THE ROLE OF

VASCULAR REACTIVITY IN

NORMOTENSIVE AND HYPERTENSIVE RATS

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By

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To My
Parents & Family
"—— for the present impasse in hypertension is probably chiefly due to the fact that certain fundamentally important aspects of vascular behaviour are appreciated by contemporary science either dimly or not at all."

G.W. Pickering, 1945.
An increase in reactivity of the blood vessels is a feature in many types of hypertension and may be of importance in the pathogenesis of high blood pressure. The literature on experimental hypertension, the renin-angiotensin system and vascular reactivity in human and various experimental models of hypertension are reviewed.

Vascular responsiveness was examined using an isolated hindlimb preparation of rat and perfused with the animal's own blood. Plasma renin concentration was inversely related with dietary sodium in normal rats. Pressor responsiveness to angiotensin II was reciprocally related to endogenous levels of renin in the normotensive rats and the differences were abolished by blockade of the renin-angiotensin system. The results favour the hypothesis that prior occupancy and or "up" regulation of endogenous receptors determine the pressor responsiveness to angiotensin II in normotensive animals. Inhibition of converting enzyme with captopril significantly decreased the formation of angiotensin II and potentiated the vascular responses to pressor agents.

Removal of a kidney and either acute administration of salt and deoxycorticosterone or clipping the remaining kidney significantly enhanced pressor responsiveness in hypertensive animals. Hypersensitivity was not present in the early phase of 2-kidney 1-clip Goldblatt hypertension but it was demonstrated in the chronic phase of hypertension. These differences may be explained by occupancy and availability of angiotensin II receptors and medial hypertrophy. Relief of ischaemia restored arterial pressure and hindlimb vascular resistance to normal at one day but hypersensitivity to pressor agents was still present in the chronic and to the same extent in the early renovascular hypertension. This may be due to subnormal vascular tone when structural changes are still present. 60 days after unclipping, pressor reactivity returned to normal suggesting regression of structural vascular changes. These studies suggest that structural hypertrophy is a consequence of hypertension and may have an additional effect after prolonged hypertension.
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# CONTENTS

Abstract 1

Acknowledgements 2

CHAPTER - 1 HISTORICAL REVIEW 3

1.1 Early landmarks 4

1.2 Early breakthrough and concepts in experimental approach to essential hypertension 10

CHAPTER - 2 THE RENIN-ANGIOTENSIN SYSTEM 15

2.1 General aspects 16

2.2 Components of the renin-angiotensin system 16

   2.2.1 Renin substrate 18
   2.2.2 Renin 18
   2.2.3 Angiotensin I 24
   2.2.4 Angiotensin converting enzyme 25
   2.2.5 Angiotensin II 26
      Actions of angiotensin II 27
      (a) Cardiovascular system 27
      (b) Nervous system 27
      (c) Kidney 28
      (d) Adrenal cortex 29
      (e) Adrenal medulla 29
   2.2.6 Angiotensin III 30

2.3 Inhibitors of the renin-angiotensin system 30

   2.3.1 Antibodies 31
   2.3.2 Renin inhibitors 32
   2.3.3 Competitive antagonists of angiotensin II 32
   2.3.4 Converting enzyme inhibitors 34

CHAPTER - 3 VASCULAR SMOOTH MUSCLE IN HYPERTENSION 38

3.1 Studies in humans 39

3.2 Studies in animals 41

   3.2.1 Mineralocorticoid hypertension 42
   3.2.2 Experimental renovascular hypertension 45
   3.2.3 Spontaneously hypertensive rats 50

3.3 Extraneous factors influencing vascular smooth muscle reactivity 54
3.3.1 Sympathetic nervous system 55
3.3.2 Sodium balance 57
3.3.3 A slow pressor effect of angiotensin II 58
3.3.4 Enhanced sensitivity to angiotensin II 59
3.3.5 Local vascular humoral systems 61
(a) Vascular renin 61
(b) Renal vasopressor substances 62
(i) Kallikrein-kinin system 63
(ii) Prostaglandins 64
(iii) Antihypertensive renomedullary lipids 65
3.3.6 Other vasopressor substances 66
3.3.7 Non-specific changes in pressor sensitivity 67

3.4 Purpose of the experimental studies 69

CHAPTER - 4  METHODS AND MATERIALS 72

4.1 Experimental model 73
4.2 Induction of renal hypertension 73
4.3 Preparation of the constricting clip 74
4.4 Surgical procedures 74
4.5 Left renal artery stenosis 74
4.6 Right nephrectomy 75
4.7 Surgical correction of hypertension 75
4.8 Indirect measurement of blood pressure by a light plethysmographic method
(a) Method 77
(b) Accuracy of the method 79
(c) Incidence of hypertension in the animals 79
4.9 Measurement of plasma renin concentration 79
4.9.1 Basic principles of renin assay 80
4.9.2 Plasma collection and storage 82
4.9.3 The renin assay 82
(a) Incubation 83
(b) Radioimmunoassay 83

HINDQUARTER PERFUSION STUDIES 85
4.10 Animal preparation 85
4.11 Tracheostomy 86
4.12 Cannulation of the left carotid artery 86
4.13 Direct measurement of blood pressure 87
4.14 Cannulation of the left jugular vein 88
4.15 Cannulation of the aorta 88
4.16 The extra-corporeal circuit 90
4.17 Perfusion of the hindquarters 92
4.18 Calibration of the perfusion pump 93
4.19 Determination of dose–responses 93

EXPERIMENTAL STUDIES 97
4.20 Influence of dietary sodium and the kidney in normotensive rats 97
4.21 Mineralocorticoid and 1-kidney 1-clip Goldblatt hypertension 99
4.22 Early and chronic renovascular hypertension 100
4.23 Surgical reversal of renovascular hypertension 101
4.24 Statistical analysis 102

CHAPTER - 5 RESULTS 104
5.1 Influence of dietary sodium and the kidney on vascular reactivity in normotensive animals 105
  5.1.1 Blood pressure and renin 105
  5.1.2 Effect of captopril on hindlimb perfusion pressure 105
  5.1.3 Conversion of angiotensin I to angiotensin II 109
  5.1.4 Increase in hindlimb perfusion pressure by angiotensin I 110
  5.1.5 Angiotensin II pressor reactivity 114
  5.1.6 Noradrenaline pressor responses 116

5.2 Pressor responsiveness to angiotensin II and noradrenaline in steroid and 1-kidney 1-clip Goldblatt hypertension 118
  5.2.1 Blood pressure and plasma renin 118
  5.2.2 Effect of captopril on hindlimb perfusion pressure 118
  5.2.3 Angiotensin I and inhibition of conversion to angiotensin II by captopril 123
  5.2.4 Angiotensin II pressor reactivity 123
  5.2.5 Noradrenaline pressor responses 125

5.3 Pressor response studies in early and chronic 2-kidney 1-clip Goldblatt hypertension 128
  5.3.1 Blood pressure and renin 128
  5.3.2 Effect of captopril on hindlimb perfusion pressure 133
  5.3.3 Angiotensin I and inhibition of conversion to angiotensin II by captopril 133
  5.3.4 Angiotensin II pressor reactivity 133
  5.3.5 Noradrenaline pressor responses 135
5.4 Effect of surgical reversal on pressor responsiveness in early and chronic 2-kidney 1-clip Goldblatt hypertension

5.4.1 Blood pressure 137
5.4.2 Plasma renin concentration 137
5.4.3 Body weight 141
5.4.4 Hindlimb vascular resistance 141
5.4.5 Angiotensin I and inhibition of conversion to angiotensin II by captopril 148
5.4.6 Angiotensin II pressor reactivity 148
5.4.7 Noradrenaline pressor responses 152

CHAPTER - 6 DISCUSSION 157

6.1 Influence of dietary sodium and the kidney on vascular reactivity in normotensive animals 158

6.1.1 Blood pressure and sodium balance 158
6.1.2 Hindlimb perfusion pressure and conversion to angiotensin II 159
6.1.3 Vascular reactivity 160

6.2 Pressor responsiveness to angiotensin II and noradrenaline in steroid and 1-kidney 1-clip Goldblatt hypertension 167

6.2.1 Blood pressure and plasma renin 167
6.2.2 Hindlimb perfusion pressure and captopril 168
6.2.3 Vascular reactivity 169

6.3 Pressor response studies in early and chronic 2-kidney 1-clip Goldblatt hypertension 175

6.3.1 Blood pressure and plasma renin 175
6.3.2 Vascular reactivity 177

6.4 Effect of surgical reversal on pressor responsiveness in early and chronic 2-kidney 1-clip Goldblatt hypertension 181

6.4.1 Blood pressure and plasma renin 181
6.4.2 Vascular reactivity 182

CHAPTER - 7 CONCLUSIONS 186

REFERENCES 189
CHAPTER - 1

HISTORICAL REVIEW
HISTORICAL REVIEW

1.1 Early Landmarks

Schaarschmidt and Nicolai in Germany in the mid-eighteenth century first ascribed essential hypertension in humans (as we know it today) to a "spastic constriction of the vascular bed" and noted that the circulation was in a state of "vehement agitation" (Backer, 1953).

In 1836, Richard Bright published his report of autopsies upon patients who died with various diseases characterised during life by dropsy and by albumin in the urine. He made two remarkable observations - that the kidneys were invariably abnormal, usually shrunken and with a granular surface appearance; and that there was often a considerable hypertrophy of the heart. He considered that the disease of the kidney might be primary and somehow responsible for the condition of the heart when he wrote "the hypertrophy of the heart seems in some degree to have kept pace with the advance of disease in the kidneys" (Bright, 1836). He incorrectly interpreted cardiac hypertrophy as a compensatory mechanism in response to the increased force necessary to propel blood through the diseased kidneys. Nevertheless, Bright's findings stimulated numerous investigators to search for the cause of what is referred to as Bright's disease.

In 1868, Johnson in England first described arteriolar medial hypertrophy which confirmed the earlier observations made by Bright of left ventricular hypertrophy and arterial thickening. Johnson's findings were soon confirmed by Ewald in 1877 who suggested that the initial rise in blood pressure may give rise to a widespread autoregulatory constriction of the arterioles (whole-body autoregulation) and subsequently to generalised medial hypertrophy.
Brown-Sequard in 1892 hypothesised that many organs of the body were capable of releasing specific signal substances into the general circulation which were not waste products but had specific effects upon target tissues and organs. He arrived at this conclusion after observing the temporary improvement in the condition of nephrectomised animals which had been given renal extracts (Olmsted, 1946).

However, the major advances in experimental renovascular hypertension came about with the discovery of renin by Tigerstedt and Bergman in 1898 and the consistent production of hypertension in an animal model by Goldblatt and his colleagues in 1934.

At the turn of the nineteenth century, Tigerstedt and Bergman (1898) aware of the association between nephritis and hypertension observed by Bright, attempted to test the Brown-Sequard hypothesis with respect to the role played by the kidney. These investigators homogenised fresh rabbit kidney in saline and injected the supernatant fluid intravenously into other rabbits. At each attempt, injection of the kidney extract into the recipient rabbits caused a rise in blood pressure that started within 10 seconds, peaked at 2 minutes and...
disappeared in 20 minutes. They observed a biphasic pressor response, the nature of which they were unable to explain. They further demonstrated that the same pressor substance was only present in the renal venous and not in the arterial blood or urine. They demonstrated that the new pressor substance, which they called renin, was water soluble, non-dialyzable, heat labile and that it was only present in the renal cortex. Repeated injections of renin had a lesser effect upon blood pressure (i.e. tachyphylaxis occurred). They further established that the pressor action of renin may be mediated by an indirect effect on vascular smooth muscle.

