHR compared to WKY rats at 12-weeks of age, but no difference in efferent arteriolar resistance was observed (Arendshorst and Beierwaltes, 1979b). Urinary sodium and water excretion in SHR reaches levels seen in WKY rats by 8-9 weeks of age (Dilley *et al.*, 1984).

Changes in renal haemodynamics have been observed prior to any significant alterations in blood pressure in the SHR. Thus posing the question as to whether it is indeed the kidney that is the primary influencing factor in the predisposition to hypertension in the SHR. Reductions in GFR and RBF, observed in the developmental phase (6- to 10-weeks of age) of SHR hypertension, are normalised once hypertension has reached the maintenance phase (adulthood). RVR on the other hand remains elevated (Dilley *et al.*, 1984).

1.5.4 Renal transplantation - SHR

Coburn *et al* (1972) were the first to investigate the influence of renal grafts from SHR donors on chronic blood pressure levels in normotensive recipients, and *vice versa*. To avoid immunological rejection of the renal grafts, F_1 hybrids bred from hypertensive and normotensive parents were chosen as the recipients (Rettig *et al.*, 1993). It was found that the blood pressure of the recipients from SHR donors increased progressively for several weeks following renal transplantation, reaching the same level as the donor strain, and remaining at that level permanently (Kawabe *et al.*, 1979). When these experiments were performed using WKY rat kidneys, the blood

pressure of the F_1 hybrids was either reduced or did not change (Kawabe *et al.*, 1979; Rettig *et al.*, 1993).

The above mentioned studies were performed in adult rats, at which time hypertension was already established. Therefore, although it strongly supports the involvement of the kidney in the development of hypertension, we still cannot conclusively say that it is the sole cause. The development of hypertension in the recipients may in fact be due to secondary renal damage, due to the high perfusion pressure before transplantation (Rettig *et al.*, 1990). In fact, Rettig *et al* (1993) went on to show that normotensive donor kidneys exposed to high perfusion pressure did promote hypertension in otherwise normotensive recipients.

It is always an issue as to whether an increase in blood pressure has a direct effect on the kidney to produce changes in the renal vasculature or whether alterations in renal structure occur first, due to some other factor, which then produce an increase in blood pressure. In-order to distinguish between the primary and secondary renal mechanisms involved in the post-transplantation hypertension, Rettig *et al* (1990, 1993) sought to avoid high renal perfusion pressure in the donor kidneys. Stroke-prone SHR (SHR-SP) were treated with ramipril, an angiotensin converting enzyme inhibitor (ACEi), for the duration of their life. This antihypertensive treatment reliably maintained systolic blood pressure below 120 mmHg. Renal grafts were removed at 20-weeks of age and transplanted in age-matched F_1 hybrid recipients. Despite the prevention of hypertension in the SHR-SP, the recipients of the renal grafts still developed post-transplantation hypertension (Rettig *et al.*, 1993), compared to renal grafts from WKY rats, indicating therefore, that there is a strong genetic component to the predisposition of hypertension in the recipients. There was also no difference in GFR and RBF between recipients of renal grafts from either SHR-SP or WKY rat donors.

These studies thereby support not only a renal component but also a genetic component to the development of hypertension. Similar results have also been shown in human subjects. A study performed in the U.S.A, involved six black essential hypertensive patients with nephrosclerosis and renal failure, who received renal transplants from normotensive donors (Curtis *et al.*, 1983). After an average follow up period of 4.5 years, all recipients were normotensive, without receiving antihypertensive treatment and had evidence of reversal of hypertensive damage to the heart (Curtis *et al.*, 1983). This study also supports the involvement of the kidney in the predisposition to human essential hypertension.

1.5.5 Renal transplantation in other genetic rat models

Renal transplantation studies have also been performed in other genetic rat models of essential hypertension (Rettig *et al.*, 1993; De Jong and Ganten, 1994). In both the MHS and the Dahl-S rats, hypertension also appears to follow the kidney (Rettig *et al.*, 1993; De Jong and Ganten, 1994), as is the case with the SHR and WKY rat models. Immunological graft rejection is not a problem with these two strains of rats as they are genetically very similar to their respective normotensive controls. Results demonstrated that a genetic defect residing in the kidney appears to play a major role in the aetiology of hypertension, in the MHS and Dahl-S rat strains (Rettig *et al.*, 1993; De Jong and Ganten, 1994).

1.5.6 Structural changes in the vasculature of the SHR

It has been demonstrated that the SHR display an increase in the wall to lumen ratio of both the mesenteric and renal vasculature (Mulvany *et al.*, 1980; Lee *et al.*, 1983; Nordborg *et al.*, 1983; Smeda *et al.*, 1988b). These structural changes were thought to be a secondary response to an increase in arterial pressure, however there is evidence which suggests that these structural changes occur prior to a significant increase in arterial pressure (Nordborg *et al.*, 1983; Lee, 1985; Smeda *et al.*, 1988b). One group has shown that as early as 15-days of age, both mesenteric and renal vessels demonstrate significant increases in wall to lumen ratios in SHR compared to WKY rats (Nordborg *et al.*, 1983). Other groups have found no difference until much later when arterial pressure is significantly different to normotensive controls (Bohlen and Lobach, 1978; Limas *et al.*, 1980; Mulvany *et al.*, 1980). Recently Sabbatini *et al* (2000) examined the afferent and efferent arterioles of 26-week old SHR and WKY, and demonstrated an increase in wall to lumen ratio in both vessels. Often the increase in wall to lumen ratio has been interpreted as synonymous with growth, but this is not necessarily the case. Increased wall to lumen ratio can also be due to either hypertrophy (increase in the size of the smooth muscle cells) or remodelling (re-arrangement of the same smooth muscle cells around a narrower lumen, (Heagerty *et al.*, 1993). Whether this increase in wall to lumen ratio is the cause or effect of the development of hypertension is still open to debate, but it is a very important characteristic shared by the SHR and human essential hypertensive patients (Folkow, 1982).

There are a number of possible causes for the structural changes, some of which have been explored unsuccessfully and some require further study. Certainly in our laboratory we have shown that although blood pressure can be reduced with angiotensin type 1 receptor blockade or enalapril treatment, the hypertrophy generally associated with the renal arterial vasculature of the SHR is not reversed (Kett *et al.*, 1995; Kett *et al.*, 1996b; Anderson *et al.*, 1997). Other studies have also examined the effects of antihypertensive treatment on the SHR pre-glomerular vasculature and found that although blood pressure is reduced the increased wall to lumen ratio is still present (Mulvany, 1987; Smeda *et al.*, 1988a; Tsoporis and Leenen, 1988; Ledingham *et al.*, 2000; Sabbatini *et al.*, 2000). These studies do not support the involvement of an increased blood pressure in the hypertrophy of the SHR vasculature.

As mentioned previously, Poiseuille's law states that resistance of a vessel is proportional to the fourth power of the vessel radius. Since the kidneys receive 20% of total cardiac output, given Poiseuille's relationship, any reduction in vessel lumen dimensions has important consequences on total peripheral resistance (Korner and Angus, 1992; Heagerty *et al.*, 1993). The encroachment of the vessel walls on the lumen, as is the case in the SHR, would result in an increased resistance to blood flow under resting conditions (Korner *et al.*, 1989) and renders the vessel more responsive to vasoactive stimuli (Korner and Angus, 1992). This constant increase in vascular resistance would mean a reduction in both RBF and GFR. However, RBF and GFR are normalised, but arterial pressure remains elevated (Arendshorst and Beierwaltes, 1979a; Göthberg *et al.*, 1983; Harrap and Doyle, 1986; Smeda *et al.*, 1988b). Therefore, it is quite possible for structural changes in the renal vasculature to be responsible for the development of hypertension (at least in the SHR).

1.6 How might the renal nerves lead to hypertension in the SHR?

The renal sympathetic nerves may lead to the development of hypertension in the SHR in a number of ways. As discussed in *Section 1.4 The sympathetic nervous system in hypertension*, the SHR show an increase in renal sympathetic nerve activity (Judy *et al.*, 1976; Ricksten *et al.*, 1981; Thorén, 1987) and circulating levels of noradrenaline are also increased (Donohue *et al.*, 1988). This increase in renal nerve activity can act directly on the renal vasculature by stimulating adrenergic receptors to produce vasoconstriction (see 1 in *Figure 1.3*). An increase in

RSNA also leads to an increase in renin release (see 2 in *Figure 1.3*), thereby leading to an increase in angiotensin II formation, which again can act directly to produce renal vasoconstriction or feedback to facilitated release of noradrenaline (Dominiak *et al.*, 1987; Dendorfer *et al.*, 1998). Increased RSNA can also lead to a reduction in sodium and water excretion (Block *et al.*, 1952; see 3 in *Figure 1.3*) subsequently increasing cardiac output (Guyton *et al.*, 1972). Initially of special interest to this thesis, renal vasoconstriction could also be due to hypertrophy and/or remodelling of the smooth muscle cells. There is evidence to suggest that catecholamines and angiotensin II can act as growth promoting factors on cultured VSMCs (Chen *et al.*, 1995; Kubo *et al.*, 2000), and may be capable of producing such changes *in vivo*.

All three pathways illustrated in *Figure 1.3* lead to constriction of the renal vasculature, increases in total peripheral resistance and perhaps cardiac output, reduction in RBF and GFR and ultimately leading to development of hypertension. Fink and Brody (1980) have previously discussed similar pathways.

The portion of this schema of interest to me is pathway 1, whereby an increase in RSNA and circulating noradrenaline can lead to hypertrophy and/or remodelling of the VSMCs. I have hypothesised that an increase in RSNA, via either direct or indirect actions, leads to VSMC growth in young SHR. As mentioned previously young SHR have elevated levels of NGF, angiotensin II and both circulating and tissue stores of noradrenaline. Cultured VSMC obtained from SHR have also been shown to produce angiotensin II locally, whereas VSMC from WKY rats do not (Kubo *et al.*, 2000). Apart from NGF, angiotensin II and noradrenaline, there also exists other growth factors and vasoactive substances which could also act in concert to produce VSMC growth and vascular constriction within the kidney (Gibbons, 1995).

Angiotensin II can promote VSMC growth via angiotensin type 1 receptor activation and induction of the proto-oncogenes *c-fos* and *c-myc* (Gibbons, 1995; Kubo *et al.*, 2000). Therefore, in the SHR, elevated levels of angiotensin II produced by an increase in RSNA could lead to VSMC growth *in vivo*. Angiotensin II has also been shown to induce expression of the proliferative factors such as, platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), also leading to VSMC growth (Itoh *et al.*, 1993).

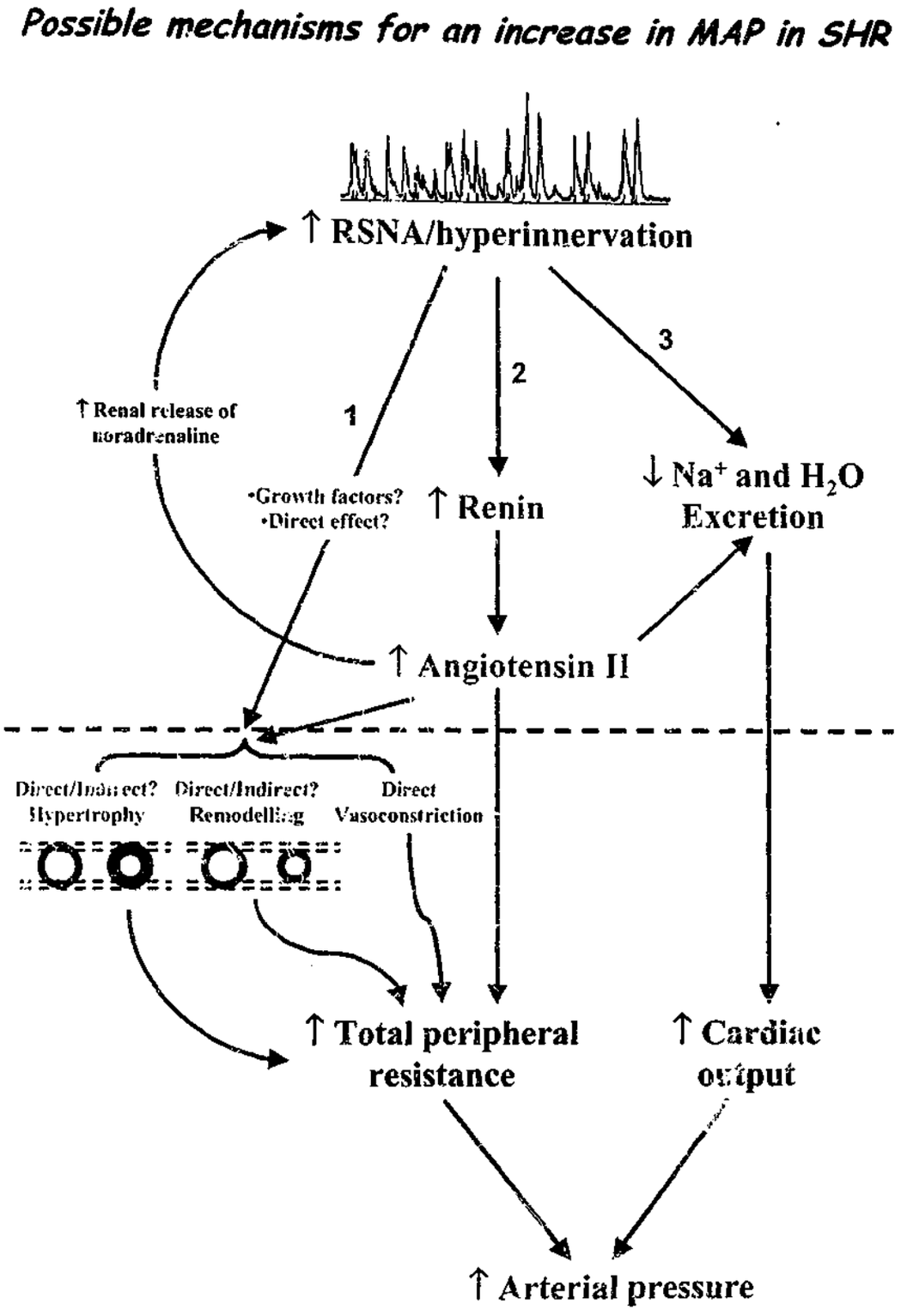

Figure 1.3 Schematic diagram illustrating the possible involvement of an increase in renal sympathetic nerve activity (RSNA), hyperinnervation of the renal vasculature and the renin-angiotensin system in the development of hypertension in SHR (below the dashed line are the possible pathways leading to an increase in arterial pressure).

The evidence reviewed in this chapter has thus far demonstrated the importance of the kidney, the sympathetic nervous system and more specifically the renal sympathetic nerves in the development of hypertension in the SHR. As covered above (*Section 1.6 How might the renal nerves lead to hypertension in the SHR?*) the renal sympathetic nerves can have both direct and indirect effects on the VSMCs of the renal vessels leading to hypertrophy and/or remodelling in the SHR. The hypertrophy/remodelling would translate into an increase in renal vascular resistance, previously hypothesised to mainly reside in the pre-glomerular vasculature of the SHR. As the kidney receives a large proportion of total cardiac output, any change in renal vascular resistance would have a greater contribution to total peripheral resistance and thereby lead to the development of hypertension.

1.7 General hypothesis

Given the review of the literature above the main hypothesis, to be examined in this thesis therefore, is that narrowing of the intra-renal arteries (like main renal artery stenosis), due to wall hypertrophy/remodelling (see *Figure 1.4*), is the cause of essential hypertension (Anderson, 1994; Anderson *et al.*, 1995). Recent evidence has led us to further hypothesise that it is in fact the renal nerves that are responsible for the development of the hypertrophy/remodelling of the intra-renal vasculature and therefore the development of essential hypertension (Tomoda *et al.*, 1997).

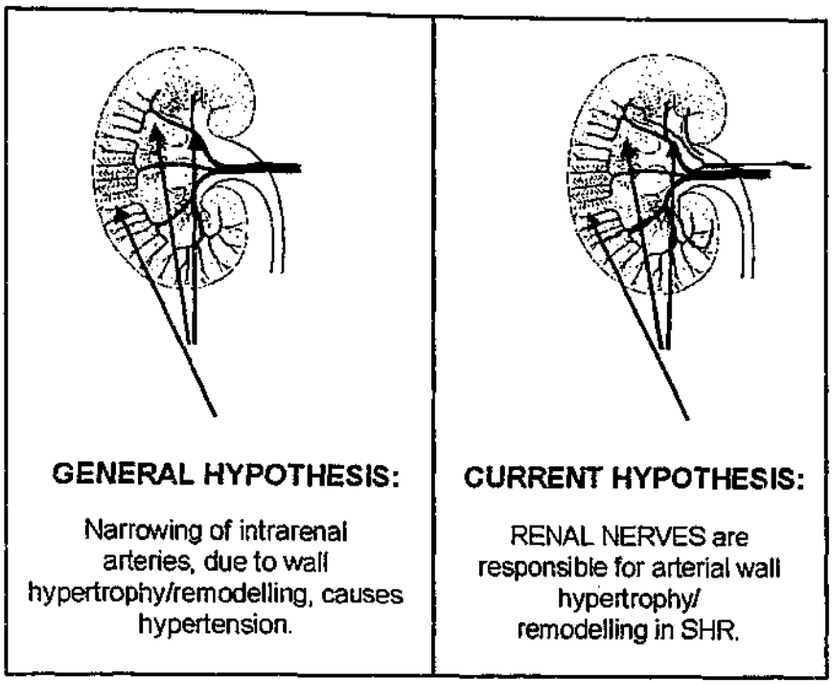

Figure 1.4 A schematic diagram defining the general hypothesis. The renal sympathetic nerves may be responsible for producing structural changes in the renal vasculature, which then behave in a manner similar to narrowing of the main renal artery.

1.7.1 Specific aims

1. To investigate the role of the renal nerves in the development of hypertension. In the first experiment I aimed to establish that in our SHR colony and using my methods and protocol I could affect the development of hypertension in this strain. Bilateral renal denervation was performed on 6-week old SHR and their arterial pressure followed over a 4-week period, until 10-weeks of age.

2. To examine the influence of the renal nerves on the lumen diameter of the pre-glomerular vasculature and in particular the afferent arteriole in the SHR. Chronic unilateral renal denervation was performed on 6-week old SHR and following vascular casting the lumen diameters of the afferent and efferent arterioles, and interlobular artery were examined four weeks later.

3. To determine the influence of the renal nerves on the lumen and wall dimensions of the larger renal vessels, in particular the interlobular and arcuate arteries of the SHR, which are known to show hypertrophy at this age (6 to 10-weeks). Chronic unilateral renal denervation was performed on 6-week old SHR and WKY rats. Using unbiased

stereological techniques the interlobular and arcuate artery wall and lumen dimensions were examined four weeks later.

4. To investigate the functional significance of the renal nerves, *in vivo*, on the hypertrophy/remodelling of the renal vasculature in the SHR. Chronic unilateral renal denervation was performed on 6-week old SHR and four weeks later whole kidney renal function studies were performed and glomerular capillary pressure measured using micropuncture techniques.

CHAPTER 2

CHAPTER 2

General methods

This chapter outlines the methods used generally throughout this thesis. In each subsequent chapter, methods specific to that chapter will be covered in detail.

2.1 Animals and experimental groups

Male SHR and WKY rats derived from the original Okamoto strain, were obtained from the Baker Medical Research Institute at 6-weeks of age, and housed two per cage in a room maintained at 23-25°C with a 12 h light/dark cycle. Standard rat chow and water were supplied *ad libitum*. All experiments were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation as being in accord with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NH&MRC, 1997).

2.1.1 Experimental groups

Chapter 3: Effect of chronic bilateral renal denervation on development of hypertension in the SHR.

Group 1 – Sham operated SHR at 6-weeks of age (SHR_S, n = 8)

Group 2 – SHR that underwent bilateral renal denervation at 6-weeks of age (SHR_{BDx}, n = 8)

Chapter 4: Effect of chronic unilateral renal denervation on the renal arterioles of the SHR kidney – vascular casting.

Group 1 – Sham operated SHR at 6-weeks of age (SHR_S, n = 11)

Group 2 – SHR that had undergone unilateral renal denervation at 6-weeks of age (SHR_{Dx}, n = 10)

Chapter 5: ***Stereological analysis of the renal vasculature of the SHR and WKY rat following chronic unilateral denervation.***

Group 1 – Sham operated WKY at 6-weeks of age (WKY_S, n = 7)

Group 2 – WKY that had undergone unilateral renal denervation at 6-weeks of age (WKY_{Dx}, n = 7)

Group 3 – Sham operated SHR at 6-weeks of age (SHR_S, n = 6)

Group 4 – SHR that had undergone unilateral renal denervation at 6-weeks of age (SHR_{Dx}, n = 8)

Chapter 6: ***Micropuncture analysis of the renal haemodynamics following chronic unilateral renal denervation in SHR.***

Group 1 – Sham operated SHR at 6-weeks of age (SHR_S, n = 7)

Group 2 – SHR that had undergone unilateral renal denervation at 6-weeks of age (SHR_{Dx}, n = 9)

2.2 Surgical denervation of the kidney (Chapters 3, 4, 5, & 6)

At 6-weeks of age, rats were subjected to unilateral or bilateral renal denervation or sham operation. The rats were anaesthetised using a mixture of sodium pentobarbitone (30 mg/kg body weight; Nembutal, Rhone Merieux Australia Pty Ltd, Qld, Australia), and methohexitone sodium (40 mg/kg bodyweight; Brietal Sodium, Eli Lilly Australia Pty Ltd, NSW, Australia) and saline (0.9% NaCl), administered via an intra-peritoneal (i.p.) injection at a dose of 0.1 ml/100g body weight. Rats were then placed on a heated table for surgery.

The left kidney, renal artery and vein were exposed via a flank incision. Denervation was performed by clearing all the way around the kidney and then carefully stripping away the adventitia and any visible renal nerves from the renal vessels (Tomoda *et al.*, 1997). The vessels were then painted with a 10% phenol in alcohol solution, this was left for approximately one minute and then rinsed away with copious amounts of saline. For the bilaterally denervated animals, this same procedure was performed on the right kidney. The sham-operation involved exposing the left kidney and renal vessels for the same length of time as for the denervation procedure, except that the vessels were not stripped of the adventitia and not painted with 10%

phenol. The same procedure was performed on the right kidney for the group acting as the control for the bilaterally denervated animals. Following surgery, rats were allowed to recover in their own box on a heating pad. Once the rats were conscious, buprenorphine (0.01 mg/kg; Temgesic, Reckitt and Colman Pharmaceuticals, NSW, Australia) was administered subcutaneously.

2.3 Conscious blood pressure measurement

2.3.1 Indirect tail-cuff method (Chapters 3 & 4)

Systolic blood pressure was measured in conscious SHR and WKY rats using a multi-channel tail plethysmography system that used photoelectric sensors to detect tail pulses (IITC Life Science Instruments, Woodland Hills, California, U.S.A.). Rats were placed in perspex cylinders appropriate to their body size and placed in the test chamber, maintained at 27-28°C (no more than 8 rats were assessed at one time). After a 15-20 min stabilisation period measurements began. Three pressure measurements were taken for each rat, per pass. Two passes were carried out per day and thus, six pressure measurements in total were taken for each rat on any given day. The systolic blood pressure for the session was taken as the mean of the six measurements. Diastolic blood pressure, mean arterial pressure and heart rate were also measured using the tail-cuff technique.

2.3.2 Direct tail artery method (Chapters 3, 4, 5, & 6)

At 10-weeks of age, conscious mean arterial pressure (MAP) and heart rate (HR) were measured directly via a catheter inserted acutely into the tail artery. Rats were anaesthetised with methohexitone sodium (50 mg/kg body weight, i.p.), and the tail artery catheterised with a teflon catheter attached to a polyvinyl chloride catheter (SV65; ID = 0.86 mm, OD = 1.52 mm; Dural Plastics and Engineering, NSW, Australia). Following a 1 hour recovery period, conscious MAP was measured by attaching the tail artery catheter to a pressure transducer (Cobe, Arvarda, U.S.A.) and HR measured by a tachometer (Model 173; Baker Medical Research Institute, Melbourne, Australia), activated by the pressure pulse. The output was then recorded on a Grass polygraph and relayed to a computer equipped with an A-D acquisition card. Conscious MAP and HR were recorded as 2 s averages for 15 min. An average MAP was obtained for this period prior to perfusion fixation (*Chapters 4 and 5*).

2.4 Perfusion fixation of the kidney (Chapters 4 & 5)

Kidneys were perfusion fixed in-order to study the renal vasculature, at maximal dilatation and at pressures equivalent to arterial pressure.

Following the measurement of conscious tail artery pressure, rats were anaesthetised with sodium pentobarbitone (40 mg/kg body weight, i.p.). The abdominal aorta and inferior *vena cava* were exposed via a midline incision. The superior mesenteric and iliac arteries were isolated and tied off near their junction with the aorta. A loose ligature was placed around the aorta above the level of both renal arteries. The lower abdominal aorta was cannulated with a polyethylene catheter (PE 160 ID = 1.14 mm, OD = 1.57 mm; Clay Adams, division of Becton Dickinson and Company, NJ, U.S.A.) for retrograde perfusion, and connected to the perfusion apparatus via a 3-way stopcock. At a mean pressure corresponding to that measured on the morning of the experiment (plus 30 mmHg, which allows for the inherent resistance of the perfusion apparatus), the kidneys were cleared of blood with a rinsing solution (0.1M PO_4 buffer plus 0.24 mg/ml sodium nitroprusside for maximum dilatation; pH 7.4 at room temperature). At the commencement of perfusion the *vena cava* was severed at the junction of the renal vein to allow release of the perfusate, and the aortic ligature was tightened. This resulted in immediate blanching of the kidneys. The kidneys were then perfusion-fixed with 4% paraformaldehyde in 0.1M PO_4 buffer for 30-60 sec (*Chapter 4*) or 10% buffered formalin for 5 min (*Chapter 5*).

Following perfusion fixation either methacrylate resin was injected into the kidneys, for *Chapter 4* (*Section 4.2 Methods*), or both kidneys were removed and stored in 10% buffered formalin until they were processed for unbiased stereological analysis (*Section 5.2 Methods*).

2.5 Tissue noradrenaline assay (Chapters 3,4 & 6)

Tissue noradrenaline content was used as an indicator of the level of innervation at 10-weeks of age. In *Chapter 3*, tissue noradrenaline levels were measured in both left and right kidneys from 3 sham operated SHR and 5 bilaterally denervated SHR. In *Chapter 4*, four rats (2 sham operated and 2 unilaterally denervated), were used for measurement of tissue noradrenaline content in both left and right kidneys. In *Chapter 6*, four animals from each group were used for noradrenaline content determination in both left and right kidneys.

At 10-weeks of age, following measurement of conscious tail artery pressure, rats were anaesthetised with sodium pentobarbitone (40 mg/kg i.p.) and the kidneys rapidly excised, weighed and frozen in liquid nitrogen, and then stored at -80°C until homogenisation. Kidneys were homogenised in 0.4M perchloric acid (1 ml/0.5 g tissue wet weight). The homogenate was centrifuged at 3000 rpm for 30 min, and the supernatant was then collected and stored at -80°C for subsequent catecholamine analysis. At the time of assay samples were thawed at room temperature and catecholamines extracted from the supernatant by alumina adsorption (Medvedev *et al.*, 1990). Following extraction, the catechols were separated by high-performance liquid chromatography and the amount quantified with the use of electrochemical detection (Medvedev *et al.*, 1990).

Mean absolute levels of noradrenaline were obtained for each of the groups.

2.6 Immunohistochemistry (Chapters 3 & 5)

Immuno-staining of kidney sections for tyrosine hydroxylase was used as a visual indicator of the presence or absence of renal sympathetic nerves. Following either perfusion fixation (*Chapter 5*) or immersion fixation (*Chapters 3*) in 10% buffered formalin, kidney slices were embedded in paraffin and 12 µm sections obtained. Slides were then de-waxed and underwent a series of re-hydration steps. Following this, the slides were washed in phosphate buffered saline (PBS; 3 x 10 min), after which they were incubated at room temperature for 1 hour with avidin (1:500; Vector Laboratories Inc., Burlingame, CA, U.S.A.). An overnight incubation at room temperature with a mouse monoclonal antibody generated against tyrosine hydroxylase (TH; 1:100; DiaSorin, Stillwater, Minnesota, U.S.A.), diluted in 10% normal horse serum in PBS, completed the first day of the procedure. On the following day the slides were washed again in PBS (3 x 10 min), and incubated for 2 hours with a biotinylated secondary antibody to mouse IgG (1:200; Vector Laboratories Inc., Burlingame, CA, U.S.A.). After washing in PBS once again (3 x 10 min), the slides were incubated with an avidin-biotin complex for 1 hour, following a further wash in PBS (5 min) 0.5 mg/ml diaminobenzidine was added to each slide for 10 min. At the end of this period hydrogen peroxide (final concentration 0.075%) was added and the reaction allowed proceeding for 5 min before washing in PBS. Sections were then dehydrated in ethanol, coverslipped, and examined under a light microscope (*Figure 2.1*).

