THE RENAL MEDULLARY CIRCULATION
AND BLOOD PRESSURE CONTROL

Anabela G Correia
B.Sc. (Hons) Monash University

Department of Physiology
Monash University
Clayton, Australia

February 2001

Submitted in total fulfilment of the requirements of the degree of
Doctor of Philosophy

Man's main task in life is to become what he potentially is.
The most important product of his efforts, is his own personality.

Erich Fromm.

To my dad ....

TABLE OF CONTENTS

CHAPTER 1   LITERATURE REVIEW

CHAPTER 2   GENERAL METHODS

CHAPTER 3   METHODS FOR RENAL MEDULLARY INFUSION OF VASOACTIVE COMPOUNDS: METHODOLOGICAL CONSIDERATIONS

CHAPTER 4   EFFECTS OF RENAL MEDULLARY AND INTRAVENOUS NORADRENALINE ON RENAL ANTIHYPERTENSIVE FUNCTION

CHAPTER 5   ROLES OF PRESSURE DIURESIS/NATRIURESIS AND INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM, IN THE DEPRESSOR RESPONSE TO INCREASED RENAL ARTERY PRESSURE

CHAPTER 6 EFFECTS OF ACTIVATION OF VASOPRESSIN-V₁-RECEPTORS ON REGIONAL KIDNEY BLOOD FLOW AND GLOMERULAR ARTERIOLE DIAMETERS

CHAPTER 7 GENERAL DISCUSSION

SUMMARY

The experiments described in this thesis have examined the hypothesis that the renal medullary microcirculation plays a significant role both in the long and short-term regulation of arterial pressure. This hypothesis is based on the notion that increased renal arterial pressure stimulates the activation of three renal antihypertensive mechanisms; renin release, pressure diuresis/natriuresis, and stimulation of release of the putative renal medullary depressor hormone. It is hypothesised that the latter two mechanisms are dependent on poor autoregulation of the renal medullary microcirculation, for their expression.

In Chapter 3, a method was devised and tested, for the delivery of vasoactive agents (noradrenaline) to the renal medullary interstitium of rabbits. Both chronic and acutely implanted catheters were tested, and the renal distribution of radiolabel was determined after infusion of [^{3}H]-noradrenaline for 20 min into the outer and inner medullary interstitium. Autoradiographic analysis determined that after outer medullary interstitial infusion of [^{3}H]-noradrenaline, radiolabel was most concentrated in the outer medulla and inner cortex. Therefore this technique was deemed suitable for the delivery of vasoactive substances to the renal medulla, as the infused substance appears to concentrate in the regions, which house the vasculature most likely to contribute to the regulation of medullary blood flow.

Employing the technique developed in Chapter 3, we were then able to test the effect of reducing medullary blood flow on the renal antihypertensive responses to increased renal artery pressure. Renal arterial pressure was increased step-wise from ~65 to ~180 mmHg using an extracorporeal circuit to attached to a roller pump. Medullary interstitial infusion of noradrenaline (300 ng/kg/min) reduced medullary blood flow ~30% and blunted both the pressure diuresis/natriuresis response, and the fall in arterial pressure during increased renal artery pressure. Evidence available at that time suggested that the acute depressor response to increased renal artery pressure resulted chiefly from release of an unidentified hormonal factor from the renal medulla. Intravenous infusion of the same dose of noradrenaline produced a small reduction in cortical blood flow only, and did not blunt the renal antihypertensive responses to increased renal artery pressure. Thus medullary blood flow appears to play an important role in the short-term control of arterial pressure by modulating the pressure diuresis/natriuresis response and perhaps also the release of the putative renal medullary depressor hormone. However our conclusion regarding the putative renal medullary depressor hormone remained controversial, particularly since the observation was made that the

diuresis/natriuresis increased exponentially with step increases in renal arterial pressure. This prompted the experiments carried out in Chapter 5.

In Chapter 5, we tested the role of the pressure diuresis/natriuresis mechanism, and the inhibition of renin release, in mediating the acute depressor response to increased renal artery pressure. As in Chapter 4, an extracorporeal circuit was established in anaesthetised rabbits, and we tested the effects of increasing renal artery pressure on systemic arterial pressure, the pressure-natriuresis mechanism, and levels of plasma renin activity. Furthermore, we tested the effects on the depressor response to increased renal artery pressure on blocking the systemic haemodynamic effects of pressure diuresis/natriuresis by infusing compound sodium lactate at a rate equivalent to urine flow, and 'clamping' the renin-angiotensin system. Four groups of rabbits were studied. In a control group, renal artery pressure was maintained at ~65 mmHg. In the remaining three groups renal artery pressure was increased to ~160 mmHg. In two of these groups, compound sodium lactate was infused at a rate equivalent to urine flow. In addition, in one of these groups the renin-angiotensin system was 'clamped' by simultaneous intravenous infusion of enalaprilat and angiotensin II. We found that the depressor effect of increased renal artery pressure is abolished if the systemic haemodynamic effects of pressure natriuresis/diuresis are blocked by preventing cardiac output from falling. Based on these findings, we conclude that the depressor response, to increased renal artery pressure in the extracorporeal circuit preparation in anaesthetised rabbits, occurs chiefly due to negative salt and water balance secondary to the pressure diuresis/natriuresis mechanism. In supplementary experiments (described in Chapter 7) we obtained data indicating that similar mechanisms mediate the acute depressor response to unclipping the renal artery in 1-kidney, 1-clip hypertensive rats.

The vascular elements that regulate medullary blood flow *in vivo* remain unknown. In the experiments described in Chapter 6 we attempted to determine the vascular sites within the kidney, responsible for reducing medullary blood flow in response to activation of the V_1-receptors. The vasopressin V_1-agonist [Phe^2, Ile^3, Orn^8]-vasopressin was infused intravenously into anaesthetized rabbits and its renal effects were monitored using laser-Doppler flowmetry. This reduced renal medullary blood flow approximately 30% without reducing cortical blood flow. Since medullary blood flow is supplied entirely from the efferent arterioles of juxtamedullary glomeruli, we tested whether vasoconstriction of juxtamedullary glomerular arterioles contributes to V_1-receptor mediated reductions in medullary blood flow. On completion of the infusion (30 min), kidneys were immediately perfusion fixed at the final recorded MAP, and filled with methacrylate casting material. The diameters of afferent and efferent arterioles in the outer, mid and juxtamedullary cortex of the left kidneys were determined by scanning electron microscopy. We were, unable to

detect any significant differences, between V_1-antagonist and vehicle treatments on afferent or efferent arteriole diameters in and region of the cortex. These results do not support a role for juxtamedullary arterioles in producing V_1-receptor mediated reductions in MBF, suggesting that downstream vascular elements (e.g. outer medullary descending vasa recta) might possibly be involved.

In conclusion, through the development of new techniques and experimental approaches, we are gaining a better understanding of how the renal medullary circulation contributes to the regulation of arterial pressure (both in the short and the long term), and how the medullary circulation is regulated. Many issues surrounding the extent and level of involvement of so called 'renal medullary antihypertensive mechanisms', particularly the putative renal medullary depressor hormone, require further investigation. The results of the studies conducted and described in this thesis have contributed to our understanding of these issues. However, there is a clear need for further research in this area. In the future, vital information derived from such studies will move us towards prevention or cure of hypertension, rather than just its control.

Declaration

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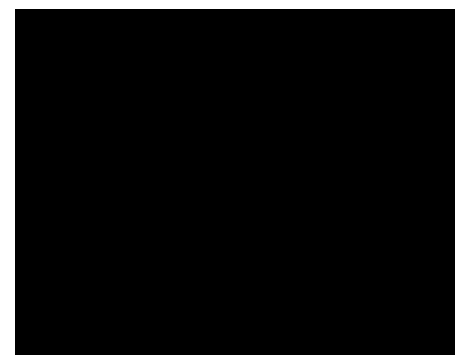
Anabela G. Correia

Monash University,

Clayton, Australia.

Department of Physiology

Acknowledgements

Sincere thanks, to all who contributed to and influenced this thesis. Particularly....

Dr. Roger Evans
Providing me with the 'PhD' opportunity and challenge
Teaching me how to ask a question, and find its truth
Sharing your gift
Inspiration
Thank-you for being by teacher, and my friend

Associate Professor Göran Bergsrtöm
Intellectual input and advice throughout
the past 3 years, particularly with Chapter 4
Teleological explanations
Opportunity and time spent in Göteborg
Strong rebuttals to my version of the 'medullipin' story

Dr. Andrew Lawrence
Autoradiography technique
Advice and input in Chpater 3

Dr. Kate Denton
Renal fixation and casting techniques
Advice and input in Chapter 6

Professor Wawrick Anderson
Advice and direction with experiments in Chapter 5

Professors Ross Young, and Bob Bywater
Sitting in on my lab presentations and agreeing to be on my PhD committee

Anna Madden
Teaching me how to set-up and implement the extracorporeal circuit
AAS, the tricky aspects of data analysis and being a friend
(arriving to work on time)

Katrina Worthy
Processing renin samples, AAS, and being so reliable

Gunta Jaudzems and Joan Clark
Teaching me how to use the scanning electron microscope

OJAY girls
Years of gossip, entertainment, and serious distraction

Famous Five
Sonia Alfaiate, Christine Morley, Kirsty Scott, Peta Austeberry, and Jacinda Cottee
Your support (in my life) can never be measured. May your personalities continue to amaze and amuse me.

My Family
Samuel: Free entry into Seven, and reminding me what life is really about;
following your heart and never asking why.
Mum: for tireless support, endless phone calls, and giving me strength.
Dad: for being my best friend, knowing the right thing to say, and showing me how to grab stars from the sky.

PUBLICATIONS PRODUCED DURING CANDIDATURE

Published papers arising directly from this thesis

A.G. Correia, G. Bergström, A.J. Lawrence, and R.G. Evans (1999) Effects of renal medullary interstitial infusion of norepinephrine in anesthetized rabbits. *American Journal Physiology* 277 (*Regulatory Integrative Comp. Physiol. 46*): R112-R122.

A.G. Correia, G. Bergström, A.C. Madden, and R.G. Evans (2000) Effects of renal medullary and intravenous norepinephrine on renal antihypertensive function. *Hypertension* 35: 965-970.

A.G. Correia, K.M. Denton, and R.G. Evans (2001) Effects of activation of vasopressin V_1-receptors on regional kidney blood flow and glomerular arteriole diameters. *Journal of Hypertension* 19: in press.

Published papers arising from work performed during PhD candidature

R.G. Evans, **A.C. Correia**, S.R. Weeks, and A.C. Madden (2000) Responses of regional kidney blood flow to vasoconstrictors in anaesthetized rabbits: dependence on agent and renal artery pressure. *Clinical and Experimental Pharmacology and Physiology* 27: 1007-1012.

Conference proceedings

A.G. Correia, G. Bergström, A.J. Lawrence, and R.G. Evans (1997) Influencing renal medullary blood flow in the rabbit by local infusion of noradrenaline; effect of catheter position. *Proceedings of the High Blood Pressure Council of Australia*: (19th Annual scientific meeting, Freemantle), 19, 50.

A.G. Correia, G. Bergström, A.C. Madden, and R.G. Evans (1998) Effects of medullary interstitial infusion of noradrenaline on the response to increased renal arterial pressure in anaesthetised rabbits. *Proceedings of the High Blood Pressure Council of Australia*: (20th Annual Scientific Meeting, Melbourne), 20, 104.

A.G. Correia, K.M. Denton, and R.G. Evans (1999) Do cortical vascular sites mediate reduced medullary perfusion during activation of vasopressin V_1-receptors in the kidney? *Proceedings of the High Blood Pressure Council of Australia*: (21st Annual Scientific Meeting, Melbourne), 11, 21.

A.G. Correia, K.M. Denton, and R.G. Evans (1999) Effects of Vasopressin V_1-receptor activation on renal cortical and medullary blood flow, and glomerular arteriole dimensions. *Journal of Hypertension*: 18 (Suppl. 4), S69.

A.G. Correia, A.C. Madden, G. Bergström, and R.G. Evans (1999) Effects of renal medullary interstitial, and intravenous norepinephrine, on renal antihypertensive function. *Journal of Hypertension*: 18 (Suppl. 4), S216.

A.G. Correia, G. Bergström, W.P. Anderson and R.G. Evans (2001) Role of pressure diuresis/natriuresis in the acute depressor response to increased renal artery pressure. Submitted to the 24th *International Congress of Physiological Sciences* (Christchurch, New Zealand).

LIST OF ABBREVIATIONS

α	Alpha	l	Liter(s)
β	Beta	M	Medulla
°C	Degrees Celsius	MAP	Mean arterial pressure
<	Less than	μg	Microgram(s)
>	Greater than	mg	Milligram(s)
%	Percent	μl	Milliliter(s)
%$\triangle$	Percent change	min	Minute(s)
±	Plus/minus	MBF	Medullary blood flow
ANOVA	Analysis of variance	ml	Milliliter(s)
AP	Arterial pressure	mm	Millimeter(s)
AVR	Ascending vasa recta	mM	Millimole(s0
Beats/min	Beats per min	mmHg	Millimeters of mercury
C	Cortex	NaCl	Sodium chloride
Ci	Curie	ng	Nanogram(s)
cm	Centimeter	OD	Outside diameter
CO	Cardiac output	OS	Outer stripe
CVP	Central venous pressure	P	Papilla
CBF	Cortical blood flow	PBF	Papillary blood flow
DVR	Descending vasa recta	PRA	Plasma renin activity
FE_{Na+}	Fractional excretion of sodium	PU	Perfusion units
FE_{VOL}	Fractional excretion of urine	RAP	Renal artery pressure
FF	Filtration fraction	RBF	Renal blood flow
g	Gram(s)	RVR	Renal vascular resistance
GFR	Glomerular filtration rate	SAP	Systemic arterial pressure
Hct	Haematocrit	SHR	Spontaneously hypertensive rat
hr	Hour(s)	SV	Stroke volume
HR	Heart rate	SVR	Systemic vascular resistance
ID	Inside diameter	TPR	Total peripheral resistance
IM	Inner medulla	U_{VOL}	Urine flow
IU	International units	$U_{Na+}V$	Urinary sodium excretion
i.v.	Intravenous	v/v	Volume per unit volume
Kg	Kilogram	w/v	Weight per unit volume
		WKY	Wistar-Kyoto rat

Chapter One

LITERATURE REVIEW

1.0 Hypertension and the community

Essential hypertension, or high blood pressure of unknown aetiology, is a leading cause of human cardiovascular morbidity and mortality in most developed countries. It remains largely asymptomatic until late in its course when organ and vessel damage become irreversible. Despite the numerous investigations undertaken to clarify the various mechanisms involved in the regulation of blood pressure, the primary determinants of essential hypertension remain largely unknown. Both environmental and genetic factors contribute to the development of hypertension but while the role of some environmental exposures, such as high salt consumption or stress, appears likely, the underlying determinants, genetic or otherwise, remain unknown.

Cardiovascular disease is a prevailing health problem amongst Australia's population, killing more people alone than any other disease, thus producing enormous strain on the health care system. In 1997, deaths related to cardiovascular disease claimed more lives than those related to cancers and other ailments *(Armstrong et al., 1999)*.

Since the recognition of the prevalence of hypertension and its impact on the community, researchers have taken various approaches to elucidate the factors underlying the development of hypertension. A major recurrent theme has been the dependency of hypertension on interactions between genetic predisposition and environmental factors. Although research directed towards treatment of hypertension will always be required, it is important to direct more attention toward research focused on generating knowledge of the underlying cause, so that a cure can be offered, ahead of treatment.

The kidney appears to play major roles in the genesis of essential hypertension, as demonstrated by experiments that show that hypertension can be 'transplanted'; so that when a kidney from a

hypertensive subject is donated to a normotensive recipient, the recipient develops hypertension. The reverse is also true *(Churchill and Churchill, 1992)*. Hypertension is common in patients with renal disease, but may occur in the absence of reduced renal function (i.e., glomerular filtration rate). By gaining a more complete understanding of the regulation of kidney function, and its role in the regulation of arterial pressure, we can move towards prevention and cure, rather than just treatment of this costly condition.

The renal medulla appears to play an important regulatory role in the long-term maintenance of arterial blood pressure. Briefly, it has been proposed that the renal medulla, through its poor autoregulatory capacity, has the ability to detect changes in the level of arterial pressure, via associated changes in medullary blood flow (MBF) *(Roman and Smits, 1986; Cowley et al., 1992)*. Sodium and water reabsorption appears to be highly dependent on the level of MBF, through mechanisms that remain to be completely defined *(Cowley, 1997)*. This allows the kidney to adjust the level of sodium and water excretion through the pressure diuresis/natriuresis mechanism and return arterial pressure to normal, by adjusting the excretion of salt and water. Furthermore it is hypothesized that the renal medulla possesses its own endocrine function. According to this hypothesis the renal medulla releases a putative vasodepressor substance into the circulation, presumably in response to increased renal artery pressure (RAP) *(Muirhead, 1991; Cowley et al., 1992; Thomas et al., 1994; Bergström and Evans, 1998; Bergström and Evans, 2000)*.

The global aim of the experiments detailed in this thesis was to develop a greater understanding of the role of the renal medulla, and the medullary microcirculation in particular, in blood pressure regulation, and to attempt to determine the vascular elements responsible for MBF regulation. In this chapter, a synopsis of the experimental aims and major findings of each of the studies undertaken in this thesis will be given. This will then be followed by a review of the literature and relevant background information concerning the kidney, with particular attention paid to the renal medulla and its involvement in blood pressure regulation.

1.1 Synopsis of experimental aims and findings

The experiments described in Chapter 3 (Method for local delivery of vasoactive compounds to the renal medullary interstitium), involved the development of methods for the local delivery of vasoactive compounds to the renal medulla, so that blood flow in this region could be manipulated both in acute and chronic experimental settings in conscious and anaesthetized rabbits. Briefly, [^{3}H]-noradrenaline was delivered to the renal medullary interstitium via acutely and chronically

positioned catheters. The kidneys were then removed, 'snap frozen', and sectioned and processed for the determination of radiolabel concentration throughout the renal cortex, outer and inner medulla, and the papilla, using autoradiography. Taken together with our previous study investigating these techniques *(Correia, 1997)*, these experiments demonstrated that vasoactive compounds could be targeted to the renal outer medullary interstitium to reduce blood flow to this region. Specifically, our present experiments demonstrated that infused radiolabel was concentrated within a short distance from the infusion site. Our experiments also demonstrated that the catheters were suitable for use in both chronic and acute experimental settings.

Having established a method for selectively reducing MBF by targeting vasoactive compounds to the renal medulla (Chapter 3; *Correia, 1997*) our next aim (Chapter 4; Effects of renal medullary and intravenous noradrenaline infusion on renal antihypertensive function) was to employ this technique to investigate the role of MBF in renal antihypertensive mechanisms. An extracorporeal circuit was established in anaesthetized rabbits, which allows RAP to be progressively increased without direct effects on the systemic circulation. Thus, this technique allows activation of renal antihypertensive mechanisms; reduced renin release, pressure diuresis/natriuresis, and perhaps also release of a putative renal medullary depressor hormone. To examine the role of renal medullary perfusion in these mechanisms, we tested the effects of infusion of noradrenaline, either into the renal medullary interstitium (selective reduction of MBF) or intravenously (selective reduction of cortical blood flow (CBF)), on responses to increased RAP in anaesthetized rabbits. When RAP was increased in a stepwise fashion urine flow and sodium excretion increased exponentially and plasma renin activity (PRA) and mean arterial pressure fell. Medullary interstitial but not intravenous noradrenaline blunted the increased diuresis and diuresis/natriuresis, and the depressor response to increased RAP, suggesting that reduced medullary blood flow caused by noradrenaline infusion may blunt these renal antihypertensive mechanisms.

The depressor response to increased RAP observed in the extracorporeal circuit model used in the experiments described in Chapter 4 has been attributed to release of a depressor hormone from the renal medulla *(Christy et al., 1991)*. However, in studies performed in Chapter 4 (Effects of renal medullary and intravenous noradrenaline renal antihypertensive function), it was observed that the pressure-natriuretic and diuretic responses increased exponentially in conjunction with increases in RAP and that this was blunted by medullary interstitial infusion of noradrenaline. Given the important role of salt and fluid balance in the control of arterial pressure, it seemed likely that the antidiuretic/anti-natriuretic effect of medullary interstitial noradrenaline played some role in its ability to blunt the depressor response to increased renal artery pressure. Therefore, the aim of the

studies described in Chapter 5 (Roles of pressure diuresis/natriuresis and inhibition of the renin-angiotensin system in the depressor response to increased renal artery pressure) was chiefly to determine the extent of the involvement of the pressure diuresis/natriuresis mechanism in the depressor response to increased RAP in this model. Briefly, an extracorporeal circuit was established in rabbits equipped with ascending aortic flow probes and RAP was increased and maintained at 160 mmHg. During the period of increased RAP the excreted urinary volume was measured on a minute per minute basis. In some animals, the excreted urine volume was then returned intravenously during this period, in the form of compound sodium lactate. In other animals the renin-angiotensin system was also "clamped" by intravenous enalaprilat (2 mg/kg plus 10 µg/kg/min) and MAP and RBF were returned to their original 'control' levels by an intravenous angiotensin II infusion (40-50 ng/kg/min). The results of these experiments demonstrated that the depressor response to increased RAP is abolished when cardiac output is maintained by maintaining salt and fluid balance. Thus, neither the renin-angiotensin system, nor a putative renal medullary depressor hormone, appear to play major roles in the depressor response to increased RAP in this experimental model.

There is now considerable information regarding the effects of vasoactive hormones on MBF. However, there is little information, particularly from *in vivo* studies, about the precise vascular sites responsible for hormonal control of MBF. In the experiments described in Chapter 6 (Effects of activation of vasopressin V_1-receptors on regional kidney blood flow and glomerular arteriole diameters), renal MBF was selectively reduced (~30%), by an intravenous infusion of the V_1-agonist [Phe^2, Ile^3, Orn^8]-vasopressin. Kidneys were then perfusion fixed *in vivo*, filled with methacrylate, and removed and processed for determination of afferent and efferent arteriolar diameters throughout the outer, mid and juxtamedullary cortex, by scanning electron microscopy. Although [Phe^2, Ile^3, Orn^8]-vasopressin selectively reduced MBF by ~30%, we could detect no effect of this agent on vessel diameter throughout each of the kidney regions (outer cortex, mid cortex, and juxtamedullary cortex). These observations raise the possibility that reductions in MBF in response to activation of V_1-receptors might be mediated by vascular elements downstream from the efferent arteriole, possibly the outer medullary descending vasa recta.

1.2   The kidney and blood pressure control mechanisms

When RAP is acutely increased in animal models, renal antihypertensive mechanisms are stimulated which act in concert to restore arterial pressure to normal levels. Renal renin release is reduced, so that the activity of the pro-hypertensive renin-angiotensin system is inhibited. Urinary excretion of salt and water increases exponentially with the increased RAP, so reducing cardiac output. Thirdly, there is now considerable evidence for the release of a putative renal medullary depressor hormone in response to increased RAP. Each of these systems, and their involvement in blood pressure regulation, will be discussed below (see Figure 1.1).
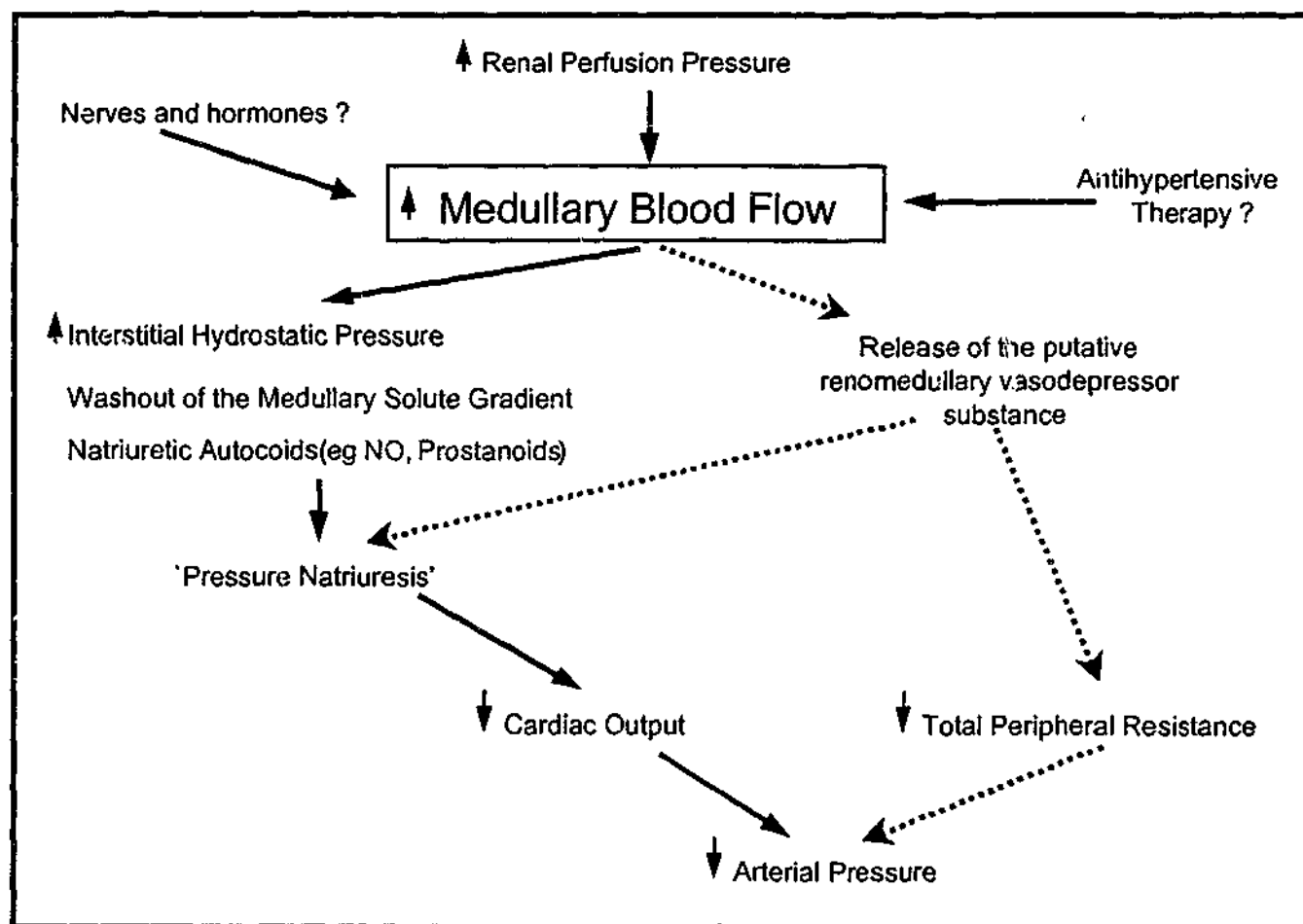
Figure 1.1   Proposed sequence of events by which increased RAP initiates renal antihypertensive mechanisms. Arrows with solid lines indicate the hypothesized mechanisms mediating pressure diuresis/natriuresis. Arrows with dotted lines indicate the hypothesized control mechanisms for the release of the renal medullary depressor hormone. Factors that modify medullary blood flow (i.e. nerves and hormones) should therefore modulate these antihypertensive responses. Figure modified from *(Bergström and Evans, 2000)*.

1.2.1   The renin-angiotensin system

1.2.1.1   Circulating renin-angiotensin system

The renin-angiotensin system plays a significant role in the long-term regulation and maintenance of arterial blood pressure and sodium balance. Within the kidney the renin-angiotensin system plays a major regulatory function which responds to changes in systemic arterial pressure or extracellular fluid volume, through actions of angiotensin II at specific receptors within the renal vasculature, mesangial and tubules to alter both renal vascular resistance and salt excretion *(Admiraal et al., 1990)*. Hypernatremia, hypovolemia, hypotension, and activation of the sympathetic nervous system stimulate the renin-angiotensin system.

The classical description of the renin-angiotensin system focuses on the circulating hormonal system, where renin is released from specialized smooth muscle cells of the juxtaglomerular apparatus of the afferent arteriole *(Johns et al., 1987; Hackenthal et al., 1990)*. The main stimuli for renin release are salt depletion, plasma volume reduction, and decreased arterial pressure *(Navar et al., 1996)*. Others may include; renal sympathetic nerve activation   (β-adrenergic stimuli stimulation), a reduction in circulating angiotensin II, vasopressin, and low plasma potassium concentrations *(Mene and Dunn, 1992)*.

Once released into the circulation renin cleaves angiotensinogen of hepatic origin to generate angiotensin I, which is thereafter converted to angiotensin II through the action of angiotensin-converting enzyme. This action is predominant in the lungs, which contain an abundance of the angiotensin-converting enzyme in the luminal side of the capillary endothelium, but also occurs in other organs (see below).

Having entered the systemic circulation, angiotensin II acts to raise arterial pressure, by affecting both vascular tone (acute) and structure (chronic) *(Guyton and Hall, 1996; Navar et al., 1996)*. Within the kidney angiotensin II contributes to blood volume regulation through the enhancement of sodium reabsorption either by direct stimulation of tubular sodium transport mechanisms, or indirectly, through aldesterone release from the adrenal cortex which also promotes renal tubular sodium reabsorption *(Mene and Dunn, 1992; Guyton and Hall, 1996)*. Angiotensin II also has important effects on regional renal haemodynamics, including the glomerular microcirculation, as well as trophic functions where it acts on many renal and vascular cell types, to regulate cell growth. Furthermore, direct links with other systems, such as kallikrein-kinins and the

prostaglandins, and the sympathetic nervous system suggest that the renin-angiotensin system is part of an inter-related neuro-humoral system involved in the control of kidney perfusion and renal cellular function *(Mene and Dunn, 1992)*.

In the circulation, the vasoconstrictor actions of angiotensin II are short lived due to its rapid metabolism. However, it does produce concentration-dependent vasoconstriction of resistance vessels in virtually all organs, although skeletal muscle and lung vessels display reduced sensitivity to angiotensin II *(Palmer et al., 1987; Mene and Dunn, 1992)*.

Aldosterone is secreted by the zona glomerulosa of the adrenal cortex, in response circulating levels of angiotensin II. Its major physiological role is the stimulation of sodium transport from the ascending limb of the loop of Henle, in exchange for potassium or hydrogen. Thus, it regulates the balance of sodium and potassium concentration in the blood *(Sherwood, 1993)*.

An indication of the significance of the renin-angiotensin system in the regulation of renal function, and therefore arterial pressure, came from Siragy and colleagues who blocked components of the intra-renal renin-angiotensin system by simultaneous renal artery infusions of an angiotensin converting enzyme inhibitor and an angiotensin II receptor antagonist. This treatment produced increases in glomerular filtration rate (~60%), and renal plasma flow (~100%), a ten fold increase in urinary sodium excretion, and an approximately six fold increase in urine flow in conscious, uni-nephrectomized dogs *(Siragy et al., 1990)*. Similarly, intra-renal infusion of an angiotensin converting enzyme inhibitor in the anaesthetized dog significantly increased glomerular filtration rate, urinary sodium excretion and urine volume *(Levens, 1990)*. These and other studies provide evidence that angiotensin II tonically inhibits salt and water excretion, and constricts the renal vasculature, so increasing arterial pressure.

1.2.1.2 Tissue renin-angiotensin system

The concept of tissue specific renin-angiotensin systems is now recognized, and it is now accepted that both renal and non-renal tissues express local angiotensin II biosynthetic ability. Such systems have been identified in the brain, and in peripheral tissues such as the kidney, systemic vasculature, adrenal glands, and heart, all of which locally produce angiotensin II *(Navar et al., 1996)*. These systems chiefly act directly within the organ, or may even secrete angiotensin II into the circulation to act on angiotensin II receptors which have been localized on various tissues including vascular smooth muscle cells, epithelial cells, and cells of the adrenal cortex and medulla, heart, and brain

(Navar et al., 1996). The kidney itself has a well developed tissue renin-angiotensin system. Recent evidence indicates that this system is regulated independently of the classic circulating renin-angiotensin system, and plays a role in the regulation of renal function *(Navar, 1986; Navar et al., 1996).*

1.2.1.3 Paracrine and autocrine effects of angiotensin within the kidney

As discussed above, the actions of the renin-angiotensin system on renal function and blood pressure control may not be entirely dependent on circulating angiotensin II being delivered to the kidney. In the kidney also, some cells house all the necessary components for the production of angiotensin II, which may be synthesized intracellularly, and then secreted *(Navar et al., 1996).* Immunohistochemical studies have shown that renin and angiotensin II are both present in juxtaglomerular cells of the afferent arteriole within the same secretory granules *(Hackenthal et al., 1990)* and that monolayer cell cultures of juxtaglomerular cells contain renin, angiotensin-converting enzyme, and both angiotensin I and II *(Rightsel et al., 1982).* Proximal tubular cells may also be able to synthesize their own angiotensin II from angiotensin I *(Yanagawa et al., 1991).* These cells may also secrete angiotensin II into the surrounding interstitial environment, contributing to the elevated interstitial angiotensin II levels *(Navar et al., 1996).*

The presence of functional angiotensin-converting enzyme in the kidney has been shown by experiments demonstrating the conversion of ~20% of circulating angiotensin I to angiotensin II during passage through the kidney *(Rosivall et al., 1983; Rosivall et al., 1984; Navar et al., 1996).* This activity has been localized to the vascular endothelial cells of the renal arteries, afferent and efferent arterioles, and glomerular and peritubular capillaries, but is also present on both the brush border (luminal) and basolateral membranes of the proximal tubule cells *(Navar et al., 1996).*

Collectively, data suggests an intrarenal generation of angiotensin II, providing it with both paracrine and/or autocrine functions as well as the classical endocrine hormone functions. In turn, angiotensin II acts at angiotensin receptors found throughout the kidney on glomerular mesangial cells, pre and postglomerular arterioles, vasa recta bundles of the inner stripe of the outer medulla, medullary interstitial cells, and several tubular segments of the nephron *(Brown and Venuto, 1988; Navar et al., 1996).*

1.2.1.4 Renin-angiotensin system and hypertension

The renin-angiotensin system appears to be involved in the development of renovascular hypertension and may be implicated in the pathogenesis of essential hypertension *(Waeber et al., 1986)*. One proposition is that in hypertensive patients the basal level of arterial pressure is set at a higher level because the kidney requires a higher perfusion pressure for the suppression of renin release *(Guyton and Hall, 1996)*. Angiotensin-converting enzyme inhibitor drugs and AT_1 antagonists are beneficial in the treatment of certain cases of hypertension. By blocking the generation or actions, respectively of angiotensin II, they blunt the ultimate salt and fluid conserving actions and arteriolar constrictor effects of the renin-angiotensin system. There is however no obvious correlation between measured plasma renin activity and blood pressure in human essential hypertension or in the spontaneous-hypertensive rat (SHR), two forms of hypertension characterized by relatively low levels of plasma renin activity *(Folkow, 1982)*.

1.2.2 Pressure diuresis/natriuresis

According to the classical 'Guytonian' view of the role of the kidney in long-term blood pressure control, whenever arterial pressure is elevated, sodium and water excretion is increased, until arterial pressure is returned to control levels *(Guyton et al., 1972; Cowley, 1992)*. This mechanism is believed to be non-adaptive and largely responsible for the long term control of arterial pressure *(Cowley et al., 1992)*. According to this hypothesis, hypertension can only occur if the excretory ability of the kidney is impaired. The mechanism(s) responsible for pressure diuresis/natriuresis remain uncertain, however evidence indicates that it may be dependent on a poor autoregulatory capacity of the renal medullary circulation relative to whole kidney blood flow, since increased sodium excretion in response to increased RAP occur without changes in total renal blood flow (RBF) or glomerular filtration rate (GFR) *(Cowley et al., 1992; Roman and Zou, 1993; Cowley, 1997;Cowley and Roman, 1997;Tornel and Madrid, 2000;)*.

According to this theory, an increase in RAP produces a concurrent increase in inner MBF, which triggers a sequence of events which act in concert to inhibit tubular sodium reabsorption, *(Cowley et al., 1992)*. Thus, increased RAP results in increased MBF, and so vasa recta blood flow, which in turn leads to (i) washout of the medullary solute gradient, (ii) increased vasa recta capillary pressure and so increased renal interstitial hydrostatic pressure, and (iii) presumably also the release of diuretic/natriuretic autocoids such as nitric oxide and prostaglandins *(Cowley et al., 1992; Cowley and Roman, 1997; Bergström and Evans, 2000)*.

1.2.2.1 Pressure diuresis/natriuresis and hypertension

The kidneys of hypertensive patients *(Omvik et al., 1980)* and genetically hypertensive rats *(Roman and Cowley, 1985b; Roman and Zou, 1987; Khraibi and Knox, 1988; Roman and Kaldunski, 1988b;)* require an elevated level of MAP in order to achieve similar levels of sodium and water excretion, as do the stroke-prone strain of spontaneously hypertensive rat (SHR) *(Nagaoka et al., 1981)*. The reason for the inability of 'hypertensive kidneys' to effectively excrete sodium and water when perfused at normotensive pressures remains uncertain, however an intrinsic impairment of the pressure diuresis/natriuresis mechanism, rather abnormalities of the neural and/or endocrine control of the kidney seem likely *(Liard, 1977; Cowley and Roman, 1983)*.

Under experimental conditions in which the neural and hormonal backgrounds are controlled, through renal denervation, and maintenance infusion of vasopressin, aldosterone and noradrenaline, increases in RAP of ~50 mmHg produce a nine-fold increase in urine flow and sodium excretion in the normotensive Wistar-Kyoto normotensive rat (WKY), compared to only a four-fold increase in the SHR *(Roman and Cowley, 1985b)*. Not only does this response occur independently of external influences, but also of changes in RBF, and glomerular filtration rate (GFR), which remained similar between the two groups *(Arenshorst, 1979; Roman and Cowley, 1985b)*. Therefore, under these conditions, the diuretic and natriuretic responses to elevations in RAP are blunted in SHR when compared to WKY normotensive rats, which is believed to contribute to the functional resetting of the kidney toward higher perfusion pressures necessary for the development of hypertension *(Roman and Cowley, 1985b)*. Furthermore, these studies indicate that the level of RAP markedly influences tubular reabsorption, indicating that small changes in arterial pressure may have a greater influence on sodium and water excretion than had been previously recognized *(Roman and Cowley, 1985a)*.

Reduced renal MBF is the most apparent renal haemodynamic abnormality in the development of hypertension in SHR, since total RBF, CBF, and GFR remain similar in young and adult SHR and WKY rats *(Khraibi and Knox, 1988; Roman and Kaldunski, 1988b)*. On the other hand, MBF is reduced in SHR, even before hypertension has developed, and therefore renal medullary vascular resistance is elevated in the SHR model *(Roman and Kaldunski, 1988b; Lu et al., 1994)*. Based on this evidence, it is hypothesized that in order for hypertension to occur, MBF must be reduced, which in turn contributes to the haemodynamic resetting of the pressure diuretic/natriuretic relationship *(Cowley, 1992)*. This hypothesis was tested *in vivo* using a chronic infusion technique, which allowed delivery of captopril (5 mg/kg/day) to the renal medullary interstitium to increase

MBF, which was in turn monitored by chronic laser-Doppler flowmetry. The results confirmed that a chronic increase in MBF in SHR, produced a left-ward shift in the pressure natriuretic relationship and lowered arterial pressure *(Lu et al., 1994)*.

The role of changes in MBF in the development of hypertension has not been widely studied. Ganguli et al. reported that papillary blood flow (PBF), measured using the albumin accumulation technique, was lower in 17 week old SHR compared with WKY *(Ganguli et al., 1976)*. Relationships between RBF, CBF, PBF and RAP were compared in 3-5, 6-9, and 12-16 week old SHR and WKY *(Roman and Zou, 1987)*. It was found that MAP was ~19 mmHg greater in 3-5 week old SHR than in age matched WKY rats, and that PBF measured at equivalent RAP was significantly lower (about 30%) in all age groups of SHR compared with values measured in WKY *(Roman and Zou, 1987)*. These results are in general agreement with previous observations made in adult SHR, which support the notion that an irregularity in inner MBF is responsible for the abnormal pressure diuresis/natriuresis in hypertensive rats, and that this response is not attributable to a defect in whole kidney haemodynamics *(Arenshorst, 1979; Roman and Kaldunski, 1988b)*.

1.2.2.2 Autoregulation of renal medullary blood flow

Autoregulation is the intrinsic ability of an organ to maintain constant blood flow despite changes in arterial perfusion pressure. The question of whether or not MBF is autoregulated has provoked much research and debate *(Pallone et al., 1990)*. Several studies, employing various techniques to measure MBF, support the notion that it is efficiently autoregulated *(Cohen et al., 1983; Majid and Navar, 1996; Harrison-Bernard et al., 1996; Majid et al., 1997)*, while both earlier and more recent reports indicate the converse (see below).

Thurau et al. studied autoregulatory behavior in the medulla by measuring the transit time of Evans Blue dye *(Thurau, 1964)*. As blood pressure increased, transit time decreased, indicating an increase in MBF with increased MAP. Later studies using the H_2 washout technique *(Aukland, 1968)*, the transit time of labeled red cells *(Graensgoe and Wolgast, 1972)*, or the laser-Doppler technique *(Roman and Smits, 1986)*, however have produced varied results. Cohen et al.. demonstrated autoregulation in the medulla up to a MAP of 120 mmHg, but at higher pressures MBF increased *(Cohen et al., 1983)*, even though total RBF continued to be autoregulated up to higher pressures (~140-150 mmHg) *(Zimmerhackl et al., 1985b)*.

Recent studies by Cowlev, Roman, and colleagues, employing laser-Doppler flowmetry have indicated that blood flow to the inner medulla in volume expanded rats is poorly autoregulated. In these experiments RAP was altered in a stepwise fashion in hydropenic rats, from ~150 mmHg *(Roman and Smits, 1986; Roman and Cowley, 1988)*. They found that MBF reduced almost linearly . with the fall in perfusion pressure even though blood flow in the deep cortex and outer medulla remained efficiently autoregulated *(see also Roman and Zou, 1993; Cowley and Roman, 1997)*. This is difficult to reconcile with the evidence that MBF is predominantly derived from the efferent arterioles of juxtamedullary glomeruli, the pre-glomerular vasculature of which exhibits autoregulatory capacity *(; Casellas and Moore, 1990; Cohen et al., 1983; Gonzalez et al., 1994; Bouriquet and Casellas, 1995)*. Further investigations employing laser-Doppler flowmetry, demonstrated that the superficial and deep cortex are autoregulated as well as is whole kidney blood flow in volume expanded rats, but outer and inner MBFs are not well autoregulated *(Mattson et al., 1993; Roman and Zou, 1993)*.

The lack of inner MBF autoregulation in volume expansion is difficult to understand, since the juxtamedullary glomeruli, which supply the renal medullary blood vessels, demonstrate efficient autoregulation of single nephron glomerular filtration rate *(Goransson and Sjoquist, 1984)* and glomerular capillary pressure *(Carmines et al., 1990)*. The most plausible explanation for this is that blood flow may be redistributed within the medullary post-glomerular circulation in response to changes in RAP. The increase in shear stress resulting from an increase in flow through these vessels may release vasoactive agents, which may act on the contractile elements in the vascular network between the descending and ascending vasa recta in the outer medulla to redistribute post-glomerular blood flow *(Pallone et al., 1990)*. A study by Fenoy and Roman supports this explanation, showing that local release of nitric oxide may play a role in the lack of autoregulation of PBF in rats *(Fenoy and Roman, 1992)*. In support of this, other studies have shown that PBF is influenced by humoral factors *(Kiberd et al., 1987a; Kiberd et al., 1987b; Zimmerhackl et al., 1987)*, and renal sympathetic tone *(Hermansson et al., 1984)*, and that plasma levels of vasopressin, angiotensin, atrial natriuretic factor, and renal nerve activity are all affected by changes in blood volume *(Roman and Cowley, 1988)*. Videomicroscopy studies indicate that the number of perfused vasa recta capillaries and the velocity of RBC in these vessels increased significantly after RAP is elevated *(Roman and Cowley, 1988)*. The increase in PBF is caused by an increase in blood flow through perfused capillaries as well as the recruitment of flow in non-perfused capillaries *(Roman and Cowley, 1988)*. This study therefore confirmed, using another method, that PBF is poorly autoregulated in volume-expanded rats.

Conversely, studies in dogs, by Majid and Navar also employing laser-Doppler technology found that MBF measured throughout different regions of the renal medulla (outer and inner medullary blood flows were measured) was well autoregulated *(Majid and Navar, 1996; Majid et al., 1997)*. Differences in experimental preparations, as well as the precise techniques used to measure MBF may have contributed in part to these inconsistencies in the literature. The resolution of this issue remains a key goal of research aimed at understanding the role of the medullary circulation in the control of blood pressure.

1.2.2.3 Medullary blood flow and tubular sodium reabsorption

The concept that changes in renal MBF can alter tubular reabsorption of sodium first emerged nearly 40 years ago. Early and Friedler observed that the natriuretic response to intravenous saline was associated with a fall in urine osmolality and the renal extraction of para-aminohippurate *(Early and Friedler, 1965a; Early and Friedler, 1965b)*. With the assumption that para-aminohippurate is only extracted in the renal medulla and that increased MBF reduces para-aminohippuric acid (PAH) extraction, they concluded that volume expansion (or increased RAP) produces increases in MBF, which inhibits water reabsorption in the thin descending loop of Henle, secondary to loss of the medullary solute gradient. They suggested that the fall in water reabsorption from the thin descending limb of Henle's loop reduces the sodium concentration of the fluid entering the ascending limb of Henle's loop, and so reduces sodium reabsorption in this portion of the nephron *(Early and Friedler, 1965a; Early and Friedler, 1965b)*.

Similar findings have since been produced using the micropuncture technique, which have demonstrated that elevations in RAP inhibit sodium reabsorption in the proximal tubule of superficial nephrons and even more so in the proximal tubule and thin descending limb of the loop of Henle of deep nephrons *(Haas et al., 1986; Roman and Cowley, 1988)*. Other suggested sites include the proximal tubule, thick ascending limb of Henle's loop and the collecting duct *(Arenshorst, 1979; Roman and Cowley, 1988)*. The pressure-diuresis phenomenon has also been studied in isolated blood-perfused kidney preparations, which have provided results consistent with these conclusions. However, these experiments have been difficult to interpret because autoregulation of RBF and GFR is not as efficient *in vitro* as it is in the intact animal *(see Roman and Cowley, 1985a)*.

Thus, increased MBF dissipates the medullary urea gradient, which likely contributes to the pressure natriuretic response through the inhibition of water reabsorption in the thin descending

loop of Henle, and therefore increased reabsorption in this nephron segment *(Roman and Zou, 1993)* and by increasing the conductance of the paracellular pathway to ions in the proximal tubule, thin descending loop of Henle and thin ascending loop of Henle, (which in the rat, are all highly permeable to sodium and chloride) *(see Roman and Zou, 1993)*. Furthermore, as discussed below, elevations in vasa recta capillary pressure may inhibit water re-uptake from the medullary interstitium and increase medullary interstitial pressure, which may participate in the pressure-diuresis/natriuresis response by inhibiting tubular reabsorption of sodium and water *(see Cowley, 1997)*.

1.2.2.4 Renal interstitial hydrostatic pressure

It is proposed that increased MBF is accompanied by increases in vasa recta capillary pressure, and a transient inhibition of water uptake from the renal medullary interstitium, thereby increasing renal interstitial hydrostatic pressure (RIHP) *(Roman and Cowley, 1988; Roman and Zou, 1993)*. Elevations in RAP in male Sprague-Dawley rats significantly increase RIHP, diuresis/natriuresis and diuresis, but this response is blunted in the SHR *(Roman and Cowley, 1985b; Roman, 1986; Khraibi and Knox, 1988)*. The blunted effect of RAP on RIHP in the SHR may be responsible for the blunted pressure diuresis/natriuresis and diuresis response observed in these rats, since this relationship is shifted to higher pressures, when compared to WKY rats *(Norman et al., 1978)*. Furthermore, evidence exists in support of the notion that the brisk diuresis/natriuresis and diuresis observed in WKY rats, is related to the transmission of RAP to the renal interstitium due to poor MBF autoregulation, leading to a significant increase in RIHP, decreases in sodium reabsorption in various segments of the nephron, and thus diuresis/natriuresis and diuresis *(Roman and Cowley, 1985b)* (see Figure 1.2).

Intra-renal infusions of vasodilators such as acetylcholine, bradykinin *(Mertz et al., 1984)*, and prostaglandin E_2 produce significant increases in sodium excretion which are associated with increases in MBF and RIHP *(Knox et al., 1983; Haas et al., 1984; Granger and Scott, 1988; Cowley et al., 1995)*. Furthermore, this rise in RIHP may be prevented by renal de-capsulation, which greatly blunts renal vasodilation-induced increased in sodium excretion *(Haas et al., 1984; Granger and Scott, 1988)*. Thus, alterations in MBF, either secondary to changes in RAP or tone in vascular elements that regulate MBF, appear to be closely linked with changes in RIHP and tubular sodium reabsorption. On the other hand, there are a number of aspects of this hypothesis that merit further testing. In particular, the evidence that alterations in MBF shift the pressure diuresis/natriuresis relationship arises from experimental models that allows RAP to be set to levels

at or below systemic arterial pressure. It remains to be seen whether the influence of MBF on pressure diuresis/natriuresis also holds true when RAP is increased to levels above systemic arterial pressure (hypertensive levels), which might better reflect the antihypertensive function of the pressure diuresis/natriuresis mechanism. This can be achieved using an extracorporeal circuit model developed in our laboratory, which allows RAP to be set to any level, above or below RAP *(Christy et al., 1991; Evans et al., 1995)*. Thus, a major aim of the experiments described in this thesis was to examine the effects of reducing MBF on pressure diuresis/natriuresis in this experimental model.
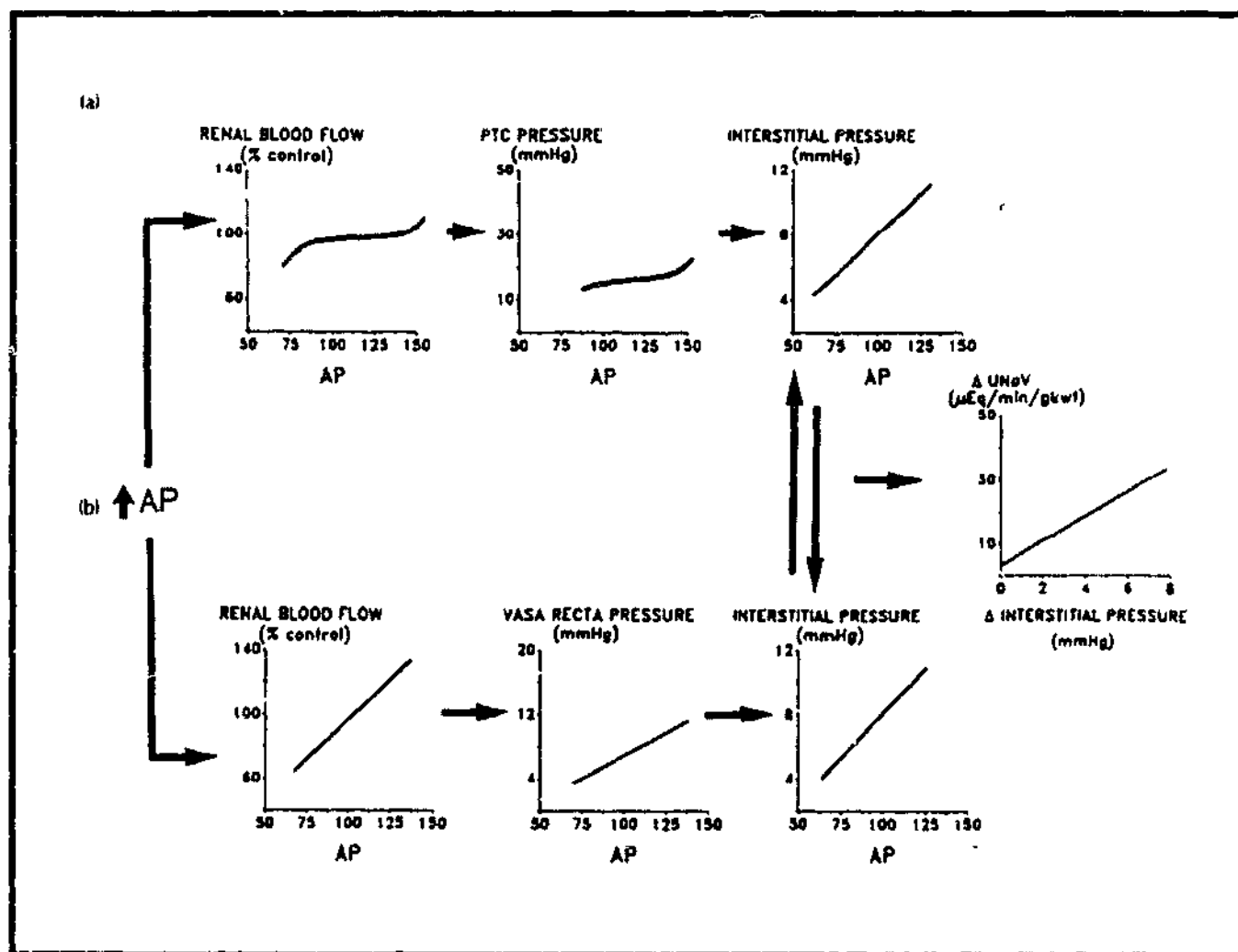
Figure 1.2   Summary of proposed mechanisms responsible for pressure diuresis/natriuresis. (a) Renal cortical responses to changes of renal arterial pressure (AP) show good autoregulation of cortical blood flow and absence of measurable changes of peritubular capillary pressure (PTC) as arterial pressure is changed. (b) Renal medullary responses indicate lack of autoregulation of medullary blood flow with vasa recta capillary pressure and medullary interstitial fluid pressure rising with arterial perfusion pressure. This rise of interstitial pressure transmitted throughout the kidney is associated with an increase in renal excretion of sodium and water. $U_{Na}V$, urinary sodium excretion; gkwt, g kidney weight. From *(Cowley, 1992)*.

1.2.3 The putative renal medullary depressor horr ne

A proposed third renal antihypertensive mechanism, the putative renal medullary depressor hormone, has been the center of much debate and extensive research. This depressor substance dubbed 'medullipin' by the late Eric Muirhead, is thought to be released from the renal medullary interstitial cells, in response to increased RAP *(Muirhead, 1991; Muirhead, 1993)*. However, despite strong evidence for the release of such a depressor substance from the medulla, the physiology and identity of this hormone and its involvement in the aetiology of hypertension require elucidation.

The idea that the renal medulla possesses the ability to release a depressor substance in response to increases in RAP arose initially from three lines of experimental evidence. The first line of evidence was initiated by Grollman *et al.*, who compared the effects of ureteal ligation, with uretocaval anastomoses. Ureteal ligation causes atrophy of the renal medulla, and development of a 'hydronephrotic kidney' *(Grollman et al., 1949)*. Dogs prepared with ureterocaval anastomoses, and therefore deprived of excretory ability failed to develop hypertension, whereas those with ureteral ligation did. Muirhead and colleagues later discovered that the renal papillae of the ligated kidney preparations were destroyed whereas the papillae of the anastomosed preparations were intact *(Muirhead et al., 1960a)*. This finding prompted Muirhead to propose that cells in the renal papilla were involved in the prevention of hypertension in dogs with ureterocaval anastomoses *(see Muirhead, 1980)*. This hypothesis was supported by the finding that transplants of the renal medulla (and no other region of the kidney, i.e. the cortex) prevented renoprival hypertension *(Muirhead et al., 1960b)*.

He then went on to report findings using transplants of fragmented renal medulla, cortex and other tissues (liver and spleen) in experimental models of hypertension (in rats), including accelerated renoprival hypertension, one-kidney, one clip hypertension, malignant hypertension, and extreme salt loaded renoprival hypertension *(Muirhead et al., 1975)*. Renomedullary tissue was antihypertensive in all instances whereas renocortical and other tissues were not *(see Muirhead, 1980)*. Similar transplantation studies from other laboratories have confirmed these findings *(Solez et al., 1976; Susic et al., 1978)*.

Muirhead's next observation was that the main cells surviving the transplants were in close association with the capillaries *(Muirhead et al., 1972a)*. His next challenge was to isolate these

cells and determine their content. Using tissue culture techniques he was able to grow these cells and determine that they contained abundant lipid droplets *(Muirhead et al., 1972b)*. He was finally able to link the antihypertensive effect of these renomedullary interstitial cells *in vivo* by showing that transplantation of these cultured cells into hypertensive rats produced antihypertensive effects *(Muirhead et al., 1975)* (see above). In other studies, Muirhead was able to provide lipid extracts, from cultured renal medullary interstitial cells, whole renal medulla, and the venous effluent of kidneys perfused at high pressure, that had blood pressure lowering effects *(Muirhead et al., 1991a; Muirhead et al., 1991b)*. The ultimate goal of complete chemical characterization of the active principle(s) in these lipid extracts has remained elusive.

The second line of evidence came from studies in rats, which demonstrated that arterial pressure could be rapidly 'normalized' following the removal of the renal arterial clip from renal hypertensive rats *(see Bing et al., 1981)*. This rapid reduction in MAP, according to several studies, cannot be explained by either normalization of structural vascular changes, prostaglandins, the renal kallikrein-kinin system, the renin-angiotensin system, vasopressin, endogenous opioids or the pressure diuresis-natriuresis mechanism *(Russell et al., 1982a; Russell et al., 1982b; Muirhead et al., 1985)*. On the other hand, when rats were pretreated with 2-bromoethylamine to chemically ablate the renal medulla, the reversal of the hypertension following renal artery unclipping occurred much more slowly, suggesting that the depressor substance was released from the renal medulla *(Bing et al., 1981)*.

Thirdly, cross-circulation studies in rats, in which RAP could be increased by means of a pump, cross-circulated in series with an intact 'assay rat', demonstrated that a humoral depressor mechanism which lowered MAP was activated in the 'assay rat' *(Karlström et al., 1989)*. A depressor response could also be obtained under similar cross-circulation conditions, using spontaneously hypertensive rats, although much higher perfusion pressures were required for the activation of the depressor response, than in normotensive (WKY) rats *(Karlström et al., 1991)*. There is at least indirect evidence for a role of MBF in this depressor response, since it is blunted by blockade of nitric oxide synthesis and electrical stimulation of the renal nerves; treatments that can reduce MBF *(Rudenstam et al., 1992; Bergström, 1995; Bergström et al., 1995; Rudenstam et al., 1995; Bergström et al., 1996)*.

Our group has confirmed and extended these findings using a novel approach applied in rabbits and dogs. In these studies, an extracorporeal circuit was established to perfuse the kidney *in situ* with the animals' own blood. A pump was used to circulate blood drawn from the aorta, and return it to

the vena cava (rabbits, or the iliac vein in dogs), and the renal artery. By altering flow in the venous limb of the circuit using a Starling resistor (but not altering total flow through the circuit), RAP and RBF can be set and maintained to any level, without producing direct effects on the systemic circulation. Using this model, powerful hypotensive responses were observed in response to the doubling of RAP (170-190 mmHg) in both anaesthetized dogs and rabbits. It was also found that the depressor response to increased RAP was abolished when the animals were pre-treated with 2-bromoethylamine to chemically ablate the renal medulla *(Christy et al., 1991)*, (Figure 1.3). It was ...gued in these experiments that the falls in MAP in these experiments were not due to volume depletion triggered by the pressure-natriuretic-diuretic response, as all experimental animals were in positi.. ..id balance at all times *(Christy et al., 1991)*. Other experiments indicate that the depressor response is not due to prostaglandins, platelet activating factor, or suppression of the renin-angiotensin system *(Christy et al., 1993)*, nitric oxide release *(Evans et al., 1995; Thomas et al., 1995)*, or products of cytochrome P450 metabolism of arachidonic acid *(Evans et al., 1998b)*.
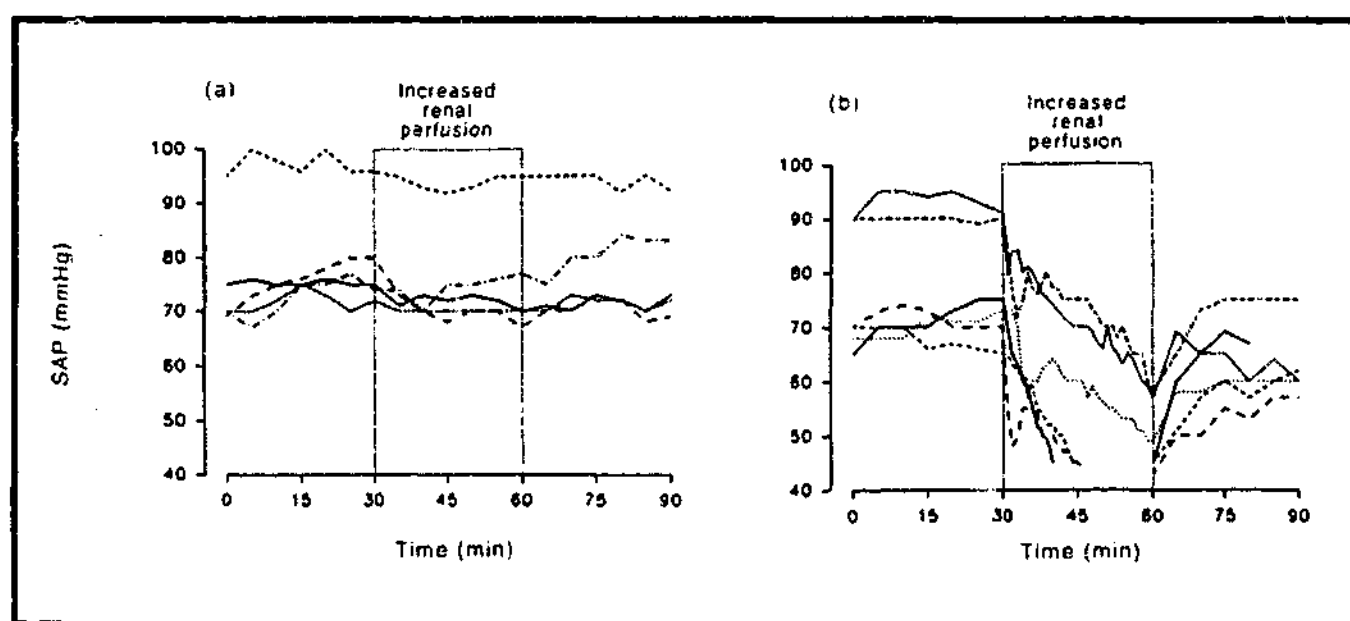
Figure 1.3 Mean systemic arterial pressure (SAP) before, during and after increasing renal perfusion pressure, using the extracorporeal circuit. Responses are shown for individual rabbits. (a) Response in rabbits pretreated with 2-bromoethylamine to produce 'chemical medullectomy', (b) response in normal rabbits. Adapted from *(Christy et al., 1991)*.

Although there is no direct evidence linking the factor(s) responsible for mediating these depressor responses of increased RAP to 'medullipin', the depressor substance is presumed to be housed in the renal medullary interstitial cells. This remains to be shown definitively. The characteristic component of these cells are their lipid inclusions, visible using both light and electron microscopy.

These cells are interposed by cytoplasmic extensions between the tubules and blood vessels of the renal medullary intersititum *(Lemley and Kriz, 1991)*, closely resembling the rungs of a ladder *(Kriz, 1981)*, and anchor through cytoplasmic processes into the basement membrane of Henle's loop and the vasa recta *(Kriz, 1981)*.

Pitcock and colleagues *(Pitcock et al., 1982; Pitcock et al., 1984; Pitcock et al., 1985)*, have reported various morphological changes in renal medullary interstitial cells in some forms of genetic hypertension, and a reduced number of these cells (and fewer lipid granules) in Dahl salt-sensitive rats compared to salt-resistant rats. They also reported that Dahl salt-resistant rats, but not Dahl salt-sensitive rats, respond to an increased salt diet with an increase in the number and size of renal medullary interstitial granules.

While the components of the lipid inclusions have not been fully characterized, histochemical studies have shown them to comprise largely of saturated and unsaturated lipids. Three possible antihypertensive factors have been identified from isolated renal interstitial cells and also from renal venous effluent from isolated kidneys perfused at high pressures *(Muirhead et al., 1991a)*: prostaglandin E_2, antihypertensive polar renomedullary lipid (APRL; known to be identical to platelet activating factor, PAF) and the antihypertensive neutral renomedullary lipid, collectively known as medullipin I. Other components of these cells are; triglycerides, cholesterol esters and free fatty acids as well as precursors of prostaglandins, (mainly prostaglandin E_2), and glycosaminoglycans, mainly hyaluronic acid *(see Thomas et al., 1996)*. Medullipin I is inactive, but appear to require 'activation' by cytochrome P-450 during the passage through the liver to form medullipin II, which has powerful depressor actions *(Muirhead et al., 1991b)*. Despite intense efforts in determining the molecular structure of both medullipin I and II, their identities remain unknown.

One possible stimulus for the release of this putative renal medullary depressor hormone is increased MBF in response to increased MAP. If MBF is poorly autoregulated (see discussion above), the medullary microcirculation is in an ideal position to detect increased MAP, and in turn initiate a cascade of events leading to release of this putative hormone. This hypothesis has to date received little attention, and is a major focus of the experiments described in this thesis. We can only speculate at present as to the precise mechanisms that might allow increased MBF to stimulate release of the putative renal medullary depressor hormone. They might include changes in intravascular sheer stress, inner medullary concentrations of ions or metabolites, medullary interstitial osmolarity or hydrostatic pressure, or medullary oxygen levels.

Although the experiments described above provide evidence of a depressor substance released from the renal medulla in response to increased RAP, this evidence remains indirect. One of the major focuses of the experiments described in this thesis is to more stringently test the hypothesis that this hormone exists, and is released in response to increased RAP. Specifically, this hypothesis can only be maintained if the depressor response to increased RAP can be demonstrated under conditions where the other major renal antihypertensive mechanisms, inhibition of renin release and pressure diuresis/natriuresis, are controlled for.

1.3   The renal circulation

The significance of local flow changes in the kidney was first suggested by Trueta and colleagues *(see Ofstad and Aukland, 1985)*, who claimed that 'redistribution' of intrarenal blood flow could cause cortical hypoxia and tubular necrosis, as well as other types of renal abnormalities. Subsequently, several hypotheses implied that important renal functions such as the maintenance of the corticomedullary osmotic gradient (important for the concentration of urine and the excretion of sodium chloride) might be regulated in part through regulation of local blood flow in the medulla. Hence, the notion that the renal medullary circulation plays a significant role in the regulation and maintenance of salt and fluid balance and circulatory homeostasis was born.

The unique arrangement of the cortical and medullary microcirculation is shown diagrammatically in Figure 1.4. The cortical and medullary microcirculations are virtually 'in-series' and 'in parallel' simultaneously. They are in series because all blood that flows to the medulla must first pass through the cortex. On the other hand, however, MBF is derived from a sub-population of glomeruli at the corticomedullary junction, so redistribution of blood flow between these juxtamedullary glomeruli and those in other cortical regions could theoretically lead to large changes in MBF yet little or no change in total CBF. As will be described in more detail below, there are also more subtle mechanisms that could possibly allow for differential control of CBF and MBF. First, however, we must consider the structural aspects of the renal circulation in detail.
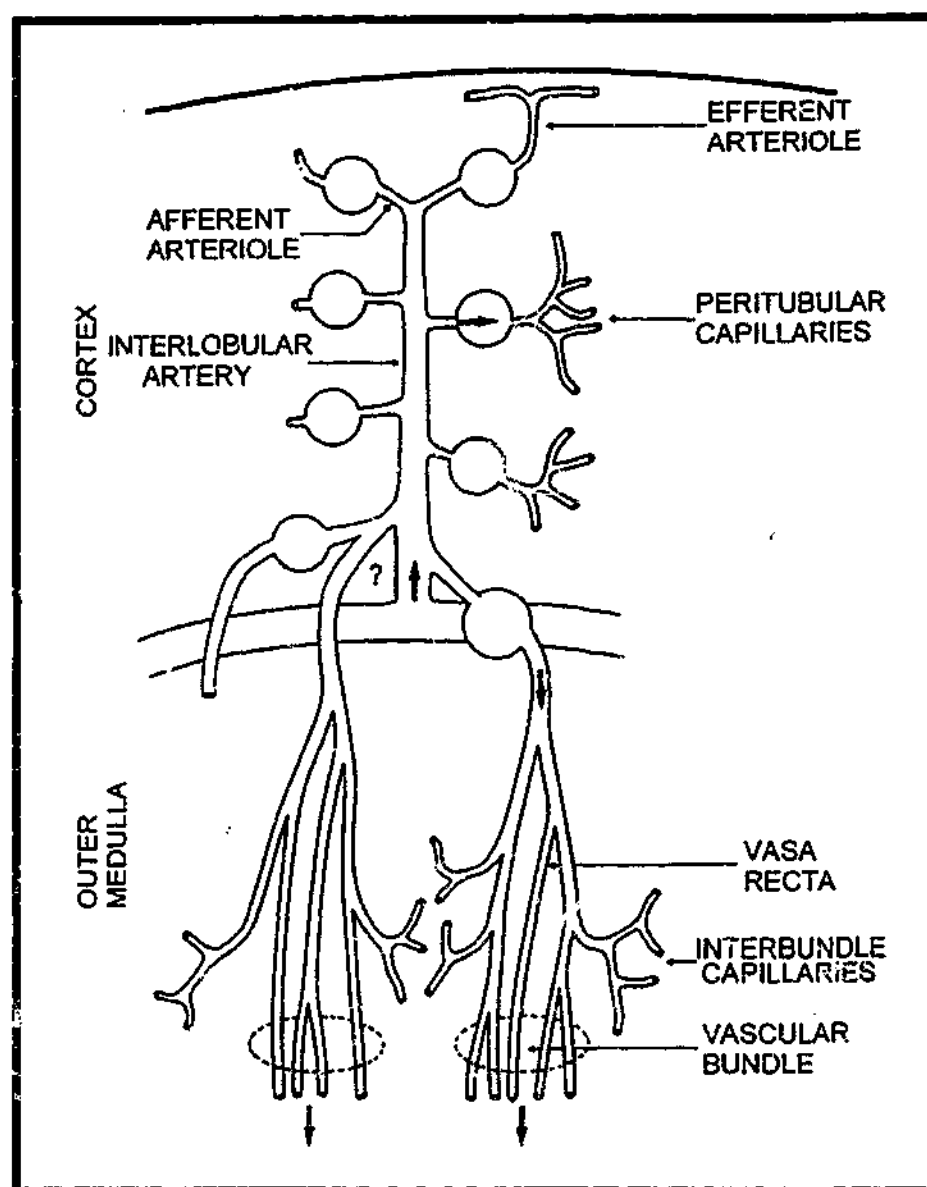
Figure 1.4 The renal circulation, and the microcirculatory resistance segments in the cortex and outer medulla. The kidney is divided into the cortex, which contains glomeruli, nephron segments (not shown) and a dense capillary plexus (which branches from peritubular capillaries; not shown). An arcuate artery gives rise to interlobular arteries from which afferent arterioles originate at an angle that varies with cortical location. Blood is supplied to the renal cortex and medulla principally from the efferent flow of superficial and juxtamedullary glomeruli, respectively. Efferent arterioles of juxtamedullary glomeruli give rise to descending vasa recta (DVR) in the outer stripe of the outer medulla. In the inner stripe of the outer medulla, the DVR and ascending vasa recta (AVR) returning from the inner medulla (IM) (not shown) form vascular bundles. The DVR from the bundle periphery supply the interbundle capillary plexus of the inner stripe, while those in the center supply blood to the inner medulla. The extent to which blood flow to the medulla is supplied by pre-glomerular 'shunt' pathways is uncertain. Taken from *(Pallone et al., 2000)*.

1.3.1 Cortical microcirculation

The cortical circulation begins with the branching of the renal artery into (6-10) interlobar arteries. These then ascend within the renal pelvis, penetrate the renal parenchyma, change direction, and follow an arc-like direction near the corticomedullary border as arcuate arteries. The arcuate arteries give rise to the interlobular arteries which ascend through the cortex toward the renal capsule and give rise to afferent arterioles. The afferent arterioles enter the glomeruli, and exit as efferent arterioles to form a peritubular capillary network *(Beeuwkes, 1980; Pallone et al., 1990; Bertram, 2000)*.

1.3.1.1 Afferent arterioles

The angles at which the afferent arterioles arise from the interlobular arteries are dependent on their position in the cortex. Those of the outer cortex enter the glomeruli in line with the parent vessel, but those that supply the juxtamedullary glomeruli leave at a recurrent angle. Most afferent arterioles are branches of the cortical radial (interlobular) arteries, although some juxtamedullary afferent arterioles are direct branches of arcuate arteries *(Bertram, 2000)*.

The afferent arteriole enters the glomerulus at the vascular pole and branches to form the glomerular tuft. The wall of the afferent arteriole is composed of one to three layers of muscle cells and elastic tissue *(Pallone et al., 2000)*. These vascular components gradually diminish near the glomerulus, as some muscle cells are replaced by renin containing granular cells of the juxtaglomerular apparatus *(Pallone et al., 2000)*. The afferent arterioles of juxtamedullary nephrons contain fewer granular cells than those of the mid and outer cortical regions, possibly implying a lower renin content.

In some species, muscular intra-arterial cushions exist at the origin of juxtamedullary afferent arterioles. Their purpose is uncertain, but two possible roles have been hypothesized; their position is well suited to the modulation of MBF, since it is accepted that the majority of MBF arrives from these vessels. The second possible function of these cushions is a possible skimming role which acts to reduce the haematocrit of medullary blood *(Pallone et al., 1990)*.

1.3.1.2 Efferent arterioles

Efferent arterioles begin inside the glomerular tuft. As with afferent arterioles, those of the juxtamedullary region are larger in size and have 2-4 layers of smooth muscle cells, compared with the smaller arterioles of the mid and outer cortical regions, which contain only 1-2 layers *(Bertram, 2000)*. This has been documented across many species, including; rabbits, dogs, rats, and humans *(Pallone et al., 1990; Pallone et al., 1998)*. Juxtamedullary efferent arterioles are also longer, and cross the corticomedullary junction to enter the outer stripe of the outer medulla where they give rise to the descending vasa recta *(Pallone et al., 2000)*.

1.3.2 Renal medullary blood flow

1.3.2.1 Medullary microcirculation

Although the renal medulla receives only ~10% of total RBF it serves the important functions of supplying oxygen and nutrients and removing carbon dioxide and metabolic end products from its surrounding tissue. MBF also plays a significant role in blood pressure regulation through urine concentration and dilution *(Ullrich et al., 1962; Thurau, 1964; Fadem et al., 1982)*, and may stimulate the release of the putative depressor hormone *(Bergström and Evans, 1998)*.

Blood enters the renal medulla chiefly via the efferent arterioles of juxtamedullary glomeruli, although alternate routes have been postulated *(Kriz, 1981; Kriz, 1982; Pallone et al., 1990)*. These efferent arterioles have larger diameters and a thicker endothelium than those of the cortex. They are surrounded by two to four layers of smooth muscle cells, and are accompanied by sympathetic innervation. As these vessels descend further into the renal medulla, the smooth muscle cells are gradually replaced by pericytes, so that most, if not all contractile function, along with sympathetic innervation is probably lost at the junction of the outer and inner stripe *(Pallone et al., 1990)*. On the other hand, contractile protein expression has been detected in inner medullary pericytes *(Park et al., 1997a)*, although its functional significance remains to be determined.

1.3.3 Outer medulla

The renal medulla is structurally divided into three sections; the outer medulla, which has two sections of its own, the outer and inner stripe, and the inner medulla *(Edwards, 1956; Smith, 1956)*.

In the outer stripe, the efferent arterioles divide into as many as thirty descending vasa recta, to resemble a 'horse tail' arrangement. At intervals these descending vasa recta branch off to form a capillary plexus, which is most dense in the inner stripe, less dense in the outer stripe and even less dense in the inner medulla *(Kriz, 1981; Jamison and Kriz, 1982; Zimmerhackl et al., 1987)*.

The most prominent characteristic of the medullary circulation is the distinct division of the vascular and interbundie regions of the inner stripe *(Pallone et al., 1990)*, which spatially separates blood perfusing different areas of the medulla. As the descending vasa recta travel further towards the papilla, the endothelium thins and becomes fenestrated *(Schwartz et al., 1976; Jamison and Kriz, 1982; Pallone et al., 1990)*. The capillaries forming the plexus are also lined with a thin fenestrated endothelium, and gradually become ascending vasa recta, which serve to remove blood from the medulla and return it to the interlobular and arcuate veins. The capillaries forming the plexus and the ascending vasa recta are morphologically indistinguishable from systemic capillaries *(Schwartz et al., 1976; Jamison and Kriz, 1982)*, except that they have larger diameters *(Steinhausen et al., 1981; Zimmerhackl et al., 1985a; Zimmerhackl et al., 1985b)*.

The inner stripe of the outer medulla remains the most constant (across species) of the medullary circulation. It is present in all species, and its complexity appears to be related to urine concentrating ability. The least complicated arrangement is found in rabbits, guinea pigs, dogs, cats, some species of monkey, and humans. This 'simple' vascular arrangement comprises solely of descending and ascending vasa recta *(Kriz, 1981)*, with most of the ascending vasa recta originating from within the bundles of the inner medulla *(Pallone et al., 1990)*, and ascending directly to the cortex.

The 'complex' vascular bundle is more common in species with greater urine concentrating ability, including rats, mice, *Meriones*, and *Psammomys obesus (Kaissling et al., 1975; Pallone et al., 1990)*. This complex arrangement incorporates the descending thin limbs of nephrons with short loops of Henle together with the descending and ascending vasa recta, such that the ratio of ascending to descending vasa recta is ~4:1 in the *Psammomys* (see *Pallone et al., 1990)*. In some species, this complex bundle becomes even more complex, through the positioning of the descending thin limbs of Henle's loop within the bundles, such as in the rat, and the incorporation of extensive invaginations of the pelvis in the *Psammomys*. The structural arrangements act to ensure optimum opportunities for countercurrent exchange between descending and ascending vasa recta *(see Pallone et al., 1990)*.

1.3.4 Inner medulla

The number of microvessels in the medulla decreases from the corticomedullary junction to the papillary tip such that the vascular cross sectional area of the medulla decreases exponentially *(Knepper et al., 1977; Kriz, 1981)*. Soon after the junction of the inner and outer medulla, the vascular bundles disappear and individual descending and ascending vasa recta become dispersed amongst the tubular structures of the inner medulla *(Lemley and Kriz, 1987)*. The proportion of medullary tissue occupied by the interstitium is now increased from ~5% in the outer medulla to almost 30% near the papillary tip *(Knepper et al., 1977)*. The inner medullary interstitial cells are arranged between these structures and it has been postulated that they act to provide structural support, and may act to inhibit the axial diffusion of solutes, preventing dissipation of corticomedullary gradients *(Kriz, 1981; Bankir and deRouffignac, 1985)*.

As in the outer medulla, ascending vasa recta outnumber descending vasa recta and have larger diameters. In *vivo* observations *(Zimmerhackl et al., 1985d)* and mass balance calculations *(Pallone et al., 1984; Zimmerhackl et al., 1985d)*, have established that the ascending vasa recta out-number the descending vasa recta by a ratio of approximately 2.3:1 in the Munich-Wistar rat, and 1.7:1 in the hamster *(Pallone et al., 1990)*.

Descending vasa recta have a non-fenestrated endothelium and are selectively permeable, but gradually develop fenestrations as they turn to become ascending vasa recta. In the inner medulla, the fenestrations cover ~50% of the surface area, but as they ascend closer to the cortex, these fenestrations decrease to cover ~15-30% of the ascending vasa recta wall. The highly fenestrated structure of the ascending vasa recta endothelium suggests a high permeability to water and small solutes, and the increase in size and number of the ascending vasa recta (relative to descending vasa recta) implies a larger surface area for transcapillary exchange and a slower blood flow rate, thereby optimizing the time available for countercurrent exchange and reabsorption. At the corticomedullary border, the ascending vasa recta of the medulla empty into the arcuate veins or into the basal parts of interlobular veins. However, in some species (with high urine concentrating ability) such as the rat, guinea pig, and especially the desert rodent *Psammomys obesus*, some of the venous medullary vessels continue to ascend within the medullary rays of the cortex and finally empty into middle or even upper parts of interlobular veins *(see Pallone et al., 1990)*.

1.3.5   Renal medullary interstitial cells

Renal medullary interstitial cells are unique to the renal medulla and are most prevalent towards the papillary tip *(Lemley and Kriz, 1991)*. They are best known for their ability to synthesize large quantities of vasodepressor lipids or lipid precursors, including PGE_2 and perhaps also medullipin I *(Muirhead et al., 1972b; Muirhead, 1991)* The number of lipid droplets varies from species to species, and amongst individuals within the same species, and from cell to cell within the same individual. They are also frequently absent from cells *(Kriz, 1981)*. It has been suggested that the number of lipid droplets is correlated with the salt and water balance of the animal, however, these data are controversial *(Mandal et al., 1974; Pitcock et al., 1982)*. The renal medullary interstitial cells typically bridge the interstitium between medullary blood vessels and thin limbs of Henle's loops, forming a ladder-like arrangement with the long axis of the cells perpendicular to the long axis of the papilla *(Pallone et al., 1990)*. This anatomic arrangement suggests a number of functions. The most frequently considered possible function of the medullary interstitial cells has been the production of the medullary prostaglandins and the storage of prostaglandin precursors *(Muirhead et al., 1972b)*. However, these cells are not the only and possibly not the most important source of renal prostaglandins, so this role remains open for speculation. Morphologically, due to their position a structural role has also been considered, particularly in the prevention of vascular collapse in situations where volume reabsorption is dependent on interstitial hydraulic pressures that exceed those within the (ascending) vasa recta lumen *(Pallone et al., 1998)*. In addition, due to their orientation it has been proposed that they may hinder axial diffusion in the medulla thereby limiting dissipation of the solute concentration gradient *(Kriz, 1981)*. With respect to this latter point, it is interesting to note that the kangaroo rat, which has the ability to produce highly concentrated urine, has the greatest abundance of papillary renal medullary interstitial cells known *(see Pallone et al., 1990)*. A further function for these cells has been postulated – contraction. Renal medullary interstitial cells have contractile elements and cytoplasmic fibrils which are anchored to adjacent blood vessels and Henle's loop *(see Hughes et al., 1995; Park et al., 1997a)*. This putative contractile function might add weight to the argument that these cells play a regulatory role in urine production, as well as regulation of other renal medullary functions.

1.4   Possible sites involved in the regulation of renal medullary blood flow

The medullary circulation is complex and there are many possible sites which could govern or contribute to the control of MBF, either actively or passively *(Pallone et al., 1990; Bergström and Evans, 2000; Pallone et al., 2000)*. These are discussed in detail below (see Figure 1.5).

Since it is accepted that MBF is delivered almost entirely from the efferent arterioles of juxtamedullary glomeruli, and that these constitute only about 10% of all glomeruli, vasoconstriction or vasodilation of either the afferent or efferent arteriole(s) of these glomeruli could theoretically produce a change in flow to the renal medulla but little or no change in total RBF *(Pallone et al., 1990)*. Furthermore, it has been suggested that 5-10% of the juxtamedullary glomeruli possess shunt pathways, which run between afferent and efferent arterioles, allowing blood supplying the renal medulla via these shunts to bypass the juxtamedullary glomeruli. Therefore MBF could theoretically be altered without any change in glomerular perfusion and filtration in deep nephrons. However these shunt pathways comprise only a small percentage of total MBF and their functional significance requires clarification *(Pallone et al., 1990)*.

Roughly 20% of juxtamedullary glomerular efferent arterioles supply only the inner cortex, and do not enter the medulla *(Chou et al., 1990)*. Differential control of blood flow to the two different types of glomeruli would alter blood distribution between the inner cortex and medulla, although this seems unlikely, because they represent such a small percentage of the overall blood flow to this region.

The outer medullary descending vasa recta contain contractile elements (smooth muscle cells), are innervated by the sympathetic nervous system, and respond to vasoactive agents *in vitro (Harrison-Bernard and Carmines, 1994; Silldorff et al., 1995)*. Contraction of these vessels will produce a change in conductance of the vessels supplying the medulla, and may provide a mechanism by which blood, when passing through the vascular network in the outer medulla, can be directed either into the inner medulla or shunted back to the inner cortex *(Pallone et al., 1990)*.

As described in previous sections of this chapter, renal medullary interstitial cells are irregular star shaped cells with long cytoplasmic projections, interposed between vasa recta and the thin limbs of the loop of Henle throughout the medulla, forming a ladder like arrangement. It is believed that these medullary interstitial cells might participate in the control of MBF by constriction of their cytoplasmic processes and thereby the interstitium and vasculature *(Park et al., 1997a; Bergström*

et al., 1998). A further possible paracrine role of these cells in control of MBF might result from local secretion of vasoactive substances, including PGE_2 and medullipin I *(Zusman and Keiser, 1977; Muirhead, 1991).*

A further hypothesized mechanism which might be involved in the regional distribution of MBF is the occurrence of passive changes in the vasculature responsible for the venous outflow from the medulla, as the proximity of tubular structures to the venous drainage of the renal medulla might alter outflow resistance in this region of the vasculature *(Pallone et al., 1990).* Renal kinins might also participate in this response, since kinins are the chief mediators of increased MBF in response to captopril treatment in rats, perhaps by reducing the vasa recta outflow resistance *(Mattson and Roman, 1991).*

Afferent arterioles of juxtamedullary glomeruli are also distinctive by virtue of having specialized structures at their origin from the interlobular artery. These, intra arteriolar 'cushions', contain a smooth muscle cell like structure which protrudes into the lumen of the afferent vessel. They are found in rat, cat, and dog, but not in rabbit guinea pig, hamster, pig, sheep, ox, or man *(Zimmerhackl et al., 1985b).* Although the function of these cushions is uncertain, their location makes them ideal candidates for the regulation of blood flow in the medulla *(Pallone et al., 1990).*

As the descending vasa recta reach further towards the renal papilla, the smooth muscle cells are gradually replaced by pericytes which form an incomplete layer around these vessels. Pericytes are considered as 'hybrid' cells as they contain microfilaments, suggesting a contractile capacity *(Hughes et al., 1995; Park et al., 1997a).*

Despite this abundance of information regarding the sites that could theoretically regulate MBF, there is surprisingly little information about the vascular sites that regulate MBF *in vivo.* In one sense this is hardly surprising, given the inaccessibility of the medullary microcirculation. One of the aims of the experiments described in this thesis, therefore, was to begin to investigate this issue. Although the sites responsible for the control of MBF *in vivo* remain to be definitively determined, there is now little doubt that circulating and locally acting hormones, and the renal sympathetic nerves, can differentially regulate CBF and MBF. The diversity of responses of CBF and MBF to these regulatory factors is discussed below.
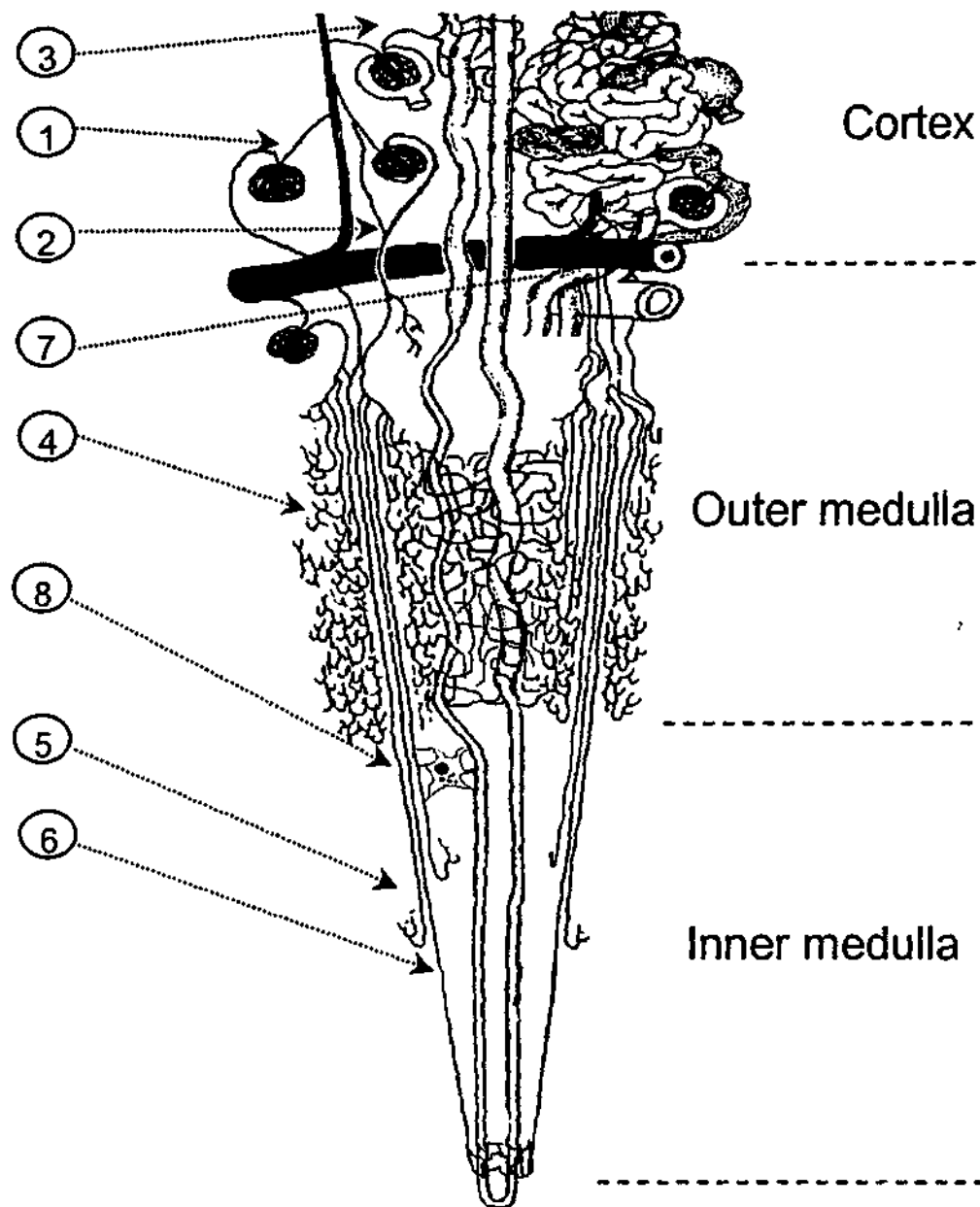
Figure 1.5   Schematic representation of the microcirculation of the renal medulla. The descending vasa recta are shown on the left side while the ascending vasa recta and venous vasculature are shown on the right. Numbered arrows represent potential sites for control of medullary blood perfusion.: (1) the afferent and efferent arterioles of juxtamedullary glomeruli; (2) shunt pathways running between afferent and efferent arterioles, some connect afferent and efferent arterioles in parallel with the glomeruli while others replace the glomerular circulation; (3) the efferent arterioles of some of the juxtamedullary glomeruli only supply the inner cortex (~20%) while others descend into the medulla; (4) the outer medullary descending vasa recta and the vascular network in the outer medulla; (5) passive components of vascular resistance in the inner medulla; (6) putative contractile elements in inner medullary vasa recta; (7) passive changes in the venous outflow resistance from the medulla; (8) renal medullary interstitial cells. Taken from *(Bergström and Evans, 2000)*.