The discovery that the rise in blood pressure could be brought about by a circulating humoral substance lay dormant for the next 40 years primarily because other investigators failed to confirm the observations made by Tigerstedt and Bergman. However, the interest was revived after the work of Harry Goldblatt into renovascular hypertension in the 1930's. Goldblatt on the basis of his own pathological observations realised that intrarenal arterial and arteriolar narrowing was almost invariably associated with high blood pressure in man (Goldblatt, 1947). To test the possibility that the renal vascular abnormality might be primary i.e. the cause rather than the result of hypertension, Goldblatt and his co-workers in their classic work (Goldblatt, Lynch, Hanzal and Summerville, 1934) measured systolic blood pressure of conscious dogs by a carotid or van Leersum loop method before and after constriction of both renal arteries. In a series of experiments using adjustable silver clamps, they demonstrated that initial constriction of only one renal artery resulted in a slight rise in blood pressure which then fell to normal but partial constriction of both renal arteries resulted in persistent elevation of
blood pressure (2-kidney hypertension). A rise in blood pressure was also produced by constriction of only one of the renal arteries and removal of the contralateral kidney (1-kidney hypertension). Thus varying the degree of constriction of the renal arteries resulted in a benign or malignant form of hypertension. Goldblatt speculated that in this condition "there may be an accumulation or new formation of some substance ——— which may effect a pressor action like that of a hormone" (Goldblatt et al, 1934). It was also shown that constriction of other blood vessels such as the femoral and splenic arteries had no effect on blood pressure. Thus, one of the greatest advances made in experimental hypertension by clamping of the renal arteries was the induction of pathological vascular lesions which resembled those seen in the humans (essential or primary hypertension).

Further studies by Goldblatt and co-workers (Goldblatt, 1947) demonstrated that neural factors were not responsible since complete sympathectomy did not prevent the development of elevated pressure following renal artery constriction, that hypertension could be induced in species other than the dog, and that adrenal medulla was not essential but the adrenal cortex played a significant role. Goldblatt’s group further investigated the role of humoral mechanisms of the kidney by ligating the renal veins and maintaining renal artery constriction adequately to produce hypertension. They found that, although the blood pressure returned to normal, uraemia subsequently developed. Thus the discovery of a reproducible technique of inducing hypertension in experimental models, without appreciably affecting renal excretory function and which also closely resembled human renovascular hypertension, provided a great impetus to the modern study of hypertension.
After these experiments it was generally believed that an ischaemic kidney released a vasopressor substance(s). This stimulated a large volume of research. While many investigators confirmed the findings of Goldblatt and his group (Page, 1935; Prinzmetal and Friedman, 1936), others reported negative results in the search for the circulating humoral agent(s) in hypertensive animals (Collins and Hoffbauer, 1937; Prinzmetal, Friedman, and Oppenheimer, 1938). Harrison and co-workers (Harrison, Blacock and Mason, 1936) attracted by the postulated role of the ischaemic kidneys demonstrated that injections of renal extracts obtained from hypertensive rats elicited a pressor response in the majority of rats whereas extracts from normal rats produced little or no response at all. Pickering and Prinzmetal (1938) confirming Tigerstedt and Bergman's work attributed the failure of other workers to demonstrate the vasopressor substance to the vasodepressor effects of anaesthesia. The use of alcohol extraction procedures also accounted for several negative reports in the early decades of the twentieth century. Thus finally an attempt was being made to associate the original findings of renin by Tigerstedt and Bergman with the renovascular hypertensive model developed by Goldblatt and co-workers.

The thesis that some form of pressor agent was present in the renal venous blood was confirmed by elegant renal transplantation studies of Houssay and Fasciolo (1937). They reported that transplants of an ischaemic kidney into the neck of a nephrectomised dog raised the blood pressure of the recipient animal whereas control experiments using normal dogs as kidney donors did not change the blood pressure. Further work demonstrated that the renal venous pressor agent produced vasoconstriction in the perfused hindlimb of the toad, particularly if the blood was removed from dogs with acute hypertension of only few days duration.
In 1939, Braun-Menendez, Fasciolo, Leloir and Munoz showed that the pressor substance, unlike renin, was soluble in acetone, thermostable, dialyzable and that it could be derived from renin by incubating it with plasma. They called this vasopressor agent hypertensin. At the same time, Page together with Helmer (Page, 1939; Page and Helmer, 1940) found that renin failed to cause vasoconstriction when perfused in saline through the vasculature of an isolated rabbit ear preparation and vasoconstrictive property was instead restored by substitution of fresh blood as the perfusate. These investigators, unaware of the other group’s findings, also showed that incubating renin produced a substance which they named angiotonin (Page and Helmer, 1940). It was soon realised that hypertensin and angiotonin were the same substance and the two groups later agreed upon a common terminology, renaming the pressor substance angiotensin, and calling the plasma substrate, angiotensinogen (Braun-Menendez and Page, 1958).

To complete the chain of events, the precise site of renin formation was not then known. Goormaghtigh (1939) using different fixing agents showed that certain cells lining the renal afferent arterioles, close to the glomeruli, contained acidophil or basophil granules which suggested an endocrine function. Upon constriction of a renal artery in dogs or rabbits, these cells increased in number and size which made him suspect they gave rise to a "hypertensive substance present in the ischaemic kidney". Although these findings were disputed at the time, they were confirmed initially by Cook, Gordon and Peart (1957) and conclusively by Edelman and Hartcroft (1961) using fluorescent antibody techniques.

The next major advances in the renin-angiotensin story occurred when Skeggs and his colleagues isolated and sequenced the decapeptide
angiotensin I (Skeggs, Kahn and Shumway, 1951). Later purification and elucidation of the amino acid sequence of angiotensin I from horse (Skeggs, Marsh, Kahn and Shumway, 1954) and ox serum (Elliot and Peart, 1956) was reported. However, the decapeptide from the two sources differed in the amino acid sequence. In the horse, isoleucine occupied position 5 in the angiotensin molecule whereas in the ox, this position was taken up by valine (see Figure 2.1). Purification of "the hypertensin-converting enzyme" (Skeggs et al, 1951) showed that it was responsible for the production of the vasoactive octapeptide, angiotensin II from angiotensin I and that it was several times more potent than noradrenaline (Peart, 1956). Angiotensin II was soon synthesized by Bumpus and co-workers (Bumpus, Schwarz and Page, 1957). Finally, the development of a radioimmunoassay for measurement of plasma renin activity by Haber and his colleagues (Haber, Koener, Page, Kliman and Purnode, 1969) replaced difficult and less reliable bioassay procedures. Knowledge of the physiology and components of the renin-angiotensin system enabled antagonists of angiotensin II to be developed (Marshall, Vine and Needleman, 1970; Pals, Masucci, Sipos and Denning, Jr., 1971). At the same time the demonstration by Bakhle (Bakhle, 1971) that the venom peptides from Bothrops jararaca were potent inhibitors of the angiotensin converting enzyme and the synthesis of the nonapeptide converting enzyme inhibitor by Ondetti and his group (Ondetti, Williams, Sabo, Plusoec, Weaver and Kocy, 1971) also produced a powerful tool for analysing the role of the renin-angiotensin system in the pathogenesis of hypertension.

1.2 Early breakthrough and concepts in experimental approach to essential hypertension

In the 1930's, investigators as well as searching for the humoral
origin of hypertension also made attempts to localise the increased peripheral resistance and to define its nature (Pickering, 1936; Prinzmetal and Wilson, 1936).

Pickering (1936) concluded from his work in humans that the increase in resistance was not due to an increase in the blood viscosity but it was to be found in the smallest precapillary vessels. He noted that resistance remained elevated even after vasodilatation and even suggested the possibility of an altered vessel design. Similar results and conclusions were made by Prinzmetal and Wilson (1936). However, these findings, important from the haemodynamic point of view, were still opposed by the postulated stable humoral agent.

Extending Goldblatt's work, Wilson and Byrom (1939,1941) showed that the 'clamped' kidney was relatively protected from damage due to the raised pressure and that the opposite (or "intact") kidney showed focal destructive vascular lesions (fibrinoid arterial necrosis). Extirpation of the ischaemic kidney or removal of the constricting clip did not abolish hypertension if there was extensive damage to the contralateral kidney. This fundamental observation indicated that it was mainly the mechanical stress that initiated the effects on the blood vessel walls and not a circulating toxin. The authors put forward the concept of a "vicious circle" set in motion (Wilson and Byrom, 1941) by the stenosed kidney because the structural changes produced in the 'normal' kidney by the rise in blood pressure tended to perpetuate and exacerbate hypertension.

Further experiments on the rabbits by Pickering (Wilson and Pickering, 1938; Pickering, 1945) showed that renal hypertension could not be explained simply in terms of humoral hypothesis. It was shown...
that the renin content of the ischaemic kidney in rabbits (Goldblatt 1-kidney 1-clip hypertension) was raised in the first week of hypertension but fell to normal in the chronically hypertensive animals. Further, early removal of the ischaemic kidney returned the blood pressure to normal, but this was not always the case if hypertension had lasted 2-3 weeks. Grollman (1944) made similar observations in rabbits and put forward a theory of deficiency of some depressor factor normally produced by the kidney. Thus, later Pickering (1945) concluded that in chronic experimental hypertension "a new non-renal factor plays an important and perhaps the chief role in maintaining the raised pressure". Suspecting the extra-renal factor to be the blood vessels themselves, Pickering (1945) found a relationship between the occurrence of vascular lesions and the level of blood pressure measured in conscious rabbits. However, in anaesthetised rats, Wilson and Byrom (1941) found no such relationship which suggested that lesions were due to combination of vasoconstriction and blood pressure. This apparent different interpretation caused confusion and led to the suggestion that the renin-angiotensin system may in some way exert "vasculotoxic" effects. This controversy has persisted to the present day although 20 years later Giese (1966) clearly showed that vessel wall lesions occur, irrespective of the pressor agents, where vascular smooth muscle is distended by high blood pressure and not where vasoconstriction is maintained.

Byrom and Dodson (1949) attempted to clarify the non-renal factor in chronic hypertension. They assumed that the 1-kidney 1-clip model eliminated the possibility of secondary initiating changes previously suggested by Wilson and Byrom (1941). Removal of the constricting clip in the chronically hypertensive rats lowered the blood pressure to normal levels. This was, however, in contrast to Wilson and Byrom's
findings who had shown that removal of the clip or the ischaemic kidney with the other kidney intact in chronic hypertension resulted in persistently elevated blood pressure due to the vascular changes in the untouched kidney.

Further investigations into the surgical reversal of hypertension by Floyer (1951) showed that in the 2-kidney 1-clip model, removal of the constricting clip in rats with hypertension of more than 8 weeks failed to lower blood pressure in 50% of the rats. Removal of the contralateral, previously untouched kidney but not the unclipped kidney, however, returned the blood pressure to normal. Total nephrectomy in the 1-kidney model with early (7–10 days) and chronic (>8 weeks) hypertension resulted in persistent hypertension. In a control group of normal rats, bilateral nephrectomy resulted in an insignificant number developing hypertension ("renoprival hypertension") confirming the previous report of Braun-Menendez and Von Euler (1947). Total adrenalectomy caused a lowering of blood pressure in both models of Goldblatt hypertension. If, however, 1% saline was substituted for drinking water after bilateral adrenalectomy, hypertension persisted. Floyer came to the conclusion that the adrenal glands were in some manner responsible for the maintenance of persistent hypertension in the rat and that they were linked to the control of salt metabolism. This was developed into the hypothesis that electrolyte balance in the muscle cells and presumably other cells of the cardiovascular system might be the factor controlling blood pressure and was based on the demonstration that increased intracellular sodium was present in all tissues of hypertensive rats (Laramore and Grollman, 1950; Tobian and Binion, 1952). Selye (Selye, Hall and Rowley, 1943) further emphasised the involvement of salt as a
factor in hypertension using deoxycorticosterone acetate. In a later study, Floyer (1955) concluded that the kidney maintained normal blood pressure by renal inactivation of the extra-renal pressure mechanism which was independent of its excretory function and could be reactivated by unclipping to restore the blood pressure to normal.

Apart from the search that ensued in search for the extra-renal mechanism, a quite different approach was made by Folkow and co-workers (Folkow, Grimby, and Thulesius, 1958). They examined the relationship between vascular resistance, smooth muscle tone, and blood vessel wall distensibility in resistance vessels. Folkow has argued that blood vessels that are chronically exposed to a raised distending pressure develop medial hypertrophy with a concomitant increase in the vessel wall thickness to lumen ratio. Thus the theory of structural changes in the resistance vessels eventually confirmed Johnson's remarkable observations of medial hypertrophy in arterioles over a century ago (Johnson, 1868).