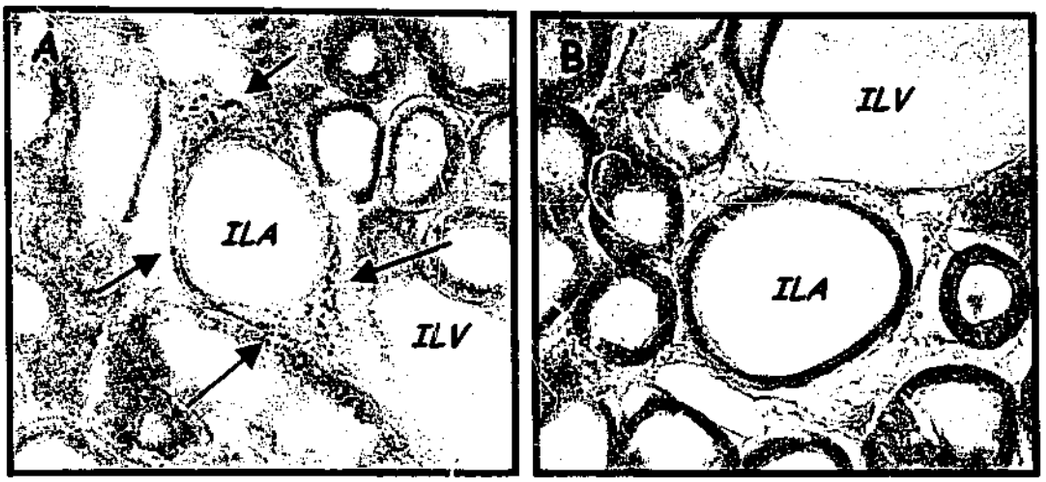

Figure 2.1 Light micrographs of typical interlobular arteries (ILA) from a sham operated kidney (A) demonstrating tyrosine hydroxylase staining for sympathetic nerve fibres (arrows) and a denervated (B) SHR kidney.

CHAPTER 3

CHAPTER 3

Effect of chronic bilateral renal denervation on the development of hypertension in the SHR

3.1 Introduction

The specific aim of this chapter was to examine whether chronic bilateral renal denervation, in my hands, attenuated the blood pressure development over a 4-week period in the SHR.

As reviewed in *Chapter 1*, chronic bilateral renal denervation, during the developmental phase of hypertension in the SHR has been shown to delay the onset of hypertension (Liard, 1977; Kline *et al.*, 1978; Winternitz *et al.*, 1980; Norman Jr and Dzielak, 1982). In a detailed examination of this, Norman and Dzielak (1982) performed bilateral renal denervation in SHR and Wistar-Kyoto (WKY) rats every 3-weeks for 16-weeks, starting at 4-weeks of age. This study demonstrated that repeated bilateral renal denervation blocked the expected progressive elevation of arterial pressure in SHR by 30-40%, while not influencing the arterial pressure of WKY rats (Norman Jr and Dzielak, 1982).

The above mentioned studies clearly demonstrated the importance of the renal sympathetic nerves in the development of SHR hypertension, but little about the potential mechanisms involved. Previous studies however, did not use the same protocol I had planned to use, and therefore it was important to demonstrate that my protocol would also attenuate the development of hypertension in the SHR.

3.2 Methods

Male SHR (n = 16), derived from the original Okamoto strain and genetically monitored (obtained from the Baker Medical Research Institute), were housed in a room maintained at constant temperature (23-25°C) with a 12h light/dark cycle. Rats were housed two per cage, and had free access to standard rat chow and tap water. All experiments were in accord with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH&MRC, 1997), and approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation.

3.2.1 Surgery and animal monitoring

At 6-weeks of age, rats were subjected to either bilateral renal denervation (SHR_{BDx}; n = 8) or sham operation (SHR_S, n = 8; refer to *Section 2.2 Surgical denervation of the kidney*) and monitored for the following four weeks. Once a week, body weight was recorded and conscious blood pressure was measured using tail-cuff sphygmomanometry (*Section 2.3.1 Indirect tail-cuff method*). At 10-weeks of age, conscious mean arterial pressure was measured directly from the tail artery (*Section 2.3.2 Direct tail artery method*). Blood samples were then collected for analysis of plasma renin activity (1ml blood sample, measured by radio-immunoassay; Oliver *et al.*, 1990) and creatinine concentration (0.25ml blood sample, Beckman Synchron CX5 Clinical System Analyzer using a modified Jaffé method; Heinegard and Tiderstom, 1973). The rat was then anaesthetised with sodium pentobarbitone (40 mg/kg body weight, i.p.). Both kidneys were quickly excised and either rapidly frozen in liquid nitrogen for later noradrenaline assay (SHR_{BDx}; n = 3 and SHR_S; n = 5, *Section 2.5 Tissue noradrenaline assay*), or immersion fixed in 10% buffered formalin for immunohistochemical analysis of tyrosine hydroxylase (SHR_{BDx}; n = 5 and SHR_S; n = 3, *Section 2.6 Immunohistochemistry*).

3.2.2 Statistics

All data points are expressed as the between animal mean ± sem. Repeated measures ANOVA was used to analyse the tail-cuff data, testing whether the effect of denervation over time was parallel to the sham operated group. Tissue noradrenaline content of the SHR_{BDx} animals was analysed as the change from control, with control being the sham operated left kidney in the SHR_S group. One way ANOVA was used to

compare the parameters in Table 3.1 and 3.2 (sham vs denervated). A $P \leq 0.05$ was considered to be of statistical significance.

3.3 Results

The two groups were of similar body weight at 6-weeks of age (prior to surgery, SHR_S 123 ± 7 and SHR_{BDx} 124 ± 4 g), and at 10-weeks of age (*Table 3.1*). There were no significant differences observed for either kidney or ventricle weights between the two groups at 10-weeks of age. Plasma creatinine levels were also similar in the two groups. Plasma renin activity, although slightly reduced in the SHR_{BDx}, was not significantly different between groups ($P = 0.09$, *Table 3.1*).

Table 3.1 Variables at 10-weeks of age.

	SHR_S (n = 8)	SHR_{BDx} (n = 8)	*P*
Body weight (g)	268 ± 7	258 ± 6	0.30
Haematocrit (%)	45.8 ± 0.7	46.3 ± 0.4	0.54
Right kidney (g wet weight)	1.18 ± 0.05	1.18 ± 0.04	0.99
Left kidney (g wet weight)	1.17 ± 0.05	1.20 ± 0.06	0.65
Right ventricle (g/ 100 g body weight)	0.074 ± 0.004	0.071 ± 0.004	0.66
Left ventricle (g/ 100 g body weight)	0.261 ± 0.009	0.247 ± 0.007	0.21
PRA (ng AI/ml plasma/hr)	7.2 ± 1.5	4.4 ± 0.5	0.09
Plasma creatinine (μmol/L)	37.1 ± 2.0	36.2 ± 1.2	0.73

Variables measured at 10-weeks of age in bilaterally renal denervated (SHR_{BDx}) or sham operated (SHR_S) rats. Plasma renin activity (PRA) is expressed as ng of angiotensin I (AI) per ml of plasma. One-way ANOVA was used to test for a difference between the two groups.

3.3.1 Indirect measurement of tail-cuff blood pressure

Systolic blood pressure (SBP) and heart rate (HR) were measured from 7- to 9-weeks of age via tail-cuff sphygmomanometry (*Section 2.3.1 Indirect tail-cuff method*). SBP was significantly lower in the SHR_{BDx} group when compared to the SHR_S group over this 3-week period ($P = 0.05$, *Figure 3.1*). Both groups demonstrated a significantly greater SBP ($P = 0.05$) over the 3-weeks following surgery. During this

period, HR fell in the SHR_{BDx} and the SHR_S groups but no significant difference was observed between groups.

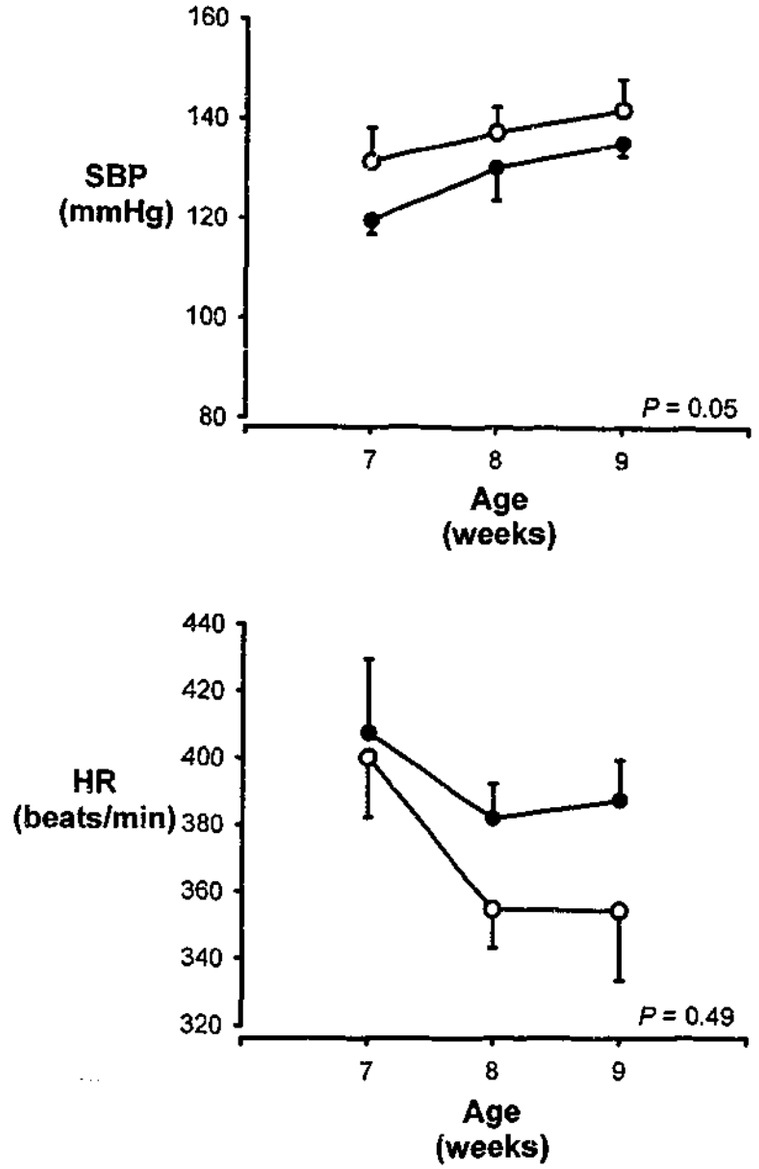

Figure 3.1 Conscious systolic blood pressure (SBP) and heart rate (HR) measured via tail-cuff sphygmomanometry in bilaterally denervated SHR (SHR_{BDx}; closed circles) and sham operated SHR (SHR_S; open circles) over the three week period following surgery. *P* values were obtained using repeated measures ANOVA, testing whether the effect in the SHR_{BDx} group over time was parallel to the SHR_S group.

Mean arterial pressure (MAP) and diastolic blood pressure (DBP), also measured via tail-cuff sphygmomanometry were not significantly different between the

groups from 7 to 9-weeks of age, however MAP tended to be lower in the SHR_{BDx} group ($P = 0.06$, *Figure 3.2*).

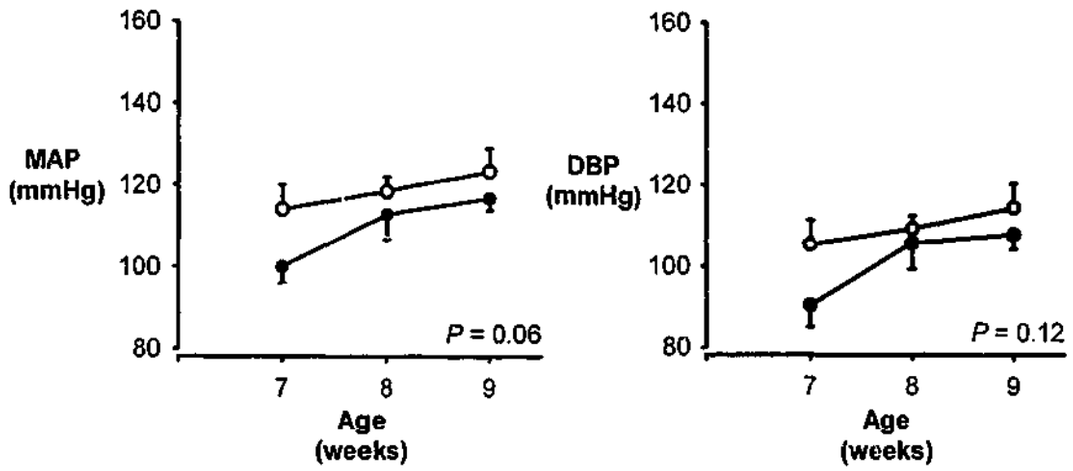

Figure 3.2 Conscious mean arterial pressure (MAP) and diastolic blood pressure (DBP) measured via tail-cuff sphygmomanometry in bilaterally denervated SHR (SHR_{BDx}; closed circles) and sham operated SHR (SHR_S, open circles) over the three week period following surgery. The *P* value was obtained using repeated measures ANOVA, testing whether the effect in the SHR_{BDx} group over time was parallel to the SHR_S group.

3.3.2 Direct measurement of tail artery blood pressure

Conscious MAP was also measured directly via acute catheterisation of the tail artery at 10-weeks (*Figure 3.3*). The SHR_{BDx} group had a significantly reduced MAP at 10-weeks of age compared to the SHR_S group (128.2 ± 2.4 and 137.2 ± 3.2 mmHg respectively; $P = 0.04$). Heart rate was not significantly different between the SHR_{BDx} group (342 ± 9 beats/min) and the SHR_S group (319 ± 7 beats/min) at 10-weeks of age.

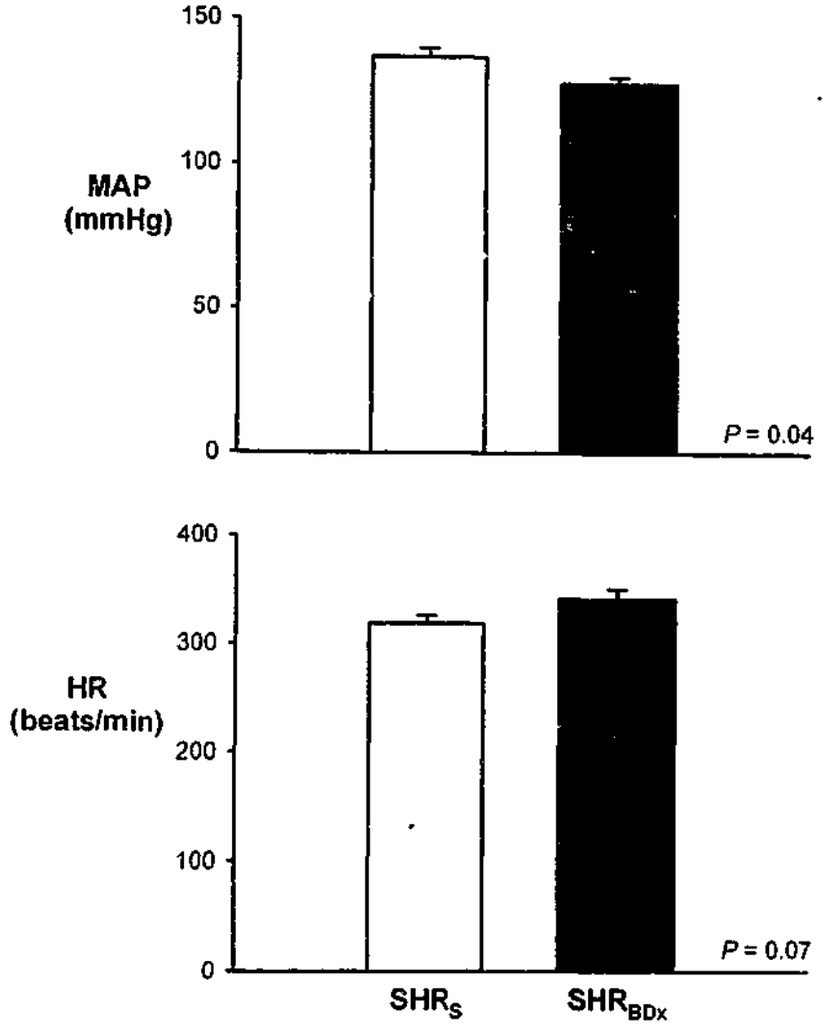

Figure 3.3 Conscious mean arterial pressure (MAP) and heart rate (HR) measured at 10-weeks of age, via tail artery catheter, in the bilaterally denervated group (SHR_{BDx}, solid bar) and the sham operated group (SHR_S, open bar). *P* values were obtained using a one-way ANOVA, testing for an effect of denervation.

3.3.3 Tissue noradrenaline content

Tissue noradrenaline content in the left kidney of the SHR_{BDx} group was 76% lower than the left kidney of the SHR_S group ($P = 0.01$, *Table 3.2*). The noradrenaline content of the right kidney of the SHR_{BDx} group was 82% less than the same kidney of the SHR_S group ($P = 0.004$, *Table 3.2*).

Table 3.2 Tissue noradrenaline content at 10-weeks of age.

	SHR_S (n = 5)	SHR_{BDx} (n = 3)	*P*
Left kidney (ng NA/g wet weight)	77.1 ± 5.4	18.6 ± 10.1	0.01
Right kidney (ng NA/g wet weight)	92.1 ± 13.6	16.8 ± 3.9	0.004

Tissue noradrenaline (NA) content in both left and right kidneys of bilaterally denervated SHR (SHR_{BDx}) and sham operated SHR (SHR_S) at 10-weeks of age. Values are the mean ± sem. *P* values were obtained from a one-way ANOVA, testing for an effect of treatment.

3.3.5 Immunohistochemistry

Immunohistochemistry was performed on both left and right kidneys from the SHR_{BDx} and the SHR_S groups (n = 5 and 3 respectively), staining for tyrosine hydroxylase. All sections examined from the SHR_S group showed positive staining for tyrosine hydroxylase (*Figure 3.4A*) while those from the SHR_{BDx} group did not show tyrosine hydroxylase staining at any level of the renal circulation, including the branches of the renal artery entering the kidney (ie no evidence of re-innervation, *Figure 3.4B*).

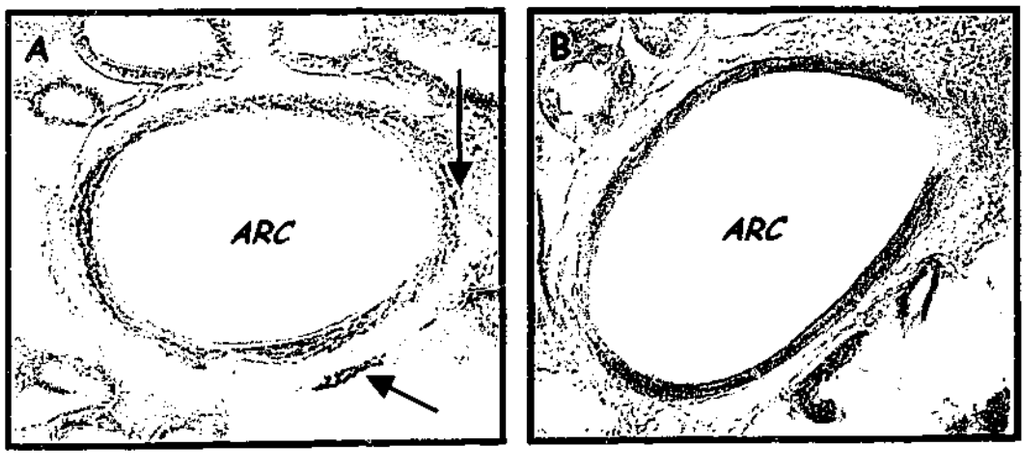

Figure 3.4 Light micrographs showing typical arcuate arteries (ARC) from a sham operated SHR kidney (A) demonstrating tyrosine hydroxylase staining for sympathetic nerve fibres (arrows) and a denervated SHR kidney (B).

3.4 Discussion

The main aim of this chapter was not only to verify previous studies, but also to see whether in my hands, this protocol (bilateral renal denervation at 6-weeks and then following through until 10-weeks of age) led to a lower arterial pressure in this colony of SHR. Bilateral renal denervation at 6-weeks of age did lead to a lower mean arterial pressure in 10-week old SHR, compared to the sham operated group. These findings are consistent with those previously published, whereby bilateral renal denervation at an early age was found to blunt or delay the development of hypertension in this strain (Liard, 1977; Winternitz *et al.*, 1980; Norman Jr and Dzielak, 1982).

Two techniques were used in-order to verify that both kidneys were denervated in the SHR_{BDx} group at 10-weeks of age. Although tissue noradrenaline content is not directly equivalent to noradrenaline released by the sympathetic nerve terminals it has been demonstrated to correlate well with the degree of sympathetic innervation (Head *et al.*, 1985; Donohue *et al.*, 1988). Donohue *et al* (1988) demonstrated an increase in tissue noradrenaline content in the kidney of SHR compared to WKY rats, at all ages, these results are consistent with the morphological study of Gattone *et al* (1990). Therefore, you would hypothesise that upon renal denervation, tissue noradrenaline content would be significantly lower in these kidneys, as has been demonstrated previously in the literature (Donohue *et al.*, 1988; Yoshida *et al.*, 1995; Tomoda *et al.*, 1997). Indeed levels measured in these experiments were significantly lower in the SHR_{BDx} group compared to the SHR_S, and thus both kidneys in the SHR_{BDx} group were denervated. These results were also supported by the immunohistochemistry results, which showed no visible tyrosine hydroxylase staining in the five SHR_{BDx} kidneys, while the SHR_S kidneys showed abundant staining.

Since renal denervation was performed at 6-weeks of age and the rats not sacrificed until 10-weeks of age, the process of re-innervation should be addressed. Previous studies have shown that in the SHR, renal noradrenaline content increases progressively from week 1 to week 6 following renal denervation, indicating sympathetic re-innervation (Winternitz *et al.*, 1980). Morphological evidence of re-innervation has also been demonstrated 28 days after kidney transplantation in humans (Gazdar and Dammin, 1970). Thus, while in the current study the kidneys had failed to re-innervate 4-weeks post-surgery, the literature suggests that this process may have complicated MAP measurements had the rats been studied over a longer period.

In conclusion, the experiments described in this chapter have provided strong evidence for the involvement of the renal sympathetic nerves in the development of hypertension in the SHR, which will be examined in more detail in the subsequent chapters.

CHAPTER 4

CHAPTER 4

Effect of chronic unilateral renal denervation on the renal arterioles of the SHR kidney - vascular casting

4.1 Introduction

As I have reviewed in *Chapter 1*, one of the main factors in the maintenance, and perhaps also the development, of hypertension in the SHR is hypothesised to be an increase in the vascular resistance of the pre-glomerular vasculature, due to hypertrophy and/or remodelling. The fact that the structural changes appear to develop early in life (Smeda *et al.*, 1988b), raises the possibility that they contribute to the pathogenesis of hypertension in this model. As yet the mechanisms, which may be responsible for the development of renal vascular hypertrophy or remodelling, are unknown.

In *Chapter 3*, I demonstrated that bilateral renal denervation of SHR at 6-weeks of age, led to a lower mean arterial pressure at 10-weeks of age compared to sham operated animals, thus confirming the importance of the renal sympathetic nerves in the degree of hypertension that develops in this strain (Liard, 1977; Wintemitz *et al.*, 1980; Norman Jr and Dzielak, 1982). Recently the renal sympathetic nerves have been implicated in the increase in pre-glomerular vascular resistance and possibly the pre-glomerular vascular hypertrophy (Tomoda *et al.*, 1997) which has been a common observation in the SHR (Smeda *et al.*, 1988b; Kimura *et al.*, 1991; Skov *et al.*, 1992; Kett *et al.*, 1995). Tomoda *et al* (1997) concluded that an increase in the pre-glomerular and a decrease in the post-glomerular lumen diameters must have occurred. However, a limitation of the Tomoda *et al* (1997) study, was that vessel lumen diameter and pre- and post-glomerular resistances were obtained indirectly.

Given the indirect evidence mentioned above, the main aim of the current study was to directly measure the lumen diameter of the afferent and efferent arterioles and interlobular arteries, in the maximally dilated kidney, using the technique of vascular casting (Gattone and Evan, 1986; Denton *et al.*, 1992). The overall hypothesis being tested in these experiments was that the renal sympathetic nerves are involved in the hypertrophy and/or remodelling observed in the pre-glomerular vessels of the SHR. Specifically I hypothesised that unilateral renal denervation at 6-weeks of age would lead to an increase in lumen diameter, at maximal dilatation, of the pre- and post-glomerular vasculature, of the superficial and mid-cortical glomeruli, at 10-weeks of age.

4.2 Methods

Male SHR were obtained from the Baker Medical Research Institute at 6-weeks of age and assigned randomly to undergo either unilateral renal denervation (n = 10) or sham-operation (n = 11, *Section 2.2 Surgical denervation of the kidney*). Two rats from each group were used to measure renal tissue noradrenaline content at 10-weeks of age (*Section 2.5 Tissue noradrenaline assay*; Medvedev *et al.*, 1990; Lambert *et al.*, 1997).

All rats were monitored for the following four weeks until they were 10-weeks of age. During this period body weights were measured once a week and arterial pressure twice a week via tail-cuff sphygmomanometry (*Section 2.3.1 Indirect tail-cuff method*). At 10-weeks of age, the rats were briefly anaesthetised with methohexitone sodium (50 mg/kg, i.p.) for insertion of a tail artery catheter. Following a 60 min recovery period conscious tail artery pressure was measured (*Section 2.3.2 Direct tail artery method*). The rat was then re-anaesthetised with sodium pentobarbitone (40 mg/kg i.p.) and prepared for perfusion fixation of the kidneys (*Section 2.4 Perfusion fixation of the kidneys*). Following perfusion fixation a mixture of methacrylate and accelerator (20:1; Mercox CL-2B, SPI supplies, Structure Probe, PA, U.S.A.) was injected into both kidneys (approximately 0.55ml; Gattone and Evan, 1986; Denton *et al.*, 1992). The renal arteries were immediately clamped and the resin was allowed to cure *in situ* for 1 hour before the kidneys were removed and stored in 4% paraformaldehyde in 0.1M PO_4 buffer overnight at room temperature. The following day, the kidneys were decapsulated, weighed and sliced coronally (each slice being 1mm thick; *Figure 4.1*).

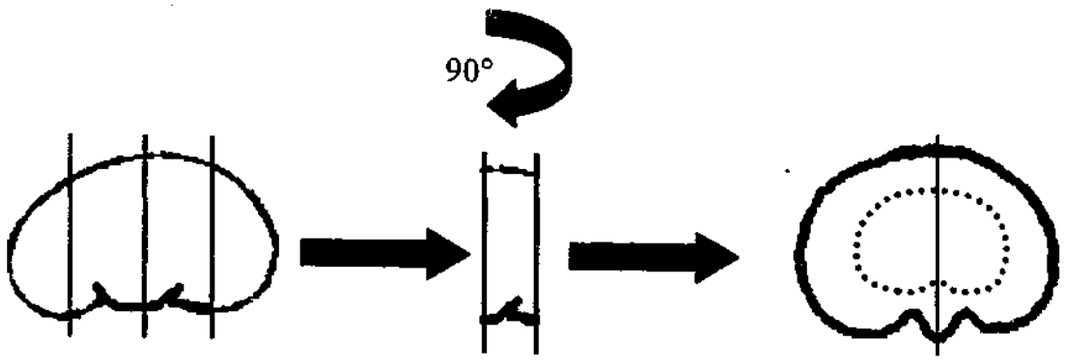

Figure 4.1 Schematic diagram demonstrating the way in which each kidney was sliced following perfusion fixation and injection of casting resin.

These pieces of kidney were then placed in 20% potassium hydroxide (KOH) to digest the tissue, leaving only the methacrylate cast of the renal vasculature. To speed digestion, the containers were placed in a water bath at 55°C. The KOH solution was changed twice daily with alternate washes in distilled water (dH_2O) to rinse away any digested tissue. This procedure usually took 24 hours to complete. The wet casts were weighed and a rough estimate of kidney weight was then obtained [(kidney + cast) – cast]. The casts were washed with 5% percent sodium hypochlorite, to ensure that no mould grew while they were in storage, allowed to dry completely on filter paper, then stored in sterile containers.

4.2.1 Scanning electron microscopy

The clean vascular casts were mounted on stubs using double-sided carbon tape and conductive carbon cement (LEIT-C nach Göcke; Neubaur Chemikalien; West Germany). They were gold coated (BAL-TEC; Balzers SCD-005 Sputter Coater) prior to examination on a Hitachi S-570 scanning electron microscope (SEM) at 10 kV. Glomeruli were classified as superficial or mid-cortical (Kriz and Kaissling, 1992), with approximately 10 micrographs taken per glomerular type at a final magnification of x670, that is a total of 20 glomeruli examined per kidney (both left and right kidneys in each animal). The glomeruli were classified using the following criteria (*Figure 4.2*); (1) superficial glomeruli were those that had afferent arterioles arising from terminal branches of the interlobular arteries and had efferent arterioles that extended to the

surface of the kidney before dividing, (2) mid-cortical glomeruli were those where the efferent arterioles branch abruptly close to the glomerulus but do not extend towards the surface as for the superficial glomeruli (Kriz and Kaissling, 1992). Kidneys and vascular casts were coded and randomised prior to mounting for SEM.