1.5 Differential control of cortical and medullary blood flow by nerves and hormones

Intravenous infusion of vasoconstrictor agents can produce diverse effects on regional kidney blood flow in intact conscious rabbits *(Evans et al., 2000b)*. This provides support for the notion that circulating and locally acting hormones, as well as renal sympathetic innervation, can differentially regulate CBF and MBF under physiological conditions. There is accumulating evidence that the medullary microcirculation plays an important role in the long-term control or arterial pressure, via its influence on tubular sodium handling *(Cowley, 1997)* and perhaps also through its endocrine function *(Bergström and Evans, 1998)* (see above). It seems likely therefore, that hormones and nerves can influence the long-term control of blood pressure by their individual and interactive effects on the intrarenal distribution of blood flow.

1.5.1 Renal nerves

The kidney is innervated with post-ganglionic sympathetic nerve fibers, which contribute to the modulation of renal renin release, tubular function, and renal vascular resistance *(Malpas et al., 1996; DiBona and Kopp, 1997; Malpas and Evans, 1998; Pallone et al., 1998)*. However, until recently the influence of renal nerves on regional kidney blood flow, and in particular blood flow in the renal medulla, has been little understood.

Noradrenaline is the chief neurotransmitter of the sympathetic nervous system. It is stored in vesicles aggregated in sympathetic nerve varicosities, and released by a calcium-dependent process of exocytosis during depolarization of the post-ganglionic sympathetic cell membrane *(see Esler et al., 1985)*. Several lines of evidence support a physiological role for the sympathetic nervous system in the regulation of renal function *(see Bradley and Hjemdahl, 1984)*. Within the kidney, noradrenaline is released from the sympathetic nerve endings, which innervate the smooth muscle cells of the renal vasculature, the juxtaglomerular cells, and some tubular segments in the cortex and outer medulla *(Bradley and Hjemdahl, 1984; Hesse and Johns, 1985)*, and distal tubules. Neuronally released noradrenaline has profound effects on the regulation of RBF, sodium reabsorption, and renin secretion *(DiBona and Kopp, 1997)*.

In vitro, noradrenaline causes contraction of outer medullary descending vasa within a concentration range of 10^{-9}-10^{-6} M *(Edwards, 1983; Yang et al., 1995)*. Vasoconstriction of outer medullary descending vasa recta by noradrenaline may therefore reduce medullary perfusion, and thereby increase sodium and water reabsorption. In the isolated perfused rabbit (superficial

microvessels) *(Edwards, 1983)*, and hamster *(Click et al., 1979)* kidney, noradrenaline has a direct vasoconstrictor effect on the interlobular artery and the afferent and efferent arterioles. Direct microscopic observations of afferent and efferent arterioles have confirmed their ability to constrict in response to noradrenaline *(Yang et al., 1995)*.

Renal nerves are found on the afferent and efferent arterioles of juxtamedullary glomeruli, and along the descending vasa recta throughout the outer, but not inner medulla *(Chou et al., 1990)*. Physiological studies on the influence of the renal innervation on MBF have been scant and sometimes contradictory, but it seems that MBF may be reduced in response to increases in nerve activity *(Rudenstam et al., 1995)*.

Recent studies by our group, utilizing electrical stimulation of the renal sympathetic nerves and simultaneous recordings of RBF, CBF, and MBF in anesthetized rabbits, suggest that the renal nerves differently influence CBF and MBF *(Leonard et al., 2000)*. MBF appears to be less sensitive than CBF to a mean increase in renal sympathetic nerve activity, but is capable of responding to higher frequencies of stimulation *(Leonard et al., 2000; Navakatikyan et al., 2000)*. The relative insensitivity of the medullary microcirculation to renal sympathetic nerve activity also appears to extend to reflex increases in sympathetic drive, since chemoreceptor stimulation (hypoxia or chemical stimulation) can reduce CBF but not MBF in anaesthetized rabbits *(Leonard et al., 2001)*, and conscious rats *(Ledderhos et al., 1998)*. Collectively, these observations are largely consistent with those of Rudenstam et al., who found a biphasic dependence on the frequency of stimulation (in rats), such that papillary perfusion is increased at 2 Hz but decreased during stimulation at 5 Hz *(Rudenstam et al., 1995)*. On the other hand they do differ from some earlier studies using methods other than laser-Doppler flowmetry, for example, Hermansson and colleagues found renal denervation to produce increases in MBF *(Hermansson et al., 1984)*.

The physiological mechanisms underlying a differential sensitivity of CBF and MBF to renal sympathetic nerve activity still requires further investigation. Regional differences in innervation density, the post-junctional responsiveness to noradrenaline or in the amount of vascular smooth muscle *(Kriz, 1981; Pallone et al., 1998)* may contribute *(see Leonard et al., 2000)*. There is both structural and functional evidence that the renal innervation could differentially regulate regional kidney perfusion by actions on the juxtaglomerular vasculature. Barajas and colleagues *(Barajas et al., 1984)* reported that, in rats, afferent and efferent arterioles are endowed with dense adrenergic innervation. McKenna and Angelakos provided similar observations in the dog and specifically pointed out the innervation of vasa recta in the outer medulla *(McKenna and Angelakos, 1968)*.

Studies of the regional responses of afferent and efferent arterioles to renal nerve stimulation were performed by Chen and Fleming *(Chen and Fleming, 1993)* in the hydronephrotic kidney. They found that the afferent arterioles of juxtamedullary glomeruli were less responsive to renal nerve stimulation than their outer cortical counterparts. Furthermore, juxtamedullary afferent and efferent arterioles have greater diameters than their outer and mid cortical counterparts *(see Pallone et al., 1990)*, and according to Poiseuille's relationship an equivalent level of smooth muscle fiber shortening, will produce greater changes in vascular resistance in the smaller vessels outside the juxtamedullary region. Thus, differences in responsiveness to nerve stimulation per se, as well as differences in vessel geometry, probably contribute to the lesser sensitivity of MBF.

There is now good evidence that increased sympathetic nerve activity, particularly that directed at the kidney, plays a role in the pathogenesis of hypertension. Elevated renal sympathetic nerve activity manifested as increased renal vascular resistance and decreased RBF has been demonstrated in human essential hypertension *(Plato and Osborne, 1996)*. Greater increases in RBF are observed in response to intrarenal arterial administration of phentolamine (α- antagonist) in human essential hypertensive patients than in normotensive control subjects *(Hollenberg et al., 1975)*. Also, SHR exhibit greater renal sympathetic nerve activity than age matched normotensive WKY rats as evidenced by both whole nerve and single fiber recordings *(Judy et al., 1976; Judy and Farell, 1979)*. The onset of hypertension in these models may be delayed or blunted by renal denervation *(Liard, 1977; Norman and Dzielak, 1982; Plato and Osborne, 1996)*.

In further support of the importance of renal sympathetic drive in the long-term control of arterial pressure intrarenal noradrenaline infusions in chronically instrumented dogs *(Katholi et al., 1977; Cowley and Lohmeier, 1979; Reinhardt et al., 1995)* and rats *(Kleinjans et al., 1984; Smits et al., 1987)* caused significant reductions in RBF and increases in plasma renin activity, and resulted in sustained hypertension. Additionally, similar doses of noradrenaline administered intravenously were not capable of producing chronic arterial hypertension, which also appears to be the case in humans *(see Katholi et al., 1977)*.

1.5.2 *Arginine vasopressin*

In the kidney, receptors for arginine vasopressin (V_{1a} and V_2 subtypes) are located predominantly in the medulla *(Ostrowlski et al., 1992)*. The V_{1a} receptor subtype is primarily associated with vascular structures in the inner stripe of the outer medulla *(Ostrowlski et al., 1992)* and mediates

vasoconstriction through activation of phospholipase C *(Howl and Wheatley, 1995)*, while the V_2 receptor mostly exists on tubular structures and stimulates recruitment of aquaporin molecules into the cell membrane, by stimulation of adenylate cyclase *(Howl and Wheatley, 1995; Park et al., 1997b)*.

V_1 receptor activation in the kidney produces a variety of responses, including selective reduction in MBF, and diuresis and diuresis/natriuresis *(Franchini et al., 1997; Evans et al., 1998a)*. For example, in anesthetized rats and rabbits, acute renal medullary interstitial or intravenous infusion of the vasopressin V_1-agonist, [Phe^2, Ile^3, Orn^8]-vasopressin, selectively reduces MBF and paradoxically increases urine flow and sodium excretion *(Nakanishi et al., 1995b; Ledderhos et al., 1995; Evans et al., 1998a)*. It may also act to inhibit the release of the putative renal medullary depressor hormone in response to increased RAP *(Bergström and Evans, 1998)*. Chronic infusion of [Phe^2, Ile^3, Orn^8]-vasopressin either intravenously *(Cowley et al., 1994)* or directly into the renal medulla *(Szczepanska-Sadowska et al., 1994)* causes sustained hypertension, in normotensive animals, which may be reversed by renal medullary administration of a V_1 antagonist [Cowley, 1994 #403; *(Szczepanska-Sadowska et al., 1994)*. Also, chronic antagonism of arginine vasopressin receptors attenuates the development of hypertension in SHR *(Feng and Arendshorst, 1996)*.

Physiological concentrations of arginine vasopressin also decrease MBF without changing the much larger cortical flow *(Zimmerhackl et al., 1985c; Kiberd et al., 1987a; Nakanishi et al., 1995b)*. This action seems to be chiefly mediated through activation of the V_1-receptor, although there have been reports that V_2-receptor activation can produce both increases and decreases in MBF *(Nakanishi et al., 1995b; Kiberd et al., 1987a)*. *In vitro* studies of isolated perfused vessel preparations have found that the afferent arterioles of juxtamedullary glomeruli are most sensitive to the vasoconstrictor effects of arginine vasopressin compared with the other vessel segments studied *(Turner and Pallone, 1997; Harrison-Bernard and Carmines, 1994)*. On the other hand, arginine vasopressin does constrict both outer medullary descending vasa recta as well as upstream pre- and postglomerular arterioles *(; Harrison-Bernard and Carmines, 1994; Navar et al., 1996; Turner and Pallone, 1997)*

1.5.3 Endothelin

As the level of renal MBF appears to be an important determinant of long-term arterial pressure, the relative insensitivity of the medullary microcirculation to the vasoconstrictive effects of endothelin-1 may have important implications for the influence of endothelins on the long term control of

arterial pressure. Endothelin-1 is the most potent vasoconstrictor known to exist, and has been implicated in the pathogenesis of kidney disease and animal models of hypertension *(Fujita et al., 1995a; Fujita et al., 1995b; Hocher et al., 1996)*. Small increases in the circulating concentration of endothelin-1 produces hypertension in rats, dogs, and sheep *(Mortensen and Fink, 1992; May et al., 1993; Wilkins et al., 1993)*. Similarly, endothelin blockade lowers arterial pressure in normotensive human subjects *(Haynes et al., 1996)* and rabbits *(Evans et al., 2000c)*.

Within the kidney endothelin receptors have been identified on collecting ducts, vascular bundles and renal medullary interstitial cells *(Terada et al., 1992a)* and endothelin constricts afferent and efferent arterioles, arcuate and interlobular arteries *(Navar et al., 1996; Pallone et al., 1998)* and outer medullary descending vasa recta *(Silldorff et al., 1995)*.

In contrast to these *in vitro* observations, endothelin-1 appears preferentially to reduce blood flow in the renal cortex, with little or no effect on MBF, in anaesthetized rats and dogs, and rabbits *(see Evans et al., 1998c; Evans et al., 2000b; Evans et al., 2000c;)*. Bolus injection of endothelin-1 (a mixed endothelin ET_A and ET_B receptor agonist) have been shown to selectively reduce CBF while transiently increasing MBF, and these effects were inhibited by blocking ET_A and ET_B receptors respectively *(Gurbanov et al., 1996)*. These findings are consistent with the hypothesis that endothelins do not constrict vascular elements controlling MBF when delivered to the vessel lumen *in vivo*. However, the results of *in vitro* studies *(Silldorff et al., 1995)*, and those in the hydronephrotic kidney *(Endlich et al., 1996)*, suggest that endothelin-1 may produce vasoconstrictor effects on outer medullary descending vasa recta when applied to the adventitia.

1.5.4 Angiotensin II

Despite the fact that a large number of studies have investigated the impact of angiotensin II on regional RBF, its effects on MBF remain unclear. A high density of angiotensin II receptors have been located on the inner stripe of the outer medulla in association with vasa recta bundles, suggesting that angiotensin II could have a direct effect on vasa recta function.

In vitro, angiotensin II constricts outer medullary descending vasa recta *(Pallone, 1994)*. Consistent with this, renal arterial infusion of low doses of angiotensin II reduces PBF in dogs *(Chou et al., 1990)*. However, the effect of angiotensin II on the medullary circulation may be dependent on the dose and the species to which it is administered. For example, studies in rats and rabbits have shown that exogenous angiotensin II either does not alter MBF *(Parekh et al., 1996; Zou, 1996;*

Evans et al., 2000b; Parekh and), or can increase MBF *(Nobes et al., 1991; Evans et al., 1998a; Evans et al., 2000a).*

However, despite the controversy regarding the effects of angiotensin II on the medullary circulation resulting from its administration, pharmacological blockade of angiotensin II formation by inhibition of angiotensin-converting enzyme has generally resulted in an increase in MBF, independent of effects on CBF *(Roman and Kaldunski, 1988a)*, and changes to vasa recta diameter *(Cupples et al., 1988; Navar et al., 1996).* Under most experimental conditions this effect appears to be largely due to increased bradykinin levels, although there is some experimental support for a role of reduced angiotensin II formation, particularly in salt-replete dogs *(Mattson and Roman, 1991; Omoro et al., 1999; Omoro et al., 2000).*

1.5.5 *Atrial natriuretic peptide*

Atrial natriuretic factor is released from the heart in response to volume expansion, and is believed to play a role in sodium and water homeostasis *(Cowley, 1992).* Atrial natriuretic peptide increases PBF in rats *(see Chou et al., 1990; Pallone et al., 1990)* and enhances the diuresis and diuresis/natriuresis associated with elevations in RAP, suggesting a possible role in blood pressure control mediated by alterations in medullary haemodynamics *(Pallone et al., 1990).* However, although much research has been directed towards the mechanism by which atrial natriuretic peptide produces these effects it remains unknown whether the increased MBF occurs as an indirect effect secondary to increased volume reabsorption in the collection duct, or a direct effect mediated by vasodilation of the renal microvasculature *(see Roman et al., 1991; Bergström and Evans, 2000).* Much of the evidence suggests that both mechanisms operate, and this notion is supported by the observation that atrial natriuretic peptide vasodilates resistance vessels in the juxtamedullary circulation *(Pallone et al., 1990).*

1.5.6 *Prostaglandins*

Prostaglandins have been implicated in the control of both CBF and MBF *(Pallone et al., 1990).* They are synthesized by renal medullary interstitial cells and by medullary collecting duct cells *(Zimmerhackl et al., 1985b).* PGE_2 and PGI_2 are vasodilators, and appear to sustain MBF by counteracting the effects of other vasoactive agents. Prostaglandin synthesis inhibitors consistently decrease MBF in anaesthetized animals *(Chou et al., 1990; Roman and Lianos, 1990; Roman et al., 1991; Parekh et al., 1996).* The mechanism of their actions on MBF remains to be determined,

however it is believed that prostaglandins selectively dilate the vasculature in the juxtamedullary region. This notion is supported by the observation of redistribution of CBF toward the inner cortex and juxtamedullary nephrons during stimulation of prostaglandin production, while during inhibition of prostaglandin production, blood flow is directed towards the superficial cortex *(Itskovitz et al., 1973; Larsson and Anggard, 1974; Lemley et al., 1984)*. There is little information regarding the influences of specific prostaglandins on MBF (i.e. PGE_2, PGI_2, $PGF_{2\alpha}$).

1.5.7 Kinins

Kinins are formed from circulating kininogen by the enzyme kallikrein, located in the cells of the distal and collecting tubules. Bradykinin is a vasodilator that appears to increase MBF. Within the kidney, bradykinin B_2 receptors are found on tubular structures and on the renal medullary interstitial cells (not the vasculature), and are most concentrated in the outer stripe *(Manning and Snyder, 1989)*. B_2-kinin receptor antagonists decrease PBF without affecting CBF or GFR, and further studies using these antagonists have demonstrated a role for endogenous bradykinin in increasing PBF during volume expansion *(Fenoy and Roman, 1992)*. Increasing intrarenal kinin levels, either by inhibition of kinin breakdown or intrarenal infusion of bradykinin, selectively increases inner CBF and PBF *(Bailie and Barbour, 1975)*, and this effect can be blocked by inhibition of nitric oxide synthesis with L-NAME *(Mattson and Cowley, 1993)*. Additionally, sodium excretion is reduced by B_2-receptor antagonism during volume expansion, indicating a role for endogenous kinins in baseline PBF and the natriuretic response to volume expansion *(see Navar et al., 1996)*.

Bradykinin also appears to play a role in the increased MBF following inhibition of angiotensin-converting enzyme *(Omoro et al., 2000)*. The site at which endogenous bradykinin acts to increase inner MBF remains to be determined. As well as an involvement with the pressure diuresis/natriuresis mechanism, there is evidence of interactions between the kinin and angiotensin systems in the control of MBF. In the isolated perfused rat kidney, angiotensin II increases bradykinin levels, which in turn attenuates the vasoconstrictor effects of angiotensin II *(Gardes et al., 1990)*. Thus there is compelling evidence that renal kinins may influence the distribution of RBF and the excretion water and electrolytes through paracrine mechanisms.

1.5.8 Adenosine

Intra-renal infusion of adenosine in dogs produces an initial decrease followed by an increase in RBF, which (during the latter response) produces a preferential increase in blood flow to the inner cortex *(Spielman et al., 1980; Hall et al., 1985)*. The effect of adenosine on MBF has only recently been examined. High doses (15 µg/min) of adenosine administered to the renal artery of rats almost doubled blood flow to the ascending and descending vasa recta. However, lower doses (2-15 µg/min) produced a marked diuresis and diuresis/natriuresis, but no effect on vasa recta blood flow *(Miyamoto et al., 1988)*. *In vitro*, low concentrations (1 pM- 10 nM) of adenosine produce vasoconstriction, presumably via activation of A_1-receptors, while A_2-receptor activation produced by higher concentrations of adenosine (>100 nM) causes vasodilation *(Silldorff et al., 1996)* of outer medullary vasa recta. These results led to the notion that A_1-receptors mediate reduced MBF, while A_2-receptors mediate increased MBF. More recent studies using laser-Doppler flowmetry have confirmed this hypothesis *(Agmon et al., 1993)*, and demonstrated a potential role of adenosine acting at A_2- receptors in the physiological regulation of MBF *(Zou et al., 1999)*.

1.5.9 Nitric oxide

Evidence now exists in support of an important role for nitric oxide in the control and modulation of renal MBF and in the regulation of arterial pressure. All isoforms of nitric oxide synthase have been detected in the renal inner and outer medulla *(Mattson and Higgins, 1996)*. The first indication that endothelial cells of vasa recta were capable of producing nitric oxide was provided by Biondi et al., who demonstrated that cyclic guanosine monophosphate (cGMP) production in both renal cortical and medullary slices increases in response to endothelium dependent (i.e. acetylcholine, bradykinin) vasodilators, and that the highest cGMP levels are found in the inner and middle portions of the inner medulla *(Biondi et al., 1990)*. Nitric oxide synthase III mRNA has been localized in the inner medullary collecting duct and glomeruli with smaller signals in the inner medullary thin limb, vasa recta, and arcuate artery *(Terada et al., 1992b)*. These data suggest that the renal medulla is a major site of constitutive synthesis of nitric oxide in the kidney.

Experimental evidence from both *in vivo* and *in vitro* studies indicate that the renal medullary microcirculation is more dependent on nitric oxide than the cortical circulation in terms of resting nitrergic vasodilator tone *(Bergström et al., 1996; Navar et al., 1996)*. *In vitro*, isolated descending vasa recta from the inner stripe of the outer medulla contract when superfused with

nitric oxide inhibitors *(Yang et al., 1995)*. The effects of blockade of nitric oxide synthase on renal MBF have been documented in a number of studies, with reduced MBF being observed after both acute and chronic administration. Systemic blockade of nitric oxide synthesis reduces both CBF and MBF in anaesthetized rabbits *(Bergström and Evans, 1998)* and rats *(Parekh et al., 1996)*. On the other hand, Lockhart et al. found that systemic inhibition of nitric oxide synthase with L-NMMA in volume expanded rats increased blood pressure and selectively decreased MBF without changing CBF *(Lockhart et al., 1994)*. Furthermore, intravenous infusion of N^G-nitro-L-argnine methyl ester in conscious rats reduces MBF but not CBF *(Nakanishi et al., 1995a)*. Long term administration of nitric oxide synthase inhibitors produces sustained hypertension *(Mattson et al., 1992; Mattson et al., 1994)* that appears to be related to a shift of the renal pressure diuresis/natriuresis relationship to higher levels of arterial pressure *(Majid et al., 1993; Cowley et al., 1995; Evans et al., 1995)*. In support of this finding, acute inhibition of nitric oxide synthase is associated with a fall in RBF *(Baylis et al., 1993; Majid et al., 1993)*, and GFR *(Baylis et al., 1993)*, and increases in both pre- and post-glomerular vascular resistance *(Salom et al., 1992)*. Collectively, these data suggest that nitric oxide plays an important role in regulating MBF.

1.6 Summary and aims of the studies undertaken in this thesis

The renal medulla appears to play an important role in the long-term control of arterial pressure *(Cowley et al., 1995)*. The precise mechanism(s) involved, remain uncertain, however considerable evidence supports the notion that the poor autoregulatory capacity of the renal medulla plays a key role in mediating pressure diuresis/natriuresis *(Cowley et al., 1995)*. It is also possible that the relationship between RAP and MBF plays a key role in mediating the release of the putative renal medullary depressor hormone *(Bergström and Evans, 1998)*. On the other hand, definitive evidence for the existence of this hormone is still lacking. If the level of MBF plays important roles in regulating these renal antihypertensive mechanisms, circulating and locally acting hormones, and the renal nerves, might influence long-term control of arterial pressure through their effects on MBF. There is certainly good evidence for differential control of CBF and MBF by nerves and hormones, although little definitive information is available about the vascular sites that regulate MBF *in vivo*. The main focus of the experiments performed in this thesis has therefore been to determine the role of the renal medullary circulation in the renal antihypertensive responses to increased RAP, and to obtain more information of the vascular sites responsible for the regulation of MBF.

The specific aims of each experimental study were;

- To develop a method for the local delivery of vasoactive compounds (noradrenaline) to the renal medulla, and specifically, to determine the regional distribution of infused radiolabelled noradrenaline through the utilization of autoradiographic techniques (Chapter 3).

- To investigate the effect of reducing MBF on the renal antihypertensive response to increased RAP. This was addressed using an extracorporeal circuit in anaesthetized rabbits. We tested the effects of reduced MBF (medullary interstitial noradrenaline) and CBF (intravenous noradrenaline) on the pressure diuresis/natriuresis response, the inhibition of plasma renin activity, and the depressor response to increased RAP (Chapter 4).

- To determine the role of the pressure diuresis/natriuresis mechanism and inhibition of renal renin release in the depressor response to increased RAP (Chapter 5).

- To determine *in vivo*, the roles of juxtamedullary afferent and efferent arterioles in mediating reduced MBF in response to activation of vasopressin V_1- receptors.

Chapter Two

GENERAL METHODS

2.0 Introduction

In this chapter the methods employed in the experiments conducted in this thesis are described in general terms. Experimental protocols, and methods unique to particular studies, are described in the experimental chapters themselves (Chapters 3-6).

Studies investigating the regional renal distribution of [^{3}H]-noradrenaline, following renal medullary interstitial infusions administered via either acute or chronically positioned catheters in anaesthetised rabbits are described and discussed in Chapter 3. The common methods employed in these experiments are outlined within Sections 2.2.1, 2.2.3, 2.3.2, and 2.6, of this chapter.

Studies investigating the renal antihypertensive responses to increased RAP are described in Chapters 4 and 5, and the methods common to these experiments are described below Sections 2.2.1 and 2.2.3. Section 2.2.6 is specific to protocols in Chapter 4, and 2.3.3 to Chapter 5.

Studies investigating the role of the afferent and efferent glomerular arterioles in the control of regional blood flow are described in Chapter 6. The general methods employed in these studies are outlined in Sections 2.2.2, 2.2.5, 2.2.6, 2.4, and 2.5 below.

2.1 Rabbits: breeds and housing

All experiments were performed on rabbits bred specifically for experimental purposes (Monash University Central Animal Services, Gippsland and Baker Medical Research Institute, Prahran). Prior to experimentation, rabbits were housed individually in special purpose built cages, where they had visual but not physical contact with rabbits in adjoining cages. Water was made available

ad libitum. Rabbits were either meal fed (100 g of pellets, Lucerne chaff and oat chall in a 4:1:1.2 mixture; Chapters 5 and 6), or food was available *ad libitum* (Chapters 3 and 4). All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Department of Physiology/Central Animal Services Animal Ethics Committee. At the conclusion of the experiment, rabbits were killed with an intravenous overdose of pentobarbitone sodium (300 mg).

2.2 Surgical procedures for acute non-recovery experiments

2.2.1 General surgical preparation for acute studies in anaesthetised rabbits

Catheters were placed in both central ear arteries and marginal ear veins under local anaesthesia (1.0% lignocaine; Xylocaine; Astra Pharmaceuticals, North Ryde, NSW, Australia). Rabbits were then anaesthetised with pentobarbitone sodium (90-150 mg i.v. Nembutal; Boehringer Ingelheim, Artarmon, NSW, Australia) which was immediately followed by endotracheal intubation and artificial ventilation (Model 55-3438 Respirator, Harvard Instruments; MA, USA). Throughout surgery and subsequent experimentation, anaesthesia was maintained by intravenous pentobarbitone infusion (30-50 mg/hr). To replace lost fluids, and maintain plasma volume during surgery rabbits received an intravenous infusion of Hartmann's solution (compound sodium lactate; Baxter Healthcare Pty. Ltd., Toonagabbie, NSW, Australia) at a rate of 0.18 ml/kg/min. Surgery was performed on a heated table (Baker Medical Research Institute, Model 165) and oesophageal temperature was maintained between 36 and 38 °C throughout the experiments using a servo-controlled infrared lamp (Digi-Sense temperature Controller; Cole Palmer Instrument Company, Chicago, IL, USA).

2.2.1.1 Renal preparation

Renal denervation (Chapter 3) or nephrectomy (Chapters 4, and 5) of the right kidney occurred via a right retroperitoneal incision. Renal denervation was performed by manually stripping all visible nerves from the renal artery and vein, and painting the area with 10% w/v phenol (C_6H_5O; Sigma Chemical Co. St Louis, USA) in ethanol. To perform a right nephrectomy the renal artery and vein were cleared and tied in two places along their length away from the kidney with 3-O silk suture (Dynek. Pty. Ltd. Sydney, Australia), the vessels were then cut between the ties, and the kidney removed. The right retroperitoneal wound was also closed with 3-O silk suture (non-sterile, Dynek Pty. Ltd.).

The left kidney was exposed and denervated (as above) via a left flank retroperitoneal incision and the rabbit was placed and supported in an upright crouching position. Ureters were cannulated for urine collection (Silastic tubing, 0.5 mm ID, 0.95 mm OD; Dow Corning Co. Midland, Michigan, USA). For experiments using laser-Doppler flowmetry (Section 2.2.6) the left kidney was freed from the peritoneal lining and surrounding fat and placed dorsal side up in a stable cup (cushioned by gauze soaked in Hartmann's solution).

2.2.2 Transit-time ultrasound flowmetry for renal blood flow

The transonic ultrasound flowprobe uses large crystals which generate ultrasonic waves so that the transit time of these waves between upstream and downstream signals can be calculated. Due to its availability in many sizes, its main advantage is that it may be used to measure blood flow irrespective of the vessel size. Absolute flow values can be obtained and several probes can be placed in the same animal, both in acute and chronic studies *(Welch et al., 1995; Evans et al., 1997)*. The technical limits, are the set sizes of the probes and their cost.

In experiments other than those involving the extracorporeal circuit, a transit time ultrasound flow probe (type 2SB, Transonic Systems, Incorporated, Ithaca, NY, USA) was placed around the renal artery, and coupled acoustically with Nalco absorbent gel (Nalco Chemical Company, Maperville, IL, USA). For measurements of RBF in extracorporeal circuit experiments (Chapters 4 and 5), an in-line flow probe (type 4N, Tansonic Systems) was incorporated into the renal limb of the circuit.

2.2.3 Construction and implantation of acutely positioned medullary interstitial catheters

Catheters constructed from two 30 gauge needles, were positioned 2 cm apart on the midline aspect of the kidney, on either side of the medullary laser-Doppler flow probe (Section 2.2.6). Their tips were advanced 8.5 mm below the cortical surface, so that they lay in the outer medullary interstitium or 10.5 mm below the cortical surface for inner medullary interstitial infusion. To maintain catheter patency, sodium chloride solution (154 mM; 10 μl/kg/min) was infused via each catheter until the experimental procedures commenced.

2.2.4 General preparation and establishment procedures for the extracorporeal circuit

A right nephrectomy was performed to remove the confounding influence of the right kidney, which would otherwise be perfused at systemic arterial pressure (Section 2.2.1.1). The rabbit was positioned in an upright crouching position and a left flank incision was made for exposure of the abdominal aorta, vena cava and renal artery. These vessels were then cleared for cannulation and establishment of the circuit. The renal artery was denervated (as above; Section 2.2.1.1), except phenol was not used, as this was found to vasoconstrict the renal artery and so impede its cannulation. The left ureter was cannulated (silastic catheter., 0.5 mm ID, 0.95 mm OD), and the rabbit received an intravenous bolus dose of heparin (15,000 IU in 3.0 ml; Monoparin, Fisons Pharmaceuticals, NSW, Australia) prior to the circuit being established.

The circuit dead space (24 ml) was filled with 10% (wt/vol) dextran 40 in 154 mM NaCl (Gentran 40, Baxter Healthcare, NSW, Australia) containing 50 IU/ml heparin (Monoparin, Fisons Pharmaceuticals, NSW, Australia). First the aorta was cannulated, distal to the branching of the renal artery (2.60 mm ID, 3.00 mm OD), followed by the vena cava (1.58 mm ID, 2.16 mm OD). A roller pump (Masterflex, model 7521-45, Barnant Co., Barrington, IL. USA) was used to circulate blood through the circuit. The circuit was initially set at to withdraw blood from the distal aorta and returned to the vena cava at a rate of 70 ml/min. The renal artery was then cannulated (0.80 mm ID, 1.60 mm OD), and the pump rate increased to 90-110 ml/min and blood was now also returned to the renal artery. Averaged across all experiments, renal ischemic time was 4.6 ± 0.17 min. Blood flow to the renal artery was controlled by a starling resistor on the venous return limb and a windkessel was used on the renal artery limb to dampen the pulse pressure. RAP was measured directly via a side arm catheter proximal to the cannula inserted into the renal artery (PE 10, 0.28 mm ID, 0.61 mm OD) (Figure 2.1).

Once the circuit was established, RAP was set at ~ 65 mmHg for a 60 –90 minute equilibration period. At the beginning of this period, rabbits were given a bolus dose of [^{3}H]-inulin (4 µCi. NEN Research Products, Boston, MA, USA), administered in 1.0 ml of 154 mmol/L NaCl. An infusion of 10% (vol/vol) polygeline (Haemaccel, Hoechst; Melbourne, Australia) containing 200 IU/ml sodium heparin and 0.3 µCi/ml [^{3}H]-inulin was then initiated (replacing the maintenance infusion of Hartmann's solution). This infusion (0.18 ml/kg/min) continued throughout the duration of the experiment.
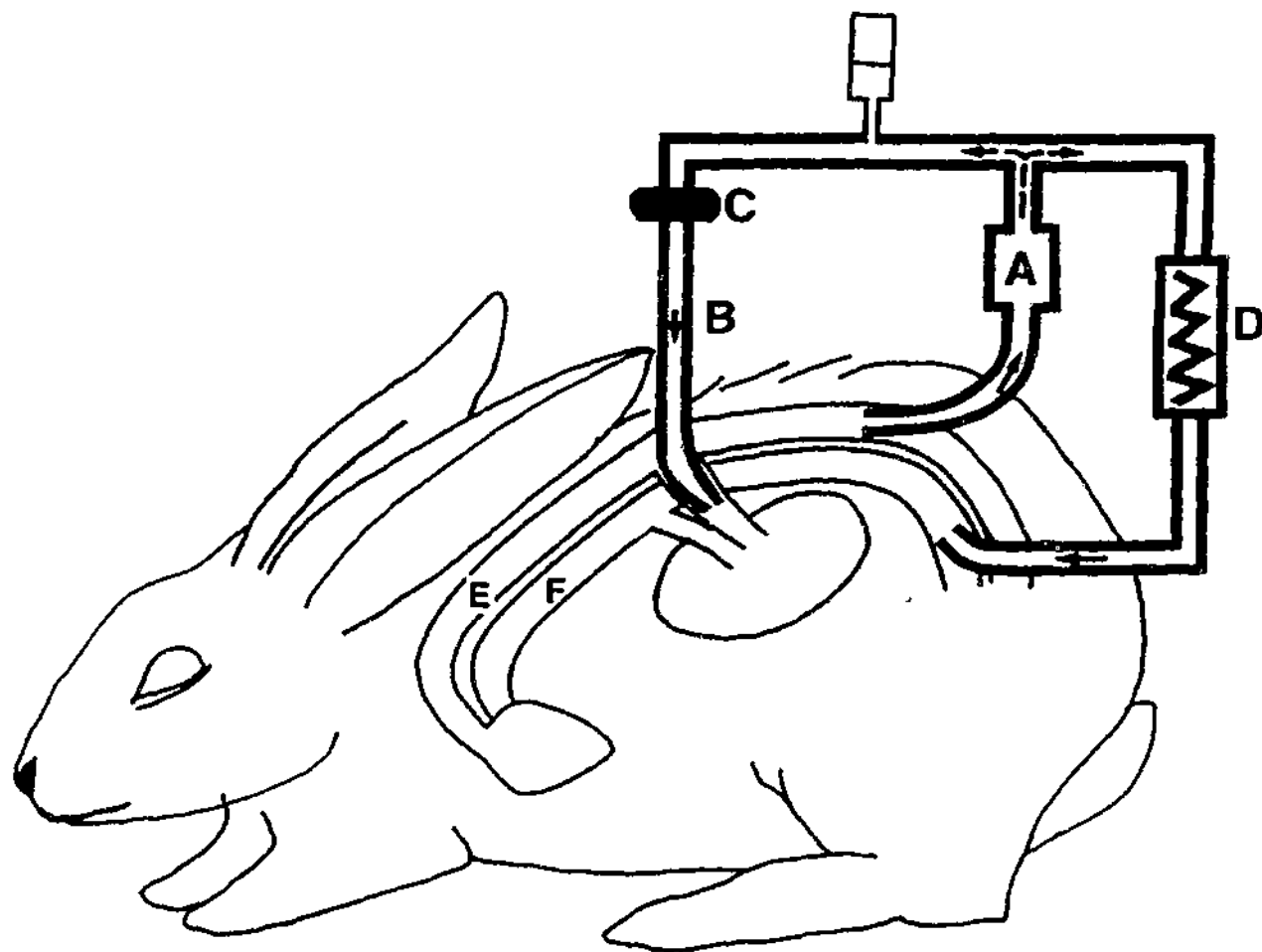
Figure 2.1   Diagram of the extracorporeal circuit used to perfuse the left kidney. Blood is drawn from the distal abdominal aorta and returned to the rabbit via the renal artery or inferior vena cava. (A), Pump draws blood from aorta (E) at constant rate. (B), Renal perfusion pressure, and (C), RBF can be set at any level by adjusting resistance of the Starling resistor (D) on the venous return limb. Systemic arterial pressure is measured via rear artery catheter. (F), vena cava. Taken from *Anderson et al., 1995.*

2.2.5   *Preparation for renal fixation*

Rabbits were prepared for surgery as outlined above. A left flank incision was made and the left kidney, aorta (superior and inferior), vena cava, and left ureter were exposed. The left and right ureters were cannulated with silastic tubing (OD 0.037 in, ID 0.02 in; Dow Corning, Midland, MI, USA). The left kidney was denervated by manually stripping all visible nerves, and placed in a stable cup for the positioning of laser-Doppler flow probes so that MBF and CBF could be measured. A transit-time ultrasonic flow probe (2SB, Transonic Systems, Ithaca, NY, USA) was

placed around the left renal artery for RBF measurements. A heparin bolus (5,000 IU i.v., Fisons Thornleigh, NSW, Australia) was administered, and an infusion of 30 IU/min was commenced. A large bore cannula (3 mm, OD; 2 mm, ID) was placed in the aorta distal to the renal arteries, which was later connected to a perfusion apparatus. The abdominal aorta was also exposed rostral to the renal arteries, and a ligature was placed around it so that it was readily occluded during the fixation process.

2.2.6 Laser-Doppler flowmetry

The laser-Doppler technique was first used to measure regional kidney blood flow in rats by Stern et al. (1977), and the technique has since been extensively evaluated *(Stern et al., 1977; Stern et al., 1979; Hansell, 1992; Roman and Smits, 1986; Takezawa et al., 1987; Hansell et al., 1990; Fenoy and Roman, 1991)*. For example Roman and Smits demonstrated a linear relationship between the laser-Doppler flow signal obtained from the renal cortex of rats with whole kidney blood flow, and a linear relationship ($r = 0.92$) between the laser-Doppler blood flow signal from the papilla of rats with papillary blood flow rates determined by the accumulation of ^{51}Cr-labeled red blood cells *(Roman and Smits, 1986; Smits et al., 1986)*.

The laser-Doppler flowmeter emits a beam of monochromatic light, which travels through a fibre optic probe, to illuminate the tissue under study ($\sim$1mm^3). The laser beam is scattered by reflective components within the tissue, and a portion of the light is reflected back via the probe's receiving fibre, and received by a photo detector inside the flowmeter. Two different signals are recorded; one is proportional to the velocity of moving particles in the measured volume and the second signal is the reflected amplitude, which is proportional to the amount of moving particles in the measured volume. These signals together produce a laser-Doppler flow signal, which is represented as a flux value. Although the technique does not enable measurement of absolute flow, it provides a continuous measurement and relative index of tissue perfusion within various regions of the kidney *(Bonner et al., 1981; Vongsavan and Matthews, 1993)*.

The technique also provides a means with which to study blood flow simultaneously, in multiple regions in both conscious and anaesthetised preparations. The laser-Doppler flowprobes are relatively non-invasive, and produce little tissue damage *(Evans et al., 2000)*. Histological damage is confined to within 200 µm of the fibre track, and produces no disruption to the microcirculatory region beyond the fibre tip where flow is determined *(Hansell, 1992)*. The technique also allows for

inter animal and between group comparisons of tissue red blood cell flows *(Roman et al., 1991; Cowley, 1997)*.

However, laser-Doppler flowmetery does have some limitations. For example, it cannot calibrated against other methods, is unable to distinguish the direction of flow (i.e. between ascending and descending vasa recta in the renal medulla), or determine whether a change in flow has occurred due to capillary recruitment *(Roman et al., 1991)*. Despite these concerns, agreement between laser-Doppler and videomicroscopy *(Fenoy and Roman, 1991)* and ^{51}Cr-labeled red blood cell accumulation *(Roman and Smits, 1986)* has been demonstrated.

In the experiments described in Chapter 3, three laser-Doppler flow probes were used to simultaneously monitor cortical and medullary perfusion. A 19gauge needle (Thermo Medical Co. Elkton, MD, USA) was used to pierce the renal capsule (midline aspect) for the insertion of a single fibre laser-Doppler flow probe (0.5 mm diameter; University of Linköping, Sweden) 10 mm below the cortical surface, using a micromanipulator (Narishige, Japan). Two cortical laser-Doppler (single fibre) flow probes were also positioned 0.5 mm below the cortical surface, on opposite sides of the dorsal aspect of the kidney. Insertion of laser-Doppler flow probes resulted in minimal bleeding, which stopped within a few minutes. Blood flow is measured in front of each laser-Doppler flow probe (approximately 1 mm^3), away from any tissue damage *(Lu et al., 1993)*. On completion of experimental preparation, the rabbits wounds were covered with gauze soaked in Hartmann's solution, which was then covered with silicone gel (Wacker Chemie, Munich, Germany; 10 parts RTV-E 604A, 1 part RTV-E 604B, 1 part KATLY.DL) to minimize fluid loss during both the equilibration and experimental periods.

Alternatively, in experiments described in Chapters 4 and 6, a 26 gauge needle type probe (DP4s, Moor Instruments Ltd., Millwey, Devon, England) was advanced 10 mm below the mid-region of the lateral surface of the kidney, so that it lay in the outer medulla using a micromanipulator (Narashige, Tokyo, Japan). A laser-Doppler flow probe (Standard plastic straight probe (DP2b), Moor Instruments Ltd.) was placed on the dorsal surface of the kidney, for measurement of superficial MBF. The wounds of these rabbits were covered in gauze soaked in Hartmann's solution and coated with a layer of melted agar which cooled to form a seal over the wound and helped to minimize fluid loss during experimental procedures.

2.3 Recovery surgery

2.3.1 General

Prior to any incisions being made, rabbits were shaved in the operative area, and the region was washed with an aqueous antiseptic solution (0.05% w/v chlorohexidine acetate 0.5% w/v; Centrimide; Baxter Healthcare, NSW, Australia). Rabbits subjected to medullary interstitial infusion of noradrenaline (Chapter 3) and those in which CO was to be measured (Chapter 5), underwent preliminary surgery 7-21 days prior to acute experimentation. A marginal ear vein was catheterized (Jelco, 24 gauge, Johnson & Johnson Medical, NSW, Australia), and anaesthesia was induced with propofol (10 mg/kg Diprivan; ICI, Melbourne, Australia). Following endotracheal intubation, general anaesthesia was maintained with halothane (1-4%, Fluothane, ICI, Victoria, Australia). Prior to any incision being made, rabbits received an intramuscular injection (0.2 ml) of a broad spectrum antibiotic (Tribressen; Trimethoprim 80 mg/ml, Sulfadiazine 400 mg/ml; Jurox Pty. Ltd. Silverwater, NSW, Australia), and a subcutaneous injection (0.1 ml) of the narcotic analgesic buprenophine hydrochloride (0.065 mg; Temgesic; Reckitt and Colman Pharmaceuticals, West Ryde, NSW, Australia). Additional analgesia was provided by instillation of 1% w/v lignocaine (Xylocaine; Astra Pharmaceuticals, North Ryde, NSW, Australia) at wound sites. Throughout surgery rabbits received an intravenous infusion of NaCl (0.1 ml/min) to maintain extracellular fluid volume. Surgery was performed on a heated table, under sterile conditions.

2.3.2 Implantation of chronic medullary interstitial catheters

Rabbits were prepared as above (Section 2.3.1). Chronically positioned catheters were implanted 7-14 days prior to the acute experiment. A left flank incision was made, and the kidney was gently exteriorized. The tip of a single polyethylene catheter (ID 0.28 mm, OD, 0.61 mm; Critchley Electrical) was introduced into the ventral side of the kidney, slightly rostral to the midline aspect, at an angle directed toward the renal pelvis. The catheter was then advanced so that its tip lay either 8.5 (n=8) or 10.5 mm (n=9) below the surface of the kidney. Correct insertion resulted in minimal bleeding, which stopped almost immediately. A small piece of nylon mesh (1.5 cm diameter; Hilton Hosiery, Coolaroo, Victoria, Australia) attached to the catheter was anchored to the surface of the kidney with cyanoacrylate glue (Loctitie; Caringbah, NSW, Australia). The catheter was tunneled subcutaneously so that its end lay between the shoulder blades, and a pre-primed osmotic mini pump (Alzet 2ML2; 5 µl/h for 14 days, Alza Co., Palo Alto, CA, USA) filled with 154 mM saline was attached to the end of the catheter to maintain catheter patency.

In preliminary studies it was found that the depth of 8.5 mm (outer medullary interstitial catheters) corresponds approximately with the junction of the inner and outer stripes of the outer medulla, whereas the depth of 10.5 mm placed the catheter in the inner medulla. When possible, gross postmortem examination of the kidneys was performed and the catheters were always found to be positioned correctly. Furthermore, no evidence of gross disruption of kidney tissue or scar tissue due to implantation of the medullary interstitial catheters was found from examination of the frozen sections taken for further analysis.

2.3.3 *Implantation of ascending aortic flowprobes*

Rabbits were prepared as above (Section 2.3.1) and instrumented with flow probes around the ascending aorta 2-3 weeks prior to the date of the acute experiment. This period allows the formation of scar tissue around the probe, which provides acoustic coupling of the probe and vessel. An incision was made above the left second intercostal space, and the heart was exposed via thoracotomy. The second and third ribs were spread using a retractor, for access to the ascending aorta. Once the pleural cavity was open, rabbits were artificially respired (Phipps and Bird; Richmond, Virginia). A space around the ascending aorta was cleared and a transit-time ultrasonic flow-probe (6SB, Transonic Systems, Ithaca, NY, USA) was positioned around the aorta, so that when secured the probe lay parallel with the vessel. The lungs were inflated by occluding the expiratory tube from the ventilator and the ribs were brought together and secured with a single suture (3.0 propylene, Johnson and Johnson Medical, Sydney, NSW, Australia).

The wound was closed with a series of sutures, first the two separate muscle layers (5.0 Surgilene; Davis and Geck, Wayne, NJ, USA), the first acting as a seal for the wound made above the intercostal space, which was made at a right angle to the ribs. The second muscle layer was made in line with the muscle fibres and ran parallel to the ribs. The subcutaneous layer (5.0 Surgilene), and finally the cutaneous layer (3.0 silk; Davis and Geck, Wayne, NJ, USA) were then closed. Care was taken not to tighten sutures too firmly, to avoid post operative tissue necrosis. Supporting stitches were also made around the cutaneous layer to hold the wound in place, and Neosporin antibiotic cream (Polymyxin Sulphate, zinc bacitracin, neomycin sulphate; Glaxo Wellcome; Boronia, Victoria, Australia) was applied to the closed wounds to prevent infection.

The flow probe cable was tunneled subcutaneously so that its plug lay between the shoulder blades, for retrieval and connection to a flow-meter on the experimental day (Transonic systems, model

T208). This wound was closed with a silk suture (3.0 Dynek, Pty. Ltd., Australia). An infant feeding tube filled with sterile NaCl solution had also been tunneled through the same wound once the probe was in position. Once all wounds were closed, the lungs were re-inflated, and the infant feeding tube was connected to a 20-ml syringe, used to drain out any air still remaining in the pleural cavity.

Throughout surgery, halothane anaesthesia was gradually reduced to promote spontaneous breathing once the lungs were re-inflated and, the respirator was turned off. Not all rabbits responded immediately, in which case the respirator was turned back on for 1-2 breaths and off again until rabbits resumed breathing on their own.

2.3.4   Post-operative care of rabbits

On completion of surgical procedures, rabbits were kept in a warm environment and closely monitored until fully conscious, and all signs of anaesthesia had worn off (2-4 hours). They were then returned to their individual housing, which was lined with straw. Normal eating and drinking habits returned almost immediately. Rabbits were monitored daily for signs of discomfort or irritation, and wounds were checked to ensure that sutures and supporting stitches were still intact. Any wound break-down was repaired under local anaesthesia (0.5% Lignocaine; Astra Pharmaceuticals, North Ryde, NSW Australia) and an antibiotic (Tribressen; Trimethoprim 80 mg/ml, Sulfadiazine 400 mg/ml; Jurox, NSW, Australia) was injected into the wound and Neosporin antibiotic cream was re-applied to the exterior, to prevent infection.

2.4   Renal fixation and casting

Rabbits were prepared for surgery as outlined above (Section 2.2.5). One liter of 2.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3-7.4) at room temperature was perfused retrogradely through the distal aorta at a pressure equivalent to MAP (during the final minute of the experiment for each rabbit). The aorta was clamped rostral to the kidneys, and the vena cava vented as soon as perfusion of the fixative commenced. Immediately following the fixation process, a mixture of methacrylate and accelerator (20:1) (Mercox CL-2B-5;MIII, Japan) was perfused into the aorta (and so the left and right kidneys) at the same "physiological" pressure. Both kidneys were then clamped above the renal hilus, and the methacrylate solution was allowed to harden *in situ* for 30 min.

2.5 Analysis of methacrylate casts

2.5.1 *Preparation of methacrylate casts for scanning electron microscopy*

Only the left kidneys were examined. Following removal from the rabbit, each kidney was stored individually in the remaining fixative to allow complete polymerization of the methacrylate (24-48 hours). To eliminate the tissue from the vascular casts, each kidney was sliced coronally (2-3 cm thick) and placed in fresh containers of potassium hydroxide (KOH) solution (20% w/v) for 1 week, or until the tissue was completely dissolved. The casts were rinsed daily in distilled water and fresh KOH solution was added, so that all the dissolved tissue surrounding the casts was removed. The containers were incubated in a water bath at 55 °C. Once the tissue had been completely dissolved, the remaining casts were rinsed in distilled water, and placed in 5% w/v sodium hipchlorite for 1 hour at room temperature. The casts were then rinsed several more times in distilled water, and were air dried in individual containers, on filter paper. At this stage the casts were coded so that the observer was blinded to treatment.

Dried vascular casts were mounted on scanning electron microscope stubs and gold coated (S150B sputter coater, Bal-Tec, Liechtenstein) for 4 min under 30 mA of current, which was increased to 6 min at 20 mA for larger samples.

2.5.2 *Scanning electron microscopy*

Once coated, samples were ready for examination under the scanning electron microscope (Hitachi S-570, Hitachi City, Japan) at 20 kV. Diameters of afferent and efferent arterioles of inner and outer cortical glomeruli were assessed from scanning electron microscope micrographs (saved to a CD and printed, magnification 350 X). Diameter measurements were made along each vessel from its site of entry or exit to or from the glomerulus to its first branching point and measured at 25 μm intervals using a digitizing tablet (Summagraphics; resolution 100 lines/mm, accuracy (0.25 mm, Calgraph, Fullerton, CA, USA)) and the MEASURE program (Capricorn Scientific Software, Victoria, Australia).

Six afferent and efferent vessels were randomly selected for analysis from each region of the cortex (superficial, mid, and juxtamedullary). However, not all data sets were complete. Afferent arterioles were identified by locating their origin on interlobular arteries, and efferent arterioles began at the glomerulus and branched into peritubular capillaries. Glomeruli pertaining to different regions of

the cortex were classified as; (i) superficial glomeruli were attached to afferent arterioles at the junction to the interlobular artery, and had efferent arterioles which were thinner and tended to branch less than those in other regions, (ii) mid-cortical glomeruli were defined as those having shorter afferent arterioles attached to the interlobular arteries and their efferent arterioles were often also short, and branched several times to form peritubular capillaries, (iii) juxtamedullary glomeruli were defined as those having afferent arterioles which branched close to the arcuate arteries, and their efferent arterioles were often noticeably thicker and longer. Occasionally their branching into the unique "horsetail" arrangement of the vasa recta *(Kriz, 1982; Pallone et al., 1990)* could be observed.

2.6   Autoradiography

Frozen kidneys which had received medullary interstitial infusion of [^{3}H]-noradrenaline (Chapter 3) were sliced into 50 μm coronal sections at −19 °C using a cryostat (Leica, CM 1800), and mounted onto glass slides (subbed with 10% gelatin; BDH Chemicals Ltd. Poole, England). These sections were left to dry overnight at room temperature in trays, which were covered with foil to prevent dust from settling on them. Once dried, the slides were mounted on to sheets of Hyperfilm-[^{3}H] (high performance autoradiography film, Amersham International, Sweden) in cassettes. The cassettes were stored in the dark for a suitable time to allow development (6-8 weeks). Developed autoradiograms were quantified using an MCID M4 image analysis system (Imaging Research, St. Catherines, Ontario, Canada) as previously described *(Ashworth-Preece et al., 1997)*. Each kidney section was divided into four regions, cortex, outer medulla (outer stripe), medulla (excluding the outer stripe and papilla), and the papilla (defined as the portion of the inner medulla that protrudes into the renal pelvis), for separate quantification.

2.7   Measurement of haemodynamic and renal variables

2.7.1   *Measurement of systemic and renal haemodynamics in anaesthetised rabbits*

Systemic arterial pressure was measured by connecting an ear artery catheter (Chapters 3,4, and 6) or side branch of an abdominal aortic catheter (Chapter 5), to a pressure transducer (Cobe; Arvada, CO, USA) calibrated at the level of the rabbit's heart using a mercury manometer. Heart rate (HR; beats/min) was measured via a cardiotachometer activated by the arterial pressure pulse. RAP (mmHg) in protocols involving the extacorporeal circuit (Chapters 4 and 5) was measured via a side-arm catheter, 3 mm proximal to the tip of the cannula inserted into the renal artery. In these

studies left RBF (ml/min) was measured as blood flow through the renal limb of the circuit, using an in-line transit time ultrasound flow probe (type 4N, Transonic Systems Inc, Ithaca, NY, USA). In rabbits not connected to the extracorporeal circuit, left RBF was measured by a perivascular transit time ultrasound flow probe placed around the renal artery (type 2SB, Transonic Systems Inc.) which was coupled acoustically with Nalco gel (Nalco Chemical Company, Maperville, IL, USA). Cardiac output (Chapter 6) was measured using the previously implanted ascending aortic transit-time ultrasound flow probe (6SB, Transonic Systems, Ithaca, NY, USA). The transit time flow probes were connected to a flowmeter (Transonic systems, model T208). Laser-Doppler flow probes were connected to a laser-Doppler flowmeter (DRT4, Moor Instruments Ltd., Devon, England) These signals were amplified, recorded, and digitized, as previously described *(Bergström and Evans, 1993)*, to provide 60 second means. CBF and MBF were expressed as perfusion units (equivalent to the instrument output in mV x 10). Signals were amplified and recorded on a Neotrace pen recorder (Neomedix Systems, Sydney, Australia) and monitored on-line using an analogue-to digital converter on an Olivetti M280 computer which provided 20 second and 60 second means of each variable.

2.7.2 Processing of blood and urine samples

Arterial blood samples were centrifuged (Model C312; Jouan) at 4 °C for 10 minutes at 3,000 rpm to separate plasma from red blood cells. Haematocrit was determined by the capillary tube method using a mirco-haematocrit reader (Hawksley and Sons, England) before the samples were centrifuged. The volumes of the timed urine samples were measured gravimetrically (Denver Instrument XL-410, Denver, USA). Twenty μl triplicates of plasma and urine samples were taken and added to (5ml) scintillation vials containing 2 ml of scintillation fluid (Ecoscint A; National Diagnostics, Atlanta, Georgia, USA). The remaining plasma was aspirated and the samples frozen and stored at −20 °C for later analysis. The concentration of radiolabel in each of the samples was measured by liquid scintillation counting (Model LS 500 TA, Beckman; Beckman Instruments; Fullerton, CA, USA). Sodium concentrations were measured by flame photometry (Instrumentation Laboratory 943, Milan, Italy).

2.7.3 Plasma renin activity

Arterial blood (2.5 ml) for measurement of plasma renin activity was collected into chilled tubes containing 20 μl of an inhibitor cocktail (0.21 % (w/v) $NaH_2PO_4.2H_2O$, 0.74% (w/v) Na_2HPO_4, 1.0% neomycin sulphate, 3.72% (w/v) ethylenediaminetetraacetate, and 1.0% (v/v) 2,3 dimercaptol-

1-propanol: a renin inhibitor, pH 7.4). Plasma renin activity was measured as the rate of angiotensin I generated from endogenous angiotensinogen *in vitro* and is therefore expressed as ng Angiotensin I/ml/hr. Angiotensin I was measured by radioimmunoassay using the method outlined by Oliver *et al. (Oliver et al., 1990)*. The radioimmunoassay for angiotensin I was performed over two consecutive days. On the first day plasma samples were slowly thawed in iced water and 50 μl of the sample was added to 50 μl of ice cold incubation buffer (0.53% (w/v) Na_2HPO_4, 2.5% (w/v) $Na_2PO_4.H_2O$, 3.7% (w/v) Na_2ethylenediaminetetraacetate, 0.1% (w/v) neomycin sulphate, 0.24% (w/v) 1, 10, phenanthroline; pH 6.2) and the sample was then incubated at 37 °C for two hours. Incubation was stopped and 300 μl of ice cold assay buffer (0.64% (w/v) Na_2HPO_4, 0.37% (w/v) Na_2ethylenediaminetetraacetate, 0.1 % (w/v) neomycin sulphate, 0.1% (w/v) casein, 0.9% (w/v) NaCl and 0.08% (w/v) $NaH_2PO_4.2H_2O$; pH 7.4) was then added to the sample. Fifty μl of the incubated sample was then added to 100 μl of the primary antibody (1:30,000 dilution; angiotensin I antibody raised in rabbit; Baker Medical Research Institute, Prahran, Victoria, Australia). One hundred μl of the trace (^{125}I-angiotensin I, 10,000 counts per min: Amersham, UK) was then added to the sample and all tubes were then vortexed and left at 4 °C for 20-24 hours. On the second day, 2 ml of dextran- 10 coated charcoal (2.5% (w/v) activated charcoal, 0.25% (w/v) concentrated HCl; 1:5 dilution with cold 0.9% (w/v) NaCl) was added to each sample. The samples were then centrifuged (Minifuge GL, Heraeus Christ, Hanover, Germany) at 4500 rpm for 20 min at 4 °C. The supernatant was aspirated and the number of disintegrations in the pellet were then determined over a one minute period (model 550, Auto gamma counter: Packard, Downers Grove, IL, USA).

2.8   Calculations

2.8.1   *Calculations of renal clearance variables*

2.8.1.1 *Renal plasma flow*

RBF (determined by transit-time ultrasound flowmetry) was multiplied by the proportion (by volume) of the blood that is plasma 1- haematocrit.

In rabbits receiving [^{3}H]-PAH infusion, this was determined by dividing the urine volume by the plasma PAH concentration (determined by scintillation counting) to determine the amount of plasma flowing through the kidneys, and multiplying it by the urinary PAH concentration, since PAH is filtered and not reabsorbed to determine the overall renal plasma flow.

2.8.1.2 Glomerular filtration rate

[^{3}H]-Inulin is a polysaccharide which is freely filtered and not reabsorbed from or secreted into the tubules. Since all the glomerular filtrate formed is cleared of inulin, the volume of plasma cleared of inulin per minute equals GFR, which is calculated as the urinary concentration of inulin divided by the plasma concentration of inulin, all multiplied by the urinary volume per min. In some experiments these values were divided by the kidney dry weight to achieve units as ml/min/g dry kidney weight.

2.8.1.3 Effective renal blood flow

Para-aminohippuric acid (PAH) is freely filtered but not reabsorbed, but differs from inulin in that it is actively secreted from the peritubular capillaries into the tubules. Thus, PAH is virtually completely (90-95%) removed from all of the plasma that flows through the kidneys. The plasma clearance for PAH is therefore used to estimate renal plasma flow. Effective RBF was calculated as renal plasma flow divided by 1- haematocrit.

2.8.1.4 Filtration fraction

Filtration fraction (FF) is the fraction of the plasma flowing through the glomerulus that is filtered, and was calculated as GFR ([^{3}H]-inulin clearance) divided by renal plasma flow [^{14}C]-PAH clearance or determined from transit-time ultrasound flowmetry corrected for haematocrit. In this thesis it is presented as a percentage value.

2.8.15 Fractional sodium excretion

This was calculated as the clearance of sodium divided by the clearance of [^{3}H]-inulin (GFR), and is expressed as a percentage. The clearance of sodium was derived by dividing the concentration of sodium excreted by the kidney (mM) by the concentration of sodium in the urine by the concentration of sodium in the plasma, and multipying this figure by urine flow. This value is expressed as percentage in this thesis.

2.8.1.6 Fractional urine excretion

This was calculated as the excreted urine volume (measured either volumetrically or gravimetrically) divided by the clearance of [^{3}H]-inulin (GFR). This volume is expressed as a percentage.

2.8.2 Calculation of vascular lumen resistance and conductance values

The relative vascular resistances of the arterioles were calculated according to Poiseuille's equation;

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

Where   R = calculated vessel resistance

             $\eta =$ viscosity of the fluid (blood),

             $l =$ length of the vessel, and

             $r =$ radius of the vessel.

In the calculations presented in Chapter 6, l was assumed to be constant, so that values of vessel resistance are expressed per unit vessel length. Blood viscosity was also assumed to be constant and treated in the same way as l. A mean value for the radius was calculated for each vessel from the measurements made along the vessel length including the one closest to the glomerulus, and relative resistances were therefore expressed as $R = 1 / \pi r^4$. Vessel conductance for the entire vessel length was calculated as 1 / R.

2.9   Overview of statistical analyses

Full details of the specific statistical analyses are described in the relevant chapters. Listed below the statistical methods used in each of the experimental chapters.

Chapter 3	-	analysis of variance
Chapter 4	-	paired t test, repeated measures analysis of
		two way analysis of variance
Chapter 5	-	paired t test, unpaired t test
Chapter 6	-	paired t test, 2-way analysis of variance, 3-way
		analysis of variance

All statistical tests were performed using the computer software package SYSTAT *(Wilkinson, 1990)*, except t-tests which were performed using EXCEL spreadsheets (Microsoft office 1995) and P values ≤ 0.05 were considered to be statistically significant.

To protect against the increased risk of comparison-wise Type 1 error resulting from compound asymmetry, all of the P values derived from repeated measures analysis of variance were conservatively adjusted using the Greenhouse-Geisser correction factor *(Ludbrook, 1994)*.

Chapter Three

METHODS FOR RENAL MEDULLARY INFUSION OF VASOACTIVE COMPOUNDS: METHODOLOGICAL CONSIDERATIONS

3.0 Summary

1. There is accumulating evidence implicating the renal medullary microvasculature in the long term regulation of arterial pressure. To study the role of the medullary microcirculation in arterial pressure regulation we required a technique for selectively altering MBF.

2. The aims of the current study were to develop and validate techniques for the delivery of vasoactive compounds to the renal medulla, to determine the optimal position of the catheter tip for maximum distribution of the radiolabel in the renal medulla and to determine whether chronically implanted medullary interstitial catheters would remain patent at least 6 weeks after implantation.

3. [^{3}H]-Noradrenaline was infused into either the renal medullary interstitium of anaesthetised rabbits via catheters that were positioned in the outer medulla or the inner medulla, and were either chronically or acutely positioned. The intrarenal distribution of radiolabel was determined by autoradiography.

4. In a supplementary study, catheters were chronically positioned in the outer medullary interstitium so that conscious rabbits could receive a saline infusion for up to six weeks.

5. Radiolabel concentration was eight times greater in the medulla than the cortex, of kidneys receiving outer medullary interstitial infusion of [^{3}H]-noradrenaline.

6. Inner medullary interstitial infusion of the radiolabel resulted only in low levels of radiolabel within the kidney.

7. When tested 6 weeks after implantation, chronically implanted outer medullary interstitial catheters remained patent.

8. We conclude that outer medullary interstitial infusion is a useful technique for the delivery of rapidly metabolized vasoactive agents to the renal medulla, that the level of distribution is

largely dependent on the infusion site, and that this technique is adaptable to chronic infusion in conscious rabbits.

3.1   Introduction

Increasingly more evidence is being presented which implicates a role of the renal medullary microvasculature in the regulation of arterial pressure. Although the renal medulla receives only ~10% of total RBF, evidence indicates that the level of renal MBF may be an important determinant of sodium and water reabsorption, *(Cowley et al., 1995; Cowley, 1997)*, and may also play a role in the release of the putative renal medullary depressor hormone *(Bergstöm and Evans, 1998)*. The renal medullary microcirculation may therefore be well placed to transduce changes in arterial pressure into homeostatic responses that restore normal arterial pressure.

One technique for studying the role of the renal medullary microcirculation in the long-term control of arterial pressure has been the infusion of vasoactive agents into the renal medulla. Cowley and colleagues *(Cowley et al., 1992; Lu et al., 1992; Lu et al., 1994; Mattson et al., 1994; Szczepanska-Sadowska et al., 1994; Cowley et al., 1995; Cowley, 1997)*, have employed this technique in rats, combined with laser-Doppler flowmetery. Their studies have shown that chronic medullary interstitial infusion of vasoconstrictor agents such as N^G-nitro-L-arginine methyl ester *(Mattson et al., 1994)* and the vasopressin V_1-agonist [Phe^2, Ile^3,Orn^8]-vasopressin *(Szczepanska-Sadowska et al., 1994)*, at doses that reduce MBF but not CBF, results in the development of sustained hypertension. Conversely, medullary interstitial infusion of the vasodilator captopril in SHR, which increases MBF but not CBF, ameliorates their hypertension *(Lu et al., 1992)*.

In longitudinal studies such as those described above, there are considerable advantages to employing larger species such as the rabbit which would allow simultaneous long term recordings of hormonal status *(Evans et al., 1994)*, cardiac output *(Evans et al., 1993)*, RBF *(Tomoda et al., 1996)*, regional kidney blood flow *(Evans et al., 2000)*, and renal sympathetic nerve activity *(Malpas and Evans, 1998)*.

In a previous study *(Correia, 1997)*, we performed a number of experiments aimed at validating techniques for the delivery of vasoactive agents to the renal medulla of rabbits. Noradrenaline was chosen as the vasoactive agent because it is readily available in tritiated form and rapidly metabolized *in vivo*, minimizing the confounding effects of spillover into the systemic circulation. These studies attempted to correlate the systemic and renal haemodynamic effects of infused [^{3}H]-

noradrenaline with the regional distribution of the intrarenal infused radiolabel. In brief, we found that outer medullary interstitial infusion of noradrenaline (300 ng/kg/min), using acutely or chronically positioned catheters reduced both CBF (15%) and MBF (23-31%). Inner medullary infusion did not affect renal haemodymanics, whereas intravenous infusion of the same dose selectively reduced CBF (15%) without significantly affecting MBF. During outer medullary infusion of [^{3}H]-noradrenaline, much of the radiolabel spilled over into the systemic circulation (~40% with chronically positioned catheters). Nevertheless, tissue solubilization analysis showed the concentration of radiolabel was about seven fold greater in the infused medulla than the cortex. Inner medullary infusion resulted in much of the radiolabel being excreted in urine. We concluded that outer medullary interstitial infusion in rabbits provides a useful method for targeting compounds to the renal medulla, but given the considerable systemic spillover with outer medullary infusion, its utility is limited to substances that are rapidly metabolized *in vivo (Correia, 1997).*

Although our previous analysis of the regional distribution of radiolabel within the kidneys after outer and inner medullary infusion of [^{3}H]-noradrenaline indicated some localisation of the radiolabel within the medulla, the absence of autoradiographic data meant that no information regarding local tissue distribution was obtained, and furthermore any differences between the two infusion sites could not be quantified. The present study had two aims. The first was to use tissue obtained in our previous study, to more closely characterize and quantify the anatomical distribution of radiolabel after medullary interstitial infusion of [^{3}H]-noradrenaline. To achieve this, we used autoradiographic techniques. Our second aim was to establish whether chronically positioned medullary interstitial catheters remained patent 6 weeks after implantation.

3.2 Methods

In order to test the regional kidney distribution of [^{3}H]-noradrenaline after medullary interstitial infusion (16-24 nCi/kg/min in 100 ng/kg/min noradrenaline), twenty six rabbits of a multicolored English strain of either sex and weighing 2.3-3.1 kg (mean 2.7 kg) were used in acute experimental studies. Of these rabbits, nine received the infusion via acutely positioned catheters, while the remaining 17 received the infusion via chronically implanted catheters, positioned either in the outer medullary (n=8) or inner medullary (n=9) interstitium. After medullary interstitial infusion of [^{3}H]-noradrenaline, the left (infused) kidneys of these rabbits, were removed and subjected to autoradiographic analysis for quantitative determination of concentration of the radiolabel throughout the kidney.

To investigate the adaptability of these techniques to chronic longitudinal studies, a supplementary study was conducted using six rabbits, also of the same strain and of either sex, weighing 2.3 – 2.8 kg (mean 2.6 kg). These rabbits received a chronic infusion of saline for up to 6 weeks via catheters that were chronically implanted such that their tips lay in the outer medullary interstitium. On completion of these studies, the infused kidneys were removed, and Evans Blue dye was injected for visual determination of catheter patency.

3.2.1 Experimental preparation

The surgical preparation of rabbits receiving a 20 min [^{3}H]-noradrenaline infusion, administered via either acute, or chronically positioned catheters is described in detail in Chapter 2, Sections 2.2.3, and 2.3.2. Briefly, following the induction of anaesthesia, both kidneys were denervated, and both ureters were cannulated. A transit-time ultrasound flow probe was placed around the renal artery, and the tips of three single-fiber laser-Doppler flow probes (0.5 mm diameter) were placed 0.5 (cortical), 0.5 (cortical) and 10 mm (medullary), respectively, below the cortical surface. Haemodynamic data from these studies have been reported elsewhere *(Correia, 1997)*.

3.2.2 Implantation of medullary interstitial catheters

3.2.2.1 Acutely positioned catheters (9 rabbits)

Catheters, constructed using 30 gauge needles, were placed 2 cm apart on the midline aspect of the kidney, on either side of the medullary laser-Doppler flow probe, with their tips positioned 8.5 mm below the cortical surface (in the outer medullary interstitium). Sodium chloride (154 mM; 10 µl/kg/min) was infused via each catheter throughout the experiment.

3.2.2.2 Chronically positioned catheters (23 rabbits)

Chronically positioned catheters were implanted 7-14 days prior to the experiment, under halothane anaesthesia and employing sterile conditions. This procedure is explained in detail in Chapter 2, Section 2.3.2. Briefly, a left flank incision was made, and the kidney was gently exteriorized. The tip of a single polyethylene catheter was introduced into the ventral side of the kidney, at an angle directed toward the renal pelvis. The catheter was then advanced so that its tip lay either 8.5 (8 rabbits subjected to one acute infusion of [^{3}H]-noradrenaline, and a further 6 rabbits subjected to longitudinal study) or 10.5 mm (9 rabbits, all subjected to acute infusion of [^{3}H]-noradrenaline)

below the kidney surface. A small piece of nylon mesh attached to the catheter was anchored to the kidney surface with cyanoacrylate glue. The catheter was tunneled subcutaneously so that its end lay between the shoulder blades, and was kept patent by attachment to an osmotic pump (Alzet 2ML2; 2.5 μl/h for 28 days, Alza Co., Palo Alto, CA, USA) filled with 154 mM saline.

3.2.3 Experimental protocols

3.2.3.1 Renal medullary interstitial infusion of [³H]-noradrenaline

On the experimental day, rabbits were anaesthetisedand prepared as outlined above. Following a 1 hour equilibration period [³H]-noradrenaline (16-24 nCi/kg/min in 100 ng/kg/min noradrenaline) was infused into the medullary interstitium for 20 min. This entire dose was delivered directly to the outer (n=8) or inner medullary interstitium (n=9) of rabbits that had chronically implanted catheters, and was divided so that one half of the dose was equally distributed between the two acutely positioned outer medullary catheters (n=9). Urine produced by both kidneys was collected during the 2 min before the infusion commenced and for each 2 min period throughout the [³H]-noradrenaline infusion. Ear arterial and renal venous blood samples (0.5 ml each) were collected at the mid point of each urine collection period. Data derived from these measurements, which characterize the disposition of radiolabel by the kidney during the infusions, have been reported elsewhere *(Correia, 1997)*. At the completion of the 20 min [³H]-noradrenaline infusion, the infused kidney was quickly retrieved, de-capsulated, and halved coronally. Kidney halves were frozen in liquid nitrogen and stored at -70°C for subsequent analysis.

3.2.3.2 Chronic renal medullary interstitial infusion (6 rabbits)

Following a 2-week recovery period, the osmotic mini pump positioned between the rabbits' shoulder blades was changed to a fresh saline (154 mM NaCl) infusion. Osmotic pump volumes were 2.0 ml for each pump and pumped at a rate of 2.5 μl/h over 28 day periods. Prior to their implantation, osmotic pumps were primed by being filled with sterile saline, and kept in sterile containers also containing saline. On completion of these experiments (6 weeks after catheter implantation), kidneys were removed, and Evans Blue dye was injected through the catheter. Patency of the catheter was determined by dissection and visual inspection of the kidney.

3.2.4   Autoradiography

These methods are described in detail in Chapter 2, Section 2.6. Briefly, coronal 50 μm sections of the frozen left kidney were cut on a cryostat at -19°C and mounted on glass slides (subbed with 10% gelatin; BDH Chemicals, Poole, UK). These sections were left to dry for 1-2 h at room temperature. Subsequent to drying, slides were apposed to tritium sensitive film (Amersham Hyperfilm, Sweden) in the presence of tritium microscales (Amersham) for 6-8 weeks. Developed autoradiograms were quantified using an MCID M4 image analysis system (Imaging Research, St. Catherines, Ontario, Canada) as previously described *(Ashworth-Preece et al., 1997)*. Each kidney section was divided into four regions; cortex, outer stripe of the outer medulla (outer stripe), medulla (excluding the outer stripe and papilla), and the papilla (defined as the portion of the inner medulla that protrudes into the renal pelvis), for separate quantification.

3.2.5   Statistical analyses

The levels of radioactivity in the various kidney regions for the infused kidneys were subjected to ANOVA, the factors comprising rabbit, and the kidney region (cortex (C), outer medulla (OS), medulla (M), and papilla (P)).

Comparisons made between specific kidney regions and different infusion sites (outer medullary vs. inner medullary) were conducted using paired and unpaired t-tests respectively.

3.3   Results

3.3.1   Intra-renal distribution of radiolabel: acute catheters

Autoradiographic analysis of the coronal kidney sections demonstrated that the levels of radioactivity in the cortex of the infused kidney were 3.4 ± 0.6, 5.6 ± 0.8, and 8.0 ± 0.8 fold lower than those in the outer stripe, medulla, and papilla, respectively (Figures 3.1a and 3.2a).

3.3.2   Intra-renal distribution of radiolabel: chronic catheters

Autoradiographic analysis of the coronal kidney sections demonstrated that with outer medullary infusion, the levels of radioactivity in the cortex of the infused kidney were 10.3 ± 2.3, 14.0 ± 3.3, and 8.8 ± 2.8 fold lower than those in the outer stripe, medulla, and papilla, respectively. After inner

medullary infusion, the levels of radioactivity in the cortex of the infused kidney were 6.6 ± 1.6, 8.7 ± 2.2 and 8.2 ± 2.4 fold lower than those in the outer stripe, medullary, and papillary regions, respectively (Figures 3.1 and 3.2).

3.3.3 Comparison of intra-renal distribution

Densiometric analysis of outer medullary infused kidneys revealed that radiolabel infused via chronically positioned catheters resulted in greater concentrations of radiolabel in the outer stripe of the outer medulla (180 ± 20 dpm/mm^2) and in the medulla (209 ± 33 dpm/mm^2), than was the case with acutely positioned catheters (79 ± 11, and 140 ± 19 dpm/mm^{2} respectively). Radiolabel concentrations in the cortex and papilla were similar with the two infusion sites (Figure 3.2).

3.3.4 Intra-renal distribution of radiolabel using chronically positioned catheters: effect of infusion site

A comparison of radiolabel concentration throughout the kidneys receiving their infusions via chronically positioned catheters in the outer or inner medullary interstitium demonstrated that outer medullary interstitial infusion resulted in greater concentrations of radiolabel being isolated in the outer stripe of the medulla (179 ± 20 dpm/mm^2), and the medulla (209 ± 33 dpm/mm^2), when compared to inner medullary infusion (74 ± 15, and 88 ± 16 dpm/mm^2). Radiolable concentration in the cotex and papilla were similar with the two infusion sites (Figure 3.2).

3.3.5 Chronic medullary interstitial infusion

At post mortem, Evans Blue dye (~0.5 ml) was infused into the medullary interstitial catheter and was found to stain areas within the renal medulla (Figure 3.3).
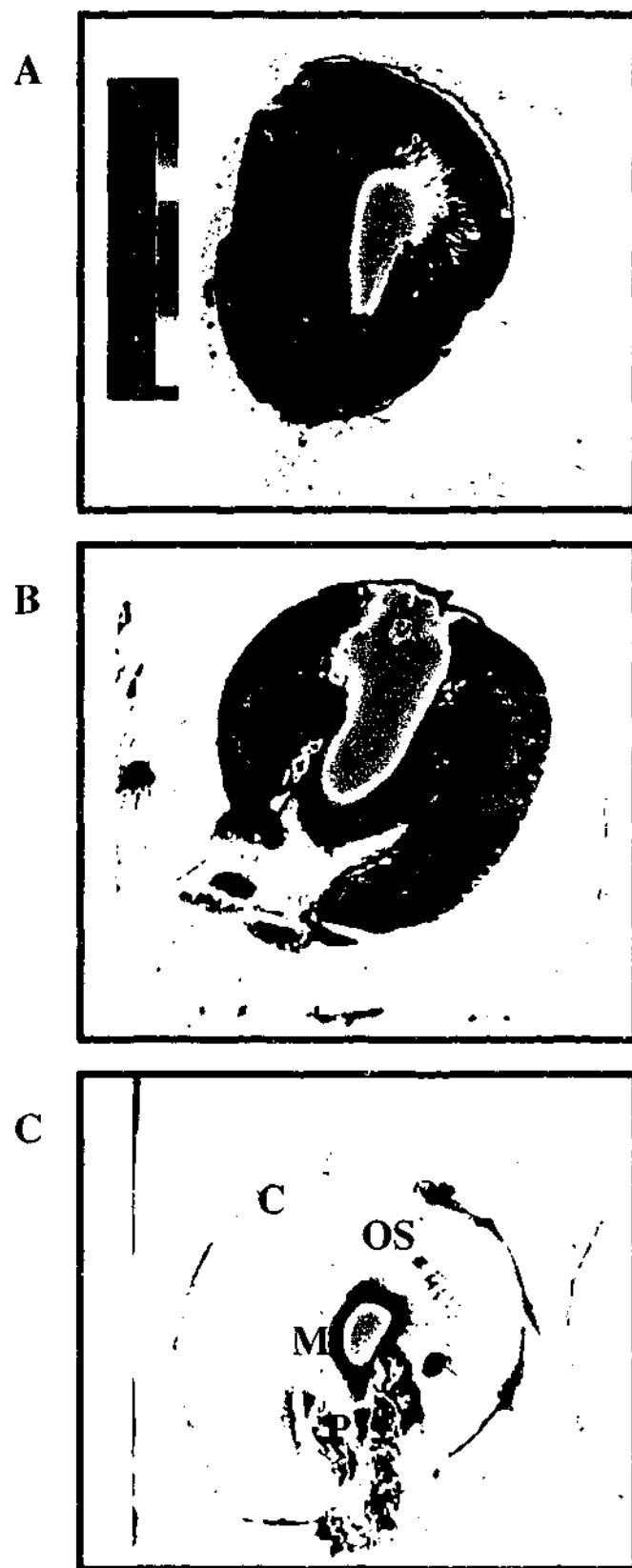
Figure 3.1 Typical autoradiograms, showing radiolabel distribution throughout kidneys following [³H]-noradrenaline (16-24 nCi/kg/min in 100 ng/kg/min noradrenaline) infusion via two acutely positioned catheters (A), or chronically positioned catheters, so that the tip lay in the outer medullary interstitium (B), or in the inner medullary interstitium (C). C, cortex; OS, outer stripe of outer medulla; M, medulla; P, papilla.
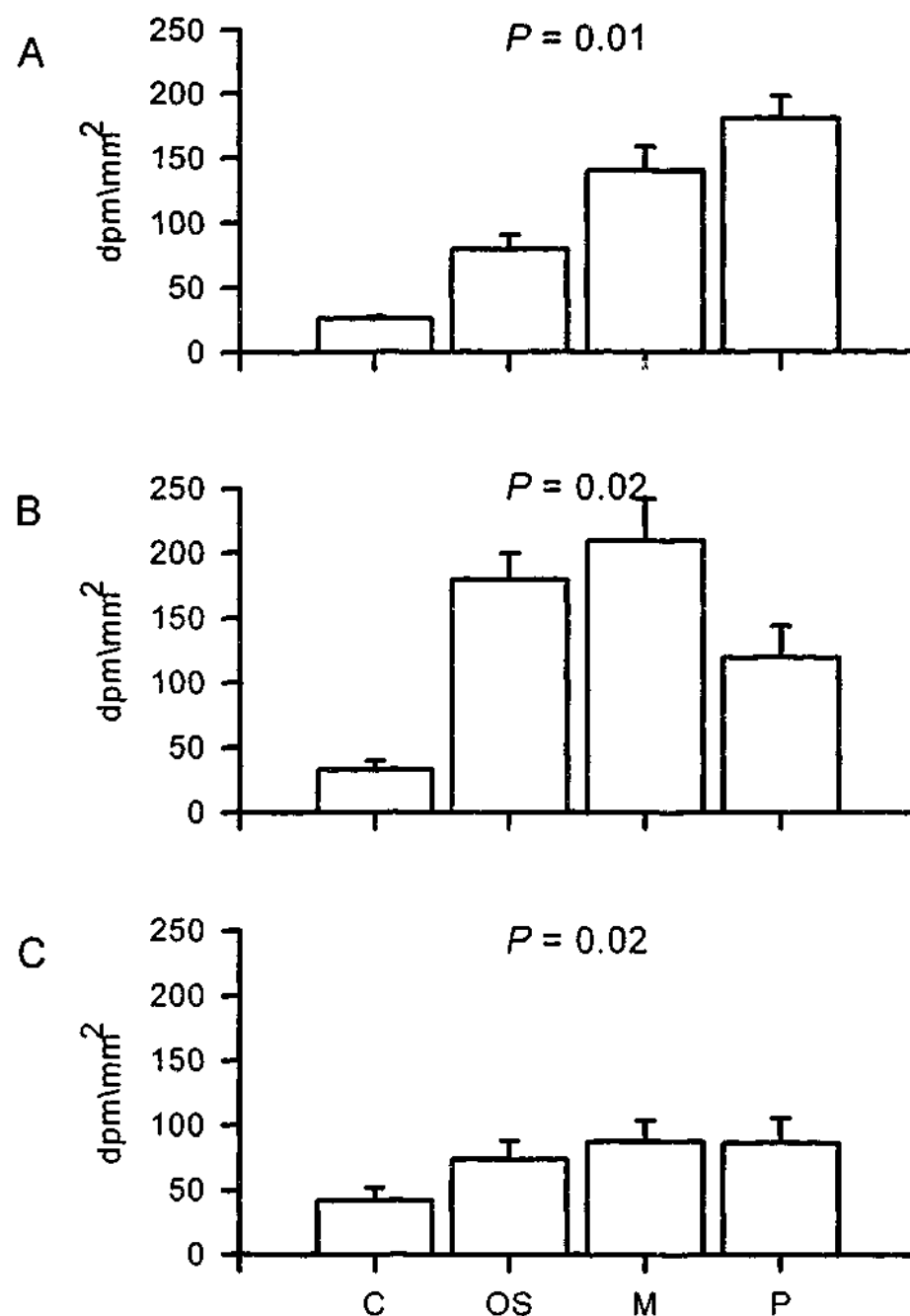
Figure 3.2 Density of radiolabel expressed as disintegrations per minute per square mm (dpm/mm^2), determined from autoradiographic analysis of infused kidneys at end of 20 min infusions of [^{3}H] noradrenaline (16- 24 nCi/kg/min noradrenaline in 100 ng/kg/min noradrenaline), administered via; A, 2 acutely positioned catheters in outer medullary interstitium (n=5), B, chronically positioned catheter in outer medulla (n=7), C, chronically positioned catheter in inner medulla (n=9). Columns and error bars represent means ± SE. *P* values represent outcomes of a partitioned ANOVA testing for a difference between levels of radioactivity in the cortex compared with other three kidney regions. C, cortex; OS, outer stripe of outer medulla; M, medulla; P, papilla. See Methods for definitions of these regions.
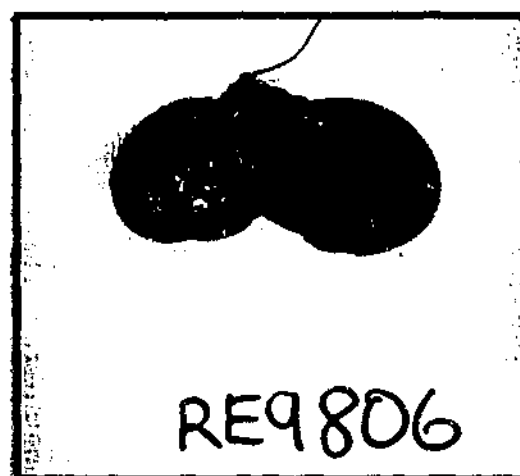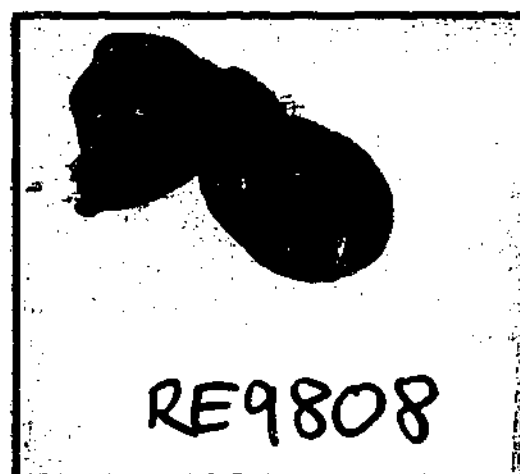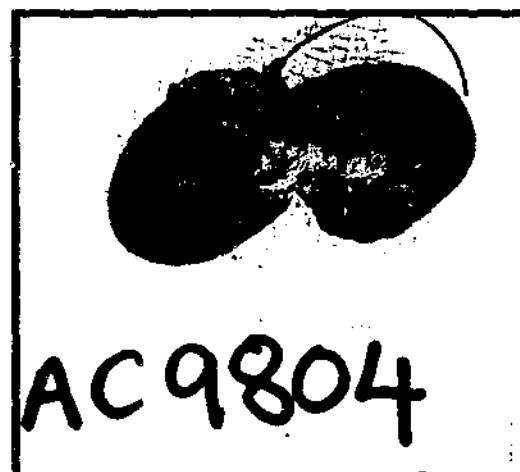
Figure 3.3 Photographs of kidneys following chronic infusion catheters, positioned in the outer medullary interstitium. Evans Blue Dye infused at post mortem shows that the dye reached the renal medulla. Thus, these catheters remained patent for at least 6 weeks.

3.4 Discussion

The aim of this study and our previous study *(Correia, 1997)* was to design and validate techniques for the delivery of pharmacological agents into the renal medullary interstitium of rabbits, and to d∷ermine the renal distribution and disposition of the infused radiolabelled substance. Similar techniques have been developed in rats *(Lu et al., 1992; Lu et al., 1994; Mattson et al., 1994; Szczepanska-Sadowska et al., 1994)*, and have helped provide considerable information regarding the role of the renal medulla, and in particular the renal medullary microcirculation, in the control of blood pressure *(Cowley et al., 1992; Cowley et al., 1995; Cowley, 1997)*.

Taken together with our previous results *(Correia, 1997)*, the results of the present study suggest that in the rabbit, outer medullary interstitial infusion of pharmacological agents, administered via either acutely or chronically implanted catheters, provides a useful method for targeting pharmacological agents to the renal medulla, for alteration of MBF. In this respect, the key finding of the present study was that infusion of [^{3}H]-noradrenaline into the outer medulla resulted in a much greater concentrations of the infused radiolabel in the medulla than in the cortex.

Previously *(Correia, 1997)*, we found that medullary interstitial infusion of noradrenaline delivered to the outer medulla of the rabbit kidney, via either acutely or chronically positioned catheters caused an increase in MAP and reductions in HR, total RBF and both CBF and MBF. The reduction in CBF was significantly less than that of MBF ($P < 0.001$, for acutely positioned catheters) and appeared to have occurred chiefly due to the systemic spillover and re-circulation of noradrenaline (~40%) during infusion. This conclusion was based on our observation of similar reductions in CBF when the same dose of noradrenaline was administered intravenously. Inner medullary interstitial infusion of noradrenaline caused a slight increase in MAP, but had no significant effect on either total RBF or CBF or MBF. We concluded that the absence of renal haemodynamic effects of noradrenaline via this route was due to the fact that most of the infused noradrenaline (measured as [^{3}H]) was excreted in the urine. We found that intravenous infusion of noradrenaline (300 ng/kg/min) also increased MAP, and while it reduced total RBF and CBF similarly to outer medullary interstitial infusion, did not affect MBF. This suggests that the intravenous route might provide a useful control for the systemic and renal cortical effects of outer medullary interstitial infusion of noradrenaline.

A tissue solubilization technique also employed in our previous study demonstrated considerable variation associated with the levels of radiolabel in the kidney tissue, particularly during outer

medullary infusion of radiolabel *(Correia, 1997)*. It was clear, however, that much greater levels of the radiolabel remained in the kidney during outer, compared with inner medullary infusion of [^{3}H]-noradrenaline. However, although these results provided useful information regarding the renal distribution of radiolabel in gross tissue sections, we were unable to assess the tissue distribution of radiolabel in detail.