In the 1950's and 1960's, it was finally appreciated that hypertension is not a single disease but a multifactorial one (Page, 1949; Pickering, 1968). Page's "mosaic theory" (Page, 1949,1982) has recently been re-emphasised in a recent review by Folkow (1982), although it is more properly a descriptive system than a scientific predictive theory.
CHAPTER - 2

THE RENIN–ANGIOTENSIN SYSTEM
The pharmacological activities of the renin-angiotensin system are better understood today and their inhibition by drugs in vivo has provided a powerful tool for assessing the physiological and pathological roles of the system in hypertension. This chapter therefore briefly reviews the renin-angiotensin system.

2.1 General aspects

The renin-angiotensin system is a cascade of enzymes. The system has been shown to play an integral and an important role in blood pressure homeostasis by its ability to alter peripheral vascular resistance as well as to produce changes in volume and electrolyte balance of body fluids. The system is also linked with autonomic vascular control mechanisms, to the kallikrein-kinin system and to the prostaglandins. The renin-angiotensin system yields a most potent vasopressor octapeptide, angiotensin II, via an enzymatic pathway (Figure 2.1) and functions as the main effector hormone.

Recently the renin-angiotensin system has been excellently reviewed by Peart (1975), Peach (1977) and Swales (1979). Because of the nature and enormity of the subject, only the major important physiological components of the system will be described.

2.2 Components of the renin-angiotensin system

Angiotensin is a polypeptide formed as a result of the reaction between the proteolytic enzyme renin and a plasma substrate (Figure 2.1). The components of this system are renin substrate, renin, angiotensin I, angiotensin converting enzyme, angiotensin II, peptidases and various smaller peptide fragments.
FIGURE 2.1: Pathways for peptide formation. R=alpha globulin. The structure of angiotensin II is that in humans, dogs, rats and many other mammals. Bovine and ovine angiotensin II have valine instead of isoleucine at position 5.
2.2.1 Renin substrate

Renin substrate (also known as angiotensinogen) is a glycoprotein largely synthesized by the liver and is widely distributed in the extracellular fluid. The amount of renin substrate and also its biological properties vary from species to species. The regulation of its secretion is of significance because its availability affects the level of plasma renin activity. Its rate of secretion into the peripheral circulation is variable such that a constant level of plasma substrate is maintained despite alterations in plasma renin concentrations (Blair-West, 1976). The control mechanisms of substrate formation and release are not clearly understood but its rate of production is known to increase or decrease in some physiological and pathological situations.

2.2.2 Renin

Renin is a glycoprotein found predominantly in the kidney and has been a focus of considerable research (Inagami and Murakami, 1977). Renin catalyses the formation of a decapeptide, angiotensin I, from renin substrate in plasma or lymph (Figure 2.1).

I. Storage and synthesis - Within the kidney, renin is confined to the outer zone of the renal cortex. There is no renin in the renal medulla where glomeruli are not present. In 1939, Goormaghtigh proposed that renin is synthesized, stored and secreted by the myoepitheloid cells, also known as the juxtaglomerular (JG) cells. These cells are groups of large epithelial like cells which contain cytoplasmic granules and are believed to be modified smooth muscle cells. They are located in the media of the afferent arteriole. The macula densa is an area of modified tubular epithelium where the distal tubule comes into
PLATE 2A: The juxtaglomerular apparatus.
close apposition with the glomerulus, to form a triangle with the afferent and efferent arteriole, the space in the adjacent connective tissue being filled with the lacis cells. This complex together with the JG cells is called the juxtaglomerular apparatus (Plate 2A). The JG cells have also been shown to be innervated by adrenergic nerve fibres (Nilsson, 1965), the function of which is described later.

II. Extra-renal renins (The iso-renin angiotensin system) - Enzymes with renin-like activity have also been described in the uterus, placenta, brain and brainstem, adrenal glands, large arteries and veins of various species and in the submaxillary glands of the white mouse (Ganten, Hutchinson, Schelling, Ganten, and Fisher, 1976). The physiological role of these enzymes has not been ascertained in detail.

III. Properties of renin - Renin exhibits a high degree of substrate specificity to cleave the plasma substrate molecule at leu-leu bond (Figure 2.1) to generate angiotensin I. The reaction takes place chiefly in the systemic circulation and probably within the blood vessel walls (Gould, Skeggs and Kahn, 1964; Thurston, Swales, Bing, Hurst, and Marks, 1979). The half-life for disappearance of renin in circulation varies from 10-90 minutes depending upon the species and conditions of the investigation. In normal rats, the average half-life of renin in circulation was 11 minutes. Inactivation of renin primarily involves the liver with additional renal loss (Schneider, Davis, Baumber, and Johnson, 1970).

Renin has been found to be in both active and inactive forms. However, activation has been shown to occur without a change in the molecular weight of the enzyme (Day, Lüetscher, and Zager, 1976) or alternatively there might be a conformational change in the renin
molecule. The significance of the role of inactive forms of renin in vivo has not been elucidated to date and the present concept of inactive renin being a hormone precursor has not been verified (Leckie, 1981).

IV. Control and mechanisms of renin release - Secretion of renin by the kidney is influenced and controlled by multiple interrelated factors which work directly or indirectly on the kidney. Renin secretion is increased by stimuli which decrease the extracellular fluid volume and blood pressure or increase the sympathetic output. Principal stimuli that promote renin release include constriction of the aorta or renal artery, haemorrhage, dehydration, diuretics, sodium and potassium deprivation, malignant hypertension and cardiac failure in some cases. It is now clear that in many situations, the rate of renin release is altered by simultaneous changes in several variables which act through one or more of the three major groups of mechanisms (Davis and Freeman, 1976). These include:

(a) Intrarenal receptors.

i) The renal vascular receptors are located in the media of the afferent arteriole and they might act as stretch receptors so that changes in renal perfusion pressure would bring about alteration of renin release (Tobian, Tomboulian, and Janecek, 1959).

ii) The other regulatory sensor is the macula densa; the nature of the serving mechanism is still debatable.

Present evidence suggests the view that renin secretion is inversely proportional to the rate of transport of sodium or possibly chloride ions in the tubular fluid at the level of macula densa.
(b) The renal sympathetic nerves and the circulating catecholamines.

There are several types of evidence suggesting an important role for the regulation of renin release by the sympathetic nervous system. The smooth muscle cells and the JG cells of the afferent and efferent arterioles have been shown to be innervated by nonmyelinated adrenergic nerve fibres (Plate 2A). There is also some evidence that the adrenergic nerve terminals innervate cells close to the macula densa. The experimental verification of the functional significance of these nerves, however, is met with great difficulty.

Renin secretion is increased when there is an enhanced activity of the sympathetic nervous system. Renin is released with direct electrical stimulation of the sympathetic nerve supply to the kidney or when catecholamines are infused (Johnson, Davis, and Witty, 1971). Renal denervation has been shown to decrease JG cell granularity and renin secretion.

The sympathetic effects on renin secretion are mediated via beta-adrenergic receptors since their stimulation augments renin release whereas alpha-adrenergic receptors are probably inhibitory (Vandongen and Peart, 1974). One difficulty in identifying the action of the alpha-adrenoceptor upon renin secretion in vivo is that a specific receptor-mediated effect may be swamped by blood pressure changes induced elsewhere in the circulation.

(c) Humoral agents.

A number of humoral agents have been shown to influence renin secretion under experimental conditions albeit the true extent of their importance in the normal regulation of renin release remains unsubstantiated.
i) Catecholamines - The role of circulating catecholamines has been described above.

ii) Sodium - The rate of plasma renin secretion varies inversely with plasma sodium concentration. This occurs without a change in the perfusion pressure in the kidney. The mechanism(s) whereby plasma sodium influences renin release remains to be established.

iii) Potassium - Plasma potassium level also exhibits an inverse relationship to renin release. This inverse relationship appears to be a consequence of an increased sodium transport to the macula densa and secondary to decreased sodium reabsorption in the proximal tubule.

iv) Angiotensin II - DeChamplain and colleagues (DeChamplain, Genest, Veyrat, and Boucher, 1966) reported that systemic infusion of nonpressor quantities of angiotensin II suppressed plasma renin activity in man. Angiotensin II has also been shown to suppress renin secretion in vitro. The physiological role of the circulating angiotensin II as an endogenous inhibitor of renin release is yet not clear. A number of studies where angiotensin II antagonist, converting enzyme inhibitors, or angiotensin II antibodies were used strongly indicate that this inhibitory action, perhaps directly on the JG cells, may provide a negative feedback role for circulating angiotensin II and which tends to stabilise renin secretion.

v) Antidiuretic hormone (vasopressin) - Infusion of exogenous vasopressin has been shown to inhibit renin release (Vander, 1968) whilst stimulating the release of endogenous vasopressin decrease renin secretion. The mechanism responsible appears to be a direct action of this peptide on the JG cells (Bunag, Page, and McCubbin, 1967).
vi) Prostaglandins - Recent work, in vivo and in vitro, suggest that prostaglandin synthesis in renal cortical structures (possibly the wall of the afferent arteriole) represent an important step in the mechanism regulating the release of renin. In addition, prostaglandins formed in the renal cortex seem to participate in the control of renal blood flow and glomerular filtration rate and may, thereby, affect secretion of renin. It appears from experiments in both renal slices and intact kidney preparations that prostaglandins \( \text{PGI}_2 \) and \( \text{PGE}_2 \) are involved in stimulation of renin secretion (Weber and Siess, 1980).

In addition to those factors mentioned above, there may be other hormone substances, possibly an unidentified hormone or a circulating plasma factor, that may influence renin secretion.

2.2.3 Angiotensin I

The decapeptide angiotensin I is formed as a result of reaction between renin and renin substrate which takes place mainly within the circulation and possibly in other tissues where renin substrate and renin are both present. In addition to the hydrolysis of angiotensin I by the converting enzyme, it can be degraded by plasma and tissue aminopeptidases to form a nonapeptide, des-Asp\(^1\)-angiotensin I. This is then susceptible to further hydrolysis by the converting enzyme to yield the heptapeptide des-Asp\(^1\)-angiotensin II (angiotensin III) which can modulate converting enzyme activity by product inhibition (Figure 2.1).

Release of renin is inhibited by angiotensin I, angiotensin II, and des-Asp\(^1\)-angiotensin II and the latter peptide has also been implicated in the steroidogenic actions of the renin-angiotensin system (Freeman, Davis, Lohmeier, and Spielman, 1977).
Although angiotensin I is generally regarded as an inactive precursor of angiotensin II, it does seem to have a direct pressor action at some sites. It has been shown to act directly on the adrenal medulla to release catecholamines (Peach, 1971) to facilitate the release of noradrenaline from peripheral sympathetic neurones (Johnson, Marshall, and Needleman, 1974) and to have a direct action on the central nervous system (Peach, 1977). Furthermore, angiotensin I has also been implicated in the regulation of intrarenal blood flow distribution (Itskovitz and McGiff, 1974).

Present evidence suggests that there is no specific "receptor site" for angiotensin I. At physiologic concentrations, the decapeptide exerts little or no action although at high concentrations, angiotensin I can directly stimulate all tissues that respond to angiotensin II.

2.2.4 Angiotensin converting enzyme

Angiotensin converting enzyme, ACE (Kininase II; dipeptide carboxy hydrolase) is an exopeptidase that catalyses the removal of a carboxy-terminal dipeptide residue from a variety of polypeptide substrates in a non-specific manner. The enzyme hydrolyses the bond between the eighth and ninth aminoacids, phenylalanine and histidine, of the angiotensin molecule (Figure 2.1) to form the octapeptide angiotensin II (Oparil, Sanders, and Haber, 1970). The same enzyme is also responsible for the degradation of nonapeptide bradykinin to inactive peptide fragments. It has been shown that converting enzyme is identical to kininase II which is a bradykinin degrading enzyme (Erdos, 1975). Thus, converting enzyme can modulate blood pressure in one of two ways, viz. activate generation of a potent vasopressor agent angiotensin II and, inactivate potent vasodepressor peptide,
bradykinin.

Converting enzyme activity has been found in a wide variety of tissues from many species. The enzyme is present in plasma but the rate of conversion of angiotensin I to angiotensin II is too slow to account for the immediate action of the decapetide in vivo (Ng and Vane, 1967). Converting enzyme is found predominantly in the vascular endothelium of the lungs. Pulmonary vessels are now regarded as one of the main sites for the generation of angiotensin II (Aiken and Vane, 1970). In addition, the enzyme has also been demonstrated in the brain, kidney, heart, liver, uterus and the peripheral vascular tissue where in particular angiotensin I may be locally converted to angiotensin II.