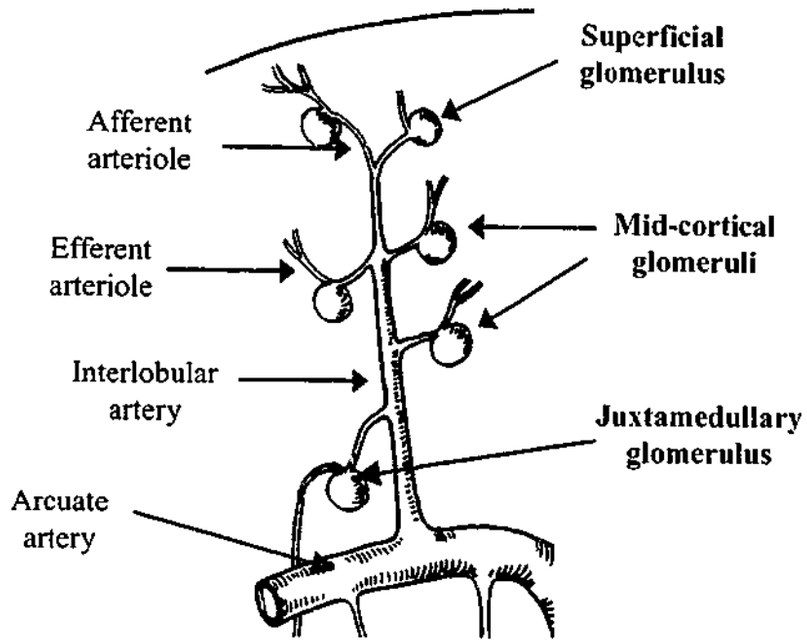

Figure 4.2 Schematic diagram demonstrating the renal vasculature and glomerular types. Modified from (Fourman and Moffat, 1971).

4.2.2 Measurements & calculations

Vessel dimensions (see *Figure 4.3*): Lumen diameter of both the afferent and efferent arterioles were measured at 25 μm intervals starting from the "entry" or "exit" points of the vessels (Measure Version 2.15; Capricorn Scientific Software, Victoria, Australia). The "entry" point was defined as the point prior to branching of the afferent arteriole into the glomerular capillaries. The "exit" point of the arterioles was defined as the point post branching of the glomerular capillaries. The length of the afferent arteriole was measured from the entry point into the glomerulus back to the first branch point (the interlobular artery) and an average diameter was obtained for each vessel. The efferent arteriole length was measured from the exit point from the glomerular tuft for 75 μm. It was not possible to measure the efferent arterioles all the way to the first branch point because this branch point was not always obtained in all micrographs. In

four animals analysed, efferent arterioles were not visible due to differences in filling of the kidneys (eg obscured by peri-tubular capillaries or not filled satisfactorily), or the vessels were sometimes broken during processing. Therefore, an average lumen diameter for each efferent arteriole, over the first 75 μm, is presented in the results. Interlobular artery lumen diameter was measured at a single point, just prior to where it branched to form the afferent arteriole. In the results section, data are presented in two ways. Firstly the data obtained from the superficial and mid-cortical glomeruli were combined and group mean ± sem are presented. Secondly the data obtained from the superficial and mid-cortical glomeruli are examined separately, with again the group mean ± sem presented.

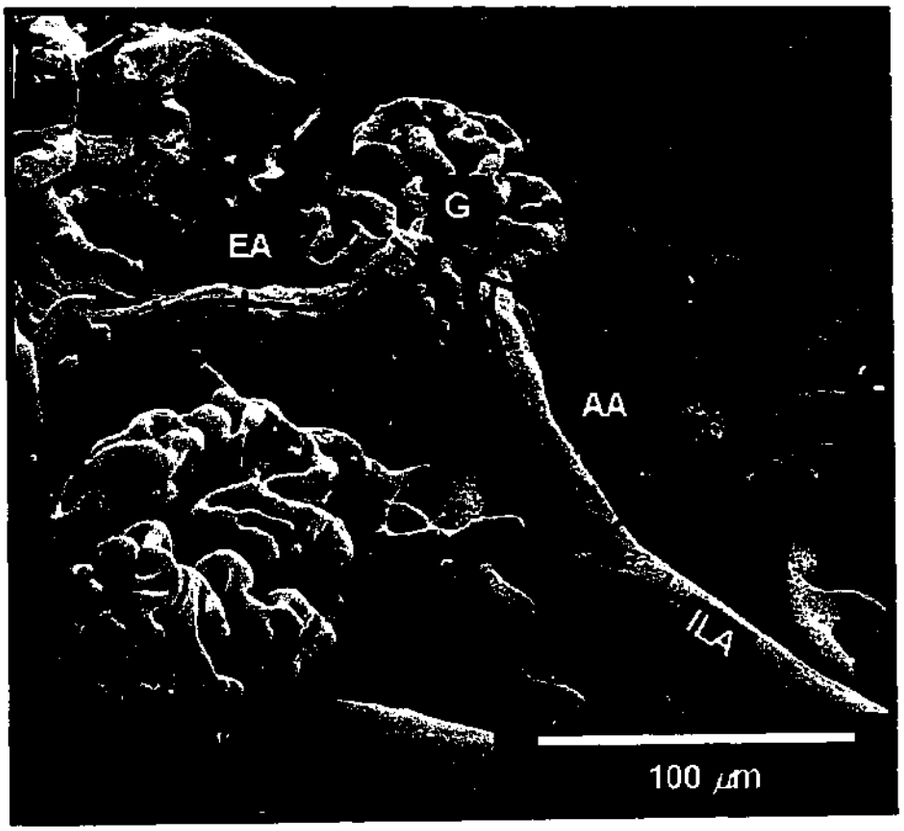

Figure 4.3 Scanning electron micrograph demonstrating the measurements made for each glomerulus (G). Afferent arteriole (AA) length was measured from where it branched from the interlobular artery (ILA) to the point prior to branching into the glomerular tuft. Efferent arteriole (EA) length was measured post branching of the glomerular tuft, to identify the first 75 μm. Diameter was measured at 25 μm intervals along the length of both the afferent and efferent arterioles.

4.2.2.1 Calculations

Resistance can be calculated from lumen diameters with the use of Poiseuille's equation, which states:

$$R \propto 8\eta l / \pi r^4$$

where R = resistance, η = viscosity of the fluid, l = length of the vessel (μm) and r = radius of the vessel lumen (μm). In this chapter relative resistance per unit length, assuming constant viscosity and length, was calculated as:

$$R \propto 1/\pi r^4$$

Glomerular tuft volume was also estimated, by tracing the circumference of the tuft. Diameter was estimated, from the micrograph, with the use of a computer package (Measure). Glomerular tuft volume was calculated from the mean diameter using the following equation:

$$V = 4/3 * \pi r^3$$

where V = glomerular tuft volume (μm^3), r = radius (μm). This calculation has the limitation that it assumes the glomerulus is spherical.

4.2.3 Statistics

The computer program SYSTAT (Wilkinson, 1990) was used for all statistical analyses. For the data in *Table 4.1 and 4.2* the group mean was obtained and a one-way ANOVA was performed testing for a difference between the unilaterally denervated group (SHR_{Dx}) and the sham operated group (SHR_S). Repeated measures ANOVA was used to test for an effect of treatment over time on tail-cuff pressure measurements (*Figure 4.4*). For all other results the mean was obtained for both the left and the right kidney of both groups. A one-way ANOVA was used to test for a difference between the left and right kidney for each group.

4.3 Results

4.3.1 Chronic unilateral renal denervation

The two groups had similar body weights prior to unilateral renal denervation or sham operation (SHR_S 154 ± 7 and SHR_{Dx} 150 ± 6 g) and at 10-weeks of age (*Table 4.1*). At 10-weeks of age (4-weeks post-surgery) heart rate (HR), left or right ventricle to body weight ratios and kidney weights were not significantly different between groups (*Table 4.1*).

Mean arterial pressure (MAP), measured directly via tail artery cannulation, was 145.9 ± 2.0 mmHg in the SHR_{Dx} and 139.9 ± 1.7 mmHg in the SHR_S at 10-weeks of age ($P = 0.04$, *Table 4.1*). Tail-cuff pressures were also measured (once a week) for 4-weeks post-surgery (*Figure 4.4*). Repeated measures ANOVA showed no significant difference in blood pressure over the four weeks ($P > 0.20$ for all parameters measured via tail-cuff).

Table 4.1 Haemodynamic and weight data at 10-weeks of age.

	SHR_S (n = 11)	SHR_{Dx} (n = 10)	*P*
Mean arterial pressure (mmHg)	139.9 ± 1.7	145.9 ± 2.0	0.04
Heart rate (beats/min)	337 ± 5	344 ± 7	NS
Body weight (g)	290 ± 5	283 ± 4	NS
Left ventricle (g/100g body weight)	0.33 ± 0.01	0.34 ± 0.01	NS
Right ventricle (g/100g body weight)	0.09 ± 0.01	0.09 ± 0.01	NS
Left kidney (g)	1.82 ± 0.03	1.81 ± 0.08	NS
Right kidney (g)	1.80 ± 0.03	1.80 ± 0.04	NS

Values are group mean ± sem for the sham operated animals (SHR_S) and unilaterally denervated animals (SHR_{Dx}). Values were obtained at 10-weeks of age on the day of perfusion fixation (ie four weeks following surgery). *P* values were derived using a one-way ANOVA, and values > 0.05 were not significant (NS).

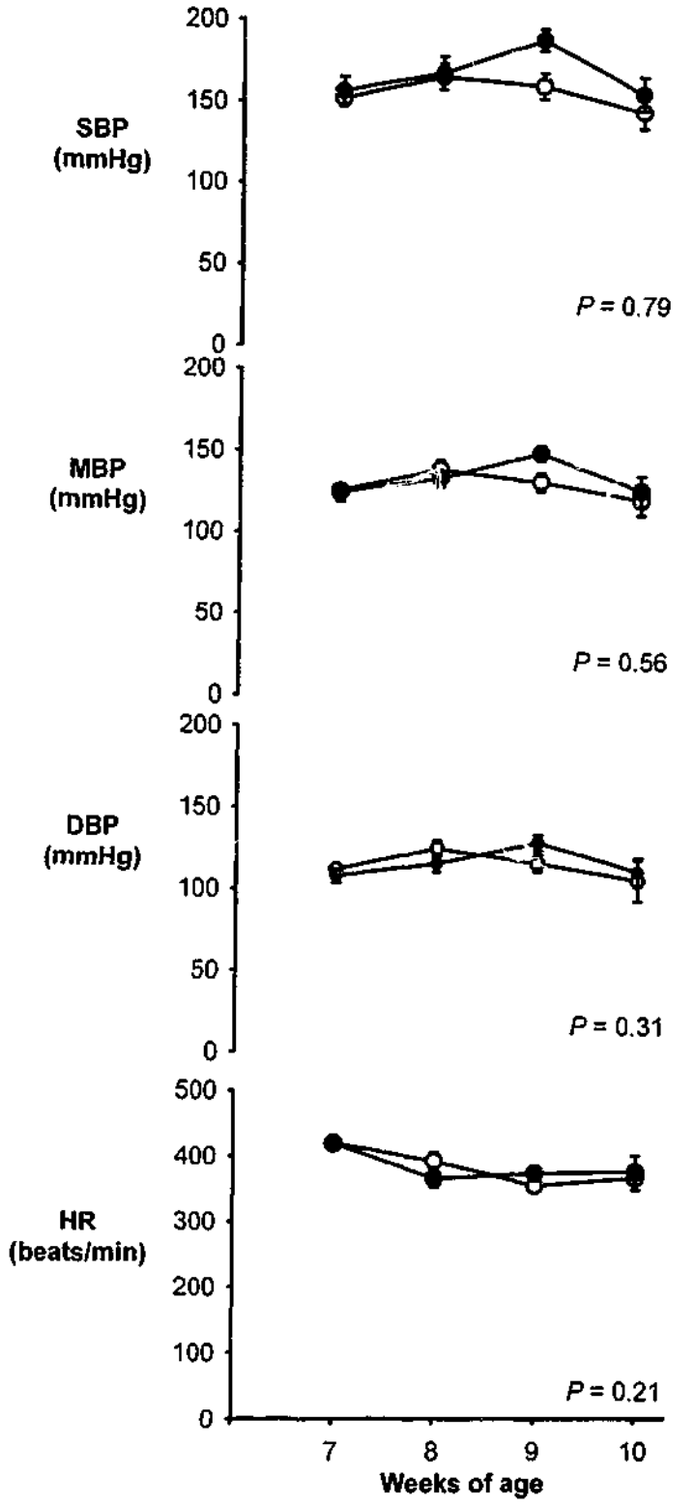

Figure 4.4 Systolic, mean and diastolic blood pressures (SBP, MBP and DBP respectively) and heart rate (HR) measured via the indirect tail-cuff method (mean ± sem). Measured from 7- to 10-weeks of age in unilaterally denervated (closed circles) and sham operated SHR (open circles). *P* values are the main effect of treatment from repeated measures ANOVA.

4.3.2 Structural variables

4.3.2.1 Combined data (superficial and mid-cortical glomeruli)

Afferent arteriole

Afferent arteriole lumen diameter was not significantly different between the left and right kidneys in either the SHR_S (15.7 ± 0.5 and 15.1 ± 0.5 μm respectively) or the SHR_{Dx} (14.8 ± 0.2 and 14.5 ± 0.3 μm respectively) groups (*Figure 4.5*). Calculated relative resistance, per unit length, of the afferent arteriole in the left and right kidneys of the SHR_S (0.105 ± 0.012 and 0.134 ± 0.029 units x 10^{-3}) and the SHR_{Dx} (0.121 ± 0.008 and 0.142 ± 0.013 units x 10^{-3}) groups were not significantly different (*Figure 4.5*). Afferent arteriole length also did not vary between left and right kidneys in either group (*Figure 4.5*).

Efferent arteriole

Average efferent arteriole lumen diameter measured for the first 75 μm length of vessel was not significantly different (*Figure 4.6*) in the left and right kidneys of the SHR_S (12.3 ± 0.8 and 13.1 ± 0.7 μm respectively) and the SHR_{Dx} (12.0 ± 0.3 and 11.5 ± 0.3 μm respectively). Relative resistance calculated for this portion of the efferent arteriole was not significantly different between the left and right kidneys of the SHR_S group ($P = 0.43$, *Figure 4.6*). The efferent arteriole of the left denervated kidney (0.385 ± 0.147 units x 10^{-3}) did have a significantly lower relative resistance than the right innervated kidney (0.261 ± 0.060 units x 10^{-3}) of the SHR_{Dx} group ($P = 0.05$, *Figure 4.6*).

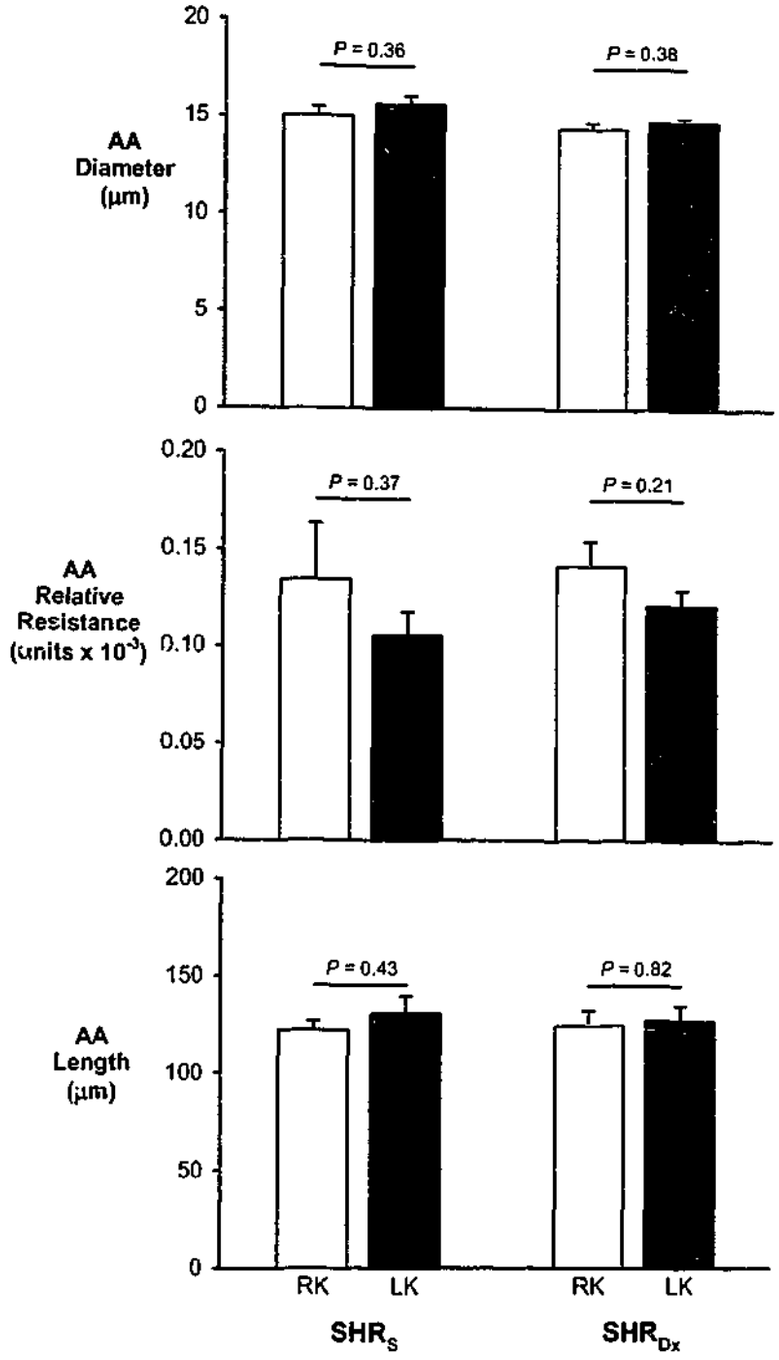

Figure 4.5 Combined data representing the between animal mean ± sem for the afferent arteriole (AA) lumen diameter, relative resistance and length, in the left and right kidneys (LK and RK respectively), of the unilaterally denervated (SHR_{Dx}) and the sham operated (SHR_S) groups. The left kidney in the SHR_{Dx} group is the denervated kidney (red bar). *P* values were obtained from a one-way ANOVA comparing the left and right kidneys in the respective groups.

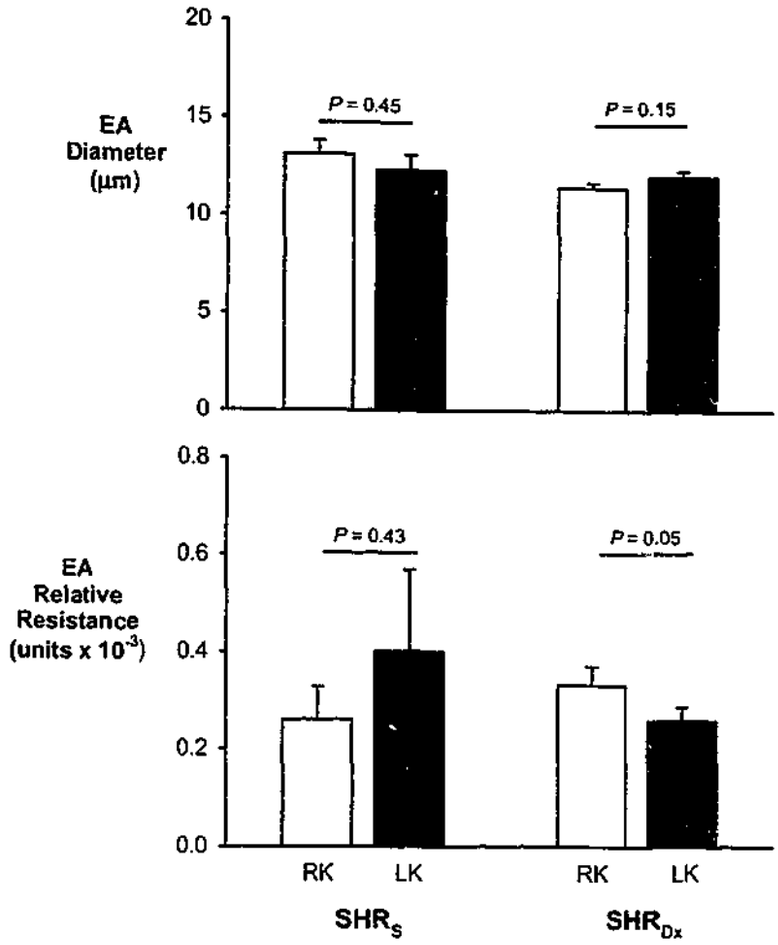

Figure 4.6 Combined data representing the between animal mean ± sem for the efferent arteriole (EA) lumen diameter and relative resistance, in the left and right kidneys (LK and RK respectively), of the unilaterally denervated (SHR_{Dx}) and the sham operated (SHR_S) groups. The left kidney in the SHR_{Dx} group is the denervated kidney (red bar). *P* values were obtained from a one-way ANOVA comparing the left and right kidneys in the respective groups.

Interlobular artery

Interlobular artery lumen diameter was not significantly different between the left and right kidney in either the SHR_S (23.7 ± 0.7 and 24.8 ± 0.8 µm respectively) or the SHR_{Dx} (24.2 ± 0.9 and 25.1 ± 0.7 µm respectively) groups (*Figure 4.7*). Relative resistance, of the interlobular artery, in the left and right kidney of the SHR_S (0.029 ± 0.003 and 0.022 ± 0.003 units x 10^{-3}) and the SHR_{Dx} (0.026 ± 0.004 and 0.022 ± 0.003 units x 10^{-3}) groups were not significantly different (*Figure 4.7*). Calculated glomerular tuft volume was not significantly different between the left and right kidney in either group (*Figure 4.7*).

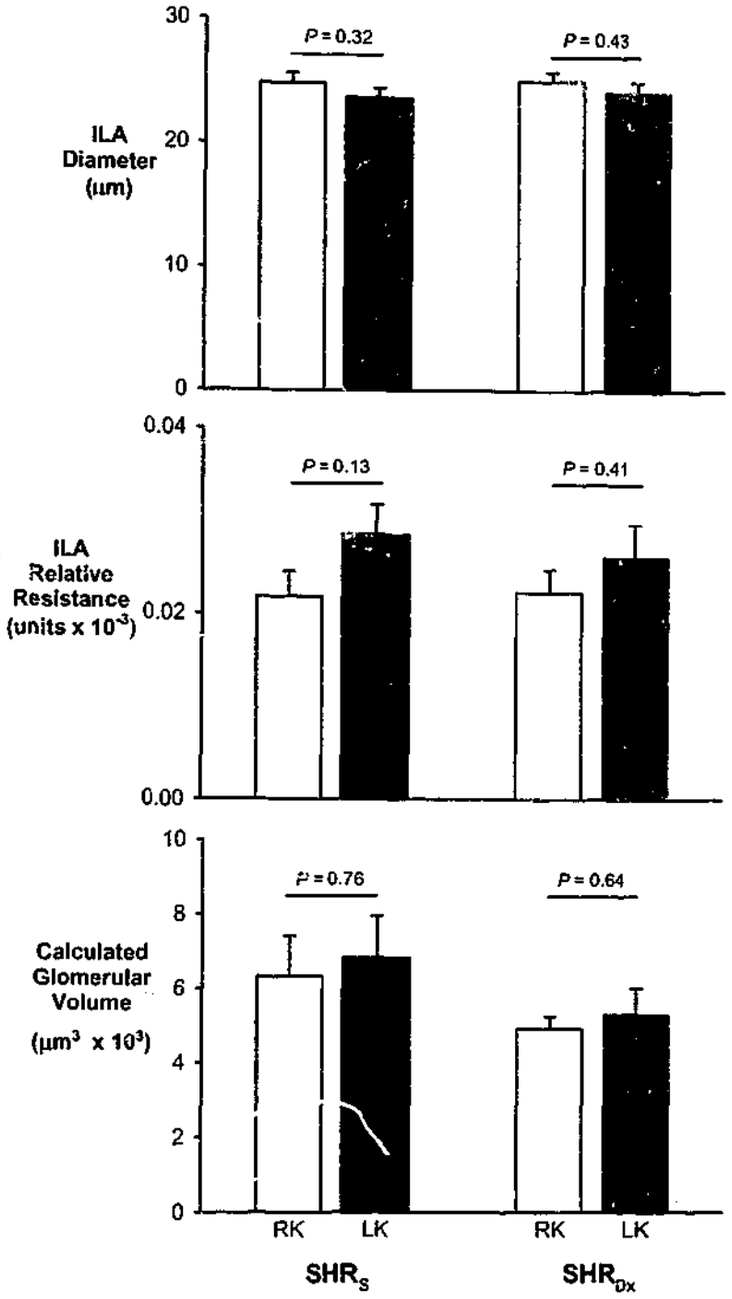

Figure 4.7 Combined data representing the between animal mean ± sem for the interlobular artery (ILA) lumen diameter, relative resistance and calculated glomerular tuft volume, in the left and right kidneys (LK and RK respectively), of the unilaterally denervated (SHR_{Dx}) and the sham operated (SHR_S) groups. The left kidney in the SHR_{Dx} group is the denervated kidney (red bar). *P* values were obtained from a one-way ANOVA comparing the left and right kidneys in the respective groups.

4.3.2.2 Regional effects

The data was also divided in-order to determine whether the structural changes were occurring in a specific region of the kidney.

Glomeruli of the superficial cortex

No significant difference was observed in the lumen diameters of the AA, EA or ILA of the superficial glomeruli in either the left or right kidneys in either group (*Table 4.2*). Calculated relative resistance also was not significantly different in the AA, EA or ILA (*Table 4.2*).

Mid-cortical glomeruli

No significant difference was observed in the lumen diameters of the AA, EA or ILA of the mid-cortical glomeruli in either the left or right kidneys in either group (*Table 4.3*). Calculated relative resistance also was not significantly different in the AA, EA or ILA (*Table 4.3*).

Table 4.2 Vascular dimensions of the glomeruli in the superficial cortex.

	SHR_S			SHR_{Dx}		
	LK	**RK**	***P***	**LK**	**RK**	***P***
Afferent arteriole						
Lumen diameter (μm)	16.1 ± 0.5	15.9 ± 0.8	NS	15.4 ± 0.3	14.7 ± 0.4	NS
Relative resistance (units x 10^{-3})	0.093 ± 0.012	0.111 ± 0.024	NS	0.104 ± 0.009	0.130 ± 0.013	NS
Efferent arteriole						
Lumen diameter (μm)	12.2 ± 0.6	13.6 ± 1.1	NS	12.1 ± 0.3	11.6 ± 0.4	NS
Relative resistance (units x 10^{-3})	0.411 ± 0.125	0.254 ± 0.059	NS	0.279 ± 0.021	0.346 ± 0.055	NS
Interlobular artery						
Lumen diameter (μm)	22.6 ± 0.7	24.6 ± 1.2	NS	22.6 ± 0.9	25.1 ± 1.2	NS
Relative resistance (units x 10^{-3})	0.032 ± 0.005	0.020 ± 0.003	0.05	0.028 ± 0.004	0.020 ± 0.003	NS
Glomerular tuft volume (μm^3 x 10^3)	7.40 ± 1.44	6.20 ± 1.09	NS	5.37 ± 0.87	4.95 ± 0.32	NS

Vascular dimensions of the glomeruli of the superficial cortex in the left and right kidneys (LK and RK respectively) of the unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. Values are the mean ± sem. *P* values are the outcome of a one-way ANOVA testing for a difference between left and right kidneys. *P* values > 0.05 were not significant (NS).

Table 4.3 Vascular dimensions of the mid-cortical glomeruli

	SHR_S			SHR_{Dx}		
	LK	**RK**	***P***	**LK**	**RK**	***P***
Afferent arteriole						
Lumen diameter (μm)	15.1 ± 0.5	14.6 ± 0.5	NS	14.1 ± 0.3	14.1 ± 0.3	NS
Relative resistance (units x 10^{-3})	0.124 ± 0.017	0.151 ± 0.035	NS	0.145 ± 0.009	0.159 ± 0.019	NS
Efferent arteriole						
Lumen diameter (μm)	12.7 ± 0.5	12.9 ± 0.6	NS	12.4 ± 0.3	11.9 ± 0.7	NS
Relative resistance (units x 10^{-3})	0.238 ± 0.050	0.267 ± 0.067	NS	0.242 ± 0.022	0.349 ± 0.078	NS
Interlobular artery						
Lumen diameter (μm)	26.8 ± 1.2	25.5 ± 1.1	NS	26.2 ± 1.9	24.7 ± 0.7	NS
Relative resistance (units x 10^{-3})	0.021 ± 0.005	0.022 ± 0.004	NS	0.027 ± 0.006	0.025 ± 0.002	NS
Glomerular tuft volume (μm^3 x 10^3)	8.23 ± 0.45	6.39 ± 1.08	NS	4.83 ± 0.40	4.88 ± 0.45	NS

Vascular dimensions of the mid-cortical glomeruli in the left and right kidneys (LK and RK respectively) of the unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. Values are the mean ± sem. *P* values are the outcome of a one-way ANOVA testing for a difference between left and right kidneys. *P* values > 0.05 were not significant (NS).

4.3.2.4 Renal noradrenaline content

Renal noradrenaline content was measured in two rats from each group. Overall, the left kidney of the SHR_{Dx} group appeared to have a lower noradrenaline content compared to the left kidney of the SHR_S group. One animal had an unusually low noradrenaline level in the right kidney.

Table 4.4 Tissue noradrenaline content.