Therefore in the current study, autoradiographic analysis was employed, using tissue from our previous study so that the regional renal distribution of the infused radiolabel could be more precisely determined. Using this technique, we were able to analyze the distribution of radiolabel in specific regions; the cortex, outer stripe of the outer medulla, inner medulla, and the papilla. These data confirm and extend those from our previous study *(Correia, 1997)*, and clearly show that the concentration of infused radiolabel was much greater in the medulla of the infused kidney, than in the cortex or papilla, after outer medullary interstitial infusion. Furthermore, outer medullary interstitial infusion achieves high concentrations of this agent at regions of the kidney likely to contain the vascular sites important in the control of MBF. Since MBF is derived entirely from the efferent arterioles of juxtamedullary glomeruli *(Kriz, 1982)*, of the juxtamedullary cortex, then vasoconstriction of either the afferent or efferent arterioles of these glomeruli should result in reduced MBF. Contractile elements are present in the outer medullary descending vasa recta in the outer medulla, but for the most part disapear as these vessels form the "typical" horsetail arrangement *(Pallone et al., 1990; Harrison-Bernard and Carmines, 1994)*. Since high concentrations of radiolabel were detected in the outer medullary and medullary regions of the kidney following both tissue solubilization and autoradiographic analysis, it seems likely that the infusion could have acted on these vascular elements to produce a vasoconstriction and therefore reduction in blood flow to this region of the kidney. Indeed, our previously reported measurements of regional kidney blood flow during this experiment confirm this hypothesis *(Correia, 1997)*.

An important aspect of our previous study was the information we obtained about renal handling, and therefore disposition, of the infused substance with respect to the infusion site. Outer medullary infusion of the radiolabel caused much of the infusion to be spilled over into the systemic circulation (~40%), whereas during inner medullary infusion approximately 60% of the infused radiolabel was excreted by the infused kidney associated with much lower concentrations of the radiolabel in the kidney *(Correia, 1997; present study)*. The reason for this difference between the outer medullary and inner medullary infusions remains to be determined but may relate in part to the presence of mechanisms for tubular secretion of noradrenaline *(Kopp et al., 1983)* and to the relatively lower levels of blood flow in the inner medulla compared with the outer medulla *(Pallone*

et al., 1990). It is unlikely to reflect leakage of [³H]-noradrenaline due to damage to the papillary tissue from implantation of the catheter, because no such damage was observed in the frozen sections submitted for autoradiography.

Renal handling of infused substances appears to differ between rats and rabbits *(Lu et al., 1992; Correia, 1997)*. For example during outer medullary interstitial infusion in rabbits, a large percentage (~40%) of the infusion spilled over into the systemic circulation, whereas inner medullary infusion in rats resulted in a much greater localization of the infused radiolabel being concentrated in the medulla and papilla (92%; outer zone, inner zone, and papilla), with limited spillover and re-circulation to the renal cortex, resulting in the infused kidney retaining a radioactivity level forty seven times greater than the contralateral kidney *(Lu et al., 1992)*. An important point to note is that although autoradiography provides a more accurate method of quantification, the data presented are only representative of the 50 μm sections of tissue subjected for analysis, even though these sections were collected from along the line of the catheter and therefore infusion site. In the current study, because only the infused kidney was subjected to autoradiography, half of each of the infused and contralateral kidneys were subjected to tissue solubilization. Although this is a more 'crude' method of analysis, it does provide a complete indication of the regional distribution throughout the entire kidney (infused and contralateral) *(Correia, 1997)*.

In our rabbit studies, although it was found that the infused kidney retained a much greater concentration of the radiolabel than the contralateral kidney, this difference was only twenty two and twenty four times greater for the medulla and papilla respectively, during outer medullary infusion (acute catheters), *(Correia, 1997)*. A further possible explanation for this variance, aside from less spillover in the rat, may have related to the differences in methods of analysis employed in these studies, autoradiography *(Lu et al., 1992)* versus the tissue solubilization technique *(Correia, 1997)*. Our present experiment allowed us to test this hypothesis, since we now have data using both methods from our experiment. A comparison of these two techniques in the rabbit, indicates similar results in terms of regional distribution of radiolabel throughout the infused kidney, suggesting that species differences in the handling of infused substances are likely.

A further possible explanation for the apparent differences between the handling of substances infused into the medullary interstitium in these two species may arise due to varying abilities of the renal medulla to "trap" substances infused into the interstitium and could reflect differences in medullary structure between the two species *(Kriz, 1981)*. For example, the rabbit renal medulla has

a "simple" structure, with relatively small vascular bundles containing only ascending and descending vasa recta. In the more "complex" rat renal medulla, larger vascular bundles are found that also contain descending thin limbs of short loops of Henle *(Pallone et al., 1990)*. Conversely, our results are in agreement with those of Cowley and colleagues *(Lu et al., 1992; Cowley et al., 1995)*, in rats, at least to the extent that in both species during medullary interstitial infusion of a radiolabelled small molecule ($[^{14}C]$-clentiazem in their case and $[^{3}H]$-noradrenaline in the present study), the radiolabel within the infused kidney was mostly concentrated in the medulla and papilla with very little radiolabel in the cortex of the infused kidney or in the contralateral kidney.

A strength of our previous study was the estimation, during medullary interstitial infusion of $[^{3}H]$-noradrenaline, of the amount of radiolabel spilled over from the infused kidney into the renal vein, the amount of this radiolabel that re-entered the kidney via the renal artery, and the amount of radiolabel excreted by both the infused and contralateral kidneys. Although it should be acknowledged that much of the radiolabel in these biological fluids reflect metabolites of $[^{3}H]$-noradrenaline, we argue that most small, uncharged molecules should be handled similarly by the kidney during medullary interstitial infusion (in rabbits). In the case of noradrenaline and other molecules that are rapidly metabolized *in vivo*, the proportion of the radiolabel that represents intact $[^{3}H]$-noradrenaline must become less in proportion with the distance traveled from the infusion site. A limitation of our studies is that although we were able to determine the level of radiolabel exiting and re-circulating from and to the kidney, we were unable to determine the concentration of "active", non metabolized noradrenaline. Nevertheless, the dose related pressor effects which were observed indicate that significant quantities of intact noradrenaline do spill over during o.ter medullary interstitial infusion. This demonstrates that the technique must be limited to substances that are rapidly metabolized, or to experimental settings where the effect of spillover can be controlled for. Using this technique, we were also able to establish that during inner medullary interstitial infusion of the same dose of $[^{3}H]$-noradrenaline, ~60% of the infused radiolabel exited the kidney via the ureter, and therefore produced no significant systemic effect.

The aim of the current study was to develop a method for the for the delivery of substances to the renal medulla of rabbits and to establish the optimum catheter design and catheter length for these infusions. Two different catheter lengths were tested, and chronically implanted, so that their tips lay in the outer medullary (8.5 mm below the cortical surface) and inner medullary (10.5 mm below the cortical surface) interstitium. Our results indicate that catheter position within the kidney was important, since outer medullary interstitial infusion produced a greater concentration of radiolabel within the areas of the kidney likely to control regional MBF. Inner medullary infusion resulted in

much of the radiolabel bein exited from the kidney, and produced no change in regional kidney blood flows *(Correia, 1997).*

Having established that a catheter tip positioned in the outer medullary interstitium is optimal for infusions intended to manipulate MBF, a further comparison was made to establish any possible differences between acute and chronically positioned catheters. It was found that although both catheter types produced similar levels of radiolabel concentration throughout the kidney, chronically positioned catheters achieved significantly higher concentrations throughout the outer stripe, when compared to acutely implanted catheters. On the other hand, a disadvantage of the chronically implanted catheter is the need for a preliminary surgical procedure.

Another important finding of our current study was that catheters chronically implanted in the kidney remain patent *in vivo* for up to six weeks following implantation. This result combined with the acquired knowledge of how the rabbit kidney handles infused substances with respect to regional distribution (current study) and disposition *(Correia, 1997)* has provided us with the information required for the adaptation of this technique to longitudinal studies in rabbits.

This technique is therefore suitable for acute and chronic medullary interstitial infusion of substances that are rapidly metabolized *in vivo*. With the caveats discussed above in mind, there are considerable advantages to employing a larger species to longitudinal studies. In the case of the conscious rabbit, it is possible to obtain long-term and simultaneous data regarding hormonal status *(Evans et al., 1994),* cardiac output *(Evans et al., 1993),* RBF *(Tomoda et al., 1996),* renal sympathetic nerve activity *(Malpas and Evans, 1998),* and more recently MBF and CBF *(Evans et al., 2000),* therefore providing more complete data regarding the responses to short and long term changes in renal MBF.

3.5 Conclusions

Taken together with those of our previous study *(Correia, 1997)*, the findings of the current study show that outer medullary interstitial infusion is a useful method for targeting vasoactive agents to the renal medulla. This is true not only for acutely positioned catheters, but also for chronically implanted catheters, that remain patent for at least 6 weeks. On the other hand, at least in the rabbit, this technique is apparently limited to the use of compounds which are readily metabolized, since the infused radiolabel is circulated and redistributed throughout the animal and the kidney. In the experiments described in the next chapter, we used this technique to investigate the effects of reduced MBF on renal antihypertensive mechanisms.

Chapter Four

EFFECTS OF RENAL MEDULLARY AND INTRAVENOUS NORADRENALINE ON RENAL ANTIHYPERTENSIVE FUNCTION

4.0 Summary

1. Evidence suggests that increasing RAP activates three renal antihypertensive mechanisms; reduced renin release, pressure diuresis/natriuresis, and the release of a putative renal medullary depressor hormone.

2. In previous studies, we found that intravenous noradrenaline infusion (300 ng/kg/min) selectively reduces CBF, whereas medullary interstitial infusion of the same dose selectively reduces MBF. This provides a useful tool for studying the role of MBF in a variety of different experimental settings.

3. Therefore in the current study, medullary interstitial and intravenous infusions of noradrenaline were employed to study the role of MBF responses to increased RAP.

4. In order to test the involvement of MBF in renal antihypertensive mechanisms, an extracorporeal circuit was established in anaesthetised rabbits. This circuit enables RAP to be altered independently of the systemic circulation. A right nephrectomy was performed, and the left ureter was cannulated for urine sample collection. CBF and MBF were determined by laser-Doppler flowmetry, and total RBF was measured by transit-time ultrasound flowmetery.

5. The experiment consisted of two phases. During Phase I, RAP was set at ~65 mmHg and rabbits received either an intravenous or medullary interstitial infusion of noradrenaline (300 ng/kg/min). During phase two, these infusions were continued, and RAP was increased in stepwise fashion from ~65 to ~160 mmHg.

6. With RAP at ~65 mmHg, intravenous and medullary interstitial noradrenaline infusions similarly increased MAP (by 12 – 17% of baseline), and reduced total RBF (by 16 – 17%) and CBF (by 13 – 19%), but only medullary interstitial noradrenaline reduced MBF (by 28%).

7. When RAP was increased to ~160 mmHg, urine output and sodium excretion increased exponentially, while plasma renin activity and MAP fell.

8. Medullary interstitial but not intravenous noradrenaline attenuated the increased diuresis and natriuresis and the depressor response to increased RAP.

9. Our findings indicate that noradrenaline can act within the renal medulla to attenuate the pressure diuresis/natriuresis response and perhaps also release of the putative renal medullary depressor hormone.

4.1 Introduction

It has been hypothesized that the level of renal MBF is an important determinant of urinary sodium excretion, and may be the key initiating factor in the pressure diuresis/natriuresis response *(Cowley, 1997)*. In turn, the impact of MBF on the pressure natriuretic mechanism provides an explanation for the effects of chronic changes in MBF on the long-term control of arterial pressure *(Cowley, 1997)*. Thus, in rats, chronic reductions in MBF shift the pressure diuresis/natriuresis relationship toward higher pressures and lead to hypertension in normotensive animals. Conversely, chronic increases in MBF shift the pressure diuresis/natriuresis relation toward lower pressures and ameliorate hypertension in spontaneously hypertensive rats *(Cowley, 1997)*.

From studies using an extracorporeal circuit in anaesthetised rabbits *(Bergstöm and Evans, 1998)*, our group have obtained preliminary evidence indicating that influences on the release and/or actions of the putative renal medullary depressor hormone might also contribute to the impact of MBF on the long-term control of arterial pressure. In this model, three major renal antihypertensive mechanisms can be studied simultaneously. Thus, when RAP is acutely increased in this model, plasma renin activity (PRA) is reduced (indicating reduced renal renin release), urine flow and urinary sodium excretion increase exponentially (pressure diuresis/natriuresis), and systemic MAP is reduced. The depressor response to increased RAP appears to be largely independent of the reduced activity of the renin-angiotensin system, in view of the fact that it is little affected by blockade of angiotensin-converting enzyme *(Christy et al., 1993)*. Presently available data also indicates that it is largely independent of the associated diuresis and diuresis/natriuresis, in view of the fact that haemoconcentration is not observed *(Christy et al., 1991; Christy et al., 1993; Bergstöm and Evans, 1998)*. There is, however, clear evidence for a role of the renal medulla, in that the depressor response is abolished by chemical medullectomy *(Christy et al., 1991)*.

In a recent study performed in our laboratory, the depressor response to increased RAP was blunted by medullary interstitial infusion of [Phe^2, Ile^3, Orn^8]-vasopressin, a treatment that selectively reduced MBF *(Bergstöm and Evans, 1998)*. These results indicate a possible role of MBF in the release of this putative hormone. However, it was difficult to determine whether this effect of medullary interstitial infusion of the V_1-agonist was specifically due to reduced MBF or to some other action of the infused agent. For example, this treatment also reduced total RBF and CBF. It was also no possible to exclude roles of non-flow-mediated extra-vascular actions on V_1-receptors in the kidney, or even extra-renal V_1 receptors, which might blunt the release and/or actions of the putative renal medullary depressor hormone *(Bergstöm and Evans, 1998)*.

The aim of the current study was to more directly test the role of the medullary microcirculation in modulating the antihypertensive responses to increased RAP. The development and validation of the technique of renal medullary interstitial infusion of noradrenaline now makes this possible *(Correia, 1997;* Chapter 3). In particular, we showed that medullary interstitial infusion of noradrenaline reduces MBF twice as much as CBF, whereas intravenous noradrenaline reduces only CBF, in rabbits. Therefore, the effects of medullary interstitial infusion and intravenous infusion of noradrenaline were compared, on the antihypertensive responses to increased RAP. The use of this experimental design makes it possible to control for the effects of noradrenaline exerted outside the renal medulla. The results of this study provide further support for the hypothesis that MBF plays a critical role in the long term regulation of arterial pressure, through its impact on pressure natriuretic /diuretic mechanisms and perhaps also via its effects on the release of the putative renal medullary depressor hormone.

4.2 Methods

4.2.1 Experimental preparation

Twenty-nine male New Zealand White rabbits, weighing 2.50 – 2.94 (mean 2.62) kg, were studied. On the experimental day, catheters were placed in both central ear arteries and marginal ear veins, the rabbits were anaesthetized, a right nephrectomy was performed, and an extracorporeal circuit was established. To test the effects of noradrenaline infusion on systemic and renal haemodynamics, rabbits received either an intravenous (n=7) or medullary interstitial (n=6; outer medulla) infusion of noradrenaline, or its vehicle. Intravenous noradrenaline infusion was previously shown *(Correia, 1997)* to cause a selective reduction in CBF with no significant effect on MBF, whereas outer medullary interstitial infusion causes a marked reduction in MBF.

Therefore these infusions were continued throughout the remainder of the experiment during which RAP was progressively increased from ~65 to ~160 mmHg, in order to test the effects of reduced CBF and MBF on responses to increased RAP.

4.2.2 Extracorporeal circuit

Rabbits were prepared for surgery, according to Section 2.2.1 of Chapter 2. Briefly, a right nephrectomy was first performed to remove any confounding influence of the contralateral kidney, and an extracorporeal circuit was then established, as described in detail in section 2.2.4 of Chapter 2. This circuit allows RAP to be set to any level, above or below systemic arterial pressure without changing total flow through the circuit or directly affecting systemic haemodynamics. Regional kidney blood flow was monitored throughout the experiment by the positioning of laser-Doppler flow probes in the outer medullary interstitium and on the superficial cortical surface, as described in detail in Section 2.2.6 of Chapter 2. In fourteen rabbits, medullary infusion catheters were acutely positioned laterally, 10 mm either side of the laser-Doppler flow probe, and advanced so that their tips lay at the junction of the outer and inner stripes of the outer medulla (8.5 mm below the cortical surface) (Section 2.2.3, Chapter 2).

Once the extracorporeal circuit was established, RAP was set at ~ 65 mmHg for a 60 min equilibration period. A bolus dose of [^{3}H]-inulin (4 μCi, NEN Research Products) was administered in 1.0 ml of 154 mM NaCl. An infusion of 10% (vol/vol) polygeline (Hemaccel, Hoechst, Melbourne, VIC, Australia) containing 200 IU/ml sodium heparin and 0.3 μCi/ml [^{3}H]-inulin was then initiated (0.18 ml/kg/min) which continued for the duration of the experiment.

4.2.3 Measurements

Systemic (MAP and HR) and renal haemodynamics (RAP, RBF, CBF, MBF) were measured throughout the experiment, and are described in detail in Sections 2.7.1, Chapter 2. Briefly, RAP was measured in a side-arm catheter, 3 mm proximal to the tip of the cannula inserted into the renal artery while MAP was measured via a catheter in a central ear artery. Blood flow through the renal limb of the circuit was measured with an in-line ultrasonic flow probe (type 4N, Transonic Systems Inc). These signals were amplified, recorded, and digitized, as described in Section 2.7.1 of Chapter 2.

Plasma renin activity and urinary concentrations of [^{3}H]-inulin and sodium were determined as detailed in Sections 2.7.2 (processing of blood and urine samples) and 2.7.3 (plasma renin activity). [^{3}H]-inulin clearance was used to estimate glomerular filtration rate (GFR). At the completion of each experiment, the left kidney was removed, decapsulated and desiccated, and its dry weight was determined. All values of renal blood flow, GFR, urine flow, and urinary sodium excretion are therefore expressed per gram of dry kidney weight (expressed as g [mean 1.77 ± 0.03 g]).

4.3   Experimental protocols

4.3.1   General

Each experimental protocol consisted of 2 phases. Phase 1, which followed the 60 minute equilibration period, tested the effects of either outer medullary interstitial (Protocol 1) or intravenous (Protocol 2) infusion of noradrenaline on systemic and renal haemodynamics. The second phase of each protocol involved testing the effect of these treatments on the responses to increased RAP (Figure 4.2).

4.3.2   Effects of outer medullary interstitial noradrenaline: protocol 1

After 10 minutes of stable baseline recordings, outer medullary interstitial infusion of either noradrenaline (300 ng/kg/min; n=6) or its vehicle (154 mM NaCl, 20 µl/kg/min; n=8) commenced and continued for the remainder of the experiment. Twenty minutes later, RAP was set at ~ 65, 85, 110, 130, and 160 mmHg for consecutive 20 minute periods and, once set, was not readjusted. Urine produced by the left kidney was collected during the final 15 minutes of each period. Arterial blood (1 ml) for clearance measurements was collected from an ear artery catheter at the midpoint of each 15 minute clearance period, and samples (1 ml) for determination of plasma renin activity were collected at the midpoint of the first, third, and fifth clearance periods. Blood volume was replaced by an equivalent volume of 10% polygeline solution (Hemaccel). At the end of the fifth clearance period, RAP was returned to ~65 mmHg for a further 20 min.

4.3.3   Effects of intravenous noradrenaline: protocol 2

This protocol was identical to protocol 1, except noradrenaline (300 ng/kg/min; n=7) or its vehicle (20 µl/kg/min; n=8) were administered intravenously via an ear vein catheter.
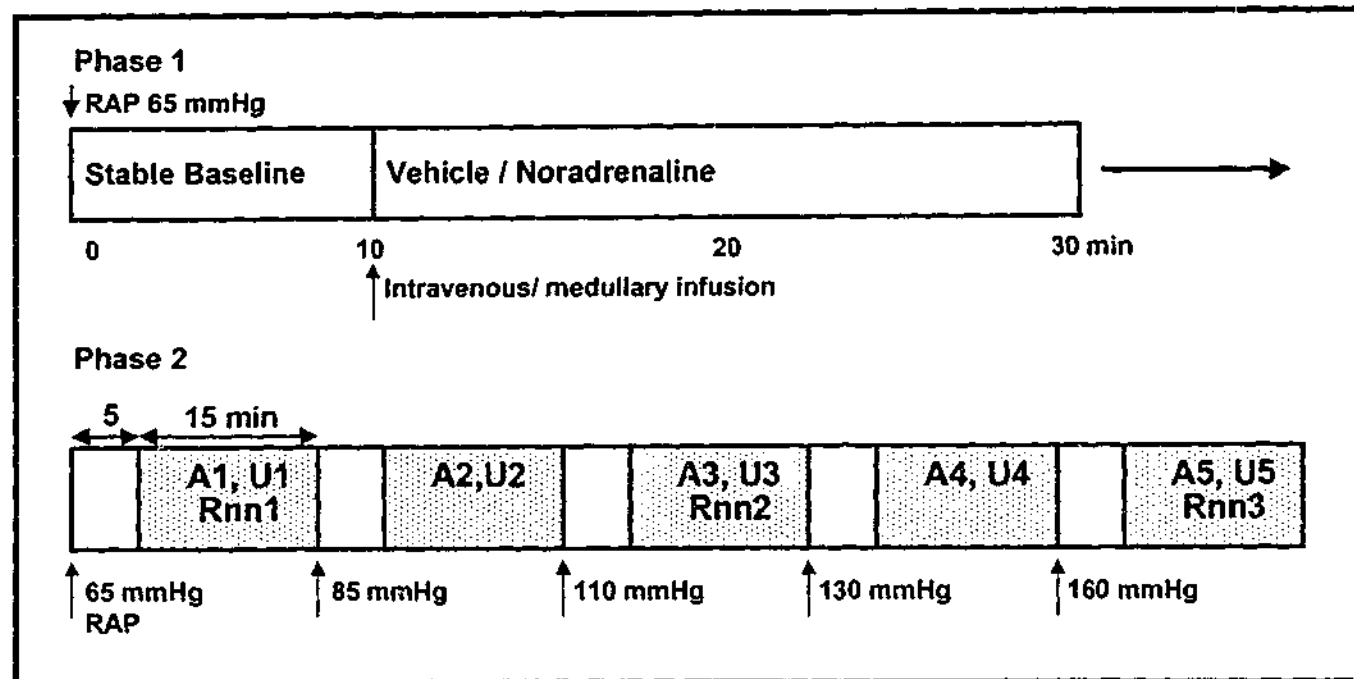
Figure 4.1 Schematic representation of experimental protocol. During a 60-min equilibration period, renal artery pressure (RAP) was set and maintained at 65 mmHg. Phase 1; following 10 min of stable baseline recordings, rabbits received a 20-min infusion of either noradrenaline (300 ng/kg/min) or vehicle (20 µl/kg/min) delivered either intravenously or to the outer medulla. This infusion was continued until the end of the experiment. During Phase 2; RAP was re-set and maintained at 65, 85,110,130 and 160 mmHg for consecutive 20-min periods. During the final 15 min of each 20-min period urine was collected for urinary clearance measurements (shaded areas; U1-U5), 1 ml blood samples were collected at the mid point of each collection period (A1-A5) for renal clearance measurements and haematocrit, and at every second period a further 1 ml blood sample was collected for determination of plasma renin activity (Rnn1-Rnn3).

4.4 Statistical analysis

4.4.1 Phase I

These data were first submitted to repeated measures analysis of variance, to determine whether responses to noradrenaline differed from those of the saline vehicle. To test whether each of the noradrenaline or vehicle treatments altered baseline systemic and renal haemodynamics, average levels of each variable during the period 10 to 20 minutes after the initiation of the infusion were compared with the levels during the 10 minute control period by paired *t* test.

4.4.2 Phase II

These data were analyzed by ANOVA adapted for repeated measures analysis of variance. To test whether increasing RAP altered each variable, a 1-way analysis was first performed on all vehicle treated rabbits to provide the main effect of increasing RAP (P_{RAP}). The interaction term between RAP and treatment (vehicle or noradrenaline) was then determined from 2-way analyses for each route (intravenous and medullary interstitial). This tested for effects of noradrenaline infusion on the responses to increased RAP.

4.5   Results

4.5.1   Phase I: Effects of renal medullary interstitial and intravenous noradrenaline infusions

4.5.1.1 Effects of renal medullary interstitial noradrenaline on systemic and renal haemodynamics

Renal medullary interstitial infusion of noradrenaline (300 ng/kg/min) was accompanied by progressive haemodynamic changes that reached steady state by 10 minutes after the infusion began (Figure 4.2). The changes included increases in RAP (by $19 \pm 4\%$ of its baseline level during the period 10 to 20 minutes after beginning the infusion) and MAP (by $17 \pm 4\%$) and reductions in RBF ($16 \pm 3\%$), CBF ($13 \pm 2\%$), and MBF ($28 \pm 9\%$) but no significant change in HR ($1 \pm 2\%$ change). Medullary interstitial infusion of the vehicle had no significant effect on any of these variables (Table 4.2).

4.5.1.2 Effects of intravenous noradrenaline on systemic and renal haemodynamics

Intravenous noradrenaline (300 ng/kg/min) was also accompanied by reductions in RBF (by $17 \pm 9\%$ of its baseline value) and CBF (by $19 \pm 3\%$) and by increases in MAP ($12 \pm 4\%$) and RAP ($4 \pm 1\%$). However, unlike renal medullary noradrenaline, intravenous noradrenaline had no significant effect on MBF ($1 \pm 8\%$ change). Intravenous infusion of the vehicle was accompanied by small variations in MAP ($4 \pm 1\%$), HR ($1 \pm 1\%$), and RBF ($-4 \pm 2\%$) but no significant changes in RAP, CBF, or MBF (Figure 4.3, Table 4.1).

4.5.2 Phase I1: Effects of increasing renal artery pressure in anaesthetised rabbits

4.5.2.1 Renal haemodynamic variables (vehicle infusion)

As shown in Figure 4.4, as RAP was increased from 66 ± 1 to 158 ± 3 mmHg, there were progressive increases in RBF (from 13 ± 1 to 29 ± 2 ml/min/g) and GFR (from 0.8 ± 0.1 to 3.0 ± 0.4 ml/min/g) (P_{RAP} <0.001). Renal vascular resistance and filtration fraction responded biphasically. As RAP was increased from ~65 to ~110 mmHg, renal vascular resistance increased from 5.9 ± 0.8 to 7.7 ± 2.3 mmHg/ml/min/g before decreasing to 6.9 ± 0.6 mmHg/ml/min/g when RAP was increased to ~ 160 mmHg (P_{RAP} =0.05). Filtration fraction also responded in a similar manner, increasing from $3.5 \pm 1.1\%$ to $9.3 \pm 1.9\%$ as RAP was increased from ~65 to ~110 mmHg, before decreasing to $8.0 \pm 1.4\%$ when RAP was increased to ~160 mmHg (P_{RAP} < 0.001) (Figure 4.4, Table 4.2).

4.5.2.2 Regional renal blood flows (vehicle infusion)

As shown in Figure 4.5, as RAP was increased from 66 ± 1 to 158 ± 3 mmHg, CBF increased progressively from 235 ± 31 to 329 ± 45 perfusion units (P_{RAP} =0.01) whereas although MBF did not change significantly.

4.5.2.3 Renal excretory variables (vehicle infusion)

As shown in Figure 4.6, as RAP was increased form ~65 to ~160 mmHg, there were progressive increases in urine volume (from 0.09 ± 0.02 to 1.24 ± 0.09 ml/min/g) and urinary sodium excretion (from 12 ± 2 to 161 ± 13 μmol/min/g) and in the fractional excretions of urine (from $12 \pm 1\%$ to 43 $\pm 3\%$) and sodium (from $11 \pm 2\%$ to $40 \pm 3\%$) (P_{RAP} <0.001).

4.5.2.4 Systemic haemodynamic variables (vehicle infusion)

As shown in Figure 4.7, as RAP was increased from ~65 to ~160 mmHg, MAP fell progressively from 78 ± 3 to 50 ± 5 mmHg and at an increasing rate of 0.04 ± 0.06 to 0.96 ± 0.15 mmHg/min (P_{RAP} <0.001). Haematocrit decreased gradually from $22.1 \pm 0.9\%$ to $21.6 \pm 0.9\%$ as RAP was increased from ~65 to ~110 mmHg and increased thereafter to $22.5 \pm 0.9\%$ when RAP was increased to ~ 160 mmHg (P_{RAP} =0.04). Heart rate tended to decrease (from 266 ± 5 to 253 ± 8 beats/min) as RAP increased to ~ 160 mmHg (P_{RAP} =0.05).

4.5.2.5 Plasma renin activity (vehicle infusion)

Plasma renin activity fell as RAP was increased, averaging 14 ± 3, 12 ± 2, and 7 ± 3 ng angiotensin I/ml/h when RAP was ~65, 110, and 160 mmHg, respectively (P_{RAP} =0.04), Figure 4.8.

4.5.3 Effects of medullary interstitial and intravenous noradrenaline on responses to increased renal artery pressure

The RAP-dependent increases in RBF and CBF were significantly attenuated by medullary interstitial noradrenaline (Figures 4.4and 4.5). RAP dependent increases in urine volume and urinary sodium excretion (Figure 4.5) and decreases in MAP (Figure 4.6) were significantly attenuated, but no significant effect on plasma renin activity was observed. Medullary interstitial noradrenaline also significantly altered the response of haematocrit to increased RAP, attenuating the increase in haematocrit as RAP was increased to ~110 mmHg. Intravenous infusion of noradrenaline did not significantly influence any of these responses to increased RAP (Figures 4.3 – 4.7).

4.5.4 Effects of resetting renal artery pressure to ~65 mmHg

When RAP was reset to ~ 65 mmHg, RBF returned to levels similar to those observed during the initial period (most leftward point in Figure 4.4) in vehicle-treated rabbits (-3 $\pm$ 4% different from its previous level, during the period 15 to 20 minutes after RAP was reset to ~65 mmHg) and in rabbits treated with medullary interstitial noradrenaline (-13 $\pm$ 4%) and intravenous noradrenaline (39 $\pm$ 27%). MAP rose when RAP was reset to ~65 mmHg but did not completely recover to its previous level in vehicle-treated rabbits (-28 $\pm$ 5%) and in rabbits treated with outer medullary noradrenaline (-14 $\pm$ 6%) and intravenous noradrenaline (-30 $\pm$ 10%).
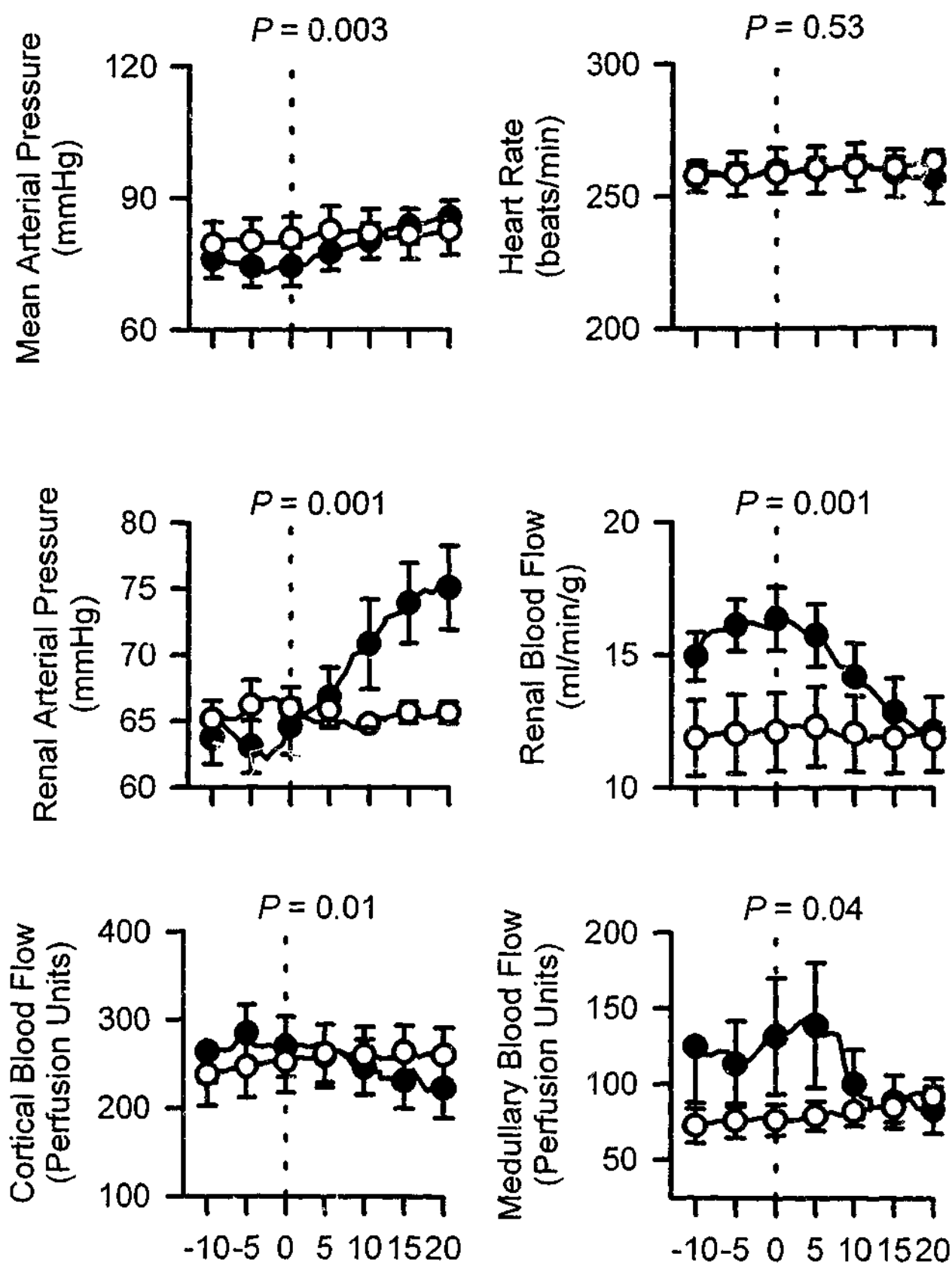
Time from Commencement of Medullary Noradrenaline Infusion (min)

Figure 4.2 Effects of renal medullary interstitial infusion of noradrenaline (300 ng/kg/min) on systemic and renal haemodynamic variables. (O) Vehcile (20 μl/kg/min; 154 mM NaCl; n=8). (●) Noradrenaline (300 ng/kg/min; n=6). Lines show 1 min means, while symbols show 5 min means ± SEM for each variable. *P* values test whether the response to noradrenaline differed from that of its vehicle ($P_{Time*Treatment}$).
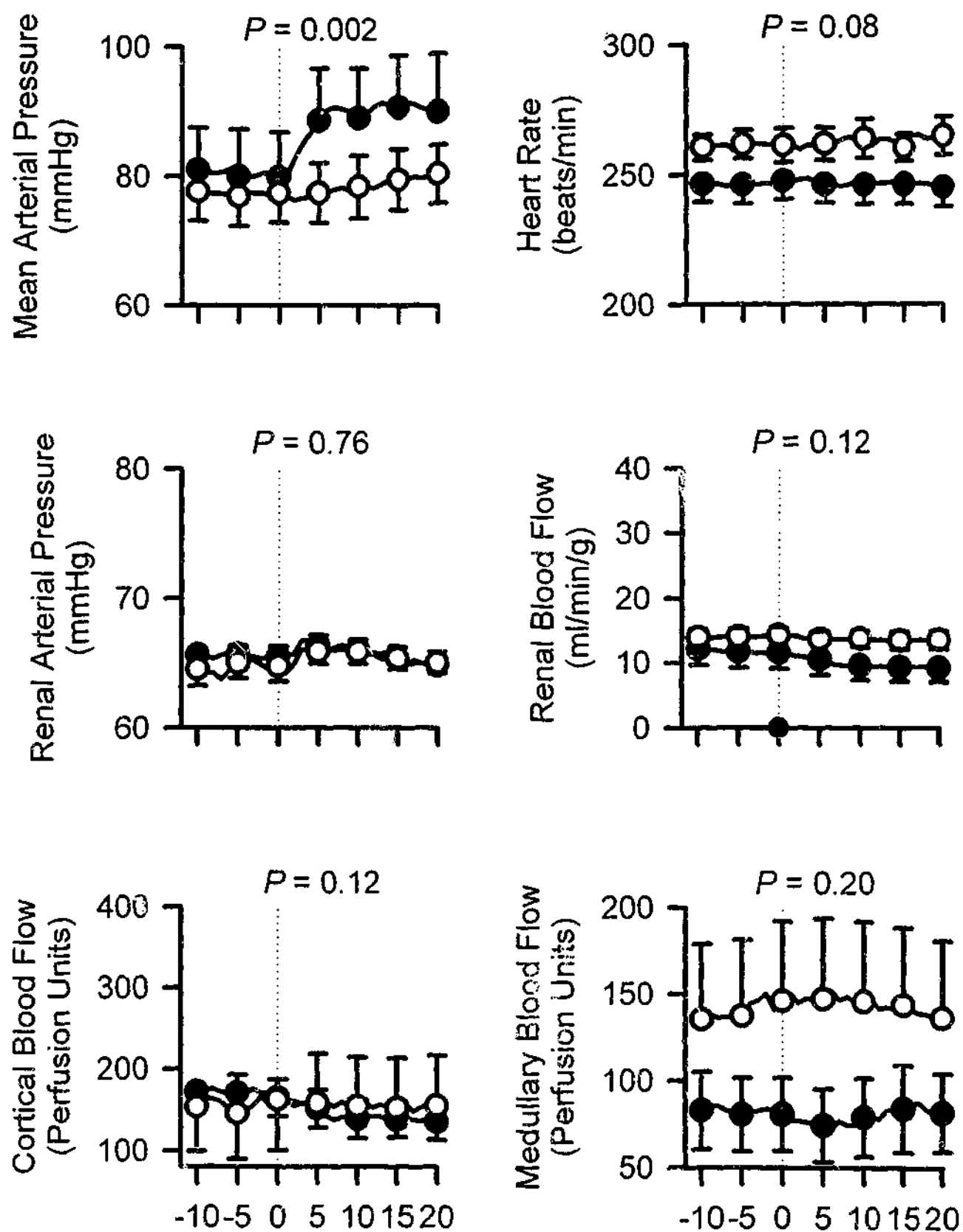
Time from Commencement of Intravenous Noradrenaline Infusion (min)

Figure 4.3 Effects of intravenous noradrenaline (300 ng/kg/min), on systemic and renal haemodynamic variables. (O) Vehicle (20 µl/kg/min; 154 mM NaCl; n=8). (●) Noradrenaline (300 ng/kg/min; n=7). Lines, symbols, error bars, and *P* values are as for Figure 4.2.
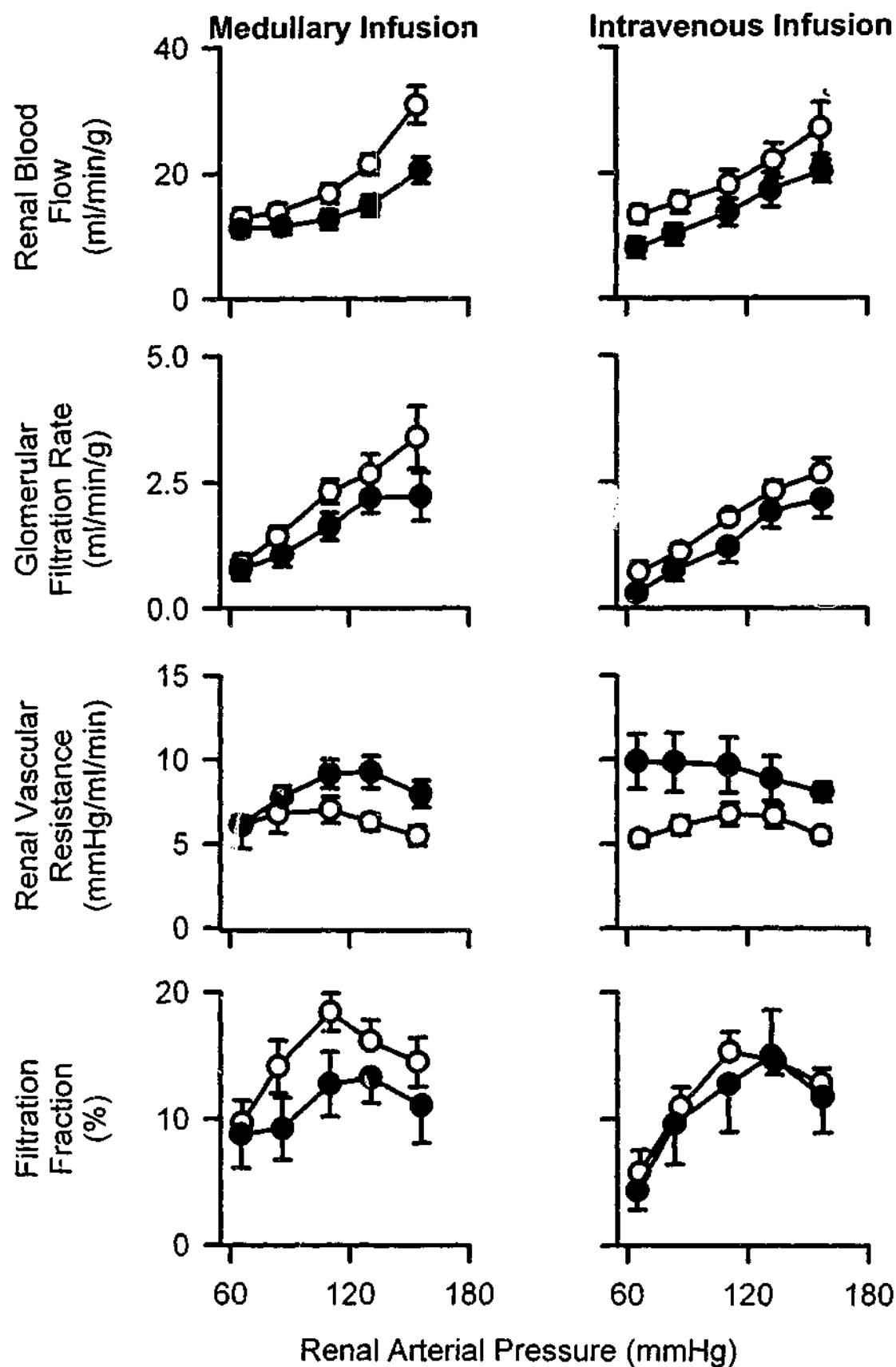
Figure 4.4 Effects of renal outer medullary interstitial and intravenous infusion of noradrenaline on renal haemodynamic variables. Symbols are as for Figure 4.2, and are the mean value for each 15 min at each level of RAP. Error bars are as for Figure 4.2. Outcomes of repeated measures analyses of variance, testing for the effects of increasing RAP ($P_{Treatment}$) and RAP dependent ($P_{Pressure \cdot Treatment}$), and independent ($P_{Treatment}$) effects of these treatments are given in Table 4.2. ($P_{Pressure \cdot Treatment}$) tested whether noradrenaline treatment altered the response to increased RAP.
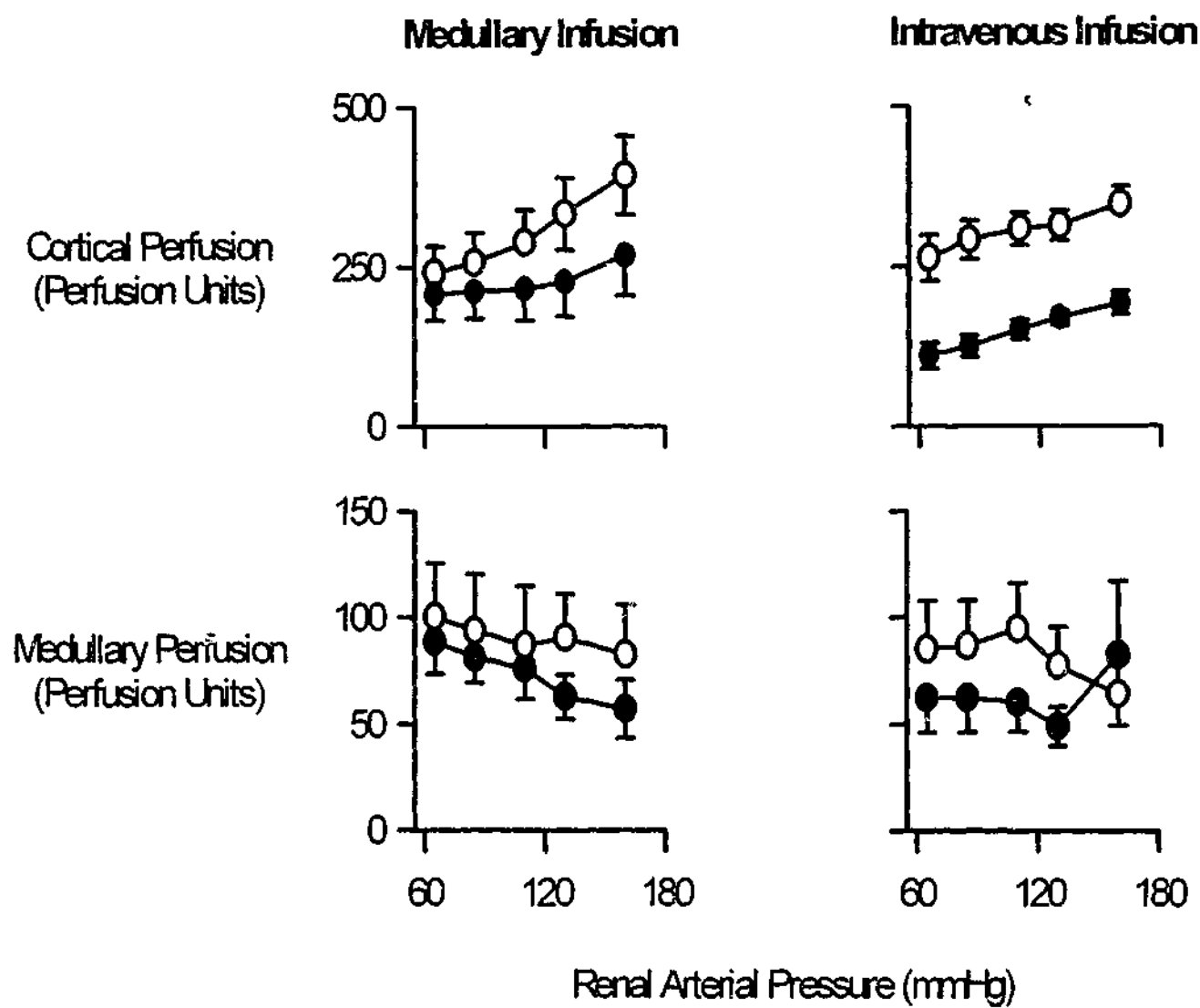
Figure 4.5   Effects of renal outer medullary interstitial and intravenous infusion of noradrenaline on responses of renal cortical and medullary perfusion to progressively increasing RAP. Symbols, error bars, and *P* values (given in Table 4.2) are as for Figure 4.4.
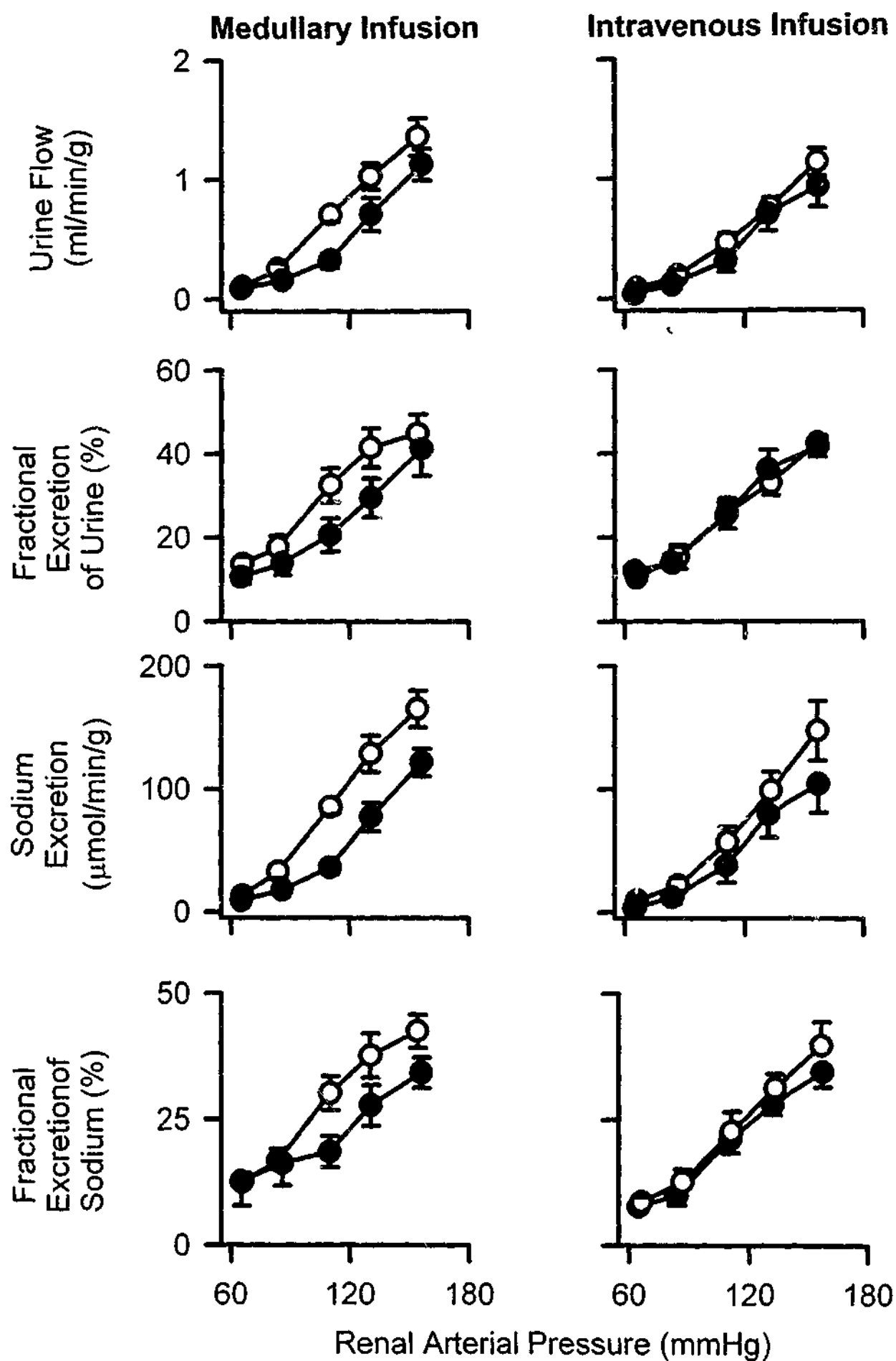
Figure 4.6   Effects of renal outer medullary interstitial and intravenous infusion of noradrenaline on renal excretory responses to progressively increasing RAP. Symbols, error bars, and *P* values (given in Table 4.2) are as for Figure 4.4.
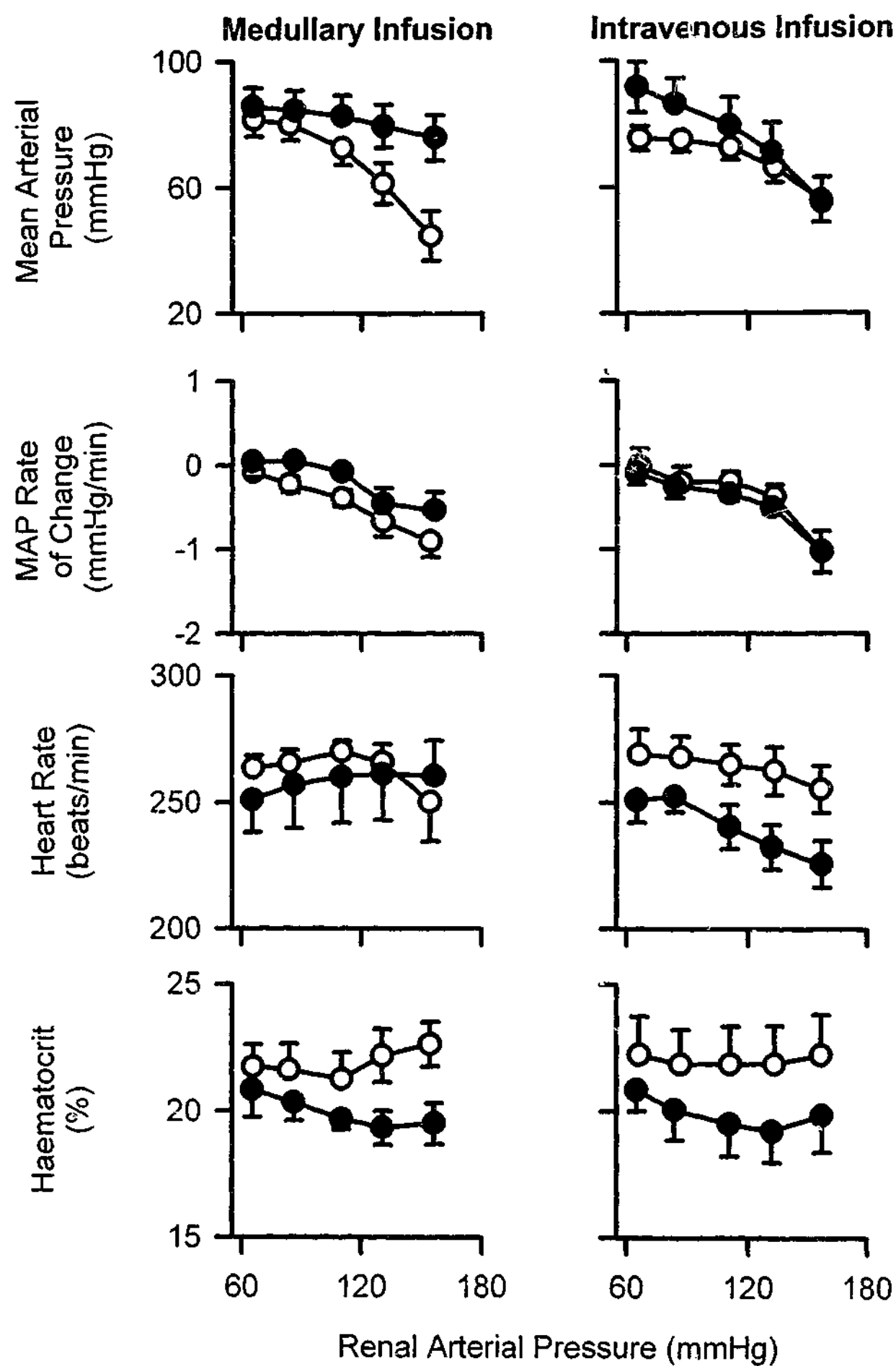
Figure 4.7 Effects of renal outer medullary interstitial and intravenous infusion of noradrenaline on systemic haemodynamic responses to progressively increasing RAP. Symbols, error bars, and *P* values (given in Table 4.2) are as for Figure 4.4

Medullary Infusion **Intravenous Infsuion**

Plasma Renin
Activity
(ng Ang 1/ml/h)

Renal Arterial Pressure (mmHg)
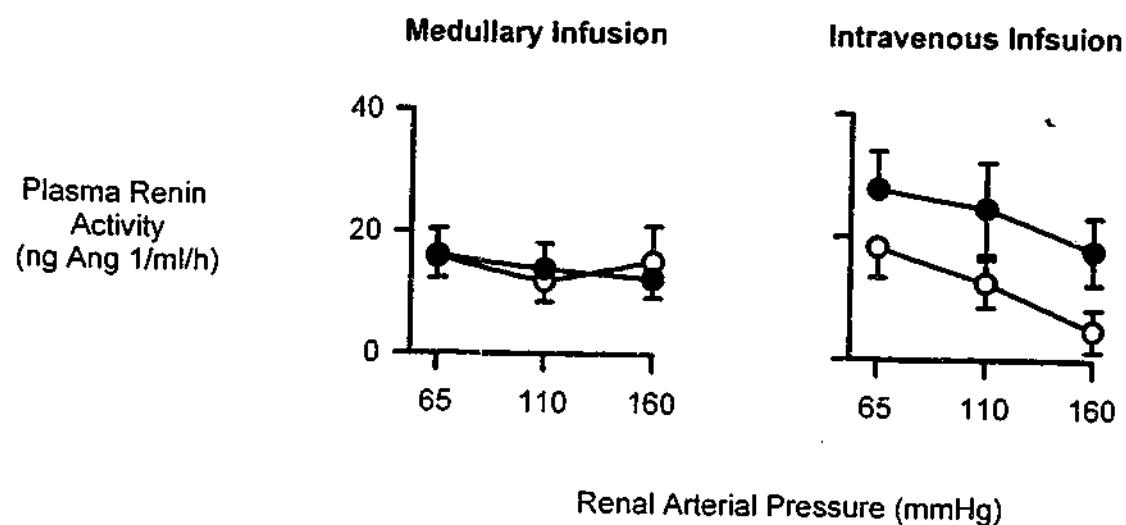
Figure 4.8 Effects of renal outer medullary interstitial and intravenous infusion of noradrenaline on responses of plasma renin activity to progressively increasing RAP. Symbols, error bars, and *P* values (given in Table 4.2) are as for Figure 4.2.

Table 4.1   Effects of medullary interstitial and intravenous noradrenaline on systemic and renal haemodynamics (Data presented as percentage change).

	Medullary Interstitial Infusion		Intravenous Infusion	
	Vehicle	Noradrenaline	Vehicle	Noradrenaline
MAP	2.4 ± 1.4	17 ± 4	3.5 ± 0.6	12 ± 4
HR	- 0.7 ± 2.3	2 ± 1	1.3 ± 0.5	-0.02 ± 3.0
RAP	- 0.3 ± 2.0	19 ± 4	0.8 ± 1.0	-17 ± 9
RBF	0.8 ± 2.5	-16 ± 3	-3.7 ± 5.8	-17 ± 9
CBF	0.3 ± 2.3	-13 ± 2	0.3 ± 5.8	-19 ± 3
MBF	3 ± 10	-28 ± 9	-0.4 ± 4.1	-1.3 ± 7.5

MAP, mean arterial pressure; HR, heart rate; RAP, renal arterial pressure: RBF, renal blood flow: CBF, cortical blood flow; MBF, medullary blood flow.

Table 4.2   Outcomes of repeated measures analyses of variances for the data depicted in Figures 4.4-4.8.

Variable	Pressure	Medullary Noradrenaline		Intravenous Noradrenaline	
		Treatment	Press*Treat	Treatment	Press*Treat
Renal Haemodynamics: Figure 4.4					
RAP	<0.001	0.39	0.51	0.39	0.57
RBF	<0.001	0.05	0.003	0.10	0.76
GFR	<0.001	0.53	0.76	0.33	0.72
RVR	0.05	0.15	0.01	0.16	0.27
FF%	0.001	0.44	0.06	0.65	0.82
Regional Kidney Perfusion: Figure 4.5					
CBF	0.005	0.31	0.01	<0.001	0.62
MBF	0.34	0.47	0.55	0.49	0.13
Renal Excretory Function: Figure 4.6					
U_{VOL}	<0.001	0.01	0.04	0.29	0.58
FE_{VOL}	<0.001	0.20	0.07	0.91	0.68
$U_{Na+}V$	<0.001	0.01	0.03	0.32	0.39
FE_{Na+}	<0.001	0.14	0.16	0.44	0.84
Systemic Haemodynamics: Figure 4.7					
MAP	<0.001	0.02	0.05	0.38	0.11
DMAP/dt	<0.001	0.03	0.92	0.52	0.89
HR	0.05	0.75	0.19	0.05	0.40
Hct	0.04	0.12	0.02	0.27	0.17
Plasma Renin Activity: Figure 4.8					
PRA	0.04	0.98	0.39	0.22	0.29

Each P value was derived from a repeated measures ANOVA which tested whether noradrenaline treatment altered the responses to increased RAP. Press*Treat, Pressure*Treatment. RAP= renal arterial pressure, RBF = renal blood flow, GFR = glomerular filtration rate, RVR = renal vascular resistance, FF = filtration fraction, CBF = cortical blood flow, MBF = medullary blood flow, U_{VOL}= urine flow, FE_{VOL}= fractional excretion of urine, $U_{Na+}V$= urinary sodium excretion, FE_{Na+}= fractional excretion of sodium, MAP = mean arterial pressure, HR = heart rate, Hct= haematocrit, PRA= plasma renin activity.

4.6   Discussion

We have recently shown in anaesthetised rabbits that medullary interstitial infusion of noradrenaline (300 ng/kg/min) reduces MBF more than CBF, and that intravenous infusion of the same dose only reduces CBF *(Correia, 1997)*. Consistent with this observation, medullary interstitial infusion of [^{3}H]-noradrenaline resulted in greater levels of radiolabel in the medulla than in the cortex (Chapter 3). In the present study, we used these findings as a tool to examine the role of MBF in modulating the renal antihypertensive responses to increased RAP. Our major finding was that medullary interstitial noradrenaline, but not intravenous noradrenaline, attenuated both the pressure diuresis/natriuresis response and the depressor response to increased RAP. These observations provide further support for the hypothesis that MBF plays an important role in the control of arterial pressure, both through its involvement in the mechanisms mediating pressure diuresis/natriuresis and possibly also in the mechanisms mediating release of the putative renal medullary depressor hormone.

Consistent with our previous observations in a conventional anaesthetised rabbit preparation *(Correia, 1997)*, in the extracorporeal circuit model, infusion of noradrenaline increased MAP and reduced RBF and CBF similarly by the two routes. This indicates significant systemic spillover into the renal cortex, consistent with our previous extensive characterization of this method *(Correia, 1997;* Chapter 3). However, our results also indicate that these renal cortical and extra-renal effects of noradrenaline can be effectively controlled for by intravenous infusion. The striking difference between the effects of noradrenaline infused by the two routes was that medullary interstitial infusion of noradrenaline reduced MBF by ~30%, whereas intravenous noradrenaline had little or no effect on MBF. Thus, our present experimental design provided a good paradigm for examining the effects of reduced MBF on the renal antihypertensive responses to increased RAP. We can also be fairly confident that these infusions provided relatively constant renal haemodynamic effects, inasmuch as in all experimental groups, RBF levels were similar at the end of the experiment, when RAP was reset to ~65 mmHg, compared with RBF levels during the initial period at this level of RAP.

One surprising finding in this study was that MBF did not increase as RAP was increased. On face value, these data suggest that MBF is well autoregulated in the extracorporeal circuit model. On the other hand, data concerning MBF throughout the course of Phase II of the experiment may have been confounded by changes in kidney size in response to changes in RAP. Since the medullary laser-Doppler flow probe was held and supported in a stable position independent of the kidney, as

the kidney increased in size, it is likely that it was forced to move back along the line of the flow probe. This action would have resulted in MBF being measured in different regions of the medulla throughout the course of the experiment, tending towards the papilla as RAP is increased. Evidence suggesting poor autoregulation of MBF in response to increased RAP *(Roman and Zou, 1993)* would indicate that throughout the course of phase II MBF should have increased. Since blood flow to the renal papilla is substantially less than that in the outer medulla *(Pallone et al., 1990)*, it remains possible that the relative stability of MBF in the face of increased RAP reflects a technical limitation of the laser-Doppler flowmetery technique under the conditions of our experiment.

Our major finding (i.e. medullary interstitial, but not intravenous, infusion of noradrenaline attenuates both the pressure diuresis/natriuresis response and the depressor response to increased RAP) provides evidence for a role of the renal medulla in renal antihypertensive mechanisms. Because intravenous infusion of noradrenaline did not significantly affect these responses, we can confidently exclude roles of noradrenaline mediated outside the kidney that are related, for example, to its systemic pressor effect, modulation of hormone release from extra-renal sites, or inhibition of the peripheral response to the putative renal medullary depressor hormone. We can also probably exclude contributions mediated solely in the cortical microvasculature, inasmuch as RBF and CBF were similarly reduced by medullary interstitial and intravenous infusions of noradrenaline. Roles for the renin-angiotensin system also appear unlikely in view of the fact that plasma renin activity in rabbits receiving medullary interstitial infusions of noradrenaline was indistinguishable from that in vehicle-treated control rabbits.

4.6.1 Pressure diuresis/natriuresis

Medullary interstitial, but not intravenous noradrenaline attenuated the diuretic and natriuretic responses to increased RAP. This effect is also likely to account for the statistically significant influence of medullary interstitial noradrenaline on haematocrit responses to increased RAP, because the reduced diuresis/natriuresis would attenuate haemoconcentration at high levels of RAP. Tubular elements probably play a key role in mediating the attenuated diuresis/natriuresis, because medullary interstitial noradrenaline did not significantly affect the relation between GFR and RAP. Our results indicate a role of the renal medulla in mediating the effects of medullary interstitial infusion of noradrenaline on the pressure diuresis/natriuresis response, but our present experiment does not definitively demonstrate that these effects were mediated by the actions of noradrenaline on MBF. In particular, a direct effect of noradrenaline on tubular function in the medulla cannot be

discounted, because tubular adrenoceptors are certainly known to directly influence fluid and sodium reabsorption in the kidney *(Hesse and Johns, 1984; Gellai, 1990).*

On the other hand, our present results are consistent with the large body of work by Cowley and colleagues *(Cowley, 1997)* showing that treatments that alter MBF, but not those that influence CBF alone, profoundly influence the pressure diuresis/natriuresis response. They have argued that the chief initiating factor in the pressure diuresis/natriuresis response is increased MBF and that this leads to a rise in renal interstitial hydrostatic pressure, which in turn inhibits tubular sodium reabsorption *(Cowley, 1997).*   ‹

However, there is still considerable controversy regarding this hypothesis *(Majid et al., 1997),* so its further critical evaluation is important. In this respect, the present study is significant because it has used an experimental model, with an extracorporeal circuit, that differs from conventional models for studying pressure diuresis/natriuresis, in which RAP is altered by adjustable clamps on the aorta or renal artery *(Roman and Cowley, 1985; Majid et al., 1997).* In our laboratory, other experiments performed using this experimental model have previously shown that another treatment that reduces MBF, blockade of nitric oxide synthesis with N^G-nitro-L-arginine, also attenuates the pressure diuresis/natriuresis response *(Evans et al., 1995).* Conversely, manipulating CBF only by intrarenal infusion of endothelin-1, or systemic administration of endothelin antagonist, has little effect on pressure diuresis/natriuresis responses in this model *(Weekes et al., 2000).*

Importantly, our experimental model allows RAP to be set at levels considerably greater than MAP, so that the pressure diuresis/natriuresis response can be investigated over a wide range of RAP. The renal vascular responses to increased RAP in the extracorporeal circuit model differ from those in conventional preparations *(Roman and Cowley, 1985),* in that RBF and CBF increases considerably as RAP is increased. However, as has been argued previously, autoregulation in this model is seen as an increase in renal vascular resistance in response to increased RAP, but its effect on RBF is limited by the fixed rate of the pump and high resistance of the vena caval limb *(Christy et al., 1993).*

4.6.2   Putative renal medullary depressor hormone

As has been observed previously *(Bergström and Evans, 1998),* increased RAP was accompanied by pressure-dependent reductions in MAP. This response has been extensively characterized previously and appears to be unrelated to the accompanying inhibition of the renin-angiotensin

system *(Christy et al., 1993)*, or increase in urinary volume and urinary sodium excretion *(Christy et al., 1991; Bergstöm and Evans, 1998)*. On the basis of the finding that the depressor response is abolished by chemical medullectomy, it has been proposed that this response to increased RAP is mediated chiefly by release of an as yet to be characterized depressor hormone from the renal medulla *(Christy et al., 1991; Thomas et al., 1996)*. It may be that this putative hormone is identical, or similar, to "medullipin", which has been isolated but not yet fully chemically characterized *(Brooks et al., 1994)*.

Previous studies have shown that some *(Rudenstam et al., 1992; Bergstöm et al., 1995; Bergstöm and Evans, 1998)*, but not all stimuli that reduce MBF *(Evans et al., 1995)*, attenuate the depressor response to increased RAP. In the present study, we found that the depressor response to increased RAP was greatly blunted by medullary interstitial, but not intravenous, infusion of noradrenaline. Thus, our results provide the most direct evidence yet obtained, suggesting that the level of MBF influences the release of the putative renal medullary depressor hormone.

Nevertheless, we cannot as yet completely exclude the possibility that some other action of noradrenaline in the renal medulla, such as the direct action on renal medullary interstitial cells, the proposed site of storage and release of medullipin *(Thomas et al., 1996)*, inhibits the release of the putative renal medullary depressor hormone. However, given our previous finding that medullary interstitial infusion of [Phe^2, Ile^3, Orn^5]-vasopressin reduces MBF and attenuates the depressor response to increased RAP *(Bergstöm and Evans, 1998)*, a role for the medullary microvasculature seems worth of further investigation. To this end, future studies should replicate this experimental paradigm with other pharmacological agents that might selectively decrease and increase MBF.

4.7 Conclusions

Our findings indicate that noradrenaline can act within the renal medulla to attenuate the pressure diuresis/natriuresis response and the release of the putative renal medullary depressor hormone. At present, we cannot be certain that this effect of noradrenaline is mediated by the accompanying reduced MBF, but we have strong circumstantial evidence that this is so. Any vasoactive agent is likely to have extravascular effects that might influence the antihypertensive responses to increased RAP. Therefore, the only way we can dissect out the relative role of effects on MBF from other actions mediated within the renal medulla is to examine the effects of a range of agents that alter MBF. Our experience so far with extracorporeal circuit models such as that used in the present study is that only treatments that alter MBF influence these renal medullary antihypertensive

mechanisms *(Rudenstam et al., 1992; Bergstöm et al., 1995; Evans et al., 1995; Bergstöm and Evans, 1998;)*. Therefore, it seems likely that the medullary microvasculature plays a key role in the mechanisms controlling blood pressure in the long term, not only via actions on the renal handling of salt and water but also by influencing the release of the putative renal medullary depressor hormone.

On the other hand, to date there have been no studies that have directly tested whether the depressor response to increased RAP is completely independent of the changes in urinary salt and water excretion, and the renin-angiotensin system, that accompany increased RAP. This is the subject of the next chapter of this thesis.

Chapter Five

ROLES OF PRESSURE DIURESIS/NATRIURESIS AND INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM, IN THE DEPRESSOR RESPONSE TO INCREASED RENAL ARTERY PRESSURE.

5.0 Summary

1. Increasing RAP activates renal antihypertensive mechanisms; reduced renin release, pressure natriuresis/diuresis, and perhaps also release of the putative renal medullary depressor hormone, which act together to reduce MAP.

2. Evidence indicates the involvement of the renal medulla and MBF in the long-term regulation of systemic arterial pressure. Treatments which reduce MBF shift the pressure diuresis/natriuresis relationship to higher pressures, and also blunt the depressor response to increased RAP.

3. The aim of this study was to determine the extent to which these antihypertensive mechanisms are linked. That is, we tested the involvement of the pressure diuresis/natriuresis mechanism and inhibition of renal renin release in the depressor response to increased RAP.

4. Ascending aortic flowprobes were implanted 2-3 weeks prior to the acute experiment so that cardiac output could be monitored throughout the establishment of the extracorporeal circuit, and the experimental manipulation of RAP.

5. We tested the effects of increasing RAP on systemic MAP, the pressure diuresis/natriuresis mechanism, and levels of plasma renin activity. Furthermore, we tested the effects on the depressor response to increased RAP by blocking the systemic haemodynamic effects of the pressure diuretic/natriuretic response by an infusion of compound sodium lactate at a rate equivalent to urine flow, and 'clamping' the renin-angiotensin system by simultaneous administration of enalaprilat and angiotensin II.

6. Four groups of rabbits were studied. In a control group, RAP was maintained at ~65 mmHg. In the remaining three groups RAP was increased to ~160 mmHg during which time urine volume was measured each minute. One group received no other treatment, but in the other two

compound sodium lactate was infused at a rate equivalent to urine flow. One of these groups also received intravenous infusions of enalaprilat and angiotensin II to 'clamp' the renin-angiotensin system.

7. Our results show that the depressor effect of increased RAP is abolished if the haemodynamic effects of the pressure natriuretic/diuretic response are blocked by preventing cardiac output from falling by infusion of compound sodium lactate. No further effect of enalaprilat/angiotensin II pretreatment was observed.

8. We conclude from these studies that the fall in MAP seen when RAP is increased in this extracorporeal circuit preparation in the anaesthetised rabbit, occurs chiefly due to negative salt and water balance secondary to the pressure diuresis/natriuresis mechanism, and not due to inhibition of renal renin release or the release of a putative renal medullary depressor hormone.

5.1   Introduction

As observed in the experiments described in Chapter 4, an acute increase in RAP in anaesthetised animals stimulates the activation of renal antihypertensive mechanisms which act in concert to restore arterial pressure to normal levels. Renal renin release is reduced, so that the activity of the pro-hypertensive renin-angiotensin system is inhibited *(Navar et al., 1996)*. Urinary excretion of salt and water increases exponentially with the increased RAP, so reducing cardiac output *(Cowley et al., 1995)*. Thirdly, evidence now exists to support the release of a putative depressor hormone from the renal medulla in response to increased RAP *(Muirhead, 1993; Thomas et al., 1996; Bergström et al., 1998)*.

Evidence provided by Muirhead et al *(Muirhead, 1980; Muirhead, 1993)*, in a series of elaborate experiments indicates the presence of a putative vasodepressor hormone, which is housed in the renal medullary interstitial cells, and released in response to an increase in RAP. The identity of this depressor substance has proven difficult to determine, contributing to the difficulty in confirming and extending Muirhead's experimental findings. Several physiological models have been employed in an effort to elucidate both the chemical identity of the medullary depressor substance and to determine the physiological processes mediating its release and activation within the circulatory system. One of the experimental approaches used has been to employ experimental models which allow perfusion of the kidney *in vivo*, and allow the effects of increased RAP on systemic haemodynamics to be observed.

Perhaps the simplest of these models is that produced in renal hypertensive rats, in which arterial pressure is 'normalized' following the removal of the renal arterial clip *(Muirhead and Brooks, 1980)*. In these models, the depressor response to unclipping is blunted by chemical medullectomy by BEA-pretreatment *(Bing et al., 1981; Taverner et al., 1984)*, and by pre-treatment with a cytochrome P450 inhibitor *(Zou et al., 1995)*, consistent with the hypothesis that it is dependent at least in part on the release of 'medullipin' from the renal medulla.

In our laboratory, we have used a technique based on the same general principle, in which a pump is used to circulate blood drawn from the aorta, and return it to the vena cava (rabbits, or the iliac vein in dogs), and the renal artery *(Anderson et al., 1995)*. RAP can be set and maintained at any pressure, independently of the systemic circulation by altering the resistance in the venous limb of the circuit, using a Starling resistor.

A major advantage of this experimental paradigm is the perfusion of the kidney *in situ* at any level above or below systemic arterial pressure *(Anderson et al., 1995)*. Using this model, powerful hypotensive responses are observed in response to increased RAP in both anaesthetised dogs and rabbits *(Christy et al., 1991; Thomas et al., 1996)*. The depressor response appears not to be due to prostaglandins, platelet activating factor, or suppression of the renin-angiotensin system *(Christy et al., 1993)*, nitric oxide release *(Evans et al., 1995; Thomas et al., 1995)*, or products of cytochrome P450 metabolism of arachidonic acid *(Evans et al., 1998b)*. It has further been argued that the depressor response to increased RAP in this model is not due to hypovolaemia secondary to the associated pressure diuretic/natriuretic response, as all experimental animals were said to be in positive fluid balance at all times *(Christy et al., 1991; Christy et al., 1993)*.

On the other hand, the depressor response to increased RAP in this model has been attributed to a depressor hormone housed in the renal medulla, since this response was prevented in animals subjected to prior chemical medullectomy *(Christy et al., 1991)*. This concept has also been examined in cross-circulation studies in rats in which RAP is increased by means of a pump to a kidney, cross-circulated in series with an intact 'assay rat' *(Karlström et al., 1989)*. In this model, increased RAP in the pump-perfused kidney reduces MAP in the 'assay rat' *(Bergström, 1995)*. This depressor response could also be obtained under similar cross-circulation conditions, using spontaneously hypertensive rats, although much higher perfusion pressures were required for the activation of the depressor response than in normotensive (WKY) rats *(Karlström et al., 1991)*.

Although the experiments described above provide evidence of a depressor substance released from the renal medulla in response to increased RAP, this evidence remains indirect. Studies described in the previous chapter (Chapter 4) provided the observations that increases in RAP from ~65 to ~160 mmHg were accompanied by an exponential increase in renal sodium and water excretion, and a progressive fall in MAP, and that these responses were both blunted by selective reduction of MBF (medullary interstitial noradrenaline) but not CBF (intravenous noradrenaline). The objective of the current experiments was to directly determine the extent of the involvement of the pressure diuresis/natriuresis mechanism in the depressor response to increased RAP. As discussed above, previous studies using this preparation have provided indirect evidence that the depressor response to increased RAP is independent of the accompanying increased salt and water excretion and reduction in plasma renin activity. However, this hypothesis remains to be tested directly, which is the chief aim of the present study.

As in the experiments described in the previous chapter (Chapter 4), an extracorporeal circuit was established in anaesthetised rabbits so that the left kidney could be perfused *in situ* and RAP could be increased to levels above systemic arterial pressure. Four groups of rabbits were studied, all of which were instrumented with ascending aortic flow probes three to four weeks prior to the experimental day. We determined the magnitude of the depressor response to increasing RAP from ~65 to ~160 mmHg for 30 min, and how this response was affected by (i) infusion of compound sodium lactate at a rate to replace urine flow and so maintain cardiac output ('cardiac output clamp') and (ii) additionally 'clamping' the renin-angiotensin system by combining angiotensin-converting enzyme blockade with an intravenous infusion of angiotensin II. We also made detailed observations of the haemodynamic status of animals during establishment of the extracorporeal circuit, so as to determine whether they diverge greatly from normal physiological conditions.

5.2 Methods

5.2.1 Experimental preparation

Twenty four New-Zealand White, male rabbits were studied (body wt 2.10 -2.93 kg; mean 2.53 ± 0.03 kg). On the experimental day, rabbits were prepared in a similar manner to the experiments described in Chapter 4 with an extracorporeal circuit being established so that RAP could be controlled and altered independently of direct effects on systemic haemodynamics. Rabbits were randomly assigned to four groups. In the first group, RAP was maintained at a constant control level of ~65 mmHg, and urine volume was collected and recorded each minute during the control,

experimental and recovery phases (30 min each). In group two, after the 30 min control period, RAP was increased to ~160 mmHg for 30 min and, urine volume was also collected and recorded (volumetrically). RAP was also increased to ~160 mmHg in groups 3 and 4, during the 30 min experimental phase of the experiment. These animals also received an intravenous infusion of compound sodium lactate at a rate equivalent to urine flow ('cardiac output clamp'). The protocol for Group 4 differed slightly, in that angiotensin converting enzyme was blocked to prevent production of angiotensin II (by enalaprilat (2.0 mg/kg plus 10 µg/kg/min), and angiotensin II was infused intravenously to restore MAP and RBF to their control levels ('renin-angiotensin system clamp'). This experiential model allowed us to directly determine the relative roles of pressure diuresis/natriuresis and inhibition of the renin/angiotensin system in the depressor response to increased RAP (Figure 5.1). Furthermore, as the extracorporeal circuit remains a useful tool for manipulating RAP in anaesthetised rabbits, preliminary observations were made in these studies between conscious, anaesthetised, and extracorporeal circuit states. These comparisons were made to enable a better understanding of the circulating conditions present in the extracorporeal circuit model.
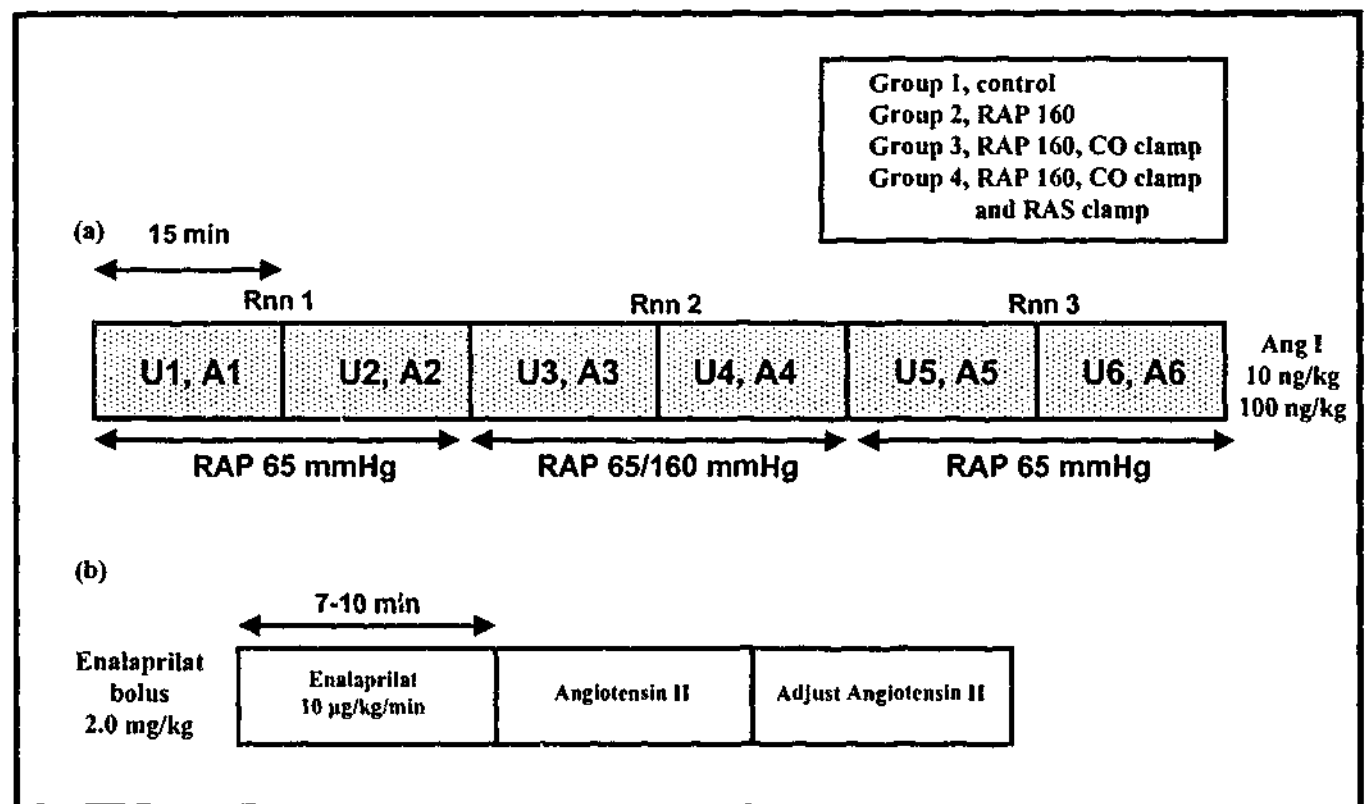
Figure 5.1   Schematic diagram of the experimental protocol. Following establishment of the extracorporeal circuit, renal artery pressure (RAP) was set and maintained at 65 mmHg for a 60 min equilibration prior to the start of the experiment. Four groups of rabbits were studied (n=6 in each group). In all rabbits were RAP was set and maintained at 65 mmHg for a 30 min period, and was

either maintained at 65 mmHg (group 1) or increased to 160 mmHg (groups 2-4) for a further 30 min, before being returned again to 65 mmHg. Each period comprised two consecutive 15 min periods during which urine was collected (U1-U6). Ear arterial blood samples (1.0 ml) were collected for plasma sodium determination and haematocrit at the mid-point of each period. A further 1 ml arterial blood sample was collected at the mid-point of each 30 min period for determination of plasma renin activity (Rnn 1-3). The excreted urine volume (U1-U6) was measured volumetrically on a minute per minute basis for all rabbits. In rabbits in groups 3 and 4 (CO; 'cardiac-output clamp') and equivalent volume of compound sodium lactate was infused intravenously. At the end of U6, nine rabbits were selected at random, and the effects of alternating doses of angiotensin I were tested on arterial pressure. Rabbits in group 4 ('renin-angiotensin system' clamp (RAS)) were given intravenous enalaprilat (2.0 mg/kg, plus 10 μg/kg/min) and angiotensin II at a dose which restored MAP and RBF to their original control levels.

5.2.2   Implantation of cardiac output flowprobes

Rabbits were instrumented with flow probes 2-3 weeks prior to the experiment to allow the formation of scar tissue around the probe, which provides acoustic coupling of the probe and vessel. Flow probes were implanted around the ascending aorta (6SB, Transonic Systems, Ithaca, NY, USA), under halothane (1-4% Fluothane; ICI, Victoria, Australia) anaesthesia and sterile conditions *(Shweta et al., 1999)*. This procedure was explained in detail in Sections 2.3.1 and 2.3.3 in Chapter 2 and will only be described briefly here. Firstly an incision was made above the left second intercostal space and the muscle layers were opened individually. The heart was exposed via the second intercostal space so that a space around the ascending aorta could be cleared for the positioning of a transit-time ultrasound flowprobe. The lungs were then re-inflated, and the ribs were brought together and secured. The skin wound was closed with a series of sutures, so that each muscle layer was sealed and the subcutaneous and cutaneous layers could be closed. The flowprobe plug was then tunneled subcutaneously so that its end lay between the shoulder blades, and it could be retrieved and connected to a flowmeter on the experimental day. This skin wound was closed with a silk suture (3.0 Dynek, Pty. Ltd., Australia). Following surgery rabbits were kept in a warm comfortable environment and closely monitored until fully conscious (2-4 hours). They were then returned to their individual housing and cared for as described in Section 2.3.4, Chapter 2.

5.2.3 Minor procedures on the experimental day

On the experimental day, prior to induction of anaesthesia, MAP, HR and CO were measured in conscious rabbits for 30 min. To prepare for this, the flow probe plug was exteriorized under local analgesia (0.5% Lignocaine; Astra Pharmaceuticals) for connection to a transit-time ultrasound flowmeter (Transonic systems, model T208) to provide ascending aortic flow. Catheters were placed in the central ear arteries and ear veins. MAP and HR were measured by connecting an ear artery catheter to a pressure transducer (Cobe, Arvarda, CO, USA). HR was measured by a tachometer activated by the pulse pressure. During these preparations and the recordings each rabbit was individually housed in a 15 x 40 x 18 cm box, fitted with a wire mesh lid.

5.2.4 Surgical preparation and establishment of the extracorporeal circuit

This has been described in detail in Chapter 2, and was similar to that for the experiments described in Chapter 4. Induction of general anaesthesia was by intravenous pentobarbitone sodium (90-150 mg). Artificial ventilation was commenced, and an incision was made in the neck for exposure of the jugular vein. A catheter (SV 50; 0.75 mm ID, 1.45 mm OD; Dural Plastics and Engineering, Dural, NSW, Australia) was then introduced into the jugular vein and advanced ~5 cm for measurement of central venous pressure (CVP). The extracorporeal circuit was then established (see below). After its establishment, RAP was set and maintained at ~65 mmHg. A bolus dose of [^{3}H] inulin (4 µCi) was then administered in 1.0 ml of 154 mN NaCl. The infusion of Hartmann's solution (0.18 ml/kg/min) that was given throughout the surgery was replaced with 10% vol/vol polygeline (Haemaccel) solution containing 200 IU/ml sodium heparin, and 0.3 µCi/ml [^{3}H] inulin. On completion of the surgical preparations, the rabbit's wounds were covered with gauze soaked in 154 mM NaCl solution to minimize fluid loss.

5.2.5 Measurements

Systemic (MAP, HR, CVP and CO) and renal (RAP and RBF) haemodynamics were measured throughout the experiment, as described in Section 2.7. Plasma renin activity, and urinary concentrations of [^{3}H] inulin and sodium were determined as detailed in Sections 2.7.3 and 2.7.2. At the completion of each experiment, the left kidney was removed and desiccated, and its dry weight determined. All values of RBF, GFR, U_{VOL}, and U_{Na}^+V are therefore expressed per gram of dry kidney weight (expressed as g [mean 1.65 ± 0.05g]).

5.3   Experimental protocols

5.3.1   General

MAP, HR and CO were measured in conscious rabbits for 30 min prior to induction of anaesthesia. Haemodynamic variables were also monitored during establishment of the extracorporeal circuit, to provide detailed information about the status of the circulation under these conditions, relative to normal circulatory conditions in conscious and anaesthetised rabbits. Following establishment of the extracorporeal circuit and a 60 min equilibration period, rabbits were randomly assigned into one of the 4 experimental groups (n=6 for each group). RAP was first set to 65 mmHg for a 30 min control period in all groups. RAP was then either set at 160 mmHg for 30 min (3 groups) or remained at 65 mmHg (1 control group). This period was then followed by a 30 min recovery period (RAP ~65 mmHg). In all rabbits, urine output was determined each minute during the 90 min experimental period. The three groups in which RAP was increased to 160 mmHg received either (i) no treatment, (ii) a 'cardiac output clamp', consisting of intravenous infusions of compound sodium lactate (Hartmann's solution) to replace urine output each minute during the period when renal artery pressure was increased, or (iii) a 'renin-angiotensin system clamp', consisting of enalaprilat (2.0 mg/kg plus 10 µg/kg/min) to block angiotensin converting enzyme and an intravenous infusion of angiotensin II (40-50 ng/kg/min) to restore MAP to its pre-enalaprilat level *(Christy et al., 1993)*, as well as the 'cardiac output clamp'. The bolus dose of enalaprilat was administered intravenously after 30 min of stable baseline recordings after establishment of the extracorporeal circuit (that is, at the mid-point of the 60 min equilibration period), and the infusion of angiotensin II commenced 10 min later.

5.3.2   Responses to angiotensin I

To test the effectiveness of the 'angiotensin II clamp', following the recovery period 9 rabbits (4 from the 'renin-angiotensin system clamp' group and 5 from the other groups) received bolus intravenous doses of angiotensin I (10 and 100 ng/kg). These doses were given in random order approximately 20 min apart.

5.3.3   Statistical analyses

5.3.3.1 Systemic and renal haemodynamic variables in conscious rabbits, and during establishment of the extracorporeal circuit

Data collected from rabbits during the preparative phase of the experiment were analyzed as two separate groups; those that received the enalaprilat/angiotensin II treatment (group 4) and those that did not (groups 1-3). Average levels were determined for conscious (15 min before induction of anaesthesia), anaesthetised (15 min before establishing the extracorporeal circuit) and extracorporeal circuit states (final 15 min of the 60 min equilibration period). Analysis of variance was used to make comparisons between each state (conscious, anaesthetised and "circuit established") within each of the two groups, and also between the two groups. These analyses of variance were partitioned so we could make specific contrasts between the various states (Table 5.1). To protect against the increased risk of type 1 error as a result of these multiple comparisons, *P* values were adjusted using the Dunn-Sidak correction *(Ludbrook, 1994)*.