2.2.5 Angiotensin II

The octapeptide angiotensin II (Asp-Val-angiotensin II or Asp-Ileu-angiotensin II) is the principal effector hormone of the renin-angiotensin system. It is regarded as the most potent naturally occurring vasopressor agent known. In terms of its pressor activity on arteriolar smooth muscle, it is estimated to be 10 times more potent than noradrenaline on a weight basis and 50 times more stronger on a molar basis whereas venous smooth muscle is far more responsive to noradrenaline than to angiotensin II. The hormone has a rapid in vivo half-life of only 1-2 minutes and is cleared from the circulation in the peripheral vascular bed by tissue amino-peptidases and endopeptidases (Peach, 1977).

Angiotensin II has a variety of actions which are concerned with the regulation of the arterial pressure and volume status of the individual (Peach, 1977) but only the major activities in relation to hypertension will be reviewed here.
Actions of angiotensin II -

(a) Cardiovascular system:

i) Heart - Angiotensin II has been shown to have both positive inotropic and chronotropic effects on the heart. In vivo, the rise in the blood pressure after injection of angiotensin II is accompanied by reflex bradycardia and a fall in cardiac output.

ii) Vascular smooth muscle - Physiological effects of angiotensin II in contraction of smooth muscle is of great importance in determining resistance of vessels. In addition, stimulation of the sympathetic nervous system by the pressor agent may also aid vasoconstriction and raise the blood pressure.

iii) Vascular permeability - Angiotensin II when applied in high concentration to large arteries produce widening of the gap between the lining of the endothelial cells. This effect has been postulated to be mediated by the local synthesis of prostaglandins (Robertson and Khairallah, 1972).

iv) Regional haemodynamics - Blood flow responses to angiotensin II differ from one segment of the vascular bed to another and between different vascular regions.

(b) Nervous system:

i) Pressor response - Direct injection of angiotensin II into the brain stem or infusion into the vertebral arteries produces peripheral vasoconstriction (Scroop and Lowe, 1969). This central pressor action of angiotensin II is mediated by area postrema of the medulla oblongata and the subfornical region of the third ventricle with the effect of
raising the peripheral resistance through increased sympathetic
efferent activity (Buckley and Jandhyala, 1977).

ii) Dipsogenic response - Administration of low doses of angiotensin II into the central nervous system or intravenously in physiological amounts into the systemic circulation stimulates drinking (Fitzsimons, 1972). The effects can be blocked by competitive antagonist of angiotensin II, sar-ala-angiotensin II or saralasin.

iii) The neurosecretory cell of the supraoptic nucleus are sensitive to angiotensin II and on stimulation release antidiuretic hormone, ADH (Malvin, 1971). However, intravenous administration of angiotensin II does not increase the levels of ADH (Severs and Daniels-Severs, 1973).

iv) Sympathetic nervous system - When the sympathetic nerves are stimulated angiotensin II augments the release of noradrenaline into the synaptic cleft and so enhance the vasoconstrictor action of the sympathetic activity (Malik and Nasjletti, 1976; Zimmerman, 1978).

There are several possible explanations for the indirect effects of angiotensin. It has been suggested that

a) angiotensin acts at the site of excitation-contraction coupling in smooth muscle, thereby facilitating adrenergic discharge, or

b) vascular smooth muscle becomes sensitized as a result of stimulation of alpha-adrenergic receptor sites, so that other stimulating agents interacting with different sites likewise become sensitized.

(c) Kidney:

The renal vascular bed is highly sensitive to angiotensin II and the
effects may be an intrarenal vascular effect.

It is thought that normal circulating levels of angiotensin II play a direct renal role in the control of sodium, potassium and water homeostasis. Angiotensin II exerts a direct stimulating effect on sodium reabsorption independent of additional changes such as on aldosterone or glomerular filtration rate (Johnson and Malvin, 1977). Mendelsohn (1979) has demonstrated the local occurrence of angiotensin II in the rat kidney and suggested the possibility of an intra-renal renin-angiotensin system with angiotensin II acting as a local regulating hormone. The role of this system in the control of renal function has been recently reviewed (Levens, Peach, and Carey, 1981).

(d) Adrenal cortex:

Angiotensin II exerts an important action on the cells of the zona glomerulosa located in the adrenal cortex. Here it stimulates the secretion of aldosterone by promoting the conversion of cholesterol to pregnolone rather than promoting the release of preformed stores of aldosterone. In addition, angiotensin II as a component of the renin-angiotensin-aldosterone axis is also probably a major factor in body’s ability to respond to changes in sodium balance (Boyd and Peart, 1971; Tait and Tait, 1979). The by-product of angiotensin II, the heptapeptide des-Asp^1-angiotensin II or ‘angiotensin III’ is also believed to be involved in steroidogenesis.

(e) Adrenal medulla:

Angiotensin II acts directly on the chromaffin cells of the adrenal medulla to release catecholamines. This is probably brought about by depolarising the chromaffin cell membrane with a resultant influx of extracellular calcium into the cell and thus result in secretion. This
is probably not of physiological importance in the pressor response to angiotensin II (Peach, 1977).

2.2.6 ANGIOTENSIN III

The biological fate of the angiotensins is probably the most complicated aspects of the renin-angiotensin system. Angiotensin II is removed from the circulation by enzymatic mechanisms, specificity of which is unknown, although some peptide is taken up at the receptor sites. The number of possible metabolites of angiotensin II is considerable since most tissues contain enzymes with variable characteristics, capable of degrading the octapeptide.

Goodfriend and Peach (1975) have described angiotensin III which is a seven amino acid peptide. This peptide appears to be equally important as angiotensin II in aldosterone stimulating activity, but without its full pressor properties. It has been estimated that angiotensin III has about 40% of the direct pressor effect of that of angiotensin II.

It is yet not clear whether the heptapeptide, angiotensin III, is produced in sufficient amounts to play a substantial role in the regulation of blood pressure.

2.3 Inhibitors of the renin-angiotensin system

The chemistry of the renin-angiotensin system had been well established by the 1950’s and 1960’s. Since then, major advances have been made using natural and synthetic inhibitors of renin, converting enzyme and angiotensin II which have provided a deeper insight in understanding the role of the renin-angiotensin system in regulation of blood pressure and salt and fluid balance. This subject has been
recently reviewed by Antonaccio and Cushman (1981).

2.3.1 Antibodies

Studies with antibodies against renin, angiotensin I and angiotensin II constitute some of the oldest and least satisfying approaches to inhibition of the renin-angiotensin system. Although early studies with antisera to kidney extracts were promising in that they inhibited the development of hypertension in response to renal ischaemia (Goldblatt, Haas, and Lamfrom, 1951; Johnson and Wakerlin, 1940), subsequent studies produced variable and conflicting results (Haber and Burton, 1979). Active and passive immunisation to renin, angiotensin I or angiotensin II has prevented renal hypertension or lowered blood pressure in some studies whereas no effect was observed by others (Peart, 1975).

The variability of results with antibodies is probably related to many factors, including specificity and affinity of the antibody, levels of angiotensin II, feedback control of renin release, distribution and half-life of the antibodies (Ganten and Gross, 1979).

It has only been since 1977 that hog (Inagami and Murakami, 1977), rat (Matoba, Murakami, and Inagami, 1978), human (Yokosawa, Inagami, and Haas, 1978) and dog (Dzau, Slater, and Haber, 1979) renins have been purified to homogeneity. Goat antibody to purified dog renin was found to be very effective in decreasing the blood pressure of conscious normotensive sodium depleted dogs as well as Goldblatt 1-kidney 1-clip hypertensive dogs (Dzau, Kopelman, Barger, and Haber, 1980). The use of such specific antibodies in the future may help to clarify some of the confusing literature in the area.
2.3.2 Renin inhibitors

Naturally occurring inhibitors of renin-substrate reaction can be divided into three main groups - (i) pepstatin and analogues of pepstatin, (ii) lipids and phospholipids, and (iii) renin-substrate analogues. However, in vitro tests have indicated limited use of these inhibitors.

2.3.3 Competitive antagonists of angiotensin II

The most specific development has been that of competitive inhibitors of the angiotensin II once the identification of its amino acid sequence and synthesis of the polypeptide were accomplished. Bumpus and his colleagues (Bumpus et al, 1957) modified position 1-8 of the octapeptide and the important aspect of this work has been superbly summarized by Khosla (Khosla, Smeby, and Bumpus, 1974).

Synthesis of angiotensin II analogues with only partial agonist activity and which inhibited the responses to angiotensin II have provided a better understanding of the renin-angiotensin system (Marshall et al, 1970; Pals et al, 1971). The structure-activity relationships among angiotensin II antagonists has been excellently reviewed by Bumpus (1977). Generally, the following structural modifications of the angiotensin II molecule have provided to be the most effective for obtaining the antagonistic activity:

\[
\text{Asp - Arg - Val - Tyr - Ile - His - Pro - Phe}
\]

\text{ANGIOTENSIN II}

\[
\text{Sar - Arg - Val - Tyr - Val - His - Pro - Ala}
\]

\text{SARALASIN}
(i) substitution of the C-terminal aromatic -Phe residue with aliphatic amino acid (Ala, Ile, Leu, etc) which reduces the intrinsic activity but not the affinity to bind in or near the receptor site for native angiotensin II,

(ii) substitution of the N-terminal Asp- residue with an unnatural amino acid (Sar, MeAspNH, etc) slows the dissociation rate from angiotensin II receptors and imparts resistance to action of aminopeptidases.

An aromatic side chain at -Tyr- and an imidazole moiety at -His- are apparently essential for receptor affinity.

The most widely studied competitive antagonist of angiotensin II, in both animal and clinical studies, has been the analogue sarcosine-alanine-angiotensin II, otherwise known as saralasin. This compound was selected for extensive testing because it seemed to have less agonistic effect on vascular smooth muscle than competitive antagonists of angiotensin II. Saralasin blocks the pressor response to exogenous renin, angiotensin I and angiotensin II in a dose dependent fashion in intact animals (Pals et al, 1971). However, it lowers blood pressure only in high renin situations such as in salt restricted normotensive or hypertensive animals. Furthermore, the half-life of saralasin is 4-5 minutes compared with only 15 seconds for angiotensin II (Pettinger, Keeton, and Tonaha, 1975).

One disturbing feature of this peptide is its agonist properties when it is initially infused (Dunn, Carvalho, Kem, Higgins, and Frohlick, 1976). When renin is low e.g. in DOC-salt hypertensive rats, angiotensin II antagonists caused marked pressor effects (Mann, Phillips, Dietz, Haebara, and Ganten, 1978). Pressor effects of
saralasin have also been noted in salt loaded normotensive and "low-renin" hypertensive patients (Ganten and Gross, 1979).

Thus, none of the angiotensin II antagonists developed so far is completely devoid of agonist activity, none has oral activity, and all have only a relatively short duration of action. All of these properties are undesirable and severely limit the clinical usefulness of these compounds for the treatment of hypertension.

2.3.4 Converting enzyme inhibitors

Ferreira in 1965 found that the venom of the Brazilian pit viper, Bothrops jararaca, contained a substance that potentiated and prolonged the effects of bradykinin and suspected that it might also contain inhibitors of the angiotensin I converting enzyme. These peptides potentiated the effects of bradykinin and the mixture was consequently termed "bradykinin potentiating factor" or BPF. Later Bakhle (1968) reported that BPF also inhibited converting enzyme activity in dog lung homogenates and Yang et al (Yang, Erdos, and Levine, 1970) subsequently demonstrated that angiotensin I converting enzyme was identical to kininase II, an important kinin degrading peptidase.

Shortly after, work from two laboratories (Ferreira, Bartelt, and Greene, 1970; Ondetti et al, 1971) elucidated the structure of the bradykinin potentiating peptides, BPF, present in the venom. Further work on the peptides that could inhibit converting enzyme showed that a nonapeptide with several proline substitutions would be the most active, and this substance was designated SQ 20,881 and termed teprotide. Teprotide was found to be a poorer substrate and most useful in vivo, being a competitive inhibitor of angiotensin I converting enzyme.
TEPROTIDE (SQ 20,881)

This compound was attractive because as well as blocking the converting enzyme activity, it appeared that bradykinin blood concentrations were not increased at the drug concentration necessary for converting enzyme inhibition. However, measurements of circulating bradykinin are unreliable and this distinction between the two effects is doubtful. Teprotide is a straight chain peptide and, like the angiotensin II antagonists, must be given intravenously. It has a pharmacological half-life of just under one hour, thereby limiting its therapeutic usefulness.