Rat #	Left kidney (ng NA/g wet weight)	Right kidney (ng NA/g wet weight)
R6 - sham	52.5	59.2
R7 - sham	70.7	63.1
R9 – unilaterally denervated	7.1	13.0
R10 – unilaterally denervated	20.1	57.2
% from control	22	57

Data are expressed as the tissue noradrenaline (NA) content ng/g wet weight at 10- weeks of age from sham operated and unilaterally denervated rats. Percentage of control was calculated as the percent of the mean NA level in the left or right kidney of the denervated animals compared to the corresponding mean left or right kidney of the sham operated animals.

4.4 Discussion

The hyperinnervation of the renal vasculature and overactivity of the renal sympathetic nerves have been hypothesised to initiate the development of hypertrophy and/or remodelling of the renal vasculature commonly observed in the SHR (see *Chapter 1*). Of particular interest in this chapter was, what effect does removal of the renal sympathetic nerves have on the lumen diameter of the pre-glomerular arterioles? Bilateral renal denervation in SHR has been demonstrated to not only reduce arterial pressure (Liard, 1977; Winternitz *et al.*, 1980) but also lead to an increase in the lumen diameter of the afferent arteriole (Gattone *et al.*, 1984). This increase in lumen diameter would be expected to translate to a reduction in the calculated relative resistance in these vessels, which may be antihypertensive due to a possible increase in glomerular

capillary pressure. However as discussed in *Chapter 1* (*Section 1.4.1.2 Bilateral renal denervation*), one major issue arises from these previous studies (Liard, 1977; Winternitz *et al.*, 1980; Gattone *et al.*, 1984). An increase in arterial pressure will itself lead to the development of vessel hypertrophy (Lee and Smeda, 1985), and therefore reduction of arterial pressure in the SHR, with the use of bilateral renal denervation, may attenuate the hypertrophy but it would be difficult to interpret the results. Therefore, any structural differences observed in the previous studies could have occurred as a direct effect of removal of the renal sympathetic innervation or as a secondary response to the reduction in arterial pressure. Unilateral renal denervation has previously been demonstrated not to alter arterial pressure in the SHR (Tomoda *et al.*, 1997), and this model is therefore a means of studying the renal vasculature without the confounding influence of a reduction in arterial pressure.

The major finding of this chapter was that chronic unilateral renal denervation had apparently little effect on the afferent arteriole lumen diameter, inconsistent with the main hypothesis. In contrast, there was a significant reduction in the calculated relative resistance of the efferent arteriole in the left denervated kidney of the SHR_{Dx} group compared to the intact right kidney. Although a statistically significant increase in lumen diameter of the efferent arteriole was not detected, using Poiseuille's equation to calculate relative resistance there was a statistically significant reduction in relative resistance.

Poiseuille's equation is often used to calculate hydraulic resistance of blood vessels. However this law applies to "tubes" with constant diameter and straight axis, but blood vessels in many beds do not have such properties. Poiseuille's law therefore, leads to an underestimation of vascular resistance (Iordache and Remuzzi, 1995). In the systemic circulation approximately two thirds of the resistance is in the small arterioles (Guyton and Hall, 2000). As Poiseuille's equation states, resistance is inversely proportional to the fourth power of the radius, therefore very small changes in lumen diameter of a vessel will lead to large alterations in vascular resistance (Guyton and Hall, 2000). Certainly it must be kept in mind that this method has limitations, however in this chapter the renal vessels are examined under conditions of maximal dilatation to reduce the chance of limitations of Poiseuille's law.

The experiments conducted in this chapter were based on the findings of Tomoda *et al* (1997), a study in which SHR were subjected to unilateral renal denervation at 6-weeks of age. Four weeks later a functional test of renal vascular resistance was performed (an iso-osmotic colloid perfused, maximally dilated, isolated

kidney preparation), comparing denervated and sham operated kidneys. These experiments produce pressure-flow and pressure-GFR relationships which are determined by the lumen dimensions of the renal vasculature (Folkow, 1982). The authors were able to demonstrate a significant shift in the pressure-GFR relationship in the chronically denervated kidney (Tomoda *et al.*, 1997). These changes were interpreted to reflect an increase in lumen diameter of the pre-glomerular vessels and perhaps a decrease in the lumen diameter of the post-glomerular vessels must have occurred (Tomoda *et al.*, 1997). However, the current study demonstrated no significant difference in lumen diameters of either the interlobular artery or afferent arteriole following unilateral renal denervation, indicating that structural differences in the pre-glomerular vasculature may not explain the previous findings of Tomoda *et al* (1997).

It may be argued that the vascular casting technique has limitations, and that it may not be sensitive enough to detect small differences in lumen diameter. I am confident however, that the method of vascular casting can be used to detect quite small changes in renal arteriole dimensions. This method has been validated extensively (Gattone and Evan, 1986; Gattone and Sale, 1986; Kimura *et al.*, 1990; Denton *et al.*, 1992; Denton *et al.*, 2000). Importantly the estimates of lumen diameter in this chapter are comparable to those previously reported in rats using vascular casting (Evan and Dail, 1977; Gattone *et al.*, 1983a; Gattone *et al.*, 1983b; Kimura *et al.*, 1989; Kimura *et al.*, 1991; Skov *et al.*, 1992) and stereology (Kett *et al.*, 1995; Kett *et al.*, 1996b). Furthermore, previous studies using the vascular casting technique have been able to demonstrate a significant vasodilatation in SHR following bilateral renal denervation (Gattone *et al.*, 1984).

Tomoda *et al* (1997) were only able to measure renal vascular resistance indirectly, and from this they speculated as to the possible sight of structural differences. The preparation used by Tomoda *et al* (1997) is based on the original method of Gothberg *et al* (1979), which involves blocking tubuloglomerular feedback and the renal vasculature has no vasomotor tone. The renal vessels being examined are treated as "tubes" with no influence of either intrinsic or extrinsic factors. Therefore it is not surprising that the results obtained in the current chapter differ from those obtained previously using the isolated kidney preparation (Tomoda *et al.*, 1997). Recently a study examined the role of tubuloglomerular feedback in the regulation of efferent arteriole resistance *in vitro* (Ren *et al.*, 2001). The authors demonstrated that with increasing concentrations of sodium chloride, pre-constricted efferent arterioles dilate and that this response was mediated by activation of adenosine A_2 receptors.

Perhaps therefore in the Tomoda *et al* (1997) study, blockade of tubuloglomerular feedback prevents the dilation of the efferent arteriole and therefore gave the appearance of a predominantly pre-glomerular response.

Previous studies have demonstrated that there are two types of sympathetic nerves innervating the kidney, type I axons almost exclusively innervate the afferent arteriole, while type II axons are evenly distributed on the afferent and efferent arterioles (Luff *et al.*, 1991; Luff *et al.*, 1992). The afferent arteriole appears to be more densely innervated compared to the interlobular artery or efferent arteriole (Dieterich, 1974; De Michele and Amenta, 1988; Luff *et al.*, 1991; Luff *et al.*, 1992). Therefore the fact that there was no significant effect of chronic unilateral renal denervation on the pre-glomerular vasculature was unexpected. However, as I have just stated, the efferent arteriole is also innervated, primarily with type II sympathetic axons. Therefore, the fact that there was a significant reduction in the calculated relative resistance of the efferent arteriole is not so surprising. We cannot conclude with certainly whether the effect on the efferent arteriole is a direct effect of the renal sympathetic nerves or a secondary response to changes in the local haemodynamics (eg glomerular capillary pressure).

Renal noradrenaline content has been demonstrated to be reduced following renal denervation, because dissection of the nerves leads to no further release of the neurotransmitter into the surrounding tissue or circulation (Kline *et al.*, 1980; Morgunov and Baines, 1981; Mercer and Kline, 1984; Yoshida *et al.*, 1995; Tomoda *et al.*, 1997). Therefore this technique has been used as a verification of renal denervation. In this chapter renal noradrenaline content was found to be considerably lower in the denervated kidney. In *Chapter 3*, I demonstrated that a significant reduction in renal noradrenaline content was consistent with immunohistochemical analysis (no staining for tyrosine hydroxylase was evident in the denervated kidneys), and so I am confident that the animals used in this chapter were denervated.

In examining the lumen dimensions the aim was to keep the vasculature as close to *in vivo* conditions as possible. Therefore, both kidneys were fixed at a pressure equivalent to mean arterial pressure (measured on the day), and allowances were made for the inherent pressure of the perfusion apparatus (Kett *et al.*, 1995). At maximal dilatation, vascular resistance has been demonstrated to be increased in both hypertensive patients (Heagerty *et al.*, 1993) and SHR (Göthberg and Folkow, 1983). By examining the vasculature at maximal dilatation, vasomotor tone, which is the

"background degree" of vasoconstriction, is eliminated. Therefore it would be possible to examine the structural properties without interference.

My main hypothesis states that the renal sympathetic nerves are involved in the development of hypertrophy and/or remodelling in the pre-glomerular vasculature of the SHR. An increase in renal sympathetic nerve activity or hyperinnervation of the vasculature, possibly via activation of VSMC growth, may lead to encroachment on the vessel lumen and therefore increase vascular resistance. The experiments reported in this chapter demonstrated no apparent effect of unilateral renal denervation on the afferent arteriole, but an apparent reduction in relative resistance of the efferent arteriole. The experiments described in *Chapter 5*, therefore, will examine the effect of chronic unilateral renal denervation on the wall and lumen dimensions of the larger vessels of the juxtamedullary region in the kidneys of the Wistar-Kyoto rat and the SHR using unbiased stereological techniques.

CHAPTER 5

CHAPTER 5

Stereological analysis of the renal vasculature of the SHR and WKY rat following chronic unilateral denervation

5.1 Introduction

In the previous chapter I demonstrated that chronic unilateral renal denervation led to a lower efferent arteriole resistance, with a tendency for a reduction in afferent arteriole resistance. The main hypothesis, examined in this thesis, was that the renal sympathetic nerves were responsible for the hypertrophy/remodelling observed in the pre-glomerular vasculature of the spontaneously hypertensive rat (SHR). However, the technique of vascular casting, used in *Chapter 4*, does not provide us with any information about the vessel wall, rather it examined whether or not renal denervation led to changes in lumen diameter of the arterioles. In this chapter I have used unbiased stereological techniques to examine the effect of unilateral renal denervation on the larger renal vessels, in particular the proximal interlobular and arcuate artery wall and lumen dimensions. *Chapter 4* examined the smaller vessels of the superficial and mid-cortical regions, and thus the current chapter examined the effect of unilateral renal denervation on the larger renal vessels. The afferent and efferent arterioles cannot be examined with the stereological techniques to be used in this chapter because they are too small for analysis via light microscopy.

Unilateral renal denervation, as covered in detail in *Chapter 1* (*Section 1.4.1.3 Unilateral renal denervation*), was used because it has been previously demonstrated not to alter blood pressure (Tomoda *et al.*, 1997), therefore, avoiding the confounding influence of the blood pressure difference. Previous studies have utilised techniques

which lower blood pressure eg bilateral renal denervation (Gattone *et al.*, 1984), angiotensin converting enzyme inhibition (Kett *et al.*, 1995), and angiotensin II blockade (Kett *et al.*, 1996b) in-order to examine the changes in the renal vasculature in the SHR.

The aim of the current experiments therefore, was to examine the effect of unilateral renal denervation on wall and lumen dimensions of the larger renal vessels, in particular the proximal interlobular and arcuate arteries, in SHR and WKY rat kidneys.

5.2 Methods

Male Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were obtained from the Baker Medical Research Institute at 6-weeks of age and assigned randomly (see *Section 2.1.1 for group numbers*) to undergo either a unilateral renal denervation or sham operation (*Section 2.2 Surgical denervation of the kidney*). All rats were monitored for the following four weeks until 10-weeks of age, with body weight recorded weekly. At 10-weeks of age, rats were anaesthetised with methohexitone sodium solution (50 mg/kg, i.p.) and a catheter inserted in the tail artery (*Section 2.3.2 Direct tail artery method*), following a 1 h recovery period, conscious tail artery pressure was measured. Rats were then re-anaesthetised with sodium pentobarbitone (40 mg/kg, i.p.) and prepared for perfusion fixation of both left and right kidneys (*Section 2.4 Perfusion fixation of the kidney*).

5.2.1 Stereological methods & calculations

Following perfusion fixation, kidneys were coded in-order to analyse the samples in a blind fashion. The left kidney from each animal was then sliced at 1mm (*Figure 5.1*) in the horizontal plane using a razor blade slicing device (Baddeley *et al.*, 1986), generating approximately 20 slices per kidney. After estimation of kidney volume (see below), every second slice was dehydrated through graded alcohol, processed into hydroxymethacrylate (Technovit 7100, Heraeus Kulzer GmbH & Co. KG, Germany), and flat embedded into moulds. One 2 μm section was cut from each block (approximately 10 blocks per kidney) and stained with haematoxylin and eosin. Low power light microscopy was used to assess overall kidney vascular arrangement and to set up the stereological protocols. Arcuate arteries were identified along the cortico-medullary junction and differentiated from the larger interlobar arteries farther upstream as being completely surrounded by tubules rather than being next to an

epithelial surface (Kett *et al.*, 1995). The cortico-medullary junction was defined by following the arrangement of the arcuate arteries and juxtamedullary glomeruli (see *Figure 5.2*). Once this junction had been defined, the cortex was divided into equal thirds and only the interlobular arteries of the inner third (proximal) were used for analysis (Anderson *et al.*, 1997).

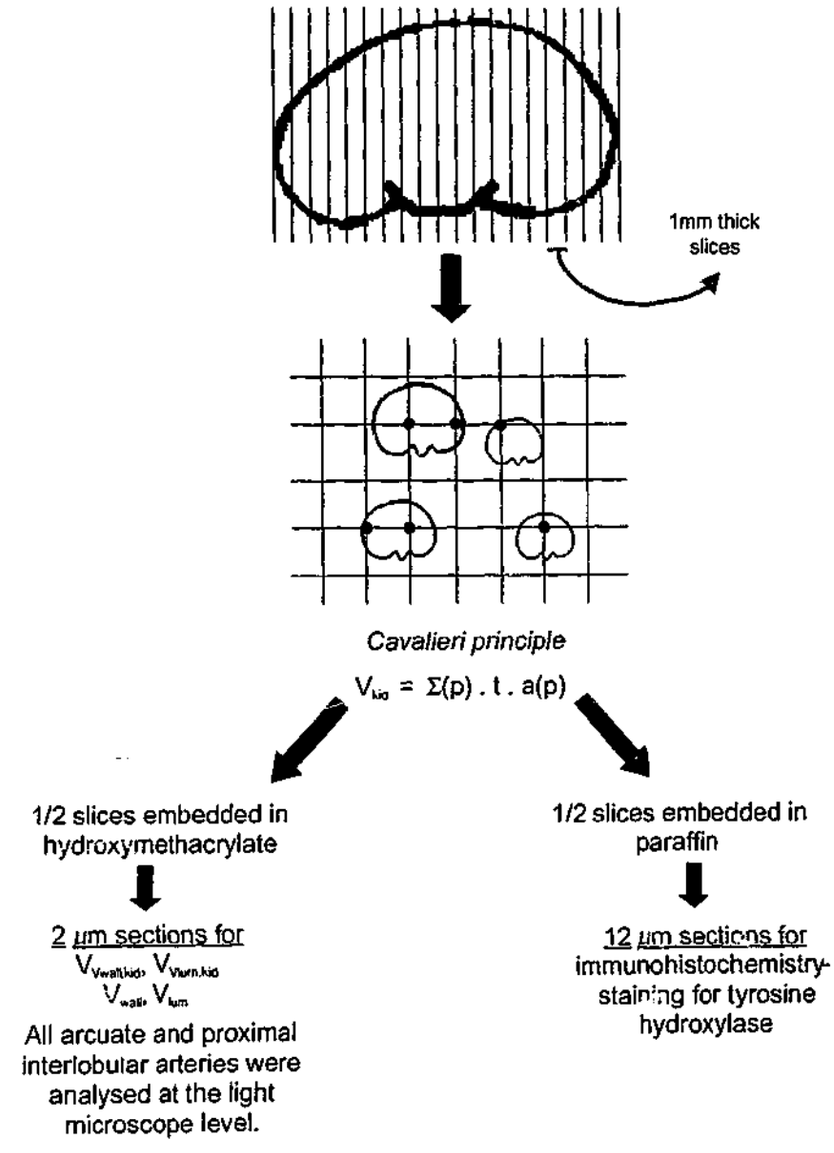

Figure 5.1 Flow diagram illustrating the slicing and handling of kidney once perfusion fixed. Kidney volume (V_{kid}) was calculated using the Cavalieri principle and is the product of the sum of all points overlaying the kidney slices ($\Sigma(p)$), slice thickness (T) and the area associated with each point of the grid (a(p)).

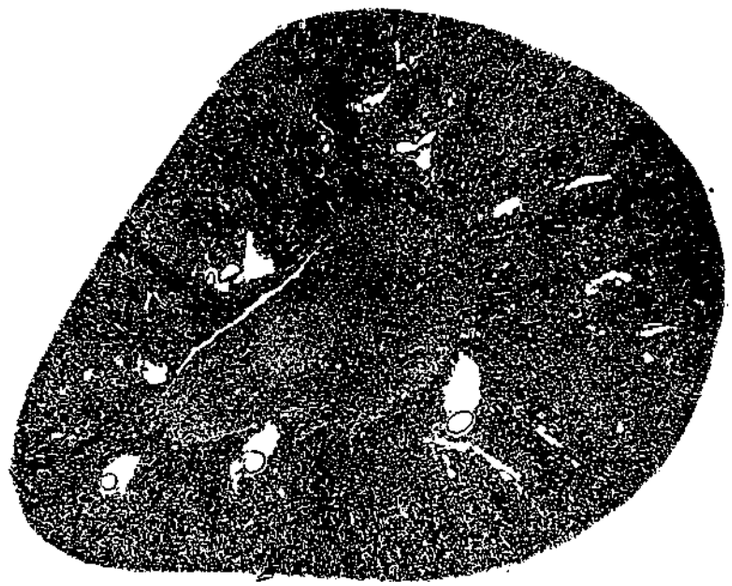

Figure 5.2a Light micrograph of 2μm thick hydroxymethacrylate section stained with haematoxylin and eosin illustrating the vessel layout at low magnification.

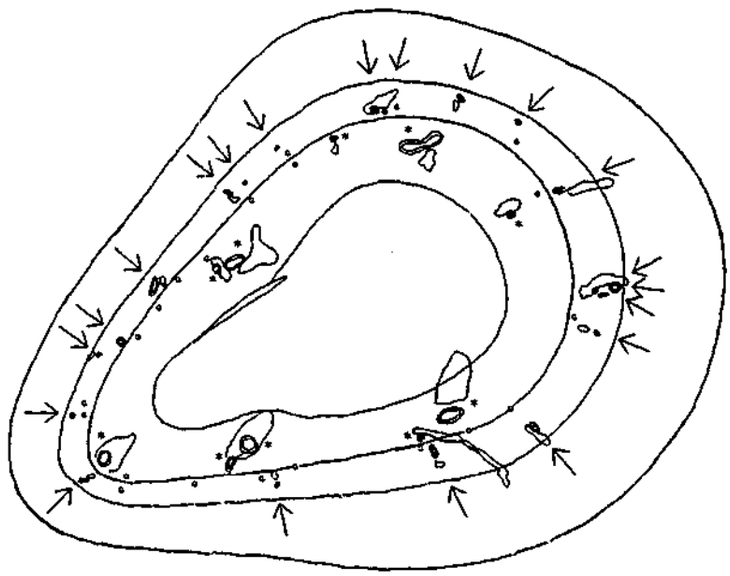

Figure 5.2b Tracing of Figure 5.2a demonstrating the selection criteria for arcuate and proximal interlobular arteries. Arcuate arteries (*), defined as those running along the cortico-medullary junction and completely surrounded by tubules. The cortex was then divided into thirds and all interlobular arteries present in the innermost third of the cortex were analysed (←). The large glomeruli (G) found near the arcuate arteries are used to define the cortico-medullary junction.

5.2.1.1 Volume Estimations

An unbiased estimate of fixed kidney volume was obtained using the Cavalieri Principle (Gundersen and Jensen, 1987; Gundersen *et al.*, 1988). In brief, 1 mm slices of fixed rat kidney were viewed with a stereoscopic microscope at a magnification of x1.25. An orthogonal grid was placed over the slices. Kidney volume (V_{kid}) was estimated by,

(1) $$V_{kid} = \Sigma P * a(p) * T$$

where ΣP is the sum of grid points overlying the cut surfaces of the kidney slices, a(p) is the area associated with each grid point (4 mm^2), and T is the thickness of each slice (1 mm).

To estimate the volume densities of the wall ($V_{Vwall,kid}$) and lumen ($V_{Vlum,kid}$) of the proximal interlobular and arcuate arteries, the hydroxymethacrylate sections were projected at a magnification of x22.5 onto a table using an Olympus BX-50 microscope modified for projection and an orthogonal grid placed over the image. The area of the kidney (A_{kid}) in the sections was calculated using,

(2) $$A_{kid} = \Sigma P * a(p)$$

where ΣP is the total number of grid points overlying the kidney sections and a(p) is the area associated with each grid point (1.235 mm^2).

For estimation of the area of the wall (A_{wall}, excluding adventitia) and lumen (A_{lum}) of the vessels the sections were projected, as above, at a magnification of x298 for the proximal interlobular and x148 for the arcuate arteries. The total area of the walls was determined by,

(3) $$A_{wall} = \Sigma P * a(p)$$

where ΣP is the total number of grid points overlying the walls and a(p) is the area associated with each grid point (1.086×10^{-3} mm^2 and 4.565×10^{-3} mm^2 for the interlobular and arcuate arteries respectively). Then the volume density of the vessel wall in the kidney ($V_{Vwall,kid}$) was determined using a standard stereological formula (Weibel, 1979),

(4) $$V_{wall}/V_{kid} = A_{wall}/A_{kid}$$

The total volume of proximal interlobular artery and arcuate artery wall contained in the kidney (V_{wall}) were then determined using,

(5) $$V_{wall} = (V_{wall}/V_{kid}) * V_{kid}$$

Equations 3, 4, and 5 were modified to estimate A_{lum}, V_{lum}/V_{kid}, and V_{lum}.

The wall-to-lumen ratio was determined using,

(6) $$Wall:Lumen = (V_{wall}/V_{kid})/(V_{lum}/V_{kid})$$

5.2.1.2 Digitiser Analysis

To estimate the wall thickness and lumen diameter of the vessels, the outer diameter of the vessel and lumen was traced at the point of minimum diameter (represents the true diameter of the vessel) and measured with a calibrated digitising tablet using MEASURE software (Capricorn Scientific Software).

5.2.1.3 Immunohistochemistry

As mentioned above (*Figure 5.1*) half of the kidney slices were embedded in paraffin for immunohistochemical analysis (*Section 2.6 Immunohistochemistry*).

5.2.2 Statistics

Values are expressed as mean ± sem (in *Table 5.1*) or mean ± SD (all Figures). A two-way ANOVA was performed with the factors being strain (WKY or SHR; P_S), treatment (sham operation or unilateral renal denervation; P_T) and the interaction between the two factors also examined (P_{S*T}).

5.3 Results

5.3.1 Haemodynamic and weight data

Body weight at 10-weeks of age was similar across all four groups and was not affected by unilateral renal denervation (*Table 5.1*). On average the WKY_S and the WKY_{Dx} (118.0 ± 6.8 and 116.1 ± 4.0 mmHg respectively) had lower mean arterial pressure (MAP) than the SHR_S and SHR_{Dx} (138.5 ± 8.9 and 142.5 ± 7.5 mmHg respectively) groups ($P_S < 0.0001$, *Table 5.1*). Unilateral renal denervation did not significantly effect MAP in either the WKY or SHR groups ($P_T = 0.69$). Heart rate was also similar in all four groups (*Table 5.1*).

Both the right and left kidneys of the two SHR groups were significantly larger when compared to the two WKY groups ($P_S \leq 0.01$, *Table 5.1*). Left ventricle to body weight ratio was significantly greater in the SHR compared to the WKY ($P_S < 0.0001$, *Table 5.1*). This ratio was not significantly different between the SHR_S/SHR_{Dx} groups (0.30 ± 0.03 and 0.32 ± 0.02 respectively) and the WKY_S/WKY_{Dx} groups (0.28 ± 0.03 and 0.27 ± 0.02 respectively, $P_{S*T} = 0.37$) following unilateral renal denervation (*Table 5.1*). Plasma renin activity was not significantly different between groups and was not significantly affected by unilateral denervation (*Table 5.1*).

5.3.2 Stereological data

Consistent with the greater kidney weight in the two SHR groups (*Table 5.1*), total kidney volume (V_{kid}, *Figure 5.3*) was also significantly greater in the two SHR groups (1651 ± 203 mm^3) compared to the WKY groups (1460 ± 211 mm^3, $P_S = 0.03$). However, there was no significant effect of chronic unilateral renal denervation on V_{kid} in either strain ($P_T = 0.27$, *Figure 5.3*).

Table 5.1 Haemodynamic and weight data at 10-weeks of age.

	SHR_S (n = 6)	SHR_{Dx} (n = 8)	WKY_S (n = 7)	WKY_{Dx} (n = 7)	P_S	P_T	P_{S*T}
Body weight (g)	260 ± 13	268 + 22	264 ± 15	268 ± 12	NS	NS	NS
Mean arterial pressure (mmHg)	138.5 ± 8.9	142.5 ± 7.5	118.0 ± 6.8	116.1 ± 4.0	< 0.0001	NS	NS
Heart rate (beats/min)	335 ± 26	341 ± 22	333 ± 42	330 ± 27	NS	NS	NS
Haematocrit (%)	46.3 ± 3.0	45.8 ± 3.0	45.6 ± 1.0	45.5 ± 0.6	NS	NS	NS
Right kidney (g)	1.55 ± 0.15	1.65 ± 0.18	1.44 ± 0.12	1.47 ± 0.06	0.01	NS	NS
Left kidney (g)	1.54 ± 0.12	1.66 ± 0.19	1.37 ± 0.13	1.41 ± 0.08	0.001	NS	NS
Left ventricle (g/per 100 g body weight)	0.30 ± 0.03	0.32 ± 0.02	0.28 ± 0.03	0.27 ± 0.02	< 0.0001	NS	NS
Plasma renin activity (ng AI/ml/h)	6.19 ± 3.11	5.64 ± 2.5	5.44 ± 1.16	4.58 ± 2.06	NS	NS	NS

Values are the mean ± sem for the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and unilaterally denervated rats (WKY_{Dx} and SHR_{Dx} respectively). *P* values were obtained from a two-way ANOVA testing for a difference between strain (P_S; WKY or SHR), treatment (P_T; sham operated or unilaterally denervated), or an interaction between strain and treatment (P_{S*T}). *P* value > 0.05 was not significant (NS).

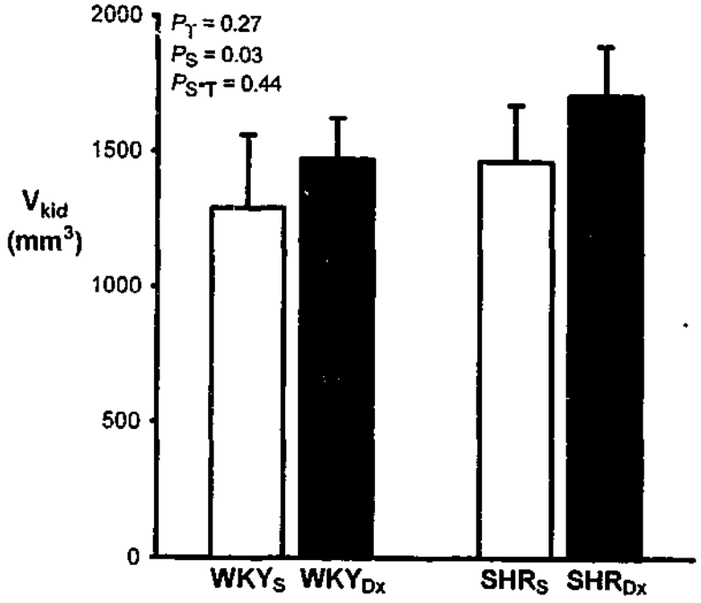

Figure 5.3 Left kidney volume (V_{kid}) of the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors (P_{S*T}).

5.3.2.1 Proximal interlobular artery

Overall chronic unilateral renal denervation had no significant effect on proximal ILA dimensions in either strain, except for a narrowing of the lumen in the SHR_{Dx} group. There was also no significant difference between the two strains in proximal ILA wall or lumen dimensions.

Volume density of the wall ($V_{Vwall,kid}$) in the SHR groups (0.49 ± 0.10 x 10^{-3} mm^3/mm^3) tended to be less than in the WKY groups (0.60 ± 0.17 x 10^{-3} mm^3/mm^3), however these values did not reach statistical significance (P_S = 0.07, *Figure 5.4*). Chronic unilateral renal denervation had no significant effect on the $V_{Vwall,kid}$ on either strain (P_T = 0.18, *Figure 5.4*).

Total wall volume (V_{wall}) was not significantly different between the two SHR and WKY groups (P_S = 0.52) and there was also no significant effect of renal denervation on either strain (P_T = 0.46, *Figure 5.4*).

Volume density of the lumen ($V_{Vlum,kid}$) in the two SHR groups ($0.52 \pm 0.19 \times 10^{-3}$ mm^3/mm^3) also tended to be less than in the WKY groups ($0.68 \pm 0.23 \times 10^{-3}$ mm^3/mm^3), however these values did not reach statistical significance ($P_S = 0.07$). Unilateral renal denervation did not significantly effect the $V_{Vlum,kid}$ in either strain, although there was a tendency for the denervated groups to have lower $V_{Vlum,kid}$ compared to the sham operated groups ($P_T = 0.07$, *Figure 5.4*).