5.3.3.2 Systemic and renal haemodynamic variables, and renal excretory responses to increased renal artery pressure

To determine the effects of increasing RAP within each of the 4 groups, paired t-tests were used to contrast the levels of variables during the final 15 min of the period of increased RAP, with the final 15 min of the control period (see Figure 5.1). To determine whether these responses differed across the 4 groups, unpaired t-tests were used to contrast the % changes in each variable between these experimental periods.

5.3.3.3 Angiotensin I infusion

Unpaired t-tests were used to contrast responses to angiotensin I between 'renin-angiotensin system clamp' rabbits and rabbits not receiving this treatment.

5.4   Results

5.4.1   *Observations during establishment of the extracorporeal circuit*

5.4.1.1 Conscious rabbit recordings

Recordings of conscious MAP, HR and CO were made for 30 min prior to induction of anaesthesia. Data for the final 15 min of this period are presented in Figures 5.2 and 5.3, which show that systemic haemodynamics had stabilized during this period. Across all 24 rabbits, MAP averaged 85 ± 2 mmHg, HR averaged 218 ± 5 beats/min, and CO averaged 153 ± 7 ml/min/kg (Figure 5.2, Table 5.1). SV and SVR were also calculated for this period and averaged 0.73 ± 0.03 ml/kg and 0.58 ± 0.04 mmHg/ml/min/kg respectively (Figure 5.3, Table 5.1).

5.4.1.2 Anaesthetised baseline recordings (A)

Baseline levels of haemodynamic variables in anaesthetised rabbits during the 15 min prior to establishment of the extracorporeal circuit are shown as (A) in Figures 5.4-5.5. When averaged across all rabbits, MAP was 25 ± 1 mmHg less, CO was 28 ± 1 ml/min/kg less, and SV was 0.21 ± 0.01 ml/kg less than in the conscious state. HR, increased (35 ± 1 beats/min) after anaesthetisia but, SVR remained unchanged (Table 5.1).

5.4.1.3 Heparin bolus (B)

Intravenous administration of a 2.5 ml bolus dose of heparin (2,500 IU) caused a transient reduction and then sustained increase in MAP. That is, it initially fell 3 ± 2 mmHg before increasing to be 6 ± 2 mmHg greater than before heparin administration. This was accompanied by reduced HR by 20 ± 3 beats/min during the first 2 min after administration of the heparin bolus, compared with the 2 min before it was administered (from 251 ± 10 to 231 ± 7 beats/min). Little or no changes were observed in CVP, CO, SV or SVR (Figures 5.4 and 5.5).

5.4.1.4 Occlusion of the aorta (C)

The most pronounced response to tying off the aorta (distal to the renal arteries) was an increase in MAP of 7 ± 2 mmHg during the first 2 min after aortic occlusion. MAP then continued to increase so that it was a further 11 ± 2 mmHg greater during the final 2 min of this period (C) when

compared to (B). This was accompanied by increases in CVP (1.0 ± 0.6 mmHg) and SVR ($0.13 \pm$ 0.02 ml/min/kg) during the first 2 min of (C) and reductions in SV (0.04 ± 0.01 ml/kg) and CO ($7 \pm$ 2 ml/min/kg), which continued to decrease a further 20 ± 2 ml/min/kg during the final 2 min of this period. Little or no change was seen in HR.

5.4.1.5 Starting the peristaltic pump (70 ml/min) (D)

When the peristaltic pump was set to withdraw blood from the abdominal aorta (70 ml/min) and return it to the vena cava, MAP fell by (12 ± 1 mmHg). SV initially decreased 0.04 ± 0.01 ml/kg in the first 2 min of this period before increasing to be 0.21 ± 0.01 ml/kg greater than in the final 2 min of the previous period. CO and SVR also behaved similarly, initially decreasing 12 ± 5 ml/min/kg and 0.06 ± 0.03 ml/min/kg respectfully, before each increasing a total of 49 ± 1 ml/min/kg and 0.37 ± 0.06 ml/kg/min by the end of that period (D) (Figures 5.4 and 5.5). CVP and HR each fell ($0.7 \pm$ 0.2 mmHg and 7 ± 4 beats/min respectively) in the first 2 min after the pump was started, but recovered slightly (during equilibrium) to only 0.1 ± 0.3 mmHg and 4 ± 3 beats/min less than that prior to the start of the period.

However, if blood flow through the extracorporeal circuit is excluded from these calculations, values of CO was found to initially fall 40 ± 3 ml/kg/min during the first 2 min of the pump being increased, before increasing to 23 ± 5 ml/kg/min greater than at the end of the previous period (C). SVR showed the opposite response, initially increasing 0.5 ± 0.2 mmHg/ml/kg/min during the first 2 min after the pump commenced. However by the end of this period (D) it had reduced 0.27 ± 0.05 mmHg/ml/kg/min to equilibrium at the end of (C) (Figure 5.6).

5.4.1.6 Increasing the peristaltic pump rate to 110 ml/min (E, F)

Once the renal artery catheter was implanted and tied in position (E), the kidney was included in the perfusion circuit, and the pump rate was increased to 110 ml/min (F). This had little or no effect on any of the measured variables (Figures 5.4 – 5.6).

5.4.1.7 Establishment of the extracorporeal circuit (G)

Point 'G' in Figures 5.4-5.6 shows the beginning of the final 15 min of the equilibration period, after the circuit had been established for at least 60 min and all variables had stabilized. The average levels of these variables during this period are shown in Table 5.1. Apart from a small difference in

MAP, which was 3 ± 1 mmHg greater during the first 2 min of this period, as compared to the final 2 min of (F), all haemodynamic variables were similar to their level when the circuit was first established.

5.4.2 Haemodynamics during conscious, anaesthetised, and extracorporeal states

Once the circuit had been established, systemic haemodynamic variables returned to similar levels to those observed in the conscious state. In rabbits which did not receive the angiotensin II block, MAP was 17 ± 1 mmHg less in the anaesthetised than the conscious state, but increased 12 ± 1 mmHg once the circuit was established. CO was also similar in the conscious and extracorporeal circuit states in these rabbits, but was 22 ± 1 ml/min/kg lower in the anaesthetised state, compared to the conscious state. SV showed a similar pattern to CO. HR was greater once anaesthesia was induced. All other variables were similar across the three states for this group of rabbits (Table 5.1).

Levels of all haemodynamic variables, including RBF and RVR were closely similar in rabbits treated with enalaprilat/angiotensin II ('renin-angiotensin system clamp') compared with those in rabbits not given this treatment (Table 5.1).

5.4.3 Renal haemodynamic responses to increased renal artery pressure

5.4.3.1 Time control (Group 1)

As depicted in Figure 5.7 and Table 5.2, RBF and RVR remained relatively stable across the 90 min course of the experiment when RAP was maintained at ~65 mmHg.

5.4.3.2 Effects of increasing renal artery pressure on renal haemodynamic variables

Group 2

When RAP was increased to ~160 mmHg RBF increased from 12 ± 2 to 39 ± 6 ml/min/g and RVR did not change significantly (Figure 5.7, Table 5.2).

Group 3 ('cardiac output clamp')

When RAP was increased to ~160 mmHg and the excreted urine volume was returned as intravenous compound sodium lactate, RBF increased $331 \pm 64\%$ from 14 ± 2 to 57 ± 8 ml/min/g (during the period 15-30 min after RAP was increased) and RVR decreased $40 \pm 6\%$ from $5.4 \pm$

0.95 to 3.1 ± 0.43 mmHg/ml/min/g. These responses were not significantly different from those seen in group 2 (Figure 5.7, Table 5.2).

Group 4 ('cardiac output clamp' plus 'renin-angiotensin system clamp')

When RAP was increased to ~160 mmHg, together with both 'cardiac output' and 'renin-angiotensin clamp', RBF increased 254 ± 50% from 12 ± 2 to 39 ± 3 ml/min/g and RVR decreased 26% from 6.1 ± 0.7 to 4.3 ± 0.4 mmHg/ml/min/g. These responses were not significantly different from those seen in group 3 (Figure 5.7, Table 5.2).

5.4.4 Renal excretory responses to increased renal artery pressure

5.4.4.1 Time control (Group 1)

As depicted in Figures 5.8 and Table 5.2, all renal excretory variables remained relatively constant, during the 90 min experimental period when RAP was maintained ~65 mmHg.

5.4.4.2 Effects of increasing renal artery pressure on renal excretory function

Group 2

When RAP was increased to ~160 mmHg GFR increased 304 ± 133% (from 1.5 ± 0.5 to 4.3 ± 0.7 ml/min/g), urine flow increased 2846 ± 50% (from 0.07 ± 0.04 to 1.47 ± 0.33 ml/min/g), sodium excretion increased 575 ± 93% (from 9 ± 5 to 186 ± 14 ml/min/g), and the fractional excretion of sodium and urine increased (from 9 ± 1 to 45 ± 3% and from 7 ± 1 to 41 ± 2% respectively). Filtration fraction did not change significantly.

Group 3 ('cardiac output clamp')

When RAP was increased and maintained at ~160 mmHg and the excreted urine volume was returned as an intravenous infusion of compound sodium lactate, all aspects of renal excretory function significantly were increased, with the exception of FF. GFR, urine flow and sodium excretion increased from 1.24 ± 0.03 to 5.4 ± 0.9 ml/min/g, from 0.07 ± 0.02 to 1.7 ± 0.2 ml/min/g, and from 7 ± 2 to 101 ± 22 μmol/min/g, respectively. The fractional excretion of sodium and urine also increased from 10 ± 3 to 37 ± 4% ($P \leq 0.001$) and from 13 ± 4 to 47 ± 4% respectively. These changes were not significantly different from those in group 2, (Figure 5.8, Table 5.2).

Group 4 ('cardiac output clamp' plus 'renin-angiotensin system clamp')

When RAP was increased to ~160 mmHg, together with both cardiac output and renin angiotensin clamps, renal excretory function responded similarly to group 3 (Figure 5.8, Table 5.2). GFR, urine flow, and sodium excretion each increased from 1.1 ± 0.1 to 6.0 ± 0.6 ml/min/g, from 0.06 ± 0.01 to 1.8 ± 0.1 ml/min/g, and from 6 ± 2 to 200 ± 12 μmol/min/g, respectively). Fractional excretion of sodium and urine also increased from 5 ± 2 to $22 \pm 3\%$ and from 10 ± 2 to $42 \pm 2\%$ respectively. Filtration fraction also significantly increased (from $9 \pm 2\%$ to $13 \pm 2\%$) but this response was not significantly different from that seen in group 3.

5.4.5 Systemic haemodynamic responses to increased renal artery pressure

5.4.5.1 Time control (Group 1)

As depicted in Figures 5.9 and 5.10 and Table 5.2, systemic haemodynamic variables remained relatively stable during the 90 min experimental period when RAP was maintained ~65 mmHg.

5.4.5.2 Effects of increasing renal artery pressure on systemic haemodynamic variables

Group 2

When RAP was increased to ~160 mmHg, MAP fell ($35 \pm 5\%$) from 83 ± 4 to 54 ± 5 mmHg. SVR also decreased from 0.63 ± 0.04 to 0.52 ± 0.05 ml/min/kg. CVP was also reduced from 2 ± 2 to 0 ± 1 mmHg.

When RAP was increased to ~160 mmHg, CO fell ($20 \pm 5\%$) from 132 ± 6 to 105 ± 4 ml/min/kg, while SV, HR and Hct remained relatively constant across the 90 min experimental period.

Group 3 ('cardiac output clamp')

When RAP was increased to ~160 mmHg and CO was maintained by intravenous infusion of compound sodium lactate, CVP was reduced 2 ± 1 to 1 ± 1 mmHg while MAP and SVR did not change significantly, CO, SV, HR and Hct also did not change significantly.

Group 4 ('cardiac output clamp' plus 'renin-angiotensin system clamp')

When RAP was increased to ~160 mmHg, together with both 'cardiac output' and 'renin angiotensin clamps', systemic haemodynamic responses were similar to those of *group 3*. CVP was

reduced from 2 ± 1 to 1 ± 1 mmHg while MAP, SVR, CO, SV, HR and Hct did not change significantly.

When RAP was increased to ~160 mmHg, systemic haemodynamic responses were similar for groups 3 and 4, but MAP was reduced significantly more in group 2 which had not received the 'cardiac output clamp', when compared to group 3 ($P = 0.001$). MAP fell 36 ± 5% in group 2, but only 8 ± 4% and 6 ± 5% in groups 3 and 4 respectively. Furthermore, CO was reduced significantly more in group 2 (20 ± 5 %) than group 3 (2 ± 2%) ($P = 0.008$), as was SV (group 2, 18 ± 4%; group 3, 3 ± 2%; $P = 0.03$).

5.4.6 Plasma renin activity

Plasma renin activity did not change significantly over the course of the experiment in any of the 4 groups (Figure 5.11, Table 5.2).

5.4.7 Effects of angiotensin I infusion on mean arterial pressure

These data are depicted in Figure 5.12. In rabbits from groups 1-3, angiotensin I administered at 10 ng/kg caused MAP to increase by 3 ± 1 mmHg from, 66 ± 10 to 69 ± 10 mmHg. At a dose of 100 ng/kg, angiotensin I caused an even greater increase in MAP of 8 ± 2 mmHg, (from 66 ± 9 to 71 ± 9 mmHg). In rabbits from group 4 ('angiotensin II clamp'), neither dose of angiotensin I significantly increased MAP.
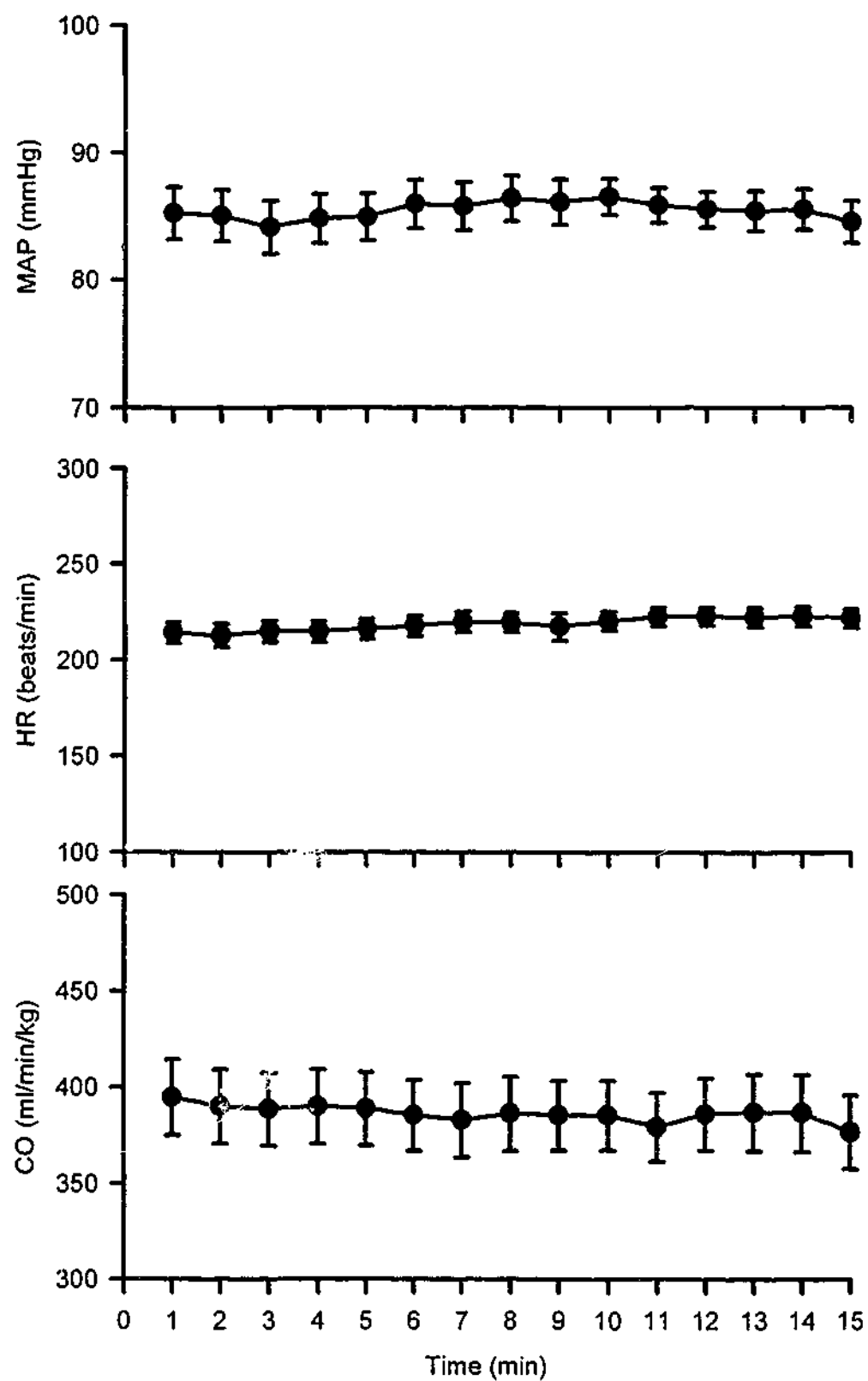
Figure 5.2 Final 15 min of stable baseline recordings of conscious rabbits prior to induction of anaesthesia. Data shown are 1 min averages, (mean ± SEM) of 24 rabbits. MAP, mean arterial pressure; HR, heart rate; CO, cardiac output.
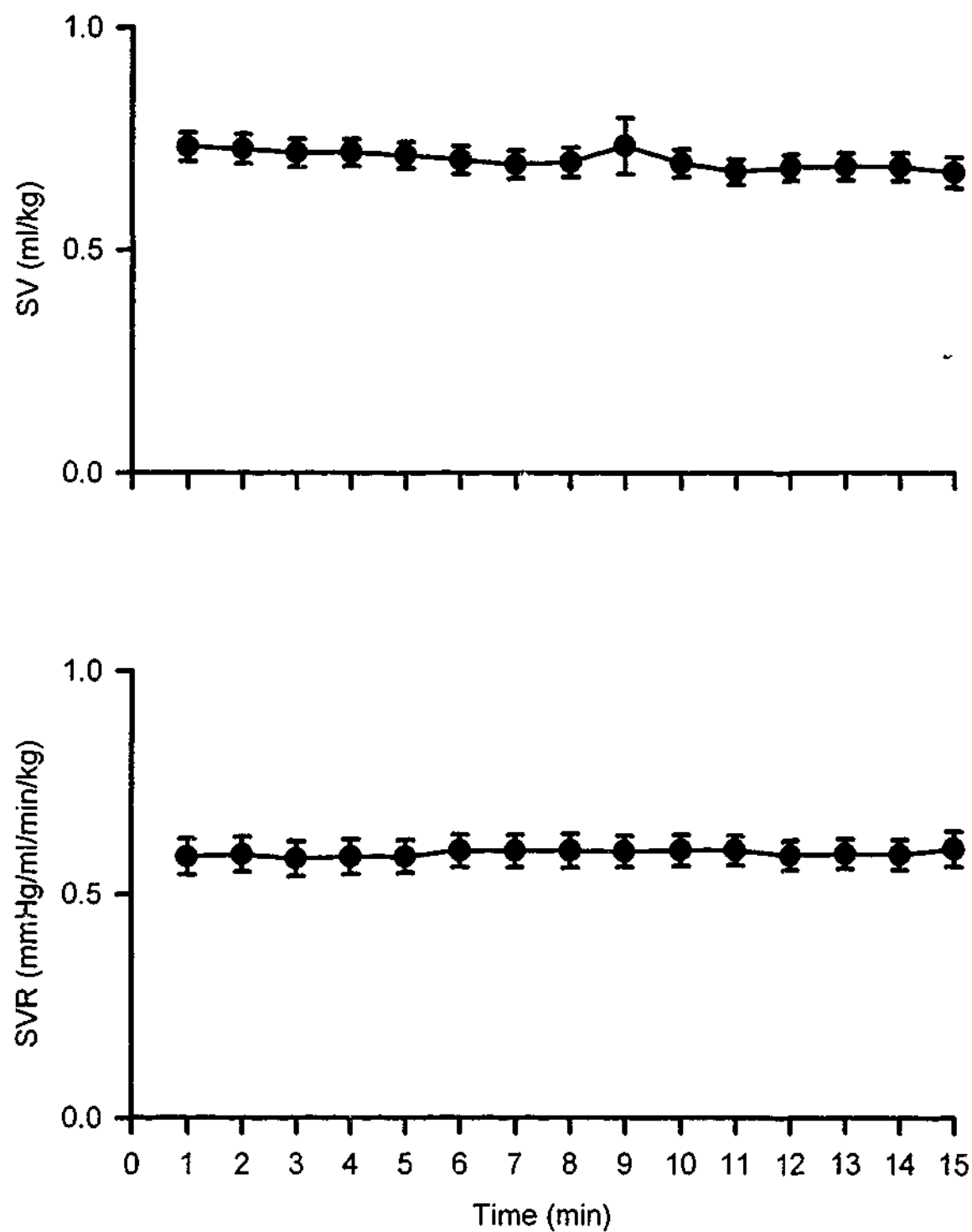
Figure 5.3   Final 15 min of stable baseline recordings in conscious rabbits prior to induction of anaesthesia. Data shown are 1 min averages, (mean ± SEM) of 24 rabbits. SV, stroke volume; SVR, systemic vascular resistance.
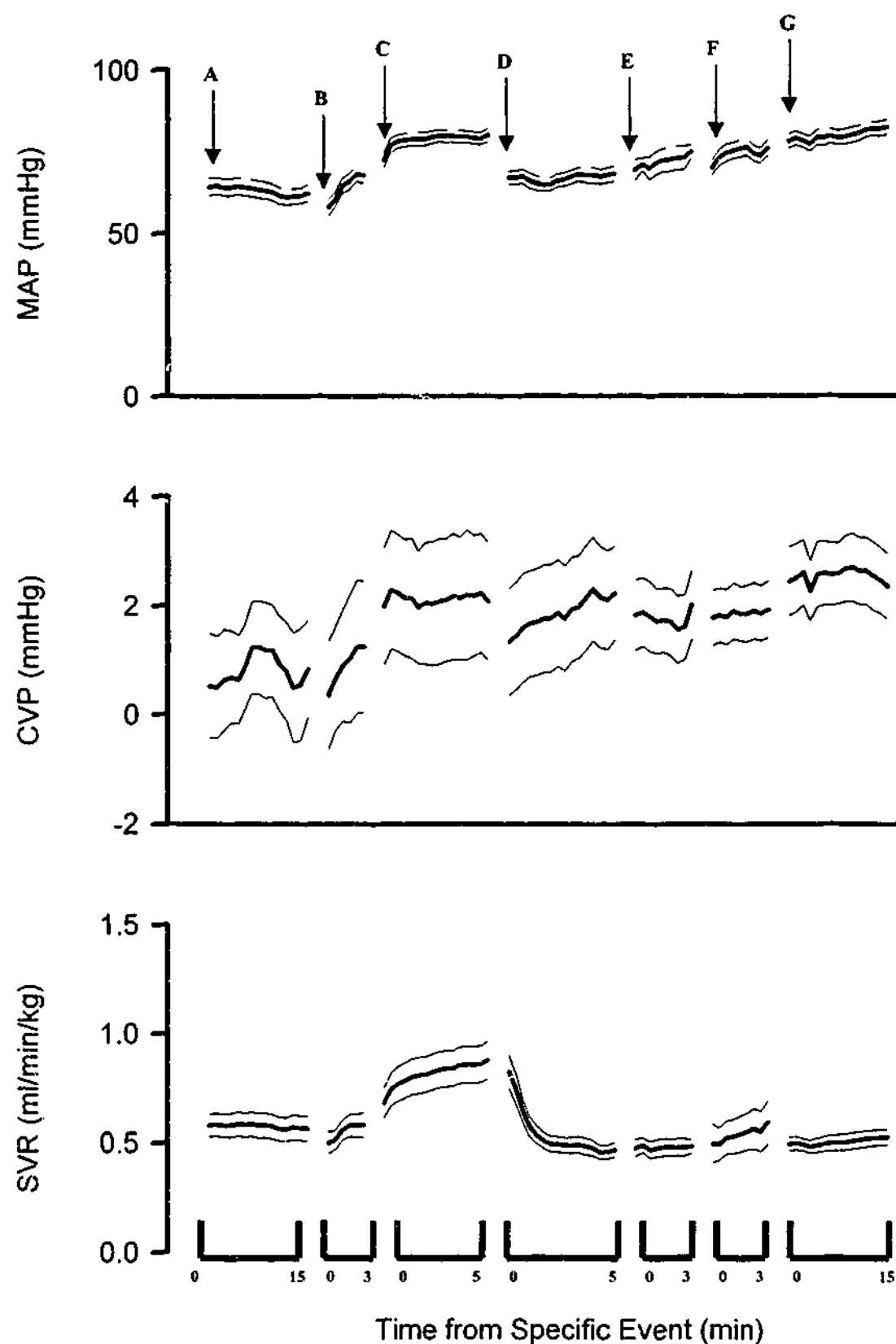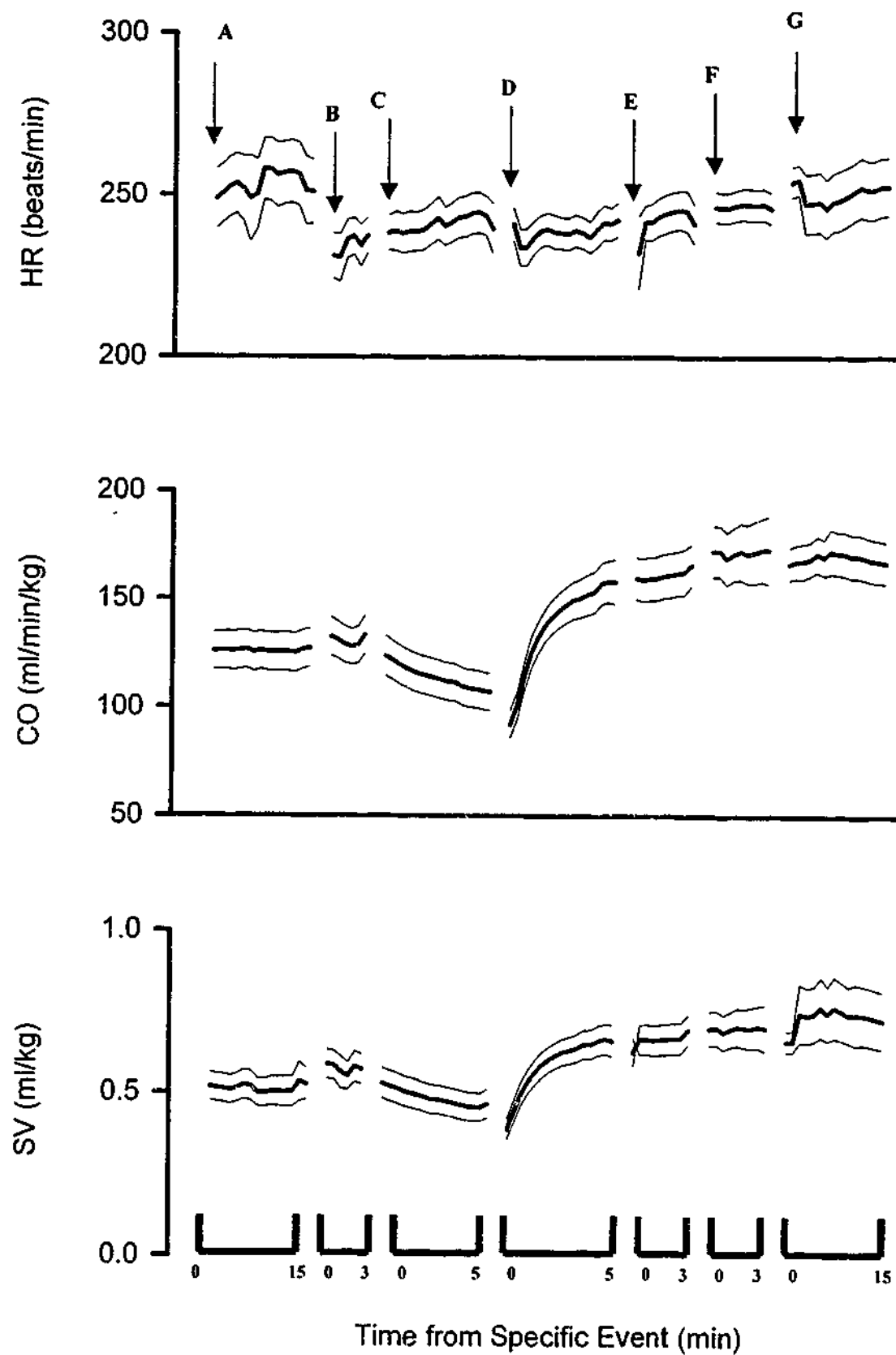
Figure 5.4 Systemic haemodynamic variables during establishment of the extracorporeal circuit in anaesthetised rabbits. MAP, mean arterial pressure; CVP, central venous pressure; SVR, systemic vascular resistance. Thick and thin lines show the mean ± SEM respectively for 24 rabbits. **A**, anaesthetised baseline recordings; **B**, heparin bolus; **C**, aortic occlusion; **D**, set peristaltic pump to 70 ml/min; **E**, tie renal artery; **F**, increase peristaltic pump to 110 ml/min; **G**, established extracorporeal circuit. Note that the time-scale (abscissa) is not standardised, to ensure clarity of presentation.

Figure 5.5 Cardiovascular variables during establishment of the extracorporeal circuit in anaesthetised rabbits. HR, heart rate; CO, cardiac output; SV, stroke volume. Lines, and events marked **A- G** are as for Figure 5.4.
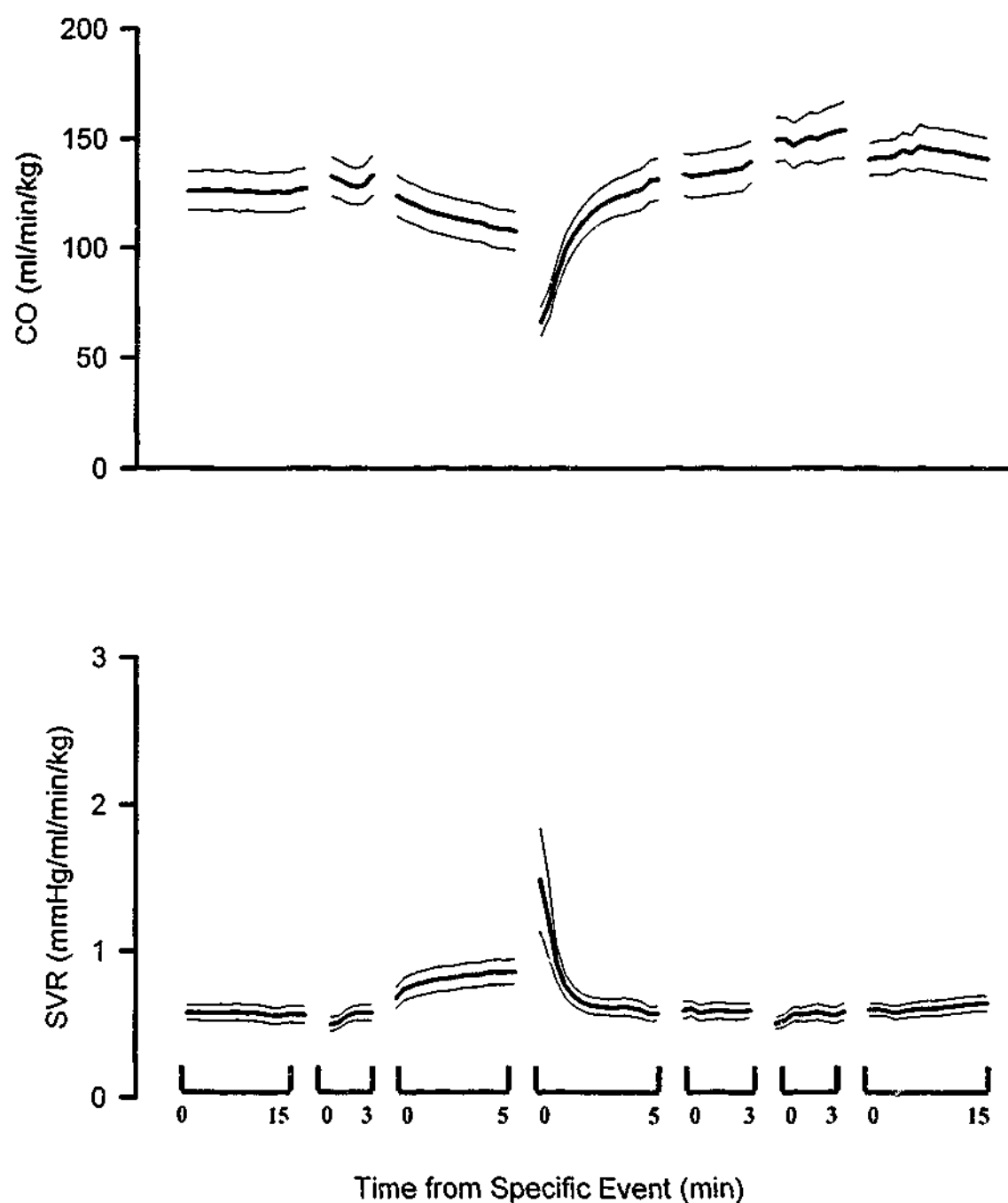
Figure 5.6   Cardiac output, and systemic vascular resistance during establishment of the extracorporeal circuit in anesthetized rabbits. For this figure, flow through the extracorporeal circuit has been excluded from the calculations. CO, cardiac output; SVR, systemic vascular resistance. Error bars, and events marked **A- G** are as for Figure 5.4.
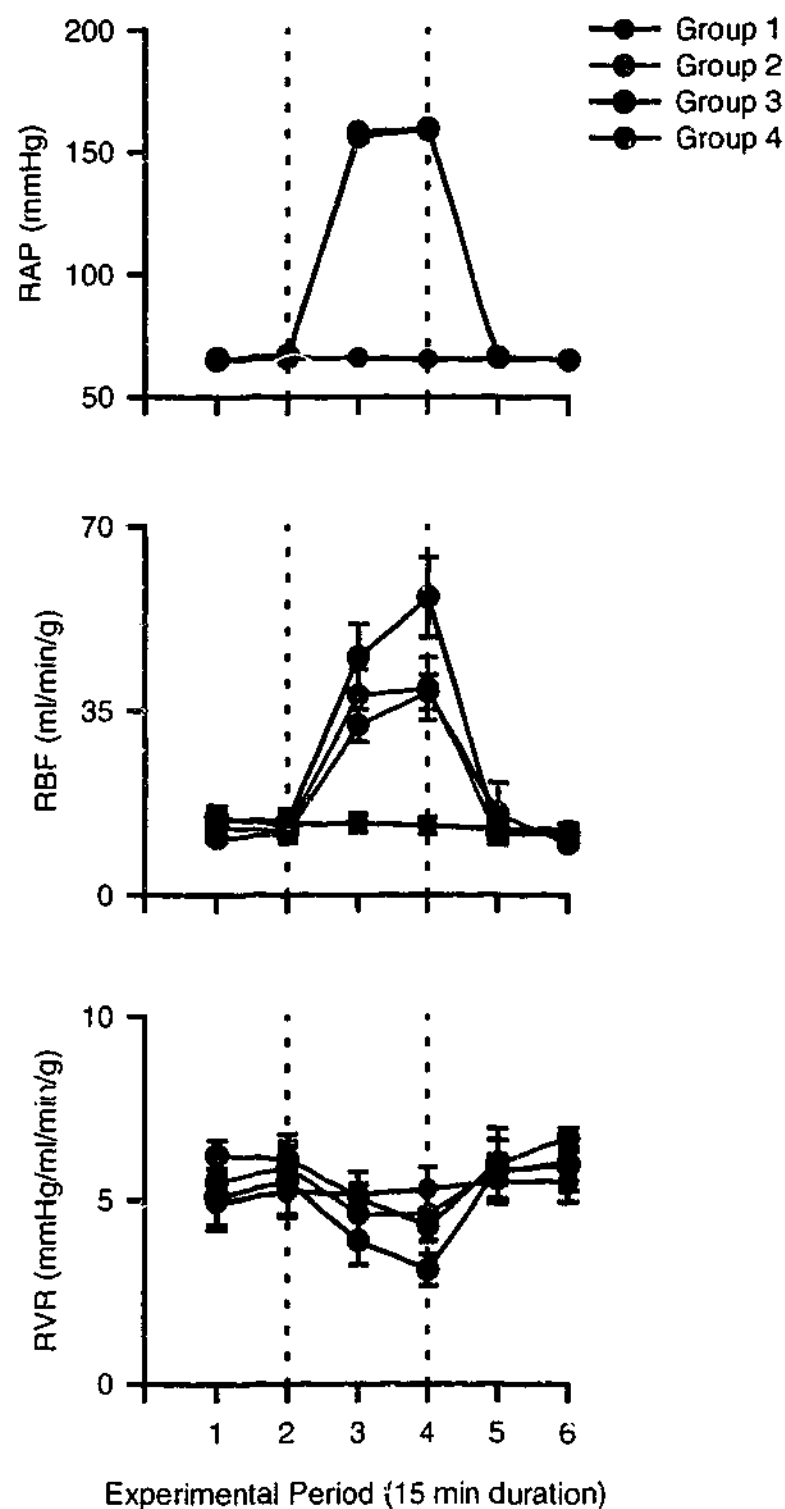
Figure 5.7 Renal haemodynamic responses to increased RAP. Each point is the average of the final 15 min during each event. Symbols and error bars are the mean ± SEM for each variable. *P* values are given in Table 5.2 and are the outcomes of a paired t-test which contrasted levels of each variable during period 4 (15 min, end of experimental phase), with period 2 (15 min, end of control). During the first 30 min (periods 1-2), renal artery pressure (RAP) was set at ~65 mmHg in all four groups. In groups 2-4, RAP was set to ~160 mmHg for 30 min (periods 3-4), before being returned to ~65 mmHg for a further 30 min (periods 5-6). In groups 3 and 4, urine volume during the period of increased RAP was returned to the animal as an equivalent volume of compound sodium lactate ('cardiac output clamp'). In group 4, the effects of increased RAP on the renin-angiotensin system were controlled for by intravenous administration of enalaprilat (2 mg/kg plus 20 μg/kg/min) and angiotensin II (40-50 μl/kg/min).
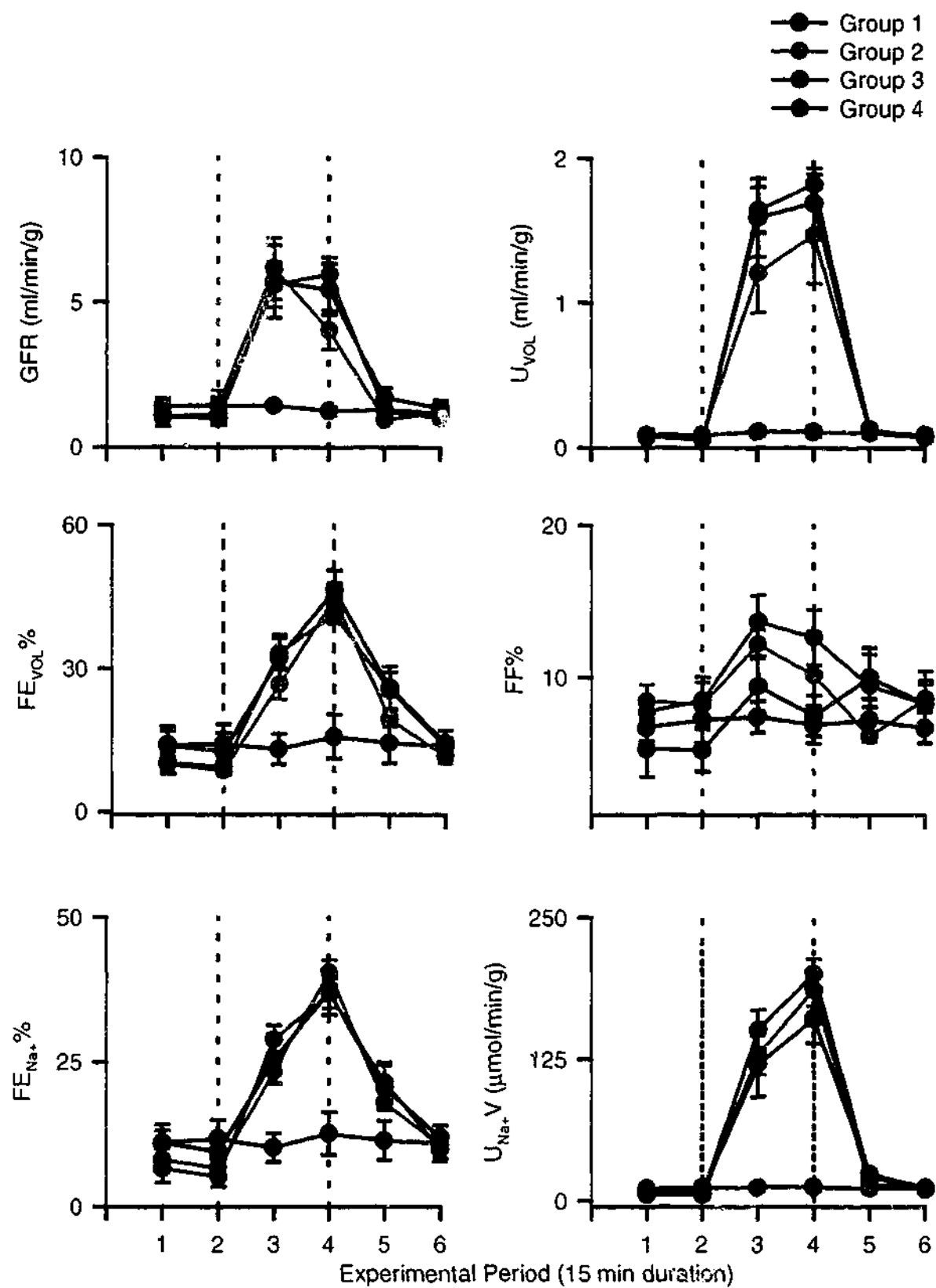
Figure 5.8 Renal excretory responses to increased RAP. Lines, symbols and error bars are as for Figure 5.7. *P* values (given in Table 5.2) are as for Figure 5.7. GFR, glomerular filtration rate; U_{VOL}, urine flow; FE_{VOL}, fractional excretion of urine; FF, filtration fraction; FE_{Na+}, fractional excretion of sodium; $U_{Na+}V$, urinary sodium excretion. GFR, U_{VOL}, and $U_{Na+}V$ are expressed per gram of dry kidney weight.
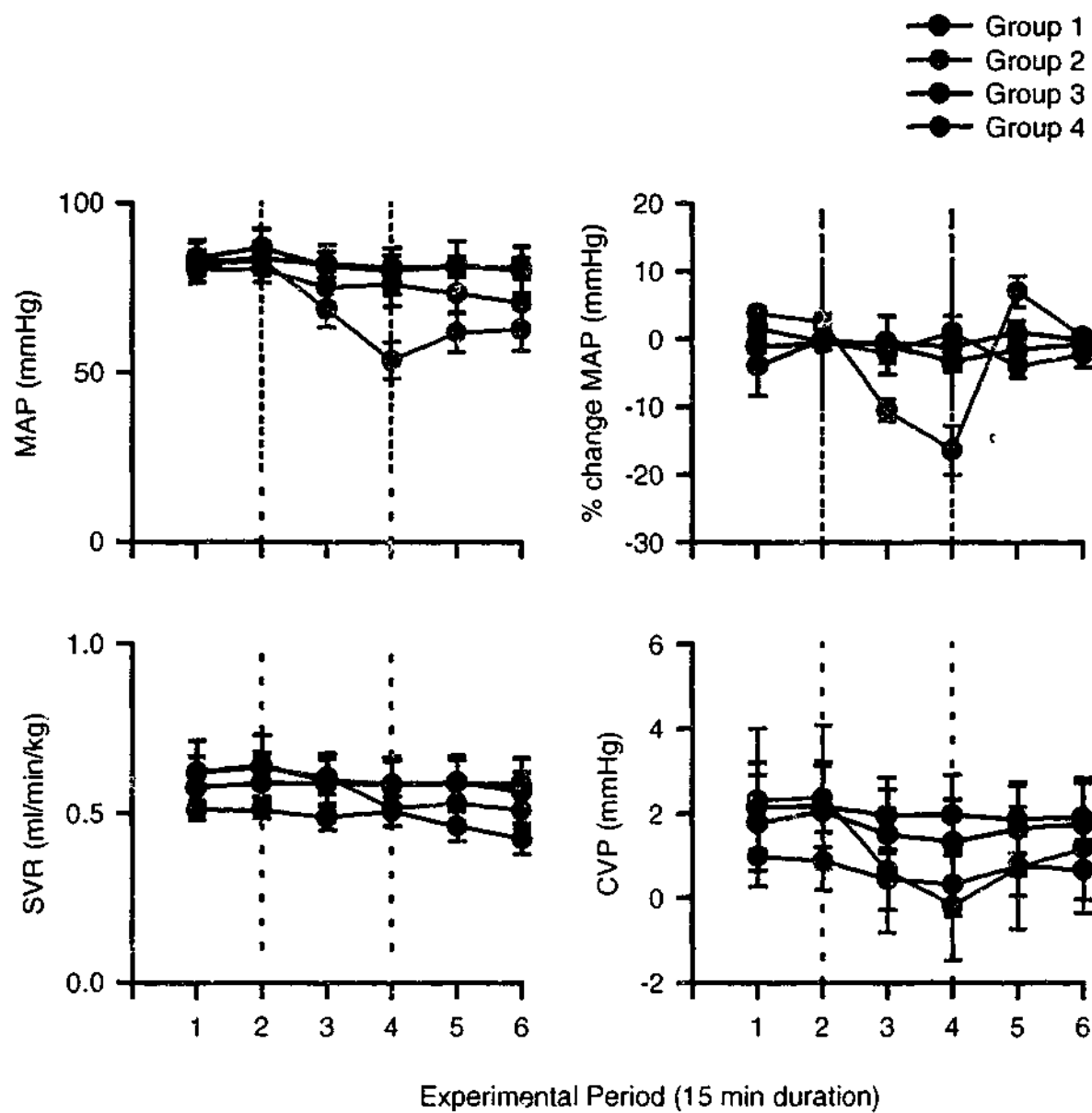
Figure 5.9 Systemic haemodynamic responses to increased RAP. Lines, symbols and error bars are as for Figure 5.7. *P* values (given in Table 5.2) are as for Figure 5.7. MAP: mean arterial pressure; SVR, systemic vascular resistance; CVP, central venous pressure.
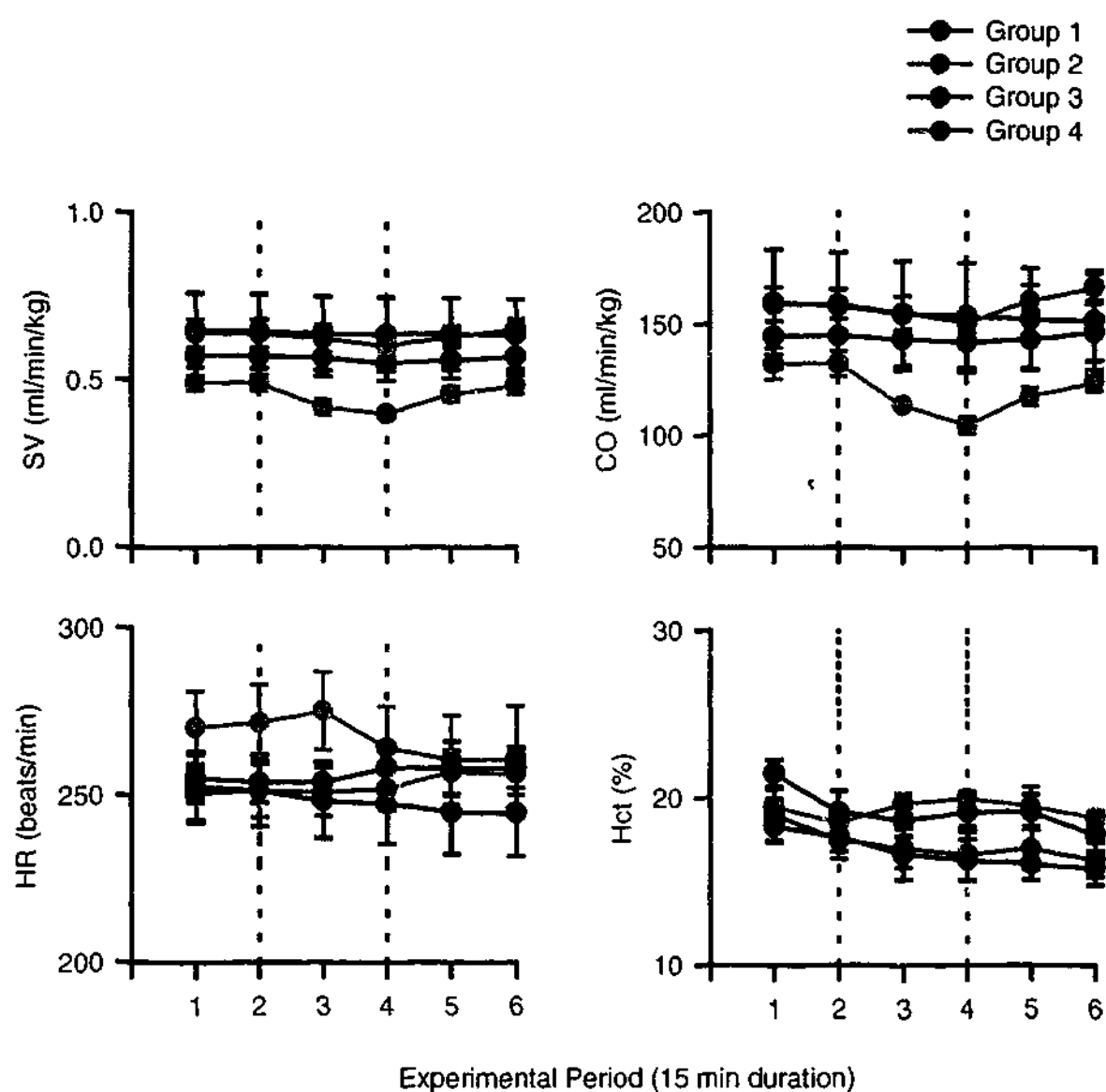
Figure 5.10 Systemic haemodynamic responses to increased RAP (continued). Lines, symbols
and error bars are as for Figure 5.7. *P* values (given in Table 5.2) are as for Figure 5.7. SV, stroke volume; HR, heart rate: CO, cardiac output; Hct, haematocrit.
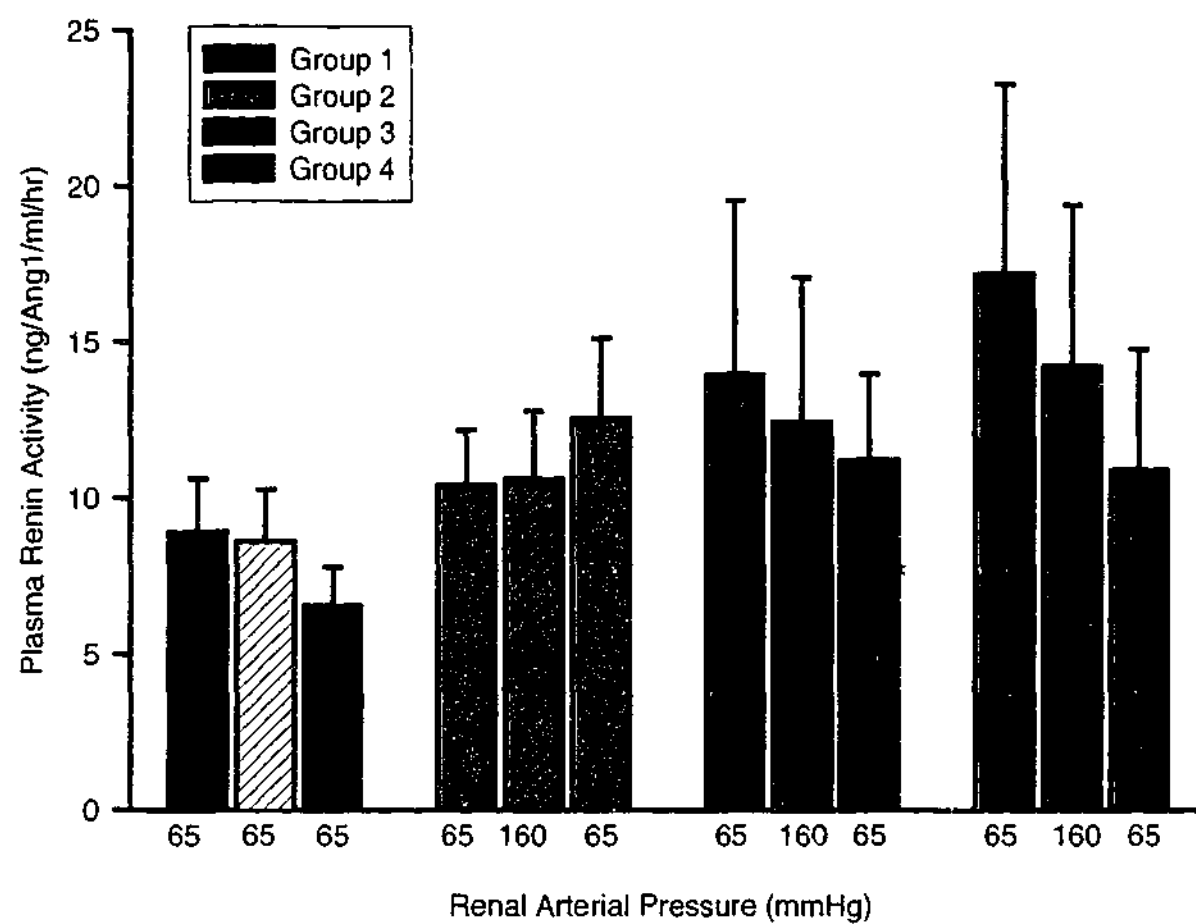
Figure 5.11 Plasma renin activity for each of the four groups. Bars and error bars are the mean ± SEM for plasma renin samples collected at the mid point of each 30 min period. *P* values (given in Table 5.2) are as for Figure 5.7.
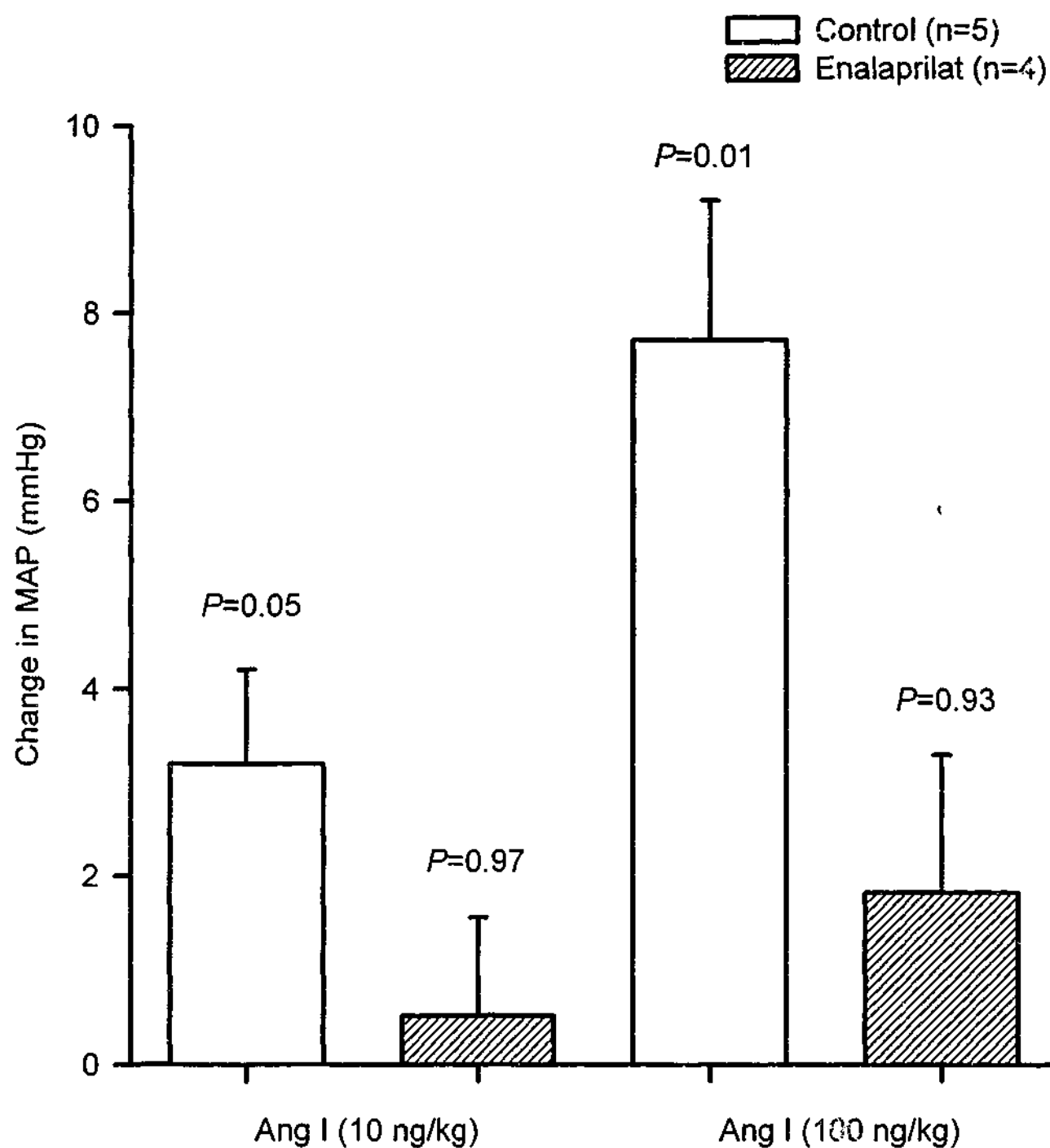
Figure 5.12 Effects of intravenous bolus doses of angiotensin I (10 ng/kg and 100 ng/kg) on mean arterial pressure (MAP) in control (empty bars; n=5) and 'renin-angiotensin system clamped' rabbits (hatched bars; n=4). Data are the % change from baseline values. *P* values are the outcomes of paired t-test which compared MAP after each angiotensin I bolus to baseline MAP values.

Table 5.1 Resting haemodynamic variables in rabbits according to state (conscious, anaesthetized or with the extracorporeal circuit established) and group (control or 'renin-angiotensin system clamp').

	MAP	HR	CO	CVP	SVR	SV	RAP	RBF	RVR
Control (n=18)									
Conscious (15 min)									
	84 ± 2	224 ± 6	157 ± 7		0.58 ± 0.04	0.68 ± 0.03			
Anaesthetised (15 min)									
	62 ± 5 ***	256 ± 6*	132 ± 11*	1.13 ± 1.84	0.54 ± 0.07	0.53 ± 0.06			
Circuit established (15 min)									
	83 ± 3 [†††]	260 ± 6*	143 ± 9	1.75 ± 0.69	0.61 ± 0.04	0.56 ± 0.04	66 ± 0.4	14.2 ± 1.2	5.5 ± 0.4
P_{STATE}	< 0.001	0.02	0.29	0.56	0.58	0.05			
'Renin-angiotensin system clamp' (n=6)									
Conscious (15 min)									
	90 ± 3	201 ± 6	151 ± 19		0.62 ± 0.06	0.77 ± 0.08			
Anaesthetised (15 min)									
	67 ± 5*	246 ± 9*	109 ± 10*	0.08 ± 1.02	0.67 ± 0.09	0.45 ± 0.04*			
Circuit established (15 min)									
	80 ± 4	250 ± 7**	157 ± 8	0.83 ± 0.75	0.51 ± 0.03	0.63 ± 0.04	66 ± 0.4	10.9 ± 0.7	6.2 ± 0.3
P_{STATE}	0.02	0.006	0.08	0.57	0.38	0.02			
P_{GROUP}	0.89	0.42	0.65	0.81	0.80	0.40	0.97	0.12	0.35

Values are given as the mean $\pm$ SE mean. MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; CVP, central venous pressure; SVR, systemic vascular resistance; SV, stroke volume; RAP, renal arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance. P_{GROUP} tests whether the mean levels of variables differed between the two groups of rabbits (df 1,22-42), P_{STATE} tests for heterogeneity according to state within each group (df 1-2, 4-42). Specific contrasts within each group were also made: * P <0.05, ** P <0.01, *** P <0.001 for difference from conscious state; [†] P <0.05, [††] P <0.01, [†††] P < 0.001 for difference between anaesthetized state and extracorporeal circuit (df 1,4-32).

Table 5.2   Outcomes of paired t-tests determining whether variables changed within each group across the course of the experiment. Data are depicted in Figures 5.7-5.11.

	Control	RAP 160	RAP 160 volume replacement	RAP 160 return volume replacement plus 'RAS clamp'
Renal Hemodynamics: Figure 5.7				
RAP	0.58	≤ 0.001	≤ 0.001	≤ 0.001
RVR	0.84	0.51	0.03	0.04
RBF	0.80	0.02	≤ 0.001	≤ 0.001
Renal Excretory Function: Figure 5.8				
U_{VOL}	0.56	≤ 0.001	≤ 0.001	≤ 0.001
FF%	0.82	0.45	0.22	0.001
GFR	0.60	0.01	0.003	0.003
$U_{Na+}V$	0.94	≤ 0.001	≤ 0.001	≤ 0.001
$FE_{Na+}\%$	0.71	≤ 0.001	≤ 0.001	≤ 0.001
$FE_{VOL}\%$	0.50	≤ 0.001	≤ 0.001	≤ 0.001
Systemic Hemodynamics: Figures 5.9 and 5.10				
MAP	0.16	0.003	0.10	0.29
%ΔMAP	0.55	0.01	0.11	0.54
SVR	0.60	0.02	0.24	0.97
CVP	0.37	0.004	0.01	0.05
CO	0.10	0.02	0.49	0.09
SV	0.34	0.09	0.07	0.10
HR	0.32	0.46	0.15	0.85
Hct	0.12	0.30	1.00	0.46
Plasma Renin Activity: Figure 5.11				
PRA	0.44	0.88	0.65	0.07

Average levels of each variable during the second (control) and fourth (15-30 min after RAP was increased in groups 2-4) experimental periods were compared by paired t-test. RAP, renal arterial pressure; RVR, renal vascular resistance; RBF, renal blood flow; U_{VOL}, urine flow; FF, filtration fraction; GFR, glomerular filtration rate; $U_{Na+}V$, urinary sodium excretion; FE_{Na+}, fractional excretion of sodium; FE_{VOL}, fractional excretion of urine; MAP, mean arterial pressure; %Δ MAP, % change in MAP; SVR, systemic vascular resistance; CVP, central venous pressure; CO, cardiac output; SV, stroke volume; HR, heart rate; Hct, haematocrit; PRA, plasma renin activity; RAS, renin-angiotensin system.

5.5   Discussion

The major novel finding of the current study was that the depressor response to increased RAP could be abolished in rabbits in which salt and fluid balance, and so cardiac output, was maintained by intravenous infusion of compound sodium lactate. We conclude that the depressor response to increased RAP in this model can be completely accounted for by reduced cardiac output, secondary to increased salt and water excretion. Therefore, neither release of the putative renal medullary depressor hormone, nor inhibition of renal renin release appear to contribute significantly to the depressor response to increased RAP in this experimental model.

Comparisons made across animals throughout the three different states; conscious, anaesthetised, and once the extracorporeal circuit had been established, demonstrated that MAP and CO were substantially reduced and HR increased in anaesthetised rabbits, when compared to the conscious state. On the other hand, CO and MAP in rabbits with an established extracorporeal circuit were closely similar to that observed in the conscious state. These observations further validate our major findings and conclusions, in that they show for the first time that systemic haemodynamics in the extracorporeal circuit model remain within a physiologically relevant range.

The 'cardiac output clamp' employed during these studies in rabbits in groups 3 and 4 worked effectively as CO was titrated and maintained throughout the experiments. The 'renin-angiotensin system clamp' was also effective in blocking the renin-angiotensin system since (i) bolus doses of angiotensin failed to increase MAP, in rabbits which had received this treatment, and (ii) resting systemic and renal haemodynamics and renal excretory variables in rabbits that had received this treatment were similar to those in rabbits from the other three experimental groups.

When RAP was increased in anaesthetised rabbits which did not receive the 'cardiac output clamp', MAP fell. This depressor response was associated with increased urine flow and sodium excretion, and reduced CVP and CO, and a small but significant reduction in SVR. Thus, it appears to result chiefly from the pressure diuresis/natriuresis mechanism that caused CO to fall due to negative salt and fluid balance. This hypothesis was confirmed by the results from the group of rabbits treated with the 'cardiac output clamp'. In this group, in which the reduction in CO was prevented by maintenance of salt and fluid balance, no significant depressor response was observed. This treatment also abolished the reduction in SVR seen in group 2, suggesting that this might be secondary to the depressor response itself, either through local autoregulatory mechanisms or inhibition of sympathetic drive due to acute central hypovolaemia *(Evans et al., 2001)*.

Our results also suggest that the renin-angiotensin system plays little or no role in mediating the depressor response to increased RAP, since responses to increased RAP in rabbits receiving both the 'cardiac output clamp' and the 'renin-angiotensin system clamp' were indistinguishable from those in the group receiving only the 'cardiac output clamp'. This hypothesis is further supported by our observations of unchanged PRA throughout the course of the experiment, indicating that although renal renin release might be inhibited by increased RAP, the relatively long circulating half-life of renin prevents substantial changes in circulating levels of this enzyme.

These present findings seem to be at odds with those of Christy et al. (1991) who first tested this circuit in rabbits. Those experiments were conducted with the intention of determining the effect of increased RAP on MAP in rabbits with intact and chemically ablated renal medullas. It was hypothesized that increases in RAP would produce a depressor response due to the release of a putative hormone from the renal medulla. Consistent with this hypothesis, they observed that the depressor response was abolished in rabbits in which the renal medulla had been ablated by BEA-treatment. However, they also observed that the pressure diuresis/natriuresis response was significantly blunted in chemically medullectomized rabbits. Therefore, the blunted depressor response may have occurred due to a reduced ability of these rabbits to excrete sodium and water, rather than removal of the source of the putative renal medullary depressor hormone.

Our present observations also seem at odds with another study performed in our laboratory. Bergström and Evans, found that selective reduction of MBF by renal medullary interstitial infusion of the V_1-agonist [Phe^2, Ile^3, Orn^8]-vasopressin also blunted the depressor response to increased RAP *(Bergström and Evans, 1998)*. This study is a little more difficult to reconcile with our present findings since in rabbits receiving the vasopressin V_1-agonist, the pressure natriuresis-diuresis response was if anything slightly greater than that of the control rabbits. However, our recent study of the disposition of [^{3}H]-noradrenaline during medullary interstitial infusion has shown that renal medullary interstitial infusion of vasoactive compounds results in spillover into the systemic circulation. In the case of [^{3}H]-noradrenaline, around 40% of the infused radiolabel spilled over into the systemic circulation *(Correia, 1997)*. Based on comparison of the systemic effects of medullary interstitial and intravenous [Phe^2, Ile^3, Orn^8]-vasopressin *(Evans et al., 1998a)*, it seems likely that this agent has comparable systemic spillover when administered into the medullary interstitium. Thus, we cannot exclude the possibility that [Phe^2, Ile^3, Orn^8]-vasopressin acted outside the kidney to blunt the depressor response to increased RAP. We can probably exclude a non-specific effect secondary to the pressor action of [Phe^2, Ile^3, Orn^8]-vasopressin, since intravenous noradrenaline,

which also had a pressor action, did not blunt the depressor response to increased RAP (Chapter 4). One possibility that merits investigation is that 'he^2, Ile^3, Orn^8]-vasopressin acts in the central nervous system, to support arterial pressure in the 'ace of falling cardiac output. In this regard it is of interest that the systemic haemodynamic response to increased RAP resembles that to haemorrhage and other maneuvers that acutely reduce central blood volume *(Evans et al., 2001)*. Furthermore, there is some evidence for roles of both central nervous system *(Johnson et al., 1998)* and peripheral *(Schadt and Hasser, 1991)* V$_1$-receptors in recovery from decompensated hypovolaemia, although vasopressin itself seems to play little role in the acute response to central hypovolaemia *(Evans et al., 2001)*.

Based on these observations it may be necessary to reinterpret previous studies which employed the extracorporeal circuit, as observations of the systemic haemodynamic responses to increased RAP have been largely interpreted in the context of the putative renomedullary depressor hormone, which according to these findings, appears to be an incorrect assumption. On the other hand, although the findings of this study provide convincing evidence that the pressure diuresis/natriuresis mechanism as the main cause of the depressor response in this experimental model, these findings in no way indicate that the depressor substance is not released or does not exist. They simply show that this experimental model is not an assay for its release.

In the experiments described in Chapter 4, we found that the depressor response to increased RAP was blunted by medullary interstitial infusion of noradrenaline, which reduced MBF. However, the pressure diuresis/natriuresis relationship was also blunted by medullary interstitial noradrenaline. Taken together with the results of the present study, it seems likely that the effect of medullary interstitial noradrenaline on the depressor response to increased RAP was due chiefly to the effect of this treatment on the pressure diuresis/natriuresis relationship, rather than (as we had previously concluded) on release of the putative renal medullary depressor hormone. This conclusion is consistent with the notion that MBF has a profound impact on long-term blood pressure control, chiefly through its role in modulating urinary salt and water excretion *(Cowley, 1997)*.

In summary, the results of this study indicate that the depressor response to increased RAP in the extracorporeal circuit model is abolished if extracellular fluid volume is maintained by a 'cardiac output clamp' indicating that in this particular model the depressor response occurs due to the effects of the pressure diuresis/natriuresis mechanism and not the putative renal medullary depressor hormone. This finding implies that the extracorporeal circuit model is not suited for

experiments aimed at studying the putative depressor hormone, even though the circuit provides a presumed stimulus for the release of this hormone; increased RAP.

5.6   Conclusions

The extracorporeal circuit model provides a useful tool with which to study renal antihypertensive mechanisms in anaesthetised rabbits, because it allows perfusion of the kidney independently of the systemic circulation. The results of these studies provide evidence that the pressure diuresis/natriuresis mechanism dominates the kidneys ability to regulate arterial pressure under these experimental conditions. This is based on our observation that the depressor response observed during increased RAP was abolished in rabbits in which the pressure diuretic/natriuretic response was blocked. Inhibition of the renin-angiotensin system appears to play little or no role in mediating the acute depressor response to increased RAP in this model, presumably because to the long circulating half-life of renin. We also conclude that release of the putative renal medullary depressor hormone makes little or no contribution to the depressor response to increased RAP in this extracorporeal circuit experimental model in anaesthetised rabbits.

Chapter Six

EFFECTS OF ACTIVATION OF VASOPRESSIN-V$_1$-RECEPTORS ON REGIONAL KIDNEY BLOOD FLOW AND GLOMERULAR ARTERIOLE DIAMETERS

6.0   Summary

1. Intravenous infusion of the vasopressin V$_1$-agonist [Phe^2, Ile^3, Orn^8]-vasopressin selectively reduces renal MBF in anaesthetised and conscious rabbits. Since MBF is derived entirely from the efferent arterioles of juxtamedullary glomeruli, the aim of the current study was to test whether vasoconstriction of juxtamedullary glomerular arterioles contributes to V$_1$-receptor mediated reductions in MBF.

2. Experiments were performed in anaesthetised rabbits. The right kidney remained innervated while the left kidney was denervated and placed in a stable cup. Regional and total kidney blood flows were measured by a perivascular flow probe placed around the renal artery, and by laser-Doppler flow probes positioned in the outer medulla, and on the cortical surface. Throughout the experiment urine was collected via catheters placed in each of the ureters, and a perfusion apparatus was connected to a large-bore catheter in the abdominal aorta for fixation of the left kidney.

3. Following a 30 min control period, rabbits received a 30 min intravenous infusion of the V$_1$-agonist [Phe^2,Ile^3,Orn^8]-vasopressin (30 ng/kg/min) or its vehicle. During this time, left and right kidney urine samples, and arterial blood samples were collected. On completion of the infusion, kidneys were immediately perfusion fixed at the final recorded MAP, and filled with methacrylate casting material.

4. The diameters of afferent and efferent arterioles in the outer, mid and juxtamedullary cortex of the left kidneys were determined by scanning electron microscopy.

5. Intravenous [Phe^2, Ile^3, Orn^8]-vasopressin increased MAP (19 ± 3%) and reduced MBF (30 ± 9%), but had no effect on CBF or total RBF. Vehicle treatment did not affect these variables. There were no significant differences in afferent or efferent arteriole diameter, in any of the cortical regions, between [Phe^2, Ile^3, Orn^8]-vasopressin and vehicle treated rabbits.

6. These results do not support a role for juxtamedullary arterioles in producing V$_1$-receptor mediated reductions in MBF, suggesting that downstream vascular elements (e.g. outer medullary descending vasa recta) might possibly be involved.

6.1   Introduction

Although only about 10% of total RBF perfuses the renal medulla, there is now evidence that the medullary microcirculation plays a key role in the long-term regulation of blood pressure, chiefly through its influence on renal salt and water handling *(Cowley, 1997)*. Therefore, in order to understand the mechanisms underlying the long-term regulation of blood pressure, we require a more complete understanding of the factors regulating MBF.

In both conscious and anaesthetised rats and rabbits, intravenous infusion of low doses of arginine vasopressin *(Zimmerhackl et al., 1985; Franchini et al., 1997)* or the selective the V$_1$-agonist - [Phe^2, Ile^3, Orn^8] vasopressin *(Evans et al., 1998; Evans et al., 2000)* can reduce MBF without affecting CBF or total RBF. There is also good evidence that arginine vasopressin plays an important role in the physiological regulation of MBF *(Franchini and Cowley, 1996)*. The precise mechanisms mediating the selective effect of arginine vasopressin on MBF remain unknown, but they could theoretically involve vasoconstriction of afferent and/or efferent arterioles of juxtamedullary glomeruli (the source of MBF), or downstream vascular elements (vasa recta) *(Pallone et al., 1990)*. On the other hand, we can exclude vasoconstriction at vascular sites upstream from the afferent arteriole, which would be expected to also reduce CBF and RBF. We can probably also exclude a role for proposed contractile elements in inner medullary descending vasa recta *(Pallone et al., 1990)*, since Zimmerhackl *et al.*, (1985), using video microscopy, were unable to detect arginine vasopressin induced changes in the diameters of these vessels, even though erythrocyte velocity within them was significantly reduced *(Zimmerhackl et al., 1985)*.

In support of the hypothesis that V$_1$-receptor mediated decreases in MBF are mediated by vasoconstriction of juxtamedullary arterioles and or outer medullary descending vasa recta, V$_1$-receptor mRNA has been located in these vascular elements *(Park et al., 1997)*. There is also good evidence from *in vitro* studies that arginine vasopressin can constrict juxtamedullary arterioles *(Edwards et al., 1989; Harrison-Bernard and Carmines, 1994;Tamaki et al., 1996)* and outer medullary descending vasa recta *(Edwards et al., 1989)*. On the other hand, in the study by Harrison-Bernard and Carmines (1994), using the blood perfused juxtamedullary nephron preparation, arginine vasopressin at physiological concentrations (>10^{-12} M) was found to constrict

afferent arterioles of juxtamedullary glomeruli but not outer medullary descending vasa recta. Furthermore, in the one study demonstrating vasoconstriction of outer medullary descending vasa recta to vasopressin *in vitro*, the concentrations required ($\sim10^{-10}$ M) were about one order of magnitude greater than those encountered in plasma under physiological conditions *(Turner and Pallone, 1997)*. Thus, on balance this evidence supports a role for vasoconstriction of juxtamedullary glomerular arterioles in mediating reduced MBF in response to activation of V_1-receptors. However, as yet there is no evidence from *in vivo* studies to confirm or reject this hypothesis.

Therefore, in the current study we directly tested this hypothesis in anaesthetised rabbits by examining the effects of an intravenous infusion of [Phe^2, Ile^3, Orn^8] vasopressin on RBF, CBF, and MBF and employing the technique of vascular casting to measure the luminal dimensions of afferent and efferent arterioles in the outer, mid, and juxtamedullary cortical regions *(Denton et al., 1992; Denton et al., 2000)*.

6.2   Methods

6.2.1   Experimental preparation

Fourteen, New-Zealand White, male rabbits were used (body weight 2.18 ± 2.62 kg; mean 2.41 ± 0.03 kg). Preparation of the rabbits on the experimental day is described in detail in Chapter 2, Sections 2.2.1, 2.2.2, 2.2.5, and 2.2.6. Briefly, catheters were placed in both central ear arteries and marginal ear veins, the rabbits were anaesthetised with pentobarbitone (90-150 mg), and prepared for measurement of RBF, CBF, and MBF, and collection of urine from both kidneys. Rabbits received an intravenous infusion of either [Phe^2,Ile^3,Orn^8]vasopressin (n=7) or its vehicle (n=7). Thirty minutes later, the left kidney was perfusion fixed and methacrylate filled for later analysis of the glomerular arterioles using scanning electron microscopy.

6.2.2   Surgery

Rabbits were prepared for surgery according to Section 2.2.1 of Chapter 2. Briefly, the left kidney was denervated and placed in a stable cup for the positioning of laser-Doppler flow probes in the inner medulla and on the superficial cortex. A transit time ultrasound flow probe was placed around the renal artery for measurement of RBF. Both ureters were cannulated for urine collection. A large bore cannula was placed in the aorta distal to the renal arteries, which was later connected to the

perfusion apparatus for fixation. The abdominal aorta was isolated above the renal arteries so it could be occluded during the fixation process.

6.2.3 Experimental protocols

On completion of the surgery, bolus doses of [^{3}H]-inulin (4 µCi; NEN Research Products, Sydney, Australia) and [^{14}C]-PAH (1 µCi; NEN Research Products) were administered intravenously, and the maintenance infusion of Hartmann's solution (0.18 ml/kg/min) was replaced with a solution containing 300 nCi/ml [^{3}H]-inulin and 83 nCi/ml [^{14}C]-PAH, in 4 parts Hartmann's solution and 1 part 10% v/v polygeline (Haemaccel; Hoechst, Melbourne, Australia). Following a 60 min equilibration period, and a 30 min control period, rabbits received a 30 min intravenous infusion of either the V₁-agonist [Phe^2,Ile^3,Orn^8] vasopressin (Peninsula Laboratories Inc., Belmont, CA, USA; 30 ng/kg/min; n=7), or its vehicle (50 µl/kg/min 154 mM NaCl; n=7) for 30 min. Urine produced by the left and right kidneys was collected during the final 20 min of both the control and infusion periods. Arterial (3.0 ml) blood samples were collected at the mid point of each period for determination of plasma renin activity, plasma sodium concentration and haematocrit (Figure 6.1).

Perfusion fixation of both kidneys commenced immediately at the end of the second urine collection. One liter of 2.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3-7.4) at room temperature was perfused retrogradely through the distal aorta at a pressure equivalent to MAP during the final 2 minutes of the [Phe^2,Ile^3,Orn^8] vasopressin or vehicle infusion for each rabbit. The upper aorta was clamped above the kidneys and the vena cava vented as soon as perfusion of the kidney commenced. Immediately following fixation, a mixture of methacrylate and accelerator (20:1) (Mercox CL-2B-5; MIII; West Chester, PA, USA) was perfused into the left and right kidneys at the same pressure as the fixative. Both kidneys were than clamped above the renal hilus, and the methacrylate resin was allowed to harden *in situ* for 30 min. The left kidneys were removed, weighed (13.37 ± 0.33 g) and stored in 2.5% paraformaldehyde for later processing.
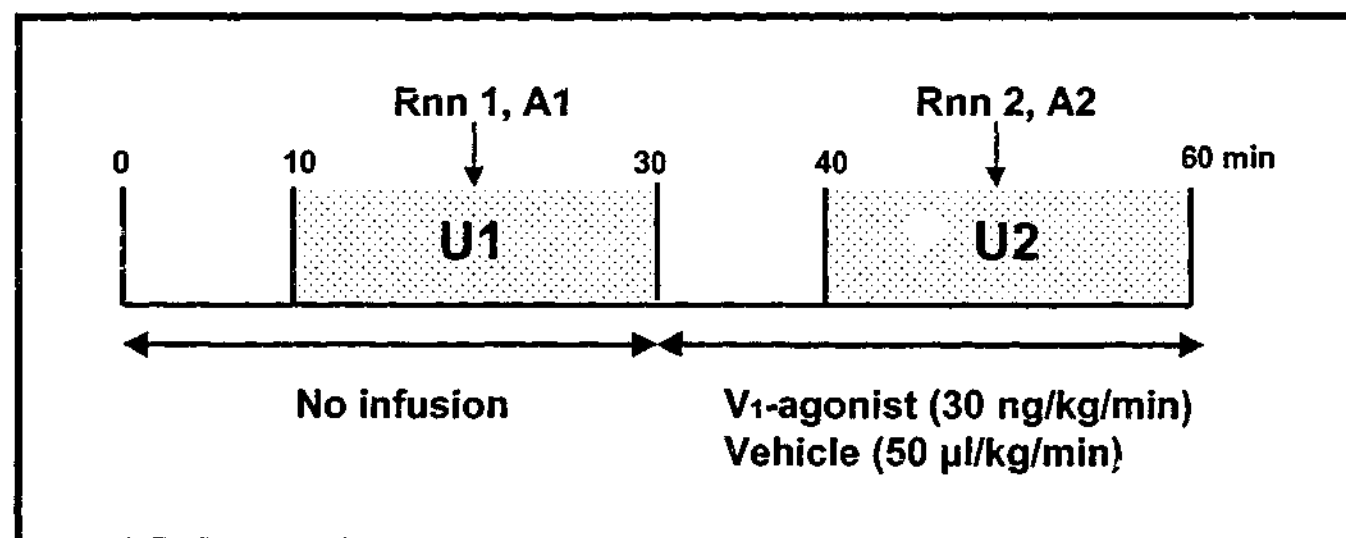
Figure 6.1 Schematic diagram of experimental protocol for study investigating the effect of [Phe^2,Ile^3,Orn^8] vasopressin infusion on afferent and efferent arteriolar diameters and systemic and renal haemodynamics. Following a 90 min equilibration period, and 10 min of stable baseline recordings, urine was collected from both the right and left ureters for 20 min (U1), at the mid point of this period, ear arterial blood samples (3.0 ml) were collected for determination of plasma renin activity (Rnn1) and plasma sodium concentration and haematocrit (A1). During the second phase of the experimental period, an infusion of either the V_1-agonist (30 ng/kg/min) or its vehicle (50 μl/kg/min) was commenced, and following 10 min the first experimental period was repeated. Shaded areas represent urine collection periods. The left kidney was perfusion fixed and filled with methacrylate at the end of the second urine collection period (U2).

6.2.3.1 Haemodynamic variables

MAP was measured throughout the experiment using a side arm catheter, 3 mm proximal to the tip of the cannula inserted into the aorta. The remaining haemodynamic variables, HR and renal haemodynamics (RBF, CBF, and MBF) were measured throughout the experiment as described in detail in Section 2.7, Chapter 2.

6.2.3.2 Analysis of urine and blood samples

Blood and urine samples were processed for measurement of plasma renin activity, haematocrit, and determinations of sodium, [^{3}H]-inulin and [^{14}C]-PAH concentrations. GFR and effective RBF were calculated as detailed in Sections 2.7.2 and 2.7.3 (Figure 6.1).

6.2.3.3 Preparation of methacrylate casts for scanning electron microscopy

Only the left kidneys were examined. Following removal from the rabbit, each kidney was stored individually in fixative to allow complete polymerization of the methacrylate (24-48 hours). The preparation of the vascular casts for scanning electron microscopy is explained in detail in Section 2.5.1 of Chapter 2. Briefly, the kidneys were sectioned and incubated for 1 week in tissue solubilizer potassium hydroxide (KOH) for removal of the tissue from the casts. The remaining casts were then washed, mounted, and gold coated (SCD 005 Sputter Coater; Bal-Tec, Liechtenstein) before being examined in a scanning electron microscope at 20 Kv (Hitachi S-570, Hitachi City, Japan).

Luminal diameters of afferent and efferent arterioles of outer, mid, and juxtamedullary glomeruli were measured from scanning electron micrographs (final magnification 660 X). Diameter measurements were made at 25 µm intervals (Figure 6.2) along each vessel from its junction with the glomerulus to its first branching point. Six afferent and efferent vessels from each region of the cortex were measured. These were selected and classified as previously described *(Denton et al., 1992; Denton et al., 2000)*. The vascular casts were coded and randomised before the micrographs were taken. Measurements along the arteriolar lengths were made using a digitizing tablet (Summagraphics; resolution 100 lines/mm, accuracy ± 0.25 mm, GTCO Calcorp, USA) and the MEASURE program (Capricorn Scientific Software, Victoria, Australia). Vessel selection and measurement procedures are described in greater detail in Section 2.5.2 (Scanning electron microscopy) in Chapter 2.

6.2.3.4 Vascular diameter, resistance and conductance

These calculations are explained in Section 2.8.2 of Chapter 2. Briefly, mean values for the radius (r) and diameter of each vessel were derived from the measurements made along the vessel length. Relative resistances (R) were calculated according to Poiseulle's relationship ($R = 1/r^4$) and expressed per unit length *(Denton et al., 1992; Denton et al., 2000)*, see Table 6.2.

6.2.4   Statistical analysis

6.2.4.1 Haemodynamic data

To test whether [Phe^2,Ile^3,Orn^8]-vasopressin or its vehicle altered baseline systemic and renal haemodynamic variables, the average levels of each variable during the first 10-30 min after commencing the infusion were compared with levels during the initial control period by paired t-test.

6.2.4.2 Glomerular arteriole dimensions

To test whether the [Phe^2,Ile^3,Orn^8]-vasopressin treatment altered vessel diameter in comparison to vehicle infusion, the average diameter of the treated vessels in each cortical region (outer, mid, juxtamedullary) were compared with those in control animals by unpaired t-test. Paired t-tests were used to test for differences between afferent and efferent arteriole diameters in the various regions of the cortex.

6.3   Results

6.3.1   Baseline levels during the control period

Systemic and renal haemodynamic variables (Figures 6.3 & 6.4), and renal excretory variables (Figure 6.4) during the control period were similar to those previously observed by us under similar experimental conditions *(Denton et al., 1992; Correia, 1997; Evans et al., 1998; Denton et al., 2000)*. There were no systemic differences in these variables between the two groups of rabbits ($P >$ 0.05).

6.3.2   Haemodynamic and renal responses to [Phe^2,Ile^3,Orn^8]-vasopressin and vehicle treatment

Intravenous [Phe^2,Ile^3,Orn^8]-vasopressin (30 ng/kg/min) increased MAP (19 ± 3%) and haematocrit (7 ± 2%), and reduced HR (16 ± 2%) and MBF (30 ± 8%), but RBF, CBF and renal vascular resistance did not significantly change (Figure 6.2, Table 6.1). Vehicle treatment did not significantly affect any of the measured variables with the exception of haematocrit, which decreased by 3 ± 1% (Figure 6.2, Table 6.1).

[Phe^2,Ile^3,Orn^8]-vasopressin treatment had no significant affect on effective RBF in either kidney. In both the left (denervated) and right (and innervated) kidney, [Phe^2,Ile^3,Orn^8]-vasopressin treatment significantly increased GFR (58 ± 13 and 109 ± 38%, respectively), urine flow (166 ± 32 and 355 ± 78%, respectively) and sodium excretion (118 ± 21 and 290 ± 82%, respectively). Following vehicle treatment there were small but statistically significant increases in GFR in the right kidney (33 ± 10%), urine flow in the left kidney (32 ± 14%), and sodium excretion in both the left (36 ± 15%) and right (42 ± 16%) kidneys, but no significant changes in effective RBF (Figure 6.4).

6.3.3 Vessel lumen diameters and calculated relative resistances

No evidence of focal constriction was observed along the length of any of the arterioles. Therefore, mean diameter was calculated for each afferent and efferent arteriole in the different regions, as an arithmetic mean of each measurement (every 25 μm) along the length of the vessel from the glomerulus.

There were no significant differences in glomerular afferent or efferent arteriole lumen dimensions between vehicle and [Phe^2,Ile^3,Orn^8]-vasopressin agonist treated rabbits (Figure 6.5). In particular, juxtamedullary afferent and efferent arteriolar diameters were closely similar in the two groups of rabbits. There was, however, a tendency for mid cortical efferent arteriolar diameter to be less in [Phe^2,Ile^3,Orn^8]-vasopressin treated than in vehicle treated rabbits ($P = 0.07$, Figure 6.5). Calculated relative resistance per unit vessel length was closely similar in vehicle and [Phe^2,Ile^3,Orn^8]-vasopressin agonist treated rabbits for all arterioles (Figure 6.4), except for mid cortical efferent arterioles in which a tendency for increased relative resistance was observed in the [Phe^2,Ile^3,Orn^8]-vasopressin treated rabbits ($P = 0.07$).

When averaged across both groups of rabbits, afferent arteriole lumen diameters in the outer, mid, and juxtamedullary cortex were 15.06 ± 0.70, 13.87 ± 0.52, and 15.48 ± 1.20 μm, respectively. Compared with these afferent arterioles, the corresponding efferent arterioles had smaller diameters in the outer cortex (12.37 ± 1.00 μm, $P = 0.02$) and mid cortex (12.13 ± 0.61 μm, $P = 0.03$), but greater diameters in the juxtamedullary cortex (17.62 ± 1.48 μm, $P = 0.02$).