In 1977, Ondetti and co-workers (Ondetti, Rubin, and Cushman, 1977) synthesised an orally active agent, captopril (D-3-mercapto-2-methylpropanoyl-L-proline, SQ 14,225), which competitively inhibited converting enzyme without acting as a substrate. Captopril is also about ten times as potent as teprotide. The other advantage of captopril is that it can be administered in both oral and intravenous forms and has a longer plasma half-life of 4.5 hours (Gavras, Brunner, Turini, Kershaw, Tifft, Cuttelad, Gavras, Vukovich, and McKinstry, 1978).

\[
\begin{align*}
\text{CH}_3 \\
\text{HS-CH}_2\text{-CH-CO-N-COOH}
\end{align*}
\]

CAPTOPRIL (SQ 14,225)

The structure of captopril is such that:

(i) the sulphydryl group of captopril appears to interact with the Zn of angiotensin I converting enzyme,
(ii) the terminal carboxy group binds to a positively charged portion of the enzyme,

(iii) the amide carboxy moiety forms a hydrogen bond, and

(iv) the proline ring and the methyl side chain help to bind the enzyme in some favourable but unknown manner.

Captopril has a greater affinity for the receptor sites than angiotensin I. Thus, because of its inhibitory effect on the converting enzyme, captopril blocks the conversion of angiotensin I to angiotensin II for biological activity, and augments the effects of bradykinin both in vivo and in vitro. However, captopril has no effect by itself on any smooth muscle tissue tested so far, nor does it inhibit contractile responses to vasoactive agents such as angiotensin II, acetylcholine, dopamine and noradrenaline.

While the mode of action of captopril was at first thought to be solely through inhibition of the converting enzyme, the long term effects of this drug in man appear to be more complicated (Laragh, Case, Atlas, and Sealey, 1980). Studies in progress will define more precisely the role of this drug in inhibition of the renin-angiotensin system.

In summary although both the angiotensin I converting enzyme inhibitors mentioned above, are effective as antihypertensive agents, one main drawback is their doubtful specificity. In addition to the selective inhibition of the formation of angiotensin II, they may, in part lower the blood pressure by potentiation of kinins. Thus after administration of the converting enzyme inhibitor, raised plasma bradykinin levels have been reported (Williams and Hollenberg, 1977).
However, this observation has not been confirmed since the specificity of kinin assays is doubtful (Carretero, Scicli, and Maitra, 1981). In addition captopril has been shown to stimulate the synthesis of prostaglandins, especially that of PGE\textsubscript{2}.

Despite blockade of the renin-angiotensin system with saralasin, intravenous administration of teprotide produced a further fall in blood pressure in rats (Thurston and Swales, 1978). Although both agents inhibit the activity of the renin-angiotensin system, this disparity may be explained by the additional properties of each inhibitor; that is, either the partial agonist properties of saralasin or the possible potentiation of kinins by the converting enzyme inhibitor. Alternatively, an unidentified vasodepressor system may be activated or possibly an unknown vasopressor system inactivated.

Thus it is evident that a fall in blood pressure by converting enzyme inhibition may not be an entirely specific response to assess the role of the renin-angiotensin system in hypertension (Swales, 1978).
CHAPTER - 3

VASCULAR SMOOTH MUSCLE IN HYPERTENSION
VASCULAR SMOOTH MUSCLE IN HYPERTENSION

Despite significant research accomplishments, diseases of the heart and blood vessels remain the leading cause of death in Westernised countries. High blood pressure is recognised as one of the major risk factors for the development of premature cardiovascular disease.

The role of vascular smooth muscle has attracted a growing interest. It has been widely accepted that the primary manifestation of hypertension is an increase in peripheral vascular resistance (Folkow and Neil, 1971). Attention has therefore been focused at the possibility that there is, at least, in some forms of hypertension, a defect in the vascular wall as the proximate cause of the increased peripheral resistance since vascular reactivity to pressor agents has also been observed to be augmented. The following account will review the evidence for vascular wall abnormality in both human and animal models with hypertension.

3.1 Studies In Humans

A number of studies have been carried out in humans, though often without proper controls to draw definite conclusions. Griesman (1952) reported a greater constriction of vessels of the nail bed with noradrenaline in hypertensive than in normal subjects. Mendlowitz and co-workers (Mendlowitz and Naftchi, 1958; Mendlowitz, 1973) also noted that the resistance vessels in the digital beds of hypertensive subjects showed an increased response to infusion of noradrenaline but this was considered to reflect a genetic abnormality in the local turnover of noradrenaline. Other early quantitative studies on hypertensive regional vascular beds by Duff (1957) and by Doyle (Doyle,
Fraser, and Marshall, 1959) were of particular importance because different concentrations of intra-arterially administered vasopressor agents were used during recordings of both pressure and blood flow. Furthermore, increased pressor responsiveness to angiotensin II was also shown in patients with essential hypertension (Kaplan and Silah, 1964). This increase in reactivity to noradrenaline and angiotensin II was either believed to represent an abnormality or it could simply reflect a physiologic adaptation to a low endogenous activity of the sympathetic nervous system or the renin-angiotensin system.

Conway (1963) postulated that structural vascular changes were responsible for the increase in vascular resistance and vascular reactivity. He found that the vascular resistance in the forearm of hypertensive patients remained greater than that in the normotensive subject even during complete vasodilatation. Sivertsson (1970) arrived at similar conclusions on studies of hand blood flow. He also looked at vascular responses to graded increase of noradrenaline infusion and showed that whereas smooth muscle sensitivity to noradrenaline in essential hypertension was largely unchanged, the increase in resistance was much greater than in controls.

The haemodynamic importance of pre-capillary media thickening in hypertension has been considered in detail by Folkow (1978). Folkow has emphasised that the increased responsiveness to noradrenaline and angiotensin II could result from structural changes in the resistance vessels and need not be due to a change in the functional properties of the smooth muscle - blood vessels that are chronically exposed to a raised distending pressure develop medial hypertrophy with a concomitant increase in the wall to lumen ratio and it has been suggested that this could be responsible for the exaggerated response
to both vasoconstrictor and vasodilator agents as a direct consequence of the altered vascular geometry.

It therefore remains unclear whether or not there is a functional or a structural abnormality of the vascular smooth muscle in essential hypertension. Far more penetrating analysis can be carried out in a variety of experimental animal models with hypertension. These might be representative of the multifactorial mechanisms underlying essential hypertension. Experimental animal models share many of the characteristics with essential hypertension since many of them have been developed by utilization of possible aetiological factors in human hypertension i.e. excessive sodium intake, increased activity of the renin-angiotensin system, increase in mineralocorticoid production and genetic abnormality. The major disadvantage, however, is that they are not necessarily the appropriate models for changes in vascular smooth muscle in human essential hypertension. Nevertheless, they are widely studied and the use of genetically hypertensive rats has been considered as one of the most reliable models for studying essential hypertension (Yamori, 1982) while the 2-kidney 1-clip Goldblatt hypertensive model, for example, is an excellent model for investigating the mechanism(s) underlying renovascular hypertension.

3.2 Studies In Animals

Many studies on the reactivity of isolated perfused vessels or vascular beds of normotensive and hypertensive animals have been made. Although most of these studies demonstrate a nonspecific increase in vascular reactivity of blood vessels in hypertension, it has been difficult to determine how much of the increase is due to hyperactivity of the vascular smooth muscle. "Vascular reactivity" in isolated
vascular beds is usually measured as the magnitude of an increase or a
decrease in resistance to flow produced by a given vasoconstrictor. The
magnitude of this response could depend either on an increase in the
reactivity of the vascular smooth muscle (functional property) or a
structural thickening of the vessel wall (structural property).
Structural and functional influences are often difficult to dissociate
in the true resistance vessels, which explains much of the current
controversies on vascular reactivity.

Accordingly, I will review the work carried out on vascular
reactivity in animal models with three different forms of hypertension.

3.2.1 Mineralocorticoid Hypertension

This type of hypertension is generally induced in animals by
administration of salt and deoxytocorticosterone which produces an
overall increase in peripheral resistance, the exact nature of which is
poorly understood. The rise in blood pressure can be brought about by a
combination of unilateral nephrectomy, access to water containing 0.9-
1.0% sodium chloride to drink and either intramuscular or subcutaneous
injection or silastic implants containing >10mg per kg body weight
deoxytocorticosterone. These procedures usually employed 4-5 weeks before
the study bring about an increase in blood volume and a rise in blood
pressure with a dramatic reduction in renin secretion by the kidney and
an increase in vascular sensitivity to vasoconstrictive agents (Selye
et al, 1943; Sturtevant, 1956; Brunner, Chang, Wallach, Sealey, and
Laragh, 1972).

Although deoxytocorticosterone-salt (DOC-salt) induced hypertension is
generally associated with an increase in vascular reactivity, conflicting results have been reported in the literature.
Using an in vitro preparation, isolated strips of aorta from DOC hypertensive rats failed to demonstrate hyperresponsiveness to noradrenaline when compared with normotensive control rats (Readleaf and Tobian, 1958; Mallov, 1959). Later report confirmed this finding where aortic rings from DOC hypertensive rats were either less reactive or showed unchanged reactivity compared with normotensive controls (Massingham and Shevde, 1971). The thoracic aorta showed a decreased response specifically to angiotensin II (Couture and Regoli, 1980a). In addition, responses of portal vein to angiotensin II and noradrenaline were also unchanged (Couture and Regoli, 1980a). Hinke (1965) on the other hand, found an increased response to noradrenaline but not to angiotensin II in the isolated tail arteries from DOC hypertensive rats.

Perfusion studies of isolated vascular beds, however, uniformly indicate hyperresponsiveness to vasopressor agents in DOC-salt induced hypertension. Using an isolated tail preparation, Beilin and associates (Beilin, Wade, Honour, and Cole, 1970) reported that DOC-salt treated rats showed increased reactivity to noradrenaline but not to angiotensin II. It was found that complete dose-response curves to angiotensin II were not possible because of prolonged tachyphylaxis usually encountered in all artificially perfused preparations. Increased cardiovascular reactivity to noradrenaline and angiotensin II was demonstrated in pithed preparation of DOC-salt hypertensive rats (Finch, 1971) whereas in another in vivo preparation, while there was an increase in angiotensin II response, the sensitivity to noradrenaline was unchanged or decreased (Couture and Regoli, 1980a). Enhanced pressor responsiveness to both angiotensin II and noradrenaline was also shown in whole body preparation of DOC-salt
hypertensive rats, so that the dose-response curve shifted to the left (Marks, Bing, Thurston, Russell, and Swales, 1982; Bing, Russell, Swales, and Thurston, 1982). Increased vascular response to exogenous noradrenaline (Finch and Haeusler, 1974) and also to angiotensin II (Couture and Regoli, 1980a) was shown in perfused vascular bed of hindquarter preparation of DOC hypertensive rats while an increased sensitivity to noradrenaline was also reported in the mesenteric vasculature of DOC-salt treated rats (Finch, 1971; Ekas and Lokhandwala, 1980).

It was demonstrated that the changes in smooth muscle sensitivity described above were independent of the rise in arterial blood pressure. Elegant studies by Bohr and co-workers (Hansen and Bohr, 1975; Berecek and Bohr, 1977) have shown that the changes in vascular reactivity occur in hindlimbs from DOC hypertensive animals protected from the increased arterial pressure by ligating one external iliac artery. It was found that changes in sensitivity associated with hypertension also developed in the protected vascular bed and could not be reversed. It was noted that structural resistance was lower in the occluded compared with the contralateral hindlimb which makes a role for the structural adaptation as suggested by Folkow less likely (Berecek and Bohr, 1977).