Total lumen volume (V_{lum}) was not significantly different between the two SHR and WKY groups ($P_S = 0.34$) and there was also no significant effect of renal denervation on either strain ($P_T = 0.15$, *Figure 5.4*).

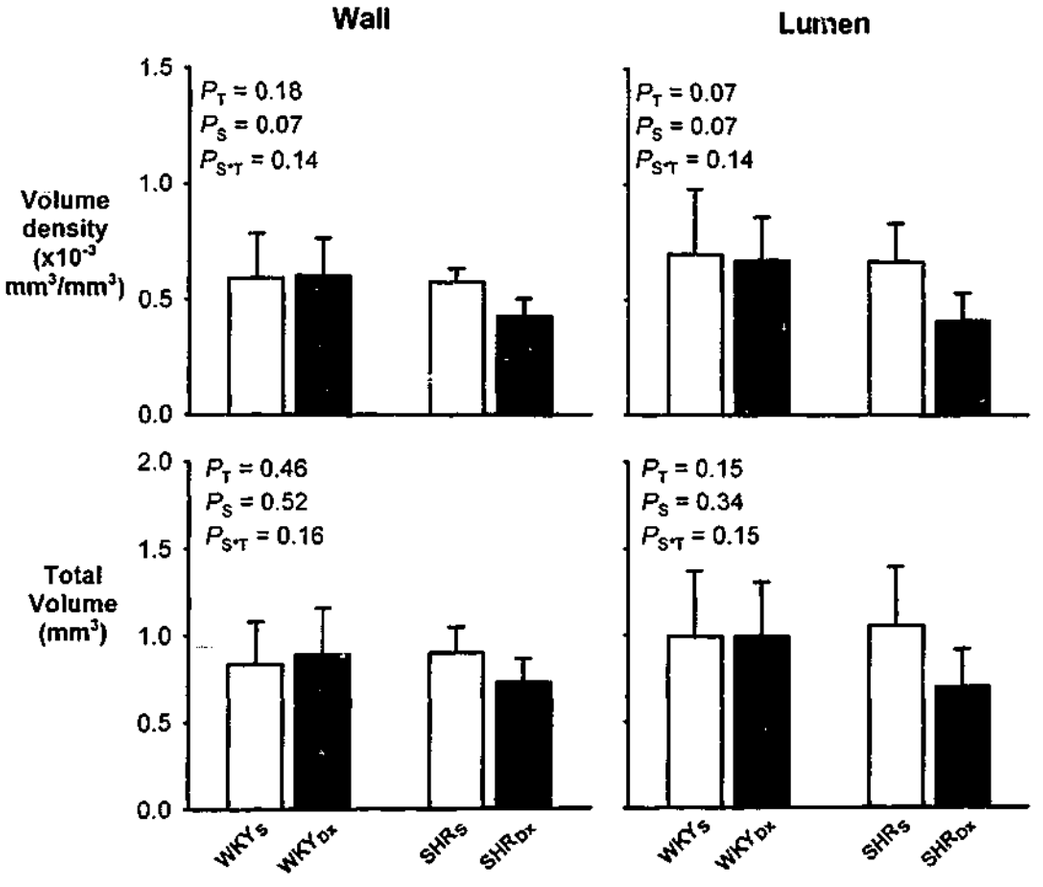

Figure 5.4 Proximal interlobular artery wall and lumen volume density ($V_{Vwall,kid}$ and $V_{Vlum,kid}$ respectively) and total volumes (V_{wall} and V_{lum} respectively) in the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors ($P_{S•T}$).

Proximal ILA wall thickness (*Figure 5.5*) was not significantly different in the SHR groups (10.2 ± 1.1 μm) compared to the WKY groups (10.3 ± 1.5 μm, $P_S = 0.92$). Unilateral renal denervation did not produce any significant effect in either strain (P_T = 0.48, *Figure 5.5*).

Proximal ILA lumen diameter was also not significantly different in the SHR groups compared to the WKY groups (P_S = 0.90). However, there was a significant effect of renal denervation, primarily a narrowing of the lumen in the SHR_{Dx} group (P_{S*T} = 0.004, *Figure 5.5*).

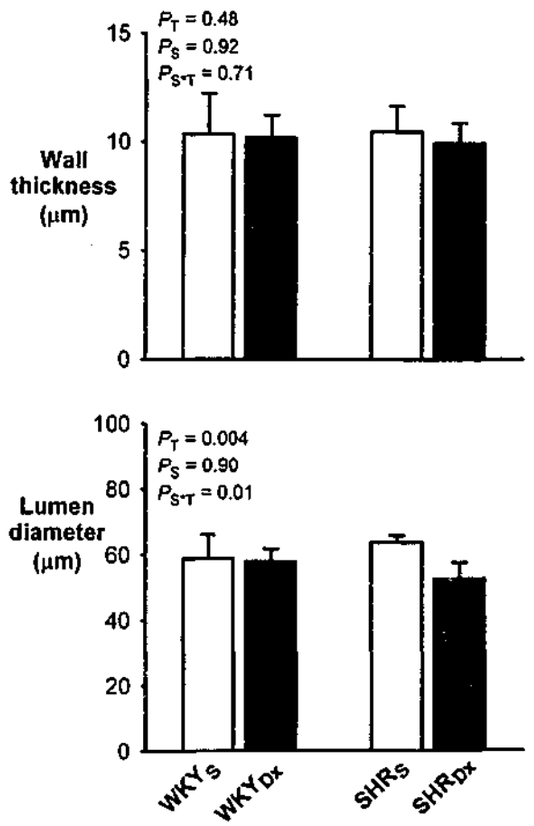

Figure 5.5 Proximal interlobular artery wall thickness and lumen diameter in the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors (P_{S*T}).

Wall to lumen ratio was not significantly different between the two strains and renal denervation did not have any statistically significant effect in either strain (*Figure 5.6*).

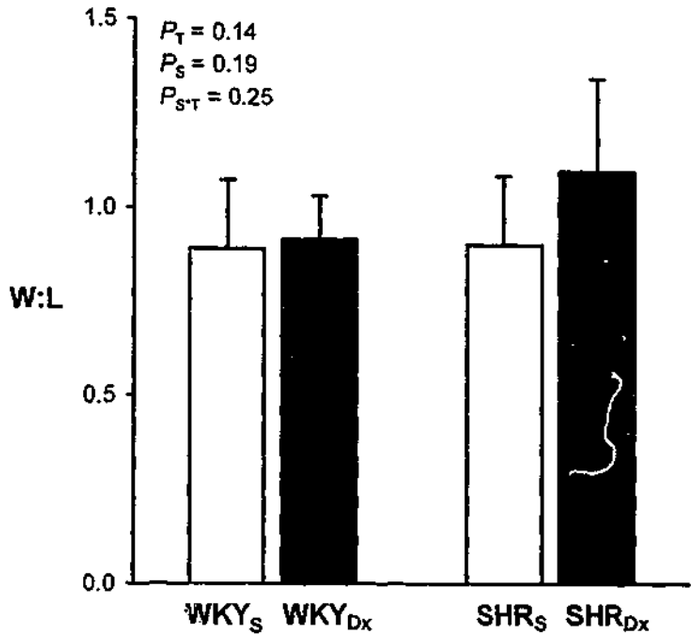

Figure 5.6 Proximal interlobular artery wall:lumen (W:L) ratio in the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors (P_{S*T}).

5.3.2.2 Arcuate artery

Chronic unilateral renal denervation had no significant effect on ARC wall or lumen dimensions in either strain. There was a significant difference between the two strains in total wall volume and wall thickness of the ARC, with the SHR demonstrating signs of vascular hypertrophy.

Arcuate artery $V_{Vwall,kid}$ in the SHR groups ($1.61 \pm 0.60 \times 10^{-3}$ mm^3/mm^3) was not significantly different to that of the WKY groups ($1.37 \pm 0.37 \times 10^{-3}$ mm^3/mm^3, $P_S = 0.19$) *Figure 5.7*). Chronic unilateral renal denervation had no significant effect on the $V_{Vwall,kid}$ in either strain ($P_T = 0.70$, *Figure 5.7*).

$\underline{V_{wall}}$ was significantly greater in the two SHR groups (2.61 ± 0.97 mm^3) compared to the WKY groups (1.97 ± 0.47 mm^3, $P_S = 0.04$), however there was no significant effect of renal denervation in either strain ($P_T = 0.99$, *Figure 5.7*).

$\underline{V_{Vlum,kid}}$ in the SHR groups (2.25 ± 0.81 x 10^{-3} mm^3/mm^3) was not significantly different to the two WKY groups (2.04 ± 0.58 x 10^{-3} mm^3/mm^3, $P_S = 0.34$). Unilateral renal denervation did not significantly effect $V_{Vlum,kid}$ in either strain (*Figure 5.7*).

$\underline{V_{lum}}$ was not significantly different between the SHR (3.67 ± 1.33 mm^3) and WKY groups (2.93 ± 0.73 mm^3, $P_S = 0.09$), renal denervation had no significant effect in either strain ($P_T = 0.86$, *Figure 5.7*).

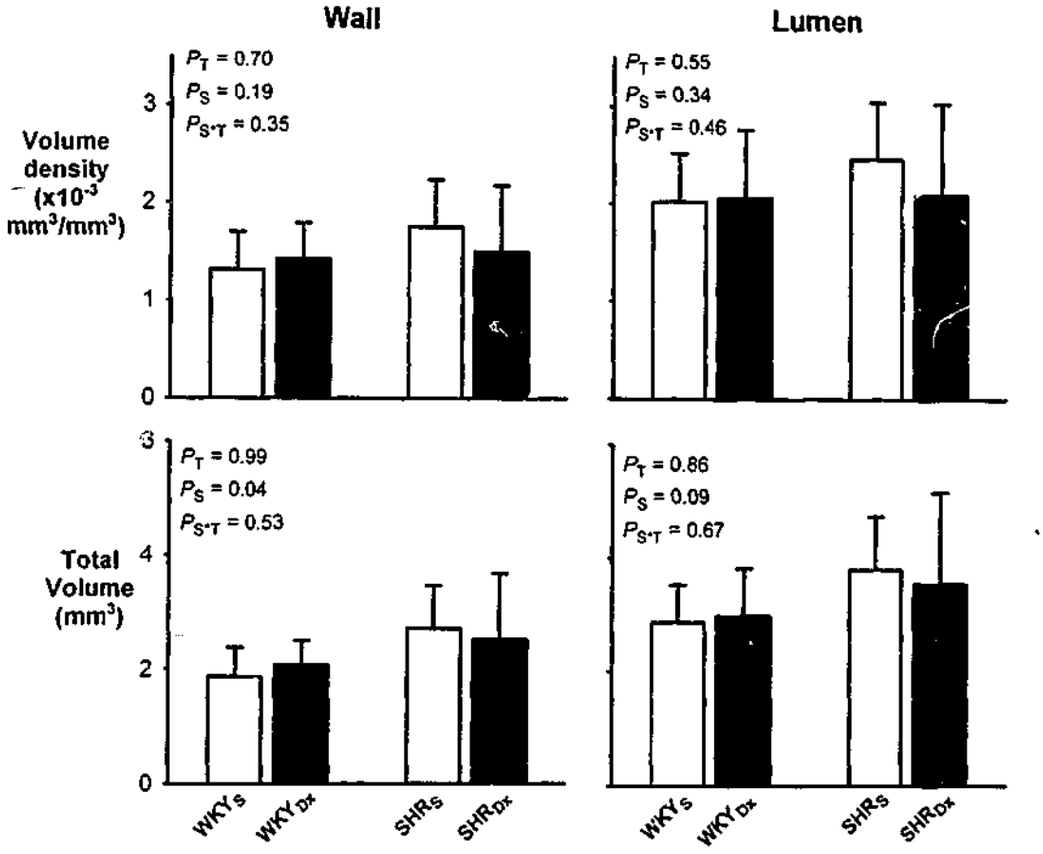

Figure 5.7 Arcuate artery wall and lumen volume density ($V_{Vwall,kid}$ and $V_{Vlum,kid}$ respectively) and total volumes (V_{wall} and V_{lum} respectively) in the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors ($P_{S•T}$).

ARC wall thickness (*Figure 5.8*) was significantly greater in the two SHR groups (19.7 ± 2.0 μm) compared to the WKY (17.8 ± 3.2 μm, $P_S = 0.04$). Unilateral renal denervation tended to reduce wall thickness in the SHR_{Dx}, however there was no significant effect in either strain ($P_T = 0.31$, *Figure 5.8*).

ARC lumen diameter was also not significantly different in the SHR groups compared to the WKY ($P_S = 0.11$), and renal denervation produced no significant effect in either strain ($P_T = 0.57$, *Figure 5.8*).

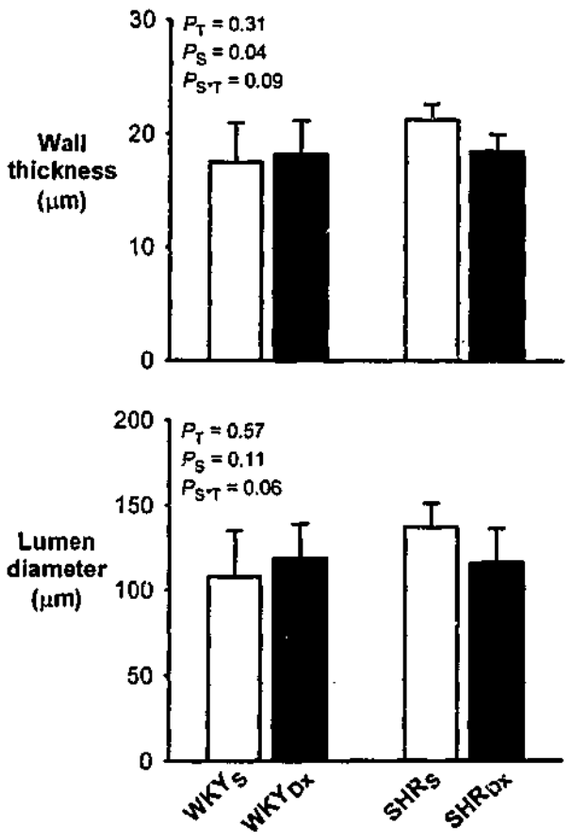

Figure 5.8 Arcuate artery wall thickness and lumen diameter in the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors ($P_{S\cdot T}$).

Wall to lumen ratio was not significantly different between the two strains and denervation did not have any statistically significant effect in either strain (*Figure 5.9*).

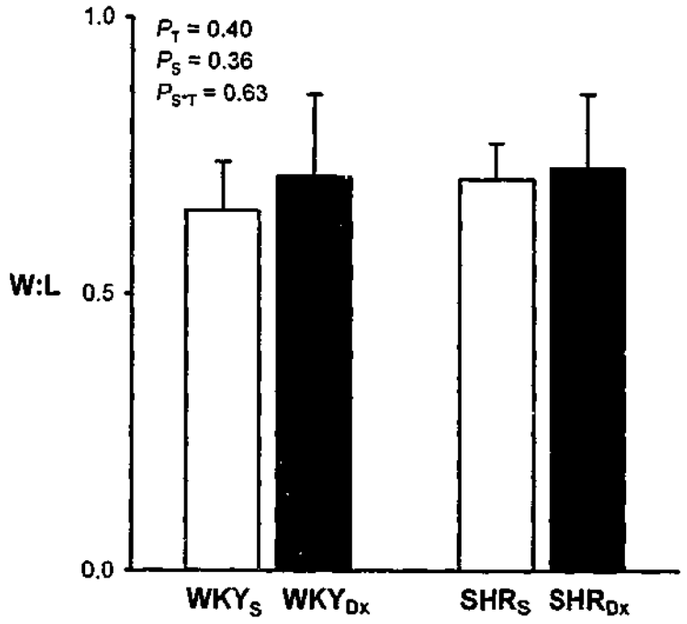

Figure 5.9 Arcuate artery wall:lumen (W:L) ratio in the sham operated Wistar-Kyoto and spontaneously hypertensive rats (WKY_S and SHR_S respectively) and those that had undergone unilateral renal denervation (WKY_{Dx} and SHR_{Dx} respectively). Values are the group mean ± SD, and *P* values were obtained from a two-way ANOVA testing for the effect of strain (P_S, WKY or SHR), treatment (P_T, sham or unilateral denervation), or the interaction between the two factors (P_{S*T}).

5.3.3 Immunohistochemistry

Immunohistochemical analysis demonstrated an absence of staining for tyrosine hydroxylase in any of the vessels examined throughout the left kidneys of both denervated groups (*Figure 5.10*). In contrast there was clear staining in all vasculature throughout the left kidneys, from the large interlobar and arcuate arteries to the superficial interlobular arteries and afferent arterioles, of both the WKY_S and the SHR_S groups (*Figure 5.10*).

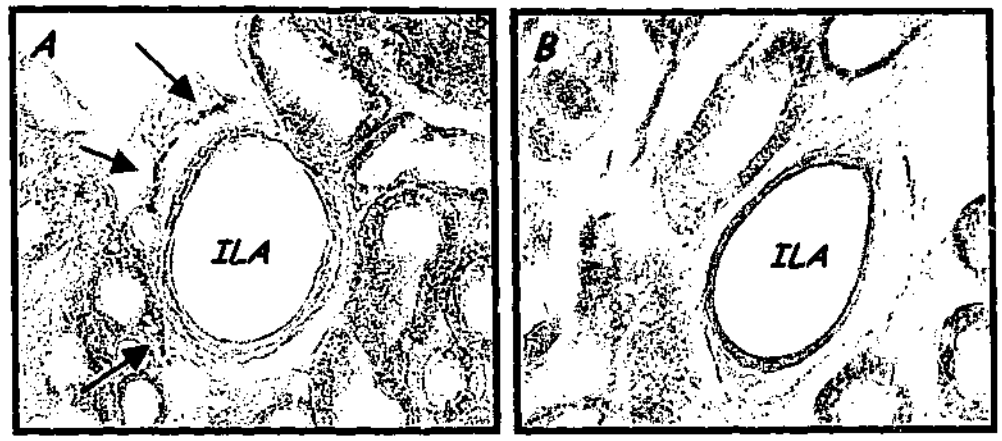

Figure 5.10 Light micrographs demonstrating typical interlobular arteries (ILA) from a sham operated kidney (A) demonstrating tyrosine hydroxylase staining for sympathetic nerve fibres (arrows) and a denervated kidney (B).

5.4 Discussion

The specific aim of the experiments performed in this chapter was to examine the role of the renal sympathetic nerves on the wall and lumen dimensions of the larger pre-glomerular vessels (arcuate and proximal interlobular arteries) in SHR and WKY rats. Vessels of this size (diameters greater than ~ 90 – 100 μm) have previously been reported to be responsible for much of the pressure difference between SHR and WKY rats in other non-renal vessels (Bohlen, 1986; Korner and Angus, 1992). Previously Tomoda *et al* (1997) performed a study in which 6-week old SHR underwent either chronic unilateral renal denervation or sham operation and then a functional test of renal vascular resistance was performed four weeks later. Consistent with previous findings (Tomoda *et al.*, 1997), chronic unilateral renal denervation in the current study, did not affect blood pressure in either strain. This was very important with respect to the experimental design and the main hypothesis, as the interpretation of the results therefore were not confounded by differences in systemic arterial pressure.

In the current study, there was no significant effect of unilateral renal denervation on the proximal ILA wall dimensions ie SHR did not respond to chronic unilateral renal denervation differently to the WKY rats. There was also no significant difference in wall to lumen ratio or volume density of both the wall and lumen of the ARC between strains. In the chronically denervated groups, there was a tendency for a thinner wall and a significantly narrower lumen in the ARC of the SHR_{Dx}. The results of this chapter demonstrated no significant difference between SHR and WKY in the

estimation of proximal ILA wall to lumen ratio, volume density or total volume of both the wall and lumen and also wall thickness or lumen diameter, these will be discussed below. However, SHR did demonstrate signs of hypertrophy in the ARC in that total wall volume and wall thickness were significantly greater than in the WKY rats. In summary, it appears that chronic unilateral renal denervation had no significant effect on the wall or lumen parameters of either the proximal ILA or ARC of SHR and WKY in this study. However, there was an interesting observation in the proximal ILA of the SHR_{Dx}, in that lumen diameter was significantly narrower. The functional consequences of these findings will be addressed in *Chapter 6*.

Until recently, methods for counting structures in three-dimensional tissues and organs were very limited. However, we now have methods which are accurate (unbiased) and precise (reproducible; Bertram, 2001). Stereology is concerned with the quantitative analysis of three-dimensional structures and therefore is well suited for the quantitative analysis of tissues and organs (Bertram, 1995; Bertram, 2001). By obtaining a small section, representative of the tissue of interest, it is possible to obtain an estimate of the whole organ. Our group has widespread experience in these techniques (Kett *et al.*, 1995; Kett *et al.*, 1996a: Kett *et al.*, 1996b), and it is an excellent tool with which the renal vasculature can be examined *in vitro* at close to *in vivo* conditions. It should be noted that the Cavalieri principle provides an estimate of perfusion fixed kidney volume, while estimates of volume density and total volume were for perfusion fixed, embedded and sectioned kidney samples. There is evidence to suggest that processing of tissue for microscopy often alters the volumes of tissue components (Weibel, 1979; Kett *et al.*, 1995). Our group has previously demonstrated that the same processing procedure as that used in this chapter produces minimal shrinkage (Kett *et al.*, 1995), and therefore no correction for shrinkage was introduced into the calculations.

The SHR and WKY used throughout this study, and indeed throughout the whole thesis come from the same colony used by our group previously (Anderson, 1994; Kett *et al.*, 1995; Kett *et al.*, 1996a; Tomoda *et al.*, 1997; Bergström *et al.*, 1998; Kett *et al.*, 2001a; Kett *et al.*, 2001b). The SHR used in this study demonstrated a significantly greater MAP at 10-weeks of age compared to the WKY rats (~ 20-25 mmHg difference), and also showed a significant level of left ventricular hypertrophy (as evidenced by the greater left ventricle to body weight ratio). Unlike our previous study (Kett *et al.*, 1995), the proximal ILA did not demonstrate any signs of hypertrophy in the SHR compared to WKY rats. Wall-to-lumen ratio, of both the

proximal ILA and ARC, were not significantly greater in the SHR compared to the WKY rats. However, there was a significant increase in V_{wall} of the ARC in the SHR, suggesting the presence of wall hypertrophy at least in the ARC. The reason for the difference in my findings compared to previous studies is unknown despite using the same colony of rats. The differences can perhaps be explained by the fact that in the current experiment the renal vessels were examined under different conditions.

In the current study, the vessels were examined under conditions of maximal dilatation and perfusion fixed at pressures equivalent to conscious MAP. Kett *et al* (1995) examined both the proximal ILA and ARC following similar perfusion fixation methods as those used in this chapter, however not under conditions of maximal dilatation, and therefore these results are not directly compatible to the data I have presented. Sharifi *et al* (1998) examined renal vessels of similar size to the ARC I examined, using a wire-myograph-mounted preparation, either at rest or pressurised to 100 mmHg, but did not have vessels from WKY rats for comparison. Smeda *et al* (1988) also examined maximally dilated renal vessels in 21-week old SHR and WKY rats, and divided the vessels according to size. The authors noted a significantly thicker wall in the cortical vessels (in vessels greater than 70 μm in diameter) of the SHR compared to age-matched WKY rats (Smeda *et al.*, 1988b), however these vessels were dissected and isolated from the surrounding kidney tissue. Therefore, the fact that I did not find a significant difference in vessel wall hypertrophy in the proximal ILA of the SHR, may mean that these parameters are dependent on the methods used (Smeda *et al.*, 1988b; Kett *et al.*, 1995; Sharifi *et al.*, 1998).

There is evidence that the length of a vessel will also affect arterial wall thickness, with the arterial wall thickening as the vessel shortens (Lew and Angus, 1992). Vessel length *in vivo* is not only dependent on the pressure within the artery, but also on the tethering of the artery to the surrounding tissue (Lew and Angus, 1992). By dissecting the renal vessels away from the dense renal parenchyma (Smeda *et al.*, 1988a; Smeda *et al.*, 1988b), it is possible to change the length, and therefore wall thickness and lumen diameter of the arteries (Lew and Angus, 1992). By examining the renal vessels in complete kidney sections, as in this chapter and Kett *et al* (1995), the connections to the surrounding parenchyma are maintained therefore reducing the risk of detecting "artifactual" changes in vessel parameters. Therefore, the method of fixation used in this chapter is perhaps a better means of examining, *in vivo*, renal vascular properties.

To date there are very few studies that have examined the renal vasculature following perfusion fixation (at *in vivo* pressures) and under conditions of maximal dilatation in SHR and WKY rats. One factor for consideration is that perhaps hypertrophy of the ILA is not uniform throughout the cortex of the SHR. It is also notable that the levels of renal noradrenaline and arterial pressure measured in the SHR used throughout this thesis were much lower than previous studies (Kett *et al.*, 1995; Tomoda *et al.*, 1997). In the previous two chapters I measured tissue noradrenaline content in rats that had undergone renal denervation or sham operation. The noradrenaline levels I obtained in my sham operated rats was much lower (~ 50% lower) when compared to levels previously reported in the literature (Kline *et al.*, 1978; Head *et al.*, 1985; Tomoda *et al.*, 1997). Renal noradrenaline content is greater in SHR compared to WKY rats (Head *et al.*, 1985), and is hypothesised to correlate to arterial pressure. It is therefore possible that my rats had lower renal noradrenaline content compared to other groups and therefore, were not as hypertensive. Perhaps this explains why the degree of hypertrophy in the renal vasculature was not as pronounced as previously reported by our group (Kett *et al.*, 1995).

Chronic unilateral renal denervation has not been used to examine the role of the renal sympathetic nerves in the hypertrophy of the renal vasculature of SHR. Only one other study has used this same denervation protocol (as used throughout this thesis), and using a functional test of lumen diameter compared the denervated kidney to a sham operated kidney (Tomoda *et al.*, 1997), but not examined renal vascular hypertrophy directly. As covered in detail in *Section 1.4.1.3 Unilateral renal denervation*, the authors suggested that an increase in pre-glomerular lumen diameter and a decrease in post-glomerular lumen diameter must have occurred in response to chronic unilateral renal denervation (Tomoda *et al.*, 1997). The results of Tomoda *et al* (1997) study are not consistent with the findings of this thesis so far. In the previous chapter I demonstrated that there was no significant effect of chronic unilateral renal denervation on the pre-glomerular vasculature (interlobular artery and afferent arteriole), but there was a significant reduction in calculated relative resistance of the efferent arteriole. In this chapter there was no evidence that chronic unilateral renal denervation significantly affects the wall of either the ARC or proximal ILA, there was however a significantly narrower lumen in the proximal ILA of the SHR_{Dx} (ie evidence of remodelling in the proximal ILA following denervation). The evidence from the experiments performed in this thesis, so far, are not in favour of the renal sympathetic nerves having a role in the development of hypertrophy of the pre-glomerular vasculature.

The reasons for the unexpected findings of this chapter are not known. However, along with the findings of the previous chapter, these results demonstrate no evidence for attenuation of hypertrophy in the pre-glomerular vasculature following chronic unilateral renal denervation in SHR. The reduced proximal ILA lumen diameter in SHR_{Dx} group is difficult to explain. My main hypothesis was that the renal sympathetic nerves were responsible for the hypertrophy/remodelling observed in the pre-glomerular vasculature of the SHR. Therefore, upon renal denervation it would be consistent with this hypothesis that proximal ILA lumen diameter would be greater, not reduced, and that wall thickness would be reduced. The original hypothesis would also be consistent with the proposed structural alterations producing an increase in glomerular capillary pressure (P_{gc}). However, the fact that a narrowing of the proximal ILA was observed in this chapter (granted, that no information with regards to the efferent arteriole is obtained here) would perhaps produce a reduction in P_{gc}. As my findings were inconsistent with the original hypothesis of this thesis, the functional consequences needed to be addressed further, and that was the specific aim of *Chapter 6*.

CHAPTER 6

CHAPTER 6

Micropuncture analysis of the renal haemodynamics following chronic unilateral renal denervation in SHR

6.1 Introduction

The previous two chapters have concentrated on the morphological effects of unilateral renal denervation, in young SHR, and demonstrated narrowing of the proximal ILA. While there was significant reduction in calculated relative resistance of the efferent arteriole, estimated from measurements of lumen diameter from vascular casts (*Chapter 4*). In the current chapter my aim was to examine the functional consequences of the significantly reduced efferent arteriole resistance and apparent narrowing of the proximal ILA with the use of micropuncture techniques to assess glomerular capillary pressure (P_{gc}) and to calculate pre- and post-glomerular conductance in the SHR *in vivo*.

Morphological analysis (as in the previous two chapters) does not provide us with information about the functional consequences of any structural alterations occurring due to chronic unilateral renal denervation. Micropuncture is a challenging procedure, but it is the only means by which P_{gc} can be measured directly or estimated from stop-flow pressure measurements. It is essential in studies evaluating the contribution of the pre- and post-glomerular vessels to changes in renal haemodynamics and glomerular function. The experiments in the previous two chapters were performed *in vitro*, and therefore the specific aim of this chapter was to characterise the renal haemodynamic effects four weeks after unilateral renal denervation in SHR, *in vivo*.