Figure 6.2 Scanning electron microscope image of juxtamedullary glomeruli. A. afferent arteriole; E, efferent arteriole; G, glomerulus; OMDVR, outer medullary descending vasa recta.
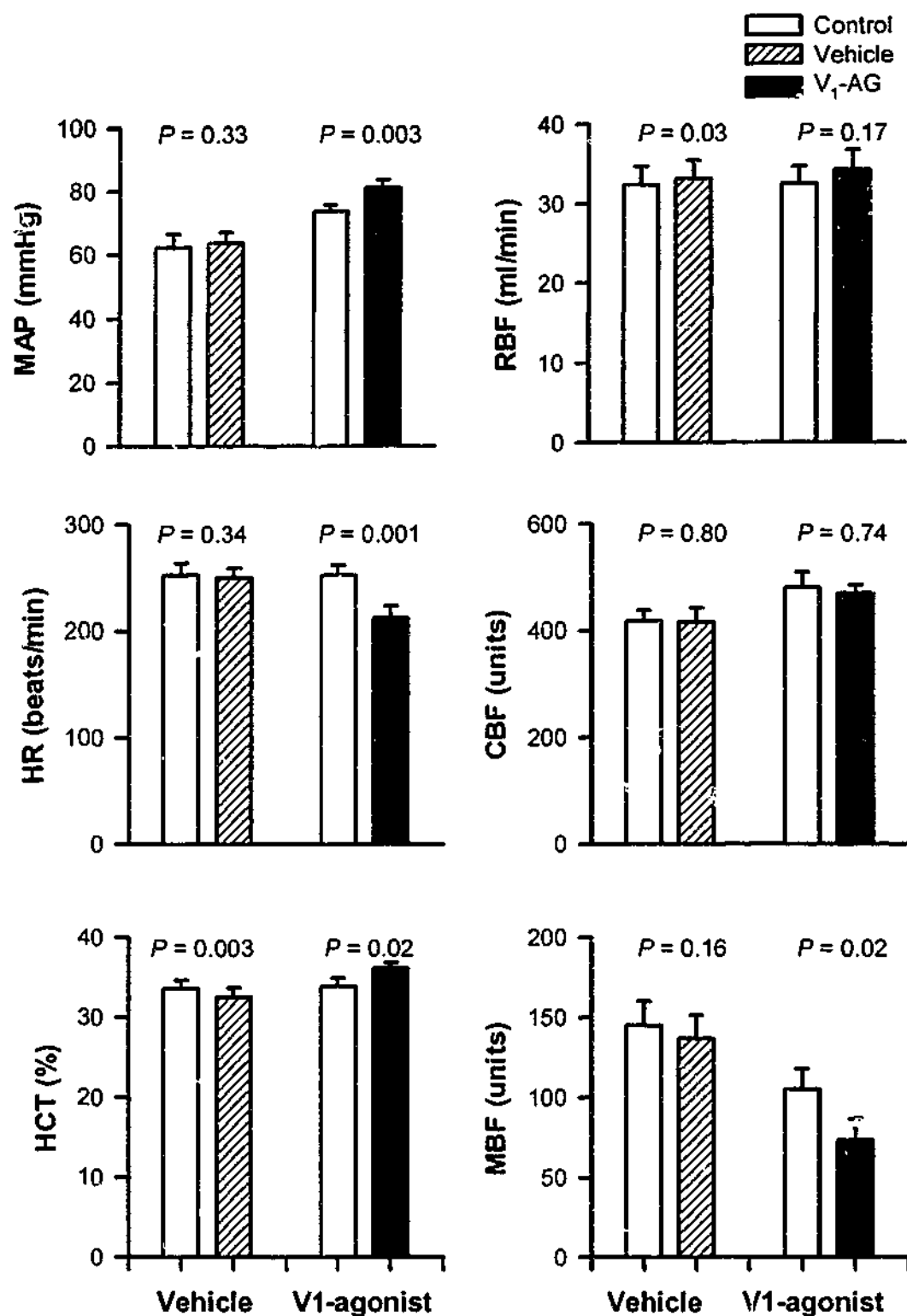
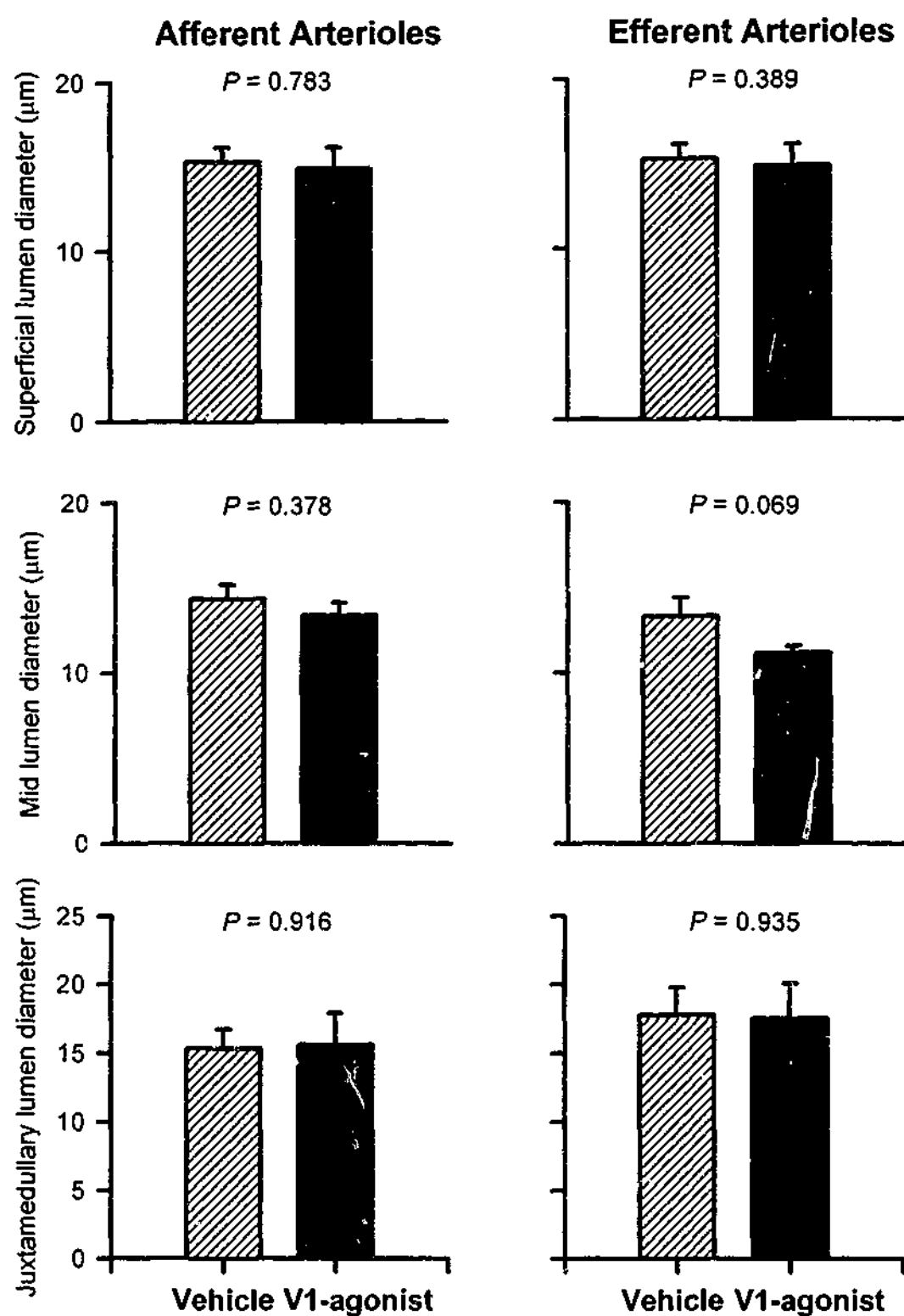**Figure 6.3** Effects of intravenous infusion of [Phe^2,Ile^3,Orn^8]-vasopressin (30 ng/kg/min) or its vehicle (154 mM NaCl, 50 µl/kg/min) on systemic and renal haemodynamic variables. MAP, mean arterial pressure; HR, heart rate, HCT, haematocrit; RBF, renal blood flow; CBF, renal cortical perfusion, and MBF, renal medullary perfusion. Columns and error bars represent the mean ± SEM of data (n=7) during the final 20 min of the control period (open columns), and the final 20 min of the period of infusion of vehicle (hatched columns) or [Phe^2,Ile^3,Orn^8]-vasopressin (filled columns). *P* values represent outcomes of paired t-tests, testing whether variables changed significantly during infusions of vehicle or [Phe^2,Ile^3,Orn^8]-vasopressin.
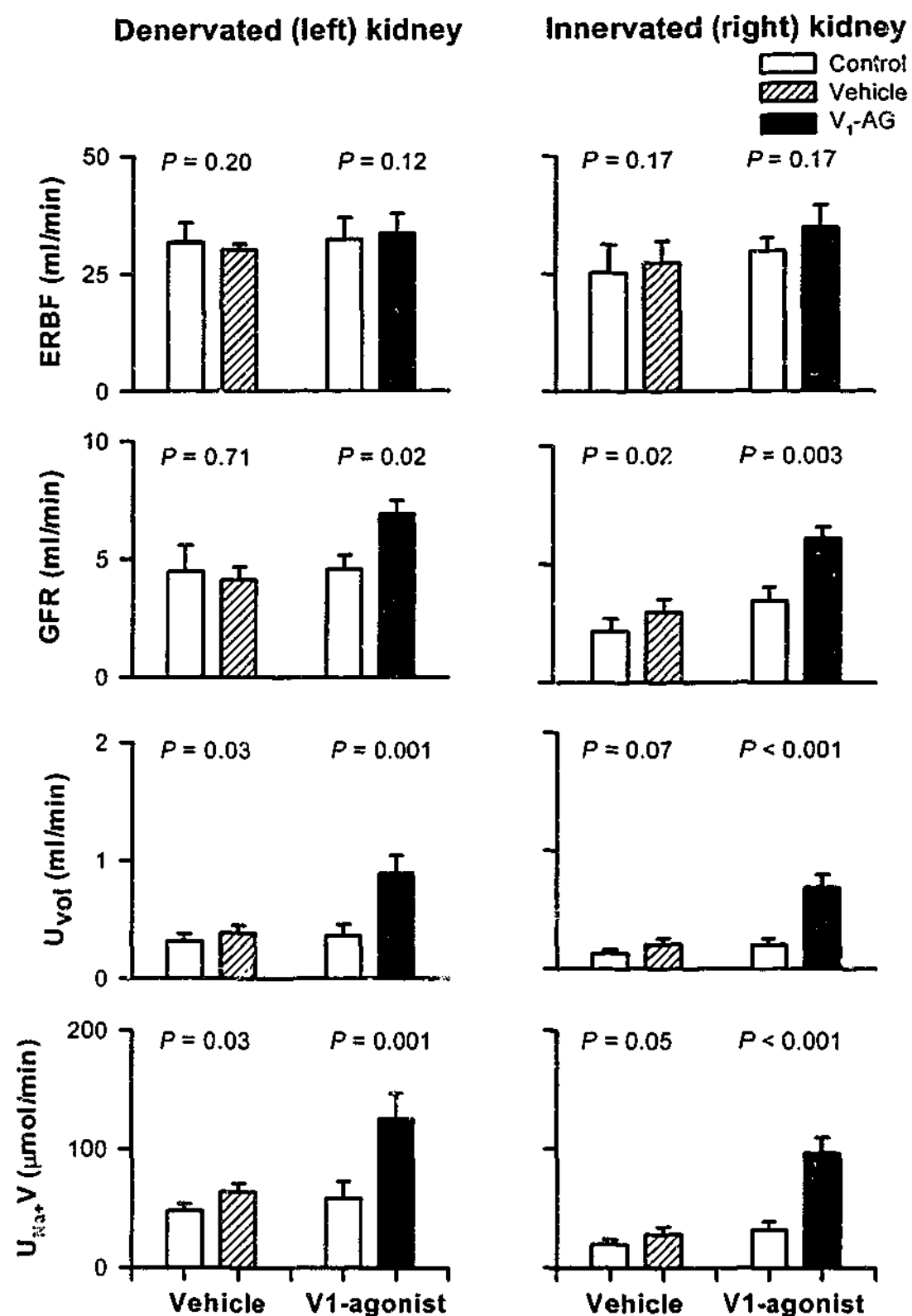
Figure 6.4   Effects of intravenous infusion of [Phe^2,Ile^3,Orn^8]-vasopressin (30 ng/kg/min) or its vehicle (154 mM NaCl, 50 μl/kg/min) on renal clearance variables in the denervated (left) kidney and the intact (right) kidney. ERBF, effective renal blood flow; GFR, glomerular filtration rate; U_{VOL}, urine flow, $U_{Na+}V$, urinary sodium excretion. Columns, error bars and P values are as for Figure 6.3.

Figure 6.5 Diameters of vascular casts of glomerular arterioles in the superficial, mid, and juxtamedullary cortex, after intravenous infusion of $[Phe^2,Ile^3,Orn^8]$-vasopressin (filled columns; 30 ng/kg/min) or its vehicle (hatched columns; 154 mM NaCl, 50 μl/kg/min). Columns and error bars represent the between rabbit mean ± SEM (n=7). P values represent the outcomes of unpaired t-tests, testing whether diameters of vessels in $[Phe^2,Ile^3,Orn^8]$-vasopressin- treated rabbits differed from the corresponding vessels in vehicle- treated rabbits.

Table 6.1   Effects of intravenous infusion of vehicle (50 µl/kg/min; n=7) or [Phe^2, Ile^3,Orn^8]-vasopressin (30 ng/kg/min; n=7) on systemic and renal haemodynamic variables. Data are presented as percentage change from baseline measurements.

	Vehicle	[Phe^2, Ile^3,Orn^8] vasopressin
MAP	-1 ± 3	19 ± 3 *
HR	-1 ± 2	-16 ± 2 ***
HCT	-3 ± 1 *	7 ± 2 *
RBF	3 ± 1	6 ± 4
CBF	-1 ± 2	-1 ± 5
MBF	-5 ± 4	-30 ± 10 *

Each value represents the mean ± SEM of the percentage difference for each variable, between the baseline levels, and the levels 10-30 min following commencement of the infusion of V₁-agonist or vehicle. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ for outcome of paired t-test. MAP, mean arterial pressure; HR, heart rate; HCT, haematocrit; RVR, renal vascular resistance; RBF, renal blood flow; CBF, renal cortical blood flow; MBF, renal medullary blood flow.

Table 6.2   Effects of intravenous infusion of [Phe^2, Ile^3, Orn^8]-vasopressin (30 ng/kg/min) or its vehicle (50 µl/kg/min) on relative resistance.

TREAT	VESSEL	OUTER CORTEX	MID CORTEX	JUXTA MEDULLARY
Vehicle	Afferent	0.051 ± 0.018	0.064 ± 0.017	0.060 ± 0.021
V₁- agonist	Afferent	0.087 ± 0.041	0.080 ± 0.022	0.128 ± 0.058
Vehicle	Efferent	0.293 ± 0.051	0.189 ± 0.039	0.124 ± 0.036
V₁- agonist	Efferent	0.417 ± 0.073	0.375 ± 0.083	0.174 ± 0.059

6.4   Discussion

As previously demonstrated *(Evans et al., 1998; Evans et al., 2000)*, intravenous infusion of the V_1 -receptor agonist [Phe^2, Ile^3,Orn^8] vasopressin selectively reduced MBF compared with CBF and RBF in rabbits. Our present aim was to determine whether this selective effect of the V_1 -receptor agonist [Phe^2, Ile^3,Orn^8]-vasopressin on MBF is mediated by selective vasoconstriction of juxtamedullary glomerular arterioles, using a previously characterized vascular casting technique *(Denton et al., 1992; Denton et al., 2000)*. We found that intravenous infusion of [Phe^2, Ile^3,Orn^8]-vasopressin reduced MBF by 30 ± 8% but we could not detect a change in the diameters of afferent or efferent juxtamedullary arterioles. These data are not consistent with a role of juxtamedullary arterioles in mediating the effects of [Phe^2, Ile^3,Orn^8]-vasopressin on MBF. Since vasoconstriction of vascular elements upstream from glomerular arterioles (i.e. interlobular or arcuate arteries) should produce reductions in CBF (and RBF) as well as MBF, our data raise the possibility of a role for downstream vascular elements in mediating MBF responses to [Phe^2, Ile^3,Orn^8]-vasopressin. The outer medullary portions of the descending vasa recta are the most likely candidates, since these have previously been shown to be responsive to both arginine vasopressin and [Phe^2, Ile^3,Orn^8]-vasopressin *in vitro (Turner and Pallone, 1997)*.

In the current study, our combination of *in vivo* measurements of RBF, CBF and MBF together with data from *ex- vivo* analysis of vascular casts from the same animals, provided a unique opportunity for direct correlation of the actions of [Phe^2, Ile^3,Orn^8]-vasopressin on regional kidney blood flow and glomerular arteriole dimensions. The laser-Doppler technique for measurement of regional kidney blood flow has previously been validated *(Hansell, 1992)*, and used extensively by others *(Franchini and Cowley, 1996; Cowley, 1997; Franchini et al., 1997)* and ourselves *(Correia, 1997; Evans et al., 1998; Evans et al., 2000;* also Chapter 4*)* to demonstrate the diversity of responses of regional kidney blood flow to hormonal agents. The vascular casting technique used in the present study involves rapid tissue fixation at physiological pressure, after which the renal vasculature is filled with methacrylate resin material. Although some artifact is associated with the vascular casting procedure, the vascular casting technique has previously been extensively validated *(Gattone et al., 1983; Gattone and Evan, 1986; Kimura et al., 1990; Denton et al., 1992; Denton et al., 2000)*. Importantly, the glomerular arteriole diameters derived from the vascular casts in the present study are comparable to those previously reported in rabbits using several techniques, including vascular casting *(Denton et al., 1992; Denton et al., 2000)*, stereology *(Kaissling and Kriz, 1979)*, and isolated arteriole preparations *(Ito and Carretero, 1990; Weihprecht et al., 1991)* We also observed regional differences in glomerular arteriole diameters, which is consistent with

previous studies in this *(Kaissling and Kriz, 1979; Denton et al., 1992; Denton et al., 2000)* and other species *(Gattone et al., 1983; Dwarkin and Brenner, 1996)*. Furthermore, previous studies using the technique of vascular casting have demonstrated vasoconstriction in glomerular arterioles in response to angiotensin II *(Denton et al., 1992; Denton et al., 2000)* and noradrenaline *(Kimura et al., 1990)* and vasodilatation in response to atrial natriuretic peptide *(Kimura et al., 1990)*. We can therefore be confident that the vascular casting technique can be used to detect changes in renal arteriole dimensions.

Consistent with the lack of effect of $[Phe^2, Ile^3, Orn^8]$-vasopressin on CBF and RBF, we observed no significant differences between vehicle and $[Phe^2, Ile^3, Orn^8]$-vasopressin treated rabbits in the diameters of afferent or efferent arterioles in superficial and mid cortical regions of the cortex. If the 30% reduction in MBF were entirely due to vasoconstriction in juxtamedullary arterioles, we would have expected (based on Poiseulle's relationship, where resistance is inversely proportional to the fourth power of the vessel radius) juxtamedullary arteriole diameter to have been about 9% less in $[Phe^2, Ile^3, Orn^8]$-vasopressin treated compared with vehicle treated rabbits. However, the diameters of juxtamedullary afferent and efferent arterioles were indistinguishable in the two groups of rabbits, and indeed if anything were numerically (~3%) greater in $[Phe^2, Ile^3, Orn^8]$-vasopressin treated than in vehicle treated- rabbits.

Our failure to detect significant differences in juxtamedullary glomerular arteriole diameter between vehicle- and $[Phe^2, Ile^3, Orn^8]$-vasopressin treated rabbits reflect some inherent insensitivity of the casting technique. However, we believe this is unlikely, since previous studies in our laboratory have detected decreases of ~1 μm in outer cortical efferent arteriole diameter in rabbits receiving renal arterial infusions of angiotensin II (1 ng/kg/min), that caused a 35% reduction in RBF *(Denton et al., 2000)*. In the current study, to decrease MBF by ~30%, mean juxtamedullary afferent arteriole diameter would have had to decrease by ~9%, which corresponds to changes in arteriolar diameter of 1.4 μm (afferent) to 1.6 μm (efferent).

Our *ex-vivo* data are therefore not consistent with a role of juxtamedullary glomerular arterioles in mediating the selective effects of $[Phe^2, Ile^3, Orn^8]$-vasopressin on MBF, and in this respect are at odds with the results of *in vitro* studies showing constriction of juxtamedullary afferent arterioles in response to physiological concentrations of arginine vasopressin *(Harrison-Bernard and Carmines, 1994)*. Nevertheless, this is the first study we are aware of that has addressed this issue under *in vivo* conditions, and it is likely that the responsiveness of renal vascular elements to activation of V₁-receptors is highly dependent upon the intrarenal hormonal milieu. Furthermore, although they

appear less sensitive than afferent arterioles under *in vitro* conditions *(Harrison-Bernard and Carmines, 1994)*, outer medullary descending vasa recta do respond to arginine vasopressin and [Phe^2, Ile^3,Orn^8]-vasopressin *(Turner and Pallone, 1997)*. Taken together, these data suggest a possible role for outer medullary descending vasa recta in mediating the selective effect of [Phe^2, Ile^3,Orn^8]- vasopressin (and so perhaps also that of arginine vasopressin) on MBF. This hypothesis will be difficult to test under *in vivo* conditions, since there are considerable technical impediments to the use of casting techniques for measuring the dimensions of outer medullary descending vasa recta. This could not be achieved in the present study, because the outer medullary descending vasa recta were either inadequately filled with casting material, or when adequately filled, they often detached from the efferent arterioles during preparation of the cast. In future studies we hope to overcome this problem, and also increase the resolution of the technique so as to investigate the role of outer medullary descending vasa recta in mediating reduced MBF during activation of renal V₁-receptors.

The present study also allowed us to make a number of interesting observations about the systemic haemodynamic and renal effects of [Phe^2, Ile^3,Orn^8] vasopressin. Intravenous infusion of this agent increased MAP, GFR, urine flow, and sodium excretion. The diuretic and natriuretic effects of [Phe^2, Ile^3,Orn^8]-vasopressin have been described previously in both rats *(Ledderhos et al., 1995)* and rabbits *(Evans et al., 1998)*. These effects appear to result chiefly from a direct tubular action of activation of V₁-receptors, and are at least in part independent of the pressor effect of [Phe^2, Ile^3,Orn^8]-vasopressin *(Ledderhos et al., 1995)*. In the present study we also found that [Phe^2, Ile^3,Orn^8] vasopressin increased GFR. This effect has not been previously described, but is consistent with the pressor effect of this agent, and the lack of evidence in our casting data for autoregulatory vasoconstriction in [Phe^2, Ile^3,Orn^8]-vasopressin treated rabbits. Indeed, the only vessels in which there was any evidence of vasoconstriction were the efferent arterioles of mid cortical glomeruli, in that the average diameters of these vessels tended to be about 11% less in [Phe^2, Ile^3,Orn^8]-vasopressin treated than in vehicle treated rabbits ($P = 0.07$). Our casting data are therefore consistent with the hypothesis that glomerular capillary pressure increases during intravenous infusion of doses of [Phe^2, Ile^3,Orn^8]-vasopressin that increase MAP. This hypothesis merits direct testing with micropuncture.

6.5 Conclusions

In conclusion, the results of the present study are not consistent with an important role of juxtamedullary glomerular arterioles in mediating the selective effect of $[Phe^2, Ile^3, Orn^8]$-vasopressin on MBF. A role for downstream vascular elements, and in particular outer medullary descending vasa recta, remains possible. However, since our present *in vitro* data are at odds with the results of previous studies, they require confirmation by future experiments.

Chapter Seven

GENERAL DISCUSSION

7.0   Introduction

The main impetus for the studies described in this thesis came from the hypothesis that the renal medullary microcirculation plays a significant role in the long term control of arterial pressure, chiefly through modulation of the pressure diuresis/natriuresis response, and possibly also the release of a putative renal medullary vasodepressor hormone. In this final chapter, I will summarize the main findings from this thesis, and discuss future directions for research aimed at answering some of the important questions that arise from my work.

7.1   Renal handling of infused [³H]- noradrenaline

Renal medullary interstitial infusion of [³H]-noradrenaline, delivered to the outer medullary interstitium, results in the infused radiolabel being most concentrated in the juxtamedullary region; the inner cortex and the outer medulla. This finding was consistent with our previous observation that MBF was selectively reduced during medullary interstitial infusion of noradrenaline, adding further weight to the argument that the juxtamedullary and outer medullary vasculature is chiefly responsible for regulating MBF *(Correia, 1997)*. Furthermore, the results of this study, taken together with our previous findings *(Correia, 1997)*, indicate that outer medullary interstitial infusion of vasoactive substances provides a useful tool for studying the role of MBF (in rabbits) in the regulation of arterial pressure.

However, the scope of these studies were limited, in that only one agent, noradrenaline, was tested. It is likely that the intrarenal distribution, and effect of the infused substance on the renal circulation (in particular MBF), will depend on its handling by the kidney. In the case of [³H]-noradrenaline, it was previously shown that approximately half of the outer medullary interstitially infused radiolabel spilled over into the systemic circulation (40 %), or exited the kidney via the

ureter (4 %), limiting this technique to substances which are rapidly metabolized and therefore produce their strongest effect within a small distance from the infusion site *(Correia, 1997)*.

If the use of this technique is extended to other substances, the physico-chemical and biological characteristics of each substance will likely influence its effects and distribution within the kidney. These characteristics might include: (i) the size and charge of the molecules involved, (ii) possible interactions with other hormones in the interstitium and circulation, such as nitric oxide and eicosanoids, (iii) any potential involvement of renal uptake mechanisms, particularly within the renal tubules, (iv) direct/indirect effects on renal water and sodium handling, (v) vasoactive actions, (vi) possible regionally specific effects within the kidney (i.e. vasopressin reduces MBF but usually not CBF, irrespective of its site of administration), (viii) systemic effects resulting from spill-over and, (vii) the rate of metabolism of the substance, and any metabolic by-products.

With these considerations in mind, and based on the observations of our previous studies *(Correia, 1997; Evans et al., 1998)*, and those of others in rats *(Lu et al., 1992;*, it may be postulated that substances of a comparable charge and size to noradrenaline might be handled in a similar manner. Thus, following renal medullary interstitial infusion of most water soluble molecules, it might be expected that approximately half of the infused substance will spillover into the systemic circulation, and a much smaller portion (~5%) will exit via the ureter. The distribution and disposition of larger, non-polar, or highly charged polar molecules, on the other hand, might be very different from that of noradrenaline. Clearly therefore, future use of this technique with other substances requires detailed information about the factors that determine their distribution within the kidney.

7.2 Role of renal medullary blood flow in renal antihypertensive responses to increased renal artery pressure

Having developed a method for the selective reduction of MBF in anaesthetised rabbits, we were then able to test the effects of reducing MBF on responses to increased RAP. This was tested through the establishment of an extracorporeal circuit, which enabled RAP to be manipulated independently of direct effects on systemic arterial pressure. In the experiments described in Chapter 4, the effects of increasing RAP were tested during both reduced CBF (intravenous noradrenaline infusion; 300 ng/kg/min), and reduced MBF (medullary interstitial noradrenaline infusion; also 300 ng/kg/min). Using this circuit we were able to test the roles of the cortical and medullary circulation in three proposed renal antihypertensive mechanisms; pressure natriuresis,

the renin-angiotensin system, and the putative renal medullary humoral depressor mechanism. With regard to the latter mechanism, at the time we performed these experiments it was believed that the depressor response to increased RAP in this model was at least in part due to release of this putative humoral depressor substance.

It was concluded that medullary interstitial noradrenaline infusion blunted both the pressure natriuresis response and the release of the putative renal medullary depressor hormone, since the depressor response in these rabbits was blunted in comparison to those receiving intravenous noradrenaline (reduced CBF). Since a reduction in CBF did not affect these responses, these findings add further weight to the hypothesis that MBF plays a significant role in the regulation of arterial pressure. This conclusion is further supported by the results of other studies using this experimental model, showing that other treatments that reduce MBF such as blockade of nitric oxide synthase *(Evans et al., 1995)*, and medullary interstitial infusion of [Phe^2, Ile^3, Orn^8]-vasopressin *(Bergström and Evans, 1998)* can inhibit one or both of these antihypertensive responses to increased RAP. In contrast, they appear to be little affected by treatments that only affect CBF, such as renal arterial infusion of endothelin-1 or ET$_A$/ET$_B$-receptor blockade *(Weekes et al., 2000)*. This conclusion is also supported by the large body of work by Cowley and colleagues, showing the pro-hypertensive effects of medullary interstitial infusions of vasoconstrictors in rats (see *Cowley, 1997*). Therefore, it seems likely that the medullary microvasculature plays a key role in the mechanisms controlling blood pressure in the long term. On the other hand, future studies are required to further delineate the precise mechanisms underlying the role of MBF in blood pressure control.

7.2.1 *Further approaches to understanding the roles of medullary blood flow in blood pressure control mechanisms*

One approach which has not been tested in the extracorporeal circuit model, and may warrant investigation, is to determine effects of selective increases in MBF, on responses to increased RAP. This would further test the hypothesis that the level of MBF modulates the pressure diuresis/natriuresis mechanism, which predicts that treatments that increase MBF should enhance the diuretic/natriuretic responses to increased RAP.

In order to selectively increase MBF, vasodilators could be infused into the renal medullary interstitium using the technique developed in Chapter 3. Renal arterial infusion may provide a method for selectively increasing CBF with the same vasodilator substance. Suitable vasodilator

substances for such studies might include acetylcholine, adrenomedullin, or nitric oxide donors. In preliminary studies, these agents have been shown to produce sustained increases in total RBF during renal arterial infusion, and do not spill-over in sufficient quantities to reduce arterial pressure *(Guild et al., 2001)*.

Future studies, therefore, should compare the effects of renal arterial and renal medullary interstitial infusions of these agents. The results of these experiments should provide the background information necessary for the design of an experiment along the lines of that described in Chapter 4, to test whether increased MBF enhances pressure diuresis/natriuresis in this extracorporeal circuit model. On the other hand, this approach has already been taken in experiments in hypertensive rats, using a more conventional model of pressure diuresis/natriuresis *(Lu et al., 1992)*. These experiments showed that when MBF is increased by medullary interstitial infusion of the calcium antagonist diltiazem, the pressure diuresis/natriuresis relationship is shifted to the left. The experiments I propose in rabbits should confirm this finding under different experimental conditions, and with an experimental design that allows the systemic and renal cortical effects of the vasodilator agent to be controlled for.

7.2.2   Autoregulation of medullary blood flow

A further unresolved issue arising from this study was our inability to detect increases in MBF during increased RAP. There is presently considerable controversy as to the degree to which MBF is autoregulated (See 1.2.2.2), but given that even CBF is relatively poorly autoregulated in this extracorporeal circuit model, our finding with regard to MBF is surprising. One possibility is that our observation reflects a technical problem with our measurement of MBF under the conditions of the extracorporeal circuit model. When RAP is increased in this model, the kidney expands. Given that the medullary probe is held in place outside the kidney, renal expansion might result in the probe moving further towards the papilla, and so into medullary regions of lower relative flow. This issue requires clarification by further experiments.

One approach might be to chronically implant a laser-Doppler flowprobe so that its tip is positioned in the outer medullary interstitium (~8 mm from surface) and the probe is adhered to the renal capsule. This technique has previously been used in rabbits in our laboratory *(Evans et al., 2000)*. Using this method in the extracorporeal circuit setup would allow a different approach to the measurement of MBF. Using this approach, if the kidney expands when RAP is increased, if anything the probe tip would retract out towards the cortex, since it is no longer held stationary by a

micromanipulator, but adhered to the renal capsule. This should allow us to determine whether the observations described in Chapter 4 were artifactual, due to movement of the probe tip when MBF is increased.

On the other hand, if our observation is correct, and MBF is well autoregulated in the extracorporeal circuit model, this might have important consequences for our understanding of the mechanisms underlying pressure diuresis/natriuresis. As outlined in detail in Chapter 1, poor autoregulation of MBF is thought to be critical for the full expression of the pressure diuresis/natriuresis mechanism *(Cowley, 1997)*. On the other hand, others *(Majid and Navar, 1996; Majid et al., 1997; Majid et al., 1998)* have observed pressure diuresis/natriuresis in the absence of measurable changes in MBF. It may be then, that the extracorporeal circuit model provides a new way to address this issue, and perhaps further resolve a role of MBF in mediating, as opposed to modulating, the pressure diuresis/natriuresis mechanism.

7.3   Factors mediating depressor responses to increased renal artery pressure

7.3.1   Role of pressure diuresis/natriuresis an the renin-angiotensin system in the depressor response to increased renal artery pressure

A probing question arising from the experiments conducted in Chapter 4 was, to what extent did the pressure diuresis/natriuresis mechanism contribute to in the depressor response to increased RAP. This question arose from the observation that renal excretion of sodium and water increased exponentially when RAP increased from ~65 to ~160 mmHg kidney dry weight. For example, urine flow increased from 0.1 to 1.5 ml/min/g, which one might predict, would lead to a fall in extracellular fluid volume, and therefore cardiac output, and arterial pressure.

The design of the studies described in Chapter 5 allowed us to test the roles of pressure diuresis/natriuresis and inhibition of renin release in the depressor response to increased RAP. In brief, these experiments demonstrated that the depressor response was chiefly the result of increased salt and water excretion, and not the actions of a putative renal medullary depressor substance, or inhibition of the renin-angiotensin system. These results suggest that, the extracorporeal circuit is not a good model of 'medullipin' release. On the other hand, the results of the experiments described in Chapter 5 do not prove that a depressor substance was not released from the medulla under the conditions of our experiment, although if it was, any effect it might have had was obscured by the powerful depressor response resulting from pressure diuresis/natriuresis and so

hypovolaemia. It is also possible that our experiment did not provide the optimal conditions for stimulation of release of this putative renal medullary depressor hormone. Specifically, we did not test the effects of gradually increasing RAP. It may be that ~160 mmHg is outside the physiological range for release of this putative depressor hormone.

7.3.2   Is the renal medullary depressor hormone released within a specific physiological range?

It remains possible that the renal medullary depressor hormone is released only within a certain physiological range of RAP, and this mechanism is blunted outside that range. In this context, it is of interest to note that Thomas et al *(Thomas et al., 1994)* found that depressor responses could be observed in this extracorporeal circuit model when RAP was increased in steps from 95 mmHg up to 185 mmHg. As in the studies described in this theses, the fall in blood pressure in these experiments was accompanied by natriuresis and diuresis that were both pressure related and progressive with each increase in RAP (95, 125, 155, and 185 mmHg). In these experiments urine excretion was 0.39 ± 0.11 ml/min when RAP was set at ~95 mmHg but increased to 2.06 ± 0.36 ml/min when RAP was ~185 mmHg. This was accompanied by reductions in MAP of 4.2 ± 0.7 (at RAP ~ 95 mmHg) and 18.1 ± 5.3 mmHg/min (at RAP ~185 mmHg) respectively. These figures are comparable to those presented in Chapter 5, in that we observed a urinary excretion rate of 1.5 ± 0.3 ml/min when RAP was set at ~165 mmHg and a fall of 16.3 ± 3.7 mmHg/min in MAP. Thus, despite previous claims these animals were not in positive fluid balance at all times during these extracorporeal circuit experiments *(Floyer, 1975; Neubig and Hoobler, 1975)*.

Therefore, an observation which was overlooked in the Thomas et al studies was that although the fall in blood pressure continued proportionally with the rise in RAP and therefore urine production, the intravenous maintenance infusions in these rabbits remained constant (10 ml/kg/hr, ~ 0.4 ml/min). In our experiments we were able to show that the fall in blood pressure could be prevented in rabbits in which these maintenance infusions were adjusted to maintain fluid balance.

Nevertheless, the experiments described in Chapter 5 should be replicated with a design involving step-wise increases in RAP, from ~65 - ~160 mmHg. The probability that the putative vasodepressor hormone produces physiological effects outside this 'physiological' range is unlikely since we have shown that the fall in arterial pressure at RAP of ~160 mmHg occurs chiefly due to negative salt and water balance and hypovolaemia.

7.3.3 Cross perfusion studies

Before the implementation of the extracorporeal circuit in larger species, such as the dog and rabbit, the most convincing physiological evidence for the release of the putative renal medullary depressor hormone came from the cross circulation studies in rats, where an isolated kidney was extracorporeally perfused with blood from an intact 'donor' rat *(Karlström and Gothberg, 1987)*. This setup is similar to the extracorporeal circuit detailed in Chapters 4, and 5 in that it allows RAP of the isolated kidney to be regulated independently of direct effects on the systemic circulation of the intact 'assay' rat, since blood flow through the circuit remains constant *(Gothberg and Karlström, 1991)*.

In this model it has been demonstrated that increased RAP in the cross perfused isolated kidney produces a reduction in blood pressure in the assay rat, possibly via release of a humoral depressor substance from the renal medulla. It was suggested that the stimulus for the release of this depressor substance was increased RAP and/or the concomitant increase in RBF *(Gothberg and Karlström, 1991)*. It was even suggested that the renal medullary depressor hormone stimulated a diuretic/natriuretic response in these rats, since urine flow and sodium excretion increased not only in the isolated kidney perfused at high pressure, but also in the recipient kidney, in which arterial pressure fell *(Karlström et al., 1988)*. This observation also raises the question as to whether the depressor response to increased RAP in this model could be due to hypovolaemia secondary to increased salt and water excretion. My calculations, based on the data in Karlström et al. (1988), are equivocal on this matter. Taking the urine produced by both the isolated recipient kidney and the kidneys of the donor rat during high pressure perfusion (about 70 µl/min) and subtracting that from the maintenance infusion (about 50 µl/min), we can predict a negative volume balance of about 20 µl/min. This seems unlikely to account for the dramatic depressor response to increased RAP in this model. On the other hand, given my present results, this possibility warrants testing by direct experimentation.

7.3.4 Unclipping effects of renal hypertensive rats

Another model employed in studies aimed at studying the physiology of the putative renal medullary depressor hormone is the rapid depressor response after unclipping the 1-kidney-1clip renal hypertensive rat *(Ledingham and Cohen, 1962; Liard and Peters, 1973)*. It is stated in these reports that the depressor response to unclipping occurs independently of urinary volume losses, even though an increase in sodium and water excretion was observed *(Liard and Peters, 1970)*.

However, it should be noted that while 'fluid loss' was prevented in these experiments by maintenance infusions, these infusions were not increased in a compensatory manner proportional with increases in renal excretory volumes, after unclipping *(Ledingham and Cohen, 1962; Liard and Peters, 1970; Liard and Peters, 1973).*

In the 2-kidney, 1-clip hypertension model it was found that the initial fall in blood pressure in response to unclipping was 'blunted' in rats in which the renal medulla had been chemically ablated, leading to the interpretation that the depressor response to increased RAP occurred due to the release of a depressor hormone from the renal medulla *(Bing et al., 1981; Taverner et al., 1984).* It should be noted however that little attention was given to the level of urinary excretion or activity of the renin-angiotensin system in these studies, system so roles for these factors in the depressor response to unclipping cannot be completely excluded.

In a study by Neubig and Hoobler, *(Neubig and Hoobler, 1975)* a volume of saline, equal to the volume of urine excreted, was infused intravenously every 15-30 min following removal of the renal arterial clip. It was found that MAP in this group of rats behaved in a similar manner to that of rats, in which volume was not maintained after unclipping. That is, similar depressor responses were observed in both groups. These observations are consistent with those of Liard and Peters (1973), who observed that ligation of the ureter did not abolish the initial (6 hr) depressor response to unclipping 1-kidney, 1-clip hypertensive rats. On the other hand, they contrast with those of Muirhead and Brooks (1980), who showed that the normalisation of arterial pressure after unclipping 1-kidney, 1-clip rats was prolonged by ureto-caval anastomosis or ureteral ligation. Thus, despite the widespread presumption that the initial depressor response to unclipping 1-kidney, 1-clip hypertensive rats reflects, at least in part, a humoral depressor mechanism arising from the renal medulla, an important role for the pressure diuresis/natriuresis mechanism in this phenomenon has still not been excluded. Thus, an experiment directly assessing this issue was called for.

7.3.5 Role of pressure diuresis/natriuresis in the depressor response to unclipping of renal hypertensive rats

I was recently (August- September 2000) granted the opportunity to undertake such a study. I took a similar approach to that described in Chapter 5, but using the unclipping model in renal hypertensive rats. These experiments were performed at the University of Göteborg (Sweden), under the supervision of Associate Professor Göran Bergström. It should be noted that my time spent in Goteborg was limited (4 weeks) and therefore insufficient for completion of the study.

During my time there I did manage to develop a suitable protocol and conduct several preliminary studies, including some experiments used in the final analysis. I would like to take this opportunity to thank Dr. Jia Jing for teaching me how to perform the procedures described below in rats, as my experience to this time was limited to rabbits, and to further thank her for completing the remaining experiments and compiling the data presented below. I also thank Associate Professor Bergström and Dr. Jing for allowing me to present the results of these studies here.

The objective of these studies was to test the role of the pressure diuresis/natriuresis mechanism in the depressor response to unclipping of renal hypertensive rats. In brief, under anaesthesia a right nephrectomy was performed and a clip was placed around the left renal artery of normotensive rats (WKY rats; body weight 150-210 g) 8 weeks prior to the date of the acute experiment. Only rats with arterial blood pressures greater than 150 mmHg were used in the acute experiment. On the experimental day, rats were weighed, anaesthetized (Inactin; 150 mg/kg, subcutaneously) and subjected to the following protocol.

Experimental preparation following anaesthesia; Once a surgical level of anaesthesia was reached (i) a tracheotomy was performed (PE 240), (ii) the tail artery was cannulated for measurement of MAP and blood sample collections for haematocrit determination, (ii) the jugular vein was cannulated (PE 50; 0.75 mm ID, 1.45 mm OD) for measurement of CVP, and the delivery of saline and haemacell for plasma volume maintenance throughout surgery and the experiment, (iii) the left ureter was cannulated for urine collection (PE 10; 0.28 mm ID, 0.61 mm OD), and (iv) scar tissue surrounding the renal artery and arterial clip was cleared for later easy removal of the clip. Following surgery, a 60 min equilibration period was allowed for blood pressure and volume levels to stabilize. A further 30 min of stable baseline measurements were then made prior to experimental manipulations.

Experimental groups; Following 30 min of stable baseline recordings, rats were subjected to one of the three following procedures (i) In a control group, the clip was manipulated but not removed, (ii) in the second group, the renal artery clip as removed, (iii) in the third group, the clip was removed and the rat received an intravenous infusion of 154 mM NaCl equivalent to urine flow. Urine flow was determined in all animals at five minute intervals for the 60 min experimental duration, which commenced immediately following removal of the renal artery clip, or sham unclipping. Two haematocrit readings were made (collected directly into capillary tubes), one at the mid-point of equilibration and another 30 min following sham/renal artery unclipping (see Figure 7.1).
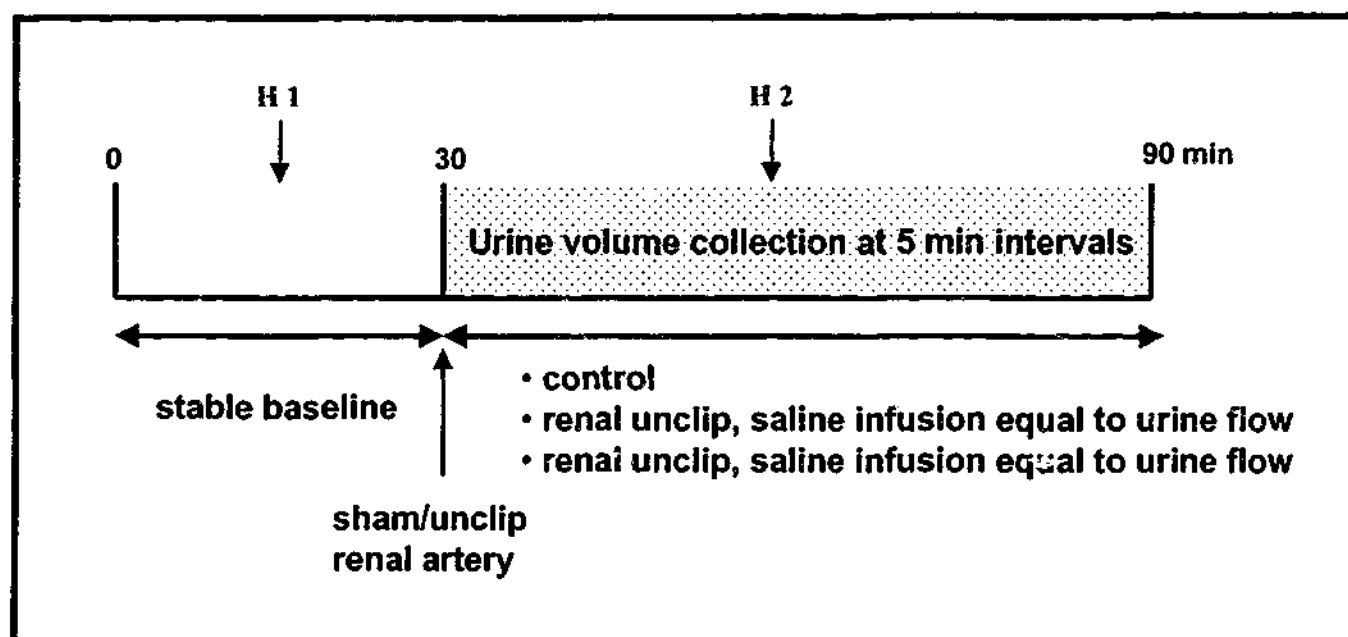
Figure 7.1 Schematic diagram of the experimental protocol. Following a 60 min equilibration period, stable baseline recordings of mean arterial pressure, central venous pressure (not shown) and heart rate were taken for 30 min prior to sham/removal of the renal artery clip. For the remaining 60 min of the experiment urine was collected and measured gravimetrically at 5 min intervals and two arterial blood samples were collected for haematocrit determination ((H1, H2); data not shown). Three groups of rats were studied (i) sham unclipping, (ii) unclipping of the renal artery, and (iii) unclipping of renal artery, and intravenous infusion of 154 mM NaCl at a rate equivalent to urine flow.

The data presented in Figure 7.2 show that, as has been shown previously, renal artery unclipping is followed by a rapidly developing depressor response *(Muirhead and Brooks, 1980)*. These data also show that his depressor response is abolished if urinary excretion is compensated for by saline infusion. Thus, like the depressor response to increased RAP in the extracorporeal circuit mode, the rapid depressor response to unclipping 1-kidney, 1-clip hypertension seems to be chiefly dependent on hypovolaemia secondary to pressure diuresis/natriuresis. Neither of these models, therefore, appear to be appropriate 'assays' for the putative renal medullary depressor hormone.

Two important points arise from these findings. The first is that the pressure diuresis/natriuresis mechanism seems to play a dominant role in blood pressure regulation, even over the relatively short time periods of these experiments. The second important point relates to the existence of the renal medullary depressor hormone. These experimental models have played a major role in

underpinning the physiological evidence supporting the concept of the renal medullary depressor hormone. Thus, we should now critically re-evaluate this concept.
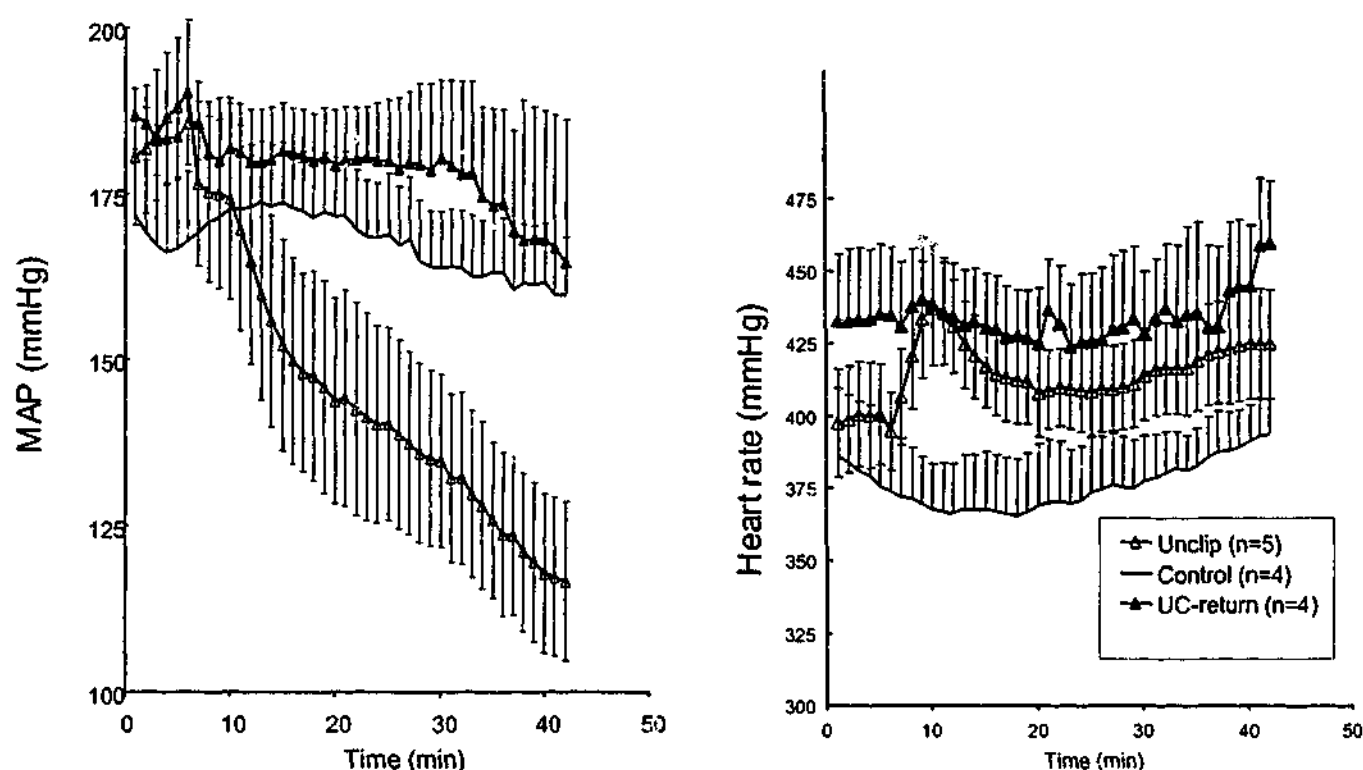
Figure 7.2 Mean arterial pressure (MAP) and heart rate responses to renal unclipping or sham unclipping of anaesthetised renal hypertensive rats. The line with no symbols represents the sham unclipping group. Open triangles represent rats in which the renal artery clip was removed. Closed triangles represent rats in which the renal artery clip was removed, and an infusion of 154 mM NaCl was administered to compensate for urinary excretion.

7.4 Vasopressin V_1-agonist effects on renal blood flow and glomerular arterioles

The experiments described in Chapter 6 concentrated on the mechanisms controlling MBF, rather than the role of MBF in blood pressure control. This study was designed to provide more information about the vascular sites responsible for the control of MBF. MBF in anaesthetised rabbits was selectively reduced) by an intravenous infusion of the V_1-agonist [Phe^2, Ile^3, Orn^8]-vasopressin. The rabbits' kidneys were then perfusion fixed, and renal casts were made by methacrylate infusion. The main findings were, that intravenous [Phe^2, Ile^3, Orn^8]-vasopressin produced a selective reduction in MBF, but we were unable to detect changes in the diameters of juxtamedullary afferent or efferent arterioles. This finding gives rise to two possible explanations, either (i) the vascular elements responsible for the reduced MBF in response to the V_1-agonist are

not in the renal cortex, but instead in the outer medulla (outer medullary descending vasa recta ?), or (ii) that the casting technique was not sensitive enough to detect changes in juxtamedullary glomerular arterioles.

The main advantage of the experimental approach taken in Chapter 6, was the combination of *in vivo* measurements of regional and total RBFs together with *ex-vivo* analysis of vascular casts from the same animals. However, this approach did present its disadvantages. Firstly due to the terminal nature of the experiment, within animal comparisons between [Phe^2, Ile^3, Orn^8]-vasopressin and vehicle infusion could not be made, and secondly due to the delicate nature of the casts and excessive handling, some degree of artifact is likely to be associated with this procedure.

It is accepted that the supply of MBF is derived (almost) entirely from the efferent arterioles of juxtamedullary glomeruli *(Pallone et al., 1990)*. Therefore it may been reasoned, that in order for [Phe^2, Ile^3, Orn^8]-vasopressin to produce a selective reduction in blood flow to the renal medulla, vasoconstriction of either the juxtamedullary arterioles or outer medullary descending vasa recta must occur. Although V_1-receptors have been located in the outer medullary descending vasa recta *(Park et al., 1997)*, and they have been shown to constrict in response to V_1-receptor activation *in vitro (Turner and Pallone, 1997)*, to date there is no information from *in vivo* studies that bears on this issue. In a large part, this is due to the inaccessibility of this region of the kidney. Clearly, resolution of this issue is of major importance for an understanding of the factors regulating MBF *in vivo*.

7.4.1 *Possible future directions in measuring in-vivo changes with ex-vivo microscopy*

If the observed reduction in MBF in response to infusion of [Phe^2, Ile^3, Orn^8]-vasopressin is chiefly due to vasoconstriction of outer medullary descending vasa recta, then based on the results presented in Chapter 6, this may be difficult to resolve using scanning electron microscopy. This arises particularly because comparisons have to be made between animals, rather than within the same vessels in the same animal. An alternate approach may be through the implementation of a more sensitive technique, which preferably allows measurements to be made in real time *in vivo*. These techniques await development.

One approach that I have trialed in preliminary studies is confocal microscopy. Although it still requires vascular casting and between-group comparisons, it does provide certain advantages over the scanning electron microscope technique. For example: (i) Following staining of the renal casts,

no further processing is required and the size of the cast is limited only by the size of the stage under the microscope lens, not the size of the stubb used in the scanning electron microscope, (ii) 3-dimentional images of vessels selected under the microscope are compiled and generated, by computer, and in most instances 2-dimentional measurements may be made, as well as 3-dimentional determination of the 'volume' of the scanned image, which can be calculated 'on screen' and does not require the use of a digitizing tablet, and (iii) perhaps the most important advantage of this method is greater image resolution.

However, a major limitation of confocal microscopy which we encountered, was that the computer program used for calculating arteriolar dimensions was unable to distinguish these vessels as a separate entities from the glomeruli. Various approaches were taken to circumvent this problem, which included mathematical formulas, and communication with the software developers, but in the end we were limited by the time restrictions of my PhD candidature, and returned to the previously validated scanning electron microscope method. However, this technique may warrant further investigation since, due to the minimal tissue handling required, and higher resolution, it may in the near future be possible to make measurements of outer medullary descending vasa recta, as well as glomerular arterioles.

7.5   Conclusions

Clearly, we have only just begun to develop an understanding of how the renal medullary circulation contributes to the regulation of arterial pressure, both in the short and the long term. Many issues surrounding the extent and level of involvement of these mechanisms, particularly the renal medullary depressor hormone still warrant further investigation. My experimental results certainly reinforce the 'Guytonian' view, that the pressure diuresis/natriuresis mechanism plays an overriding dominant role in the long-term control of arterial pressure. Further understanding of the factors underlying this mechanism, including MBF, must therefore be a central goal of hypertension research in the future. This information may help us towards prevention or cure of hypertension, rather than just its treatment.

Renal medullary interstitial infusion of norepinephrine in anesthetized rabbits: methodological considerations

ANABELA G. CORREIA,[1] GÖRAN BERGSTRÖM,[2] ANDREW J. LAWRENCE,[3]
AND ROGER G. EVANS[1]
Departments of [1]Physiology and [3]Pharmacology, Monash University,
Clayton, Victoria 3168, Australia; and [2]Department of Physiology, University of Göteborg,
Göteborg S-413 90, Sweden

Correia, Anabela G., Göran Bergström, Andrew J. Lawrence, and Roger G. Evans. Renal medullary interstitial infusion of norepinephrine in anesthetized rabbits: methodological considerations. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R112–R122, 1999.—We tested methods for delivery of drugs to the renal medulla of anesthetized rabbits. Outer medullary infusion (OMI) of norepinephrine (300 µg·kg^{-1}·min^{-1}), using acutely or chronically positioned catheters, reduced both cortical (CBF; 15%) and medullary perfusion (MBF; 23–31%). Inner medullary infusion (IMI) did not affect renal hemodynamics, whereas intravenous infusion reduced CBF (15%) without changes in MBF. During OMI of [^{3}H]norepinephrine, much of the radiolabel (~40% with chronically positioned catheters) spilled over systemically. Nevertheless, autoradiographic analysis showed the concentration of radiolabel was about fourfold greater in the infused medulla than the cortex. In contrast, during IMI, only ~5% of the infused radiolabel spilled over into the systemic circulation and ~64% was excreted by the infused kidney. The resultant intrarenal levels of radiolabel were considerably less with IMI compared with OMI. In rabbits, OMI therefore provides a useful method for targeting agents to the renal medulla, but given the considerable systemic spillover with OMI, its utility is probably limited to substances that are rapidly degraded in vivo.

hypertension; laser-Doppler flowmetry; renal blood flow; renal medulla

---

THERE IS ACCUMULATING evidence implicating the renal medullary microvasculature in the control of arterial pressure. In particular, the level of renal medullary blood flow (MBF) appears to be an important determinant of sodium and water reabsorption (4, 5) and is perhaps also important for the release of the putative renal medullary depressor hormone (2). Furthermore, although there is evidence to the contrary (16, 17), some studies have shown that renal MBF can be poorly autoregulated, at least under volume-expanded conditions (4, 5). The renal medullary microcirculation may therefore be well placed to transduce changes in arterial pressure into homeostatic responses that restore normal arterial pressure.

One technique that has been a useful tool for studying the role of the renal medullary microcirculation in the long-term control of arterial pressure has been infusion of vasoactive substances into the renal me-

dulla. Cowley and colleagues (4–6, 13, 14, 19, 23) employed this technique in rats, combined with laser-Doppler flowmetry for evaluation of regional kidney blood flow (22). They showed that chronic medullary interstitial infusion of vasoconstrictor agents such as N^G-nitro-L-arginine methyl ester (19) and the vasopressin V_1-agonist [Phe^2,Ile^3,Orn^8]vasopressin (23), at doses that reduce medullary but not cortical blood flow (CBF), results in the development of sustained hypertension. Conversely, medullary interstitial infusion of captopril in spontaneously hypertensive rats, which increases medullary but not CBF, ameliorates their hypertension (13).

In longitudinal studies such as those described above, there are considerable advantages to employing larger species. In the case of conscious rabbits, we are able to obtain long-term and simultaneous data regarding hormonal status (10), cardiac output (months; 9), renal blood flow (weeks; 25), and renal sympathetic nerve activity (weeks; 18). Therefore, in the present study we investigated methods for infusion of vasoactive agents into the renal medulla of rabbits. We chose norepinephrine (NE) as our test agent, because it is rapidly broken down in vivo, minimizing the confounding effects of spillover into the systemic circulation. We first tested whether outer medullary interstitial infusion of NE via acutely implanted needle catheters in anesthetized rabbits affected renal hemodynamics and function in the infused and contralateral kidney. We paid particular attention to the effects of the infusion on cortical and medullary perfusion, determined by laser-Doppler flowmetry. This was correlated with examination of the distribution of radiolabel after medullary interstitial infusion of [^{3}H]NE and the removal of the radiolabel from the infusion site via the renal venous and urinary excretory routes. Subsequently, similar studies were performed 7–14 days after implantation of catheters designed for chronic implantation, in which we compared the effects, distribution, and disposition of NE/radiolabel during infusion of NE into the outer and inner medulla. These studies suggest that medullary interstitial infusion of vasoactive agents provides a useful method for selectively manipulating renal MBF in rabbits. However, the positioning of the catheter tip is critical for the distribution of the infused agent and the effects observed.

METHODS

Animals

Thirty-three rabbits of a multicolored English strain of either sex and weighing 2.3–3.1 kg (mean 2.7 kg) were used.

Before experimentation all rabbits were allowed food and water ad libitum. On completion of experimental procedures, rabbits were killed with an intravenous overdose of pentobarbital sodium (300 mg). All procedures were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Animal Ethics Committee of the Department of Physiology, Monash University.

Experimental Preparation

This has been described in detail previously (8), so is described only briefly here. Catheters were inserted into both central ear arteries and marginal ear veins for collection of arterial blood, measurement of arterial pressure, and intravenous infusion. Anesthesia was induced and maintained by intravenous administration of pentobarbital sodium (90–150 mg plus 30–50 mg/h Nembutal; Boehringer Ingelheim, Artarmon, NSW, Australia) and immediately followed by endotracheal intubation and artificial respiration. Depth of anesthesia was monitored by corneal and toe pinch reflexes and also by monitoring arterial pressure and heart rate (HR). During surgery, Hartmann's solution (compound sodium lactate; Baxter Healthcare, Toongabbie, NSW, Australia) was infused intravenously (0.18 ml·kg^{-1}·min^{-1}) to replace fluid losses. On completion of the preparative surgery, this infusion was changed to a 4:1 Hartmann's- Haemaccel (polygeline and electrolyte solution; Hoechst, Melbourne, Victoria, Australia) infusion, which was maintained until completion of the experiment. Experimental manipulations commenced 90–120 min after completion of the surgery.

Both kidneys were denervated, and both ureters were cannulated. A transit-time ultrasound flow probe (type 2SB; Transonic Systems, Ithaca, NY) was placed around the renal artery, and the tips of three single-fiber laser-Doppler flow probes (0.5 mm diameter; University of Linköping, Sweden) were placed 0.5 (cortical), 0.5 (cortical), and 10 mm (medullary), respectively, below the cortical surface (see Ref. 8 for detailed description). For protocols 2 and 3 (see below), a branch of the ileolumbar vein was isolated and cannulated (polyvinyl chloride tubing, 0.8 mm ID, 1.2 mm OD; Critchley Electrical, Auburn, NSW, Australia), the tip of the cannula being advanced so that it lay in the renal vein for collection of renal venous blood. To maintain catheter patency, heparinized (50 IU/ml heparin sodium, Monoparin, Fisons) Hartmann's solution was infused at a rate of 2 ml/h.

Implantation of Medullary Interstitial Catheters

Acutely positioned catheters. Catheters, constructed using 30-gauge needles, were placed 2 cm apart on the midline aspect of the kidney, on either side of the medullary laser-Doppler flow probe, with their tips positioned 8.5 mm below the cortical surface. Sodium chloride (154 mM; 10 µl·kg^{-1}·min^{-1}) was infused (via each catheter) into the renal medulla until 60 min before the experimental procedures commenced.

Chronically positioned catheters. These were implanted 7–14 days before the experiment under halothane (1–4%, Fluothane; ICI, Victoria, Australia) anesthesia and sterile conditions (see Ref. 25). A left flank incision was made, and the kidney was gently exteriorized. The tip of a single polyethylene catheter (ID 0.28 mm, OD 0.61 mm; Critchley Electrical) was introduced into the ventral side of the kidney, slightly rostral to the midline aspect, at an angle directed toward the renal pelvis. The catheter was then advanced so that its tip lay either 8.5 ($n = 8$) or 10.5 mm ($n = 9$) below the surface of the kidney. Correct insertion resulted in minimal

bleeding, which stopped almost immediately. A small piece of nylon mesh (1.5 cm diameter; Hilton Hosiery, Coolaroo, Victoria, Australia) attached to the catheter was anchored to the surface of the kidney with cyanoacrylate glue (Loctite; Caringbah, NSW, Australia). The catheter was tunneled subcutaneously so that its end lay between the shoulder blades, and an osmotic mini pump (Alzet 2ML2; 5 µl/h for 14 days, Alza, Palo Alto, CA) filled with 154 mM saline was attached to the end of the catheter to maintain catheter patency.

In preliminary studies we found that the depth of 8.5 mm (outer medullary catheters) corresponds approximately with the junction of the inner and outer stripes of the outer medulla, whereas the depth of 10.5 mm placed the catheter in the white, inner medulla, but not in the papilla. When possible, gross postmortem examination of the kidneys was performed in this and other studies (Ref. 8, unpublished observations), and the catheters were always found to be correctly placed. Furthermore, no evidence of gross disruption of kidney tissue or scar tissue due to implantation of the medullary interstitial catheters was found from examination of the frozen sections taken for autoradiographic analysis (see below).

Experimental Protocols

Protocol 1. Acutely positioned medullary interstitial catheters: effects of medullary interstitial infusion of saline and NE on systemic and renal hemodynamics and renal excretory function (8 rabbits). Once the surgical preparations were completed (see above), bolus doses of [^{3}H]inulin (4 µCi) (NEN Research Products, Sydney, NSW, Australia) and [^{14}C]paraaminohippuric acid (PAH) (1 µCi; NEN Research Products) were administered in Hartmann's-Haemaccel solution (0.18 ml·kg^{-1}·min^{-1}) was replaced with a solution containing 300 nCi/ml [^{3}H]inulin and 83 nCi/ml [^{14}C]PAH. This infusion continued for the remainder of the experiment. Ninety minutes later, the first of 11 20-min clearance periods began. These were in turn divided into three runs, consisting of three, four, and four 20-min clearance periods, respectively. These experimental runs were separated by 90-min equilibration periods. During the first experimental run, the effects of medullary interstitial infusion of saline were tested. Thus saline (154 mM NaCl; 10 µl·kg^{-1}·min^{-1} through each of the 2 catheters) was infused into the renal medulla during the second, but not the first or third, experimental period. At the completion of this experimental run, the medullary interstitial infusion of saline was recommenced and continued for the remainder of the experiment. During the second and third experimental runs, either NE (0, 30, 100, and 300 ng·kg^{-1}·min^{-1}, respectively, during the four 20-min experimental periods) or its vehicle (20 µl·kg^{-1}·min^{-1} 154 mM NaCl) was administered into the medullary interstitium. The order in which the treatments (vehicle or NE) were administered was alternated (unpaired crossover design), so that four rabbits received NE before its vehicle and four received the vehicle before NE. The urine produced by both the left and right kidneys was collected during the 11 clearance periods, and 1-ml arterial blood samples were collected before each experimental run commenced, at the midpoint, and at the completion of each run.

Protocol 2. Acutely positioned catheters: disposition and distribution of radiolabel during intramedullary infusion of [^{3}H]NE (9 rabbits). [^{3}H]NE (16–24 nCi·kg^{-1}·min^{-1} in 100 ng·kg^{-1}·min^{-1} NE; one-half the dose via each of the 2 catheters) was infused into the medullary interstitium for 20 min. Urine produced by both kidneys was collected during the 2 min before the infusion commenced and for each 2-min

period throughout the [³H]NE infusion. Ear arterial and renal venous blood samples (0.5 ml each) were collected at the midpoint of each urine collection period.

At the completion of the 20-min [³H]NE infusion, both the infused and contralateral kidneys were quickly retrieved, decapsulated, and halved coronally. For half of each kidney, portions of the cortex, medulla, and papilla were dissected and weighed in separate preweighed 20-ml vials, to which 50 μl of tissue solubilizer (NCS II, 0.5 N solution, Amersham, Buckinghamshire, UK) was added per milligram of wet tissue weight. Radioactivity in each region of the kidneys was subsequently determined by liquid scintillation counting (see below). For five of these rabbits, the remaining kidney halves were frozen in liquid nitrogen and stored at −70°C for subsequent autoradiographic analysis (see below).

Protocol 3. Chronically positioned outer and inner medullary catheters (17 rabbits). First, increasing doses of NE (0, 30, 100, and 300 ng·kg⁻¹·min⁻¹) were infused into the inner (n = 9) or the outer (n = 8) medulla via the catheters implanted 7–14 days previously. Each dose of NE was infused for 20 min. Second, after a 20- to 40-min recovery period, during which all variables returned to their baseline levels, NE (300 ng·kg⁻¹·min⁻¹) was infused intravenously for 20 min. Finally, after a further 20–40 min was allowed for recovery from intravenous NE, [³H]NE (16–24 nCi·kg⁻¹·min⁻¹ in 100 ng·kg⁻¹·min⁻¹ NE) was infused via the chronically implanted medullary interstitial catheter for 20 min. Arterial and renal venous blood samples and urine samples were collected during the infusion, and the kidneys were harvested and processed at the completion of the infusion, as described for *protocol 2*.

Measurements

Systemic and renal hemodynamic variables. Mean arterial pressure (MAP, mmHg), HR (beats/min), left renal blood flow (transit-time ultrasound flowmetry; RBF$_{probe}$, ml/min), and cortical and medullary laser-Doppler fluxes (CBF and MBF, respectively, V) were determined as described previously (8).

Renal function (protocol 1). Clearance measurements of glomerular filtration rate (GFR, ml/min) and effective renal plasma flow (which was corrected for hematocrit to provide effective renal blood flow; ERBF, ml/min) and determinations of urine and sodium excretion were made as previously described (8). At the completion of experiments in which renal clearance measurements were made (*protocol 1*), the kidneys were removed and desiccated, and the dry weight was determined. Therefore, for this protocol, RBF$_{probe}$, ERBF, GFR, urine flow rate, and urinary sodium excretion are expressed per gram of kidney dry weight (mean = 1.66 ± 0.07 g).

Disposition of radiolabel during infusion of [³H]NE (protocols 2 and 3). The concentrations of [³H]NE and its metabolites in each of the samples were measured by liquid scintillation counting and expressed in terms of the total dose of NE infused (100 ng·kg⁻¹·min⁻¹). The rate of disposition of radiolabel by the infused and contralateral kidneys was then calculated as the concentration (above) multiplied by the flow rate (ml·kg⁻¹·min⁻¹). Urine flow was determined gravimetrically. Renal venous blood flow was taken as renal arterial blood flow determined by the transit-time ultrasound flow probe and was not corrected for urine flow.

Analysis of solubilized kidney tissue (protocols 2 and 3). Once dissolved (after at least 7 days of incubation in tissue solubilizer at room temperature), triplicate 20-μl samples of each solubilized tissue sample were added to a water-soluble scintillation fluid (ACS, Aqueous counting fluid; Amersham) and subjected to liquid scintillation counting.

Autoradiography (protocols 2 and 3). Coronal 50-μm sections of the frozen left kidney were cut on a cryostat at −19°C and mounted on glass slides (subbed with 10% gelatin; BDH Chemicals, Poole, UK). These sections were left to dry for 1–2 h at room temperature. Subsequent to drying, slides were apposed to tritium-sensitive film (Amersham Hyperfilm) in the presence of tritium microscales (Amersham) for 6–8 wk. Developed autoradiograms were quantified using an MCID M4 image analysis system (Imaging Research) as previously described (1). Each kidney section was divided into four regions, cortex, outer stripe of the outer medulla (outer stripe), medulla (excluding the outer stripe and papilla), and the papilla (defined as the portion of the inner medulla that protrudes into the renal pelvis), for separate quantification.

We chose to use both methods (analysis of solubilized kidney tissue and autoradiography) because both have inherent advantages and disadvantages and provide complementary data. For example, the results from counting solubilized tissue are potentially subject to variation due to the dissection, the degree of solubilization, and extrapolation errors from counting aliquots. On the other hand, whereas the autoradiograms provide greater sensitivity and anatomic resolution, the data they provide are representative only of the relatively thin (50 μm) sections taken.

Statistical Analyses

Data were analyzed by ANOVA using the computer software SYSTAT (26). To protect against the increased risk of comparison-wise type I error resulting from repeated-measures designs, *P* values were conservatively adjusted, where appropriate, using the Greenhouse-Geisser correction (15).

Protocol 1. The data were analyzed as the average level of each variable over each 20-min clearance period, because, at least for the renal clearance measurements, this was the maximum level of resolution. To test for an effect of medullary interstitial infusion of saline, an ANOVA was partitioned to contrast the levels of each variable during the 20-min saline infusion (the second experimental period of the first experimental run) with the 20-min periods before and after the saline infusion. To test for effects of medullary interstitial infusion of NE on systemic and renal hemodynamic variables, we used the interaction term of time and treatment (vehicle or NE). For renal clearance variables, the interaction term of time and kidney (infused and contralateral) was used to control the confounding effects of changes in arterial pressure on renal function. To test for differences between the responses of CBF and MBF during NE infusion, the main effect of kidney region (cortex and medulla) tested whether, across all three doses of NE, changes on MBF were greater than those in CBF.

Protocol 2. The levels of radioactivity in the various kidney regions were subjected to ANOVA, the factors comprising rabbit, side (infused or contralateral kidney), and kidney region.

Protocol 3. ANOVAs were partitioned to test the specific hypotheses that medullary interstitial infusion of NE dose relatedly influenced the levels of systemic and renal hemodynamic variables and that intravenous infusion of NE influenced these variables. To test for differences between outer and inner medullary interstitial [³H]NE infusion in terms of the levels of radioactivity entering the kidney via the renal artery and exiting the kidney via the urine and the renal vein, the interaction term between catheter position (outer or inner medulla) and time was used. Data concerning the levels of radiolabel in the infused and contralateral kidneys were analyzed as for *protocol 2*.

RESULTS

Protocol 1. Acutely Positioned Outer Medullary Interstitial Catheters: Effects of Medullary Interstitial Infusion of Saline and NE on Systemic and Renal Hemodynamics and Renal Excretory Function

Medullary interstitial infusion of saline. Outer medullary interstitial infusion of the vehicle (154 mM NaCl) had no significant effects on the levels of any of the systemic and renal hemodynamic or renal clearance variables in both the infused and contralateral kidney (data not shown).

Medullary interstitial infusion of NE. Medullary interstitial infusion of NE (30, 100, and 300 ng·kg⁻¹·min⁻¹) was accompanied by dose-related increases in MAP (by 3 ± 4, 10 ± 2, and 16 ± 4% of resting, respectively) and small but statistically significant reductions in HR by (1 ± 1, 3 ± 1, and 3 ± 1% of resting, respectively). In the infused (left) kidney, RBF$_{probe}$ was dose relatedly reduced by (8 ± 1, 16 ± 3, and 30 ± 4% of resting, respectively), as was CBF (by 6 ± 4, 7 ± 1, and 19 ± 4% of resting, respectively) and MBF (by 17 ± 5, 37 ± 11, and 45 ± 9% of resting, respectively). The reductions in MBF were significantly greater than those of CBF (*P* < 0.001) (Fig. 1), consistent with the notion that tissue levels of NE are greater in the medulla and/or inner cortex than in the outer cortex during medullary interstitial infusion. In later protocols, this hypothesis was more directly tested by analysis of the intrarenal distribution of radiolabel during medullary interstitial infusion of [³H]NE.

During medullary interstitial infusion of saline for four consecutive 20-min periods, the patterns of the responses of ERBF, GFR, urine flow, and sodium excretion were indistinguishable in the infused (left) and contralateral (right) kidneys. In contrast, during medullary interstitial infusion of NE, ERBF, GFR, and urine flow were reduced in the infused kidney relative to the contralateral kidney. A similar pattern of responses was observed for urinary sodium excretion, although this was not statistically significant (*P* = 0.09) (Fig. 2). Neither medullary interstitial infusion of saline nor NE appeared to affect the fractional excretion of urine (urine flow/GFR) or sodium (sodium clearance/GFR) (*P* always ≥ 0.3; data not shown).

Protocol 2. Acutely Positioned Catheters: Disposition and Distribution of Radiolabel During Intramedullary Infusion of [³H]NE

Disposition of radiolabel. The amount of radioactivity leaving the infused (left) kidney via renal venous blood and urine, the amount reentering the left kidney via the renal artery, and the amount leaving the contralateral (right) kidney in its urine increased progressively during the 20-min [³H]NE infusion. With the exception of renal arterial delivery of radiolabel, all of these variables appeared to reach steady state during the final 6 min of the infusion (Fig. 3). When averaged across the final 6 min of the [³H]NE infusion, 134.5 ± 34.6 ng·kg⁻¹·min⁻¹ of [³H]NE equivalents exited the kidney via the renal vein. Almost one-tenth (12.9 ± 1.6
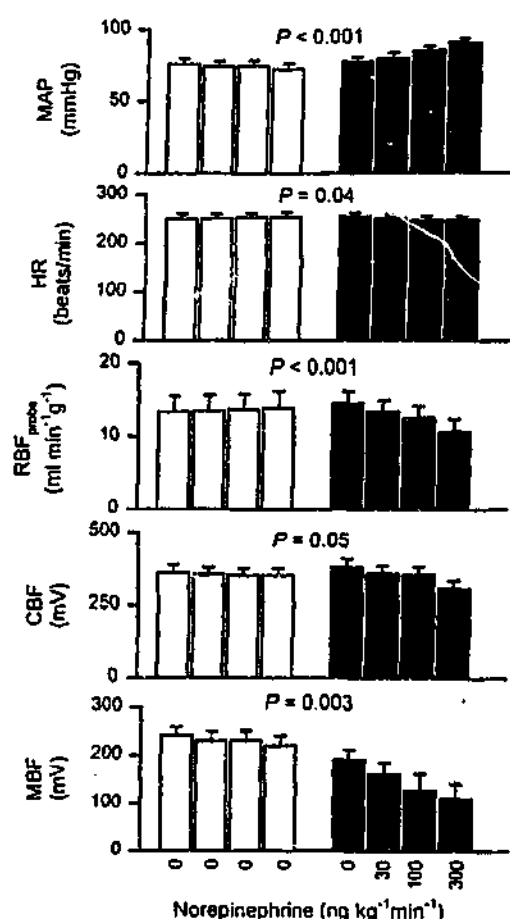
Fig. 1. Effects of medullary interstitial infusion of norepinephrine (0, 30, 100, and 300 ng·kg⁻¹·min⁻¹) or its vehicle (154 mM NaCl; 20 μl·kg⁻¹·min⁻¹) on systemic and renal hemodynamic variables. MAP, mean arterial pressure; HR, heart rate; RBF$_{probe}$, renal blood flow determined by transit-time ultrasound flow probe; CBF, renal cortical perfusion (laser-Doppler flux signal); MBF, renal medullary perfusion (laser-Doppler flux signal). Columns and error bars represent means ± SE of data from 8 rabbits (except for CBF and MBF, where n = 6 because laser-Doppler flowmetry was not employed in 2 rabbits), for average levels over each 20-min experimental period. *P* values represent outcomes of interaction terms (treatment × dose) from repeated-measures ANOVA (degrees of freedom 3, 30–42) testing for nonparallelism in responses to vehicle and norepinephrine.

ng·kg⁻¹·min⁻¹) of this radiolabel reentered the infused kidney via the renal artery. A similar amount of radioactivity probably entered the right kidney from its renal artery, because ERBF in this preparation is similar for the two kidneys (see Fig. 2). During the final 6 min of the infusion, the amount of radiolabel excreted by the left kidney (21.5 ± 4.7 ng·kg⁻¹·min⁻¹) was approximately sixfold greater than that excreted by the right kidney (3.8 ± 0.7 ng·kg⁻¹·min⁻¹).
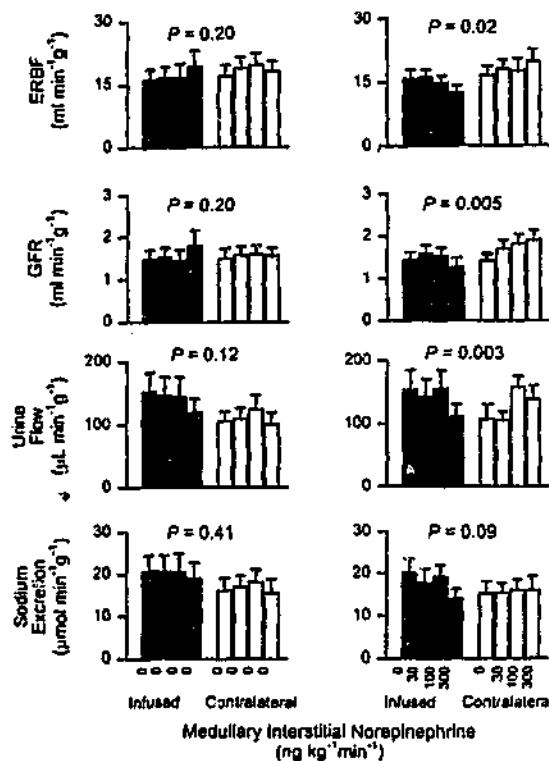
Fig. 2. Effects of medullary interstitial infusion of norepinephrine (0, 30, 100, and 300 ng·kg⁻¹·min⁻¹; *right*) or its vehicle (NaCl 154 mM; 20 µl·kg⁻¹·min⁻¹; *left*) on renal clearance variables. ERBF, effective renal blood flow determined by [¹⁴C]*para*-aminohippurate clearance; GFR, glomerular filtration rate determined by [³H]inulin clearance. Columns and error bars represent means ± SE of data from 8 rabbits. P values represent outcomes of repeated-measures ANOVA testing for nonparallelism in responses of left (infused, solid bars) and right (contralateral, open bars) kidneys.

Intrarenal distribution of radiolabel. The concentration of radioactivity, determined from the solubilized kidney-tissue, was significantly greater in the infused compared with the contralateral kidney (P value of infused vs. contralateral kidney = 0.03; Fig. 4A).

Fig. 3. Disposition of radiolabel (expressed as [³H]norepinephrine equivalents (ng·kg⁻¹·min⁻¹) see METHODS for description of calculations) during a 20-min infusion of [³H]norepinephrine (16-24 nCi·kg⁻¹·min⁻¹ in 100 ng·kg⁻¹·min⁻¹ norepinephrine) into renal outer medulla of left kidney via 2 acutely positioned catheters. Coordinates represent means ± SE of 9 sets of observations. ○, Radiolabel spilling over from infused kidney into renal vein; ●, radiolabel reentering infused kidney via renal artery; ■, radiolabel excreted by infused (left) kidney; □, radiolabel excreted by contralateral (right) kidney.
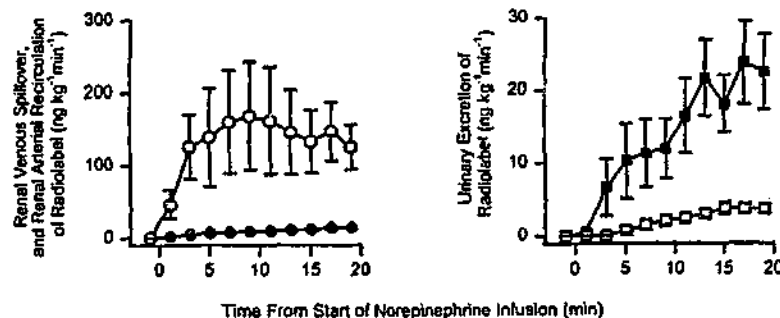
Because these data were clearly not normally distributed, as evidenced by the proportionality of the means and their attendant SEs, they were log transformed and subjected again to ANOVA. Analysis of the log-transformed data showed significant heterogeneity of variance according to region in both the infused and contralateral kidney. Within the infused kidney, this can be attributed to the fact that the radiolabel was more concentrated in the medulla (495 ± 388 ng/g) and papilla (592 ± 347 ng/g) than in the cortex (75 ± 44 ng/g). The opposite appeared to be the case in the contralateral kidney in which radiolabel was more concentrated in the cortex (49 ± 29 ng/g) than the medulla (23 ± 12 ng/g) or papilla (25 ± 10 ng/g).

Consistent with the above observations, autoradiographic analysis of the coronal kidney sections demonstrated that the levels of radioactivity in the cortex of the infused kidney were 3.4 ± 0.6-, 5.6 ± 0.8-, and 8.0 ± 0.8-fold lower than those in the outer stripe, medulla, and papilla, respectively (Fig. 4, B and C).

Protocol 3. Chronically Positioned Outer and Inner Medullary Catheters

Effects of outer and inner medullary infusion of NE. The effects of outer medullary infusion of NE (30, 100, and 300 ng·kg⁻¹·min⁻¹) via a chronically implanted catheter were similar to those observed when acutely positioned catheters were used (Fig. 5, *left*). Thus MAP was dose dependently increased by 5 ± 1, 14 ± 3, and 27 ± 7% of its resting level, respectively, whereas dose-dependent reductions were observed in CBF (by 14 ± 8, 16 ± 8, and 16 ± 6% of its resting level, respectively) and MBF (by 10 ± 4, 18 ± 6, and 24 ± 8% of its resting level, respectively).

In contrast to outer medullary infusion, the dose-dependent pressor responses to inner medullary infusion of NE (30, 100, and 300 ng·kg⁻¹·min⁻¹) were lesser in magnitude (increasing by 3 ± 3, 6 ± 3, and 8 ± 4% of its resting level, respectively), and no significant effects on CBF or MBF were observed (Fig. 5, *right*).

Effects of intravenous infusion of NE. Intravenous NE (300 ng·kg⁻¹·min⁻¹) increased MAP (by 7 ± 1% of its resting level) and reduced RBF_probe (by 15 ± 5% of its resting level) and CBF (by 14 ± 7% of its resting level) but did not significantly alter MBF (−4 ± 4% change) (Fig. 6).

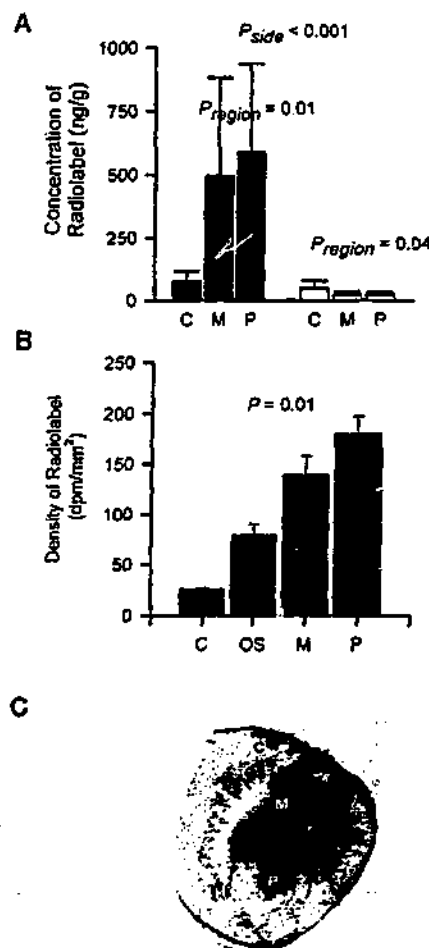Time From Start of Norepinephrine Infusion (min)

Fig. 4. A: distribution of radiolabel in infused (left, solid bars) and contralateral (right, open bars) kidneys at end of 20-min outer medullary infusion of [³H]norepinephrine (16-24 nCi·kg⁻¹·min⁻¹ in 100 ng·kg⁻¹·min⁻¹ norepinephrine) via 2 acutely positioned catheters. Radiolabel in solubilized kidney tissue is expressed as [³H]norepinephrine equivalents (ng/g). Columns and error bars represent means ± SE for 9 rabbits. P values are outcomes of ANOVA testing for differences between levels of radioactivity (log₁₀ transformed) in infused and contralateral kidneys (P_side) and for heterogeneity of variance among different kidney regions (P_region). B: levels of radiolabel, expressed as disintegrations per minute per square mm (dpm/mm²), determined from autoradiographic analysis of 5 infused kidneys. Columns and error bars represent means ± SE. P value represents outcome of a partitioned ANOVA testing for a difference between levels of radioactivity in cortex compared with other three kidney regions. C: typical autoradiogram from an infused kidney. C, cortex; OS, outer stripe of outer medulla; M, medulla; P, papilla. See METHODS for definitions of these regions.

Disposition of radiolabel during outer and inner medullary infusion of [³H]NE. The profile of renal disposition of the radiolabel during outer medullary infusion of [³H]NE was similar to that found using

acutely positioned catheters (*protocol 2*). That is, much of the infused radiolabel spilled over into the renal vein (39.1 ± 25.2 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion), but only small amounts were excreted by the infused kidney (3.7 ± 2.0 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion). Not surprisingly therefore, progressive increases were observed in the amount of radiolabel reentering the infused kidney via the renal artery (9.6 ± 0.4 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion) and the amount excreted by the contralateral kidney (1.1 ± 0.1 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion) (Fig. 7, *left*).

In contrast, during inner medullary infusion of [³H]NE, only small amounts of the radiolabel spilled over into the renal vein of the infused kidney (4.8 ± 2.7 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion), whereas most of the infused radiolabel was excreted by the infused kidney (63.6 ± 12.8 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion). Consistent with this, only small quantities of radiolabel reentered the infused kidney via the renal artery (1.6 ± 1.1 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion) or were excreted by the right kidney (0.1 ± 0.1 ng·kg⁻¹·min⁻¹ during the final 6 min of the infusion) (Fig. 7, *right*).

Intrarenal distribution of radiolabel. There was considerable variation associated with the levels of radiolabel in the solubilized kidney tissue, particularly during outer medullary infusion of radiolabel. It was clear, however, that much greater levels of the radiolabel remained in the kidney during outer medullary, compared with inner medullary, infusion of [³H]NE (Fig. 8A).

Autoradiographic analysis of the coronal kidney sections demonstrated that with outer medullary infusion, the levels of radioactivity in the cortex of the infused kidney were 10.3 ± 2.3-, 14.0 ± 3.3-, and 8.8 ± 2.8-fold lower than those in the outer stripe, medulla, and papilla, respectively. During inner medullary infusion, the levels of radioactivity in the cortex of the infused kidney were 6.6 ± 1.6-, 8.7 ± 2.2-, and 8.2 ± 2.4-fold lower than those in the outer stripe, medullary, and papillary regions, respectively (Fig. 8, B and C).

DISCUSSION

The primary objective of this study was to design and validate techniques for delivery of pharmacological agents into the renal medullary interstitium of rabbits. Similar techniques have been developed in rats (13, 14, 19, 23), and these have helped provide considerable information regarding the role of the renal medulla and in particular the renal medullary microcirculation in the control of blood pressure (4–6). Our results suggest that in the rabbit, outer medullary interstitial infusion of pharmacological agents, using either acutely or chronically implanted catheters, may be a useful method for targeting pharmacological agents to the renal medulla and in particular for altering MBF. Thus delivery of NE into the outer medullary interstitium via acutely positioned or chronically implanted catheters dose relatedly reduced MBF more than it did CBF or RBF_probe. In
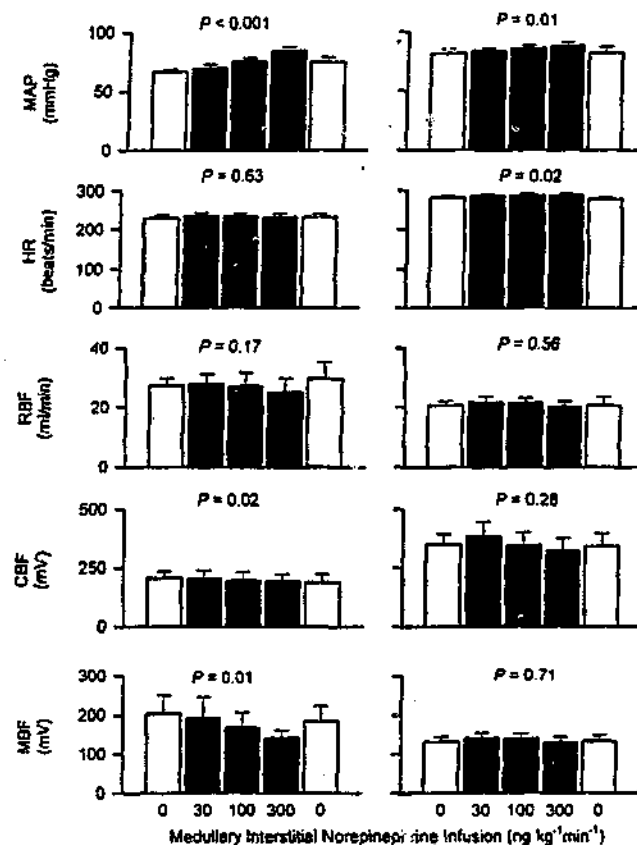
Fig. 5. Systemic and renal hemodynamic effects of outer (n = 8; *left*) and inner (n = 9; *right*) medullary interstitial infusion of norepinephrine delivered via chronically positioned catheters. Each dose (0, 30, 100, 300 ng·kg⁻¹·min⁻¹) was infused over consecutive 20-min periods. Columns and error bars represent means ± SE of each variable over final 10-min period when all variables were stable. Solid bars, periods when norepinephrine was infused; open bars, periods during which vehicle (154 mM NaCl; 20 µl·kg⁻¹·min⁻¹) was infused. P values represent outcome of a partitioned ANOVA testing for a dose-dependent effect of infusions on each hemodynamic variable. Recovery period was excluded for this analysis.