Thus, the fact that a change in vascular reactivity was observed before the rise in blood pressure could have an important bearing in the pathogenesis in this model of hypertension. In pigs with DOC implants, an increase in whole body sensitivity to noradrenaline and angiotensin II was observed as early as 2 days after implantation and before a significant rise in arterial pressure had developed (Berecek and Bohr, 1978). Vascular reactivity to noradrenaline and angiotensin
II was also shown to be increased in the renal vasculature of DOC hypertensive rats not only in the chronic stage but also in the prehypertensive stage (Berecek, Stocker, and Gross, 1980) further suggesting that changes in vascular reactivity, per se, participate in the development of this type of hypertension.

It has been suggested that an increased release of noradrenaline in the peripheral nervous system could contribute to the development and maintenance of DOC hypertension (DeChanplain, 1973). The retention and storage of noradrenaline as well as the increase in vascular reactivity is believed to be influenced by sodium intake (DeChanplain, 1973; Abboud, 1974). In vitro studies indicate significant increase in ionic turnover in the aorta from DOC hypertensive rats which precede the rise in arterial pressure (Jones and Hart, 1975). This increase in ionic permeability of the smooth muscle membrane may perhaps act to decrease the membrane potential of the vascular smooth muscle and result in increased vascular tone and responsiveness.

Plasma renin concentration has been shown to be significantly reduced in DOC-salt hypertensive rats compared with normal rats (Pettinger, Marchelle, and Augusto, 1971) and this may have some implication in determining hyperresponsiveness to angiotensin II (Bing et al, 1982).

3.2.2 Experimental Renovascular Hypertension

The most commonly used experimental model for renovascular hypertension is based upon Goldblatt's original studies of bilateral renal artery constriction in dogs (Goldblatt et al, 1934). A constricting clip or clamp is placed on one of the renal artery so that the opposite kidney may be removed (Goldblatt 1-kidney 1-clip
hypertension) or left in situ (Goldblatt 2-kidney 1-clip hypertension).

It has been suggested that during the development of renovascular hypertension, there is an enhanced reactivity of the cardiovascular system to vasopressor agents and that this contributes to the maintenance of hypertension (Skulan, Brousseau, and Leonard, 1974; Davis, 1977; Ichikawa, Johnson, Fowler, Payne, Kurz, and Keitzer, 1978).

Studies on in vitro vascular strips from hypertensive animals have produced less consistent results to the question of alterations in smooth muscle sensitivity. Early work has indicated that spirally cut aortic strips from rats with both models of Goldblatt hypertension developed same or less contractile force in response to noradrenaline than strips from normotensive control rats (Readleaf and Tobian, 1958). This was confirmed by Mallow (1959) who showed that aortic strips from 1-kidney 1-clip Goldblatt hypertensive rats failed to exhibit hyperresponsiveness to noradrenaline. In contrast, other studies have indicated that there is an increased responsiveness to noradrenaline in both aortic (Gordon and Nogueira, 1962; Field, Janis, and Triggle, 1972) and femoral artery strips (Bandick and Sparks, 1970; Bohr and Sitrin, 1970) from renal hypertensive rats. Furthermore, Holloway and Bohr (1973) have reported that the sensitivity of the femoral artery strips is increased in renovascular hypertensive rats. However, changes in vascular reactivity are not exclusively confined to the arteries. It was shown that the portal vein from 2-kidney 1-clip Goldblatt hypertensive rats was more responsive to noradrenaline than normotensive control rats (Sutter and Ljung, 1977) whereas no change to noradrenaline and angiotensin II was observed by another group (Couture and Regoli, 1980b).
Changes in vascular reactivity have been observed to occur before the rise in arterial pressure in various vascular beds perfused with either blood or artificial media of animal models with renovascular hypertension (Ogden, Brown, and Page, 1940; McQueen, 1956; Finch and Haeusler, 1974). Ogden et al (1940) using renal hypertensive rabbits found that increased vascular reactivity to vasopressin occurred before the animals developed a rise in arterial pressure. Later studies by McQueen (1956) showed that increased reactivity to noradrenaline in the isolated perfused hindquarter preparation of 1-kidney 1-clip Goldblatt hypertensive rat was evident within two days after clipping the sole kidney. In 2-kidney 1-clip Goldblatt hypertensive rats, he found that increased reactivity to noradrenaline paralleled the rise in blood pressure for up to 2 weeks. By this time, the increase in vascular reactivity was fully established and there was no further relationship between blood pressure and vascular reactivity. This is similar to the findings in the pig in which there appears to be no further increase in vascular reactivity beyond that found 1 week post DOC treatment (Berecek and Bohr, 1978). McQueen's results were later confirmed by Laverty and Smirk (1961) using a blood perfused denervated hindlimb preparation of a rat. Other evidence for increase in vascular reactivity in renal hypertensive rats was reported by Phelan (1966). In addition, sensitivity to noradrenaline was shown to be increased in the perfused hindquarter preparation of 1-kidney 1-clip Goldblatt hypertensive rat (Finch and Haeusler, 1974). In contrast, other reports documented sensitivity to noradrenaline to be unchanged in the perfused hindquarter (Lundgren, Hallback, Weiss, and Folkow, 1982) of 2-kidney 1-clip Goldblatt hypertensive rats whereas systemic sensitivity to angiotensin II was found to be decreased compared with normal rats (Marks et al, 1982).
Increases in the vasoconstrictor responses to angiotensin II and noradrenaline in blood perfused mesenteric preparations have also been demonstrated in rats (Laverty, McGregor, and McQueen, 1968; McGregor and Smirk, 1968) and in dogs with 2-kidney 1-clip Goldblatt hypertension (Greenberg, 1981). Enhanced vascular reactivity to noradrenaline in the mesenteric vasculature of 1-kidney 1-clip Goldblatt hypertensive rats was consistently demonstrated by Collis and Alps (1975, 1976).

In many of the experiments, it was found that the peripheral resistance of the denervated perfused blood vessels from renal hypertensive rats was higher than that of the control blood vessels. Such increase in peripheral resistance has been associated with the increased vascular reactivity in the perfused innervated hindlimbs of rats with renovascular hypertension (Laverty and Smirk, 1961; Finch and Haeusler, 1974). Thus Nolla-Panades (1963) demonstrated a significant correlation between the initial perfusion pressure of the hindlimb vasculature of the rat and the increase in reactivity to noradrenaline. These results are still consistent with the hypothesis that the increased reactivity and peripheral resistance may be due to an increased wall to lumen ratio of the blood vessels (Smirk, 1949; Folkow et al, 1958; Folkow, 1978).

The changes in vascular responsiveness in both models of Goldblatt hypertension may be related to the level of circulating renin. In the early stage of 1-kidney Goldblatt hypertension (1–6 weeks post clipping) increased renin activity has been demonstrated in the dog (Bianchi, Tenconi, and Lucca, 1970) whereas peripheral renin levels in the 1-kidney 1-clip hypertensive rat are usually low or normal (Bumpus, Sen, Smeby, Sweet, Ferrario, and Khosla, 1973; Bengis and Coleman,
administration of angiotensin I converting enzyme inhibitor, captopril, has been shown to lower blood pressure in this model (Sen et al, 1979), while, in the chronic phase (>16 weeks post clipping), plasma renin and angiotensin II levels are usually normal (Brown, Davis, Olichney, and Johnson, 1966; Miksche, Miksche, and Gross, 1970). In contrast, the 2-kidney 1-clip Goldblatt model of hypertension is characterised by a transient increase in renin only in the early phase (Miksche et al, 1970; Brunner, Kirshman, Sealey, and Laragh, 1971; Koletsky, Pavlicko, and Rivera-Valez, 1971; Leenen, DeJong, and DeWield, 1973; Thurston and Swales, 1976) and administration of captopril was shown to lower blood pressure.

Recently, much attention has been focused on the effect of surgical reversal of hypertension, by either unclipping or nephrectomy of the ischaemic kidney, to study the pathophysiology of renovascular hypertension at a normotensive blood pressure level. However, evidence to date indicate that the fall in blood pressure with surgical manipulation varies with the model and duration of hypertension. Thus, removal of the ischaemic kidney in the early phase of 2-kidney 1-clip hypertension, the blood pressure returns to normal within 24 hours (Wilson and Byrom, 1941; Koletsky and Rivera-Velez, 1970; Gross, 1971; Thurston and Swales, 1974; Russell, Bing, Thurston, and Swales, 1982) though, in the chronic phase persistent elevation of blood pressure has been reported (Wilson and Byrom, 1941; Koletsky and Rivera-Velez, 1970; Thurston and Swales, 1974; Thurston, Bing, and Swales, 1980). Removal of the kidney in 1-kidney 1-clip model failed to lower blood pressure (Floyer, 1955) possibly due to resultant volume expansion. Removal of the constricting clip, on the other hand, lowers blood pressure to
normal levels at any stage of hypertension within 24 hours in both phases of 2-kidney 1-clip hypertension (Gross, 1971; Thurston et al, 1980; Russell et al, 1982) and 1-kidney 1-clip model (Byrom and Dodson, 1949; Floyer, 1955) and this further throws doubt on the involvement of vascular hypertrophy as suggested by Folkow (Folkow, 1978) in renovascular hypertension. It was shown that the rapid normalisation of blood pressure in the 2-kidney 1-clip hypertensive model was followed by a gradual reversal of the enhanced reactivity (Ten Berg and DeJong, 1980; Lundgren, 1974; Lundgren et al, 1974) which takes about 2 weeks. Furthermore, the longer the duration of hypertension, the slower and more incomplete the regression of the vascular changes (Lundgren, 1974; Lundgren et al, 1974).

3.2.3 Spontaneously Hypertensive Rats

The breeding of a colony of rats specifically selected for high blood pressure (Smirk and Hall, 1958; Okamoto and Aoki, 1963) has provided experimental models with hypertension which is not attributable to renal or endocrine malfunction. These genetically hypertensive rats, named spontaneously hypertensive rats (SHR) have been studied in an attempt to discover the underlying causes in the initiation and maintenance of raised blood pressure in human essential hypertension.

Despite numerous studies on these animals, on the assumption that they provide an appropriate experimental model, especially the Japanese strain (Okamoto, 1972), the question of vascular reactivity to vasopressor agents has not been conclusively resolved and much controversy still exists in the literature.

Laverty and Smirk (1961) found that the New Zealand strain of SHR
had an increased peripheral resistance in the vasculature of the hindlimb and it was shown to be positively correlated with the blood pressure of the whole animal. Elevated resistance has also been reported in the perfused whole body (Folkow, Hallback, Lundgren, and Weiss, 1970b) and in several isolated vascular beds of the Japanese strain (Folkow, Hallback, Lundgren, and Weiss, 1970a, 1971a; Haeusler and Heafely, 1970; Finch and Haeusler, 1974). It appears that a large part, if not all, of the hypertension seen in the adult SHR is the result of alterations in the peripheral vascular beds. It has been proposed that the elevated peripheral vascular resistance results from increased reactivity to normal vasoconstrictor influences such as noradrenaline (Nosaka, Yamori, Ohta, and Okamoto, 1972; Folkow et al, 1971; Haeusler and Heafely, 1970).

Laverty (1961) found that the response to noradrenaline of the blood perfused hindlimb blood vessels was greater in the New Zealand strain of SHR than in control animals. Using the perfused isolated mesenteric blood vessel preparation, it was reported that the blood vessels from the same strain of rats react more to both noradrenaline and angiotensin II than the controls (McGregor and Smirk, 1968; Laverty et al, 1968). However, later Massingham and Shevde (1971) using the same strain of hypertensive rat reported that the rings from the thoracic aorta exhibited a reduced reactivity to noradrenaline compared with control normotensive rats.

In the Japanese strain of hypertensive rats which have been developed from the Wistar strain (Okamoto-Kyoto strain), there are similar conflicting reports of vascular reactivity to vasoactive agents.
When vascular reactivity is studied in isolated perfused vascular beds, it is uniformly found that responses to vasocoestrictor agents are exaggerated. An in vivo study by Okamoto and co-workers (Okamoto, Hazama, Takeda, Tabei, Nosaka, Fukushima, Yamori, Matsumoto, Haebara, Ichijima, and Suzuki, 1966) reported that vascular responsiveness to an intravenous injection of noradrenaline in SH rats was greater than in normotensive rats while in another study the angiotensin II response was enhanced and sensitivity to noradrenaline unchanged (Shibayama, Mizoguchi, and Sokabe, 1971; Couture and Regoli, 1980b). Studies of the peripheral vasocostrictive actions of noradrenaline and angiotensin II in isolated perfused hindquarter preparation of SH rats uniformly indicate hyperresponsiveness. Folkow and co-workers (Folkow et al, 1970a, 1970b) demonstrated that the dose-response curve to noradrenaline infusions was significantly steeper in addition to an increase in maximal pressor response when compared with normotensive control rats. There are many other reports of increased pressor responsiveness to noradrenaline in the perfused hindquarter preparation of SH rats compared with normotensive rats (Nosaka et al, 1972; Finch and Haeusler, 1974; Lais, Shaffer, and Brody, 1974; Lais and Brody, 1975, 1978; Bhattacharya, Dadkar, and Dohadwala, 1977; Lee, Walsh, and Tobia, 1980; Cheng and Shibata, 1980; Couture and Regoli, 1980b).