Four weeks following unilateral renal denervation, P_{gc} and pre- and post-glomerular conductance were calculated, in volume replete SHR. In order to examine the consequences of chronic unilateral renal denervation on renal vessel structure as opposed to the acute effects of the renal sympathetic nerves, two experimental periods were performed. Period 1 examined the renal haemodynamic response to chronic unilateral renal denervation (four weeks prior). Period 2 examined whether any renal haemodynamic differences observed between the two groups in Period 1 persisted following acute renal denervation.

6.2 Methods

Male SHR were obtained from the Baker Medical Research Institute at 6-weeks of age and assigned randomly to undergo either a unilateral renal denervation (SHR_{Dx}, n = 9) or sham operation (SHR_S, n = 7; *Section 2.2 Surgical denervation of the kidney*). All rats were monitored for the following four weeks until 10-weeks of age, during this period body weight was recorded weekly. At 10-weeks of age the rats were anaesthetised with methohexitone sodium solution (50 mg/kg, i.p.) and the tail artery was cannulated (*Section 2.3.2 Direct tail artery method*), following a 1h recovery period, conscious MAP was measured for 15 min.

6.2.1 Micropuncture preparation

Following measurement of conscious MAP the rat was then re-anaesthetised with Inactin (175 mg/kg, i.p., Research Biochemicals International, Massachusetts, USA) and placed on a warm operating table. Body temperature was maintained throughout the experiment at 36-38°C using a servo-controlled infrared lamp (Digi-Sense; Cole Palmer, Vernon Hills, Illinois, USA). Once a surgical level of anaesthesia was achieved, tracheotomy (SV 110; Dural Plastics & Engineering, New South Wales, Australia), and cannulation of the left jugular vein (PE 50; Critchley Electrical, New South Wales, Australia) were performed. After cannulation of the trachea, a stream of 100% oxygen was blown toward the tracheal tube throughout the experiment. A continuous infusion of 2% bovine serum albumin (BSA; 6 ml/h) was then commenced in-order to replace fluid loss during surgery (*Figure 6.1*). A catheter (PE 50) was placed in the bladder via a suprapubic incision in-order to collect urine from the right kidney throughout the experiment. Via a flank incision the left kidney was exposed, decapsulated, placed in a cup for micropuncture and the left ureter cannulated (PE 50)

for urine collection. A bath was constructed around the kidney (using cotton wool) and sealed with agar (Denton and Anderson, 1991). Following surgical preparation for micropuncture, the 2% BSA infusion was reduced to 3 ml/h. A saline infusion containing [^{3}H]-inulin (methoxy-[^{3}H]-inulin; NEN Life Science Products, Massachusetts, USA) and [^{14}C]-para amino hippuric acid (PAH; NEN Life Science Products, Massachusetts, USA) was commenced (0.2 ml bolus 0.4 μCi and 0.1 μCi respectively plus 1.08 ml/100g/h; 278 nCi/ml and 83 nCi/ml respectively) and the rat allowed to equilibrate for one hour (*Figure 6.1*).

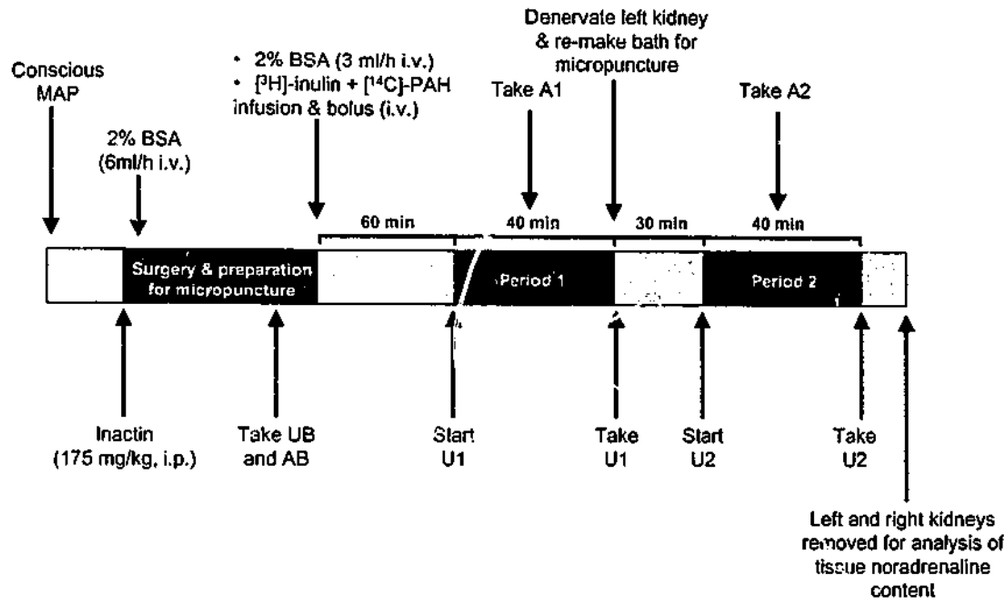

Figure 6.1 Schematic representation of the experimental protocol (see text for full explanation). During surgery rats received infusion of 2% bovine serum albumin (BSA), which was turned down once radioactive infusion had commenced. The experimental protocol consisted of two periods with urine (U1 and U2) and arterial blood samples (A1 and A2) collected. Blank urine (UB) and arterial blood samples (AB) were taken prior to commencement of the radioactive infusion.

6.2.2 Experimental protocol

The protocol consisted of two experimental periods (before and after acute denervation of the left kidney), each period consisting of a 40-min urine collection with an arterial blood sample (0.25 ml) taken at the mid-point of each period (*Figure 6.1*). Period 1 allowed examination of the effects of unilateral renal denervation performed four weeks prior to the experiment. All rats then underwent acute denervation of the

left kidney this allowed assessment of the effects from possible structural alterations occurring due to chronic unilateral renal denervation, as opposed to an acute effect of the renal sympathetic nerves (Period 2). Measurements made in each period included; MAP, HR, haematocrit, effective renal blood flow (ERBF), whole kidney GFR, FF, urine flow rate (UFR), arterial protein concentration, stop-flow pressure (SFP), proximal tubule pressure (P_t), plasma and urinary Na^+ concentrations (by ion selective electrode on a Beckman Synchron CX5 Clinical System). Micro-pressures were recorded using a 900A Micropressure System (World Precision Instruments, Florida, USA), and at least 3 recordings of each pressure were made. At the end of the experiment, the rat was given an anaesthetic overdose and kidneys excised, weighed and then rapidly frozen (n = 4 from each group) in liquid nitrogen for subsequent analysis of tissue noradrenaline levels (*Section 2.5 Tissue noradrenaline assay*).

6.2.3 Measurements & calculations

GFR and effective renal plasma flow (ERPF) were determined as the clearance of [^{3}H]-inulin and [^{14}C]-PAH respectively (Correia *et al.*, 1999). Filtration fraction (%) was determined as

$$FF = (GFR/ERPF) * 100$$

Effective renal blood flow (ml/min) was calculated as

$$ERBF = ERPF/(1 - haematocrit)$$

Renal vascular conductance (RVC; ml/min/mmHg) was calculated as

$$RVC = RBF/MAP$$

Urinary sodium excretion (U_{Na}^+V; mmol/min) was calculated as

$$U_{Na}^+V = [Na^+]/(1000 * UFR)$$

and fractional sodium excretion (FE_{Na}^+; %) calculated as

$$FE_{Na}^+ = U_{Na}^+V/(100 * GFR)$$

Arterial protein concentration (C_a, g %) was measured using the Lowry method (Lowry *et al.*, 1951) and colloid oncotic pressure calculated (Landis and Pappenheimer, 1963). Estimated glomerular capillary pressure (P_{gc}; mmHg) was calculated as

$$P_{gc} = SFP + \pi_a$$

where π_a is arterial colloid oncotic pressure (Gertz *et al.*, 1966). Pre-glomerular conductance (ml/min/mmHg) was determined as

$$\text{Pre-glomerular conductance} = RBF/(MAP - P_{gc})$$

and post-glomerular conductance (ml/min/mmHg) as

$$\text{Post-glomerular conductance} = (RBF - GFR)/P_{gc}$$

a modification of the Gomez method (Gomez, 1951). The glomerular ultrafiltration coefficient (K_f, ml/min/mmHg) was calculated as

$$K_f = GFR/(P_{gc} - P_t - \pi_{gc})$$

where glomerular oncotic pressure (π_{gc}) equals

$$\pi_{gc} = (\pi_a + \pi_e)/2$$

and efferent oncotic pressure (π_e) calculated using the Landis and Pappenheimer equation (Landis and Pappenheimer, 1963),

$$\pi_e = (1.736 * C_e) + (0.281 + C_e^2)$$

where C_e is the efferent arteriole protein concentration (g %)

$$C_e = C_a/[1 - (FF/100)]$$

When $\pi_e/\Delta P > 1$, minimum values of K_f were calculated assuming $\pi_e = \Delta P$ (Deen *et al.*, 1972), where ΔP is the hydrostatic pressure difference (mmHg)

$$\Delta P = P_{gc} - P_t$$

6.2.3.1 Stop-flow pressure (SFP) measurements

As mentioned above, P_{gc} was estimated from SFP (Gertz *et al.*, 1966). SFP was measured by placing a glass pipette (10 μm tip), filled with castor oil and Sudan Black, into a tubule and a small amount injected in-order to block flow. The oil droplet was held steady by the application of pressure to the oil pipette. A second pipette (1 - 2 μm tip) was then inserted into the tubule between the oil droplet and the glomerulus, and pressure was measured when flow was stopped in the tubule (ie pressure at which filtration ceases, see *Figure 6.2*).

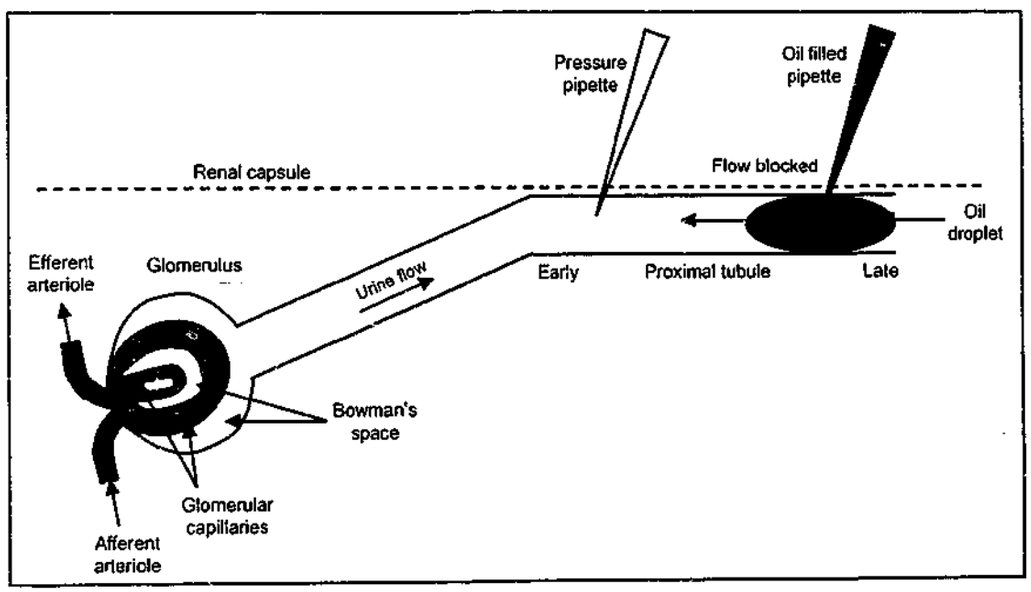

Figure 6.2 Diagrammatic representation of stop-flow pressure measurement. SFP is the pressure at which filtration ceases ie pressure is equal and opposite to net ultrafiltration pressure (see text for full description).

6.2.4 Statistics

All values are expressed as mean ± sem. The effect of chronic unilateral renal denervation (n = 9) versus sham operated animals (n = 7) was compared using one-way ANOVA (Period 1). The responses observed, after acute renal denervation (Period 2), following previous unilateral denervation were compared to those of Period 1 using two-way ANOVA, testing for an effect of chronic unilateral denervation (P_{treat}), an effect between the two experimental periods (P_{period}) and for an interaction between the two factors ($P_{treat*period}$).

6.3 Results

6.3.1 Systemic and renal haemodynamic responses to chronic unilateral renal denervation

6.3.1.1 Conscious systemic haemodynamics and weight data

Conscious MAP was similar in the SHR_{Dx} and SHR_S (*Table 6.1*). Conscious heart rate was also similar between the SHR_{Dx} and the SHR_S (*Table 6.1*). There was no significant difference in body weight, left or right ventricular weight or left and right kidney weights between the two groups at 10-weeks of age (*Table 6.1*).

Table 6.1 Systemic haemodynamic and weight data for 10-week old SHR

	SHR_S (n = 7)	SHR_{Dx} (n = 9)	*P*
Body weight (g)	272 ± 3	282 ± 5	NS
Mean arterial pressure (mmHg)	127.0 ± 1.6	127.4 ± 3.7	NS
Heart rate (beats/min)	313 ± 3	318 ± 5	NS
Left ventricle (g/100g body weight)	0.248 ± 0.007	0.242 ± 0.007	NS
Right ventricle (g/100g body weight)	0.058 ± 0.004	0.062 ± 0.003	NS
Left kidney (g, wet weight)	1.35 ± 0.03	1.51 ± 0.08	NS
Right kidney (g, wet weight)	1.14 ± 0.03	1.16 ± 0.03	NS

Values are expressed as the group mean ± sem. *P* value was obtained using a one-way ANOVA comparing the unilaterally denervated group (SHR_{Dx}) to the sham operated group (SHR_S). *P* values > 0.05 were not significant (NS).

6.3.1.2 Anaesthetised systemic haemodynamics and whole kidney function (Period 1)

Throughout the first period there was no significant difference in MAP, HR or haematocrit (*Table 6.2*) between the SHR_S or the SHR_{Dx} groups.

Table 6.2 Anaesthetised systemic haemodynamic response to chronic unilateral renal denervation.

	SHR_S (n = 7)	SHR_{Dx} (n = 9)	*P*
Mean arterial pressure (mmHg)	131.1 ± 3.6	127.3 ± 4.8	NS
Heart rate (beats/min)	275 ± 12	287 ± 11	NS
Haematocrit (%)	42.5 ± 1.3	43.3 ± 1.0	NS

Values are the group mean ± sem for the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) groups, for the first experimental period. *P* value was obtained via a one-way ANOVA testing for an effect of treatment. *P* values > 0.05 were not significant (NS).

Left kidney function

The left kidney of the SHR_{Dx} group had a slightly higher ERBF compared to the SHR_S group (6.62 ± 0.88 and 5.64 ± 0.68 ml/min respectively), but these values were not significantly different (P = 0.40, *Figure 6.3*). RVC for the left kidney was significantly greater in the SHR_{Dx} group compared to the SHR_S group (P = 0.01, *Figure 6.3*).

GFR was not significantly different between the SHR_S and SHR_{Dx} for the left kidney (0.709 ± 0.120 and 0.886 ± 0.061 ml/min respectively, *Figure 6.4*). There was no significant difference in FF between the SHR_S and SHR_{Dx} groups in the left kidney (21.2 ± 2.6 and 25.9 ± 3.3 % respectively, P = 0.29). UFR was slightly but not significantly reduced in the left kidney of the SHR_{Dx} (P = 0.11, *Figure 6.4*) compared to the SHR_S group.

Urinary sodium excretion (U_{Na}^+V) in the left kidney was not significantly different between the two groups (P = 0.68, *Figure 6.5*). Left kidney fractional sodium excretion (FE_{Na}^+) was slightly, but not significantly lower in the SHR_{Dx} compared to the SHR_S group (P = 0.39, *Figure 6.5*).

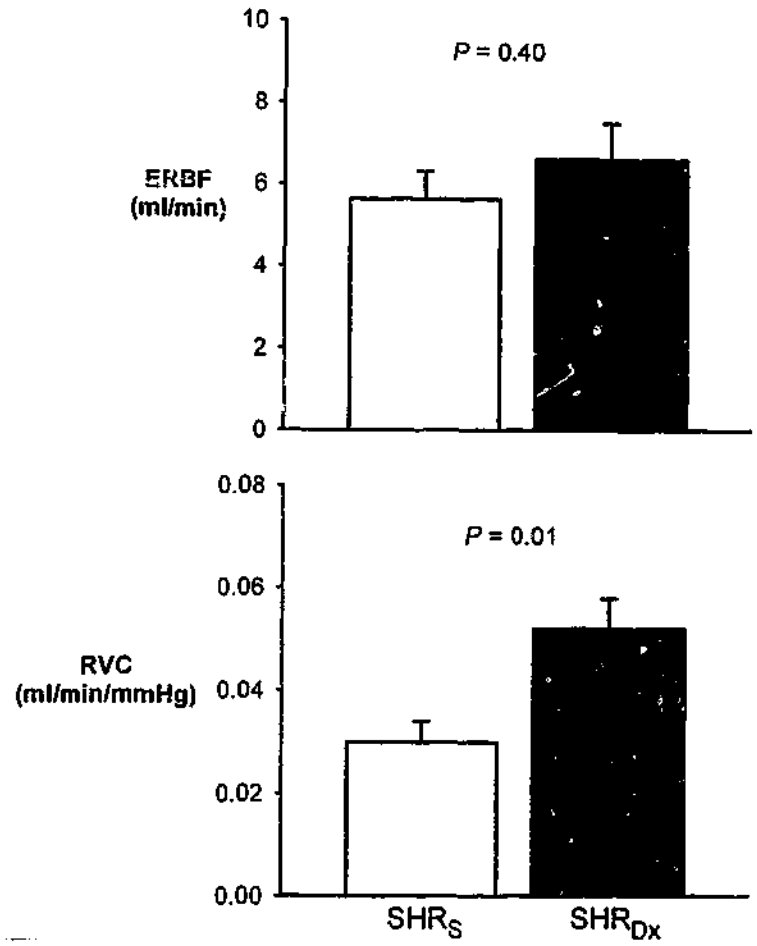

Figure 6.3 Effective renal blood flow (ERBF) and renal vascular conductance (RVC), mean ± sem, in the left kidneys of unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. *P* values were obtained using a one-way ANOVA testing for an effect of denervation.

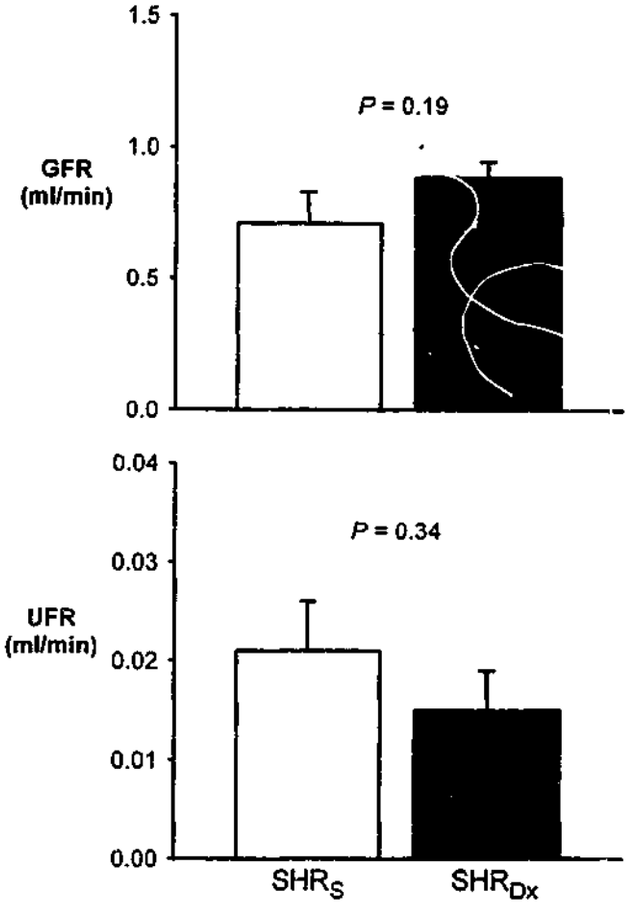

Figure 6.4 Glomerular filtration rate (GFR) and urine flow rate (UFR), mean ± sem, in the left kidneys of unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. *P* values were obtained using a one-way ANOVA testing for an effect of denervation.

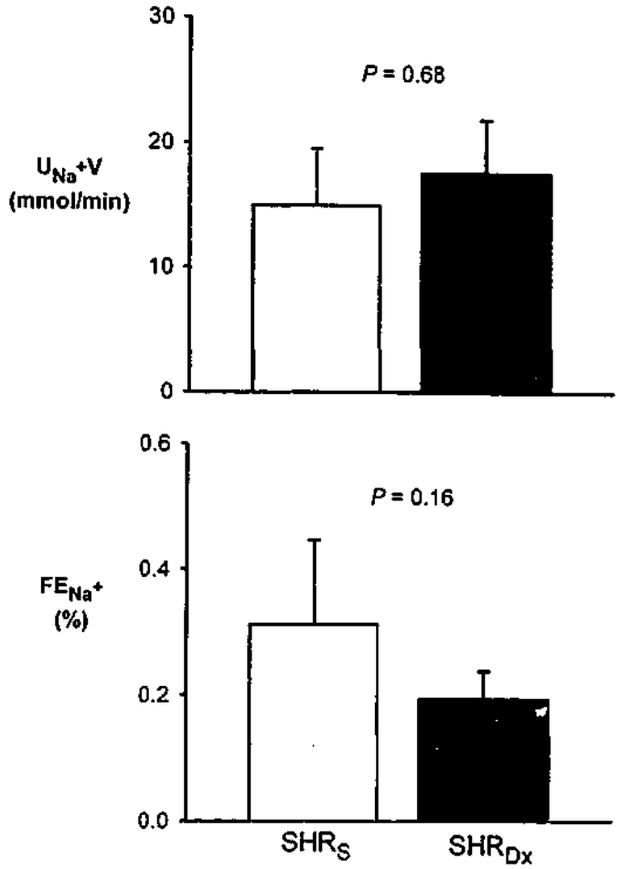

Figure 6.5 Urinary sodium excretion ($U_{Na}^{+}V$) and fractional sodium excretion (FE_{Na}^{+}), mean ± sem, in the left kidneys of unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. *P* values were obtained using a one-way ANOVA testing for an effect of denervation.

6.3.1.2 Micro-pressure measurements

Estimated P_{gc} was significantly lower in the SHR_{Dx} group (47.5 ± 0.8 mmHg) compared to the SHR_S group (50.4 ± 1.2 mmHg, *Figure 6.6*). While SFP and P_t were not significantly different between the two groups (*P* = 0.12 and 0.68 respectively, *Figure 6.6*). C_a in the SHR_S group (5.1 ± 0.2 g %) was similar to that in the SHR_{Dx} group (4.9 ± 0.1 g %, *P* = 0.35).

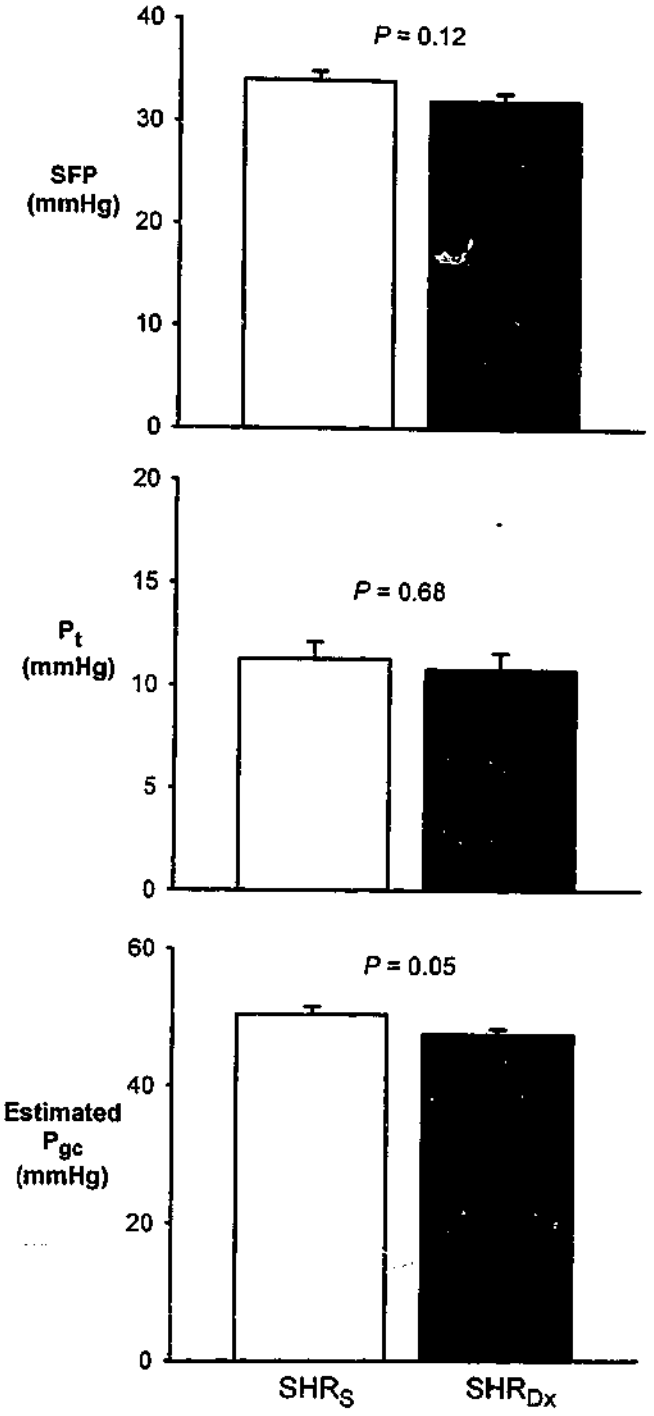

Figure 6.6 Stop-flow pressure (SFP), proximal tubular pressure (P_t) and estimated glomerular capillary pressure (P_{gc}), mean ± sem, in the left kidneys of the unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. *P* values were obtained using a one-way ANOVA testing for an effect of denervation.

Pre-glomerular conductance was not significantly different between the SHR_{Dx} group (0.084 ± 0.010 ml/min/mmHg) and the SHR_S group (0.071 ± 0.009 ml/min/mmHg, *Figure 6.7*). Post-glomerular conductance also slightly, but not

significantly, greater in the SHR_{Dx} group (0.123 ± 0.019 ml/min/mmHg) compared to the SHR_S group (0.099 ± 0.012 ml/min/mmHg, *Figure 6.7*). Pre-:post-glomerular conductance ratio was not significantly different between the SHR_S (0.732 ± 0.035) and the SHR_{Dx} group (0.726 ± 0.065, $P = 0.95$). K_f was not significantly different between the SHR_S (0.04 ± 0.01 ml/min/mmHg) and SHR_{Dx} (0.07 ± 0.02 ml/min/mmHg, $P = 0.18$) groups.

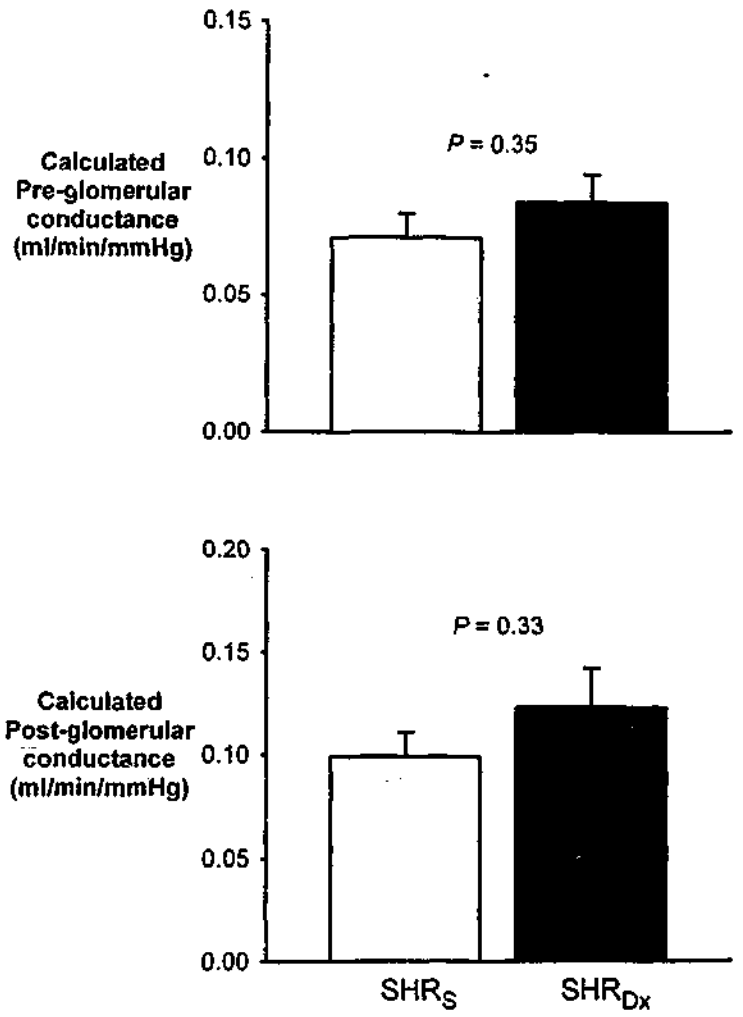

Figure 6.7 Pre- and post-glomerular conductance, mean ± sem, in the left kidneys of the unilaterally denervated (SHR_{Dx}) and sham operated (SHR_S) rats. *P* values were obtained using a one-way ANOVA testing for an effect of denervation.