Medullary Interstitial Norepinephrine Infusion (ng kg⁻¹min⁻¹)

contrast, intravenous infusion of NE reduced RBF$_{probe}$ and CBF but not MBF. When [³H]NE was infused into the outer medulla, the concentration of the radiolabel was found to be much greater in the renal medulla of the infused kidney than in its cortex or in the contralateral kidney. We therefore conclude that outer medullary interstitial infusion of NE achieves high concentrations of this agent at vascular sites important in the control of MBF, in the medulla, and/or in the inner cortex.

One of the strengths of the present study was the estimation, during medullary interstitial infusion of [³H]NE, of the amount of radiolabel spilled over from the kidney into the renal vein, the amount of this radiolabel that reentered the kidney via the renal artery, and the amounts of radiolabel excreted by the infused and contralateral kidneys. Although much of the radiolabel in these biological fluids probably reflects metabolites of [³H]NE, we argue that most small, uncharged molecules should be handled similarly by the kidney during medullary interstitial infusion. In other words, the pattern of renal disposition of radiolabel during [³H]NE infusion probably reflects that expected for stable, uncharged small molecules. In the case of NE and other molecules that are rapidly metabo-

lized in vivo the proportion of the radiolabel that represents intact [³H]NE must become less in proportion to the distance from the infusion site. In this study therefore, we should not expect complete agreement between the localization of the radiolabel and the effects of the NE infusions. Nevertheless, with this caveat in mind, we suggest that this analysis provides two important observations that illustrate the limitations of the medullary interstitial infusion technique.

First, although we were able to achieve much greater concentrations of radiolabel within the medulla compared with the cortex during outer medullary infusion of [³H]NE, we also found that much of the radiolabel (~40% using the chronically implanted catheter) spilled over into the renal vein and so recirculated systemically. This spillover is unlikely to greatly confound the interpretation of studies in which relatively unstable compounds (e.g., NE and angiotensin II) or compounds that are metabolized in the pulmonary circulation (e.g., bradykinin and endothelin-1) are infused. Nevertheless, the dose-related pressor effect that we observed clearly indicates that significant quantities of intact NE *do* spill over during medullary interstitial infusion. Furthermore, in studies where more stable compounds
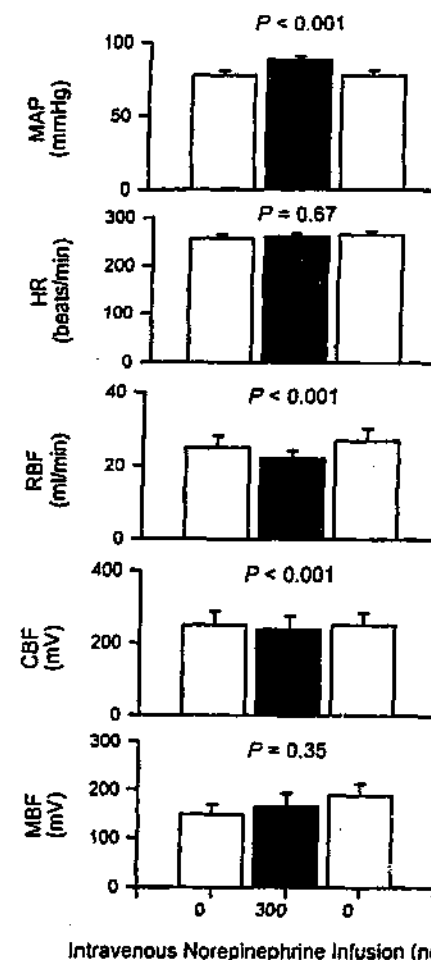

Intravenous Norepinephrine Infusion (ng kg⁻¹min⁻¹)

Fig. 6. Effects of intravenous infusion of norepinephrine (300 ng·kg⁻¹·min⁻¹) on systemic and renal hemodynamic variables (n = 17). Columns and error bars represent means ± SE of each variable during final 10 min of each 20-min infusion period. Open bars, periods before and after; solid bars, period during norepinephrine infusion. P values represent outcomes of partitioned ANOVA, testing whether levels of each variable during intravenous norepinephrine infusion differed from those preceeding and following it.

Second, we observed a major effect of the site of the medullary interstitial infusion on the way in which the kidney handled the infused [³H]NE. Thus, in contrast to the outer medullary infusion, where much of the infused radiolabel spilled over into the systemic circulation, during inner medullary infusion ~60% of the infused radiolabel was excreted by the infused kidney. The reason for this difference between the outer medullary and inner medullary infusions remains to be determined unequivocally but may relate in part to the presence of mechanisms for tubular secretion of NE (11) and to the relatively lower blood flow in the inner medulla compared with the outer medulla (20). It is unlikely to reflect leakage of [³H]NE due to damage to the papillary tissue from implantation of the catheter, because no such damage was observed in the frozen sections submitted for autoradiography. This effect of the infusion site on the disposition of the infused radiolabel probably explains why the levels of radioac-
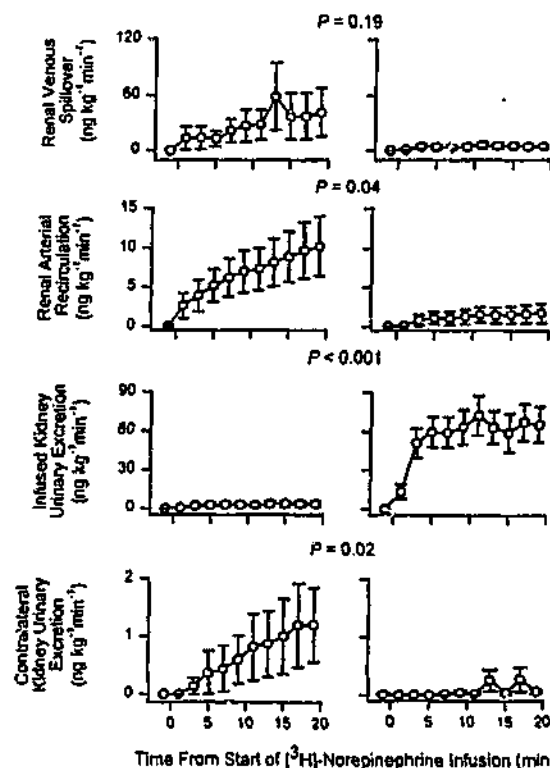

Time From Start of [³H]-Norepinephrine Infusion (min)

Fig. 7. Disposition of radiolabel (expressed as [³H]norepinephrine equivalents; ng·kg⁻¹·min⁻¹) during a 20-min infusion of [³H]norepinephrine (16–24 nCi·kg⁻¹·min⁻¹ in 100 ng·kg⁻¹·min⁻¹ norepinephrine) into outer (*left*) and inner (*right*) medulla of left kidney via chronically positioned catheters. Coordinates represent means ± SE of 8 (outer medullary infusion) or 9 (inner medullary infusion) sets of observations. P values represent outcomes of repeated-measures ANOVA, testing whether location of catheter tip (outer vs. inner medulla) influenced disposition of radiolabel.

are infused, spillover into the systemic circulation will almost certainly confound the interpretation of the results. This appears to have been the case in a recent study in which we compared the effects of outer medullary, renal arterial, and intravenous infusion of the vasopressin V₁ agonist [Phe²,Ile³,Orn⁸]vasopressin (8). The pressor and bradycardic effects of this agent were indistinguishable by all three routes, as was the reduction in MBF. Systemic spillover during outer medullary interstitial infusion therefore appears not to be a unique property of NE but also occurs with other small molecules.
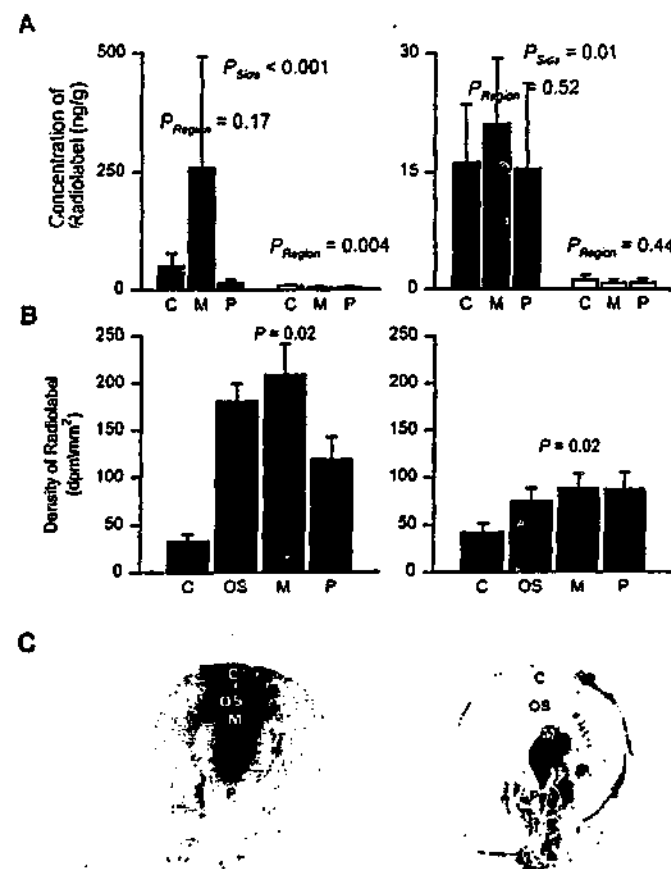
Fig. 8. A: distribution of radiolabel in infused (left, solid bars) and contralateral (right, open bars) kidneys at end of 20-min infusions of [³H]norepinephrine (18–24 nCi·kg⁻¹·min⁻¹ in 100 ng·kg⁻¹·min⁻¹ norepinephrine) into outer (left) and inner (right) medulla via chronically positioned catheters. Radiolabel in solubilized kidney tissue, expressed as [³H]norepinephrine equivalents (ng/g). Columns and error bars represent means ± SE for 7 (outer medulla) or 9 (inner medulla) rabbits. P values are as for Fig. 4. Note different scales for different routes. B: levels of radiolabel, expressed as dpm/mm², determined from autoradiographic analysis of infused kidneys. Columns, error bars, and P values are as in Fig. 3B. C: typical autoradiograms for infused kidneys.

tivity were considerably less in the ex___ ed kidneys that had received an inner medullary infusion of NE compared with those that had received the outer medullary infusion. This, in turn, may help explain why MBF was not reduced during inner medullary infusion of NE and why the pressor effect of NE was considerably less with inner medullary infusion than with outer medullary infusion of NE.

Some of the present observations are at odds with similar studies performed in rats. For example, in rats, infusion of vasoconstrictor agents into the inner medulla reduces MBF, whereas similar infusions of vasodilator agents can increase MBF (4–6, 13, 14, 19). In contrast, in the present study we found that outer medullary, but not inner medullary, infusion of NE reduced MBF. There are a number of possible explanations for this apparent species difference, the most obvious being the difference in dimensions of the renal medulla in these two species. We hypothesize therefore, that in the rat (but not the rabbit, with a ~10-fold greater kidney weight), agents infused into the inner medulla may easily diffuse the relatively short distance

to the outer medulla and inner cortex to influence vascular elements controlling MBF. It is also possible that differences in medullary countercurrent mechanisms between the two species might alter the renal handling of substances infused into the renal medulla. Indeed, Cowley and colleagues (5) argued previously that substances infused into the inner medulla are accumulated because of the efficient countercurrent exchanger in the vasa recta circulation. The results of the present study indicate that this is not the case in rabbits recei 'ng medullary interstitial infusions of [³H]NE, in which much of the infused radiolabel either spilled over into the systemic circulation (outer medullary infusion) or was excreted in the urine of the infused kidney (inner medullary infusion). These apparent differences between rats and rabbits in the ability of the inner medulla to "trap" substances infused into the interstitium could reflect the differences in medullary structure between the two species (12). For example, the rabbit renal medulla has a "simple" structure, with relatively small vascular bundles containing only ascending and descending vasa recta. In the more

"complex" rat renal medulla, larger vascular bundles are found that also contain descending thin limbs of short loops of Henle.

On the other hand, our results are in agreement with previous studies by Cowley and colleagues (5, 14) in that during medullary interstitial infusion of a radiolabeled small molecule ([¹⁴C]clentiazem in their case and [³H]NE in the present study) the radiolabel within the infused kidney was mostly concentrated in the medulla and papilla, with very little radiolabel in the cortex of the infused kidney or in the contralateral kidney. Consistent with these observations, medullary interstitial infusion of pharmacological agents in rats (23) and rabbits (present study) can have effects quite distinct from those of intravenous infusion of these agents. In the case of NE, we found that in contrast to intravenous NE, which reduced CBF and RBF$_{probe}$ without significantly affecting MBF, outer medullary infusion of NE dose relatedly reduced MBF but had considerably smaller effects on CBF and RBF$_{probe}$.

Although we cannot be certain of the precise vascular elements that mediate the reduction in MBF during outer medullary infusion of NE, we can at least suggest some likely candidates. Within the kidney, NE can mediate vasoconstriction directly by acting on vascular α_1- and α_2-adrenoceptors (7, 27) or indirectly by β-adrenoceptor-mediated stimulation of renin release (24). The fact that intravenous infusion of NE reduced CBF but not MBF suggests that an indirect stimulus, via renin release, is unlikely. NE directly constricts outer cortical afferent and efferent arterioles in situ (3) and outer medullary descending vasa recta in vitro (28). It is also possible that vascular sites in the inner medulla play some role in mediating the reduced MBF, because contractile elements have recently been identified in inner medullary descending vasa recta in rats (21). Our finding that inner medullary infusion of NE did not affect MBF does not exclude this possibility, because infusion of [³H]NE by this route resulted in relatively low levels of accumulated radiolabel in the inner medulla.

There was, however, clear evidence of cortical effects of the infused NE, because CBF and RBF$_{probe}$ were reduced, and, at least in the case of the acutely positioned catheters, GFR was also reduced. Indeed, the reductions in urine flow and sodium excretion during outer medullary infusion of NE could be completely accounted for by the reduced GFR, indicating no net change in tubular sodium and water reabsorption. This latter observation seems at odds with the notion that reduced MBF should enhance tubular salt and water reabsorption (6), but may be explained by the confounding impact of the pressor effect of the medullary interstitial NE infusion. Clearly, further studies in which renal perfusion pressure is controlled are required to delineate the direct effects of medullary interstitial NE infusion on renal excretory function.

Perspectives

The results of the present study show that NE reduces MBF when infused acutely into the outer

medullary interstitium and that this effect is dependent on the selective distribution of this compound within the renal medulla. We suggest, on the basis of the present results and the extensive previous studies by Cowley and colleagues (4–6, 13, 14, 19, 23), that the general principle that medullary interstitial infusion provides a useful technique for targeting drugs to the renal medulla (and in particular the microvasculature) can be generalized to a wide range of small molecule pharmacological agents. However, the technique appears to be limited (at least in the rabbit) by the systemic spillover that occurs with outer medullary infusion and by excretion of the infused substance with inner medullary infusion. For this reason and because the renal disposition and distribution of infused agents probably depends on their physicochemical properties, appropriate controls (intravenous and renal arterial infusions) combined with studies of the renal handling of the infused agent are probably necessary for correct interpretation of observations made with this method. Nevertheless, with these caveats in mind, the adaptation of this technique for chronic studies in rabbits, a species well suited for invasive longitudinal experimentation (see introduction), may in the future provide important information regarding the long-term consequences of alterations in MBF. In support of this contention, we recently found that chronically implanted outer medullary catheters remain patent for at least 6 wk after implantation (unpublished observations).

REFERENCES

1. Ashworth-Preece, M., E. Krstew, B. Jarrott, and A. J. Lawrence. Functional GABA$_A$ receptors on rat vagal afferent neurones. Br. J. Pharmacol. 120: 469–475, 1997.
2. Bergström, G., and R. G. Evans. Effects of renal medullary infusion of a vasopressin V₁ agonist on renal antihypertensive mechanisms in rabbits. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R76–R85, 1998.
3. Chen, J., and J. T. Fleming. Juxtamedullary afferent and efferent arterioles constrict to renal nerve stimulation. Kidney Int. 44: 684–691, 1993.
4. Cowley, A. W., Jr. Role of the renal medulla in volume and arterial pressure regulation. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1–R15, 1997.
5. Cowley, A. W., Jr., D. L. Mattson, S. Lu, and R. J. Roman. The renal medulla and hypertension. Hypertension 25: 663–673, 1995.
6. Cowley, A. W., Jr., R. J. Roman, F. J. Fenoy, and D. L. Mattson. Effect of renal medullary circulation on arterial pressure. J. Hypertens. 10, Suppl. 7: S187–S193, 1992.

7. Evans, R. G., and W. P. Anderson. Renal effects of infusion of rilmenidine and guanabenz in conscious dogs: contribution of peripheral and central nervous system α_2-adrenoceptors. *Br. J. Pharmacol.* 116: 1657–1670, 1995.

8. Evans, R. G., G. Bergström, and A. J. Lawrence. Effects of the vasopressin V_1-agonist [Phe^2,Ile^3,Orn^8]vasopressin on regional kidney perfusion and renal excretory function in anesthetized rabbits. *J. Cardiovasc. Pharmacol.* 32: 571–581, 1998.

9. Evans, R. G., J. M. Haynes, and J. Ludbrook. Effects of 5-HT-receptor and α_2-adrenoceptor ligands on the haemodynamic response to acute central hypovolaemia in conscious rabbits. *Br. J. Pharmacol.* 109: 37–47, 1993.

10. Evans, R. G., A. J. Rankin, and W. P. Anderson. Interactions of blockade of nitric oxide synthase and angiotensin-converting enzyme on renal function in conscious rabbits. *J. Cardiovasc. Pharmacol.* 24: 542–551, 1994.

11. Kopp, U., T. Bradley, and P. Hjemdahl. Renal venous outflow and urinary excretion of norepinephrine, epinephrine, and dopamine during graded renal nerve stimulation. *Am. J. Physiol.* 244 (*Endocrinol. Metab.* 7): E52–E60, 1983.

12. Kriz, W. Structural organization of the renal medulla: comparative and functional aspects. *Am. J. Physiol.* 241 (*Regulatory Integrative Comp. Physiol.* 10): R3–R16, 1981.

13. Lu, S., D. L. Mattson, and A. W. Cowley, Jr. Renal medullary captopril delivery lowers blood pressure in spontaneously hypertensive rats. *Hypertension* 23: 337–345, 1994.

14. Lu, S., R. J. Roman, D. L. Mattson, and A. W. Cowley, Jr. Renal medullary interstitial infusion of diltiazem alters sodium and water excretion in rats. *Am. J. Physiol.* 263 (*Regulatory Integrative Comp. Physiol.* 32): R1064–R1070, 1992.

15. Ludbrook, J. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc. Res.* 28: 303–314, 1994.

16. Majid, D. S. A., M. Godfrey, and L. G. Navar. Pressure natriuresis and renal medullary blood flow in dogs. *Hypertension* 29: 1051–1057, 1997.

17. Majid, D. S. A., M. Godfrey, and S. A. Omoro. Pressure natriuresis and autoregulation of inner medullary blood flow in canine kidney. *Hypertension* 29: 210–215, 1997.

18. Malpas, S. C., and R. G. Evans. Do different levels and patterns of sympathetic activation all provoke renal vasoconstriction? *J. Auton. Nerv. Syst.* 30: 1–11, 1996.

19. Mattson, D. L., S. Lu, K. Nakanishi, P. F. Papanek, and A. W. Cowley, Jr. Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am. J. Physiol.* 266 (*Heart Circ. Physiol.* 35): H1918–H1926, 1994.

20. Pallone, T. L., C. R. Robertson, and R. L. Jamison. Renal medullary microcirculation. *Physiol. Rev.* 70: 885–920, 1990.

21. Park, F., D. L. Mattson, L. A. Roberts, and A. W. Cowley, Jr. Evidence for the presence of smooth muscle alpha-actin within pericytes of the renal medulla. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R1742–R1748, 1997.

22. Roman, R. J., and C. Smits. Laser-Doppler determination of papillary blood flow in young and adult rats. *Am. J. Physiol.* 251 (*Renal Fluid Electrolyte Physiol.* 20): F115–F124, 1986.

23. Szczepanska-Sadowska, E., K. Stepniakowski, M. M. Skelton, and A. W. Cowley, Jr. Prolonged stimulation of intrarenal V_1 vasopressin receptors results in sustained hypertension. *Am. J. Physiol.* 267 (*Regulatory Integrative Comp. Physiol.* 36): R1217–R1225, 1994.

24. Takagi, M., K. Atarachi, H. Matsuoka, and T. Sugimoto. A biphasic effect of noradrenaline on renin release from rat juxtaglomerular cells in vitro is mediated by α_1- and β-adrenoceptors. *J. Endocrinol.* 132: 133–140, 1992.

25. Tomoda, F., R. A. Lew, A. I. Smith, A. C. Madden, and R. G. Evans. Role of bradykinin receptors in the renal effects of inhibition of angiotensin converting enzyme and endopeptidases 24.11 and 24.15 in conscious rabbits. *Br. J. Pharmacol.* 119: 365–373, 1996.

26. Wilkinson, L. SYSTAT: *The System for Statistics.* Evanston, IL: SYSTAT, 1990.

27. Wolff, D. W., V. M. Buckalew, Jr., and J. W. Strandhoy. Renal α_1- and α_2-adrenoceptor mediated vasoconstriction in dogs: comparison of phenylephrine, clonidine, and guanabenz. *J. Cardiovasc. Pharmacol.* 6, *Suppl.* 5: S793–S798, 1984.

28. Yang, S., E. P. Silldorff, and T. L. Pallone. Effect of norepinephrine and acetylcholine on outer medullary descending vasa recta. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H710–H716, 1995.

Effects of Renal Medullary and Intravenous Norepinephrine on Renal Antihypertensive Function

Anabela G. Correia, Anna C. Madden, Göran Bergström, Roger G. Evans

Abstract—Increasing renal arterial pressure activates at least 3 antihypertensive mechanisms: reduced renin release, pressure natriuresis, and release of a putative renal medullary depressor hormone. To examine the role of renal medullary perfusion in these mechanisms, we tested the effects of the infusion of norepinephrine, either infusion into the renal medullary interstitium or intravenous infusion, on responses to increased renal arterial pressure in pentobarbital-anesthetized rabbits. We used an extracorporeal circuit, which allows renal arterial pressure to be set to any level above or below systemic arterial pressure. With renal arterial pressure initially set at 65 mm Hg, intravenous and medullary interstitial norepinephrine (300 ng · kg^{-1} · min^{-1}) similarly increased mean arterial pressure (by 12% to 17% of baseline) and reduced total renal blood flow (by 16% to 17%) and cortical perfusion (by 13% to 19%), but only medullary norepinephrine reduced medullary perfusion (by 28%). When renal arterial pressure was increased to ~160 mm Hg, in steps of ~65 mm Hg, urine output and sodium excretion increased exponentially, and plasma renin activity and mean arterial pressure fell. Medullary interstitial but not intravenous norepinephrine attenuated the increased diuresis and natriuresis and the depressor response to increased renal arterial pressure. This suggests that norepinephrine can act within the renal medulla to inhibit these renal antihypertensive mechanisms, perhaps by reducing medullary perfusion. These observations support the concept that medullary perfusion plays a critical role in the long-term control of arterial pressure by its influence on pressure diuresis/natriuresis mechanisms and also by affecting the release of the putative renal medullary depressor hormone. *(Hypertension.* 2000;35:965-970.)*

Key Words: kidney medulla ■ laser-Doppler flowmetry ■ norepinephrine ■ natriuresis ■ renal circulation

It has been hypothesized that the level of medullary blood flow (MBF) is an important determinant of urinary sodium excretion (U$_{Na}$*V) and, indeed, may be the key initiating factor in the pressure natriuresis response.[1] In turn, the impact of MBF on the pressure natriuretic mechanism provides an explanation for the effects of chronic changes in MBF on the long-term control of arterial pressure.[1] Thus, in rare chronic reductions in MBF shift the pressure natriuresis relation toward higher pressures and lead to hypertension in normotensive animals. Conversely, chronic increases in MBF shift the pressure natriuresis relation toward lower pressures and ameliorate hypertension in spontaneously hypertensive rats.[1]

From studies using an extracorporeal circuit in anesthetized rabbits,[2] we recently obtained preliminary evidence indicating that influences on the release and/or actions of the putative renal medullary depressor hormone might also contribute to the impact of MBF on the long-term control of arterial pressure. In this model, 3 major renal antihypertensive mechanisms can be studied simultaneously. Thus, when renal arterial pressure (RAP) is acutely increased in this model, plasma renin activity (PRA) is reduced (indicating reduced renal renin release), urine flow (U$_{vol}$) and U$_{Na}$*V

increase exponentially (pressure diuresis/natriuresis), and systemic mean arterial pressure (MAP) is reduced. The depressor response to increased RAP appears to be largely independent of the reduced activity of the renin-angiotensin system, in view of the fact that it is little affected by the blockade of angiotensin-converting enzyme.[3] It also appears to be largely independent of the associated diuresis and natriuresis, in view of the fact that hemoconcentration is not observed.[1-4] There is, however, clear evidence for a role of the renal medulla, inasmuch as the depressor response is abolished by chemical medullectomy.[4]

We recently found that this depressor response to increased RAP was blunted by medullary interstitial infusion of [Phe^2,Ile^3,Orn^8]vasopressin (V$_1$-agonist), a treatment that selectively reduces MBF,[2] indicating a possible role of MBF in the release of this putative hormone. However, we were unable to determine whether this effect of medullary interstitial infusion of the V$_1$-agonist was specifically due to reduced MBF or to some other action of the agent. For example, this treatment also reduced total renal blood flow (RBF) and cortical blood flow (CBF). We also could not exclude the possibility of non-flow-mediated extravascular actions on V$_1$

receptors in the kidney or even extrarenal V$_1$ receptors, which might blunt the release and/or actions of the putative renal medullary depressor hormone.[2]

The aim of the present study was to more directly test for a role of the medullary microcirculation in modulating the antihypertensive responses to increased RAP. To this end, we made use of our recent observation that medullary interstitial infusion of norepinephrine (NE) reduces MBF twice as much as CBF, whereas intravenous NE reduces only CBF.[5] Therefore, we compared the effects of medullary interstitial infusion and intravenous infusion of NE on antihypertensive responses to increased RAP. Thus, using this experimental design, we could control for the effects of NE exerted outside the renal medulla in a way that was not possible in our previous experiment with the V$_1$-agonist.[2] Our results support the concept that MBF plays a key role in the regulation of arterial pressure, not only through its impact on pressure natriuretic/diuretic mechanisms but also via its effects on the release of the putative renal medullary depressor hormone.

Methods

Animals

Twenty-nine male New Zealand White rabbits, weighing 2.50 to 2.94 (mean 2.62) kg, were studied. Before experimentation, all rabbits were allowed food and water ad libitum. At the conclusion of the experiment, they were killed with an intravenous overdose of pentobarbital sodium. All experiments were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation.

Extracorporeal Circuit

To control and alter RAP, an extracorporeal circuit was established in anesthetized (pentobarbital at 90 to 150 mg plus 30 to 50 mg/h [Nembutal], Boehringer-Ingelheim) artificially respired rabbits as previously described.[4] Blood was withdrawn from the aorta at a rate of 90 mL/min by a roller pump (Masterflex model 7521-45, Barnant Co) and returned to the rabbit via 2 limbs, one to the renal artery and the other to the vena cava. RAP was controlled by adjusting a Starling resistor incorporated into the vena caval limb, while total flow through the circuit remained constant. For example, increasing the mechanical resistance in the vena caval limb by use of the Starling resistor diverts blood flow toward the renal limb, thus increasing RAP. The circuit dead space (24 mL) was filled with 10% (wt/vol) dextran 40 in 154 mmol/L NaCl (Gentran 40, Baxter Healthcare) containing 2 IU/mL heparin (Monoparin, Fisons Pharmaceuticals). Therefore, there was some initial hemodilution when the circuit was established; thus, hematocrit was relatively low (see Results).

To remove the confounding influence of the right kidney, which would otherwise be perfused at systemic arterial pressure, a right nephrectomy was performed in preparation for establishment of the extracorporeal circuit. The left ureter was then cannulated, and the kidney was denervated and placed in a stable cup for positioning of laser-Doppler flow probes. For measurement of MBF, a 26-gauge needle-type probe (DP4s, Moor Instruments Ltd) was advanced 10 mm below the mid region of the lateral surface of the kidney with use of a micromanipulator (Narashige). A standard plastic straight probe (DP2b, Moor Instruments Ltd) was placed on the dorsal surface of the kidney for measurement of CBF. In 14 rabbits, medullary infusion catheters were acutely positioned laterally, 10 mm either side of the laser-Doppler flow probe, and advanced so that their tips lay 8.5 mm below the cortical surface (at the junction of the outer and inner stripes of the outer medulla).[5]

The extracorporeal circuit was then established, and RAP was set at ~65 mm Hg for a 60-minute equilibration period. A bolus dose of [^{3}H]inulin (4 μCi, NEN Research Products) was administered in 1.0

mL of 154 mmol/L NaCl. An infusion of 10% (vol/vol) polygeline (Hemaccel, Hoechst) containing 200 IU/mL sodium heparin and 0.3 μCi/mL [^{3}H]inulin was then initiated (0.18 mL · kg^{-1} · min^{-1}), which continued for the duration of the experiment. Body temperature was maintained between 36°C and 38°C.[5]

Measurements

Systemic arterial pressure was measured by connecting an ear artery catheter to a pressure transducer (Cobe). Heart rate (HR) was measured by a tachometer activated by the pressure pulse. RAP was measured in a side-arm catheter, 3 mm proximal to the tip of the cannula inserted into the renal artery. Blood flow through the renal limb was measured with an in-line ultrasonic flow probe (type 4N, Transonic Systems Inc). The laser-Doppler flow probes were connected to a laser-Doppler flowmeter (DRT4, Moor Instruments Ltd). These signals were amplified, recorded, and digitized, as previously described,[2] to provide 60-second means expressed as follows: systemic MAP, mm Hg; HR, bpm; RAP, mm Hg; RBF, mL/min; and CBF and MBF, perfusion units (equivalent to the instrument output in mVX10).

PRA and plasma and urinary concentrations of [^{3}H]inulin and sodium were made as previously described.[2] [^{3}H]inulin clearance was used to estimate glomerular filtration rate (GFR). At the completion of each experiment, the left kidney was removed and desiccated, and its dry weight was determined. All values of RBF, GFR, U$_{Na}$, and U$_{Na}$*V are therefore expressed per gram of dry kidney weight (expressed as g [mean 1.77±0.03 g]).

Experimental Protocols

General

Each experimental protocol consisted of 2 phases. Phase 1, which followed the 60-minute equilibration period, tested the effects of either outer medullary interstitial (protocol 1) or intravenous (protocol 2) infusion of NE on systemic and renal hemodynamics. The second phase of each protocol involved testing the effect of these treatments on the responses to increased RAP. For technical reasons, we were unable to reliably monitor MBF during step increases in RAP, so laser-Doppler measurements are reported only for phase 1 of the experiment.

Protocol 1: Effects of Outer Medullary Interstitial NE

After 10 minutes of stable baseline readings, outer medullary interstitial infusion of either NE (300 ng · kg^{-1} · min^{-1}, n=6) or its vehicle (154 mmol/L NaCl, 20 μL · kg^{-1} · min^{-1}, n=8) was started and was continued for the rest of the experiment. Twenty minutes later, RAP was set at ~65, 85, 110, 130, and 160 mm Hg for consecutive 20-minute periods and, once set, was not readjusted. Urine produced by the left kidney was collected during the final 15 minutes of each period. Arterial blood (1 mL) for clearance measurements was collected from an ear artery catheter at the midpoint of each 15-minute clearance period, and samples (1 mL) for determination of PRA were collected at the midpoint of the first, third, and fifth clearance periods. Blood volume was replaced by an equivalent volume of 10% polygeline solution (Hemaccel). At the end of the fifth clearance period, RAP was set to ~65 mm Hg for a further 20 minutes.

Protocol 2: Effects of Intravenous NE

This protocol was identical to protocol 1, except NE (300 ng · kg^{-1} · min^{-1}, n=7) or its vehicle (20 μL · kg^{-1} · min^{-1}, n=8) was administered intravenously via an ear vein catheter.

Statistical Analysis

Phase I

To test whether each of the NE or vehicle treatments altered baseline systemic and renal hemodynamics, average levels of each variable during the period 10 to 20 minutes after the initiation of the infusion were compared with the levels during the 10-minute control period by paired t test.

Phase II

These data were analyzed by ANOVA adapted for repeated measures with the use of SYSTAT software (version 5.05). To protect against the increased risk of comparison-wise type I error resulting from compound asymmetry, probability values were adjusted by use of the Greenhouse-Geisser correction.[6] To test whether increasing RAP altered each variable, a 1-way analysis was first performed on all vehicle-treated rabbits to provide the main effect of increasing RAP (P_{RAP}). The interaction term between RAP and treatment (vehicle or NE) was then determined from 2-way analyses for each route (intravenous and medullary interstitial). This tested for effects of NE infusion on the responses to increased RAP.

Results

Effects of Renal Medullary Interstitial NE on Systemic and Renal Hemodynamics

Renal medullary interstitial infusion of NE (300 ng · kg^{-1} · min^{-1}) was accompanied by progressive hemodynamic changes that reached steady state by 10 minutes after the infusion began. The changes involved increases in RAP (by 19±4% of its baseline level during the period 10 to 20 minutes after beginning the infusion) and MAP (by 17±4%) and reductions in RBF (16±3%), CBF (13±2%), and MBF (28±9%) but no significant change in HR (1±2% change). Medullary interstitial infusion of the vehicle had no significant effect on any of these variables.

Effects of Intravenous NE on Systemic and Renal Hemodynamics

Intravenous NE (300 ng · kg^{-1} · min^{-1}) was also accompanied by reductions in RBF (by 17±9% of its baseline value) and CBF (by 19±3%) and by increases in MAP (12±4%) and RAP (4±1%). However, unlike renal medullary NE, intravenous NE had no significant effect on MBF (1±8% change). Intravenous infusion of the vehicle was accompanied by small variations in MAP (4±1%), HR (1±1%), and RBF (−4±2%) but no significant changes in RAP, CBF, or MBF.

Effects of Increasing RAP in Vehicle-Treated Rabbits

Renal Hemodynamic Variables

As shown in Figure 1, as RAP was increased from 66±1 to 158±3 mm Hg, there were progressive increases in RBF (from 13±1 to 29±2 mL · min^{-1} · g^{-1}) and GFR (from 0.8±0.1 to 3.0±0.4 mL · min^{-1} · g^{-1}) (P_{RAP}<0.001). Renal vascular resistance and filtration fraction responded biphasically. As RAP was increased from ~65 to ~110 mm Hg, renal vascular resistance increased from 5.9±0.8 to 7.7±2.3 mm Hg · mL^{-1} · min · g before decreasing to 6.9±0.6 mm Hg · mL^{-1} · min · g when RAP was increased to ~160 mm Hg (P_{RAP}=0.05). Filtration fraction also responded in a similar manner, increasing from 3.5±1.1% to 9.3±1.9% as RAP was increased from ~65 to ~110 mm Hg before decreasing to 8.0±1.4% when RAP was increased to ~160 mm Hg (P_{RAP}=0.001).

Renal Excretory Variables

As shown in Figure 2, as RAP was increased from ~65 to ~160 mm Hg, there were progressive increases in U$_{VOL}$ (from 0.09±0.02 to 1.24±0.09 mL · min^{-1} · g^{-1}) and U$_{Na}$$^+$V (from 12±2 to 161±13 μmol · min^{-1} · g^{-1}) and in the fractional
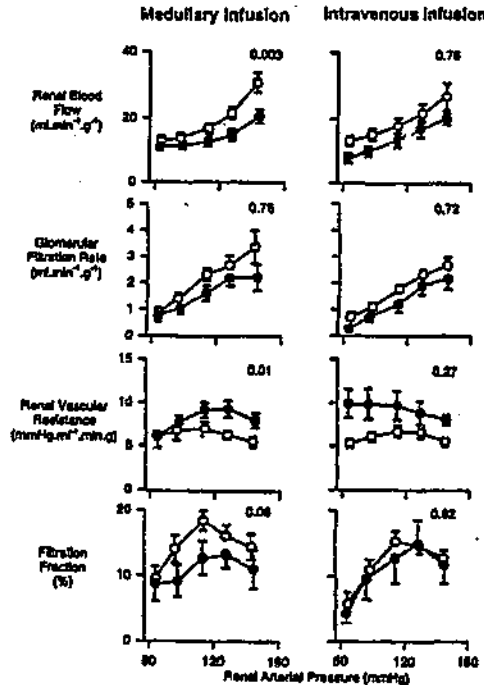

Figure 1. Effects of outer medullary interstitial infusion (n=6) and intravenous infusion (n=7) of NE on renal hemodynamic responses to progressive increases in RAP. O indicates vehicle (154 mmol/L NaCl at 20 μL · kg^{-1} · min^{-1}, n=8); ●, NE (300 ng · kg^{-1} · min^{-1}, n=6 to 8). Symbols are the mean±SEM for each 15-minute period for each variable. Probability values shown in the top right corner of each panel are the interaction terms, from repeated-measures ANOVA, between treatment (vehicle or NE) and RAP. These test whether NE treatment altered the response to increased RAP.

excretions of urine (from 12±1% to 43±3%) and sodium (from 11±2% to 40±3%) (P_{RAP}<0.001).

Systemic Hemodynamic Variables

As shown in Figure 3, as RAP was increased from ~65 to ~160 mm Hg, MAP fell progressively from 78±3 to 50±5 mm Hg and at an increasing rate of 0.04±0.06 to 0.96±0.15 mm Hg/min (P_{RAP}<0.001). Hematocrit decreased gradually from 22.1±0.9% to 21.6±0.9% as RAP was increased from ~65 to ~110 mm Hg and increased thereafter to 22.5±0.9% when RAP was increased to ~160 mm Hg (P_{RAP}=0.04). HR tended to decrease (from 266±5 to 253±8 bpm) as RAP increased toward ~160 mm Hg (P_{RAP}=0.05).

Plasma Renin Activity

PRA progressively fell as RAP was increased, averaging 14±3, 12±2, and 7±3 ng angiotensin I · mL^{-1} · h^{-1} when RAP was ~65, 110, and 160 mm Hg, respectively (P_{RAP}=0.04).

Effects of Medullary Interstitial and Intravenous NE on Responses to Increased RAP

The RAP-dependent increases in RBF were significantly attenuated by medullary interstitial NE (Figure 1). RAP-
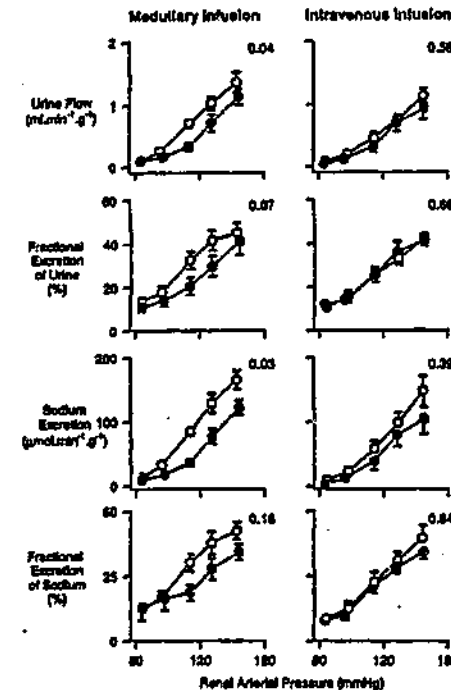
Figure 2. Effects of outer medullary interstitial and intravenous infusion of NE on renal excretory responses to progressively increased RAP. Symbols, error bars, and probability values are as described for Figure 1.

dependent increases in U$_{VOL}$ and U$_{Na}$$^+$V (Figure 2) and decreases in MAP (Figure 3) were significantly attenuated, but no significant effect on PRA was observed. Medullary interstitial NE also significantly altered the response of hematocrit to increased RAP, attenuating the increase in hematocrit as RAP was increased above ~110 mm Hg. Intravenous infusion of NE did not significantly influence any of the responses to increased RAP (Figures 1 to 3).

Effects of Resetting RAP to ~65 mm Hg

When RAP was reset to ~65 mm Hg, RBF returned to levels similar to those observed during the initial period (most leftward point in Figure 1) in vehicle-treated rabbits (−3±4% different from its previous level during the period 15 to 20 minutes after RAP was reset to ~65 mm Hg) and in rabbits treated with medullary interstitial NE (−13±4%) and intravenous NE (39±27%). MAP rose when RAP was reset to ~65 mm Hg but did not completely recover to its previous level in vehicle-treated rabbits (−28±5%) and in rabbits treated with outer medullary NE (−14±6%) and intravenous NE (−30±10%).

Discussion

We have recently shown in anesthetized rabbits that medullary interstitial infusion of NE (300 ng · kg^{-1} · min^{-1}) reduces MBF more than CBF and that intravenous infusion of the
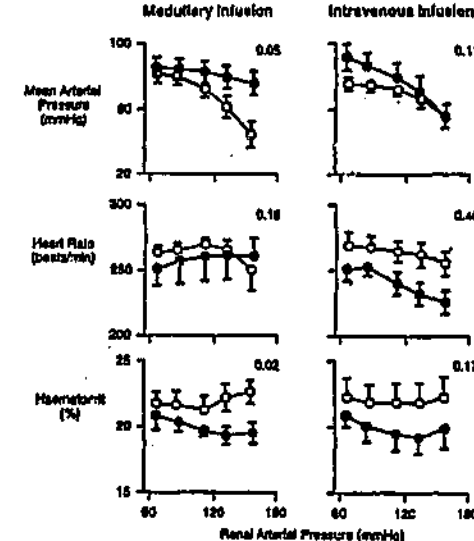
Figure 3. Effects of outer medullary interstitial and intravenous infusion of NE on systemic hemodynamic responses to progressively increased RAP. Symbols, error bars, and probability values are as described for Figure 1.

same dose reduces CBF only.[5] In the present study, we used these findings as a tool to examine the role of MBF in modulating the renal antihypertensive responses to increased RAP. Our major finding was that medullary interstitial NE, but not intravenous NE, attenuated both the pressure diuresis/natriuresis response and the depressor response to increased RAP. These observations provide further support for the hypothesis that MBF plays an important role in the control of arterial pressure, both through its involvement in the mechanisms mediating pressure diuresis/natriuresis and in the mechanisms mediating the release of the putative renal medullary depressor hormone.

Consistent with our previous observations in a conventional anesthetized rabbit preparation,[5] in the extracorporeal circuit model, infusion of NE increased MAP and reduced RBF and CBF similarly by the 2 routes. This indicates significant systemic spillover of NE infused into the renal medulla and, probably also, spillover into the renal cortex, consistent with our previous extensive characterization of this method.[5] However, our results also indicate that these renal cortical and extrarenal effects of NE can be effectively controlled for by intravenous infusion. The striking difference between the effects of NE infused by the 2 routes was that medullary interstitial infusion of NE reduced MBF by ~30%, whereas intravenous NE had little or no effect on MBF. Thus, our present experimental design provided a good paradigm for examining the effects of reduced MBF on the renal antihypertensive responses to increased RAP. We can also be fairly confident that these infusions provided relatively constant renal hemodynamic effects, inasmuch as in all experimental groups, RBF levels were similar at the end of the

experiment, when RAP was reset to ≈65 mm Hg, compared with RBF levels during the initial period at this level of RAP.

Thus, our finding (ie, medullary interstitial, but not intravenous, infusion of NE attenuates both the pressure diuresis/natriuresis response and the depressor response to increased RAP) provides evidence for a role of the renal medulla in both these renal antihypertensive mechanisms. Because intravenous infusion of NE did not significantly affect these responses, we can confidently exclude roles for NE mediated outside the kidney that are related, for example, to its systemic pressor effect, modulation of hormone release from extrarenal sites, or inhibition of the peripheral response to the putative renal medullary depressor hormone. We can also probably exclude contributions mediated solely in the cortical microvasculature, inasmuch as RBF and CBF were similarly reduced by medullary interstitial and intravenous infusions of NE. Roles for the renin-angiotensin system also appear unlikely in view of the fact that levels of PRA in rabbits receiving medullary interstitial infusions of NE were indistinguishable from those in vehicle-treated control rabbits.

Pressure Natriuresis

Medullary interstitial, but not intravenous, NE attenuated the diuretic and natriuretic responses to increased RAP. This effect likely also accounts for the statistically significant influence of medullary interstitial NE on hematocrit responses to increased RAP, because the reduced diuresis/natriuresis would attenuate hemoconcentration at high levels of RAP. Tubular elements probably play a key role in mediating the attenuated diuresis/natriuresis, because medullary interstitial NE did not significantly affect the relation between GFR and RAP. Our results indicate a role of the medullary interstitial infusion of NE on mediating the effects of medullary interstitial infusion of NE on the pressure diuresis/natriuresis response, but our present experiment does not definitively demonstrate that these effects were mediated by the effect of NE on MBF. In particular, a direct effect of NE on tubular function in the medulla cannot be discounted, because tubular adrenoceptors are certainly known to directly influence fluid and sodium reabsorption in the kidney.[7,8]

On the other hand, our present results are consistent with the large body of work by Cowley[1] showing that treatments that alter MBF, but not those that influence CBF alone, profoundly influence the pressure diuresis/natriuresis response. Cowley has argued that the chief initiating factor in the pressure natriuresis response is increased MBF and that this leads to a rise in renal interstitial hydrostatic pressure, which in turn inhibits tubular sodium reabsorption.[1] However, there is still considerable controversy regarding this hypothesis,[9] so its further critical evaluation is important. In this respect, the present study is significant because it has used an experimental model, with an extracorporeal circuit, that differs from conventional models for studying pressure natriuresis, in which RAP is altered by adjustable clamps on the aorta or renal artery.[9,10] Using this experimental model, we have previously shown that another treatment that reduces MBF, blockade of nitric oxide synthesis with N^G-nitro-L-arginine, also attenuates the pressure natriuresis response.[3,11] Importantly, our experimental model allows RAP to be set at

levels considerably greater than MAP, so that the pressure natriuresis relation can be investigated over a wide range of RAP. The renal vascular responses to increased RAP in the extracorporeal circuit model differ from those in conventional preparations,[10] in that RBF increases considerably as RAP is increased. However, as has been argued previously, autoregulation in this model is seen as an increase in renal vascular resistance in response to increased RAP, but its effect on RBF is limited by the fixed rate of the pump and high resistance of the vena caval limb.[3]

Putative Renal Medullary Depressor Hormone

As we have observed previously,[3] increased RAP was accompanied by pressure-dependent reductions in MAP. This response has been extensively characterized previously and appears to be unrelated to the accompanying inhibition of the renin-angiotensin system[3] or increase in U_{Vol} and U_{Na}^+V.[2,4] On the basis of the finding that the depressor response is abolished by chemical medullectomy,[4] we have proposed that this response to increased RAP is mediated chiefly by release of an as-yet-to-be-characterized depressor hormone from the renal medulla.[12] It may be that this putative hormone is identical, or similar, to "medullipin," which has been isolated but not yet fully chemically characterized.[13]

Previous studies have shown that some,[2,14,15] but not all, stimuli that reduce MBF[11,16] attenuate the depressor response to increased RAP. In the present study, we found that the depressor response to increased RAP was greatly blunted by medullary interstitial, but not intravenous, infusion of NE. Thus, our results provide the most direct evidence yet obtained, suggesting that the level of MBF influences the release of the putative renal medullary depressor hormone.

Nevertheless, we cannot as yet completely exclude the possibility that some other action of NE in the renal medulla, such as a direct action on renal medullary interstitial cells, the proposed site of storage and release of medullipin,[12] inhibits the release of the putative renal medullary depressor hormone. However, given our previous finding that medullary interstitial infusion of [Phe^2,Ile^3,Orn^8]vasopressin reduces MBF and attenuates the depressor response to increased RAP,[3] a role for the medullary microvasculature seems worthy of further investigation. To this end, future studies should replicate this experimental paradigm with other pharmacological agents that might selectively decrease and increase MBF.

Conclusions

Our findings indicate that NE can act within the renal medulla to attenuate the pressure natriuresis response and the release of the putative renal medullary depressor hormone. At present, we cannot be certain that this effect of NE is mediated by the accompanying reduced MBF, but we have strong circumstantial evidence that this is so. Any vasoactive agent is likely to have extravascular effects that might influence the antihypertensive responses to increased RAP. Therefore, the only way we can dissect out the relative roles of effects on MBF from other actions mediated within the renal medulla is to examine the effects of a range of agents that alter MBF. Our experience so far with extracorporeal circuit models such as that used in the

present study is that only treatments that alter MBF influence these renal medullary antihypertensive mechanisms.[3,11,14,15] Therefore, it seems likely that the medullary microvasculature plays a key role in the mechanisms controlling blood pressure in the long term, not only via actions on the renal handling of salt and water but also by influencing the release of the putative renal medullary depressor hormone.

References
1. Cowley AW Jr. Role of the renal medulla in volume and arterial pressure regulation. Am J Physiol. 1997;273:R1–R15.
2. Bergström G, Evans RG. Effects of renal medullary infusion of a vasopressin V₁ agonist on renal antihypertensive mechanisms in rabbits. Am J Physiol. 1958;275:R76–R85.
3. Christy IJ, Woods RL, Anderson WP. Mediators of the hypotensive response to increased renal perfusion in rabbits. Hypertension. 1993;21:149–154.
4. Christy IJ, Woods RL, Coumaya CA, Denton KM, Anderson WP. Evidence for a renomedullary vasodepressor system in rabbits and dogs. Hypertension. 1991;18:325–333.
5. Correia AG, Bergström G, Lawrence AJ, Evans RG. Renal medullary interstitial infusion of norepinephrine in anesthetized rabbit: methodological considerations. Am J Physiol. 1999;277:R112–R122.
6. Ludbrook J. Repeated measurements and multiple comparisons in cardiovascular research. Cardiovasc Res. 1994;28:303–311.
7. Hesse IFA, Johns EJ. The subtype of α-adrenoceptor involved in the neural control of renal tubular sodium reabsorption in the rabbit. J Physiol (Lond). 1984;352:527–538.
8. Gellai M. Modulation of vasopressin antidiuretic action by renal α₂-adrenoceptors. Am J Physiol. 1990;259:F1–F8.
9. Majid DSA, Godfrey M, Omoro SA. Pressure natriuresis and autoregulation of inner medullary blood flow in canine kidney. Hypertension. 1997;29:210–215.
10. Roman RJ, Cowley AW Jr. Characterization of a new model for the study of pressure-natriuresis in the rat. Am J Physiol. 1985;248:F190–F198.
11. Evans RG, Szenasi G, Anderson WP. Effects of N^G-nitro-L-arginine on pressure natriuresis in anaesthetized rabbits. Clin Exp Pharmacol Physiol. 1995;22:94–101.
12. Thomas CJ, Woods RL, Evans RG, Alcorn D, Christy IJ, Anderson WP. Evidence for a renomedullary vasodepressor hormone. Clin Exp Pharmacol Physiol. 1996;23:777–785.
13. Brooks B, Byers LW, Muirhead EE, Muirhead M, Pitcock JA, Maddipati KR, Maxey K. Purification of class 1 medullipins from the venous effluent of isolated normal kidneys perfused under high pressure with saline. Blood Press. 1994;3:407–417.
14. Rudenstam J, Bergström G, Göthberg G, Karlström G. Efferent renal nerve stimulation inhibits the antihypertensive function of the rat renal medulla when studied in a cross-circulation model. Acta Physiol Scand. 1995;155:183–191.
15. Bergström G, Rudenstam J, Creutz J, Göthberg G, Karlström G. Renal and haemodynamic effects of nitric oxide blockade in a Wistar assay rat during high pressure cross-circulation of an isolated denervated kidney. Acta Physiol Scand. 1995;154:241–252.
16. Thomas CJ, Anderson WP, Woods RL. Nitric oxide inhibition does not prevent the hypotensive response to increased renal perfusion in rabbits. Clin Exp Pharmacol Physiol. 1995;22:345–351.

RESPONSES OF REGIONAL KIDNEY PERFUSION TO VASOCONSTRICTORS IN ANAESTHETIZED RABBITS: DEPENDENCE ON AGENT AND RENAL ARTERY PRESSURE

Roger G Evans, Anabela G Correia, Simon R Weekes and Anna C Madden

Department of Physiology, Monash University, Clayton, Victoria, Australia

SUMMARY

1. We tested the effects of intravenous infusions of angiotensin II (AngII; 300 ng/kg per min) and the vasopressin V_1 receptor agonist [Phe^2,Ile^3,Orn^8]-vasopressin (30 ng/kg per min) on regional kidney perfusion in an extracorporeal circuit model in anaesthetized rabbits in which renal artery pressure (RAP) can be set independently of systemic mean arterial pressure. To test whether the level of RAP can influence the renal vascular response to [Phe^2,Ile^3,Orn^8]-vasopressin, we compared its effects when RAP was initially set at approximately 65 mmHg with those when RAP was set at approximately 130 mmHg.

2. When RAP was initially set at approximately 65 mmHg, a 20 min infusion of AngII increased RAP (13%) and reduced renal blood flow (RBF; 50%) and cortical perfusion (CBF; 43%). Medullary perfusion (MBF) transiently increased during the first 10 min of infusion, but was not significantly different from control levels during the final 5 min of infusion.

3. When RAP was initially set at approximately 65 mmHg, a 20 min infusion of [Phe^2,Ile^3,Orn^8]-vasopressin increased RAP (9%) and reduced RBF (21%); MBF was reduced by 57%, but CBF was reduced by only 15%. In contrast, when RAP was initially set at approximately 130 mmHg, infusion of [Phe^2,Ile^3,Orn^8]-vasopressin reduced RAP (7%) and increased RBF (13%). In these experiments, MBF was reduced by 38%, but CBF increased by 6%.

4. Our experiments show that AngII preferentially reduces CBF, while [Phe^2,Ile^3,Orn^8]-vasopressin preferentially reduces MBF. The renal vascular responses to [Phe^2,Ile^3,Orn^8]-vasopressin appear to be profoundly affected by the level of RAP, because increasing RAP from approximately 65 to approximately 130 mmHg transforms its cortical vasoconstrictor effect into cortical vasodilatation while leaving the response of the medullary microvasculature relatively unchanged. Whether renal vascular responses to other vasoactive agents (e.g. AngII) are similarly affected by the level of RAP remains to be determined.

Key words: angiotensin II, laser Doppler flowmetry, medullipin, [Phe^2,Ile^3,Orn^8]-vasopressin, renal blood flow, renal cortex, renal medulla.

INTRODUCTION

There is now considerable evidence that the renal vasculature does not respond homogeneously to vasoactive agents. For example, intravenous infusion of angiotensin (Ang)II in anaesthetized rats and rabbits reduces cortical perfusion (CBF), but either increases[1–3] or fails to reduce[4–6] perfusion of the medulla or papilla. In contrast, intravenous infusion of arginine vasopressin[7] or the V_1 receptor agonist [Phe^2,Ile^3,Orn^8]-vasopressin[2] can reduce medullary perfusion (MBF) without altering CBF. The heterogeneity of these renal vascular responses to vasoconstrictor agents may have important implications for the impact of circulating and locally acting hormones on the control of blood pressure, especially because the medullary microcirculation appears to have an important effect on the renal handling of salt and water.[8]

However, one interpretational problem with some of these experiments concerns the confounding influence of changes in systemic haemodynamics, particularly arterial pressure, that occur during intravenous infusion of vasoactive agents. To avoid this in the present study, we used an extracorporeal circuit model in anaesthetized rabbits that allows renal artery pressure (RAP) to be controlled independently of systemic arterial pressure.[9–13] This allowed us to examine the renal vascular effects of AngII and [Phe^2,Ile^3,Orn^8]-vasopressin in isolation from their effects on the extrarenal circulation. We also hypothesized that the level of RAP may affect the renal vascular response to vasoconstrictor agents, possibly by altering the intrarenal hormonal milieu. To test this hypothesis, we compared the renal haemodynamic effects of [Phe^2,Ile^3,Orn^8]-vasopressin when RAP was set at the lower end of the range found in normotensive conscious rabbits (approximately 65 mmHg)[14] with its effects when RAP was doubled (approximately 130 mmHg).

METHODS

Animals

Twenty New Zealand white rabbits (2.49–3.65 kg; mean 2.94 kg) of either sex were randomly assigned to one of four experimental groups. Rabbits were allowed food and water ad libitum until the experimental procedures began. At the conclusion of the experiment, rabbits were killed with an i.v. overdose of pentobarbitone sodium. Experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Department of Physiology/Central Animal Services Animal Ethics Committee. The experiments used an extracorporeal circuit by which RAP can be adjusted without directly altering systemic

Correspondence: Dr Roger G Evans, Department of Physiology, PO Box 13F, Monash University, Clayton, Victoria 3800, Australia. Email: roger.evans@med.monash.edu.au

vehicle with RAP initially set at approximately 65 mmHg; (ii) i.v. infusion of AngII with RAP initially set at approximately 65 mmHg; (iii) i.v. infusion of the vasopressin V_1 receptor agonist [Phe^2,Ile^3,Orn^8]-vasopressin with RAP initially set at approximately 65 mmHg; and (iv) i.v. infusion of [Phe^2,Ile^3,Orn^8]-vasopressin with RAP initially set at approximately 130 mmHg.
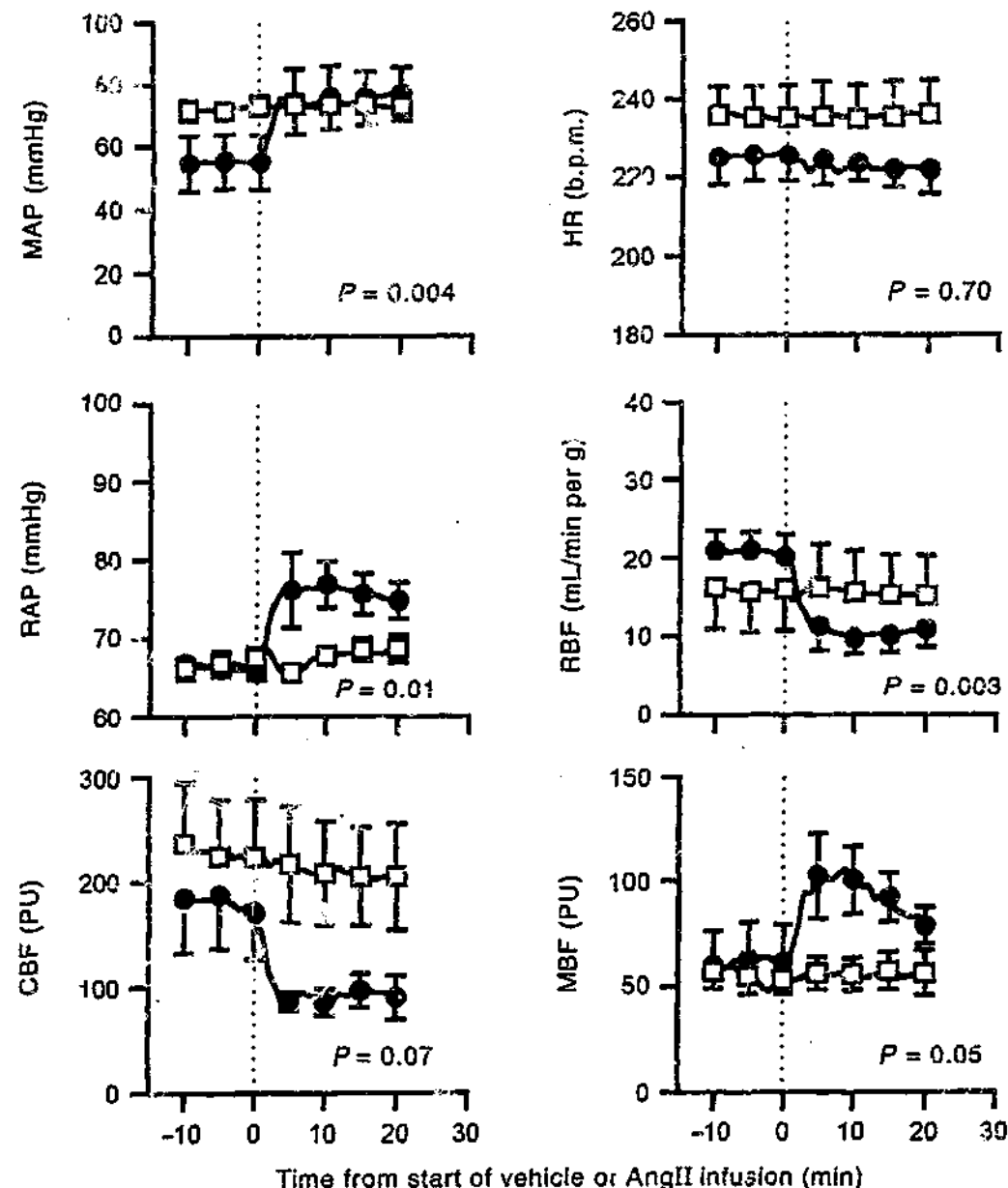
Fig. 1 Effects of i.v. infusion of vehicle (154 mmol/L NaCl at 50 μL/kg per min; □) or angiotensin II (AngII; 300 ng/kg per min; ●) on systemic and renal haemodynamic variables, with renal artery pressure initially set at approximately 65 mmHg. Lines show 1 min means. For clarity, symbols show every fifth mean ± SEM (n = 5). P values show the outcomes of the interaction terms from repeated-measures analysis of variance, testing whether the response to AngII differed from that to vehicle. MAP, mean arterial pressure; HR, heart rate; RAP, renal artery pressure; RBF, renal blood flow; CBF, cortical perfusion; MBF, medullary perfusion; PU, perfusion units.

Experimental preparations

These have been described in detail previously,[9-13] so will only be described briefly here. General anaesthesia was induced by i.v. administration of pentobarbitone sodium (90–150 mg plus 30–50 mg/h; Nembutal; Boehringer Ingelheim, Artarmon, NSW, Australia) and was immediately followed by endotracheal intubation and artificial respiration. During surgery, a balanced buffered salt solution (Hartmann's; Baxter Healthcare, Toongabbie, NSW, Australia) was infused intravenously at a rate of 0.18 mL/kg per min.

After removing the right kidney, the left kidney was denervated by stripping all visible nerves surrounding the renal artery and vein and it was then placed in a stable cup for the measurement of CBF and MBF by laser-Doppler flowmetry, as described previously.[13] The extracorporeal circuit[8-13] was then established to withdraw blood (at a constant rate of 90 mL/min) from the distal aorta by means of a roller pump and return it to the animal both through the renal artery and the vena cava. A Starling resistor incorporated into the venous limb allowed for graded reductions in the flow of blood through this limb and, so, increases in pressure and flow in the renal limb. The circuit was primed with 10% w/v dextran 40 in 154 mmol/L NaCl solution (Gentran 40; Baxter Healthcare) containing 50 IU/mL heparin (Monoparin; Fisons Pharmaceuticals, Sydney, NSW, Australia). The dead space of the circuit was 24 mL.

Immediately following establishment of the extracorporeal circuit, RAP was set and maintained at 60–70 mmHg and the infusion of Hartmann's solution (0.18 mL/kg per min) was replaced with 10% w/v polygelins (Haemoccel; Hoechst, Melbourne, Victoria, Australia) containing 200 IU/mL sodium heparin.

Recording of haemodynamic variables

Arterial pressure was measured by connecting the ear artery catheter to a pressure transducer (Cobe, Arvarda, CO, USA). Heart rate (HR) was measured by a tachometer activated by the pressure pulse. Renal artery pressure was measured in a side-arm catheter, 3 mm proximal to the tip of the cannula inserted into the renal artery, as described previously.[13] Blood flow through the renal limb was measured with an in-line ultrasonic flow probe (type 4N; Transonic Systems Inc., Ithaca, NY, USA) connected to a model T108 flowmeter (Transonic Systems Inc.) The laser-Doppler flow-probes were connected to a laser-Doppler flowmeter (DRT4; Moor Instruments Ltd, Millwey, Devon, England). These signals were amplified and recorded as described previously[13] to provide 60 s means of systemic arterial pressure (MAP), HR, RAP, renal blood flow (RBF), CBF, MBF and the concentration of moving red blood cells (CMBC) in the cortex and medulla. At the completion of each experiment, the left kidney was removed and desiccated so that its dry weight could be determined. Therefore, RBF values are expressed per g dry kidney weight.

Experimental protocols

At least 60 min after establishment of the extracorporeal circuit and once systemic and renal haemodynamic variables had stabilized, RAP was set to either approximately 65 mmHg (groups 1–3) or 130 mmHg (group 4). Following a further 30–60 min equilibration period and provided RBF had reached a relatively constant level, i.v. infusions of either saline vehicle (154 mmol/L NaCl at 50 μL/kg per min), AngII (group 2; 300 ng/kg per min; Auspep, Parkville, Victoria, Australia) or [Phe²,Ile³,Orn⁸]-vasopressin (groups 3 and 4; 30 ng/kg per min; Peninsula Laboratories, Belmont CA, USA) commenced and were maintained for a further 20 min.

Statistical analysis

We used the interaction term (time*treatment) from repeated-measures analysis of variance[19] to test whether the response to AngII differed to that of its vehicle (Fig. 1) and whether the response to [Phe²,Ile³,Orn⁸]-vasopressin was dependent on the level of RAP (Fig. 2). To protect against the increased risk of a false-positive inference, P values were conservatively adjusted using the Greenhouse–Geisser correction.[15] Paired t-tests were used to determine whether haemodynamic variables differed from their baseline level during the final 5 min of each 20 min infusion.

RESULTS

Vehicle

Levels of all variables remained relatively stable during infusion of the saline vehicle (Fig. 1; Table 1).

Angiotensin II

Intravenous infusion of AngII was accompanied by changes in systemic and renal haemodynamic variables, which, with the exception of MBF, reached equilibrium by the final 5 min of the infusion (Fig. 1). During the final 5 min period, MAP and RAP were increased 43±7 and 13±2%, respectively, compared with the 10 min control period, while RBF and CBF were reduced by 50±6 and 43±12%, respectively (Table 1). In contrast, the response of MBF was more complex and variable. During the first 10 min of infusion, MBF was increased in all five rabbits, averaging 82±33% more than during the control period. In the final 10 min of infusion, MBF returned to control levels in two of five rabbits, but remained above control levels in the other three rabbits. The CMBC was not significantly affected in either the cortex or medulla, averaging 43±31 and 12±7% greater, respectively, during the final 5 min of infusion compared with control levels.

[Phe²,Ile³,Orn⁸]-Vasopressin

When RAP was initially set at approximately 65 mmHg, i.v. infusion of [Phe²,Ile³,Orn⁸]-vasopressin was accompanied by increased MAP (by 30±5% of control levels du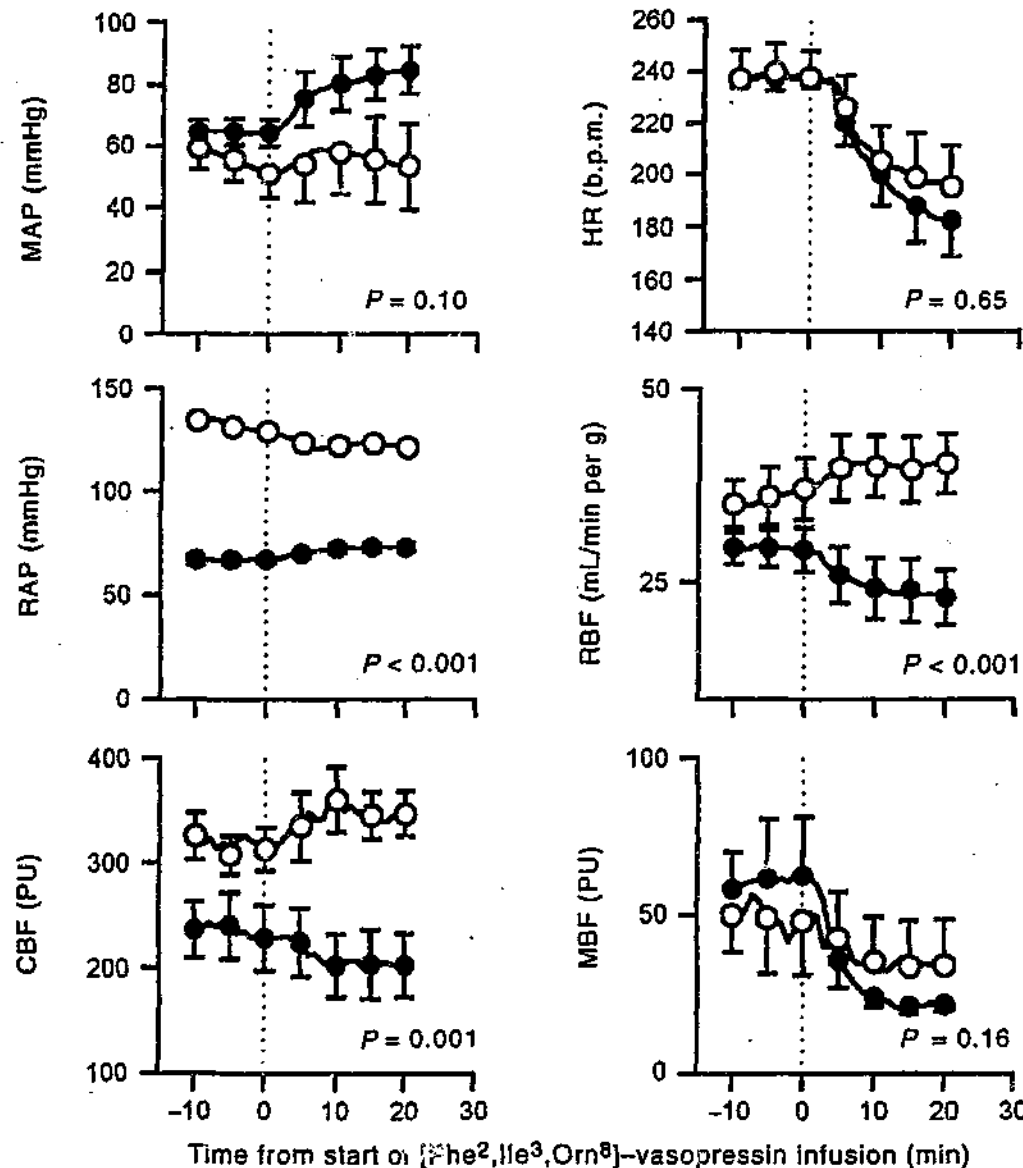ring the final 5 min of infusion) and RAP (9±2%; Fig. 2; Table 1). Renal blood flow was reduced by 21±7%. The reduction in MBF (57±10%) was considerably greater than that of CBF (15±4%). The CMBC was not significantly affected in either the cortex or medulla, averaging 2±1 and 12±8% less, respectively, during the final 5 min of infusion compared with control levels.

When RAP was initially set at approximately 130 mmHg, i.v. infusion of [Phe²,Ile³,Orn⁸]-vasopressin did not significantly affect MAP, but RAP was reduced by 7±1%. Both RBF and CBF were increased (by 13±2 and 6±2%, respectively), but MBF was reduced (38±11%). The CMBC was not significantly affected in either the cortex or medulla, averaging 3±2 and 0±5% less, respectively, during the final 5 min of infusion compared with control levels.

Fig. 2 Effects of i.v. infusion of [Phe²,Ile³,Orn⁸]-vasopressin (30 ng/kg per min) on systemic and renal haemodynamic variables, with renal artery pressure initially set at approximately 65 mmHg (●) or approximately 130 mmHg (○). Lines show 1 min means. For clarity, symbols show every fifth mean±SEM (n = 5). P values show the outcomes of the interaction terms from repeated-measures analysis of variance, testing whether the response to [Phe²,Ile³,Orn⁸]-vasopressin was dependent on the level of renal artery pressure (RAP). MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow;

Time from start of [Phe²,Ile³,Orn⁸]-vasopressin infusion (min)

Table 1 Effects of i.v. infusion of vehicle, angiotensin II and [Phe²,Ile³,Orn⁸]-vasopressin on systemic and renal haemodynamic variables

Agent	Resting RAP (mmHg)	MAP (% change)	HR (% change)	RAP (% change)	RBF (% change)	CBF (% change)	MBF (% change)
Saline vehicle (50 μL/kg per min)	65	1±5	0±2	2±4	−5±3	−9±4	−1±11
AngII (300 ng/kg per min)	65	43±7[†]	−1±2	13±2[†]	−50±6[†]	−43±12*	100±65
[Phe²,Ile³,Orn⁸]-vasopressin (30 ng/kg per min)	65	30±5[†]	−22±5[†]	9±2*	−21±7*	−15±4*	−57±10[†]
[Phe²,Ile³,Orn⁸]-vasopressin (30 ng/kg per min)	130	−9±7[†]	−18±4*	−7±1[†]	13±2[†]	6±2*	−38±11*

Values are the mean±SEM of percentage changes during the period 15–20 min after commencing each infusion from the average level during the 10 min control period (n = 5). The level to which renal artery pressure was initially set is shown in the second column. *P<0.05, [†]P<0.01 for paired t-test comparing levels before and during (15–20 min) each infusion.

RAP, renal artery pressure; MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; CBF, cortical perfusion; MBF, medullary perfusion; PU, perfusion units; AngII, angiotensin II.

DISCUSSION

The present findings with regard to the effects of i.v. infusion of AngII and [Phe³,Ile²,Orn⁸]-vasopressin in the extracorporeal circuit model were similar to those we have observed previously in anaesthetized and conscious intact rabbits.[2,16] Thus, with RAP set initially at approximately 65 mmHg, which is similar to resting MAP in conscious[14] and pentobarbitone-anaesthetized[2] rabbits, AngII (300 ng/kg per min) reduced CBF, but not MBF. Indeed, MBF transiently increased in all five rabbits studied, although the magnitude of this response was highly variable. In contrast, under the same conditions, the vasopressin V₁ receptor agonist [Phe³,Ile²,Orn⁸]-vasopressin (30 ng/kg per min) reduced MBF approximately four-fold more than it did CBF. Because none of these treatments significantly affected CMBC in either the cortex or medulla, we can be confident that the changes in CBF and MBF observed resulted predominantly from changes in red blood cell velocity (rather than concentration), secondary to alterations in vascular tone.

In our previous study in intact anaesthetized rabbits,[2] i.v. infusions of these doses of AngII and [Phe³,Ile³,Orn⁸]-vasopressin were accompanied by increases in MAP of approximately 84 and 14%, respectively. Therefore, it remained possible that the differences we observed between the effects of these agents on regional kidney perfusion resulted secondarily from their different effects on systemic haemodynamics. For example, it could be hypothesized that MBF did not fall during infusion of AngII because of the accompanying large increase in MAP. This hypothesis is supported by experimental results suggesting that the medullary microcirculation has only limited autoregulatory capacity compared with the cortex.[8] However, the present observations now permit us to exclude this possibility, because our experimental model allowed us to test the renal haemodynamic effects of these agents in isolation from their systemic haemodynamic effects. Thus, although both agents produced considerable increases in MAP, we observed similar modest increases (by approximately 10% of resting) in RAP with both. Therefore, these observations provide strong evidence that the heterogeneous effects of these agents on CBF and MBF in anaesthetized rabbits arise from differences in their direct actions within the renal vasculature.

Our observations and conclusions are also consistent with those of previous studies in rats showing that AngII and arginine vasopressin can differentially affect CBF and MBF independently of effects on arterial pressure. For example, Nobes et al. found that AngII increased papillary perfusion in anaesthetized rats, even when renal perfusion pressure was maintained at a constant level by use of an aortic clamp.[1] Similarly, Franchini et al. found that infusion of arginine vasopressin in decerebrate rats, at a dose that increased the plasma arginine vasopressin concentration from 3 to 11 pg/mL, reduced MBF without affecting CBF or mean arterial pressure.[7,17] There is also evidence that these hormones differentially regulate CBF and MBF under physiological conditions. Thus, increased NaCl intake in rats reduces plasma levels of AngII and increases CBF but not MBF.[18] Moreover, the reduced CBF in response to increased salt intake can be abolished by simultaneous infusion of subpressor doses of AngII.[18] Similarly, 48 h water restriction in rats increased plasma levels of arginine vasopressin and reduced MBF but not CBF.[19] The effects of water restriction on MBF were attenuated by medullary interstitial infusion of a V₁ receptor antagonist.[19]

The precise mechanisms underlying the disparate effects of vasoconstrictor agents on regional kidney perfusion remain unknown. However, it is likely that they include not only differences in the vascular distribution of receptors for these agents, but also differences in their effects on secondary, locally acting (mainly endothelial) factors, such as nitric oxide (NO) and prostanoids.[3,5,6] For example, in anaesthetized rats, the increase in papillary perfusion that has been observed during infusion of AngII seems to require the presence of NO, because it is abolished by blockade of NO synthesis but restored by co-infusion of sodium nitroprusside.[3] There is also evidence that both prostanoids[6,20] and NO[21,22] act in vivo to blunt medullary vasoconstrictor responses to AngII. The involvement of these local counter-regulatory mechanisms in modulating responses of regional kidney perfusion to AngII may explain, at least in part, apparent discrepancies between observations made in vivo and those made under in vitro conditions. For example, AngII has been shown to constrict both juxtamedullary afferent and efferent arterioles[23] and outer medullary descending vasa recta[24] in vitro. It may be that the activity of counter-regulatory vasodilator mechanisms (e.g. NO, prostanoids) is blunted under in vitro conditions, exposing the underlying vasoconstrictor effects of AngII on vascular elements regulating MBF.

In the group of rabbits in which RAP was set to approximately 130 mmHg, RBF and CBF (but not MBF) was considerably greater than in the group in which RAP was set at approximately 65 mmHg. However, there was clear evidence of autoregulation of RBF, because resting renal vascular resistance was 63% greater at the higher level of RAP. Under these conditions, [Phe³,Ile³,Orn⁸]-vasopressin reduced MBF by a similar magnitude, as was seen at the lower level of RAP. However, rather than reducing RBF and CBF, these were increased. Indeed, RAP was reduced during this infusion, indicating a considerable reduction in global renal vascular resistance. In contrast, the fact that MBF was still reduced by [Phe³,Ile³,Orn⁸]-vasopressin in the face of reduced global renal vascular resistance suggests a differential vascular response to activation of V₁ receptors in vascular elements regulating MBF (e.g. vasoconstriction of afferent and efferent arterioles of juxtamedullary glomeruli and/or outer medullary descending vasa recta) compared with those vascular elements regulating perfusion in the outer and mid-cortex (e.g. vasodilatation in afferent and efferent arterioles of glomeruli whose efferent arterioles lead to cortical peritubular capillaries and/or vasodilatation in upstream vascular sites).

We can only speculate, at present, as to the mechanisms responsible for this apparent conversion of the renal vasoconstrictor effect of [Phe³,Ile³,Orn⁸]-vasopressin into vasodilatation (at least in vessels other than those controlling MBF), but it likely relates to the impact of RAP on the intrarenal hormonal milieu. For example, there is some evidence for the release of a putative renal medullary depressor hormone under these experimental conditions,[9,13,25] which may be responsible for the abolition of the systemic pressor effect of [Phe³,Ile³,Orn⁸]-vasopressin we observed. It may also be responsible for the renal vasodilatation we observed in the present study, either as a direct effect of the putative hormone per se or as an interactive effect with [Phe³,Ile³,Orn⁸]-vasopressin. We believe that the former possibility is unlikely, because we have previously observed that RBF and RAP remain relatively stable in this experimental model when RAP is set to this level.[10,13] Furthermore, the fall in RAP and increases in RBF and CBF during infusion of

[Phe³,Ile³,Orn⁸]-vasopressin occurred over a similar time-course as the fall in HR and over a similar time-course to the systemic and renal haemodynamic effects of this agent observed when RAP was initially set to approximately 65 mmHg.

Increased release of numerous locally acting hormones, including NO, prostanoids and endothelins,[4,5,27] is also believed to occur when RAP is increased. The possibility that these contribute to the modulation of the renal vascular effects of [Phe³,Ile³,Orn⁸]-vasopressin by the level of RAP merits further investigation. There is certainly good evidence to implicate arginine vasopressin-induced renal vasodilatation,[28] although previous studies have suggested that this is predominantly mediated by activation of V₂ receptors.[29] In contrast, the results of the present study suggest that V₁ receptors can also mediate renal vasodilatation, at least under some experimental conditions. This hypothesis should be tested in future experiments using antagonists selective for V₁ and V₂ receptors.

In conclusion, the results of the present study show that at levels of RAP at the lower end of the range normally observed in intact conscious rabbits, the vasoconstrictor agents AngII and [Phe³,Ile³,Orn⁸]-vasopressin have disparate effects on CBF and MBF. We also found that increasing RAP can profoundly influence the nature of renal vascular responses to infused vasoactive agents, at least in the case of [Phe³,Ile³,Orn⁸]-vasopressin. This latter observation highlights the potential complex interactions between circulating and locally acting vasoactive hormones and prevailing haemodynamic factors in the regulation of intrarenal blood flow in vivo.

ACKNOWLEDGEMENTS

REFERENCES

1. Nobes MS, Harris PJ, Yamada H, Mendelsohn FAO. Effects of angiotensin on renal cortical and papillary blood flows measured by laser-Doppler flowmetry. Am. J. Physiol. 1991; 261: F998–1006.
2. Evans RG, Bergström G, Lawrence AJ. Effects of the vasopressin V₁ agonist [Phe³,Ile³,Orn⁸]vasopressin on regional kidney perfusion and renal excretory function in anesthetized rabbits. J. Cardiovasc. Pharmacol. 1998; 32: 571–81.
3. Walker LL, Rajaratne AAJ, Blair-West JR, Harris PJ. The effects of angiotensin II on blood perfusion in the rat renal papilla. J. Physiol. 1999; 519: 273–8.
4. Huang C, Davis G, Johns EJ. A study of the actions of angiotensin II on perfusion through the cortex and papilla of the rat kidney. Exp. Physiol. 1991; 76: 787–98.
5. Parekh N, Dobrowolski L, Zou A-P, Steinhausen M. Nitric oxide modulates angiotensin II- and norepinephrine-dependent vasoconstriction in rat kidney. Am. J. Physiol. 1996; 270: R630–5.
6. Parekh N, Zou A-P. Role of prostaglandins in renal medullary circulation: Response to different vasoconstrictors. Am. J. Physiol. 1996; 271: F653–8.
7. Franchini KG, Cowley Jr AW. Sensitivity of the renal medullary circulation to plasma vasopressin. Am. J. Physiol. 1996; 271: R647–53.
8. Cowley Jr AW. Role of the renal medulla in volume and arterial pressure regulation. Am. J. Physiol. 1997; 273: R1–15.
9. Christy U, Woods RL, Courneya CA, Denton KM, Anderson WP. Evidence for a renomedullary vasodepressor system in rabbits and dogs. Hypertension 1991; 18: 325–33.
10. Bergström G, Evans RG. Effects of renal medullary infusion of a vasopressin V₁ agonist on renal antihypertensive mechanisms in rabbits. Am. J. Physiol. 1998; 275: R76–85.
11. Evans RG, Szenasi G, Anderson WP. Effects of Nᴳ-nitro-L-arginine on pressure natriuresis in anaesthetized rabbits. Clin. Exp. Pharmacol. Physiol. 1995; 22: 94–101.
12. Evans RG, Day KH, Roman RJ, Hopp KH, Anderson WP. Effects of intrarenal infusion of 17-octadecynoic acid on renal antihypertensive mechanisms in anesthetized rabbits. Am. J. Hypertens. 1998; 11: 803–12.
13. Correia AG, Madden AC, Bergström G, Evans RG. Effects of renal medullary and intravenous norepinephrine on renal antihypertensive function. Hypertension 2000; 35: 965–70.
14. Evans RG, Rankin AJ, Anderson WP. Interactions of blockade of nitric oxide synthase and angiotensin converting enzyme on renal function in conscious rabbits. J. Cardiovasc. Pharmacol. 1994; 24: 542–51.
15. Ludbrook J. Repeated measurements and multiple comparisons in cardiovascular research. Cardiovasc. Res. 1994; 28: 303–11.
16. Evans RG, Madden AC, Denton KM. Diversity of responses of renal cortical and medullary blood flow to vasoconstrictors in conscious rabbits. Acta Physiol. Scand. 2000; 169: 297–308.
17. Franchini KG, Mattson DL, Cowley Jr AW. Vasopressin modulation of medullary blood flow and pressure-natriuresis-diuresis in the decerebrated rat. Am. J. Physiol. 1997; 272: R1472–9.
18. Gross V, Kurth TM, Skelton MM, Mattson DL, Cowley Jr AW. Effects of daily sodium intake and ANG II on cortical and medullary renal blood flow in conscious rats. Am. J. Physiol. 1998; 274: R1317–23.
19. Franchini KG, Cowley Jr AW. Renal cortical and medullary blood flow responses during water restriction: Role of vasopressin. Am. J. Physiol. 1996; 270: R1257–64.
20. Steinhausen M, Ballantyne D, Fretschner M, Hoffend J, Parekh N. Different responses of cortical and juxtamedullary arterioles to norepinephrine and angiotensin II. Kidney Int. 1990; 38 (Suppl. 30): S55–9.
21. Zou A-P, Wu F, Cowley Jr AW. Protective effect of angiotensin II-induced increase in nitric oxide in the renal medullary circulation. Hypertension 1997; 31: 271–6.
22. Szentiványi Jr M, Maeda CY, Cowley Jr AW. Local renal medullary L-NAME infusion enhances the effect of long-term angiotensin II treatment. Hypertension 1999; 33: 440–5.
23. Carmines PK, Morrison TK, Navar LG. Angiotensin II effects on microvascular diameters of in vitro blood-perfused juxtamedullary nephrons. Am. J. Physiol. 1986; 251: F610–18.
24. Pallone TL. Vasoconstriction of outer medullary vasa recta by angiotensin II is modulated by prostaglandin E₂. Am. J. Physiol. 1994; 266: F850–7.
25. Thomas CJ, Woods RL, Evans RG, Alcorn D, Christy U, Anderson WP. Evidence for a renomedullary vasodepressor hormone. Clin. Exp. Pharmacol. Physiol. 1996; 23: 777–85.
26. Fenoy FJ, Ferrer P, Carbonell L, Garcia-Salom M. Role of nitric oxide on papillary blood flow and pressure natriuresis. Hypertension 1995; 25: 408–14.
27. Braun C, Lang C, Hocher B, Gretz N, van der Woude FJ, Rohmeiss P. Influence of the renal endothelin system on the autoregulation of renal blood flow in spontaneously hypertensive rats. Kidney Blood Pressure Res. 1997; 20: 6–10.
28. Rudichenko VM, Beierwaltes WH. Arginine vasopressin-induced renal vasodilation mediated by nitric oxide. J. Vasc. Res. 1995; 32: 100–5.
29. Naitoh M, Suzuki H, Murakami M et al. Arginine vasopressin produces renal vasodilatation via V₂-receptors in conscious dogs. Am. J. Physiol. 1993; 265: R934–42.

Effects of activation of vasopressin-V1-receptors on regional kidney blood flow and glomerular arteriole diameters

Anabela G. Correia, Kate M. Denton and Roger G. Evans

Objectives We tested whether vasoconstriction of juxtamedullary glomerular arterioles contributes to vasopressin V1-receptor-mediated reductions in medullary perfusion (MBF).

Design and methods The left kidney of pentobarbitone anaesthetized rabbits was denervated, a perivascular flow probe placed around the renal artery and laser-Doppler flow probes positioned in the inner medulla and on the cortical surface. Rabbits then received a 30 min intravenous infusion of [Phe², Ile³, Orn⁸]vasopressin (V1-AG; 30 ng/kg per min; n = 7) or its vehicle (n = 7). Kidneys were perfusion fixed at the final recorded mean arterial pressure (MAP) and filled with methacrylate casting material. Diameters of afferent and efferent arterioles were determined by scanning electron microscopy.

Results V1-AG increased MAP (19 ± 3%) and reduced MBF (30 ± 8%) but not cortical perfusion or total renal blood flow. Vehicle-treatment did not significantly affect these variables. After vehicle- and V1-AG-treatment, juxtamedullary afferent arteriole luminal diameter averaged 15.35 ± 1.31 and 15.88 ± 1.88 μm, respectively (P = 0.92), while juxtamedullary efferent arteriole luminal

diameter averaged 17.75 ± 1.86 and 18.36 ± 2.24 μm, respectively (P = 0.93).

Conclusions V1-AG reduced MBF but did not significantly affect juxtamedullary arteriolar diameter. Our results therefore do not support a role for juxtamedullary arterioles in producing V1-receptor-mediated reductions in MBF, suggesting that downstream vascular elements (e.g. outer medullary descending vasa recta) might be involved. *J Hypertens* 19:649–657 © 2001 Lippincott Williams & Wilkins.

Correspondence and requests for reprints to Dr Roger Evans, Department of Physiology, PO Box 13F, Monash University, Victoria, 3800, Australia.
Tel: +61 3 9905 1466; fax: +61 3 9905 2566;
e-mail: roger.evans@med.monash.edu.au

Introduction

Although only approximately 10% of total renal blood flow enters the renal medulla, there is now good evidence that the medullary microcirculation plays a key role in long-term blood pressure regulation, chiefly through its influence on renal salt and water handling [1]. Therefore, in order to understand the mechanisms underlying the long-term regulation of blood pressure, we require a more complete understanding of the factors regulating medullary blood perfusion (MBF).