Studies of other vascular beds such as the isolated mesenteric and renal preparations also exhibit vascular hyperresponsiveness. Thus Haeusler and Heafely (1970) reported greater reactivity to noradrenaline of the perfused mesenteric arteries of the SH rats compared with normotensive rats. This was later confirmed where increased vascular reactivity to noradrenaline and angiotensin II was found (Bhattacharya et al, 1977). Enhanced reactivity to noradrenaline was also found in the isolated renal preparation (Folkow et al, 1971a;
Collis and Vanhoutte, 1977). However, no difference in the contractile response of isolated perfused renal artery to noradrenaline was observed between the SH rats and the normotensive Wistar rats (Haeusler and Finch, 1972).

On the other hand, the vasoconstrictor hyperresponsiveness reported in the various vascular beds of the SH rats are in direct conflict with the in vitro studies of isolated vascular preparations. A survey of reactivity of such isolated vascular strips indicate that, in general, the contractility of vascular smooth muscle to a wide variety of vasoconstrictors is usually depressed in the SH rats (Couture and Regoli, 1980b). Thus, Clineschmidt et al (Clineschmidt, Geller, Govier, and Sjoerdsma, 1970) and Hallback et al (Hallback, Lundgren, and Weiss, 1971) found no difference between aortic strips from SH and normotensive Wistar rats in their responsiveness to noradrenaline. Shibata et al (Shibata, Kurahashi, and Kuchii, 1973) found significantly less contractile response of aortic strips to noradrenaline and angiotensin II amongst other agents in SH rats compared with normotensive rats. This is consistent with the early observation of Spector et al (Spector, Fleisch, Maling, and Brodie, 1969) who reported that spirally cut aortic strips from SH rats developed less contractile force in the presence of noradrenaline than did those from normotensive animals. This type of decreased response was also found to be true for non-specific agents such as potassium chloride solution.

In addition, other reports indicate that vascular changes in the hypertensive state are not limited to arteries. Thus Greenberg and Bohr (1975), Sutter and Ljung (1977) and later Couture and Regoli (1980b) demonstrated portal veins to be more responsive to noradrenaline
compared with normal Wistar rats while the aortic strips were not so responsive. Since the portal vein is not directly exposed to the increased arterial blood pressure this suggests that local hypertension, per se, is not responsible for the altered properties of the portal vein.

The renin-angiotensin system has not been considered to play an important role in hypertension in SH rats (Sokabe, 1965; Koletsky, Shock, and Rivera-Valez, 1970; Sen, Smeby, and Bumpus, 1972). Plasma renin activity is reported to be normal or low (Laffan, Goldberg, High, Schaeffer, Waugh, and Rubin, 1978). The intervention of pharmacological treatment either by the use of hypotensive drugs (Folkow, Hallback, Lundgren, and Weiss, 1971b) or angiotensin I converting enzyme inhibitor, captopril, (Muirhead, Prewitt, Brooks, and Brosius, 1978) has both been shown to normalise blood pressure in the SH rats. However, the implication of the renin-angiotensin system in SH rats still remains a matter of controversy.

3.3 Extraneous factors influencing vascular smooth muscle reactivity

Studies of vascular smooth muscle from animals with different models of hypertension have revealed a number of changes which may be responsible for altered vascular reactivity. The following factors have attracted great interest and are by no means exclusive:

1. Sympathetic nervous system,

2. Sodium balance,

3. A slow pressor effect of angiotensin II,

4. Enhanced sensitivity to angiotensin II,
(5) Local vascular humoral systems (e.g. vascular renin, renal vasopressor substances),

(6) Vasopressor substances other than angiotensin II, and

(7) Non-specific changes in pressor sensitivity.

3.3.1 Sympathetic nervous system:

The involvement of the sympathetic nervous system in the maintenance of raised peripheral resistance in experimental models of hypertension continues to attract considerable attention. Although the arterioles and other resistance vessels are most densely innervated, all blood vessels except capillaries contain smooth muscle and receive motor nerve fibres from the sympathetic nervous system. The fibres to the resistance vessels normally regulate tissue blood flow and hence arterial pressure. In general, the alpha-adrenoceptors in the blood vessel walls cause vasoconstriction whereas the beta-adrenoceptors cause vasodilatation. These receptors may be involved in the increased peripheral sympathetic activity, for example, in the 1-kidney 1-clip Goldblatt model of hypertension (Dargie, Franklin, and Reid, 1977).

However, marked increase in the turnover or utilisation of noradrenaline has been shown in various organs of rats made hypertensive with DOC and salt. There is also evidence that the activity of the sympathetic nervous system may be modulated by afferent renal nerves in the development of hypertension since plasma noradrenaline levels were found to have increased in both 1-kidney 1-clip (Katholi, Winternitz, and Oparil, 1981) and 2-kidney 1-clip models of hypertension (Katholi, Whitlow, Winternitz, and Oparil, 1982). There was a significant inverse relationship between the degree of blood pressure elevation and endogenous levels of noradrenaline (DeChamplain,
However, a reduction in the turnover of noradrenaline was demonstrated in the hearts of SH rats (Louis, Spector, Tabei, and Sjoerdsma, 1968) and it was concluded that the changes in noradrenaline metabolism do not play a significant role in the pathogenesis of genetically determined hypertension but are secondary manifestations of the rise in blood pressure. Recently, SH rats have been reported to have an overactivity of the sympathetic nerves compared with normotensive control rats (Judy, Watanabe, Henry, Besch, Murphy, and Hockel, 1976).

Raised intracellular sodium in nerve endings has been shown to decrease the uptake of noradrenaline (Bogdanski and Brodie, 1969). This evidence has led to the suggestion that changes in sodium balance alter noradrenaline metabolism in such a way that salt loading results in more free noradrenaline becoming available to affect vascular smooth muscle (see later).

There is considerable evidence pointing to an interaction between the vasoconstrictor effect of angiotensin II and the sympathetic nervous system or noradrenaline in renovascular hypertension (Dickinson and Yu, 1967). Infusion of angiotensin II has been shown to potentiate the vascular effects of sympathetic stimulation or noradrenaline infusion (Sato and Masuyama, 1971). This potentiating effect of angiotensin II on the vascular smooth muscle has been attributed to an increased amount of noradrenaline being released from the nerve terminal (Love and Scroop, 1970; Zimmerman, Gomer, and Liao, 1972; Malik and Nasjletti, 1976) or to an inhibitory effect of angiotensin II on the re-uptake of noradrenaline (Khairallah, Davila, Panpanicolau, Glende, and Meyer, 1971) released into the synaptic cleft. If either of these mechanisms is operative, the net result would be to increase the
amount of noradrenaline being available to react with the vascular smooth muscle. Thus, a peripheral neurogenic action of angiotensin II might be to intensify the cardiovascular responsiveness to efferent sympathetic discharge. Day and Moore (1976) have provided further evidence that angiotensin II may potentiate the vasoconstrictor effects of noradrenaline and other pressor agents by inhibiting the active extrusion of sodium from the vascular smooth muscle cells, thereby causing a non-specific sensitisation.

3.3.2 Sodium balance:

The mechanism by which sodium regulates blood pressure and modifies vascular reactivity has generated intense research.

Prolonged removal of salt from the diet in both animals and in man is followed by a dramatic increase in renin production (Brown, Davies, Lever, and Robertson, 1964; Ganong and Borycska, 1967; Gocke, Gerten, Sherwood, and Laragh, 1969), by decreased vascular sensitivity to angiotensin II (Barraclough, Jones, Marsden, and Bradford, 1967; Hollenberg, Solomon, Adams, Abrams, and Merill, 1972) and by a slight (although inconsistent in all species) fall of blood pressure (Swales, Tange, and Thurston, 1975; Oliver and Cannon, 1978). The administration of salt induces opposite changes of renin and vascular sensitivity and provokes hypertension in some models (Brunner et al, 1972). Sensitivity to noradrenaline changes in the opposite direction of that to angiotensin II. Work of DeChanplain et al (DeChanplain, Krakoff, and Axelrod, 1968) suggest that excessive sodium may decrease tissue stores of noradrenaline and facilitate sympathetic transmission and the release of endogenous noradrenaline whereas sodium restriction may decrease autonomic transmission and may be associated with decreased
release of noradrenaline.

Balance studies demonstrated that the 1-kidney 1-clip Goldblatt model retained sodium whereas the 2-kidney 1-clip model developed negative sodium balance during the development of hypertension (Swales, Thurston, Queiroz, and Medina, 1972; Mohring, Mohring, Nauman, Philippi, Homsy, Orth, Dauda, Kazda, and Gross, 1975; Leenen and DeJong, 1975). Relief of renal ischaemia in the latter hypertensive model, whether by removal of the constricting clip or ischaemic kidney is associated with sodium retention in both early and chronic phases of hypertension (Thurston et al, 1980). If sodium played a significant role in maintaining elevated blood pressure, then, it follows that there would be a positive sodium balance in the hypertensive state and natriuresis upon reversal of hypertension.

Thus sodium retention may enhance peripheral resistance by an yet unknown mechanism. Fluid volume expansion would appear not to be an essential pre-requisite for the rise in blood pressure in renovascular hypertension (Swales, 1981a, 1981b).

3.3.3 A slow pressor effect of angiotensin II:

Since plasma renin and angiotensin II levels have been shown to be normal or near normal in established renovascular hypertensive animals and humans, it was suggested that the renin-angiotensin system may perhaps act, apart from a distinct acute vasoconstriction mechanism, by slowly developing a pressor mechanism (Brown, Cuesta, Davies, Lever, Morton, Padfield, Robertson, Bianchi, and Schalekamp, 1976).

Dickinson and Lawrence (1963) first demonstrated in rabbit that infusion of small subpressor doses of angiotensin II over several days produced a gradual rise in blood pressure. This was later shown to be
true also in the rat (Brown, Casals-Stenzel, Gofford, Lever, and Morton, 1981; Koletsky, Jackson, Hess, Rivera-Velez, and Pritchard, 1966) and in man (Ames, Borkowski, Sicinski, and Laragh, 1965; Oelkers, Schoneshofer, Schultze, Brown, Fraser, Morton, Lever, and Robertson, 1975). This rise in blood pressure cannot be prevented by adrenalectomy (Dickinson and Yu, 1967) but is prevented by dietary salt restriction (Cowley and McCaa, 1976). This suggests that perhaps sodium retention brought about by circulating angiotensin II may be responsible for the maintenance of hypertension. An alternate hypothesis suggests an involvement of the central nervous system. Dickinson and Yu (1967) found that infusion of subpressor doses of angiotensin II into intravertebral artery raised the blood pressure but had no effect in the general circulation. Furthermore, ablation of the area postrema in the medulla oblongata prevented this rise in blood pressure.

If there is a slowly developing pressor response to angiotensin II in the maintenance of blood pressure, then, this could either be brought by enhanced sensitivity to angiotensin II or increased local concentration of angiotensin II at vascular or nervous system sites due to increased tissue uptake (see below).

3.3.4 Enhanced sensitivity to angiotensin II:
Factors which alter the pressor responsiveness to angiotensin II can be of specific and non-specific nature. Examples of such specific factors are plasma concentrations of endogenous angiotensin II and the state of sodium balance. The site of action is either at the angiotensin receptor or at the site of the coupling of receptors to intracellular events (resulting in contraction of the smooth muscle cell). In addition, these receptors have little or no effect on the pressor responsiveness
to other vasoactive stimuli. An example of a non-specific factor in enhanced sensitivity to angiotensin II is an increase in wall to lumen ratio by medial hypertrophy of the resistance vessel (Folkow, 1978) - see below.