Right kidney function

ERBF in the right kidney was similar in both the SHR_S (8.33 ± 1.14 ml/min) and the SHR_{Dx} (8.66 ± 1.21 ml/min, $P = 0.84$) groups. RVC of the right kidney was not

significantly different between the SHR_S (0.064 ± 0.010 ml/min/mmHg) and SHR_{Dx} groups (0.069 ± 0.010 ml/min/mmHg, $P = 0.76$).

There was no significant difference in FF between the SHR_S and SHR_{Dx} groups in the right (22.7 ± 3.2 and 25.0 ± 2.3 % respectively, $P = 0.57$) kidneys. GFR in the right kidney was not significantly different between the SHR_S and SHR_{Dx} (1.03 ± 0.13 and 1.22 ± 0.21 ml/min respectively, $P = 0.46$). UFR was not significantly different between the SHR_{Dx} in the right kidneys compared to the SHR_S group ($P = 0.11$).

$U_{Na^+}V$ in the right kidney of the SHR_S group (7.60 ± 0.71 mmol/min) was not significantly different to that of the SHR_{Dx} group (20.8 ± 9.0 mmol/min, $P = 0.17$). There was no significant difference in the FE_{Na^+} in the right kidney between the SHR_S (0.24 ± 0.05 %) and the SHR_{Dx} (0.63 ± 0.27 %, $P = 0.25$).

6.3.2 Measurements following acute unilateral renal denervation (Period 2)

The specific aim of Period 2 was to examine whether the observations of Period 1 were due to possible differences in renal vessel structure between the two groups (brought about by renal denervation four weeks prior), or whether they were due to an acute effect of anaesthesia and surgical preparation increasing renal sympathetic nerve activity in the SHR_S group. Therefore, as mentioned in *Section 6.2.2 Experimental protocol*, all rats underwent acute denervation of the left kidney at the end of Period 1. Not all rats used in Period 1 made it through to Period 2. Therefore in Period 2, n = 6 in each group.

6.3.2.1 Anaesthetised systemic haemodynamics and whole kidney function (Period 2)

In the experimental period following acute denervation (Period 2), MAP and HR were not significantly different between the two groups ($P = 0.57$ and 0.33 respectively, *Table 6.3*). MAP fell between Period 1 and 2, but this was not significantly different between groups. There was also no significant difference in haematocrit between the SHR_S (42.2 ± 0.9 %) and the SHR_{Dx} group (42.4 ± 1.2 %, *Table 6.3*).

Table 6.3 Systemic haemodynamic variables following acute denervation.

	SHR_S (n = 6)	SHR_{Dx} (n = 6)	*P*
Mean arterial pressure (mmHg)	112.2 ± 2.7	115.4 ± 4.8	NS
Heart rate (beats/min)	278 ± 7	296 ± 16	NS
Haematocrit (%)	42.2 ± 0.9	42.4 ± 1.2	NS

Values are the between animal mean ± sem for the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) groups, for the second experimental period (ie following acute denervation of the left kidney). *P* values were obtained via a one-way ANOVA testing for an effect of treatment. *P* values > 0.05 were not significant (NS).

Left kidney function

ERBF was not significantly different between the SHR_{Dx} and SHR_S groups, both groups demonstrated similar levels in Period 2 (following acute renal denervation, *Figure 6.8*). ERBF was significantly reduced however, in both groups in Period 2 (P_{period} = 0.03, *Figure 6.8*). RVC was significantly greater in the SHR_{Dx} in both Period 1 and 2 (P_{treat} = 0.01, *Figure 6.8*), and the difference between the SHR_{Dx} and SHR_S groups in Period 1 was not significantly different to that observed in Period 2 ($P_{treat*period}$ = 0.79, *Figure 6.8*).

GFR was significantly reduced in both groups in Period 2 (P_{period} = 0.03, *Figure 6.9*) but there was no effect of denervation between groups (P_{treat} = 0.23, *Figure 6.9*). There was no significant difference in FF between the SHR_S and SHR_{Dx} groups in the left kidney following acute renal denervation. Following unilateral renal denervation, either chronic or acute, there was no significant effect on UFR either between groups or between experimental periods (*P* > 0.09, *Figure 6.9*).

$U_{Na}{}^{+}V$ was significantly greater in the SHR_{Dx} group in both experimental periods (P_{treat} = 0.04, *Figure 6.10*), however the difference observed between the two groups in Period 1 was equivalent to that observed in Period 2 ($P_{treat*period}$ = 0.09, *Figure 6.10*). There was no significant difference between groups or across the two experimental periods in $FE_{Na}{}^{+}$ (*Figure 6.10*). There appeared to be more variation in the SHR_{Dx} group in Period 2 for both $U_{Na}{}^{+}V$ and $FE_{Na}{}^{+}$, but these were not statistically significant (*Figure 6.10*).

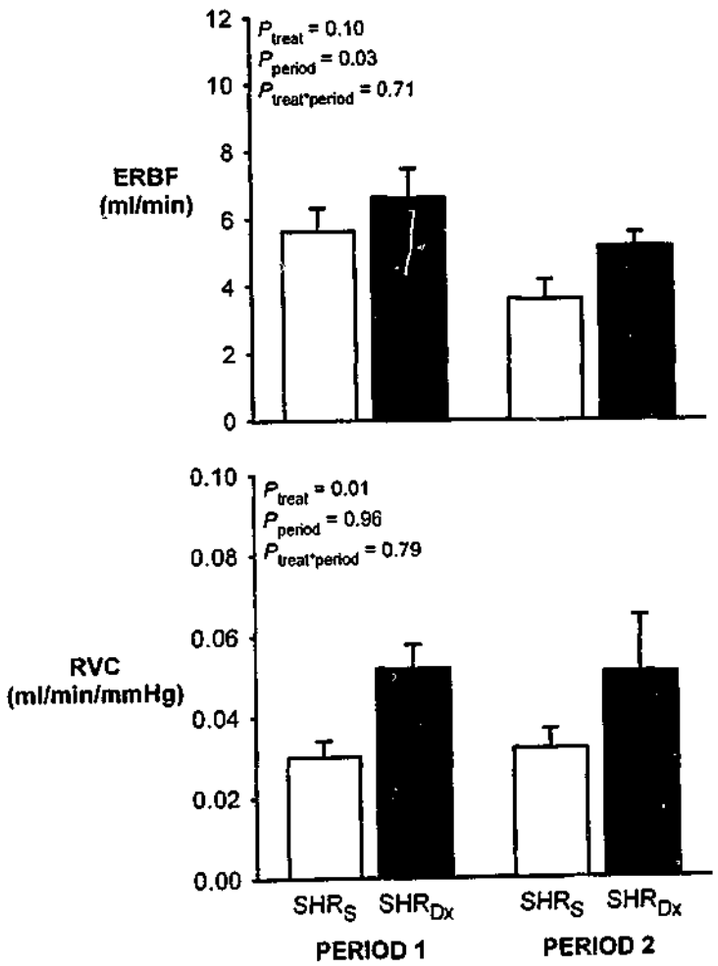

Figure 6.8 Effective renal blood flow (ERBF) and renal vascular conductance (RVC), mean ± sem, in the left kidneys of the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) rats in Period 2 (ie following acute denervation of the left kidney in both groups). *P* values were obtained using a two-way ANOVA testing for an effect of chronic denervation (P_{treat}), an effect of time (P_{period}) and an interaction between the two factors ($P_{treat*period}$).

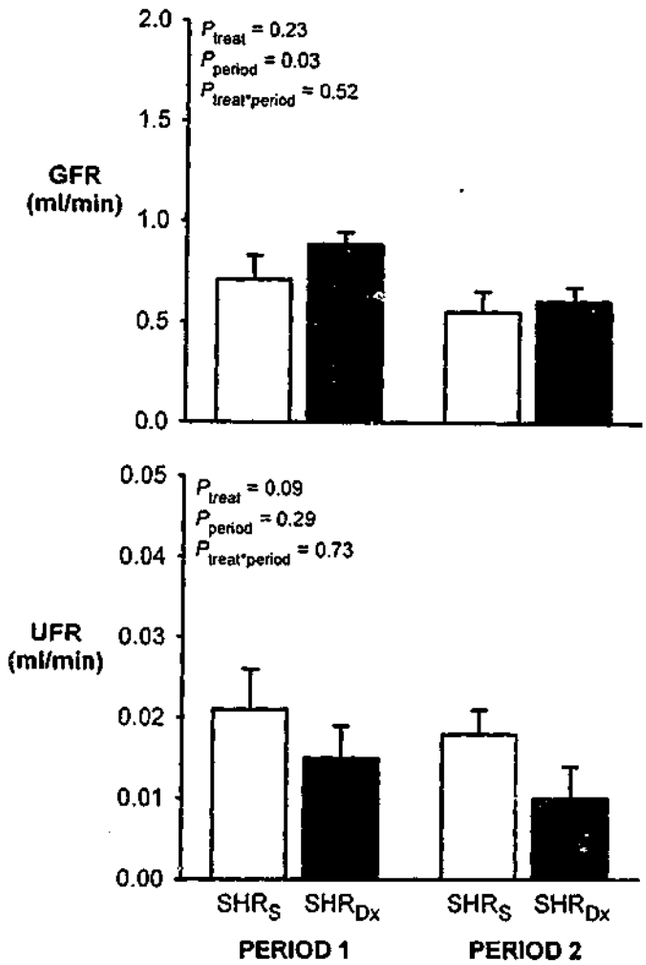

Figure 6.9 Glomerular filtration rate (GFR) and urine flow rate (UFR), mean ± sem, in the left kidneys of the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) rats in Period 2 (ie following acute denervation of the left kidney in both groups). *P* values were obtained using a two-way ANOVA testing for an effect of chronic denervation (P_{treat}), an effect of time (P_{period}) and an interaction between the two factors ($P_{treat*period}$).

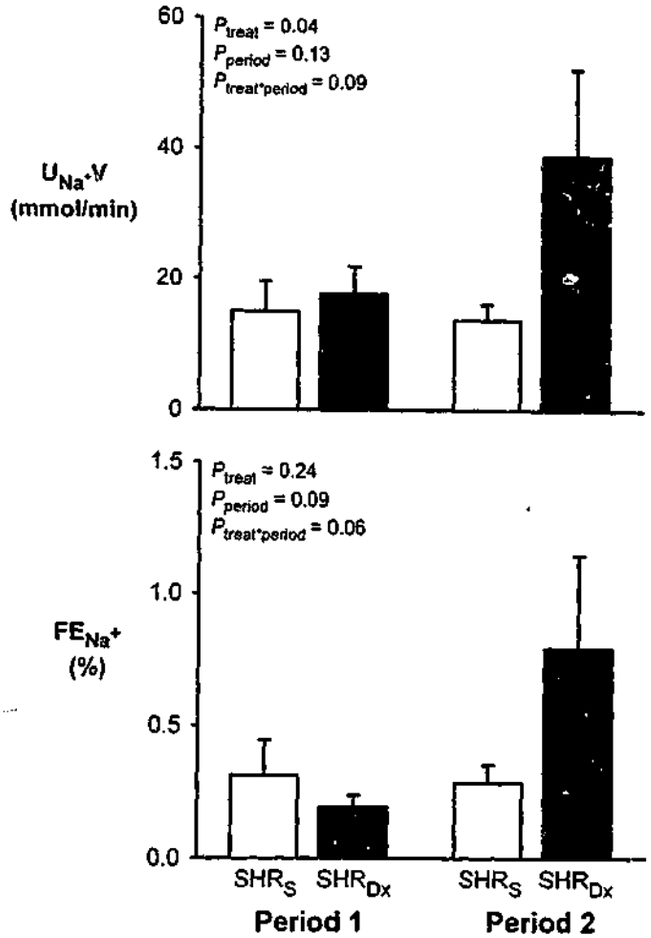

Figure 6.10 Urinary sodium excretion ($U_{Na^+}V$) and fractional sodium excretion (FE_{Na^+}), mean ± sem, in the left kidneys of the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) rats in Period 2 (ie following acute denervation of the left kidney in both groups). *P* values were obtained using a two-way ANOVA testing for an effect of chronic denervation (P_{treat}), an effect of time (P_{period}) and an interaction between the two factors ($P_{treat*period}$).

6.3.2.2 Micro-pressure measurements

Overall SFP was significantly lower in Period 2 compared to Period 1 (P_{period} = 0.02, *Figure 6.11*), however there was no significant difference between the chronically denervated animals and those previously sham operated (P_{treat} = 0.15, *Figure 6.11*). Although estimated P_{gc} was significantly lower in Period 2 (P = 0.002), there was no effect of prior chronic denervation ($P > 0.14$, *Figure 6.11*). C_a in the SHR_S group (4.7 ± 0.2 g %) was similar to that in the SHR_{Dx} group (4.9 ± 0.2 g %, P = 0.49).

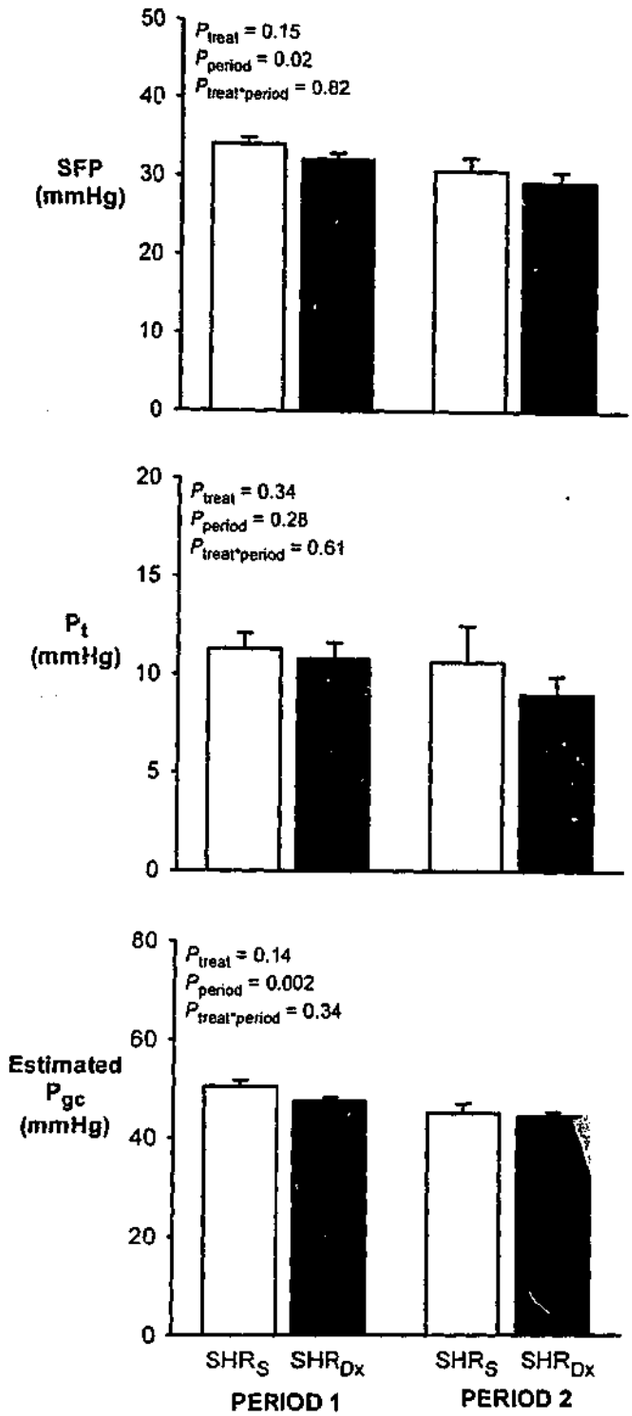

Figure 6.11 Stop-flow pressure (SFP), proximal tubular pressure (P_t) and estimated glomerular capillary pressure (P_{gc}), mean ± sem, in the left kidneys of the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) rats in Period 2 (ie following acute denervation of the left kidney in both groups). *P* values were obtained using a two-way ANOVA testing for an effect of chronic denervation (P_{treat}), an effect of time (P_{period}) and an interaction between the two factors ($P_{treat*period}$).

Pre-glomerular conductance appeared to be greater in the SHR_{Dx} group in Period 1, a difference persisting following acute renal denervation in both groups ($P_{treat*period}$ = 0.81, *Figure 6.12*). Post-glomerular conductance also appeared to be greater in the SHR_{Dx} group in Period 1 and once again this difference between the groups was observed in Period 2 ($P_{treat*period}$ = 0.79, *Figure 6.12*). Pre-:post-glomerular conductance ratio was not significantly different between the two groups in either period ($P_{treat*period}$ = 0.50). K_f was not significantly different between the two groups in either period ($P_{treat*period}$ = 0.25).

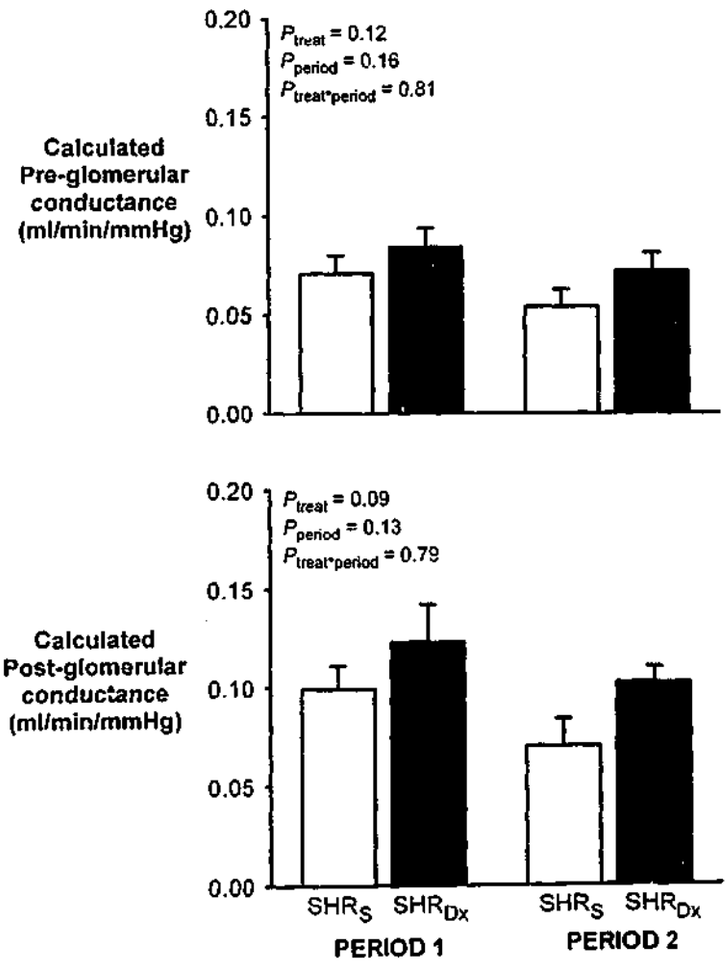

Figure 6.12 Pre- and post-glomerular conductance, mean ± sem, in the left kidneys of the sham operated (SHR_S) and unilaterally denervated (SHR_{Dx}) rats in Period 2 (ie following acute denervation of the left kidney in both groups). *P* values were obtained using a two-way ANOVA testing for an effect of chronic denervation (P_{treat}), an effect of time (P_{period}) and an interaction between the two factors ($P_{treat*period}$).

Right kidney function

Similar to the findings of Period 1, there was no significant difference following acute denervation of the left kidney in any of the renal haemodynamic measurements obtained for the right kidney.

6.3.3 Tissue noradrenaline content

Tissue noradrenaline content in the left denervated kidney was 19% of the level in the left sham operated kidney (*Table 6.4*), a value which was found to be highly significant ($P = 0.001$).

Table 6.4 Tissue noradrenaline levels at 10-weeks of age.

	SHR_S (n = 4)	SHR_{Dx} (n = 4)	*P*
Left kidney (ng NA/g wet tissue weight)	46.1 ± 1.3	8.7 ± 5.6	0.001

Tissue noradrenaline (NA) levels in the left kidney of the unilaterally denervated (SHR_{Dx}) and the sham operated (SHR_S) animals. Values are expressed as mean ± sem. *P* value was obtained using a one-way ANOVA examining the effect of treatment.

6.4 Discussion

The results of the current study demonstrate a significantly greater renal vascular conductance in the chronically denervated kidney, this is consistent with a structural alteration in the renal vasculature, following chronic unilateral renal denervation, compared to the sham operated kidneys (physiological explanations for the greater RVC are of course also possible). Estimated P_{gc} was also significantly lower in the chronically denervated kidney. If the RVC changes are attributable to structural changes, then this difference in estimated P_{gc} indicates that the predominant site of structural difference between the SHR_{Dx} and SHR_S groups appears to be the post-glomerular vessels, rather than the pre-glomerular vasculature as previously hypothesised. The findings of this chapter therefore, are consistent with those of the previous two chapters, in which the structural properties of all pre-glomerular and the

efferent arteriole were measured directly in chronically denervated and sham operated kidneys.

In *Chapter 4*, vascular casting was used to analyse the lumen diameters of the ILA, afferent and efferent arterioles. This method demonstrated no significant difference between chronically denervated and sham operated kidneys in the ILA or afferent arteriole, there was however a significant reduction in calculated relative resistance of the efferent arteriole in the denervated kidney. *Chapter 5* also demonstrated no significant effect of chronic renal denervation on the wall or lumen parameters of the ARC, however there was remodelling of the proximal ILA of the denervated kidney (no significant difference in wall thickness but a narrower lumen). In this chapter I have shown a functional difference between the sham operated and chronically denervated kidney.

The original hypothesis, proposed by Tomoda *et al* (1997) however, was that the renal sympathetic nerves were responsible for the hypertrophy/remodelling observed in the pre-glomerular vasculature of the SHR. The previous findings of Tomoda *et al* (1997) suggest that, *in vivo*, the pre-glomerular resistance may be decreased while the post-glomerular resistance may be increased following unilateral renal denervation. Although the results of this chapter do show an increase in total RVC in the chronically denervated kidney, it appears to be the case in both the pre- and post-glomerular vasculature, which is not consistent with the interpretations of the Tomoda *et al* (1997) study.

It is the surprising finding that the effect post-glomerular appeared to be the dominant effect because estimated P_{gc} was reduced despite an increase in total RVC. How can this finding be explained, considering the previous study of Tomoda *et al* (1997)? There are a number of reasons as to why there could be an effect of chronic unilateral renal denervation on the post-glomerular vasculature. Previous studies have demonstrated that there are two types of sympathetic nerves innervating the kidney, type I axons almost exclusively innervate the afferent arteriole, while type II axons are evenly distributed on the afferent and efferent arterioles (Luff *et al.*, 1991; Luff *et al.*, 1992). Efferent arterioles are innervated (even if this is a point of controversy), so there is opportunity for the nerves to have trophic effects. However, there are more nerves overall on the afferent arteriole (De Michele and Amenta, 1988). One way of explaining the findings of this study might be that the type II axons are more "important" in controlling/maintaining vessel structure. However, this is only speculation.

Obviously it is not possible to say what has caused these post-glomerular effects specifically, however, it is possible to speculate that some stereological effects might be secondary to other mechanisms. Tubuloglomerular feedback (TGF) has previously been demonstrated to be enhanced or exaggerated in SHR (Brännström and Arendshorst, 1999) and that this enhanced response is mediated by angiotensin II (Brännström *et al.*, 1996). Both acute and chronic unilateral renal denervation have also been demonstrated to re-set the TGF response (Thorup *et al.*, 1995; Thorup *et al.*, 1996). It could be hypothesised therefore that chronic unilateral renal denervation would lead to re-setting of the TGF mechanism, via reductions in renin activity and thus angiotensin II concentrations (Brännström and Arendshorst, 1999). Angiotensin II is a powerful vasoconstrictor which acts on both afferent and efferent arterioles, however it is thought to act primarily on the efferent arteriole (Ichikawa *et al.*, 1978; Stein, 1990; Steinhausen *et al.*, 1990; Navar *et al.*, 1996). Given this evidence, perhaps via in-direct methods, chronic unilateral renal denervation would lead to an increase in post-glomerular RVC which would be consistent with the findings of this chapter along with *Chapter 4*.

The current study demonstrated a reduced P_{gc} in the denervated kidney in Period 1 together with a significantly greater RVC, this can be explained by examining the haemodynamic changes in the pre- and post-glomerular vasculature. A possible explanation for the reduced P_{gc} is either a narrowing or no change of the afferent arteriole lumen diameter and an increase in efferent arteriole lumen diameter. The suggestion from these present results is that an increase in conductance (reduced resistance) of the efferent arteriole must have occurred in-order to produce a reduction in P_{gc}. Certainly the results of *Chapter 4* demonstrated a significant reduction in calculated relative resistance of the efferent arteriole, but no evidence at all of a significant effect on the pre-glomerular vasculature and therefore are consistent with the findings of the current chapter. Taken together therefore, the evidence suggests that the increased post-glomerular conductance had a structural rather than physiological basis.

P_{gc} could not be measured directly in the present experiments, as SHR do not have superficial glomeruli, therefore, SFP was used to estimate P_{gc} in this chapter. Values obtained using this method of estimating P_{gc} are comparable to those previously reported in the literature (Arendshorst, 1979; Dilley *et al.*, 1984). It is recognised that the method of measuring SFP to estimate P_{gc} has limitations, as flow in the tubule is interrupted thereby potentially causing vasodilatation of the afferent arteriole and leading to an overestimation of P_{gc}. Studies performed in Munich-Wistar rats have

demonstrated that SFP estimates reliably reflect P_{gc} (Dilley *et al.*, 1984; Arendshorst and Gottschalk, 1985). It must also be kept in mind that micropuncture is the only method of measuring P_{gc} and is therefore important for studying glomerular function.

Reno-renal reflexes may well have affected the interpretation of the *in vivo* results of this chapter. Reno-renal reflexes are defined as "neurally mediated responses occurring in one kidney as a result of interventions on the same (ipsilateral) or the opposite (contralateral) kidney" (Kopp, 1993). Previous studies have demonstrated that following unilateral renal denervation there was a resultant diuresis and natriuresis in the denervated kidney with the opposite occurring in the untouched kidney, in both rats and cats (Colindres *et al.*, 1980; DiBona and Rios, 1980; Golin *et al.*, 1987). These observations were abolished by contralateral renal denervation, and an increase in contralateral efferent sympathetic nerve activity has also been demonstrated (Kopp, 1993). It has been hypothesised that in SHR, this reno-renal reflex is impaired therefore an increase in efferent renal sympathetic nerve activity is observed in this strain (Kopp and Smith, 1989; Kopp, 1993). However there is conflicting evidence suggesting that the reno-renal reflexes in SHR are not impaired, in that an intact contralateral anti-natriuretic response is observed following unilateral renal denervation in adult SHR (Protasoni *et al.*, 1996). In the current experiments there was no significant difference, in any of the renal haemodynamic measurements, between the right kidneys of both groups. Therefore it is suggested that reno-renal reflexes were not a confounding factor in the interpretation of the results.

The measured increase in RVC and reduced estimated P_{gc} in the SHR_{Dx} group during Period 1 could have been due to an increase in lumen diameter of the renal vasculature (ie structural basis) or an acute effect of the renal sympathetic nerves. However, following acute renal denervation in both groups there was still a greater RVC observed in the SHR_{Dx} group. These results therefore, are consistent with there being a structural basis to the measured differences in RVC and P_{gc}.

As covered in detail in *Section 1.4.1.3 Unilateral renal denervation*, diuresis, natriuresis and kaliuresis are observed following chronic unilateral renal denervation without changes in GFR or RPF in euvolaemic anaesthetised rats (Wilson *et al.*, 1979). A more recent study examined the effects on anaesthetised, volume expanded SHR that had undergone unilateral renal denervation four weeks prior (Tomoda *et al.*, 1997). The authors were able to demonstrate a diuresis and natriuresis in the presence of an increase in both GFR and RBF in the left denervated kidney, even though both kidneys were exposed to the same level of systemic arterial pressure (Tomoda *et al.*, 1997). The renal

function measurements following acute renal denervation (no change in GFR, ERBF and urinary sodium excretion) in the current experiments however, are not consistent with these previous studies. The conflicting results are perhaps due to the fact that in the current experiments, the rats were volume replete and receiving 100% oxygen. Therefore, basal renal sympathetic nerve activity was low and very close to the conditions of the unilaterally denervated animals (Smith *et al.*, 1992). It was my aim throughout the experiment to ensure the preparation was stable and excellent rat homeostasis was achieved.

The role of the sympathetic nerves on the apparent increase in P_{gc} is unclear. It does not appear to be due to altered glomerular numbers or volumes, as these have been demonstrated to be within the normal range in 10-week old SHR compared to WKY rats (Kett *et al.*, 1996a).

To verify that these rats were indeed still denervated four weeks post-surgery, tissue noradrenaline content was measured (Lambert and Jonsdottir, 1998). It has previously been demonstrated that renal tissue noradrenaline levels can be as low as 5% of control four weeks post-surgery (Tomoda *et al.*, 1997). Levels measured in this chapter were comparable to those previously published, therefore verifying that the left kidney was denervated four weeks post-surgery.

In conclusion chronic unilateral renal denervation resulted in an increase in total RVC in the SHR, but a decrease in estimated P_{gc}. This indicates that while overall RVC was increased, this was not confined to the pre-glomerular vasculature as previously hypothesised. The cause of the predominantly post-glomerular response remains to be clarified.