In both conscious and anaesthetized rats and rabbits, intravenous infusion of low doses of arginine vasopressin [2,3] or the selective V1-receptor agonist [Phe², Ile³, Orn⁸]vasopressin (V1-AG) [4,5] can reduce MBF without affecting cortical perfusion (CBF) or total renal blood flow (RBF). There is also good evidence that arginine vasopressin plays an important role in the physiological regulation of MBF [6]. The precise mechanisms mediating the selective effect of arginine

vasopressin on MBF remain unknown, but they could theoretically involve vasoconstriction of afferent and/or efferent arterioles of juxtamedullary glomeruli (the source of MBF), or downstream vascular elements (vasa recta) [7]. On the other hand, we can exclude vasoconstriction at vascular sites upstream from the afferent arteriole, which would be expected to also reduce CBF and RBF. We can probably also exclude a role for proposed contractile elements in inner medullary descending vasa recta [8], since Zimmerhackl *et al.*, using video-microscopy, were unable to detect arginine vasopressin-induced changes in the diameters of these vessels, even though erythrocyte velocity within them was significantly reduced [2].

In support of the hypothesis that V1-receptor mediated decreases in MBF are mediated by vasoconstriction of juxtamedullary arterioles and/or outer medullary descending vasa recta, V1-receptor mRNA has been located in these vascular elements [9]. There is also good

evidence from in-vitro studies that arginine vasopressin can constrict juxtamedullary arterioles [10–12] and outer medullary descending vasa recta [13]. On the other hand, in the study by Harrison-Bernard and Carmines [10], using the blood-perfused juxtamedullary nephron preparation, arginine vasopressin at physiological concentrations (> 10⁻¹² mmol/l) was found to constrict afferent arterioles of juxtamedullary glomeruli but not outer medullary descending vasa recta. Furthermore, in one study demonstrating vasoconstriction of outer medullary descending vasa recta in response to arginine vasopressin *in vitro*, the concentrations required (approximately 10⁻¹⁰ mmol/l) were approximately one order of magnitude greater than those encountered in plasma under physiological conditions [13]. Thus, on balance, this evidence supports a role for vasoconstriction of juxtamedullary glomerular arterioles in mediating reduced MBF in response to activation of V1-receptors. However, as yet, there is no evidence from in-vivo studies to confirm or reject this hypothesis.

Therefore, in the current study, we directly tested this hypothesis in anaesthetized rabbits by examining the effects of an intravenous infusion of V1-AG on RBF, CBF and MBF, and employing the technique of vascular casting to measure the luminal dimensions of afferent and efferent arterioles in the outer-, mid-, and juxtamedullary-cortical regions [14,15].

Methods

Animals

Fourteen, New-Zealand White, male rabbits were used (body weight 2.18–2.62 kg; mean 2.41 ± 0.03 kg). Prior to experimentation, all rabbits were meal fed and allowed water *ad libitum*. At the conclusion of the experiment, they were killed with an intravenous overdose of pentobarbitone sodium (300 mg). All experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Department of Physiology/Central Animal Services Animal Ethics Committee.

Surgical preparation

Catheters were placed in both central ear arteries and marginal ear veins under local anaesthesia (0.5% Lignocaine; Astra Pharmaceuticals, North Ryde, NSW, Australia). Rabbits were anaesthetized with pentobarbitone sodium (90–150 mg i.v. Nembutal; Boehringer Ingelheim, Artarmon, NSW, Australia) and this was immediately followed by endotracheal intubation and artificial ventilation (Model 55-3438 Ventilator, Harvard Instruments; MA, USA). Anaesthesia was maintained during surgery and throughout the experiment by intravenous pentobarbitone infusion (30–50 mg/h). Plasma volume was maintained throughout the experiment by

an intravenous infusion of Hartmann's solution (compound sodium lactate; Baxter Healthcare Pty Ltd, Toongabbie, NSW, Australia; 0.18 ml/kg per min). Surgery was performed on a heated table, and oesophageal temperature was maintained at 36–38 °C throughout the experiment using a servo-controlled infrared lamp (Digi-Sense temperature Controller; Cole Palmer Instrument Company, Chicago, Illinois, USA).

Surgery

A left flank incision was made and the left kidney, aorta, vena cava, and ureters were exposed. The left and right ureters were cannulated with silastic tubing (outer diameter 0.94 mm, inner diameter 0.51 mm; Dow Corning, Midland, Michigan, USA). The left kidney was denervated and placed in a stable cup for the positioning of laser-Doppler flow probes. For measurement of MBF, a 26-gauge needle type probe (DP4 s, Moor Instruments Ltd, Millwey, UK) was advanced 10 mm below the mid-region of the lateral surface of the kidney, using a micro-manipulator (Narashige, Tokyo, Japan). A laser Doppler flow probe (Standard plastic straight probe, DP2b; Moor Instruments Ltd) was placed on the dorsal surface of the kidney, for measurement of CBF. A transit-time ultrasound flow probe was placed around the renal artery for measurement of RBF (type 2SB, Transonic Systems Inc., Ithaca, New York, USA). A bolus of heparin sodium (5000 IU i.v. Fisons, Thornleigh, NSW, Australia) was administered, and an infusion of 30 IU/min continued for the remainder of the experiment. A large-bore cannula (outer diameter 3 mm, inner diameter 2 mm) was placed in the aorta distal to the renal arteries, which was later connected to the perfusion apparatus. The abdominal aorta was also isolated above the renal arteries, so that it could to be occluded during the fixation process.

Upon completion of the surgery, bolus doses of [³H]-inulin (4 μCi; NEN Research Products, Sydney, Australia) and [¹⁴C]-paraminohippuric acid (PAH; 1 μCi; NEN Research Products) were administered intravenously, and the maintenance infusion of Hartmann's solution (0.18 ml/kg per min) was replaced with a solution containing 300 nCi/ml [³H]-inulin and 83 nCi/ml [¹⁴C]-PAH, in four parts Hartmann's and one part 10% v/v polygeline (Haemaccel, Hoechst, Melbourne, Australia).

Experimental protocol

Following a 60 min equilibration period, and a 30 min control period, rabbits received a 30 min intravenous infusion of either V1-AG (Peninsula Laboratories Inc., Belmont, California, USA; 30 ng/kg per min; n = 7), or its vehicle (50 μl/kg per min 154 mmol/l NaCl; n = 7). Urine produced by the left and right kidneys was collected during the final 20 min of both the control

and infusion periods. Ear arterial (3 ml) blood samples were collected at the mid-point of each period for determination of plasma sodium concentration and haematocrit.

Perfusion fixation of both kidneys commenced immediately after completion of the second urine collection. One litre of 2.5% paraformaldehyde in 0.1 mmol/l phosphate buffer (pH 7.3–7.4) at room temperature was perfused retrogradely through the distal aorta at a pressure equivalent to mean arterial pressure (MAP) during the final 2 min of the V₁-AG or vehicle infusion for each rabbit. The upper aorta was clamped above the kidneys and the vena cava vented as soon as perfusion of the fixative commenced. Immediately following the fixation process, a mixture of methacrylate and accelerator (20 : 1) (Mercox CL-2B-5; SPI, West Chester, PA, USA) was perfused into the left and right kidneys at the same pressure as the fixative. Both kidneys were then clamped above the renal hilus, and the methacrylate resin was allowed to harden *in situ* for 30 min. The left kidneys were removed, weighed (13.37 ± 0.33 g) and stored in 2.5% paraformaldehyde for later processing.

Haemodynamic variables

Arterial pressure was measured throughout the experiment using a side-arm catheter, 3 mm proximal to the tip of the cannula inserted into the aorta. This was connected to a pressure transducer (Cobe, Arvada, CO, USA), and heart rate was measured by a tachometer (Model 173; Baker Medical Research Institute, Melbourne, Victoria, Australia), activated by the pressure pulse. Left RBF was measured by connecting the transit-time ultrasound flow probe to an ultrasonic volume flow meter (Model T108, Transonic Systems Inc.). The laser-Doppler flowprobes were connected to a laser-Doppler flowmeter (DRT4, Moor Instruments Ltd). The signals were amplified and recorded on a Neotrace pen recorder (Neomedix Systems, Sydney, Australia) and relayed to an IBM compatible computer equipped with an analog-to-digital converter that provided 20 s means of arterial pressure (mmHg), heart rate (beats/min), RBF (ml/min), CBF and MBF ((Perfusion Units, PU), equivalent to the instrument output in mV × 10).

Analysis of urine and blood samples

Haematocrit was measured by the capillary tube method, and the remaining blood was centrifuged at 4 °C for 10 min at 3000 r.p.m. Plasma and urine samples were aspirated and frozen for later analysis. [³H]-inulin clearance was used to estimate glomerular filtration rate (GFR) and [¹⁴C]-PAH clearance was corrected for haematocrit to provide effective renal blood flow (ERBF), as previously described [16]. Sodium and potassium concentrations were measured by atomic

absorption spectrophotometry (Avanta, GBC Scientific Equipment, Dandenong, Victoria, Australia).

Preparation of methacrylate casts for microscopy

Only the left kidneys were examined. Following removal from the rabbit, each kidney was stored individually in fixative to allow complete polymerization of the methacrylate (24–48 h). To eliminate the tissue from the cast, each kidney was sliced coronally (2–3 cm thick) and placed in potassium hydroxide (20% w/v KOH) for 1 week (fresh KOH every 24 h). The containers were incubated in a water bath at 55 °C. Once the tissue had been digested away, the casts were rinsed in distilled water and placed in 5% w/v sodium hipochlorite for 1 h. The clean vascular casts were then dried, mounted and gold-coated (SCD 005 Sputter Coater; Bal-Tec, Liechtenstein) before being examined in a scanning electron microscope at 10 Kv (Hitachi S-570, Hitachi City, Japan).

Luminal diameters of afferent and efferent arterioles of outer-, mid- and juxtamedullary-cortical glomeruli were measured from scanning electron microscope micrographs (final magnification × 660). Diameter measurements were made at 25 μm intervals along each vessel from its junction with the glomerulus to its first branching point. Six afferent and efferent vessels from each region of the cortex (outer, mid-cortical and juxtamedullary) were measured. These were selected and classified as previously described [14,15]. The vascular casts were coded and randomized before the micrographs were taken. Measurements were made using a digitizing tablet (Summagraphics; resolution 100 lines/mm, accuracy (0.25 mm, Calgraph, Fullerton CA, USA) and the MEASURE program (Capricorn Scientific Software, Victoria, Australia).

Vascular diameter and resistance

Mean values for the radius (*r*) and diameter of each vessel were derived from the measurements made along the vessel length. Relative resistances (*R*) were calculated according to Poiseuille's relationship ($R = 1/r^4$) and expressed per unit length [14,15].

Statistical analysis

All data are reported as the mean ± SEM. Hypotheses were tested using Student's paired and unpaired (as appropriate) *t*-tests. $P < 0.05$ was considered statistically significant.

Results

Baseline levels during the control period

Systemic and renal haemodynamic variables (Figs 1 and 2), and renal excretory variables (Fig. 2) during the control period were similar to those previously observed by us under similar experimental conditions [4,14–16].

Fig. 1
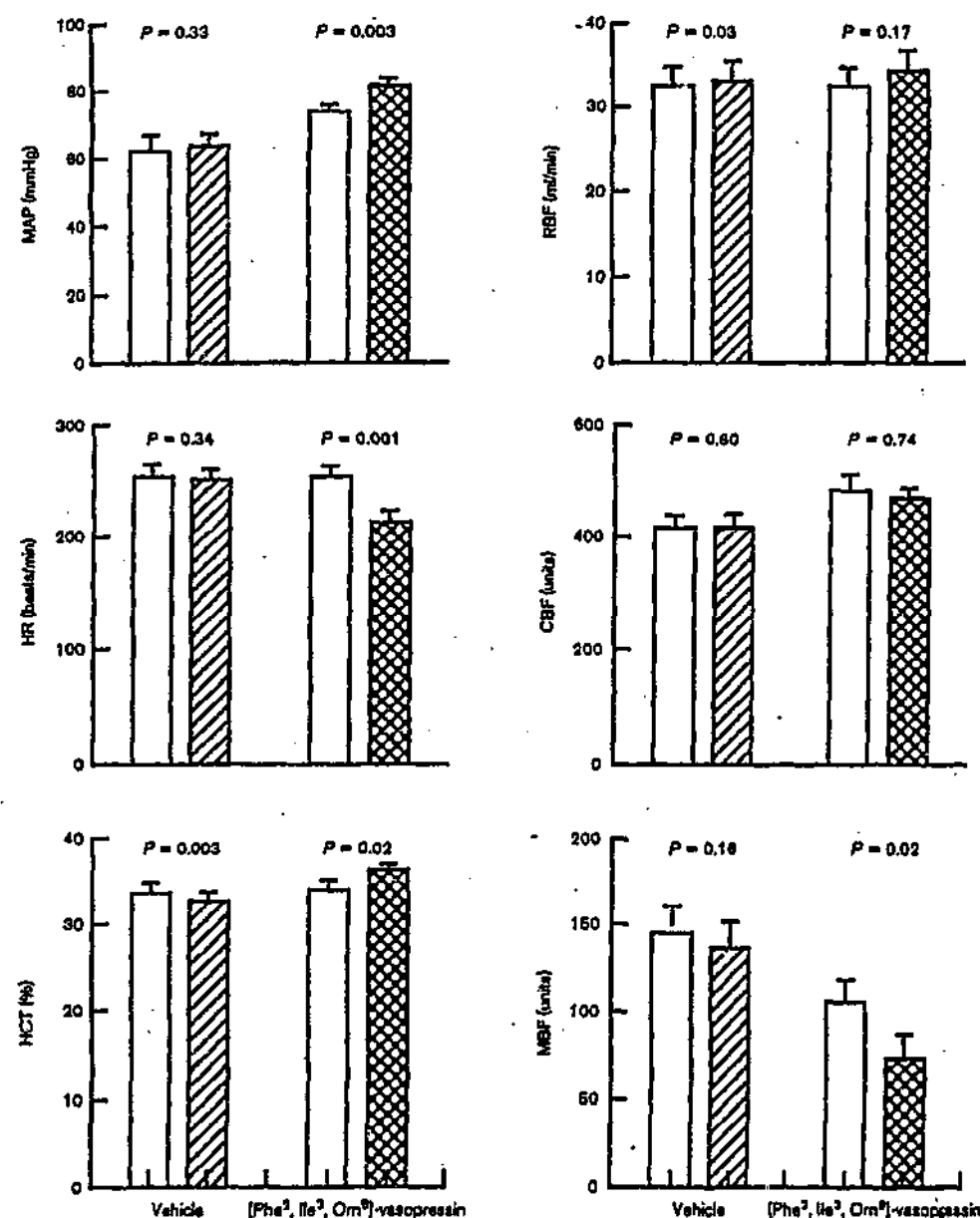
Effects of intravenous infusion of [Phe², Ile³, Om⁸]vasopressin (30 ng/kg per min) or its vehicle (154 mmol NaCl, 50 μl/kg per min) on systemic and renal haemodynamic variables. MAP, mean arterial pressure; HR, heart rate; HCT, haematocrit; RBF, renal blood flow; CBF, renal cortical perfusion; MBF, renal medullary perfusion. Columns and error bars represent the mean ± SEM of data (n = 7) during the final 20 min of the control period (open columns), and the final 20 min of the period of infusion of vehicle (hatched columns) or [Phe²,Ile³,Om⁸]vasopressin (cross-hatched columns). P-values represent outcomes of paired t-tests, testing whether variables changed significantly during infusions of vehicle or [Phe²,Ile³,Om⁸]vasopressin.

Fig. 2

Denervated (left) kidney | **Intact (right) kidney**
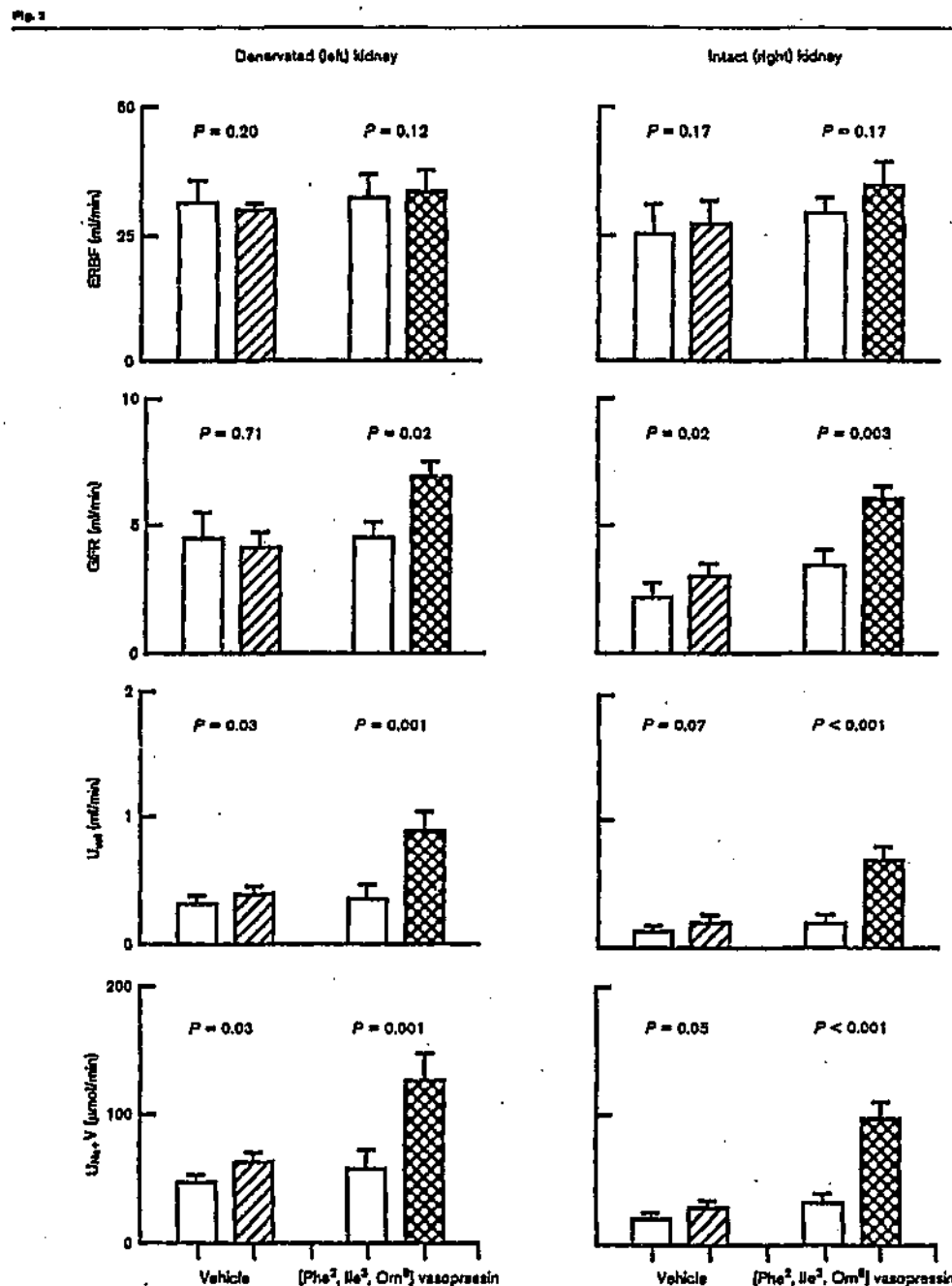
Effects of intravenous infusion of [Phe², Ile³, Orn⁸]vasopressin (30 ng/kg per min) or its vehicle (154 mmol/l NaCl, 50 µl/kg per min) on renal clearance variables in the denervated (left) kidney and the intact (right) kidney. ERBF, renal blood flow; GFR, glomerular filtration rate; U_Vol, urine flow; U_Na⁺V, urinary sodium excretion. Columns, error bars and P-values are as for Fig. 1.

There were no systematic differences in these variables between the two groups of rabbits ($P > 0.05$).

Haemodynamic and renal responses to V₁-AG and vehicle-treatment

Intravenous V₁-AG (30 ng/kg per min) increased MAP ($19 \pm 3\%$) and haematocrit ($7 \pm 2\%$), and reduced heart rate ($16 \pm 2\%$) and MBF ($30 \pm 8\%$), but RBF, CBF and renal vascular resistance (data not shown) did not significantly change (Fig. 1). Vehicle-treatment did not significantly affect any of the measured variables with the exception of haematocrit, which increased by $3 \pm 1\%$ (Fig. 1).

V₁-AG-treatment had no significant effect on ERBF in either kidney. In both the left (denervated) and right (intact) kidney, V₁-AG-treatment significantly increased GFR (58 ± 13 and $109 \pm 38\%$, respectively), urine flow (166 ± 32 and $355 \pm 78\%$, respectively) and sodium excretion (118 ± 21 and $290 \pm 82\%$, respectively). Following vehicle-treatment, there were small but statistically significant increases in GFR in the right kidney ($33 \pm 10\%$), urine flow in the left kidney ($32 \pm 14\%$), and sodium excretion in both the left ($36 \pm 15\%$) and right ($42 \pm 16\%$) kidneys, but no significant changes in ERBF (Fig. 2).

Vessel lumen diameters and calculated relative resistances

No evidence of focal constriction was observed along the length of the any of the arterioles and a mean diameter was calculated for each afferent and efferent arteriole in the different regions, as an arithmetic mean of each measurement (every 25 µm) along the length of the vessel from the glomerulus.

There were no significant differences in glomerular arteriole lumen dimensions between vehicle- and V₁-AG-treated rabbits (Fig. 3). In particular, juxtamedullary afferent and efferent arteriolar diameters were closely similar in the two groups of rabbits. However, there was a tendency for mid-cortical efferent arteriolar diameter to be less in V₁-AG-treated than in vehicle-treated rabbits ($P = 0.07$, Fig. 3). Calculated relative resistance per unit vessel length was closely similar in vehicle- and V₁-AG-treated rabbits for all arterioles (data not shown, $P \geqslant 0.40$), except for mid-cortical efferent arterioles in which a tendency for increased relative resistance was observed in the V₁-AG-treated rabbits ($P = 0.07$).

When averaged across both groups of rabbits, afferent arteriole lumen diameters in the outer, mid and juxtamedullary cortex were 15.06 ± 0.70, 13.87 ± 0.52, and 15.48 ± 1.20 µm, respectively. Compared with these afferent arterioles, the corresponding efferent arterioles had smaller diameters in the outer cortex ($12.37 \pm$

1.00 µm, $P = 0.02$) and mid-cortex (12.13 ± 0.61 µm, $P = 0.03$), but greater diameters in the juxtamedullary cortex (17.62 ± 1.48 µm, $P = 0.02$).

Discussion

Intravenous infusion of V₁-AG selectively reduces MBF compared with CBF and RBF in rabbits [4,5]. Our present aim was to determine whether the effect of V₁-AG on MBF is mediated by vasoconstriction of juxtamedullary glomerular arterioles. Intravenous V₁-AG reduced MBF by $30 \pm 8\%$, but we could not detect reductions in afferent or efferent juxtamedullary arteriolar diameter. These data are not consistent with a role of juxtamedullary arterioles in mediating the effects of V₁-AG on MBF. Since vasoconstriction of vascular elements upstream from glomerular arterioles (e.g. interlobular or arcuate arteries) should result in reductions in CBF (and RBF) as well as MBF, our data raise the possibility of a role for downstream vascular elements in mediating MBF responses to V₁-AG. The outer medullary portions of the descending vasa recta are the most likely candidates, since these are responsive to both arginine vasopressin and V₁-AG in vitro [13].

In the current study, our combination of in-vivo measurements of RBF, CBF and MBF, together with data from ex-vivo analysis of vascular casts from the same animals, provided a unique opportunity for direct correlation of the actions of V₁-AG on regional kidney blood flow and glomerular arteriole dimensions. The laser Doppler technique has previously been validated [17] and used extensively to demonstrate the diversity of responses of regional kidney blood flow to hormonal agents [1,3–6,16]. Although some artefact is associated with the vascular casting procedure, it has previously been extensively validated [14,15,18–20]. Importantly, our estimates of glomerular arteriole diameters are comparable to those previously reported in rabbits using several techniques, including vascular casting [14,15], stereology [21], and isolated arteriole preparations [22,23]. We also observed regional differences in glomerular arteriole diameters, which is consistent with previous studies in this [14,15,21] and other species [19,24]. Furthermore, previous studies using the vascular casting technique have demonstrated vasoconstriction in glomerular arterioles in response to angiotensin II [14,15], noradrenaline [18] and vasodilatation in response to atrial natriuretic peptide [18]. We can therefore be confident that it can be used to detect changes in renal arteriole dimensions.

Consistent with the lack of effect of V₁-AG on CBF or RBF, we did not detect differences between vehicle- and V₁-AG-treated rabbits in arteriolar diameters in superficial and mid-cortical regions. If the 30% reduction in MBF were entirely due to vasoconstriction in

Fig. 3
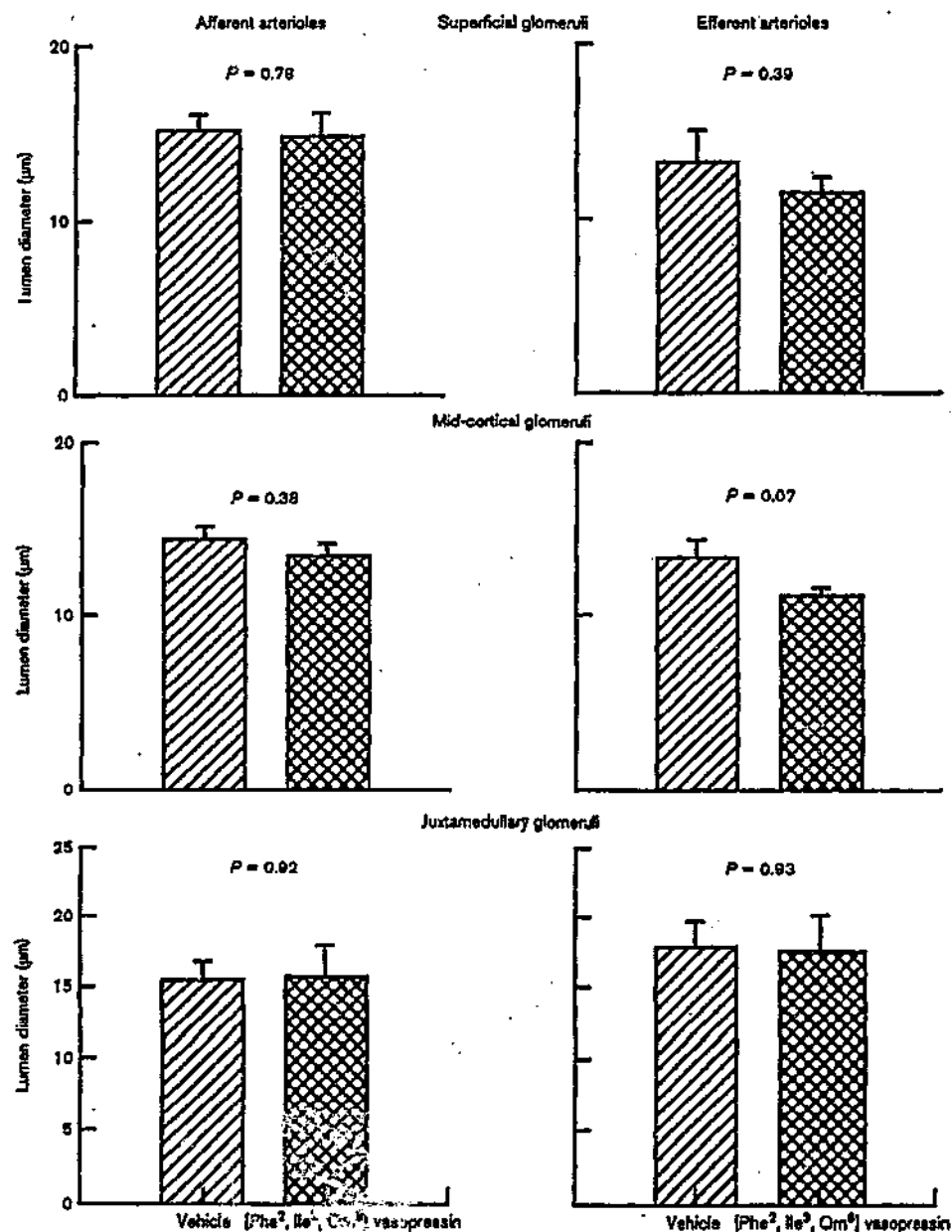
Diameters of vascular casts of glomerular arterioles in the superficial, mid- and juxtamedullary cortex, after intravenous infusion of [Phe^2,Ile^3,Orn^8]vasopressin (cross-hatched columns; 30 ng/kg per min) or its vehicle (hatched columns; 154 mmol/l NaCl, 50 μl/kg per min). Columns and error bars represent the between rabbit mean ± SEM ($n = 7$). P-values represent the outcomes of unpaired t-tests, testing whether diameters of vessels in [Phe^2,Ile^3,Orn^8]vasopressin-treated rabbits differed from the corresponding vessels in vehicle-treated rabbits.

juxtamedullary arterioles, we would predict (based on Poiseuille's relationship, where resistance is inversely proportional to vessel radius to the power of 4) juxtamedullary arteriole diameter to have been approximately 9% less in V$_1$-AG-treated compared to vehicle-treated rabbits. However, juxtamedullary arteriole diameters were indistinguishable in the two groups of rabbits and, if anything, were numerically (approximately 3%) greater in V$_1$-AG-treated than in vehicle-treated rabbits.

Our failure to detect V$_1$-AG-induced reductions in juxtamedullary glomerular arteriole diameter might reflect some inherent insensitivity of the casting technique. However, we believe this is unlikely, since we previously detected decreases of approximately 1 μm in outer cortical efferent arteriole diameter in rabbits receiving renal arterial infusions of angiotensin II (1 ng/kg per min), that cause a 35% decrease in RBF [15]. By comparison, in the current study, a 9% decrease in juxtamedullary arteriolar diameter (predicted from a 30% decrease in MBF) corresponds to reductions in arteriolar diameter of 1.4 μm (afferent) to 1.6 μm (efferent).

Our in-vivo data are therefore at odds with the results of in-vitro studies showing constriction of juxtamedullary afferent arterioles in response to physiological concentrations of arginine vasopressin [10]. Nevertheless, this is the first study we are aware of that has addressed this issue under in-vivo conditions, and it is likely that the responsiveness of renal vascular elements to activation of V$_1$-receptors is highly dependent upon the intrarenal hormonal milieu. Furthermore, although they appear less sensitive than afferent arterioles under in-vitro conditions [10], outer medullary descending vasa recta do respond to arginine vasopressin and V$_1$-AG [13]. Taken together, these data suggest a possible role for outer medullary descending vasa recta in mediating the selective effect of V$_1$-AG (and perhaps also that of arginine vasopressin) on MBF. This hypothesis remains to be tested directly.

The present study also allowed us to make a number of interesting observations about the systemic haemodynamic and renal effects of V$_1$-AG. Intravenous infusion of this agent increased MAP, GFR, urine flow and sodium excretion. The diuretic and natriuretic effects of V$_1$-AG have been described previously in both rats and rabbits, and appear to result chiefly from a direct tubular action of activation of V$_1$-receptors [4,25]. In the present study, we also found that V$_1$-AG increased GFR. This effect has not been previously described, but is consistent with the pressor effect of this agent, and the lack of evidence in our casting data for autoregulatory vasoconstriction in V$_1$-AG-treated rabbits. Indeed, the only vessels in which there was any

evidence of vasoconstriction were the efferent arterioles of mid-cortical glomeruli, in that the average diameters of these vessels tended to be approximately 11% less in V$_1$-AG-treated- than in vehicle-treated rabbits ($P = 0.07$). Our data are therefore consistent with the hypothesis that glomerular capillary pressure increases during intravenous infusion of doses of V$_1$-AG that increase MAP. This hypothesis merits direct testing with micropuncture.

In conclusion, the results of the present study are not consistent with an important role of juxtamedullary glomerular arterioles in mediating the selective effect of V$_1$-AG on MBF. A role for downstream vascular elements and, in particular, outer medullary descending vasa recta, is possible. However, since our present in-vivo data are at odds with the results of previous in-vitro studies, they require confirmation by future experiments.

Acknowledgements

References

1 Cowley AW Jr. Role of the renal medulla in volume and arterial pressure regulation. Am J Physiol 1997; 273:R1–R15.
2 Zimmerhackl B, Robertson CR, Jamison RL. Effect of arginine vasopressin on renal medullary blood flow: a videomicroscopic study in the rat. J Clin Invest 1985; 76:770–778.
3 Franchini KG, Mattson DL, Cowley AW Jr. Vasopressin modulation of medullary blood flow and pressure-natriuresis-diuresis in the decerebrated rat. Am J Physiol 1997; 272:R1472–R1479.
4 Evans RG, Bergström G, Lawrence AJ. Effects of the vasopressin V$_1$-agonist [PHE^2, ILE^3, ORN^8] vasopressin on regional kidney perfusion and renal excretory function in anesthetized rabbits. J Cardiovasc Pharmacol 1998; 32:571–581.
5 Evans RG, Madden AC, Denton KM. Diversity of responses of renal cortical and medullary blood flow to vasoconstrictors in conscious rabbits. Acta Physiol Scand 2000, 169: 297–308.
6 Franchini KG, Cowley AW Jr. Renal cortical and medullary blood flow responses during water restriction: role of vasopressin. Am J Physiol 1996; 270:R1257–R1264.
7 Pallone TL, Robertson CR, Jamison RL. Renal medullary microcirculation. Physiol Rev 1990; 70:885–920.
8 Park F, Mattson DL, Roberts LA, Cowley AW Jr. Evidence for the presence of smooth muscle α-actin within pericytes of the renal medulla. Am J Physiol 1997; 273:R1742–R1748.
9 Park F, Mattson DL, Skelton MM, Cowley AW J. Localization of the vasopressin V$_{1a}$ and V$_2$ receptors within the renal cortical and medullary circulation. Am J Physiol 1997; 273:R243–R251.
10 Harrison-Bernard LM, Carmines PK. Juxtamedullary microvascular responses to arginine vasopressin in rat kidney. Am J Physiol 1994; 267:F249–F256.
11 Tamaki T, Kiyomoto K, He H, Tomohiro A, Nishiyama A, Aki Y, et al. Vasodilation induced by vasopressin V2 receptor stimulation in afferent arterioles. Kidney Int 1996; 49:722–729.
12 Edwards RM, Trizna W. Renal microvascular effects of vasopressin and vasopressin antagonists. Am J Physiol 1989; 256:F274–F278.
13 Turner MR, Pallone TL. Vasopressin constricts outer medullary descending vasa recta isolated from rat kidneys. Am J Physiol 1997; 272:F147–F151.
14 Denton KM, Fennessy PA, Alcorn D, Anderson WP. Morphometric analysis

of the actions of angiotensin II on renal arterioles and glomeruli. *Am J Physiol* 1992; 262:F387–F372.

15 Denton KM, Anderson WP, Sinnish R. Effects of angiotensin II on regional afferent and efferent arteriole dimensions and the glomerular pole. *Am J Physiol* 2000; 279: R629–R638.

16 Correia AG, Bergström G, Lawrence AJ, Evans RG. Renal medullary interstitial infusion of norepinephrine in anaesthetized rabbits: methodological considerations. *Am J Physiol* 1999; 277:R112–R122.

17 Hansell P. Evaluation of methods for estimating renal medullary blood flow. *Renal Physiol Biochem* 1992; 15:217–230.

18 Kimura K, Hirata Y, Nanba S, Tojo A, Matsuoka H, Sugimoto T. Effects of atrial natriuretic peptide on renal arterioles: morphometric analysis using microvascular casts. *Am J Physiol* 1990; 259:F936–F944.

19 Gattone VH, Evan AP, Mong SA, Connors BA, Aronoff GR, Luft FC. The morphology of the renal microvasculature in glycerol- and gentamicin-induced acute renal failure. *J Lab Clin Med* 1983; 101:183–194.

20 Gattone VH, Evan AP. Quantitative renal vascular casting in nephrology research. *Scan Elec Microsc* 1986; 1: 253–262.

21 Kaissling B, Kriz W. Structural analysis of the rabbit kidney. *Adv Anat Embryol Cell Biol* 1979; 56:1–123.

22 Weihprecht H, Lorenz JN, Briggs J, Schnermann J. Vasoconstrictor effect of angiotensin and vasopressin in isolated rabbit afferent arterioles. *Am J Physiol* 1991; 261:P273–P282.

23 Ito S, Carretero OA. An in vitro approach to the study of macula densa-mediated glomerular hemodynamics. *Kidney Int* 1990; 38:1206–1210.

24 Dworkin LD, Brenner BM. The renal circulations. In: Brenner BM (editor): *The kidney*. Philadelphia: WB Saunders; 1996, pp. 247–263.

25 Leddatras G, Mattson DL, Skelton MM, Cowley AW Jr. In vivo diuretic actions of renal vasopressin V_1-receptor stimulation in rats. *Am J Physiol* 1995; 268:R796–R807.

*Dominance of pressure natriuresis in acute depressor responses to increased renal artery pressure in rabbits and rats

Anabela G. Correia, Göran Bergström*, Jing Jia*, Warwick P. Anderson and Roger G. Evans

Department of Physiology, Monash University, Victoria, Australia and *Department of Physiology, University of Göteborg, Göteborg, Sweden

Increasing renal artery pressure (RAP) activates pressure diuresis/natriuresis and inhibits renal renin release. There is also evidence that increasing RAP stimulates release of a putative depressor hormone from the renal medulla, although this hypothesis remains controversial. We examined the relative roles of these antihypertensive mechanisms in the acute depressor responses to increased RAP in anaesthetized rabbits and rats. In rabbits, an extracorporeal circuit was established which allows RAP to be set and controlled without direct effects on systemic haemodynamics. When RAP was maintained at ~65 mmHg, cardiac output (CO) and mean arterial pressure (MAP) did not change significantly. In contrast, when RAP was increased to ~160 mmHg, CO and MAP fell 20 ± 5 % and 36 ± 5 %, respectively, over 30 min. Urine flow also increased more than 28-fold when RAP was increased. When compound sodium lactate was infused intravenously at a rate equal to urine flow, neither CO nor MAP fell significantly in response to increased RAP. In 1 kidney–1 clip hypertensive rats, MAP fell by 54 ± 10 mmHg over a 2 h period after unclipping. In rats in which isotonic NaCl was administered intravenously at a rate equal to urine flow, MAP did not change significantly after unclipping (−14 ± 9 mmHg). Our results suggest that the depressor responses to increasing RAP in these experimental models are chiefly attributable to hypovolaemia secondary to pressure diuresis/natriuresis. These models therefore appear not to be bioassays for release of a putative renal medullary depressor hormone.

The kidneys play an important role in long-term blood pressure control. Thus, when renal artery pressure (RAP) is increased, renal renin release is reduced, so that the activity of the pro-hypertensive renin–angiotensin system is inhibited (Cowley, 1992). Also, urinary excretion rates of salt and water increase with elevated RAP (pressure diuresis/natriuresis), so reducing plasma volume which, if not compensated for, leads to a reduction in cardiac output (Cowley, 1992). Thirdly, evidence now exists to support the release of a putative depressor hormone from the renal medulla in response to increased RAP (Muirhead & Pitcock, 1985; Muirhead, 1990; Thomas et al. 1996; Bergström et al. 1998). Muirhead and colleagues provided the initial evidence supporting the existence of a renal medullary depressor hormone, which they dubbed medullipin. In a series of experiments spanning four decades, they showed depressor responses to transplants of medullary tissue (particularly renal medullary interstitial cells), and to administration of extracts of medullary tissue, or venous effluent of kidneys perfused at high pressure. They concluded that medullipin could be a neutral lipid hormone or pro-hormone housed in the medullary interstitial cells, and released in response to increased RAP (Muirhead & Pitcock, 1985; Muirhead, 1990). However, neither they nor others have definitively purified and chemically identified the active principle in these extracts (Brooks et al. 1994).

Experimental models have been employed to determine the physiological processes mediating release of this putative hormone, and its biological effects. The common feature of these models is that they allow RAP to be increased in vivo, and for the resulting effects on systemic haemodynamics to be observed. For example arterial pressure falls rapidly after removal of the renal artery clip in Goldblatt hypertension (Muirhead & Brooks, 1980). The depressor response to unclipping is blunted by chemical medullectomy (bromoethylamine (BEA)-pretreatment) (Bing et al. 1981), and by inhibition of intrarenal cytochrome P450-dependent arachidonate metabolism (Zou et al. 1995), consistent with the hypothesis that it is dependent, in part, on release of a lipid hormone from the renal medulla. Another approach is to establish extracorporeal circuits in anaesthetized animals, so that RAP can be set at levels greater than systemic arterial

pressure. In rats, this has been achieved by cross-circulating an isolated kidney from a 'donor' rat with blood from an anaesthetized 'recipient' rat (Karlström & Göthberg, 1987). In rabbits and dogs, autoperfused kidney preparations have been used (Christy et al. 1991, 1993; Thomas et al. 1994, 1995, 1996; Evans et al. 1998b; Correia et al. 2000). In these models, depressor responses to increased RAP can be blunted or abolished by chemical medullectomy (BEA) (Christy et al. 1991), and treatments that reduce renal medullary perfusion (Bergström et al. 1995; Bergström & Evans, 1998; Correia et al. 2000). Thus, the experiments performed by Muirhead's group (Muirhead & Pitcock, 1985; Muirhead, 1990; Brooks et al. 1994), combined with the more recent physiological experimentation cited above, have provided strong circumstantial evidence for the existence of this putative renal medullary depressor hormone.

On the other hand, there has been no definitive demonstration that the acute depressor responses to increased RAP in these models are independent of the associated pressure diuresis/natriuresis. To address this issue, in the present study we determined the relative contributions of changes in cardiac output and total peripheral resistance to the depressor response to increased RAP in anaesthetized rabbits. In some rabbits, we aimed to maintain cardiac output constant when RAP was increased, by infusion of compound sodium lactate at a rate equal to urine flow ('cardiac output clamp'). This allowed us to eliminate the systemic haemodynamic effects of pressure diuresis/natriuresis. In a further group of rabbits subjected to the 'cardiac output clamp', we also tested whether inhibition of the renin–angiotensin system contributes to the depressor response to increased RAP. In analogous experiments in 1 kidney–1 clip (1K1C) hypertensive rats, we tested the effects of intravenous isotonic sodium chloride, administered at a rate equal to urine flow, on haemodynamic responses to unclipping.

METHODS

Rabbit extracorporeal circuit studies

Animals. Twenty-four New-Zealand White, male rabbits were studied (2.10–2.92; mean 2.53 ± 0.03 kg). They were housed individually, in purpose built cages (500 cm high, 740 cm long and 680 cm wide) with two tiers for environmental enrichment. This housing allowed visual, but not physical, contact with rabbits in adjoining cages. The rabbits were meal fed (Evans et al. 2000) and allowed water ad libitum. The experiments were approved in advance by the Monash University Department of Physiology/Central Animal Services Animal Ethics Committee.

Preliminary surgery. Each rabbit underwent a preliminary operation for implantation of an ascending aortic flowprobe (6SB, Transonic Systems Inc., Ithaca, NY, USA) via a left thoracotomy (Shweta et al. 1999). The plug of the flowprobe was buried subcutaneously for later retrieval on the day of the acute experiment. A catheter (24 gauge, Optiva, Johnson &

Johnson Medical, Brussels, Belgium) was placed in a marginal ear vein under local analgesia (1 % lignocaine, Xylocaine, Astra Pharmaceuticals, North Ryde, NSW, Australia). Anaesthesia was then induced with intravenous propofol (10 mg kg⁻¹, Diprivan, ICI, Victoria, Australia) and after endotracheal intubation, maintained with inhaled halothane (1–4 % Fluothane, ICI). Depth of anaesthesia was monitored by testing corneal and toe-pinch reflexes. Prior to commencing the surgery itself, each rabbit was given an intramuscular injection of an antibiotic mixture containing 16 mg trimethoprim and 80 mg sulphadiazine (Tribrissen, Jurox, NSW, Australia), and a subcutaneous injection of the narcotic analgesic buprenorphine (60 μg, Temgesic, Reckitt and Coleman, NSW, Australia). Lignocaine (1 %, 2–4 ml) was instilled subcutaneously into the wound sites to enhance analgesia. Thirty millilitres of 154 mmol l⁻¹ NaCl was given by intravenous drip during the surgery, which took 30–50 min. At the completion of the surgery, animals were closely monitored for the next 3–5 h, while they recovered in a padded box with a heat pad. Thereafter, the rabbit's wellbeing was monitored daily by visual inspection and determination of food and water intake, until the day of the acute experiment (2–3 weeks after the preliminary surgery).

Procedures on the day of the acute experiment. These were carried out under local analgesia (1 % lignocaine). The plug of the flowprobe was retrieved from its subcutaneous position, and catheters were placed in both central ear arteries (22 gauge, Optiva) and marginal ear veins (24 gauge, Optiva). The ear artery catheters were used for measurement of systemic arterial pressure, and for collection of arterial blood samples. The ear vein catheters were used for intravenous infusions of anaesthetic and physiological solutions (see below). Following a 30 min period to allow full recovery from these preparative procedures, systemic arterial pressure, cardiac output and heart rate were monitored for 30 min in the conscious state.

All subsequent experimental procedures were carried out under pentobarbitone anaesthesia (90–150 mg for induction plus 30–50 mg h⁻¹ for maintenance, i.v.; Nembutal, Boehringer Ingelheim, Artarmon, NSW, Australia) and artificial ventilation as previously described (Bergström & Evans, 1998). The level of anaesthesia was monitored by corneal and toe pinch reflexes, and adjusted by altering the rate of infusion of pentobarbitone and, if necessary, administration of further bolus doses of 5–10 mg. Surgical procedures included implantation of a catheter via the jugular vein for measuring central venous pressure (Shweta et al. 1999), a right nephrectomy, cannulation of the left ureter for urine collection, and establishment of the extracorporeal circuit (Bergström & Evans, 1998). At the completion of the experiment, the rabbits were humanely killed with an intravenous overdose of pentobarbitone (300 mg).

Extracorporeal circuit. Blood was withdrawn from the aorta at a rate of 110 ml min⁻¹ by a roller pump and returned to the rabbit via two limbs; one to the renal artery and the other to the vena cava (Christy et al. 1991; Bergström & Evans, 1998). RAP was controlled by adjusting a Starling resistor incorporated into the vena caval limb, while total flow through the circuit remained constant. For example increasing the mechanical resistance in the vena caval limb using the Starling resistor diverts blood flow towards the renal limb, so increasing RAP. The circuit dead space (24 ml) was filled with 10 % w/v dextran 40 in 154 mmol l⁻¹ NaCl (Gentran 40, Baxter Healthcare,

Toonagabbie, NSW, Australia) containing 50 i.u. ml^{-1} heparin (Monoparin, Fisons Pharmaceuticals, Sydney, NSW, Australia). Thus, the establishment of the circuit resulted in some initial haemodilution, and consequently a relatively low haematocrit (see Results).

Once the extracorporeal circuit was established, RAP was set at −65 mmHg for a 60 min equilibration period. A bolus dose of [³H]-inulin (4 μCi) (NEN Research Products, Sydney, NSW, Australia) was administered in 1.0 ml of 154 mmol l^{-1} NaCl. An infusion of polygeline solution (Haemaccel, Hoechst, Melbourne, Victoria, Australia) containing 200 i.u. ml^{-1} sodium heparin and 0.3 μCi ml^{-1} [³H]-inulin then commenced (0.18 ml kg^{-1}min^{-1}), which continued for the duration of the experiment. Body temperature was maintained between 36 and 38 °C. Mean arterial pressure (MAP) and central venous pressure were measured by connecting an ear artery catheter and the jugular vein catheter, respectively, to pressure transducers (Cobe, Avarda, CO, USA). Heart rate (HR) was measured by a tachometer (Model 173, Baker Medical Research Institute, Melbourne, Victoria, Australia) activated by the arterial pressure trace. Cardiac output (CO) and renal blood flow (RBF) were measured by connecting the ascending aortic flow probe, and an in-line flow probe in the renal arm of the extracorporeal circuit (Type 4N, Transonic Systems Inc.), respectively, to a compatible flowmeter (Model T208, Transonic Systems Inc.). Analogue to digital conversion of these signals, as well as measurement of plasma renin activity, plasma and urinary concentrations of [³H]-inulin and sodium, and haematocrit, were made as previously described (Bergström & Evans, 1998). [³H]-inulin clearance was used to estimate glomerular filtration rate (GFR) (Bergström & Evans, 1998). At the completion of each experiment the left kidney was removed and desicated, and its dry weight determined. All values of RBF, GFR, urine flow (U_{vol}) and sodium excretion ($U_{\text{Na}}V$) are therefore expressed per gram of dry kidney weight (g, mean 1.65 ± 0.05 g).

Experimental protocol. MAP, HR and CO were measured in conscious rabbits for 30 min prior to induction of anaesthesia. Haemodynamic variables were also monitored during establishment of the extracorporeal circuit, to provide detailed information about the status of the circulation under these conditions relative to conditions in the normal circulation of conscious and anaesthetized rabbits. Following establishment of the extracorporeal circuit and a 60 min equilibration period, rabbits were randomly assigned to one of the four experimental groups (n = 6 for each group). RAP was first set to −65 mmHg for a 30 min control period in all groups. RAP was then either maintained at −65 mmHg (group 1) or set at −160 mmHg for 30 min (groups 2–4). This period was then followed by a 30 min recovery period (RAP −65 mmHg). In all rabbits, urine output was determined each minute during the 90 min of the experiment. The three groups in which RAP was increased to −160 mmHg received either no treatment (group 2), a 'cardiac output clamp', consisting of intravenous infusions of compound sodium lactate (Hartmann's solution; composition: Na^{+} 129 mM, K^{+} 5 mM, Ca^{2+} 2 mM, Cl^{-} 109 mM, lactate 29 mM) to exactly match urine output each minute during the period when RAP was increased (group 3), or the combination of a 'cardiac output clamp' with a 'renin–angiotensin system clamp' (group 4), consisting of enalaprilat (2.0 mg kg^{-1} plus 10 μg kg^{-1}min^{-1}) and an intravenous infusion of angiotensin II (40–50 ng kg^{-1}min^{-1}; Auspep, Parkville, Victoria, Australia)

titrated to restore MAP to its pre-enalaprilat level. The bolus dose of enalaprilat was administered intravenously after 30 min of stable baseline recordings following establishment of the extracorporeal circuit (that is, at the mid-point of the 60 min equilibration period), and the infusion of angiotensin II commenced 10 min later. Inhibition of angiotensin converting enzyme was confirmed by administration of bolus intravenous doses of angiotensin I (10 and 100 ng kg^{-1}; Auspep).

Unclipping of 1K1C hypertensive rats
Animals. Male Wistar rats (200–220 g) were purchased from the Möllegård Breeding Centre (Stensved, Denmark), and housed 2–4 per cage, in a room maintained between 23 and 25 °C with a 12 h light/dark cycle. Standard rat chow (R-34, Lactamin, Vadstena, Sweden) and water were provided ad libitum. The study was performed after prior approval from the Ethics Committee for Animal Experimentation at Göteborg University.

Surgical and experimental methodology. Under ketamine (58 mg kg^{-1} I.P.; Parke Davis, Warner Lambert Nordic AB, Solna, Sweden) and xylazine (7 mg kg^{-1} I.P., Bayer Sweden AB, Göteborg, Sweden) anaesthesia, a silver clip (inner diameter 0.2 mm, width 1.5 mm) was positioned around the left renal artery and the right kidney was removed (Bergström et al. 2001). Buprenorphine (0.03 mg kg^{-1}, Temgesic, Scherring-Plough AB, Stockholm, Sweden) was administered post-operatively for analgesia.

Four to six weeks later, the terminal acute experiment was performed under sodium thiobutabarbitone (120 mg kg^{-1}, I.P., Inactin, Research Biochemicals International, Natick, MA, USA) anaesthesia. Anaesthesia was monitored throughout the surgery and experiment by periodically testing corneal and toe-pinch reflexes, and supplemented if necessary by additional intravenous bolus doses (5–10 mg kg^{-1}) of thiobutabarbitone. The trachea was cannulated (PE-240), the tail artery was cannulated (PE 50) for measurement of MAP, the right jugular vein was cannulated (PE 50), with the tip of the cannula positioned near the right atrium, for measurement of CVP and infusion of bovine serum albumin (2 % w/v in 154 mmol l^{-1} NaCl, 4 ml h^{-1}) throughout the surgery and experiment, and the left ureter was cannulated (PE-10) for collection of urine. Heparinized 154 mmol l^{-1} NaCl (5 i.u. ml^{-1}, 1.2 ml h^{-1}) was infused via the tail artery catheter to maintain its patency. At the completion of the experiment, each rat was humanely killed with an intravenous overdose of thiobutabarbitone (50 mg).

Experimental protocol. Ninety minutes after completion of the surgery, the anaesthetized rats were randomized to three different experimental groups. In group 1, the renal artery clip was manipulated but not removed, while in groups 2 and 3 the renal artery clip was removed. Group 3 was given 154 mmol l^{-1} NaCl intravenously every 5 min, at a volume equal to urine flow over the preceding 5 min, across the 2 h experimental period following removal of the renal artery clip. Thus, these experimental groups were analogous to groups 1, 2 and 3 in the rabbit extracorporeal circuit experiment. Urinary sodium concentration was measured by flame photometry as previously described (Bergström et al. 2001), in pooled samples from the 30 min control period, and each of the two 60 min periods after unclipping or sham unclipping. Haematocrit was measured in 100 μl blood samples taken 30 min before the unclipping/sham unclipping procedure and at the completion

of the experiment. All values of U_{vol} and $U_{\text{Na}}V$ are expressed per gram of wet kidney weight (g, mean 1.57 ± 0.05 g).

Statistical analyses
Data collected during the preparative phase of the rabbit experiment were subjected to analysis of variance, partitioned to make specific comparisons between each state (conscious, anaesthetized and 'circuit established'), and between animals receiving the 'angiotensin II clamp' (group 4) and control animals (groups 1, 2 and 3). P values were conservatively adjusted using the Ryan–Holm–Šidák procedure to account for the fact that six comparisons were made within this analysis (Ludbrook, 1998).

In the rabbit experiment, we compared the levels of variables during the final 15 min of the period of increased RAP, with the final 15 min of the control period. In the rat experiment, we compared the levels of variables during the final 15 min of the experiment (105–120 min after unclipping) with those during the 30 min control period. Our specific hypotheses were that the changes in these variables between these two time periods would differ between the experimental groups. We therefore used unpaired t tests to specifically compare the changes in group 1 with group 2, group 2 with group 3, and (in the rabbit experiment) group 3 with group 4. P values were conservatively adjusted using the Ryan–Holm–Šidák (Ludbrook, 1998) procedure to account for the fact that multiple comparisons were made (three for the rabbit experiment and two for the rat experiment). P < 0.05 was considered to be statistically significant.

RESULTS

Baseline haemodynamic variables in rabbits
Levels of haemodynamic variables in conscious and anaesthetized states were similar to those we have observed previously (Evans & Bergström, 1998; Shweta et al. 1999). MAP was 23 ± 3 mmHg less, stroke volume (SV) was 0.19 ± 0.06 ml kg^{-1} less, CO was 27 ± 12 ml min^{-1}kg^{-1} less, and HR was 35 ± 11 beats min^{-1} greater in the anaesthetized compared with the conscious state. Once the extracorporeal circuit was established, MAP and CO returned to levels similar to the conscious state, although HR remained elevated (by 39 ± 7 beats min^{-1}) compared with the conscious state (Table 1). Resting levels of all haemodynamic variables, including RBF and renal vascular resistance (RVR), were closely similar in rabbits treated with enalaprilat/angiotensin II (group 4; 'angiotensin clamp') compared with those in rabbits not given this treatment (Table 1). The enalaprilat treatment completely abolished increases in MAP in response to 10 and 100 ng kg^{-1} angiotensin I, which averaged 3 ± 1 % and 8 ± 2 %, respectively, in rabbits from groups 1–3, and −7 ± 3 and 1 ± 3 %, respectively, in rabbits from group 4.
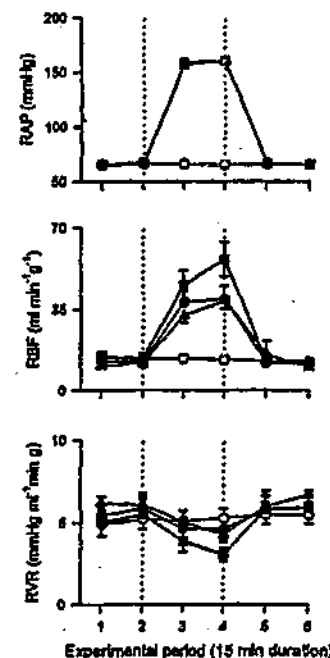

Figure 1. Renal haemodynamic responses to increased renal arterial pressure (RAP)

Symbols and error bars represent the mean ± S.E.M. of average levels during each of the six 15 min experimental periods (n = 6). In group 1 (O), RAP was maintained at −65 mmHg for the entire 90 min of the experiment. In groups 2–4, RAP was increased to −160 mmHg during periods 3 and 4 (30 min in total). Group 2 (●) received no further treatment, but groups 3 (■) and 4 (▲) received an intravenous infusion of compound sodium lactate equal to urine flow, during periods 3 and 4 ('cardiac output clamp'). In addition, group 4 had been pre-treated with the angiotensin converting enzyme inhibitor enalaprilat (2 mg kg^{-1} plus 10 μg kg^{-1} min^{-1} i.v.) and also received an intravenous infusion of angiotensin II (40–50 ng kg^{-1} min^{-1}) to restore mean arterial pressure and renal blood flow to baseline levels ('angiotensin clamp'; see Table 1). RBF, renal blood flow; RVR, renal vascular resistance. In this and subsequent figures, some symbols are obscured because the data points are coincident.

Responses to increased RAP in rabbits
Group 1. In these animals, in which RAP was maintained at −65 mmHg for the entire 90 min of the experiment, levels of renal haemodynamic (Fig. 1), renal excretory (Fig. 2) and systemic haemodynamic (Figs 3 and 4) variables, and levels of plasma renin activity (Fig. 5) remained relatively stable.

Group 2. When RAP was increased to −160 mmHg, RBF increased from 12 ± 2 to 44 ± 5 ml min^{-1} g^{-1} and RVR was reduced from 5.8 ± 0.7 to 3.8 ± 0.4 mmHg ml^{-1} min g (Fig. 1). At the same time, GFR increased from 1.5 ± 0.5 to 5.7 ± 1.0 ml min^{-1} g^{-1}, U_{vol} increased from 0.07 ± 0.04 to 1.88 ± 0.22 ml min^{-1} g^{-1}, $U_{\text{Na}}V$ increased from 9 ± 5 to 186 ± 14 μmol min^{-1} g^{-1}, and the fractional excretions of sodium and urine increased from 7 ± 1 to 41 ± 2 % and from 9 ± 1 to 45 ± 3 %, respectively (Fig. 2). Filtration fraction did not change significantly (Fig. 2).

Table 1. Resting haemodynamic variables according to state (conscious, anaesthetized or with the extracorporeal circuit established) and group

	MAP	HR	CO	CVP	SVR	SV	RAP	RBF	RVR
Control (n = 18, groups 1–3)									
Conscious	84 ± 2	224 ± 5	153 ± 7	—	0.55 ± 0.03	0.68 ± 0.03	—	—	—
Anaesthetized	62 ± 3***	256 ± 13*	132 ± 11	1.1 ± 1.1	0.55 ± 0.07	0.53 ± 0.06*	—	—	—
Circuit established	83 ± 3	260 ± 6*	143 ± 9	1.9 ± 0.7	0.61 ± 0.04	0.56 ± 0.04*	66 ± 0.4	14.2 ± 1.1	5.5 ± 0.4
'Renin-angiotensin system clamp' (n = 6)									
Conscious	90 ± 3	201 ± 6	152 ± 19	—	0.62 ± 0.06	0.77 ± 0.08	—	—	—
Anaesthetized	70 ± 5*	241 ± 9*	117 ± 6*	0.0 ± 1.1	0.61 ± 0.07	0.49 ± 0.01**	—	—	—
Circuit established	82 ± 3	253 ± 7*	157 ± 9	1.2 ± 0.7	0.53 ± 0.03	0.62 ± 0.05	66 ± 0.5	11.1 ± 0.7	6.1 ± 0.4
P_{stat}	< 0.001	< 0.001	0.006	0.22	0.99	< 0.001	—	—	—
P_{group}	0.54	0.60	0.36	0.11	0.93	0.32	0.83	0.15	0.21

Values are mean ± s.e.m. MAP, mean arterial pressure (mmHg); HR, heart rate (beats min⁻¹); CO, cardiac output (ml min⁻¹ kg⁻¹); CVP, central venous pressure (mmHg); SVR, systemic vascular resistance (mmHg ml⁻¹ min kg); SV, stroke volume (ml kg⁻¹); RAP, renal arterial pressure (mmHg); RBF, renal blood flow (ml min⁻¹ g⁻¹); RVR, renal vascular resistance (mmHg ml⁻¹ min g). P_{group} tests for differences between groups 1–3 compared with group 4 (d.f. 1,5–48). P_{stat} tests for heterogeneity according to state (d.f. 2, 45). Specific comparisons were made within each group by partitioning the analysis of variance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for difference from conscious state (d.f. 1,16–32). Because a total of six test statistics were derived for each variable (except for RAP, RVR and RBF), the Ryan-Holm–Šídák (step-down) procedure was applied to these P values to protect against the increased risk of type 1 error.

There was also a dramatic depressor response to increasing RAP. MAP fell from 83 ± 4 to 54 ± 5 mmHg, and systemic vascular resistance (SVR) decreased from 0.63 ± 0.04 to 0.52 ± 0.05 mmHg ml⁻¹ min g. CVP was also reduced from 2.4 ± 1.7 to −0.2 ± 1.3 mmHg (Fig. 3). CO fell from 132 ± 6 to 105 ± 4 ml min⁻¹ kg⁻¹ and SV fell from 0.49 ± 0.02 to 0.39 ± 0.01 ml kg⁻¹. Haematocrit increased by 1.5 ± 0.6 %. HR remained relatively constant across the 90 min experimental period (Fig. 4).
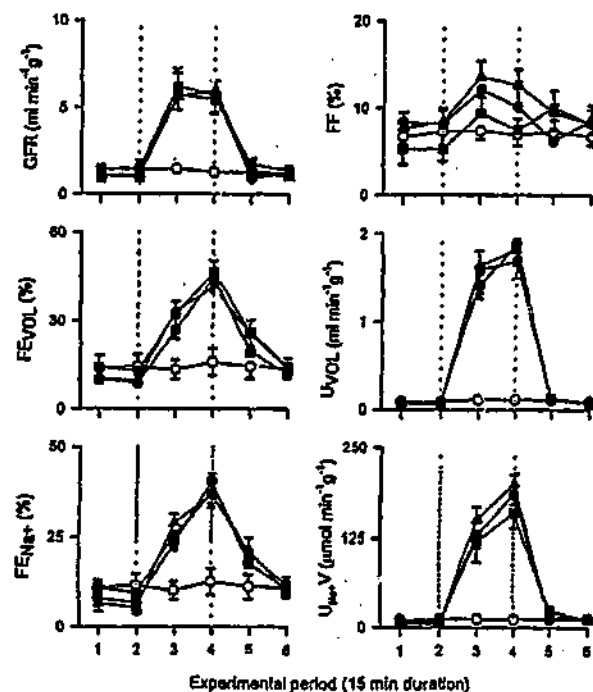
Figure 2. Renal excretory responses to increased renal artery pressure

Symbols, error bars and treatments are as for Fig. 1. GFR, glomerular filtration rate; FF, filtration fraction; FE_vol, fractional excretion of urine; U_vol, urine flow; FE_Na, fractional sodium excretion; U_Na V, sodium excretion.

Figure 3. Systemic haemodynamic responses to increased renal artery pressure

Symbols, error bars and treatments are as for Fig. 1. MAP, mean arterial pressure; SVR, systemic vascular resistance; CVP, central venous pressure. Percentage changes in MAP were calculated for the last 5 min of periods 2–6 relative to the last 5 min of the preceding period.

Group 3 ('cardiac output clamp'). When RAP was increased to ~160 mmHg and compound sodium lactate was infused at a rate equivalent to urine flow, RBF increased, and RVR fell, similarly to that seen in group 2 (P_{group} > 0.18; Fig. 1). Increases in GFR, U_{vol}, $U_{Na}V$, and the fractional excretions of sodium and urine were also similar to those observed in group 2 (P_{group} > 0.15; Fig. 2). In contrast to group 2, neither SV, CO, MAP, SVR nor haematocrit changed significantly, although CVP fell from 2.0 ± 1.2 to 1.3 ± 1.0 mmHg. Between-group comparisons indicated clear differences in responses to increasing RAP to ~160 mmHg in groups 2 and 3. Thus, the reductions in MAP (group 2, 30 ± 5 mmHg versus group 3, 7 ± 3 mmHg; P_{group} = 0.005), CO (group 2, 28 ± 7 ml kg⁻¹ min⁻¹ versus group 3, 3 ± 4 ml kg⁻¹ min⁻¹; P_{group} = 0.04) and CVP

(group 2, 2.6 ± 0.5 mmHg versus group 3, 0.7 ± 0.2 mmHg; P_{group} = 0.01) were less in rabbits receiving the compound sodium lactate infusion.

Group 4 ('cardiac output clamp' plus 'angiotensin clamp'). Responses in this group were indistinguishable from those in group 3 (Figs 1–5; P_{group} > 0.08).

Plasma renin activity in rabbits

Plasma renin activity did not change significantly over the course of the experiment in any of the four groups (Fig. 5).

Cumulative Na⁺ balance in rabbits

When RAP was maintained at ~65 mmHg (group 1), a slightly positive cumulative sodium balance (0.7 ± 0.3 mmol Na⁺) was observed during the period 30–60 min after commencing the experiment. Group 2, in which RAP was
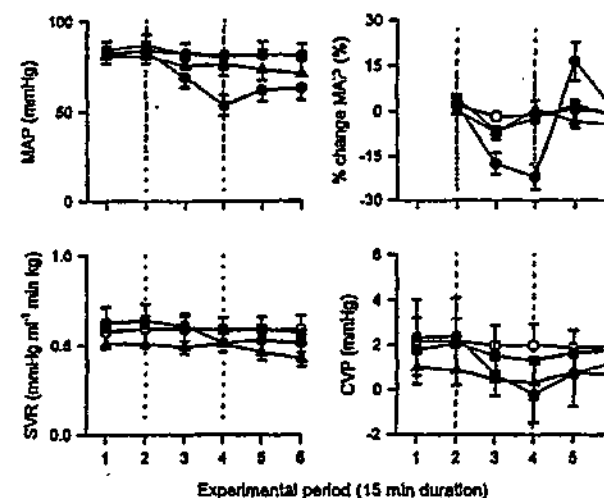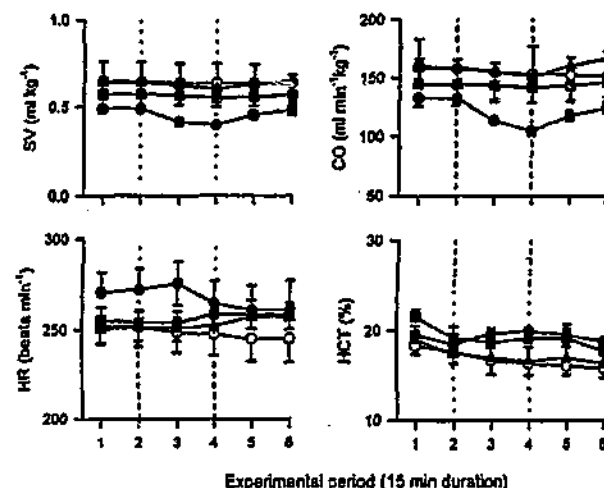

Figure 4. Systemic haemodynamic responses to increased renal artery pressure

Symbols, error bars and treatments are as for Fig. 1. SV, stroke volume; CO, cardiac output; HR, heart rate; HCT, haematocrit.

increased to ~160 mmHg during this period, developed a markedly negative sodium balance (−14.4 ± 2.2 mmol Na⁺, P_{group} = 0.004 compared with group 1). In group 3, in which RAP was set to ~160 mmHg during this period, and compound sodium lactate was infused at a rate equal to urine flow, cumulative sodium balance was not significantly different from that in group 1 (−3.7 ± 2.6 mmol Na⁺; P_{group} > 0.14). However, despite volume replacement and a stable MAP in group 4, a small but significant negative sodium balance developed (−6.7 ± 1.9 mmol Na⁺; P_{group} = 0.03 compared with group 1).

Responses to unclipping of 1K1C hypertensive rats
Compared to control levels, MAP had fallen by 54 ± 10 mmHg during the period 105–120 min after unclipping in group 2 rats. This was associated with increased U_{vol} (from 4 ± 2 to 156 ± 26 µl min⁻¹ g⁻¹) and $U_{Na}V$ (from 0.2 ± 0.1 to 17.1 ± 3.3 µmol min⁻¹ g⁻¹) during the first 60 min after unclipping. In the second hour after unclipping, U_{vol} (58 ± 11 µl min⁻¹ g⁻¹) and $U_{Na}V$ (7.6 ± 2.1 µmol min⁻¹ g⁻¹) reduced towards baseline levels. In contrast, U_{vol} and $U_{Na}V$ remained relatively stable in group 1 rats, in which the clip was manipulated but not removed, and MAP did not change (+3 ± 6 mmHg change). In group 3 rats, which received 154 mmol l⁻¹ NaCl intravenously after unclipping, at a rate exactly matched to U_{vol}, U_{vol} remained elevated for the 2 h of the study (249 ± 46 and 265 ± 57 µl min⁻¹ g⁻¹, respectively), as did $U_{Na}V$ (30.4 ± 5.9 and 32.8 ± 6.6 µmol min⁻¹ g⁻¹, respectively). MAP fell by 14 ± 9 mmHg in group 3, significantly less than that observed in group 2 (P_{group} = 0.049), and not significantly different from the response observed after sham unclipping in group 1 (P_{group} = 0.14). Neither HR nor CVP changed significantly in any of the

groups across the course of the experiment (Fig. 6). Haematocrit fell similarly in all groups, averaging 43 ± 1 % during the 30 min control period, and 37 ± 2 % at the completion of the experiment.

In group 1, cumulative sodium balance was slightly positive (+1.03 ± 0.20 mmol Na⁺). Group 2, in which the clip was removed from the renal artery, developed a negative sodium balance compared with group 1 (−0.76 ± 0.41 mmol Na⁺, P_{group} = 0.04). In group 3, in which the clip was removed and isotonic NaCl was infused at a rate equal to U_{vol}, cumulative sodium balance was positive and significantly greater than that in group 1 (+3.10 ± 0.31 mmol Na⁺, P_{group} = 0.006).

DISCUSSION

Our important new finding was that in two models that have been used for studying the putative antihypertensive hormonal function of the kidney, the acute depressor responses to increased RAP were abolished or greatly blunted when urinary fluid excretion was matched with intravenous infusions of isotonic salt solutions. We conclude that the depressor responses to increased RAP in these models can be chiefly accounted for by reduced CO, most probably secondary to increased salt and water excretion, rather than release of a putative renal medullary depressor hormone.

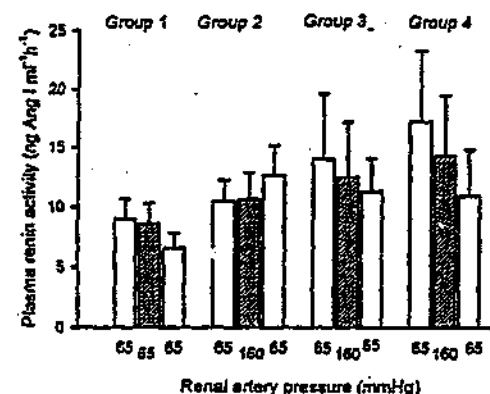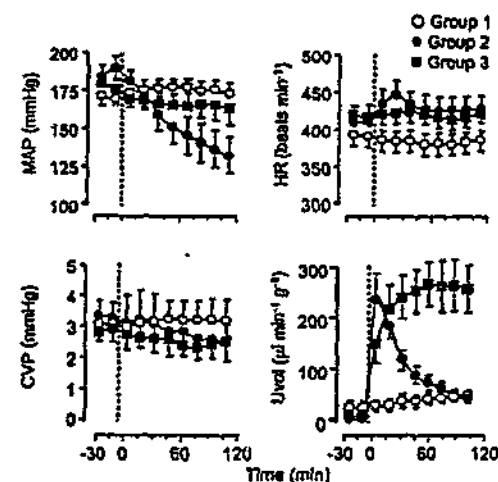Figure 5. Responses of plasma renin activity to increased renal artery pressure

Columns and error bars represent the mean ± S.E.M of plasma renin activity at the ends of periods 1, 3 (▨) and 5 (n = 6). Groups are as for Fig. 1. Ang I, angiotensin I.

Figure 6. Systemic haemodynamic responses and urine output after removing the clip from the renal artery (unclipping) of 1 kidney–1 clip hypertensive rats

Groups are: sham-unclipping (group 1; ○), unclipping (group 2; ●) and unclipping with isotonic saline administered at a rate equal to urine flow (group 3; ■). MAP, mean arterial pressure; HR, heart rate; CVP, central venous pressure; U_{vol}, urine flow (expressed per g of tissue wet weight).

Because our conclusions draw heavily on results obtained using the rabbit extracorporeal circuit model, our first aim was to assess the status of systemic haemodynamics under these experimental conditions. MAP and CO were substantially reduced and HR increased in anaesthetized rabbits, when compared to the conscious state. In contrast, CO and MAP in rabbits with an established extracorporeal circuit were closely similar to values observed in the conscious state, and remained stable when RAP was maintained at ~65 mmHg over the 90 min course of the experiment. Thus, our observations of the responses to increased RAP are unlikely to be confounded by the haemodynamic conditions of our experiment. Furthermore, the 'cardiac output clamp' (rabbits in groups 3 and 4) effectively maintained haematocrit, SV and CO in the face of increased U_{vol}, when RAP was increased to ~160 mmHg. The aim of the 'angiotensin clamp' (group 4) was to provide inhibition of endogenous angiotensin II generation, while avoiding the potentially confounding effects of hypotension and renal vasodilatation that normally attend inhibition of angiotensin converting enzyme. This aim was met, as evidenced by the abolition of responses to intravenous angiotensin I, while resting systemic and renal haemodynamics were similar to those in anaesthetized rabbits from the other three groups.

When RAP was increased from 65 to 160 mmHg in the extracorporeal circuit model, RBF and GFR increased, and RVR decreased. This apparent absence of autoregulatory behaviour probably reflects the relatively narrow range of autoregulation in this preparation, which extends only from about 80 to about 110 mmHg (G. A. Eppel & R. G. Evans, unpublished observations). The great merit of this preparation is that it allows RAP to be increased in vivo, to levels greater than systemic arterial pressure. In the present study, this allowed us to investigate the factors contributing to the acute depressor response to increased RAP.

When RAP was increased in anaesthetized rabbits that had not received the 'cardiac output clamp' (group 2), MAP fell. This depressor response was associated with increased U_{vol} and $U_{Na}V$, negative sodium balance, increased haematocrit, reduced CVP, SV and CO, and a small but significant reduction in SVR. Thus, it appears to result predominately from reduced CO mediated by pressure diuresis/natriuresis. This hypothesis was confirmed in rabbits treated with the 'cardiac output clamp' (group 3). In this group cumulative sodium balance was maintained, so that reductions in haematocrit, SV and CO were prevented, and no significant depressor response was observed. The response to increased RAP in rabbits from group 2 resembles that to haemorrhage in conscious rabbits after sino-aortic baroreceptor denervation, in which SVR falls as CO is reduced (Schadt & Ludbrook, 1991; Evans et al. 2001). In contrast, the usual response to haemorrhage

or acute central hypovolaemia in unanaesthetized rabbits (and indeed all mammals in which it has been studied), consists of two distinct phases. In the first (compensatory) phase MAP is maintained in the face of a falling CO, chiefly by reflex increases in sympathetic vasomotor drive and so SVR. This is followed by a decompensatory phase in which this reflex sympathetic activation fails, and SVR and MAP fall precipitously (Schadt & Ludbrook, 1991). However, some general anaesthetic agents blunt the compensatory phase, presumably by inhibiting baroreceptor-mediated increases in sympathetic vasomotor drive (Evans et al. 2001). Pentobarbitone anaesthesia also greatly blunts cardiovascular reflexes (Morita et al. 1987), so it is hardly surprising that SVR did not increase in response to increased RAP in rabbits from group 2. SVR actually fell in response to increased RAP in rabbits from group 2, which, like the reduction in SVR seen during haemorrhage in sino-aortic baroreceptor denervated rabbits, might be secondary to the depressor response itself, perhaps through local autoregulatory mechanisms (Schadt & Ludbrook, 1991). Consistent with this, the 'cardiac output clamp' also abolished the progressive reduction in SVR during increased RAP.

Our experiments using 1K1C hypertensive rats complement our rabbit experiments, in that they show that the depressor response to unclipping is associated with transient diuresis and natriuresis, and is greatly blunted when fluid and sodium depletion is prevented by administration of isotonic saline. However, in contrast to the rabbit experiments, our volume replacement regimen in rats resulted in a slightly positive cumulative sodium balance. This is unlikely to have confounded our observations, since administration of even large volumes of isotonic saline has little effect on MAP in normovolaemic, Inactin-anaesthetized, rats (Keeler & Wilson, 1989). Our observations therefore contrast with those of Neubig and Hoobler (1975), who found similar depressor responses to unclipping in 1K1C hypertensive rats, regardless of whether sodium balance was maintained by intravenous infusion of isotonic saline. However, they are consistent with studies showing that the normalization of arterial pressure after unclipping is delayed or blunted by a surgical uretero-caval anastamosis or saline loading (Liard & Peters, 1970; Muirhead & Brooks, 1980). All of these earlier studies were confounded by the fact that unclipping was performed under relatively long-acting anaesthesia (pentobarbitone or ether), from which the animals recovered during the experimental period. This was obviated in the present study by performing the entire study under tightly controlled and stable (Inactin) anaesthesia. We therefore conclude that the acute depressor response to increasing RAP by unclipping 1K1C hypertensive rats is most probably chiefly due to reduced extracellular fluid volume, resulting from pressure diuresis/natriuresis.

Our results also confirm and extend previous evidence indicating that the renin–angiotensin system plays little or no role in mediating the depressor response to increased RAP in the rabbit extracorporeal circuit model (Christy et al. 1993), since systemic haemodynamic responses to increased RAP in rabbits receiving both the 'cardiac output clamp' and the 'angiotensin clamp' were indistinguishable from those in the group receiving only the 'cardiac output clamp'. This hypothesis is further supported by our observation of unchanged plasma renin activity throughout the course of the experiment. Plasma renin activity does fall when RAP is increased in relatively long experimental protocols using this model (Bergström & Evans, 1998; Correia et al. 2000). However, the relatively long circulating half-life of renin probably prevented substantial changes in its circulating activity across the relatively short time-course of the present experiment.

Our present observations prompt reinterpretation of our previous studies employing the rabbit extracorporeal circuit model, because observations regarding the depressor response to increased RAP had previously been interpreted in the context of release of a putative renomedullary depressor hormone. For example the fact that the depressor response to increased RAP was abolished in chemically medullectomized (BEA-treated) rabbits (Christy et al. 1991), and in rabbits in which medullary blood flow was reduced by medullary interstitial infusion of noradrenaline (Correia et al. 2000), was taken as evidence supporting the notion that increased medullary blood flow mediates release of the putative renal medullary depressor hormone in response to increased RAP. However, these treatments also blunted the pressure diuresis/natriuresis response (Christy et al. 1991; Correia et al. 2000), which probably made important contributions to their effects on the systemic haemodynamic responses to increased RAP.

In contrast, our present results are difficult to reconcile with our previous observation that medullary interstitial infusion of the V_1-agonist [Phe^2,Ile^3,Orn^8]-vasopressin blunted the depressor response to increased RAP, since the pressure diuresis/natriuresis response during V_1-receptor stimulation was, if anything, slightly greater than that of control rabbits (Bergström & Evans, 1998; Evans et al. 1998a). Interpreted in the light of our present findings, this previous observation could possibly reflect an effect of V_1-receptor activation on the systemic haemodynamic response to hypovolaemia. This notion is consistent with the proposed roles of both central nervous system (Johnson et al. 1988) and peripheral (Schadt & Ludbrook, 1991) V_1-receptors in recovery from severe hypovolaemia. Furthermore, because significant systemic spill-over occurs when agents are infused into the renal medullary interstitum of rabbits (Evans et al. 1998a; Correia et al. 1999), [Phe^2,Ile^3,Orn^8]-vasopressin could have gained access to these sites when administered via this route.

In conclusion, the results of this study indicate that the depressor responses to increased RAP, in both the extracorporeal circuit model in rabbits and after unclipping in 1K1C hypertensive rats, is chiefly due to hypovolaemia secondary to pressure diuresis/natriuresis, and not to release of a putative renal medullary depressor hormone. This conclusion is based on our finding that the depressor responses to increasing RAP in these models are abolished or greatly blunted when urinary fluid losses are replaced by intravenous infusions of isotonic salt solutions. These models therefore are not suitable bioassays for the putative renal medullary depressor hormone, even though they provide a presumed stimulus for its release – increased RAP. This calls for reinterpretation of previous studies by ourselves (Christy et al. 1991, 1993; Bergström et al. 1995, 1998, 2001; Thomas et al. 1994, 1995, 1996; Bergström & Evans, 1998; Evans et al. 1998b; Correia et al. 2000) and others (Muirhead & Brooks, 1980; Bing et al. 1981; Muirhead & Pitcock, 1985; Karlström & Göthberg, 1987; Muirhead, 1990; Zou et al. 1995) using these models. Furthermore, although our findings do not provide direct evidence against the existence of a putative renal medullary depressor hormone, they do reinforce the dominance of pressure diuresis/natriuresis in mediating the acute antihypertensive function of the kidney.

REFERENCES

Bergström, G. & Evans, R. G. (1998). Effects of renal medullary infusion of a vasopressin V$_1$ agonist on renal antihypertensive mechanisms in rabbits. American Journal of Physiology 275, R76–85.

Bergström, G., Göthberg, G., Karlström, G. & Rudenstam, J. (1998). Renal medullary blood flow and renal medullary antihypertensive mechanisms. Clinical and Experimental Hypertension 20, 1–26.

Bergström, G., Nyström, H. C., Jia, J. & Evans, R. G. (2001). Effects of the ET$_A$/ET$_B$ antagonist, TAK-044, on blood pressure and renal excretory function after unclipping of conscious one-kidney-one-clip hypertensive rats. Journal of Hypertension 19, 659–665.

Bergström, G., Rudenstam, J., Creutz, J., Göthberg, G. & Karlström, G. (1995). Renal and haemodynamic effects of nitric oxide blockade in a Wistar assay rat during high pressure cross-circulation of an isolated denervated kidney. Acta Physiologica Scandinavica 154, 241–252.

Bing, R. F., Russell, G. I., Swales, J. D., Thurston, H. & Fletcher, A. (1981). Chemical renal medullectomy: effect upon reversal of two-kidney, one-clip hypertension in the rat. Clinical Science 61, 335S–338S.

Brooks, B., Byers, L. W., Muirhead, E. E., Muirhead, M., Pitcock, J. A., Maddipati, K. R. & Maxey, K. M. (1994). Purification of class I medullipins from the venous effluent of isolated normal kidneys perfused under high pressure with saline. Blood Pressure 3, 407–417.

Christy, L. J., Woods, R. L. & Anderson, W. P. (1993). Mediators of the hypotensive response to increased renal perfusion in rabbits. Hypertension 21, 149–154.

Christy, I. J., Woods, R. L., Courneya, C. A., Denton, K. M. & Anderson, W. P. (1991). Evidence for a renomedullary vasodepressor system in rabbits and dogs. Hypertension 18, 325–333.

Correia, A. G., Bergström, G., Lawrence, A. J. & Evans, R. G. (1999). Renal medullary interstitial infusion of norepinephrine in anesthetized rabbits: methodological considerations. American Journal of Physiology 277, R112–122.

Correia, A. G., Madden, A. C., Bergström, G. & Evans, R. G. (2000). Effects of renal medullary and intravenous norepinephrine on renal antihypertensive function. Hypertension 35, 965–970.

Cowley, A. W. Jr (1992). Long-term control of arterial blood pressure. Physiological Reviews 72, 231–300.

Evans, R. G., Bergström, G. & Lawrence, A. J. (1998a). Effects of the vasopressin V$_1$ agonist [PHE^1,ILE^2,ORN^8]vasopressin on regional kidney perfusion and renal excretory function in anesthetized rabbits. Journal of Cardiovascular Pharmacology 32, 571–581.

Evans, R. G., Day, K. H., Roman, R. J., Hopp, K. H. & Anderson, W. P. (1998b). Effects of intrarenal infusion of 17-octadecynoic acid on renal antihypertensive mechanisms in anesthetized rabbits. American Journal of Hypertension 11, 803–812.

Evans, R. G., Stevenson, K. M., Bergström, G., Denton, K. M., Madden, A. C., Gribben, R. L., Weekes, S. R. & Anderson, W. P. (2000). Sex differences in pressure diuresis/natriuresis in rabbits. Acta Physiologica Scandinavica 169, 309–316.

Evans, R. G., Ventura, S. V., Dampney, R. A. L. & Ludbrook, J. (2001). Neural mechanisms in the cardiovascular responses to acute central hypovolaemia. Clinical and Experimental Pharmacology and Physiology 28, 479–487.

Johnson, J. V., Bennett, G. W. & Hatton, R. (1988). Central and systemic effects of a vasopressin V$_1$ antagonist on MAP recovery after hemorrhage in rats. Journal of Cardiovascular Pharmacology 12, 405–412.

Karlström, G. & Göthberg, G. (1987). The humorally mediated antihypertensive system of the rat kidney: a physiological depressor mechanism? Journal of Hypertension 5 (suppl. 5), 591–594.

Keeler, R. & Wilson, N. (1989). Natriuretic response to hypervolemia is absent in rats with papillary necrosis. American Journal of Physiology 257, R422–426.

Liard, J.-F. & Peters, G. (1970). Mechanism of the fall in blood pressure after 'unclamping' in rats with Goldblatt-type hypertension. Experientia 26, 743–745.

Ludbrook, J. (1998). Multiple comparison procedures updated. Clinical and Experimental Pharmacology and Physiology 25, 1032–1037.

Morita, H., Nishida, Y., Uemura, N. & Hosomi, H. (1987). Effect of pentobarbital anesthesia on renal sympathetic nerve activity in the rabbit. Journal of the Autonomic Nervous System 20, 57–64.

Muirhead, E. E. (1990). Medullipin system of blood pressure control. News in Physiological Sciences 5, 241–244.

Muirhead, E. E. & Brooks, B. (1980). Reversal of one-kidney, one-clip hypertension by unclipping: the renal, sodium-volume relationship reexamined. Proceedings of the Society for Experimental Biology and Medicine 163, 540–546.

Muirhead, E. E. & Pitcock, J. A. (1985). The renal antihypertensive hormone. Journal of Hypertension 3, 1–8.

Neubig, R. R. & Hoobler, S. W. (1975). Reversal of chronic renal hypertension: role of salt and water excretion. Proceedings of the Society for Experimental Biology and Medicine 150, 254–256.

Schadt, J. C. & Ludbrook, J. (1991). Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. American Journal of Physiology 260, H305–318.

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 439, 150–157.

Thomas, C. J., Anderson, W. P. & Woods, R. L. (1995). Nitric oxide inhibition does not prevent the hypotensive response to increased renal perfusion in rabbits. Clinical and Experimental Pharmacology and Physiology 22, 345–351.

Thomas, C. J., Woods, R. L., Evans, R. G., Alcorn, D., Christy, I. J. & Anderson, W. P. (1996). Evidence for a renomedullary vasodepressor hormone. Clinical and Experimental Pharmacology and Physiology 23, 777–785.

Thomas, C. J., Woods, R. L., Gao, Y. & Anderson, W. P. (1994). Pressure range for release of renomedullary depressor substance in rabbits. Hypertension 23, 639–645.

Zou, A.-P., Muirhead, E. E., Cowley, A. W. Jr, Mattson, D. L., Falck, J. R., Jiang, J. & Roman, R. J. (1995). Role of changes in renal hemodynamics and P-450 metabolites of arachidonic acid in the reversal of one-kidney, one clip hypertension. Journal of Hypertension 13, 557–566.

Responses to examiners reports

I would like to thank both examiners for their thoughtful comments regarding my thesis. In response to the majority of Professor Lumbers comments, I have made specific changes by means of this addendum, which is to be attached to the back cover of the thesis. I also include here reprints of the 4 papers that have arisen directly from the work described in this thesis *(Correia et al. 1999, 2000, 2001 and 2002)*. I also include a 5th reprint, describing work that was carried out during my PhD candidature *(Evans et al. 2000)*, but was not included in my thesis because it also included the work of others. You will also note that some of the experiments described in *Correia et al. (1999)* were also not included in my PhD thesis, since they formed the basis of my B.Sc. Honours thesis *(Correia 1997)*.

For the most part, I have responded directly to each comment with a specific entry in the addendum. However, I do disagree with one or two of these comments, and so my rebuttal to these are given below.

Chapter 4: Noradrenaline infusion and renal antihypertensive mechanisms
The examiner raises the interesting issue that plasma renin activity does not seem to fall in response to increased renal artery pressure in rabbits receiving medullary interstitial infusions, but does in rabbits receiving intravenous infusions. I did not formally test whether this apparent effect is statistically significant, since it was not one of our *a priori* hypotheses. I have no plausible explanation for it, other than it might simply reflect biological variation between rabbits, so have elected not to discuss this issue in detail in the thesis.

Chapter 5: Factors mediating depressor responses to increased renal artery pressure
The examiner raises the issue of autoregulation of renal blood flow under the conditions of the extracorporeal circuit. In the extracorporeal circuit model, RAP is increased by diverting blood from the vena caval arm of the circuit towards the renal arm of the circuit using a mechanical (Starling) resistor, but without changing the total flow through the circuit. There are a number of differences in the nature of the renal vascular responses observed in this extracorporeal circuit model compared with conditions under which autoregulatory behavior is normally studied. The most commonly used method for studying renal autoregulatory behaviour involves placing adjustable clamps either on the aorta (especially in smaller species such as rats) or renal artery (especially in larger species such as dogs), so that renal artery pressure can be reduced to levels below systemic mean arterial pressure. In contrast, the model we have used in this study allows renal artery pressure to be set to any level, above or below systemic arterial pressure. We chose to set renal artery pressure at levels ranging from the lower end of normal for a conscious rabbit (65 mmHg) to levels clearly in the 'hypertensive range' (160 mmHg). Thus, as shown in the adjacent figure, our experiment examines renal vascular responses over a different range of perfusion pressures than does the 'conventional' preparation.