CHAPTER 7

CHAPTER 7

General discussion

7.1 Introduction

As covered in detail in *Chapter 1*, there is a vast body of evidence for direct involvement of the renal sympathetic nerves, or at least the catecholamines released from the nerves, in hypertrophy of VSMCs. This evidence has mainly been obtained with the use of *in vitro* preparations, whereby catecholamines have been shown to stimulate VSMC proliferation (Simpson *et al.*, 1982; Adams *et al.*, 1995; Laragh and Brenner, 1995). *In vivo* the evidence is less convincing, but one study has demonstrated that in some vascular beds denervation resulted in reduced proliferation of VSMCs (Bevan, 1975), while chemical sympathectomy, using 6-hydroxydopamine, has also been shown to reduce the number of VSMCs in rabbit aorta and the number of VSMC layers in rat mesenteric arteries (Laragh and Brenner, 1995).

Very few studies however, have examined the renal sympathetic nerves and the renal vasculature of the SHR. This is surprising considering that there is a wide of body of literature, which supports the involvement of both the kidneys and the renal sympathetic nerves in the development of SHR hypertension. Previous experiments, which have attempted to study the effects of renal denervation on the renal vasculature, have denervated both kidneys (Liard, 1977; Kline *et al.*, 1980; Winternitz *et al.*, 1980; Norman Jr and Dzielak, 1982; Saynavalammi *et al.*, 1982; Gattone *et al.*, 1984; DiBona and Jones, 1991; Greenberg and Osborn, 1994; Yoshida *et al.*, 1995). Bilateral renal denervation is known to delay the onset of development of SHR hypertension (Liard, 1977; Winternitz *et al.*, 1980; Norman Jr and Dzielak, 1982). Therefore, unilateral renal denervation is perhaps a better method of examining the role of the renal sympathetic nerves in the hypertrophy/remodelling of the SHR renal vasculature, given that pressure

per se has been implicated in structural changes of the vasculature (Folkow and Karlström, 1984).

Throughout this thesis, excluding *Chapter 3*, unilateral renal denervation was used because it was known not to alter arterial pressure in SHR (Tomoda *et al.*, 1997), and therefore interpretation of the results would not be confounded by a reduction in arterial pressure. It must be asked why unilateral renal denervation does not reduce arterial pressure to levels mid-way between those seen in intact SHR and those that had undergone bilateral renal denervation. If the renal sympathetic nerves were responsible for the development of hypertension in this strain, you would expect some reduction in arterial pressure in the UD_x model. This is quite unlike the case of Goldblatt hypertension or renal wrap hypertension in which graded increases in MAP can be achieved (Goldblatt *et al.*, 1934; Page, 1939). The fact that arterial pressure is not affected in SHR undergoing UD_x is difficult to explain, however, Tomoda *et al* (1997) also demonstrated no significant effect of UD_x on arterial pressure in SHR.

Chapter 4 examined the effects of chronic unilateral renal denervation on the lumen diameters of the interlobular artery afferent and efferent arterioles using the method of vascular casting in SHR. Four weeks following unilateral renal denervation lumen diameters were measured in the superficial and mid-cortical glomeruli. There was no evidence, from these results, for an increase in lumen diameter of either the interlobular artery, afferent or efferent arteriole (*Figure 7.1*). Poiseuille's equation, used to calculate relative resistance of a vessel, describes the relationship between the structural and functional properties of a vessel. Although it is unlikely, perhaps any changes in lumen diameter were beyond the detection limits of the technique. Interestingly there was a significant reduction in the calculated relative resistance of the efferent arteriole, possibly indicating a small difference in lumen diameter of the efferent arteriole which were undetectable using these methods (*Figure 7.1*).

Chapter 5 examined the effects of chronic unilateral renal denervation on the wall and lumen dimension of both the proximal interlobular and arcuate arteries. The results of this chapter are consistent with those of *Chapter 4*, in that there was no evidence of an increase in lumen diameter or any effect on the wall of the pre-glomerular vessels following chronic unilateral renal denervation. Unexpectedly there was a reduction in lumen diameter in the proximal interlobular artery (*Figure 7.1*).

With *Chapter 4* demonstrating no evidence of an effect of the renal sympathetic nerves on the pre-glomerular vasculature, but a significant effect on calculated relative resistance of the efferent arteriole. While *Chapter 5* demonstrated a reduction in lumen

diameter of the proximal ILA following chronic denervation, *Chapter 6* aimed to examine the functional consequences of these findings. Whole kidney renal function was measured in denervated and sham operated kidneys and micropuncture was used to estimate P_{gc}. The main findings of this chapter were a significantly greater RVC and reduced P_{gc} in the chronically denervated compared to the sham operated kidney. These findings were consistent with the renal sympathetic nerves having a significant role in efferent arteriole structure (*Figure 7.1*), similar to the findings of *Chapter 4*. The findings of *Chapter 6* are also consistent with a structural basis to the increased RVC observed in the chronically denervated kidney,. since an acute effect of the renal sympathetic nerves in the sham operated kidney was excluded.

Micropuncture studies have demonstrated that increases in renal nerve activity (via electrical stimulation) produce increases in afferent and efferent arteriole resistance (see Kon and Karnovsky, 1989). Coincident with the increased resistance is contraction of the mesangium and glomerular tuft and thus reduced glomerular ultrafiltration coefficient (K_f; Kon and Karnovsky, 1989). This observation may be synonymous with the conditions faced by the SHR kidney during the early developmental phase of hypertension in which K_f is reduced (Dilley *et al.*, 1984). Upon denervation therefore, as in this thesis, it was hypothesised that the afferent and efferent arterioles would dilate and that glomerular tuft volume would increase. However, these were not the observations of this thesis. UD_x did not significantly affect glomerular tuft volume and pre-glomerular vessel lumen diameters (*Chapter 4*). Calculated K_f was also not significantly altered, granted that a number of assumptions are made in this calculation (*Chapter 6*). Once hypertension has developed completely in SHR (at 12-weeks of age), glomerular filtration and flow are normalised due to the increased arterial pressure (Dilley *et al.*, 1984).

As I have speculated in *Figure 1.3* and again in *Figure 7.2*, the SHR renal vasculature is under the influence of a number of systems (these will be addressed further in *Section 7.3 Further possibilities and interactions*). If one system is altered in some way, eg removal of the renal nerves as used in this thesis, there is the potential for the involvement of other usually dormant systems and/or enhancement/inhibition of existing systems. However, this is only speculation at this stage.

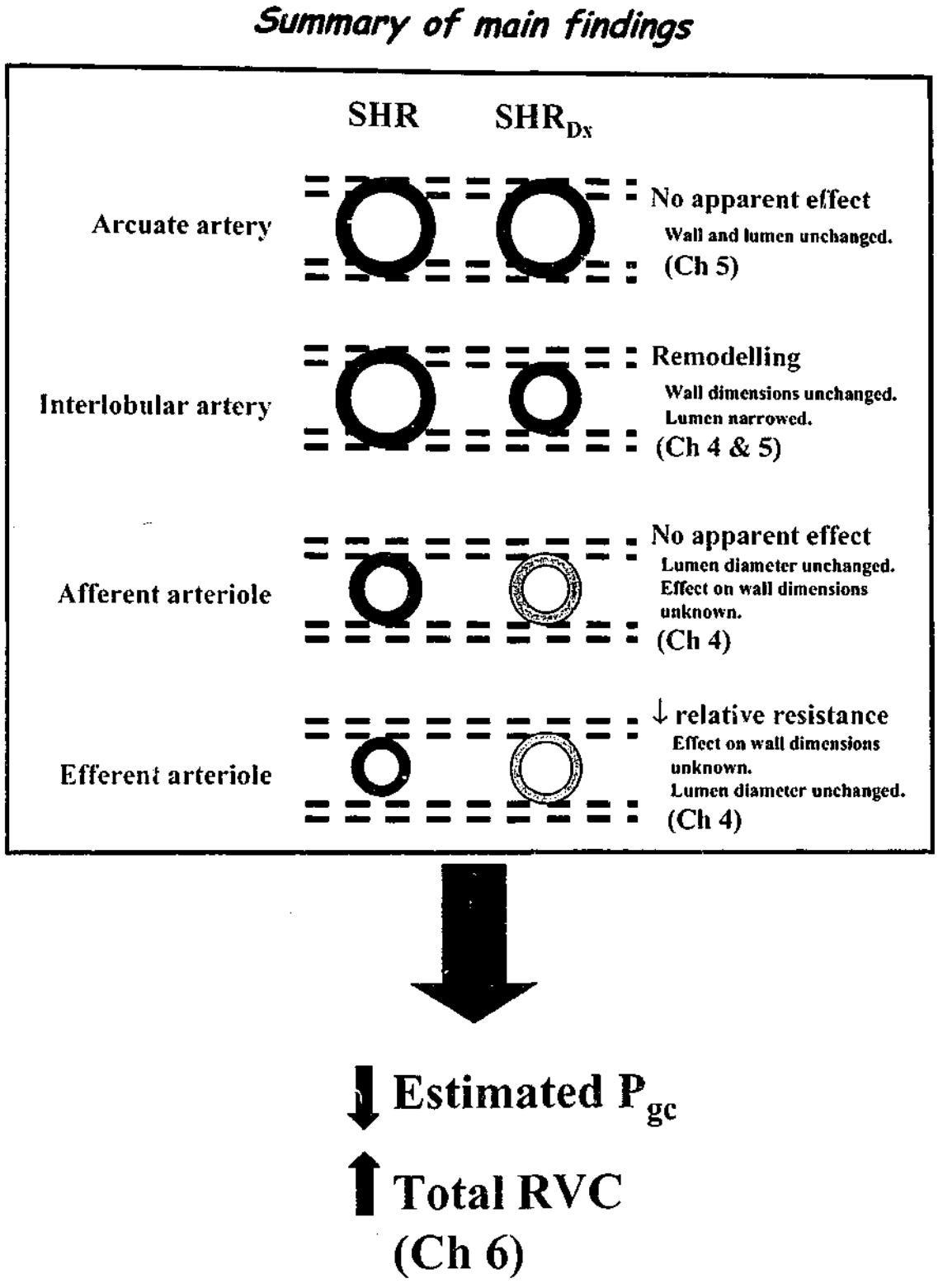

Figure 7.1 Schematic diagram summarising the main findings of this thesis, whereby alterations in renal vessel structure, in SHR that had undergone unilateral renal denervation (SHR_{Dx}), led to a reduction in estimated glomerular capillary pressure (P_{gc}) and an increase in total renal vascular conductance (RVC).

7.2 Further possibilities and interactions

In *Section 1.6* (*How might the renal nerves lead to hypertension in the SHR?*), I proposed some possible mechanisms, which might be involved in producing vasoconstriction, hypertrophy or remodelling of the renal vasculature. In light of my current findings, I now wish to re-visit the original hypothesis, and propose some further possibilities (*Figure 7.2*).

The SHR is a complicated experimental model of hypertension, however, human essential hypertension is also a complex condition. Evidence exists for a number of possible mechanisms being involved in the development of SHR hypertension. The SHR not only demonstrates an increased renal sympathetic nerve activity, but also hyperinnervation of the renal vasculature, increased renal renin content, increased renal angiotensin II levels, reduced sodium and water excretion, exaggerated tubuloglomerular feedback and increased expression of nitric oxide synthase isoforms with impaired activity of nitric oxide (Nakamura and Johns, 1995; Vicaut and Hou, 1994; Welch *et al.*, 2000; Ikenaga *et al.*, 1993; Brannström *et al.*, 1996; Dilley and Arendshorst, 1984). Human essential hypertensive patients also demonstrate increases in sympathetic nerve activity (Esler, 1995; Esler and Kaye, 1998). With respect to renin levels, a small proportion of human essential hypertensive patients demonstrate increased plasma renin levels, with an associated reduction in sodium and water excretion (Laragh and Brenner, 1995). This thesis has not provided any evidence for the pro-hypertensive involvement of the renal sympathetic nerves in the development hypertrophy/remodelling of the pre-glomerular vasculature in the SHR, but in fact an pro-hypertensive role in the post-glomerular vasculature (first evidence of this). It is highly likely that a number of mechanisms or systems interact, therefore leading to alterations in the post-glomerular vasculature to increase P_{gc} and thus renal vascular resistance. This increase in renal vascular resistance would have some contribution to total peripheral resistance and therefore an increase in arterial pressure (Guyton *et al.*, 1972).

The possible systems, which are capable of interacting with the renal sympathetic nerves and producing combined effects on the renal vasculature, are covered below.

7.2.1 Renin-angiotensin system

The renin-angiotensin system has been studied extensively in the SHR, and there is evidence that it is of great importance. Treating young SHR with angiotensin converting enzyme inhibitors (ACEi) will prevent the development of hypertension in this strain (Arendshorst *et al.*, 1990; Kett *et al.*, 1995; Bergström *et al.*, 1998). ACEi treatment of adult SHR will also reverse the hypertension observed (Tozawa *et al.*, 2000). However, very little is understood of the effect of ACEi on the renal vasculature. Some studies have demonstrated reversal of cardiovascular, renal and mesenteric hypertrophy (Li and Schiffrin, 1996; Sharifi *et al.*, 1998). However, other groups have demonstrated no significant effect of ACEi on the renal vasculature *in vitro* (Kett *et al.*, 1995; Tozawa *et al.*, 2000). The controversy surrounding the effect of ACEi on the renal vasculature may be explained by the difference in the methods used to analyse the vessels (see *Section 5.5 Discussion*).

Previous studies have demonstrated increases in renal renin content and mRNA levels in young SHR (Vicaut and Hou, 1994; Nakamura and Johns, 1995), along with increases in renal angiotensin II concentrations (Nakamura and Johns, 1995; Kubo *et al.*, 2000). Angiotensin II, via AT_1 receptors, mediates the enhanced TGF response observed in SHR (Brännström *et al.*, 1996). Angiotensin II also facilitates the exocytotic release of noradrenaline, via AT_1 receptors, and of adrenaline from the chromaffin cells of the adrenal medulla (Dendorfer *et al.*, 1998). *In vitro*, angiotensin II can also increase the secretion of nerve growth factor, while the opposite is observed following angiotensin II blockade (Charchar *et al.*, 1998). Vascular smooth muscle tone is also increased by angiotensin II via the same second messenger as noradrenaline, and thereby enhancing the effects of noradrenaline on VSMCs (Dendorfer *et al.*, 1998). This evidence therefore, suggests that angiotensin II is capable of interacting with the renal sympathetic nerves and catecholamines in-order to produce the hypertrophy/remodelling of the renal vasculature along with the enhanced TGF response seen in SHR.

In the denervated kidney therefore, I would hypothesise that since no further noradrenaline is released from nerve terminals, angiotensin II production may be reduced due to a reduction in renin secretion. As mentioned above, there is evidence suggesting that angiotensin II mediates the enhanced TGF response in SHR (Brännström *et al.*, 1996), and since angiotensin II is also more effective on the efferent arteriole, its withdrawal would also produce a greater effect on the efferent arteriole (Ichikawa *et al.*, 1978; Stein, 1990; Steinhausen *et al.*, 1990; Navar *et al.*, 1996).

Therefore, it is very possible that both the renin-angiotensin system and the sympathetic nervous system are capable of interacting to produce vascular hypertrophy in SHR kidneys. Both systems can enhance the effect of the other.

The involvement of angiotensin II needs to be further addressed however. Arendshorst *et al* (1999) recently reported that angiotensin II is a potent vasoconstrictor of both the afferent and efferent arteriole, and that the effects on the efferent arteriole are due to calcium release from intracellular stores. This suggestion by Arendshorst *et al* (1999) could be an indirect effect of the renal sympathetic nerves and may explain the findings of this thesis. Perhaps further investigation with the use of calcium channel blockers and investigation of the afferent and efferent arteriole walls would be advantageous. Certainly, Frohlich (1988) has suggested that there may be an efferent arteriole abnormality in SHR and human essential hypertension that could be reversed with calcium antagonists. In the denervated kidney, perhaps due to a reduced level of angiotensin II, calcium is unable to be released from its intracellular stores and the observation is an increase in post-glomerular conductance (one of the unexpected findings of this thesis). This also is another avenue for interaction between the renal sympathetic nerves and the renin-angiotensin system.

7.2.2 Nitric oxide

Nitric oxide is produced from the macula densa, and is a potent vasodilator (Thorup *et al.*, 2000). For example, it has previously been demonstrated that angiotensin II is capable of stimulating nitric oxide production in the renal medulla, which offsets the vasoconstrictor activity of angiotensin II (Dukacz *et al.*, 2001). Nitric oxide has also been demonstrated to be one of the most important inhibitors of the TGF response (Thorup *et al.*, 2000). However, the nitric oxide system is impaired in SHR (*Figure 7.2*), there are increased levels of expression of nitric oxide synthase isoforms but activity is impaired in some way (Ikenaga *et al.*, 1993; Welch *et al.*, 2000). It might be speculated that the inactivity of nitric oxide on the renal vasculature means that the renal vasculature is more susceptible to the vasoconstrictor actions of the renal sympathetic nerves, angiotensin II and the exaggerated TGF response in the SHR. Upon renal denervation however, nitric oxide is unlikely to have a major effect on the renal vasculature since its actions are impaired in the SHR (unknown cause).

7.2.3 Tubuloglomerular feedback

TGF is a mechanism that regulates P_{gc} in-order to maintain a constant single nephron glomerular filtration rate by altering afferent arteriole tone (Welch *et al.*, 2000). As mentioned above, the TGF response is exaggerated in SHR (Dilley and Arendshorst, 1984; Brännström *et al.*, 1996). It appears that a number of mechanisms are involved in this enhanced TGF response (*Figure 7.2*), including the renin-angiotensin system (Brännström *et al.*, 1996), nitric oxide system (Thorup *et al.*, 2000) and the renal sympathetic nerves (Thorup *et al.*, 1996; Thorup *et al.*, 2000). It has been hypothesised that denervation would lead to re-setting of the TGF mechanism, via reductions in renin activity and thus angiotensin II concentrations (Brännström and Arendshorst, 1999). As I have mentioned above, angiotensin II is a powerful vasoconstrictor, which acts on both the afferent and efferent arterioles, however it is thought to have a substantial effect on the efferent arteriole (Ichikawa *et al.*, 1978; Stein, 1990; Steinhausen *et al.*, 1990; Navar *et al.*, 1996). Perhaps therefore, via in-direct methods, chronic unilateral renal denervation would lead to an increase in post-glomerular RVC, which would be consistent with the findings of this thesis.

7.3 Future directions

The involvement of other systems in altering renal vessel structure cannot be ruled out, as this thesis has only examined one aspect. The findings of this thesis provide convincing evidence that the renal sympathetic nerves have no significant effect on the structure of the pre-glomerular vessels (with the exception of the proximal interlobular artery). Thought must therefore be given to other possible mechanisms or interactions between a number of systems as covered above. The wall of the afferent and efferent arteriole could not be examined with the techniques used throughout this thesis, therefore other means of doing so must be addressed. Although it appears that there was no apparent effect of unilateral renal denervation on the afferent arteriole lumen, there may have been some changes in the wall dimensions (eg VSMC number or arrangement). The wall dimensions of the efferent arteriole also may have changed in some way in response to unilateral renal denervation, and perhaps these smaller pre- and post-glomerular vessels need to be examined using electron microscopy. If it is possible to detect some difference in the wall dimensions of the afferent and efferent arterioles then the induction of growth factors *in vivo* also requires further study, as most information to date is a derivation of *in vitro* studies.

Interactions between the renal sympathetic nerves and other systems in the development of hypertrophy

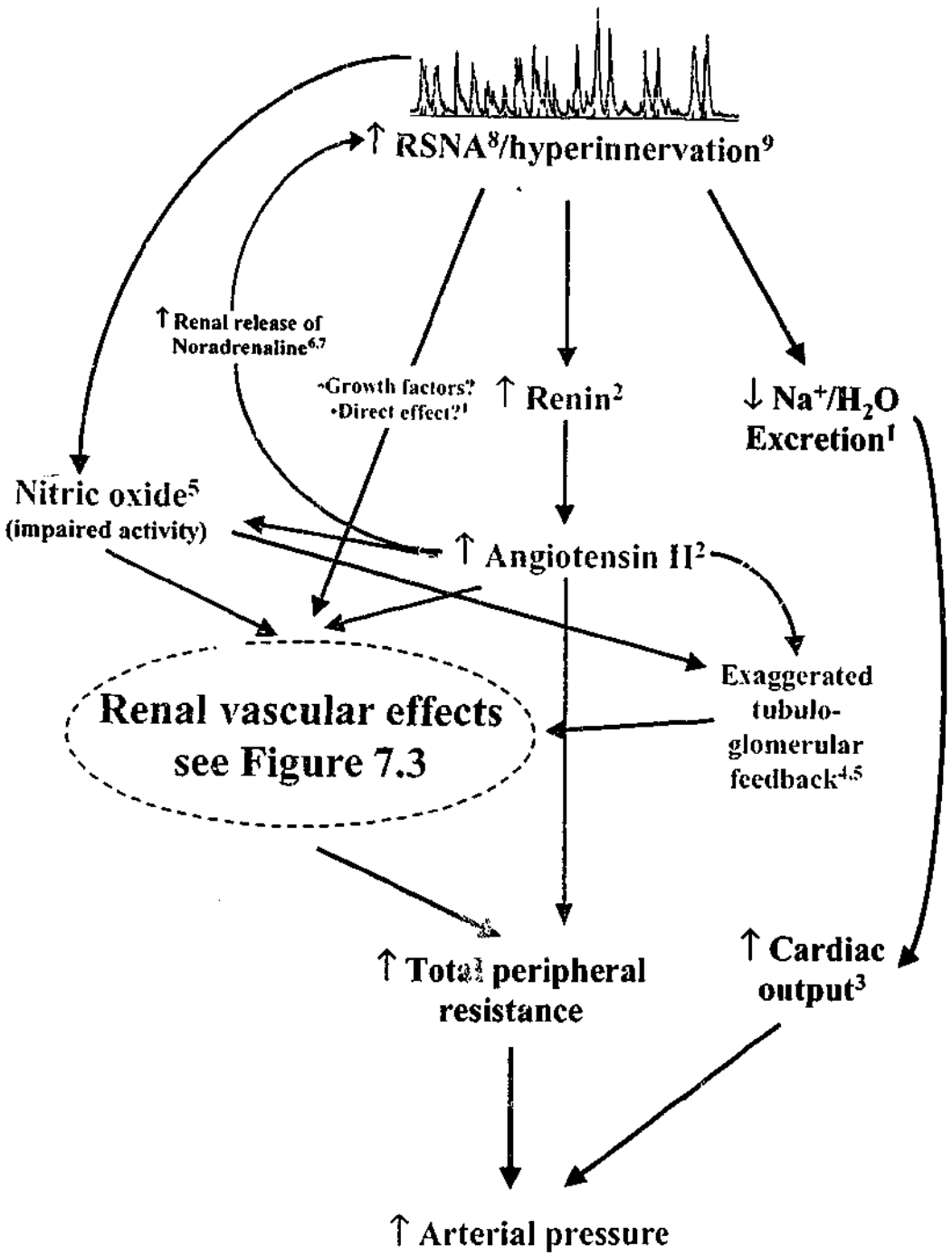

Figure 7.2 Schematic diagram illustrating some of the possible mechanisms involved in the development of hypertrophy/remodelling and the ultimate development of hypertension in SHR. Interactions between an increase in renal sympathetic nerve activity (RSNA) and other systems are hypothesised ([1]Block *et al.*, 1952; [2]Vander, 1965; [3]Guyton *et al.*, 1972; [4]Brannström *et al.*, 1996; [5]Thorup *et al.*, 2000; [6]Dendorfer *et al.*, 1998; [7]Charchar *et al.*, 1998; [8]Ricksten *et al.*, 1981; [9]Tuttle *et al.*, 1995).

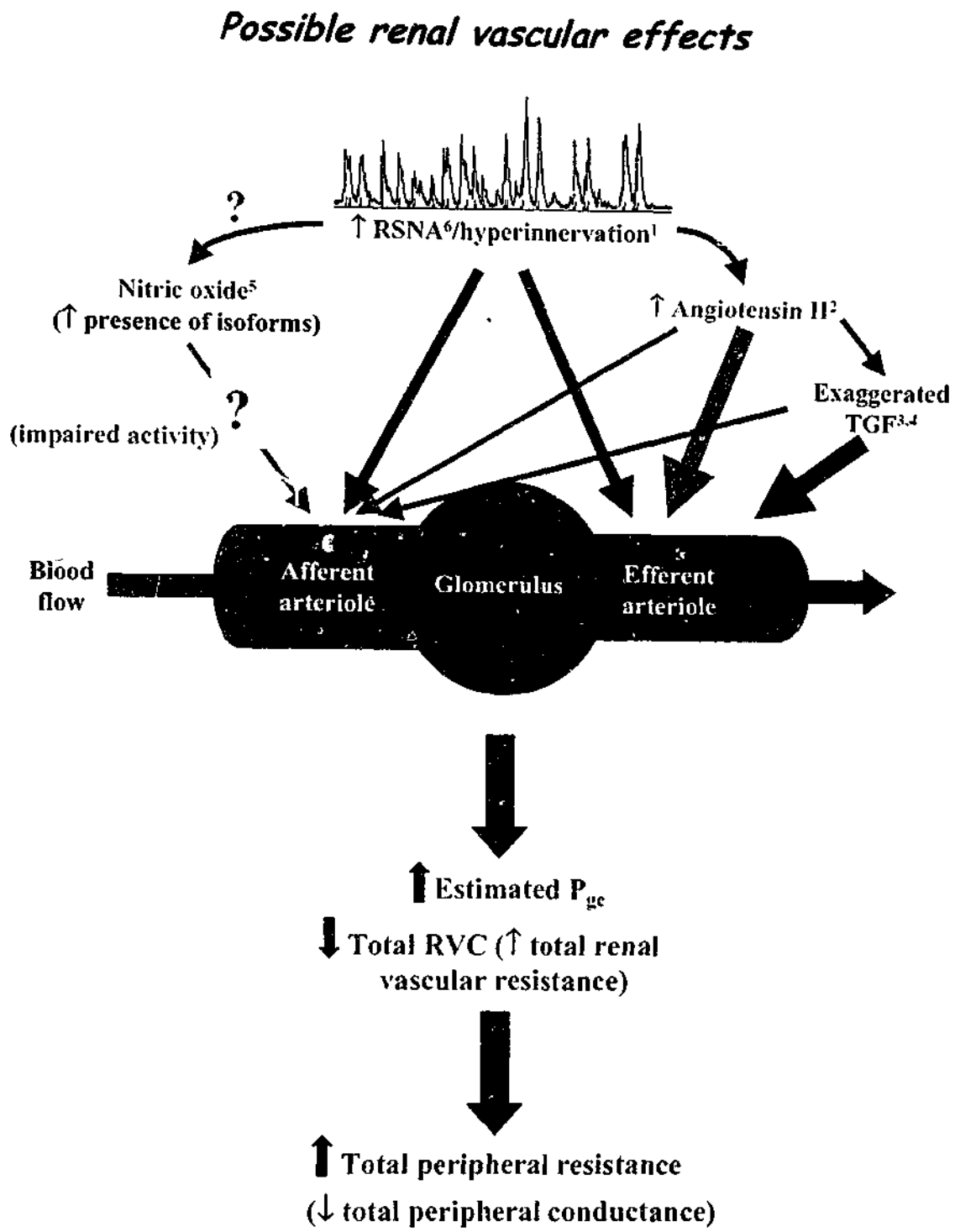

Figure 7.3 Schematic diagram illustrating the possible sites of action of the numerous systems influenced either directly or indirectly by the renal sympathetic nerves. The outcome as supported by the findings of this thesis are a reduced estimated glomerular capillary pressure (P_{gc}) and increased total renal vascular conductance (RVC) following renal denervation ([1]Tuttle *et al.*, 1995; [2]Vander, 1965; [3]Guyton *et al.*, 1972; [4]Brannström *et al.*, 1996; [5]Thorup *et al.*, 2000; [6]Ricksten *et al.*, 1981). TGF – tubuloglomerular feedback.

7.4 Conclusions

This thesis therefore has demonstrated that chronic UD_x, in SHR, resulted in an increase in total renal vascular conductance, which was due primarily to the post-glomerular component of the renal circulation since estimated P_{gc} was reduced. This requires further study, as the mechanism/s responsible are not known, but I have speculated as to some of the possibilities. The effects on the efferent arteriole could be a result of a direct effect by the renal sympathetic nerves. However, the afferent arteriole is also innervated and this does not explain the predominantly post-glomerular effect. The fact that the kidneys were not denervated completely or that re-innervation had occurred is not an issue, as either noradrenaline content and/or immunohistochemistry staining for tyrosine hydroxylase was performed in every chapter in order to verify the lack of renal nerves at 10-weeks of age. The efferent arteriole effects could also be occurring in response to secondary systems activated by the lack of renal sympathetic nerves. I have proposed some possibilities for an interaction between the renal sympathetic nerves and other regulatory systems, however they require further study.

It is evident from the findings of this thesis however, that *in vivo*, the renal sympathetic nerves have relatively little effect on the structural changes observed in the renal vasculature of the SHR. Though it is possible that the renal sympathetic nerves may act in conjunction with other regulatory systems to initiate the development of hypertrophy/remodelling in the SHR, the findings of this thesis do not support the sole involvement of the renal sympathetic nerves in the development of pre-glomerular hypertrophy and/or remodelling and therefore the development of hypertrophy. The clearest finding of this thesis is that the renal sympathetic nerves appear to increase resting post-glomerular resistance in SHR, compared to the denervated kidney, thereby increasing P_{gc}.

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