Autoregulation of Renal Blood Flow

The other important difference between these two experimental paradigms concerns the relative abilities of the renal vasculature to modulate flow in response to changes in renal artery pressure between the two preparations. In the 'conventional' preparation in the rat, where clamps are placed on the aorta, there are a number of resistive vascular beds in parallel, so that when renal vascular resistance increases in response to increased perfusion pressure, blood flow can be diverted to other (less well autoregulating) vascular beds. In contrast, in the extracorporeal circuit preparation the renal vascular bed is the only non-mechanical resistor in the system, so that renal blood flow
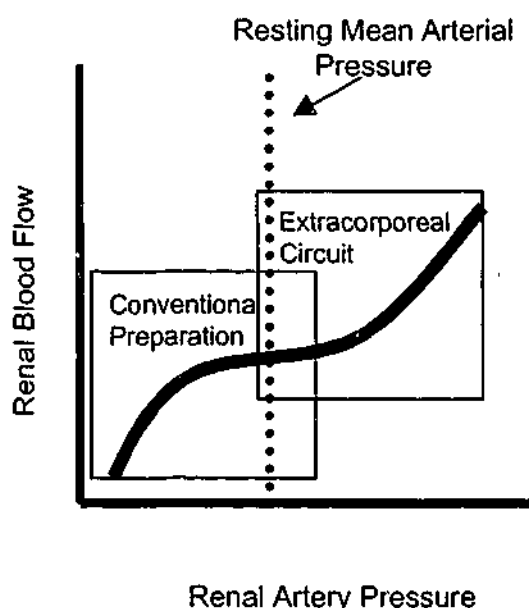
<u>must</u> increase when renal artery pressure is increased. Under these circumstances, autoregulation is seen as an increase in renal vascular resistance when renal artery pressure is increased, up to levels of around 110 mmHg (see Figure 4.4 in thesis). Nevertheless, it is correct to say that both renal blood flow and glomerular filtration rate increase when renal artery pressure is increased. Renal blood flow increases, despite the fact that renal vascular resistance increases as RAP is increased up to 110 mmHg, because the autoregulatory response is limited by the fixed rate of the pump and the high resistance of the vena caval limb. In this respect, the extracorporeal circuit model differs from the 'conventional' model for studying autoregulation, in which these variables remain reasonably stable as RAP is altered. Recently, a member of our research group (Dr Gabriela Eppel) performed a systematic study of the behaviour of total renal blood flow, and cortical and medullary blood flow, when renal artery pressure is altered with the extracorporeal circuit, and with a supra-renal aortic cuff. The major impetus for this new work, which has recently been submitted to *American Journal of Physiology*, was my findings in Chapters 4 and 5.

Chapter 6: V_1-receptors, medullary blood flow, and glomerular arterioles

The examiner questions whether previous studies have investigated the effects of medullary interstitial infusion of a vasopressin V_1-agonist. This has been done previously within our laboratory *(Evans et al. 1998a; Bergström and Evans, 1998)*. Interestingly, the effects of intravenous, renal arterial and medullary interstitial [Phe^2,Ile^3,Orn^8]-vasopressin were indistinguishable, but did not include increased GFR. The increased GFR observed in my experiment during infusion of [Phe^2,Ile^3,Orn^8]-vasopressin might be secondary to its pressor effect, as discussed on page 143 of my thesis

Other Attachments

1. **Specific Amendments to the Thesis**
2. **Reprints of papers published during PhD candidature**

Correia AG, Bergström G, Lawrence AJ, Evans RG (1999) Renal medullary interstitial infusion of norepinephrine in anaesthetized rabbits: methodological considerations. *American Journal of Physiology*, 277, R112-R122.
This paper describes work from my B.Sc. Honours thesis, and from Chapter 3 of my PhD thesis.

Correia AG, Madden AC, Bergström G, Evans RG. (2000) Effects of renal medullary and intravenous norepinephrine on renal antihypertensive function. *Hypertension*, 35, 965-970. [Chapter 4]

Evans RG, Correia AG, Weekes SR, Madden AC. (2000) Responses of regional kidney perfusion to vasoconstrictors in anaesthetized rabbits: dependence on agent and renal artery pressure. *Clinical and Experimental Pharmacology and Physiology* 27, 1007-1012.
This paper describes experiments that were performed during my PhD candidature, but were not included in my thesis.

Correia AG, Denton KM, Evans RG. (2001) Effects of activation of vasopressin-V_1-receptors on regional kidney blood flow and glomerular arteriole diameters. *Journal of Hypertension* 19, 649-657. [Chapter 6].

Correia AG, Bergström G, Jia J, Anderson WP, Evans RG. (2002) Dominance of pressure natriuresis in the acute depressor response to increased renal artery pressure in rabbits and rats. *Journal of Physiology*, 538, 901-910. [Chapter 5, plus the supplementary experiment described in Chapter 7].

Specific Amendments to the Thesis

Summary

Page (i), paragraph 1, replace 2ⁿᵈ sentence with:
This hypothesis is based on the notion that increasing renal artery pressure (RAP) sets in train three mechanisms that exert antihypertensive influences; renal renin release is reduced, thereby inhibiting the activity of the pro-hypertensive renin-angiotensin system, urinary excretion of salt and water increases and thirdly, the putative renal medullary depressor hormone may be released.

Page (i), paragraph 2, add additional sentence at the end:
These regions include the juxtamedullary cortex and outer medulla, which house vascular elements likely to be important in the control of medullary blood flow, the arterioles of juxtamedullary glomeruli, and the outer medullary descending vasa recta respectively.

Page (i), sentence beginning on the last line should read:
However, our conclusion regarding the putative renal medullary depressor hormone remains controversial, particularly since the observation was made that the diuresis/natriuresis increased exponentially with step increases in renal arterial pressure.

Page (ii), last paragraph, line 5-6 should read:
The vasopressin agonist reduced renal medullary blood flow approximately 30% without reducing cortical blood flow.

Chapter 1: Introduction

Page 6, following paragraph 2, the following discussion of the function of the macula densa should be inserted:
These mechanisms operate in concert to regulate renin release, and so the activity of the renin-angiotensin system. Increased renal sympathetic nerve activity increases renin release via activation of β-adrenoceptors on the juxtaglomerular cells. Sympathetic stimulation also has other effects on the kidney that are important in blood pressure control, and these are described in Section 1.5.1 (page 30-32)

Macula densa mechanisms
The macula densa cells occupy the epithelium of the juxtaglomerular apparatus, and detect changes in volume/solute delivery to the distal tubule *(Guyton and Hall, 1996)*. Decreased GFR will slow the rate of delivery to the loop of Henle, and thereby reduce the concentration of sodium chloride at the macula densa cells. This reduced concentration in sodium/chloride ions initiates a signal from the macula densa producing two effects; (i) a reduction in resistance of the afferent arterioles, which raises glomerular hydrostatic pressure and helps to return GFR towards normal, and (ii) an increase in renin release from the juxtaglomerular cells of the afferent arterioles (Figure 1.5). These two components of the tubuloglomerular feedback mechanism, and the position of the cells, on the juxtaglomerular apparatus, provide feedback signals to both the afferent and the efferent arterioles for efficient autoregulation of glomerular filtration rate during changes in arterial pressure.

Figure 1.5 (below)
Macula densa feedback mechanism for autoregulation of glomerular hydrostatic pressure and glomerular filtration rate during decreased renal arterial pressure (From *Guyton and Hall, 1996*).
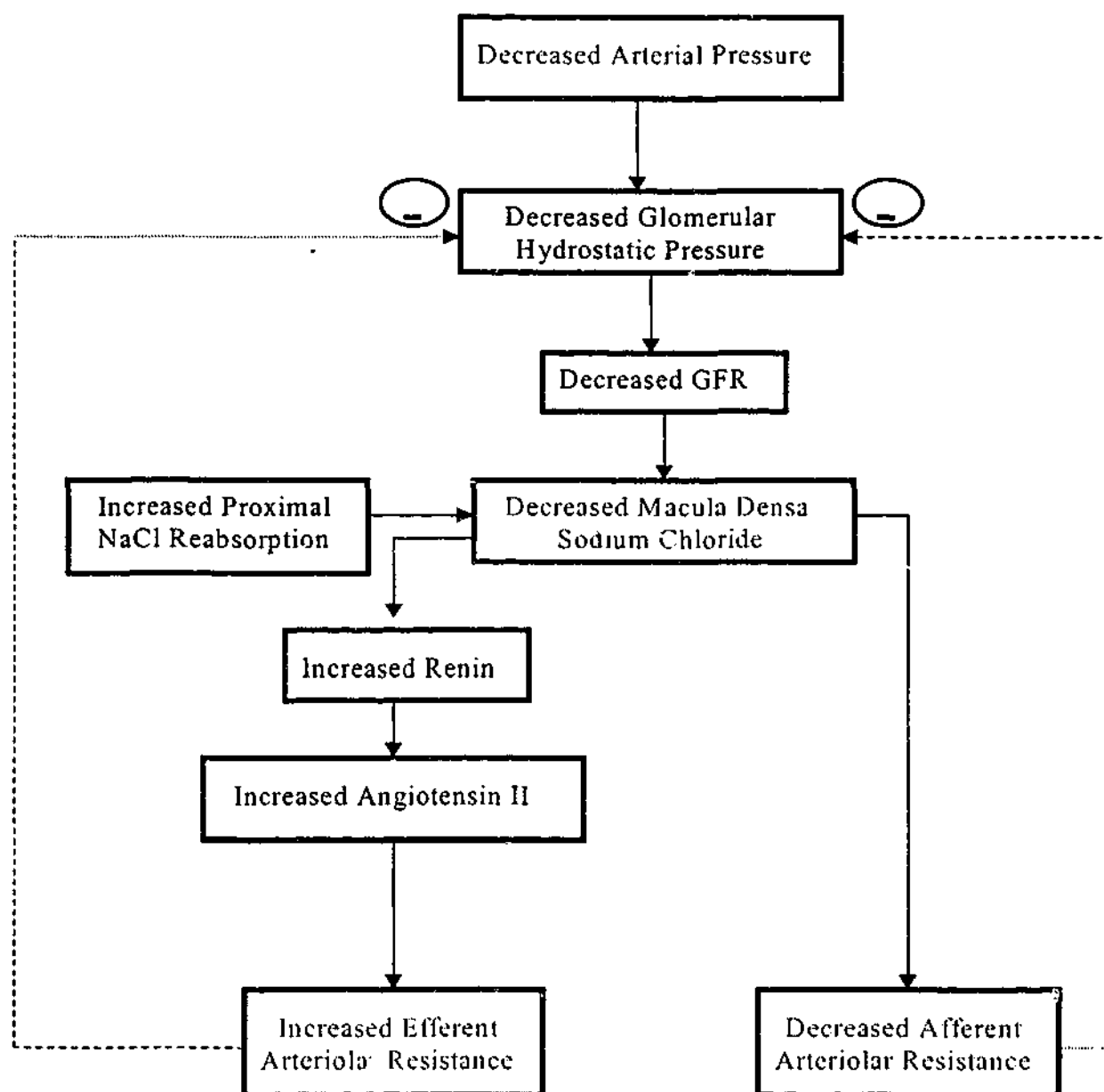
Page 6, paragraph 4, line 5:
Replace aldesterone, with aldosterone.

Page 7, insert new sentence after paragraph 3
Within the central nervous system, angiotensinergic systems stimulate thirst and salt appetite, enhance resting sympathetic drive, and can blunt responses to the loading of arterial baroreceptors *(Waeber et al. 1986., Cowley, 1992., Guyton and Hall, 1996).*

Page 8, Last paragraph, sentence starting on line 1 should read:
Collectively, data suggest that angiotensin II is generated within the kidney, providing it with both paracrine and/or autocrine functions as well as the classical endocrine hormone functions.

Page 10, paragraph 1, last sentence should read:
The reason for the inability of 'hypertensive kidneys' to effectively excrete sodium and water when perfused at normotensive pressures remains uncertain, however an intrinsic impairment of the pressure diuresis/natriuresis mechanism, rather than abnormalities of the neural and/or endocrine control of the kidney, seem likely *(Liard, 1977; Cowley and Roman, 1983).*

Page 10, last paragraph, first sentence should read:
According to studies performed by *Khraibi and Knox (1988)*, and *Roman and Kaldunski (1988)*, reduced renal MBF is the only notable change in renal haemodynamics, in young and adult SHR and WKY rats, since RBF, CBF, and GFR remain unchanged.

Page 13, third sentence of paragraph 2 should read:
With the assumption that para-aminohippurate (PAH) is only extracted in the renal cortex (which is now known to be false *(Hansell, 1992)*), and that increased MBF reduces PAH extraction, they concluded that volume expansion (or increased RAP) increases MBF, which in turn inhibits water reabsorption in the thin descending limb of the loop of Henle, secondary to the loss of the medullary solute gradient.

Page 13-14, first sentence of last paragraph should read:
Thus, increased MBF dissipates the medullary urea gradient, which may contribute to the pressure natriuretic response through the inhibition of water reabsorption in the thin descending loop of Henle, and by increasing the conductance of the paracellular pathway to ions in the proximal tubule, thin descending loop of Henle and thin ascending loop of Henle (which in the rat, are all highly permeable to sodium and chloride).

Page 16, paragraph 2
Replace 'ureteal', with ureteral

Page 17, paragraph 1, last sentence should read:
The ultimate goal of complete chemical characterisation of the active principle(s) in these lipid extracts has, however, remained elusive.

Page 18, from end of line 7 it should read:
It was argued, in these experiments that the reductions in MAP were not due to volume depletion triggered by the pressure-natriuretic-diuretic response, as all experimental animals were in positive fluid balance at all times.

Page 19, paragraph 3, first sentence should read:
While the components of the lipid inclusions have not been fully characterised, histochemical studies have shown them to consist largely of saturated and unsaturated lipids.

Page 19, paragraph3, lines 3-4 from bottom of paragraph should read:
Medullipin I is inactive, and appears to require 'activation' by cytochrome P-450 during the passage through the liver to form medullipin II, which has powerful depressor actions.

Page 24, paragraph 3, line 1 should read:
The inner stripe of the outer medulla remains the most constant component (across species) of the medullary circulation.

Chapter 2: General Methods

Page 43, paragraph 2, sentence beginning on line 7 should read:
The circuit was initially set to withdraw blood from the distal aorta, and return it to the distal vena cava, below the level of the renal veins, at a rate of 70 ml/min (Figure 2.1).

Page 46, (insert) below text
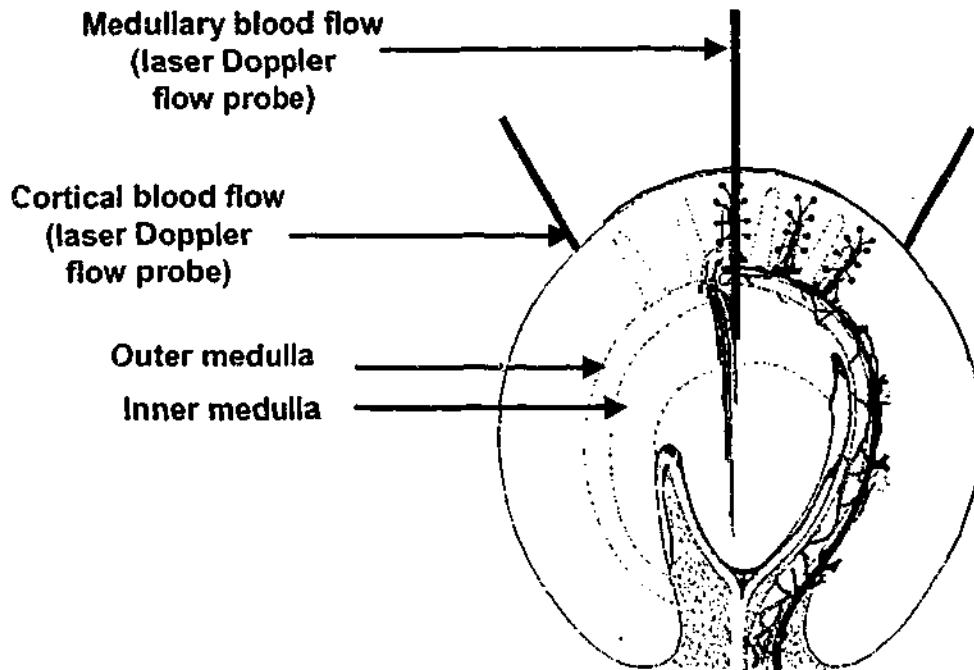
Figure 2.2
Sites of insertion of laser-Doppler flow probes, for measuring cortical and medullary blood flow in the kidney.

Page 51, paragraph 1 (continued from page 50) should read:
Six afferent and efferent vessels were randomly selected for analysis from each region of the cortex (superficial, mid, and juxtamedullary), although not all data sets were complete. Afferent arterioles were identified by locating their origin on the interlobular arteries, and efferent arterioles began at the glomerulus and branched into peritubular capillaries. Glomeruli pertaining to different regions of the cortex were classified as; (i) superficial glomeruli, which were attached to afferent arterioles at the junction of the interlobular artery. The efferent arterioles of these glomeruli were thinner and tended to branch less than those in the mid and inner cortex, (ii) mid-cortical glomeruli were defined as having shorter afferent arterioles attached to the interlobular arteries and efferent arterioles which were also short, and branched several times to form peritubular capillaries, (iii) juxtamedullary glomeruli were identified as having afferent arterioles which branched close to the arcuate arteries, and efferent arterioles which were noticeably thicker and longer. Occasionally these efferent vessels were observed to branch into the unique 'horsetail' arrangement of the vasa recta.

Page 54, paragraph 2, second last sentence should read:
The plasma clearance for PAH is therefore used to estimate effective renal plasma flow.

Page 55, bottom of page relating to statistics used in Chapter 4 should read:
paired *t* test, repeated measures analysis of variance, two-way analysis of variance

Chapter 3: Methods for renal medullary infusion of vasoactive compounds

Page 61, paragraph 2, sentence beginning on line 3 should read:
This entire dose was delivered directly to the outer (n=8) or inner medullary interstitium (n=9) of rabbits that had chronically implanted catheters. In the rabbits in which two acutely positioned catheters (n=9) were placed either side of the outer medulla, the entire dose was divided so that its delivery was equally distributed between the catheters.

Chapter 4: Effects of renal medullary and intravenous noradrenaline on renal antihypertensive function

Page 73, Point 4, sentence beginning on line 2 should read:
This circuit enables RAP to be altered independently of the systemic arterial pressure.

Page 74, Point 7 should read:
As RAP was increased to ~160 mmHg, urine output and sodium excretion increased, while plasma renin activity and MAP fell.

Page 74, sentence beginning four lines from bottom of page should read:
Presently available data also indicate that it is largely independent of the associated diuresis and diuresis/natriuresis, in view of the fact that haemoconcentration is not observed.

Page 75, paragraph 1, final sentence should read:
It was also not possible to exclude roles of non-flow-mediated extra-vascular actions on V_1-receptors in the kidney, or even extra-renal V_1-receptors, which might blunt the release and/or actions of the putative renal medullary depressor hormone.

Page 75, paragraph 2, sentence beginning on line 6 should read:
Therefore, the effects of medullary interstitial infusion and intravenous infusion of noradrenaline, on the antihypertensive responses to increased RAP, were compared.

Table 4.1, and Figures 4.2 and 4.3 have been revised (below), and are now in complete agreement with the text:

Table 4.1   Effects of medullary interstitial and intravenous noradrenaline on systemic and renal haemodynamics (Data presented as percentage change).

	Medullary Interstitial Infusion		Intravenous Infusion	
	Vehicle	Noradrenaline	Vehicle	Noradrenaline
MAP	2.4 ± 1.4	17 ± 4	3.5 ± 0.6	12 ± 4
HR	- 0.7 ± 2.3	2 ± 1	1.3 ± 0.5	-0.02 ± 3.0
RAP	- 0.3 ± 2.0	19 ± 4	0.8 ± 1.0	3.5 ± 1.0
RBF	0.8 ± 2.5	-16 ± 3	-3.7 ± 5.8	-17 ± 9
CBF	0.3 ± 2.3	-13 ± 2	0.3 ± 5.8	-19 ± 3
MBF	3 ± 10	-28 ± 9	-0.4 ± 4.1	-1.3 ± 7.5

MAP, mean arterial pressure; HR, heart rate; RAP, renal arterial pressure: RBF, renal blood flow: CBF, cortical blood flow; MBF, medullary blood flow.

Revised Figure 4.2
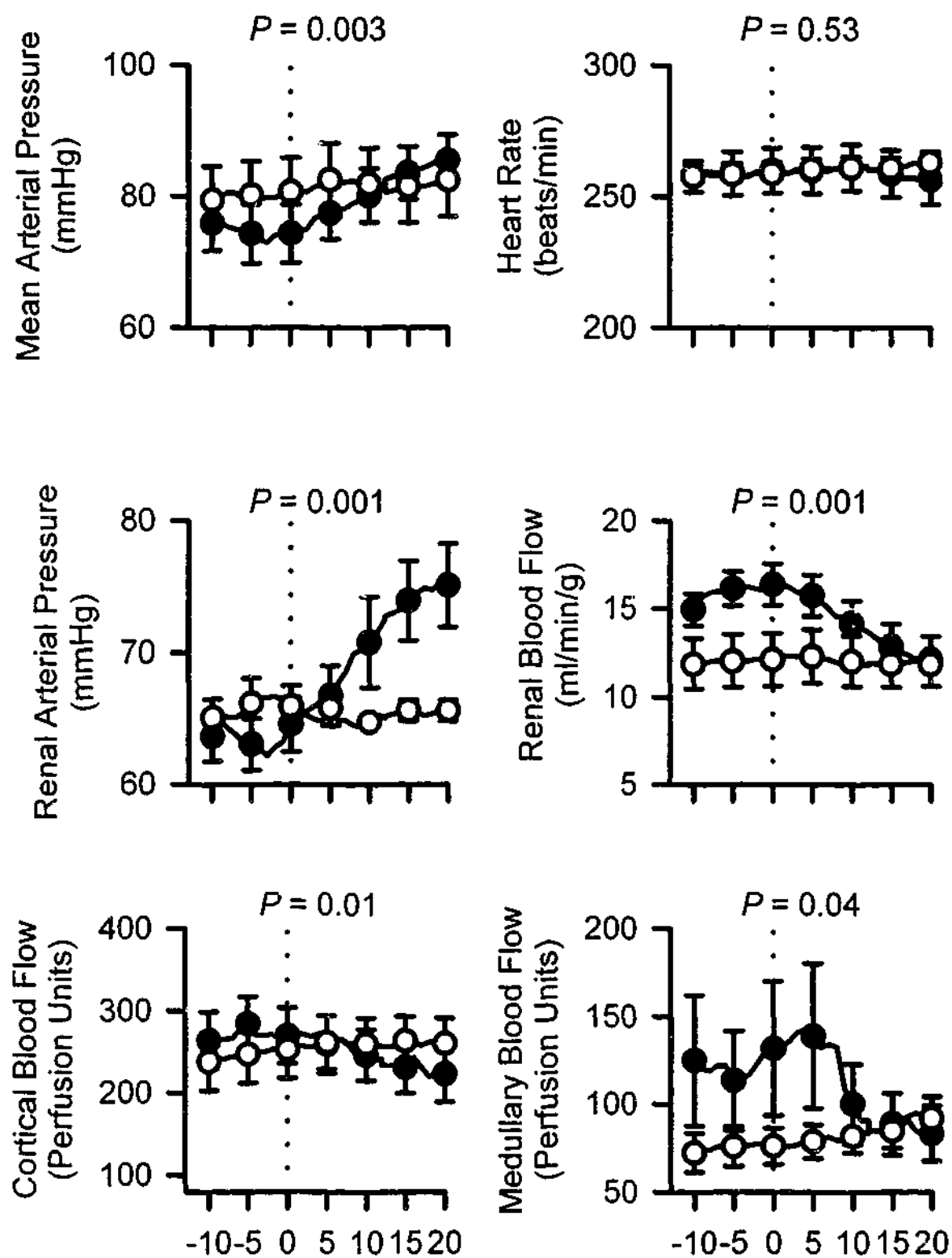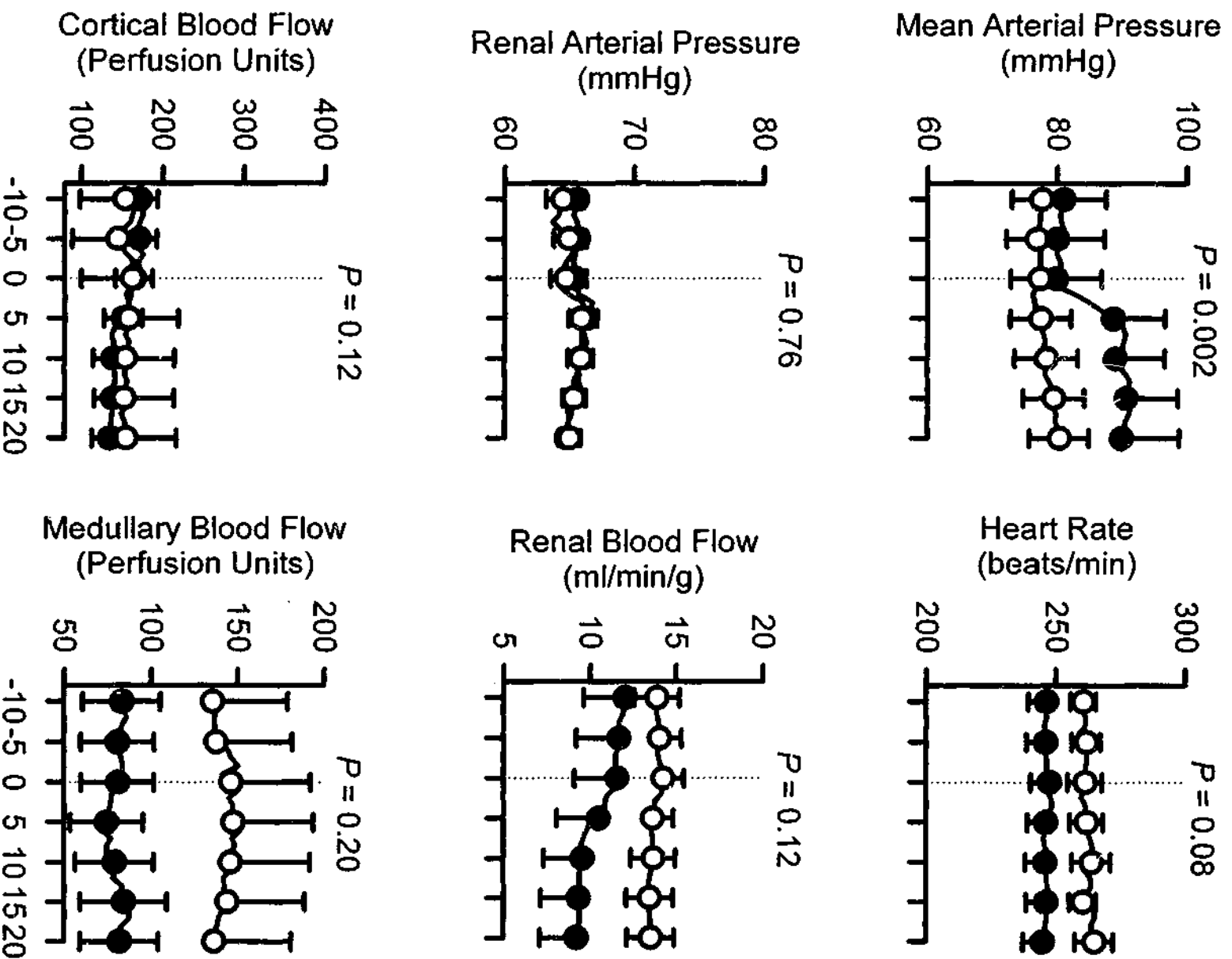
Time from Commencement of Medullary Noradrenaline Infusion (min)

Page 80, Section 4.5.2.2 should read:
As shown in Figure 4.5, as RAP was increased from 66 ± 1 to 158 ± 3 mmHg, CBF increased progressively from 235 ± 31 to 329 ± 45 perfusion units ($P_{RAP} = 0.01$), whereas MBF did not change significantly.

Page 81, section 4.5.3, sentence beginning on line 4 should read:
Medullary interstitial noradrenaline also significantly altered the response of haematocrit to increased RAP, attenuating the increase in haematocrit when RAP was increased above ~110 mmHg.

Page 81, section 4.5.4 should read:
On conclusion of the experiment, the effects of returning RAP to ~65 mmHg were recorded. RBF in vehicle-treated rabbits returned to levels similar ($-3 \pm 4\%$ different) to those observed during the initial control period. In rabbits treated with medullary interstitial noradrenaline, RBF was $-13 \pm 4\%$ and $-39 \pm 27\%$ different from its control value. MAP rose when RAP was reset to ~65 mmHg, but did not completely recover to its initial level in vehicle-treated rabbits ($-28 \pm 5\%$ different from its control level), and in rabbits treated with outer medullary noradrenaline ($-14 \pm 6\%$) and intravenous noradrenaline ($-30 \pm 10\%$).

Page 91, insert new sentence beginning on the 2nd last line:
We did not directly assess kidney size in this study, but have observed on numerous occasions the swelling of the kidney when RAP is increased.

Chapter 5: Factors mediating depressor responses to increased renal artery pressure

Page 99, 2nd paragraph, insert new text before the last sentence:
The 'renin-angiotensin' clamp enabled us to test whether inhibition of renin release contributes to the acute depressor response to increased RAP in this preparation.

Page 106, section 5.4.1.5, 1st sentence of paragraph 1 should read:
When the peristaltic pump was set to withdraw blood from the abdominal aorta (70 ml/min) and return it to the vena cava, MAP fell by 12 ± 1 mmHg.

Page 106, section 5.4.1.5, 1st sentence of paragraph 2 should read:
However, if blood flow through the extracorporeal circuit is excluded from these calculations, CO was found to initially fall 40 ± 3 ml/kg/min during the first 2 minutes of the pump rate being increased, before increasing to be 23 ± 5 ml/kg/min greater than at the end of the previous period (C).

Page 108, 1st sentence of the last paragraph should read:
When RAP was increased and maintained at ~160 mmHg and the excreted urine volume was returned as an intravenous infusion of compound sodium lactate, all renal excretory variables increased significantly, with the exception of FF, which was not significantly altered.

Page 116, Figure 5.7, insert at the end of the legend:
Note that in this figure, and also in Figures 5.8, 5.9 and 5.10, some of the symbols are obscured because they lie at identical co-ordinates to the super-imposed symbol

Page 124, paragraph 3, sentence beginning on line 2 should read:
The 'renin-angiotensin system clamp' was also effective in blocking the renin-angiotensin system since (i) bolus doses of angiotensin I failed to increase MAP, in rabbits which had received this treatment, and (ii) resting systemic and renal haemodynamics and renal excretory variables in rabbits that had received this treatment were similar to those in rabbits from the other three experimental groups.

Chapter 6: Effects of activation of vasopressin-V_1-receptors on regional kidney blood flow and glomerular arteriole diameters

Page 134, reference to figure in the last line should read:
Figure 6.3.

Page 136, legend to Figure 6.2 should read:
Scanning electron micrograph of juxtamedullary glomeruli. A, afferent arteriole; E, efferent arteriole; G, glomerulus; OMDVR, outer medullary descending vasa recta.

Page 140, Non-parametric analysis of data
Table 6.2
Effects of intravenous infusion of [Phe^2, Ile^3, Orn^8]-vasopressin (30 ng/kg/min; n=7) or its vehicle (50 µl/kg/min; n=7) on relative resistance.

TREAT	VESSEL	OUTER CORTEX	MID CORTEX	JUXTA MEDULLARY
Vehicle	Afferent	0.051 ± 0.018	0.064 ± 0.017	0.060 ± 0.021
V_1-agonist	Afferent	0.087 ± 0.041	0.080 ± 0.022	0.128 ± 0.058
Vehicle	Efferent	0.293 ± 0.051	0.189 ± 0.039	0.124 ± 0.036
V_1-agonist	Efferent	0.417 ± 0.073	0.375 ± 0.083	0.175 ± 0.059

Data are expressed as the mean ± SEM of the relative resistance (R), and were calculated according to Poisuelle's relationship ($R=1/r^4$). Resistance for vehicle and V_1-agonist infused vessels were compared for both the afferent and efferent vessels in the outer, mid, and juxta-medullary cortex. Data were subjected to both paired t-tests, and Mann-Whitney U-tests; $P > 0.05$ for all six comparisons in both tests.

BIBLIOGRAPHY

Admiraal P.J., Derkx, F.H., Danser A.H., Pieterman H. and Schalekamp M.A. (1990). Intrarenal de novo production of angiotensin I in subjects with renal artery stenosis. *Hypertension*, 16: 555-563.

Agmon Y., Dinour D. and Brezis M. (1993). Disparate effects of adenosine A_1- and A_2- receptor agonists on intrarenal blood flow. *American Journal of Physiology*, 265: F802-F806.

Anderson W.P., Woods R.L., Thomas C.J., Szenasi G. and Evans R.G. (1995). Renal medullary antihypertensive mechanisms. *Clinical and Experimental Pharmacology and Physiology*, Suppl. 2: S426-S429.

Arenshorst W.J. (1979). Autoregulation of renal blood flow in spontaneously hypertensive rats. *Circulation Research*, 44: 344-349.

Armstrong T., Bennett S., Davies J., Harlow T., Magnus P., Mathur S. and Senes-Ferrari S. (1999). Heart, stroke and vascular diseases, Australian facts. *Australian Institute of Health and Welfare, Cardiovascular Disease Series* 10: 1-55.

Ashworth-Preece M., Krstew E., Jarrot B. and Lawrence A.J. (1997). Functional $GABA_A$ receptors on rat vagal afferent neurones. *British Journal of Pharmacology*, 120: 469-475.

Aukland K. (1968). Vasopressin and intrarenal blood flow distribution. *Acta Physiologica Scandinavica*, 74: 173-182.

Bailie M.D. and Barbour J.A. (1975). Effect of inhibition of peptidase activity on distribution of intrarenal blood flow. *American Journal of Physiology*, 228: 850-853.

Bankir L. and deRouffignac C. (1985). Urinary concentrating ability: Insights from comparative anatomy. *American Journal of Physiology*, 249: R643-R666.

Barajas L., Powers K. and Wang P. (1984). Innervation of the renal cortical tubules: a quantitative study. *American Journal of Physiology*, 247: F50-F60.

Baylis C., Engels K., Samsell L. and Harton P. (1993). Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. *American Journal of Physiology*, 264: F74-F78.

Beeuwkes R. (1980). The vascular organization of the kidney. *Annual Review of Physiology.*, 42: 531-542.

Bergström G., (1995) *The renomedullary depressor mechanism and its relation to the renin-angiotensin system and nitric oxide.*, Physiology, pp. 8-12, University of Goteborg, Goteborg .

Bergström G. and Evans R.G. (1998). Effects of renal medullary infusion of vasopressin V_1-agonist on renal antihypertensive mechanisms in rabbits. *American Journal of Physiology*, 274: R76-R85.

Bergström G. and Evans R.G. (2000). Integrative aspects of the renal medullary circulation. *In:* W.P. Anderson, R.G. Evans and K.M. Stevenson (eds.), *The renal circulation: advances in organ biology.* Vol. 1: pp. 235-253, JAI Press, Stamford.

Bergström G., Gothberg G., Karlström G. and Rudenstam J. (1998). Renal medullary blood flow and renal medullary antihypertensive mechanisms. *Clinical and Experimental Hypertension,* 20: 1-26.

Bergström G., Rudenstam J., Creutz J., Gothberg G. and Karlström G. (1995). Renal and haemodynamic effects of nitric oxide blockade in a Wistar assay rat during high pressure cross-circulation of an isolated denervated kidney. *Acta Physiologica Scandinavica,* 154: 241-52.

Bergström G., Rudenstam J., Taghipour K., Gothberg G. and Karlström G. (1996). Effects of nitric oxide and renal nerves on renomedullary haemodynamics in SHR and WIstar rats, studied with laser Doppler technique. *Acta Physiologica Scandinavica,* 156: 27-36.

Bertram J.F. (2000). Structure of the renal circulation. *In:* W.P. Anderson, R.G. Evans and K.M. Stevenson (eds.), *The renal circulation.:* pp. 2-10, JAI Press Inc., Stamford, Connecticut.

Bing R.F., Russel G.I., Swales J.D., Thurston H. and Fletcher A. (1981). Chemical medullectomy; Effect upon reversal of two-kidney, one-clip hypertension in the rat. *Clinical Science,* 61: S3335-S338.

Biondi M.L., Dousa T., Vanhoutte P. and Romero J.C. (1990). Evidence for the existence of endothelium-derived relaxing factor in the renal medulla. *American Journal of Hypertension,* 3: 876-878.

Bonner R.F., Clem T.R., Bowen P.D. and Bowman R.L. (1981). Laser Doppler continuous real-time monitor of pulsatile and mean blood flow in tissue microcirculation. *In:* S.H. Chen, B. Chu and R. Nossal (eds.), *Scattering techniques, applied to supra-molecular and nonequilibrium systems.:* pp. 685-702, Plenum, New York.

Bouriquet N. and Casellas D. (1995). Chronic L-NAME hypertension in rats and autoregulation of juxtamedullary preglomerular vessels. *American Journal of Physiology,* 269: F190-F197.

Bradley T. and Hjemdahl P. (1984). Further studies on renal nerve stimulation induced release of noradrenaline and dopamine from the canine kidney in situ. *Acta Physiologica Scandinavica,* 122: 369-379.

Brooks B., Byers L.W., Muirhead E.E., Muirhead M., Pitcock J.A., Maddipati K.R. and Maxey K.M. (1994). Purification of class I medullipins from the venous effluent of isolated normal kidneys perfused under high pressure with saline. *Blood Pressure,* 3: 407-17.

Brown G.P. and Venuto R.C. (1988). Angiotensin II receptors in rabbit renal preglomerular vessels. *American Journal of Physiology,* 255: E E22.

Carmines P.K., Inscho E.W. and Gensure R.C. (1990). Arterial pressure effects on preglomerular microvasculature of juxtamedullary nephrons. *American Journal of Physiology,* 258: F94-102.

Casellas D. and Moore L.C. (1990). Autoregulation and tubuloglomerular feedback in juxtamedullary glomerular arterioles. *American Journal of Physiology,* 258: F660-F669.

Chen J. and Fleming J.T. (1993). Juxtamedullary afferent and efferent arterioles constrict to renal nerve stimulation. *Kidney International*, 44: 684-691.

Chou S.Y., Porush J.G. and Faubert P.F. (1990). Renal medullary circulation: Hormonal control. *Kidney International*, 37: 1-13.

Christy I.J., Woods R.L. and Anderson W.P. (1993). Mediators of the hypotensive response to increased renal perfusion in rabbits. *Hypertension*, 21: 149-154.

Christy I.J., Woods R.L., Courneya C.A., Denton K.M. and Anderson W.P. (1991). Evidence for a renomedullary vasodepressor system in rabbits and dogs. *Hypertension*, 18: 325-333.

Churchill P.C. and Churchill M.C. (1992). Kidney cross transplants in Dahl salt-sensitive and salt-resistant rats. *American Journal of Physiology*, 262: H1809-H1817.

Click R.L., Joyner W.L. and Gilmore J.P. (1979). Reactivity of glomerular afferent and efferent arterioles in renal hypertension. *Kidney International*, 15: 109-115.

Cohen H.J., Marsh D.J. and Kayser B. (1983). Autoregulation in vasa recta of the rat kidney. *American Journal of Physiology*, 245: F32-F40.

Correia A.G., (1997) *Methods for the deliver of pharmacological agents into the rabbit renal medulla.*, Department of Physiology, pp. 30-42, Monash University, Melbourne.

Cowley A.W.Jr. (1992). Long-term control of arterial blood pressure. *Physiological Reviews*, 72: 231-300.

Cowley A.W.Jr. (1997). Role of the renal medulla in volume and arterial pressure regulation. *American Journal of Physiology*, 273: R1-R15.

Cowley A.W.Jr. and Lohmeier T.E. (1979). Changes in renal vascular sensitivity and arterial pressure associated with sodium intake during long-term intrarenal norepinephrine infusion in dogs. *Hypertension*, 1979: 549-558.

Cowley A.W.Jr., Mattson D.L., S. L. and Roman R.J. (1995). The renal medulla and hypertension. *Hypertension*, 25: 663-673.

Cowley A.W.Jr. and Roman R.J. (1983). Renal dysfunction in essential hypertension - implications of experimental studies. *American Journal of Nephrology*, 3: 59-72.

Cowley A.W.Jr. and Roman R.J. (1997). Renal mechanisms in hypertension. *In:* A. Zanchetti and G. Mancia (eds.), *Handbook of hypertension*: pp. 740-783, Elsevier, Amsterdam.

Cowley A.W.Jr., Roman R.J., Fenoy R.J. and Mattson D.L. (1992). Effect of renal medullary circulation on arterial pressure. *Journal of Hypertension*, 10: S187-S193.

Cowley A.W.Jr., Szczepanska-Sadowska E., Stepniakowski K. and Mattson D. (1994). Chronic intravenous administration of V_1 arginine vasopressin agonist results in sustained hypertnsion. *American Journal of Physiology*, 267: H752-H756.

Cupples W.A., Sakai T. and Marsh D.J. (1988). Angiotensin II and prostaglandins in control of vasa recta blood flow. *American Journal of Physiology*, 254: F417-F424.

Denton K.M., Anderson W.P. and Sinniah R. (2000). Effects of angiotensin II on regional afferent and efferent arteriole dimensions and the glomerular pole. *American Journal of Physiology*, 279: R629-R638.

Denton K.M., Fennessy P.A., Alcorn D. and Anderson W.P. (1992). Morphometric analysis of the actions of angiotensin II on renal arterioles and glomeruli. *American Journal of Physiology*, 262: F367-72.

DiBona G.F. and Kopp U.C. (1997). Neural control of renal function. *Physiological Reviews*, 77: 75-197.

Dwarkin L.D. and Brenner B.M. (1996). *The kidney*: p. 247-238, WB Sunders, Philadelphia.

Early L.E. and Friedler R.M. (1965a). Changes in renal blood flow and possibly the intrarenal distribution of bloo? ⸮uring the natriuresis accompanying saline loading in the dog. *Journal of Clinical Investigation*, 44: 929-941.

Early L.E. and Friedler R.M. (1965b). Studies on the mechanism of natriuresis accompanying increased renal blod flow and its role in the renal response to extracellular volume expansion. *Journal of Clinical Investigation*, 44: 1857-1865.

Edwards J.G. (1956). Efferent arterioles of glomeruli in the juxtamedullary zone of human kidney. *Anatomical Record*, 125: 521-529.

Edwards R.M. (1983). Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels. *American Journal of Physiology*, 244: F526-F534.

Edwards R.M., Trizna W. and Kniter L.B. (1989). Renal microvascular effects of vasopressin and vasopressin antagonists. *American Journal of Physiology*, 256: F275-F278.

Endlich K., Hoffend J. and Steinhausen M. (1996). Localization of endothelin ET_A and ET_B receptor-mediated constriction in the renal microcirculation of rats. *Journal of Physiology*, 497: 211-218.

Esler M.D., Hasking G.J., Willet I.R., Leonard P.W. and Jennings G.L. (1985). Noradrenaline release and sympathetic nervous system activity. *Journal of Hypertension*, 3: 117-129.

Evans R.G., Bergström G., Coterill E. and Anderson W.P. (1998c). Renal haemodynamic effects of endothelin-1 and the ET_A/ET_B antagonist TAK-044 in anaesthetized rabbits. *Journal of Hypertension*, 16: 1897-1905.

Evans R.G., Bergström G. and Lawrence A.J. (1998a). Effects of the vasopressin V_1-agonist $[Phe^2,Ile^3,Orn^8]$ vasopressin on regional kidney perfusion and renal excretory function in anesthetized rabbits. *Journal of Cardiovascular Pharmacology*, 32: 571-581.

Evans R.G., Correia A.G., Weekes S.R. and Madden A.C. (2000a). Responses of regional kidney perfusion to vasoconstrictors in anaesthetized rabbits: dependence on agent and renal artery pressure. *Clinical and Experimental Pharmacology and Physiology*, 27: 1007-1012.

Evans R.G., Day K.H., Roman R.J., Hopp K.H. and Anderson W.P. (1998b). Effects of intrarenal infusion of 17-octadecynoic acid on renal antihypertensive mechanisms in anesthetised rabbits. *American Journal of Hypertension*, 11: 803-812.

Evans R.G., Haynes J.M. and Ludbrook J. (1993). Effects of 5-HT-receptor and α_2-adrenoceptor ligands on the haemodynamic respnse to acute central hypovolaemia in conscious rabbits. *British Journal of Phyarmacology*, 109: 37-47.

Evans R.G., Madden A.C. and Denton K.M. (2000b). Diversity of responses of renal cortical and medullary blood flow to vasoconstrictors in conscious rabbits. *Acta Physiologica Scandinavica*, 169: 297-308.

Evans R.G., Madden A.C. and E. C. (2000c). ET-receptor subtypes: Roles in regional renal vascular actions of exogenous and endogenous endothelins in anesthetized rabbits. *Journal of cardiovascular pharmacology*, 35: 677-685.

Evans R.G., Rankin A.J. and Anderson W.P. (1994). Interactions of blockade of nitric oxide synthase and angiotensin-converting enzyme on renal function in conscious rabbits. *Journal of Cardiovascular Pharmacology*, 24: 542-551.

Evans R.G., Stevenson K.M., Malpas S.C., Fitzgerald S.M., Shweta A., Tomoda F. and Anderson W.P. (1997). Chronic renal blood flow measurement in dogs by transit-time ultrasound flowmetry. *Journal of Pharmacological and Toxicological Methods*, 38: 33-39.

Evans R.G., Szenasi G. and Anderson W.P. (1995). Effects of N^G-nitro-L-argininge on pressure natriuresis in anasthetized rabbits. *Clinical and Experimental Pharmacology and Physiology*, 22: 94-101.

Evans R.G., Ventrua S., Dampney R.A.L. and Ludbrook J. (2001). Neural mechanisms in the cardiovascular responses to acute central hypovolaemia. *Clinical and Experimental Pharmacology and Physiology*, In press.

Fadem S.F., Hernandez-Llamas G., Ptak R.V., Rosenblatt S.G., Lifschitz M.D. and Stin J.H. (1982). Studies on themechanism of sodium excretion during drug induced vasodilation in the dog. *American Journal of Medicine*, 36: 604-610.

Feng J.J. and Arendshorst W.J. (1996). Enhanced renal vasoconstriction induced by vasopressin in SHR is mediated by V1 receptors. *American Journal of Physiology*, 271: F304-13.

Fenoy F.J. and Roman R.J. (1991). Effect of volume expansion on papillary blood flow and sodium excretion. *American Journal of Physiology*, 260: F813-F822.

Fenoy F.J. and Roman R.J. (1992). Effect of kinin receptor antagonists on renal hemodynamic and natriuretic responses to volume expansion. *American Journal of Physiology*, 263: R1136-R1140.

Floyer M.A. (1975). Renal control of interstitial space compliance: a physiological mechanism which may play a part in the etiology of hypertension. *Clinical Nephrology*, 4: 152-156.

Folkow B. (1982). Physiological aspects of primary hypertension. *Physiological Review*, 62: 347-504.

Franchini K.G. and Cowley A.W.Jr. (1996). Renal cortical and medullary blood flow responses during water restriction: role of vasopressin. *American Journal of Physiology*, 270: R1257-R1264.

Franchini F.G., Mattson D.L. and Cowley A.W.Jr. (1997). Vasopressin modulation of medullary blood flow and pressure-natriuresis-diuresis in the decerebrated rat. *American Journal of Physiology*, 272: R1472-R1479.

Fujita K., Matsumura Y., Kita S., Miyazaki Y., Hisaki K., Takaoka M. and Morimoto S. (1995a). Role of endothelin-1 and the ETA receptor in the maintenance of deoxycorticosterone acetate-salt-induced hypertension. *British Journal of Pharmacology*, 114: 925-30.

Fujita K., Matsumura Y., Miyazaki Y., Takaoka M. and Morimoto S. (1995b). Role of endothelin-1 in hypertension induced by long-term inhibition of nitric oxide synthase. *European Journal of Pharmacology*, 280: 311-6.

Ganguli M., Tobain L. and Dahl L. (1976). Low renal papillary plasma flow in both Dahl and Kyoto rats with spontaneous hypertension. *Circulation Research*, 39: 337-341.

Gardes J., Baussant T., Corvol P., Menard J. and Alhenc-Gelas F. (1990). Effect of bradykinin and kininogens in isolated rat kidney vasoconstricted by angiotensin II. *American Journal of Physiology*, 258: F1273-F1281.

Gattone V.H.d. and Evan A.P. (1986). Quantitative renal vascular casting in nephrology research. *Scan Electron Microscopy*, (Pt: 253-62.

Gattone V.H.d., Evan A.P., Mong S.A., Connors B.A., Aronoff G.R. and Luft F.C. (1983). The morphology of the renal microvasculature in glycerol- and gentamicin-induced acute renal failure. *Journal of Laboratory and Clinical Medicine*, 101: 183-95.

Gellai M. (1990). Modulation of vasopressin antidiuretic action by renal α_2-adrenoceptors. *American Journal of Physiology*, 259: F1-F8.

Gonzalez R., Fernandez-Alfonso M.S., Rodriguez-Martinez M.A., Fuertes E., Angulo J., Sanchez-Ferrer C.F. and Marin J. (1994). Pressure-induced contraction of the juxtamedullary afferent arterioles in spontaneously hypertensive rats. *General Pharmacology*, 25: 333-9.

Goransson A. and Sjoquist M. (1984). The effect of pressor doses of angiotensin II on autoregulation and intrarenal distribution of glomerular filtration rate in the rat. *Acta Physiologica Scandinavica*, 122: 615-620.

Gothberg G. and Karlström G. (1991). Physiological effects of the humoral renomedullary antihypertensive system. *American Journal of Hypertension*, 4: 569S-574S.

Graensgoe G. and Wolgast M. (1972). The pressure-flow relationship in renal cortical and medullary circulation. *Acta Physiologica Scandinavica*, 85: 228-236.

Granger J.P. and Scott J. (1988). Effects of renal artery pressure on interstitial pressure and Na excretion during renal vasodilation. *American Journal of Physiology*, 255: F828-F833.

Grollman A., Muirhead E.E. and Vanatta J. (1949). Role of the kidney in pathogenesis of hypertension as determined by a study of the effects of bilateral nephrectomy and other experimental procedures on the blood pressure of the dog. *American Journal of Physiology*, 157: 21-30.

Guild A., Austin P.A., Barrett C.J., Evans R.C., Narakatikyan M.N., Ringwood J.V. and Malpas S.C. (2001). Interactions between neural and hormonal control of the renal vasculature: a frequency domain approach. *Proceedings of the 24th International Congress of Physiological Sciences, Christchurch, N.Z.*, In Press.

Gurbanov K., Rubinstein I. and Hoffman A. (1996). Differential regulation of renal regional blood flow by endothelin -1. *American Journal of Physiology*, 271: F1166-F1172.

Guyton A.C., Coleman T.G., Cowley A.W.Jr., Sheel K.W., Manning R.D.J. and Noramn R.A.J. (1972). Arterial Pressure Regulation: Overriding dominance of the kidneys in long-term regulation and in hypertension. *The American Journal of Medicine*, 52: 584-594.

Guyton A.C. and Hall J.E. (1996). *Textbook of medical physiology*, 9 ed., W.B. Sunders Company, Philadelphia, Pennsylvania.

Haas J.A., Hammond T.G., Granger J.P., Blaine E.H. and Knox F.G. (1984). Mechanisms of natriuresis during intrarenal infusion of prostaglandins. *American Journal of Physiology*, 247: F475-F479.

Haas J.A., Granger J.P. and Knox F.G. (1986). Effect of renal perfusion pressure on sodium reabsorption from proximal tubules of superficial and deep nephrons. *American Journal of Physiology*, 250: F425-F429.

Hackenthal E., Paul M., Ganten D. and Taugner R. (1990). Morphology, physiology, and molecular biology of renin secretion. *Physiological Reviews*, 70: 1067-1116.

Hall J.E., Granger J.P. and Hester R.L. (1985). Interactions between adenosine and angiotensin II in controlling glomerular filtration rate. *American Journal of Physiology*, 248: F340-F346.

Hansell P. (1992). Evaluation of methods for estimating renal medullary blood flow. *Renal Physiology and Biochemistry*, 15: 217-230.

Hansell P., Nygre A. and Ueda J. (1990). Influence of verapamil on regional renal blood flow: A study using multichannel laser-Doppler flowmetry. *Acta Physiologica Scandinavica*, 139: 15-20.

Harrison-Bernard L.M. and Carmines P.K. (1994). Juxtamedullary microvascular responses to arginine vasopressin in rat kidney. *American Journal of Physiology*, 267: F249-F256.

Harrison-Bernard L.M., Navar G. and Cook K.A. (1996). Renal cortical and medullary microvascular blood flow autoregulation in rat. *Kidney International*, 50 (Suppl. 57): S23-S29.

Haynes W.G., Ferro C.J., O'Kane K.P.J., Sommerville D., Lomax C.C. and Webb D.J. (1996). Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. *Circulation*, 93: 1860-1870.

Hermansson K., Ojteg G. and Wolgast M. (1984). The cortical and medullary blood flow at different levels of renal nerve activity. *Acta Phsyiologica Scandinavica*, 120: 161-169.

Hesse I.F.A. and Johns E.J. (1984). The subtype of α-adrenoceptor involved in the neural control of renal tubular sodium reabsorption in the rabbit. *Journal of Physiology*, 352: 527-538.

Hesse I.F.A. and Johns E.J. (1985). The role of α-adrenoceptors in the regulation of renal tubular sodium reabsorption and renin secretion in the rabbit. *British Journal of Pharmacology*, 84: 715-724.

Hocher B., Rohmeiss P., Zart R., Diekmann F., Vogt V., Metz D., Fakhury M., Gretz N., Bauer C., Koppenhagen K., Neumayer H.H. and Distler A. (1996). Function and expression of endothelin receptor subtypes in the kidneys of spontaneously hypertensive rats. *Cardiovascular Research*, 31: 499-510.

Hollenberg N.K., Adams D.F., Solomon H., Chenitz W.R., Burger R.B., Abrams H.L. and Merrill J.P. (1975). Renal vascular tone in essential and secondary hypertension: hemodynamic and angiographic responses to vasodilators. *Medicine (Baltimore)*, 54: 29-44.

Howl J. and Wheatley M. (1995). Molecular pharmacology of V_{1a} vasopressin receptors. *General. Pharmacology*, 26: 1143-152.

Hughes A.K., Barry W.H. and Kohan D.E. (1995). Identification of a contractile function for renal medullary interstital cells. *The Journal of Clinical Investigation*, 96: 411-416.

Ito S. and Carretero O.A. (1990). An in vitro approach to the study of macula densa-mediated glomerular hemodynamics. *Kidney International*, 38: 1206-1210.

Itskovitz H.D., Stemper D., Pacholczyk D. and McGiff J.C. (1973). Renal prostaglandins: Determinants of intrarenal distribution of blood flow in the dog. *Clinical Science and Molecular Medicine*, 45: S321-S324.

Jamison R.L. and Kriz W. (1982). *Urinary concentrating mechanism: Structure and function*: p. 1-340, Oxford University Press, New York - Oxford.

Johns D.W., Carey R.M., Gomez R.A., Lynch K., Inagami T., Saye J., Geary K., Farnsworth D.E. and Peach M.J. (1987). Isolation of renin-rich rat kidney cells. *Hypertension*, 10: 488-496.

Johnson J.V., Bennett G.W. and Hatton R. (1998). Central and systemic effects of a vasopressin V_1 antagonist on MAP recovery after hemorrhage in rats. *Journal of Cardiovascular Pharmacology*, 12: 405-412.

Judy W.V. and Farell S.K. (1979). Arterial baroreceptor reflex control of sympathetic nerve activity in the spontaneously hypertensive rat. *Hypertension*, 1: 605-614.

Judy W.V., Watanabe A.M., Henry D.P., Besch H.R.J., Murphy W.R. and Hockel G.M. (1976). Sympathetic nerve activity: role in regulation of blood pressure in the spontaneously hypertensive rat. *Circulation Research*, 38(Suppl II): II-21- II29.

Kaissling B., Rouffignac C.D., Barrett J.M. and Kriz W. (1975). The structural organization of the kidney of the desert rodent *Psammomys obesus*. *Anatomy and Embryology*, 148: 121-143.

Kaissling B. and Kriz W. (1979). Structural analysis of the rabbit kidney. *Advances in Anatomy, Embryology and Cell Biology*, 56: 1-123.

Karlström G., Arnman V., Bergström G., Muirhead E.E., Rudenstam J. and Gothberg G. (1989). Renal and circulatory effects of medullipin I, as studied in the *in-vivo* cross-circulated isolated kidney and intact Wistar-Kyoto (WKY) rat. *Acta Physioogica Scandinavica*, 137: 521-533.

Karlström G., Arnman V., Folkow B. and Gothberg G. (1988). Activation of the humoral antihypertensive system of the kidney increases diuresis. *Hypertension*, 11: 597-601.

Karlström G., Bergström G., Folkow B., Rudenstam J. and Gothberg G. (1991). Is the humoral renal antihypertensive activity of the spontaneously hypertensive rat (SHR) reset to the high blood pressure? *Acta Physiologica Scandinavica*, 141: 517-530.

Karlström G. and Gothberg G. (1987). The humorally mediated antihypertensive system of the rat kidney: a physiological depressor mechanism? *Journal of Hypertension, Suppl*, 5: S91-4.

Katholi R.E., Carey R.M., Ayers C.R., Vaughan E.D.J., Yancey M.R. and Morton C.L. (1977). Production of sustained hypertension by chronic intrarenal norepinephrine in conscious dogs. *Circulation Research*, 40 (Suppl I): 1-118 - 1-126.

Khraibi A.A. and Knox F.G. (1988). Renal interstitial hydrostatic pressure during pressure natriuresis in hypertension. *American Journal of Physiology*, 255: R756-R759.

Kiberd B.A., Larson T.S., Robertson C.R. and Jamison R.L. (1987b). Effect of atrial natriuretic peptide on vasa recta blood flow in the rat. *American Journal of Physiology*, 252: F1112-F1117.

Kiberd B., Robertson C.R., Larson T. and Jamison R.L. (1987a). Effect of V_2 receptor-mediated changes on inner medullary blood flow induced by AVP. *American Journal of Physiology*, 253: F576-F581.

Kimura K., Hirata Y., Nanba S., Tojo A., Matsuoka H. and Sugimoto t. (1990). Effects of atrial natriuretic peptide on renal arterioles: morphometric analysis using microvascular casts. *American Journal of Physiology*, 259: F936-F944.

Kleinjans J.C.S., Smits J.F.M., vanEssen H., Kasbergen C.M. and Struyker-Boudier H.A.J. (1984). Hemodynamic characterization of hypertension induced by chronic intrarenal or intravenous infusion of norepinephrine in conscious rats. *Hypertension*, 25: 940-949.

Knepper M.A., Danielson R.A., Saidel G.M. and Post R.S. (1977). Quantitative analysis of renal medullary anatomy in rats and rabbits. *Kidney International*, 12: 313-323.

Knox F.G., Mertz J.I., Burnett J.C.J. and Haramadi A. (1983). Role of hydrostatic oncotic pressure in renal sodium absorption. *Circulation Research*, 52: 491-500.

Kopp U., Bradley T. and Hjemdahl P. (1983). Renal venous outflow and urinary excretion of norepinephrine, epinephrine, and dopamine during graded renal nerve stimulation. *American Journal of Physiology*, 244: E52-E60.

Kriz W. (1981). Structural organization of the renal medulla: comparative and functional aspects. *American Journal of Physiology*, 241: R3-R16.

Kriz W. (1982). Structural organization of renal medullary circulation. *Nephron*, 31: 290-295.

Larsson C. and Anggard E. (1974). Increased juxtamedullary blood flow on stimulation of intrarenal prostaglandin biosynthesis. *European Journal of Pharmacology*, 25: 326-334.

Ledderhos C., Gross V. and Cowley A.W.Jr. (1998). Pharmacological stimulation of arterial chemoreceptors in conscious rats produces differential responses in renal cortical and medullary blood flow. *Clinical and Experimental Pharmacology and Physiology*, 25: 536-40.

Ledderhos C., Mattson D.L., Skelton M.M. and Cowley A.W., Jr. (1995). In vivo diuretic actions of renal vasopressin V1 receptor stimulation in rats. *American Journal of Physiology*, 268: R796-807.

Ledingham J.M. and Cohen R.D. (1962). Circulatory changes during the reversal of experimental hypertension. *Clinical Science.*, 22: 69-77.

Lemley K.V. and Kriz W. (1991). Anatomy of the renal interstitium. *Kidney International*, 39: 370-381.

Lemley K.V. and Kriz W. (1987). Cycles and separations: the histotopography of the urinary concentrating process. *Kidney International*, 31: 538-548.

Lemley K.V., Schmitt J.L., Holliger C., Dunn M.J., Robertson R.J. and Jamison R.L. (1984). Prostaglandin synthesis inhibitors and vasa recta erythrocyte velocities in the rat. *American Journal of Physiology*, 247: F562-F567.

Leonard B.L., Evans R.G., Navakatikyan M.A. and Malpas S.C. (2000). Differential neural control of intrarenal blood flow. *American Journal of Physiology*, 279: R907-R916.

Leonard B.L., Malpas S.C., Denton K.M., Madden A.C. and Evans R.G. (2001). Differential control of intrarenal blood flow during reflex increases in sympathetic nerve activity. *American Journal of Physiology*, 280: R62-R68.

Levens N.R. (1990). Control of renal function by intrarenal angiotensin II in the dog. *Journal of Cardiovascular Pharmacology*, 16: S65-9.

Liard J.F. and Peters G. (1970). Mechanism of the fall in blood pressure after 'unclamping' in rats with goldblatt-type hypertension. *Experientia*, 26: 743-745.

Liard J.F. (1977). Renal denervation delays blood pressure increase in the spontaneously hypertensive rat. *Experientia*, 33: 339-340.

Liard J.F. and Peters G. (1973). Role of the retention of water and sodium in two types of experimental renovascular hypertension in the rat. *Pflugers Archives - European Journal of Physiology*, 344: 93-108.

Lockhart J.C., Larson T.S. and Knox F.G. (1994). Perfusion pressure and volume status determine the microvascular response of the rat kidney to N^G-monomethly-L-arginine. *Circulation Research*, 75: 829-835.

Lu S., Mattson D.L., Roman R.J., Becker C.G. and Cowley A.W.Jr. (1993). Assessment of changes in intrarenal blood flow in conscious rats using laser-Doppler flowmetry. *American Journal of Physiology*, 246: F956-F962.

Lu S., Mattson D.L. and Cowley A.W.Jr. (1994). Renal medullary captopril delivery lowers blood pressure in spontaneously hypertensive rats. *Hypertension*, 23: 337-345.

Lu S., Roman R.J., Mattson D.L. and Cowley A.W.Jr. (1992). Renal medullary interstitial infusion of diltiazem alters sodium and water excretion in rats. *American Journal of Physiology*, 263: R1064-R1070.

Ludbrook J. (1994). Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovascular Research*, 28: 303-311.

Majid D.S., Godfrey M. and Omoro S.A. (1997). Pressure natriuresis and autoregulation of inner medullary blood flow in canine kidney. *Hypertension*, 29: 210-5.

Majid D.S. and Navar L.G. (1996). Medullary blood flow responses to changes in arterial pressure in canine kidney. *American Journal of Physiology*, 270: F833-F838.

Majid D.S., Omoro S.A., Chin S.Y. and Navar L.G. (1998). Intrarenal nitric oxide activity and pressure natriuresis in anesthetized dogs. *Hypertension*, 32: 266-72.

Majid D.S., Williams A. and Navar L.G. (1993). Inhibition of nitric oxide synthesis attenuates pressure-induced natriuretic responses in anesthetized dogs. *American Journal of Physiology*, 264: F79-F87.

Malpas S.C. and Evans R.G. (1998). Do different levels and patterns of sympathetic activation all provoke renal vasoconstriction? *Journal of the Autonomic Nervous System*, 69: 72-82.

Malpas S.C., Shweta A., Anderson W.P. and Head G.A. (1996). Functional response to graded increases in renal nerve activity during hypoxia in conscious rabbits. *American Journal of Physiology*, 271: R1489- R1499.

Mandal A.K., Frolich E.D., Chrysant K., Pfeffer M.A., Yunice A. and Nordquist J.A. (1974). Ultrastructural analysis of renal papillary interstitial cells of spontaneously hypertensive rats. *Journal of Laboratory and Clinical Medicine*, 83: 256-262.

Mattson D.L. and Cowley A.W. Jr. (1993). Kinin actions on renal papillary blood flow and sodium excretion. *Hypertension*, 21: 961-965.

Mattson D.L. and Higgins D.J. (1996). Influence of dietary sodium intake on renal medullary nitric oxide synthase. *Hypertension*, 27: 688-692.

Mattson D.L., Lu S., Nakanishi K., Papenek P.E. and Cowley A.W.Jr. (1994). Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *American Journal of Physiology*, 266: H1918-H1926.

Mattson D.L., Lu S., Roman R.J. and Cowley A.W.Jr. (1993). Relationship between renal perfusion pressure and ˙ ʌod flow in different regions of the kidney. *American Journal of Physiology*, 264: R578-ʀ.ʀ ʌ3.

Mattson D.L. and Roman R.J. (1991). Role of kinins and angiotensin II in the renal hemodynamic response to captopril. *American Journal of Physiology*, 260: F670-F679.

Mattson D.L., Roman R.J. and Cowley A.W.Jr. (1992). Role of nitric oxide in renal papillary blood flow and sodium excretion. *Hypertension*, 19: 766-769.

Manning D.L. and Snyder S.H. (1989). Bradykinin receptors localized by quantitative autoradiography in kidney, ureter, and bladder. *American Journal of Physiology*, 256: F909-F915.

May C.N., Mathai M.L., McDougall J.G. and Whitworth J.A. (1993). Cardiovascular effects of long-term endothelin infusion and responses to endothelin during ACTH infusion in conscious sheep. *American Journal of Hypertension*, 6: 837-43.

McKenna O.C. and Angelakos E.T. (1968). Adrenergic innervation of the canine kidney. *Circulation Research*, 23: 345-353.

Mene P. and Dunn M.J. (1992). Vascular, glomerular, and tubular effects of angiotensin II, kinins, and prostaglandins. *In:* D.W. Seldin (ed.), *The kidney: Physiology and pathophysiology*. Vol. Second Edition: pp. 1205-1248, Raven Press, New York.

Mertz J.I., Haas J.A., Berndt T.J., Burnett J.C.J. and Knox F.G. (1984). Effects of bradykinin on renal interstitial pressures and proximal tubule reabsorption. *American Journal of Physiology*, 247: F82-f85.

Miyamoto M., Yagil Y., Larson C., Robertson R. and Jamison R.L. (1988). Effects of intrarenal adenosine on renal function and medullary blood flow in the rat. *American Journal of Physiology*, 255: F1230-F1234.

Mortensen L.H. and Fink G.D. (1992). Captopril prevents chronic hypertension produced by infusion of endothelin-1 in rats. *Hypertension*, 19: 676-80.

Muirhead E.E. (1980). Antihypertensive functions of the kidney: Arthur C. Corcoran Memorial Lecture. *Hypertension*, 2: 444-464.

Muirhead E.E. (1991). The medullipin system of blood pressure control. *American Journal of Hypertension*, 4: 556S-668S.

Muirhead E.E. (1993). Renal vasodepressor mechanisms: the medullipin system. *Journal of Hypertension*, 11 (Suppl. 5): S53-S58.

Muirhead E.E. and Brooks B. (1980). Reversal of one-kidney, one-clip hypertension by unclipping: The renal, sodium-volume relationship reexamined. *Proceedings for the Society for Experimental Biology and Medicine*, 163: 541-546.

Muirhead E.E., Brooks B., Byers L.W., Brown P. and Pitcock J.A. (1991a). Secretion of medullipin I by isolated kidneys perfused under elevated pressure. *Clinical and Experimental Pharmacology and Physiology*, 18: 409-417.

Muirhead E.E., Brooks B., Byers L.W., Brown P. and Pitcock J.A. (1991b). Medullipin System: Generation of medullipin II by isolated kidney-liver perfusion. *Hypertension*, 18 (Suppl III): III-158 - III-163.

Muirhead E.E., Brooks B., Pitcock J.A. and Stephenson P. (1972a). Renomedullary antihypertensive function in accelerated (malignant) hypertension: observations on renomedullary interstitial cells. *Journal of Clinical Investigation.*, 51: 181-190.

Muirhead E.E., Germain G.S., Armstrong F.B., Brooks B., Leach B.E. and Byers L.W. (1975). Endocrine-type antihypertensive function of renomedullary interstitial cells. *Kidney International*, 8: S271-S282.

Muirhead E.E., Germain G.S., Leach B.E., Pitcock J.A., Stephenson P., Brooks B., Brosius W.L., Daniels E.G. and Hinman J.W. (1972b). Production of renomedullary prostaglandins by renomedullary interstitial cells grown in tissue culture. *Circulation Research*, 30-31, Suppl. 2: 161-171.

Muirhead E.E., Jones F.S. and Stirman J.A. (1960a). Hypertensive cardiovascular disease of dog. *Archives of Pathology*, 70: 122-130.

Muirhead E.E., Pitcock J.A., Nasjletti A., Brown P. and Brooks B. (1985). The antihypertensive function of the kidney. Its elucidation by captopril plus unclipping. *Hypertension*, 4 (Suppl I): 1127-1135.

Muirhead E.E., Stirman J.A. and Jones F. (1960b). Renal auto-explantation and protection against hypertensive cardiovascular disease and hemolysis. *Journal of Clinical Investigation*, 39: 266-281.

Nagaoka A., Kakihana M., Suno M. and Hamajo K. (1981). Renal hemodynamics and sodium excretion in stroke-prone spontaneoulsy hypertensive rats. *American Journal of Physiology*, 241: F244-F249.

Nakanishi K., Mattson D.L. and Cowley A.W.Jr. (1995a). Role of renal medullary blood flow in the development of L-NAME hypertension in rats. *American Journal of Physiology*, 268: R317-R323.

Nakanishi K., Mattson D.L., Gross V., Roman R.J. and Cowley A.W., Jr. (1995b). Control of renal medullary blood flow by vasopressin V1 and V2 receptors. *American Journal of Physiology*, 269: R193-200.

Navakatikyan M.A., Leonard B.L., Evans R.G. and Malpas S.C. (2000). Modelling the neural control of intrarenal blood flow. *Clinical and Experimental Pharmacology and Physiology*, 27: 650-2.

Navar L.G. (1986). Physiological role of the intrarenal renin-angiotensin system. Introductory comments. *Federation Proceedings*, 45: 1411-3.

Navar L.G., Inscho E.W., Majid S.A., Imig J.D., Harrison-Bernard L.M. and Mitchell K.D. (1996). Paracrine regulation of the renal microcirculation. *Physiological Reviews*, 76: 425-536.

Neubig R.R. and Hoobler S.W. (1975). Reversal of chronic renal hypertension: Role of salt and water excretion. *Proceedings for the Society for Experimental Biology and Medicine*, 150: 254-256.

Nobes M.S., Harris P.J., Yamada H. and Mendelsohn F.A. (1991). Effects of angiotensin on renal cortical and papillary blood flows measured by laser-Doppler flowmetry. *American Journal of Physiology*, 261: F998-F1006.

Norman R.A.J. and Dzielak D.J. (1982). Role of renal nerves in onset and maintenance of spontaneous hypertension. *American Journal of Physiology*, 243: H284-H288.

Norman R.A.J., Enobakhare A.J., DeClue J.W., Douglas B.H. and Guyton A.C. (1978). Arterial pressure-urinary output relationship in hypertensive rats. *American Journal of Physiology*, 234(3): R98-R103.

Ofstad J. and Aukland K. (1985). Renal Circulation (Ch 21). *In:* D.W. Seldin and G. Giebisch (eds.), *The Kidney: Physiology and Pathophysiology*: pp. 471-494, Raven Press, New York.

Oliver J., Korner P., Woods R. and Zhu J. (1990). Reflex release of vasopressin and renin in hemorrhage is enhanced by autonomic blockade. *American Journal of Physiology*, 258: H221-H228.

Omoro S.A., Majid S.A., El-Dahr S.S. and Navar L.G. (1999). Kinin influences on renal regional blood flow responses to angiotensin-converting enzyme inhibition in dogs. *American Journal of Physiology*, 276: F271-F277.

Omoro S.A., Majid D.S.A., Dahr S.S. and Navar G. (2000). Roles of ANG II and bradykinin in the renal regional blood flow responses to ACE inhibition in sodium-depleted dogs. *American Journal of Physiology*, 279: F289-F293.

Omvik P., Tarazi R.C. and Bravo E.L. (1980). Regulation os sodium balance in hypertension. *Hypertension*, 2: 515-523.

Ostrowlski N.L., Lolait S.J., Bradley D.J., O'Carrol A.M. and Brownstein M. (1992). Distribution of V_{1a} and V_2 vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. *Endocrinology*, 131: 533-535.

Pallone T.L. (1994). Vasoconstriction of outer medullary vasa recta by angiotensin II is modulated by prostaglangin E_2. *American Journal of Physiology.*, 266: F497-F503.

Pallone T.L., Edwards A. and Kreisberg M.S. (2000). The intrarenal distribution of blood flow. *In:* W.P. Anderson, R.G. Evans and K.M. Stevenson (eds.), *The Renal Circulation.*: pp. 76-81, JAI Press Inc., Stamford, Connecticut.

Pallone T.L., Morgenthaler T.I. and Deen W.M. (1984). Analysis of microvascular water and solute exhanges in the renal medulla. *American Journal of Physiology*, 247: F303-F315.

Pallone T.L., Robertson C.R. and Jamison R.L. (1990). Renal medullary micrcirculation. *Physiological Reviews*, 70: 885-920.

Pallone T.L., Silldorf E.P. and Turner M.R. (1998). Intrarenal blood flow: Microvascular anatomy and the regulation of medullary perfusion. *Clinical and Experimental Pharmacology and Physiology*, 25: 383-392.

Palmer R.M.J., Ferrige A.G. and Moncada S. (1987). Nitric oxide release accounts for the biological activity of endothelium-deribed relaxing factor. *Nature*, 327: 524-526.

Parekh N., Dobrowolski L., Zou A.P. and Steinhausen M. (1996). Nitric oxide modulates angiotensin II- and norepinephrine-dependent vasoconstriction in rat kidney. *American Journal of Physiology*, 270: R630-5.

Parekh N. and Zou A.P. (1996). Role of prostaglandins in renal medullary circulation: response to different vasoconstrictors. *American Journal of Physiology*, 271: F653-F658.

Park F., Mattson D.L., Roberts L.A. and Cowley A.W.Jr. (1997a). Evidence for the presence of smooth muscle alpha-actin within pericytes of the renal medulla. *American Journal of Physiology*, 273: R1742-R1748.

Park F., Mattson D.L., Skelton M.M. and Cowley A.W.Jr. (1997b). Localization of the vasopressin V_{1a} and V_2 receptors within the renal cortical and medullary circulation. *American Journal of Physiology*, 273: R243-R251.

Pitcock J.A., Brown P.S. and Brooks B. (1984). Morphometric studies on the rat renal papilla of resistant and sensitive strains in partial nephrectomy salt hypertension. *Journal of Hypertension*, 2: 419-425.

Pitcock J.A., Brown P.S., Brooks B., Rapp J.P., Rightse W.A. and Muirhead E.E. (1985). The morphology and antihypertensive effect of renomedullary interstitial cells derived from Dahl sensitive and resistant rats. *Experimental and Molecular Pathology*, 43: 29-43.

Pitcock J.A., Brown P.S., Rapp J.S., Crofton J. and Muirhead E.E. (1982). Morphometric studies of the renomedullary interstitial cells of Dahl-hypertension prone and hypertension-resistant rats. *American Journal of Pathology*, 109: 123-128.

Plato C.F. and Osborne J.L. (1996). Chronic renal neuroadrenergic hypertension is associated with increased renal norepinephrine sensitivity and volume contraction. *Hypertension*, 28: 1034-1040.

Reinhardt G.A., Lohmeier T.E. and Hord C.E. (1995). Hypertension induced by chronic renal adrenergic stimulation is angiotensin dependent. *Hypertension*, 25: 549-558.

Rightsel W.A., Okamura T., Inagami T., Pitcock J.A., Takii Y., Brooks B., Brown P. and Muirhead E.E. (1982). Juxtaglomerular cells grown as monolayer cell culture contain renin, angiotensin I-converting enzyme, and angiotensin I and II/III. *Circulation Research*, 50: 822-829.

Roman R.J. (1986). Pressure diuresis mechanism in the control of renal function and arterial pressure. *Federation Proceedings.*, 45: 2878-2884.

Roman R.J., Carmines P.K., Loutzenhiser R. and Conger J.D. (1991). Direct studies on the control of the renal microcirculation. *Journal of the American Society of Nephrology*, 2: 136-149.

Roman R.J. and Cowley A.W.Jr. (1985a). Characterization of a new model for the study of pressure-natriuresis in the rat. *American Journal of Physiology*, 248: F190-F198.

Roman R.J. and Cowley A.W.Jr. (1985b). Abnormal pressure-diuresis-natiuresis response in spontaneously hyertensive rats. *American Journal of Physiology*, 248: F199-F205.

Roman R.J. and Cowley A.W.Jr. (1988). Pressure-diuresis in volume -expanded rats: Cortical and medullary hemodynamics. *Hypertension*, 12: 168-176.

Roman R.J. and Kaldunski M.L. (1988a). Influence of kinins and angiotensin II on the regulation of papillary blood flow. *American Journal of Physiology*, 255: F690-F698.

Roman R.J. and Kaldunski M.L. (1988b). Renal cortical and papillary blood flow in spontaneously hypertensive rats. *Hypertension*, 11: 657-663.

Roman R.J. and Lianos E. (1990). Influence of prostaglandins on papillary blood flow and pressure-natriuretic response. *Hypertension*, 15: 29-35.

Roman R.J. and Smits C. (1986). Laser-Doppler determination of papillary blood flow in young and adult rats. *American Journal of Physiology*, 251: F115-F124.

Roman R.J. and Zou A.P. (1987). Altered presure-natriuresis relationship in young spontaneously hypertensive rats. *American Journal of Physiology*, 9 (Suppl III): III-131 - III-136.

Roman R.J. and Zou A.P. (1993). Influence of the renal medullary circulation on the control of sodium excretion. *American Journal of Physiology*, 265: R963-R973.

Rosivall L., Carmines P.K. and Navar L.G. (1984). Effects of renal arterial angiotensin I infusion on glomerular dynamics in sodium replete dogs. *Kidney International*, 26: 263-268.

Rosivall L., Rinder D.F., Champion J., Khosla M.C., Navar L.G. and Oparil S. (1983). Intrarenal angiotensin I conversion at normal and reduced renal blood flow in the dog. *American Journal of Physiology*, 245: F408-F415.

Rudenstam J., Bergström G., Folkow B., Gothberg G. and Karlström G. (1992). Sympathetic nerve stimulation to an isolated cross-circulated kidney inhibits the pressure-induced humoral hypotensive responses but increases diuresis and natriuresis in the cross-circulating Wistar 'assay' rat. *Acta Physiologica Scandinavica*, 146: 529-30.

Rudenstam J., Bergström G., Taghipour K., Gothberg G. and Karlström G. (1995). Efferent renal sympathetic nerve stimulation *in vivo*. Effects on regional renal hemodynamics in the Wistar rat studied by laser Doppler technique. *Acta Physiologica Scandinavica*, 154: 387-394.

Russell G.I., Bing R.F., Swales J.D. and Thurson H. (1982a). Effect of pharmacological inhibition of renin, prostaglandin and kallikrein systems on surgical correction of longstanding two-kidney, one-clip hypertension in the rat. *Clinical Science*, 63: S257-S260.

Russell G.I., Bing R.F., Thurston H. and Swales J.D. (1982b). Surgical reversal of two-kidney, one-clip hypertension during inhibition of the renin-angiotensin system. *Hypertension*, 4: 69-76.

Salom M.G., Lahera V., Miranda-Guardiola F. and Romero J.C. (1992). Blockade of pressure natriuresis induced by inhibition of synthesis of nitric oxide in dogs. *American Journal of Physiology*, 262: F718-F722.

Schadt J.C. and Hasser E.M. (1991). Interaction of vasopressin and opioids during rapid hemorrhage in conscious rabbits. *American Journal of Physiology*, 260: R373-R381.

Schwartz M.M., Karnovsky M.J. and Venkatachalam M.A. (1976). Ultrastructural differences between rat inner medullary descending and ascending vasa recta. *Laboratory Investigation*, 35: 161-170.

Sherwood L. (1993). *Human physiology, from cells to systems*, 2 ed.: p. 481-490, West, New York.

Shweta A., Malpas S.C., Anderson W.P. and Evans R.G. (1999). Effects of naloxone on the haemodynamic and renal functional responses to plasma volume expansion in conscious rabbits. *Pflugers Archives - European Journal Physiology*, 439: 150-157.

Silldorff E.P., Kreisber M.S. and Pallone T.L. (1996). Adenosine modulates vasomotor tone in outer medullary descending vasa recta of the rat. *Journal of Clinical Investigation*, 98: 18-23.

Silldorff E.P., Yang S. and Pallone T.L. (1995). Prostaglanin E_2 abrogates endothelin-induced vasoconstriction in renal outer medullary descending vasa recta of the rat. *Journal of Clinical Investigation*, 95: 2734-2740.

Siragy H.M., Howell N.L., Peach M.J. and Carey R.M. (1990). Combined intrarenal blockade of the renin angiotensin system in the conscious dog. *American Journal of Physiology*, 258: F522-F529.

Smith J.P. (1956). Anatomical features of the human renal glomerular efferent vessel. *Journal of Anatomy*, 90: 290-292.

Smits G.J., Roman R.J. and Lombard J.H. (1986). Evaluation of laser-Doppler flowmetry as a measure of tissue blood flow. *Journal of Applied Physiology*, 2: 666-672.

Smits J.F., Kleinjans J.C., Janssen B.J. and Struyker-Boudier H.A. (1987). Characterization of hypertension induced by long-term intrarenal norepinephrine infusion in conscious rats. *Clinical and Experimental Hypertension A*, 9 (Suppl I): 197-209.

Solez K., D'Agostini R.J., Buono R.A., Vernon N., Wang A.L., Finer P.M. and Heptinstall R.H. (1976). The renal medulla and mechanisms of hypertension in the spontaneously hypertensive rat. *American Journal of Pathology*, 85: 555-568.

Spielman W.S., Britton S.L. and Fiksen-Olsen M.J. (1980). Effect of adenosine on the distribution of renal blod flow in dogs. *Circulation Research.*: 449-456.

Steinhausen M., Zimmerhackl B., Thederan H., Dussel R., Parekh N., Esslinger H., Hagens V.G., Komitowski D. and Dallenbach F.D. (1981). Intraglomerular microcirculation: Measurements of single glomerular loop flow in rats. *Kidney International*, 27: 17-24.

Stern M.D., Lappe D.L., Bowen P.D., Chimosky J.E., Holloway G.A.J., Keiser H.R. and Bowman R.L. (1977). Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. *American Journal of Physiology*, 32: H4441-H448.

Stern M.D., Bowen P.D., Parma R., Osgood R.W., Bowman R.L. and Stein J.H. (1979). Measurement of renal cortical and medullary blood flow by laser-Doppler spectroscopy in the rat. *American Journal of Physiology*, 236: F8F87.

Susic D., Sparks J.C., Machado E.A. and Kentera D. (1978). The mechanism of renomedullary antihypertensive action: haemodynamic studies in hydronephrotic rats with one-kidney renal-clip hypertension. *Clinical Science and Molecular Medicine*, 54: 361-367.

Szczepanska-Sadowska E., Stepniakowski K., Skelton M.M. and Cowley A.W.Jr. (1994). Prolonged stimulation of V_1 vasopressin receptors results in sustained hypertension. *American Journal of Physiology*, 267: R1217-r1225.

Takezawa K., Cowley A.W.Jr, Skelton M. and Roman R.J. (1987). Atriopeptin III alters renal medullary hemodynamics and the pressure-diuresis response in rats. *American Journal of Physiology*, 252: F992-1002.

Tamaki T., Kiyomoto K., He H., Tomohiro A., Nishiyama A., Aki Y., Kimura S. and Abe Y. (1996). Vasodilation induced by vasopressin V2 receptor stimulation in afferent arterioles. *Kidney International*, 49: 722-729.

Taverner D., Bing R.F., Fletcher A., Russell G., Swales J.D. and Thurston H. (1984). Hypertension produced by chemical renal medullectomy: evidence for a renomedullary vasodepressor function in the rat. *Clinical Science*, 67: 521-528.

Terada Y., Tomita K., Nonoguchi H. and Marumo F. (1992a). Different localization of two types of endothelin receptor mRNA in microdissected rat nephron segments using reverse transcription and polymerase chain reaction assay. *Journal of Clinical Investigation*, 90: 107-112.

Terada Y., Tomita K., Nonoguchi H. and Marumo F. (1992b). Polymerase chain reaction localization of constitutive nitric oxide synthase and soluble guanylate cyclase messenger RNAs in microdissected rat nephron segments. *Journal of Clinical Investigation*, 90: 659-665.

Thomas C.J., Anderson W.P. and Woods R.L. (1995). Nitric oxide inhibition does not prevent the hypotensive response to increased renal perfusion in rabbits. *Clinical and Experimental Pharmacology and Physiology*, 22: 345-351.

Thomas C.J., Woods R.L., Evans R.G., Alcorn D., Christy I.J. and Anderson W.P. (1996). Evidence for a renomedullary vasodepressor hormone. *Clinical and Experimental Pharmacology and Physiology*, 23: 777-785.

Thomas C.J., Woods R.L., Gao Y. and Anderson W.P. (1994). Pressure range for release of renomedullary depressor substance in rabbits. *Hypertension*, 23: 639-645.

Thurau K. (1964). Renal hemodynamics. *American Journal of Medicine*, 36: 698-719.

Tomoda F., Lew R.A., Smith I., Madden A.C. and Evans R.G. (1996). Role of brakyinin receptors in the renal effects of inhibition of angiotensin converting enzyme and endopeptidases 24.11 and 24.15 in conscious rabbits. *British Journal of Pharmacology*, 119: 365-373.

Tornel J. and Madrid M.I. (2000). Role of kinings in the control of renal papillary blood flow, pressure natriuresis, and arterial pressure. *Circulation Research*, 86: 589- ?

Turner M.R. and Pallone H.L. (1997). Vasopressin constricts outer medullary descending vasa recta isolated from rat kidneys. *American Journal of Physiology*, 272: F147-F151.

Ullrich K.J., Kramer K. and Boylan J.W. (1962). *Present knowledge of the countercurrent system in the mammalian kidney.*: p. 1-37, Grune and Stratton, New York.

Vongsavan N. and Matthews B. (1993). Some aspects of the use of laser Doppler flow meters for recording tissue blood flow. *Experimental Physiology*, 78: 1-14.

Waeber B., Nussberger J. and Brunner H.R. (1986). The renin angiotensin system: role in experimental and human hypertension. *In:* A. Zanchetti and R.C. Tarazi (eds.), *Handbook of Hypertension.* Vol. 8: pp. 489-519, Elsevier, Amsterdam.

Weekes S.R., Madden A.C., Bergström G., Anderson W.P. and Evans R.G. (2000). Effects of renal arterial endothelin-1 and endogenous endothelins on regional kidney blood flow and renal antihypertensive mechanisms in anesthetized rabbits. *Kidney Blood Pressure Research*, 23: 366-75.

Weihprecht H., Lorenz J.N., Briggs J.P. and Schnermann J. (1991). Vasoconstrictor effect of angiotensin and vasopressin in isolated rabbit afferent arterioles. *American Journal of Physiology*, 261: F273-82.

Welch W.J., Deng X., Snellen H. and Wilcox C.S. (1995). Validation of miniature ultrasonic transit-time flow probes for measurement of renal blood flow in rats. *American Journal of Physiology*, 268: F175-F178.

Wilkins F.C.J., Alberola A., Mizelle H.L., Opgenorth T.L. and Granger J.P. (1993). Chronic pathophysiologyic circulating endothelin levels produce hypertension in conscious dogs. *Journal of Cardiovascular Pharmacology*, 22(suppl.8): S325-S327.

Wilkinson L., SYSTAT: The system for statistics, Evanston, IL (1990).

Yanagawa N., Capparelli A.W.,Jr. Jo O.D., Friedal A., Barrett J.D. and Eggena P. (1991). Production of angiotensin and renin-like activity by rabbit proximal tubular cells in culture. *Kidney International*, 39: 938-941.

Yang S., Silldorff E.P. and Pallone T.L. (1995). Effect of norepinephrine and acetylcholine on outer medullary descending vasa recta. *American Journal of Physiology*, 269: H710-H716.

Zimmerhackl B., Parekh N., Kucherer H. and Steinhausen M. (1985a). Influence of systemically applied angiotensin II on the microcirculation of glomerular capillaries in the rat. *Kidney International*, 27: 17-24.

Zimmerhackl B., Robertson C.R. and Jamison R.L. (1985b). The microcirculation of the renal medulla. *Circulation Research*, 57: 657-667.

Zimmerhackl B., Robertson C.R. and Jamison R.L. (1985c). Effect of arginine vasopressin on renal medullary blood flow; A videomicroscopic study in the rat. *Journal of linical Investigation*, 76: 770-778.

Zimmerhackl B., Robertson C.R. and Jamison R.L. (1985d). Fluid uptake in the renal papilla by vasa recta estimated by two different methods simultaneously. *American Journal of Physiology*, 248: F347-F353.

Zimmerhackl B.L., Robertson C.R. and Jamison R.L. (1987). The medullary microcirculation. *Kidney International*, 31: 641-647.

Zou A.P., Muirhead E.E., Cowley A.W.,Jr. Mattson D.L., Falck J.R., Jiang J. and Roman R.J. (1995). Role of changes in renal hemodynamics and P-450 metabolites of arachidonic acid in the reversal of one-kidney, one clip hypertension. *Journal of Hypertension*, 13: 557-566.

Zou A.-P., Nithipatikom K., Li P.-L. and Cowley A.W.Jr. (1999). Role of renal medullary adenosine in the control of blood flow and sodium excretion. *American Journal of Physiology*, 276: R790-R798.

Zusman R.M. and Keiser H.R. (1977). Prostaglandin biosynthesis by rabbit renomedullary interstitial cells in tissue culture. *Journal of Clinical Investestigation.*, 60: 215-223.