Alteration of sodium balance in the rat described earlier has shown that pressor response to angiotensin II is impaired by sodium depletion but enhanced by sodium loading (Slack and Ledingham, 1976). The pressor action of other vasoconstrictors, such as noradrenaline, is hardly affected by manipulation of sodium balance (Hollenberg et al, 1972; Samwer, Schreiber, Molzahn, and Oelkers, 1974). Such modulating action of sodium must occur either by alteration of the receptor complex or modification of the response produced by the complex. Several hypotheses explain the modification of the interaction between angiotensin II and its vascular receptor which results in an altered responsiveness to the hormone.

(a) Altered 'receptor' affinity - It has been reported that the pressor response to angiotensin II may be determined by endogenous angiotensin II. Such 'receptor affinity' for angiotensin II can be varied with changes in sodium balance or following bilateral nephrectomy - salt restriction decreasing receptor sensitivity, salt loading or bilateral nephrectomy increasing receptor responsiveness (Brunner et al, 1972). However, the basis for this concept is doubtful (Swales et al, 1975) as it is based on the volume of antiserum needed to inhibit the angiotensin II response.

(b) 'Prior receptor occupancy' - This hypothesis proposes that prior occupancy of receptors by endogenous angiotensin II determines the pressor responsiveness to exogenous angiotensin II (Thurston and Laragh, 1975). Thus the reduced pressor response to angiotensin II
observed in salt deprived rats can be increased to levels observed in salt loaded rats by blocking formation of endogenous angiotensin II by converting enzyme inhibition (Thurston and Laragh, 1975) or bilateral nephrectomy (Swales et al, 1975). Increased receptor occupancy by high endogenous angiotensin II levels probably explains the reduced pressor responsiveness to exogenous angiotensin II observed in acute stage of 2-kidney 1-clip hypertensive rats (Aoki and Masson, 1969) and the enhanced pressor response seen in DOC-salt hypertensive rats probably due to decreased receptor occupancy (Thurston, 1976; Marks et al, 1982).

(c) Variation in receptor number - The availability of the number of angiotensin II receptors in vascular tissue may perhaps explain the variability in the pressor response of exogenously administered angiotensin II. Recent work by Gunther et al (Gunther, Grimbrone, and Alexander, 1980) has provided a strong evidence for variation in the number of vascular angiotensin II receptors. Using a rat mesenteric artery preparation, they have described a significant decrease in receptor number with salt depletion whereas opposite effect was seen when angiotensin II formation was inhibited by captopril.

3.3.5 Local vascular humoral systems:

Apart from circulating substances, pressor substances locally generated within the vessel wall have been described.

(a) Vascular renin

It has been hypothesised that blood pressure is maintained by local generation of angiotensin II within the resistance vessel walls (Swales, 1976). Arterioles are, however, inaccessible but larger
arteries have been shown to contain renin and it is reasonable to extrapolate such findings to the arteriole. This concept is, then, compatible with the maintenance of peripheral vascular tone and blood pressure (Swales, 1976).

Indirect experimental evidence suggest that the half-life of vascular renin is much longer than that of plasma renin (Schaechtelin, Regoli, and Gross, 1964; Swales et al, 1975). This was confirmed by direct measurement of aortic renin concentration in rats with 2-kidney 1-clip hypertension—plasma renin had reached its nadir one hour after bilateral nephrectomy, while aortic renin was still elevated at six hours (Thurston, Hurst, Bing, and Swales, 1978). The role of plasma and vascular renin was further dissociated with the use of converting enzyme inhibitor, teprotide (SQ 20,881). This lowered blood pressure by blockade of the renin-angiotensin system for up to six hours after nephrectomy, suggesting that arterial rather than plasma renin was responsible for blood pressure maintenance.

(b) Renal vasopressor substances

It is well established that the kidney exerts both a prohypertensive and an antihypertensive action (Braun-Menendez, 1958). It was originally believed that the kidney carried out its antihypertensive action by removal of sodium and reduction in volume load through natriuresis and diuresis of the excretory process. However, recently much evidence has accumulated, mainly through the work of Muirhead (Muirhead, 1974, 1980), in support of a non-excretory antihypertensive action of the kidney.

Currently, there are three important renal vasodilator systems which may account for the antihypertensive function of the kidney and thus
play a vital role in the control of blood pressure:

(i) the kallikrein-kinin system;

(ii) renal prostaglandins, and

(iii) the antihypertensive renomedullary lipids.

(i) Kallikrein-kinin system:

Recently, this system has been extensively reviewed (Levinsky, 1979; Carretero and Scicli, 1980, 1981) and present evidence suggests that it is an important component of an intrarenal humoral regulatory complex that includes the renin-angiotensin system, aldosterone and prostaglandins.

Kallikreins generate potent vasodilator peptides of which bradykinin is the most important. Our current knowledge indicates that kinins are unlikely to serve as the circulatory antihypertensive hormone from the kidney since large amounts of bradykinin is inactivated by angiotensin I converting enzyme in the pulmonary circulation (Erdos, 1976).

It is believed that the antihypertensive role of angiotensin I converting enzyme (ACE) inhibitor, captopril, may inhibit the destruction of the vasodilator peptides, kinins, in addition to blocking the formation of the vasoconstrictor peptide, angiotensin II. Present evidence suggest that the increase in the concentration of kinins may participate in the antihypertensive effect of converting enzyme inhibition which directly, or through the release of prostaglandins could affect local and peripheral vascular resistance and sodium and water excretion (Johnson, Yasujima, and Clappison, 1981).
In conclusion, the precise role for the locally generated kinins in the regulation of blood pressure is unclear. It has been suggested that bradykinin by its ability to induce the synthesis of prostaglandin could mediate in the antihypertensive function (McGiff, 1980).

(ii) Prostaglandins:

The possible significance of prostaglandins as antihypertensive principal received support mainly from the demonstration of their synthesis in the kidney and in the blood vessel wall. The biosynthesis and metabolism of prostaglandins in the kidney has been extensively reviewed (Dunn and Hood, 1977).

The renal medulla and papilla are the richest sources of prostaglandins. PGE$_2$ and PGF$_{2\alpha}$ are predominantly synthesised by the interstitial and tubular cells of the medulla and papilla whereas PGI$_2$ is synthesised by the microsomal enzymes of arterial walls in the renal cortex. The prostaglandins formed by the kidney may enter the renal tubule or pass into the vascular compartments of the blood vessels. Both PGE$_2$ and PGF$_{2\alpha}$ are rapidly degraded by a single passage through the pulmonary circulation whereas PGI$_2$, on the other hand, appear to be completely destroyed by a single passage through the kidney (Weeks and Crompton, 1979).

Present evidence indicate that the prostaglandin levels are controlled by hormones which stimulate biosynthesis rather than inhibit their degradation (Nasjletti and Malik, 1982). Both angiotensin II and noradrenaline stimulate release of PGE$_2$ from most tissues and angiotensin II has been found to stimulate the release of PGI$_2$ from the kidney, heart, lung and arterial blood vessels. Noradrenaline has been shown to release PGI$_2$ from the isolated perfused mesenteric artery of
rabbit but it is considerably less potent than angiotensin II.

Multiple roles of the prostaglandins has made it difficult to attribute a specific function. Infusion of prostaglandins may result in species specific responses and, in addition, different vascular beds may constrict or dilate even within one species (Gerber and Niles, 1979).

(c) Antihypertensive renomedullary lipids:

Work by Grollman (Grollman, Muirhead, and Vanatta, 1949) implicated renal medulla in the antihypertensive action of the kidney. This was later confirmed by Muirhead and co-workers using autotransplants of fragmented renal medulla (Muirhead, Stirman, and Jones, 1960). Further experiments indicated an antihypertensive role of renomedullary interstitial cells. Transplants of these cells grown in culture were shown to lower the blood pressure in both models of Goldblatt hypertension though the 2-kidney 1-clip model was not so responsive (Muirhead, Germain, Armstrong, Brooks, Leach, Byers, Pitcock, and Brown, 1975). On the other hand, chemical necrosis of the renal medulla further aggravated the raised blood pressure in Goldblatt 2-kidney 1-clip hypertension (Heptinstall, Salyer, and Salyer, 1975).

It has been shown that the antihypertensive response is not associated with diuresis or natriuresis even in sodium volume expanded hypertensive rats. Since there was no change in cardiac output, the vasodepressor response is dependent upon a potent vasodilator action due to a decrease in the peripheral vascular resistance (Prewitt, Leach, Byers, Brooks, Lands, and Muirhead, 1979). So far the mechanism(s) which signal the medulla to release antihypertensive substances is not known but it seems that sodium overload may stimulate
secretion whereas renal artery constriction appears to depress the function of the renomedullary interstitial cells (Susic, 1980). Extensive work by Muirhead and his associates (1974, 1980) has isolated two antihypertensive renomedullary lipids from the renal medulla which are distinct from the prostaglandins. These antihypertensive substances, named as "antihypertensive neutral renomedullary lipid" (ANRL) and "antihypertensive polar renomedullary lipid" (APRL) lower blood pressure in a dose-dependent manner and vary in magnitude and duration of their vasodepressor effect (Muirhead, 1980). The release of antihypertensive renomedullary lipids by the interstitial cells is at present a possible mechanism by which the kidney may mediate an antihypertensive action.

3.3.6 Other vasopressor substances:

The failure to implicate the renin-angiotensin system to play a major role in the pathogenesis of many forms of hypertension has aroused interest to search for additional vasopressor agents. Several alternative humoral pressor agents have been suggested though it should be noted that some laboratories have failed to reproduce original results possibly due to artifacts in the biochemical assays. The best characterised possibilities that have received most attention include:

(i) "Nephrotenasin" - This is a renal pressor substance demonstrated in the plasma of dogs with renovascular hypertension (Grollman and Krishnamurty, 1971). This pressor material has been claimed to be distinct from renin, angiotensin I and angiotensin II. Susic and Sparks (1975) also found nephrotenasin in plasma of dogs with 2-kidney 1-clip Goldblatt hypertension and in rats with chemical induced medullectomy.

(ii) "Renopressin" - This is a pressor substance which was isolated
from the renal cortex of 1-kidney 1-clip Goldblatt hypertensive rabbits (Skeggs, Kahn, Levine, Dorer, and Lentz, 1977). However, rat kidney does not appear to have renopressin (Morris and Roper, 1982) so that its location may be species dependent. Renopressin produces a slow sustained pressor response over several days and the blood pressure can be lowered to normal by specific antibodies. However, the mechanism by which renopressin raises blood pressure is unknown.

(iii) "Corticotensin" - This is a pressor substance separated from the hog renal cortex and when injected, it was shown to have an immediate pressor effect on blood pressure (Fasciolo, Risler, and Toter, 1972).

(iv) "Tonin" - This is a protease which hydrolyses renin substrate with direct formation of angiotensin II, and conversion of angiotensin I to angiotensin II. This enzyme has been claimed to be present in increased amounts in human and some forms of experimental hypertension (Boucher, Garcia, Demassieux, Gutkowska, and Genest, 1978). Rats with 1-kidney 1-clip Goldblatt hypertension were shown to lower blood pressure when injected with rabbit tonin antiserum but this effect was not shown in 2-kidney 1-clip hypertensive model.

(v) "Sensitising factor" - This substance was isolated from the plasma of both 1-kidney perinephretic hypertensive dogs and 1-kidney 1-clip Goldblatt hypertensive rats. It was shown to enhance pressor responsiveness to angiotensin II in nephrectomised rats and to noradrenaline in isolated rat mesenteric arterioles (Huang, Cardona, and Michelakis, 1978).

3.3.7 Non-specific changes in pressor sensitivity:

(a) Structural changes in resistance vessels - Folkow (1978) has put
forward the hypothesis that the increase in vascular resistance is brought about by medial hypertrophy of the vessel wall so that there will be a decrease in the internal diameter of the blood vessel and a resultant increase in the wall to lumen ratio. This alteration of the vascular geometry will for a given shortening of smooth muscle produce a greater decrease in lumen size. This will result in an enhanced constrictor response to a given concentration of the vasoconstrictor without altering its threshold dose. Thus, it is argued that enhanced vascular reactivity is due to an increase in wall to lumen ratio and independent of specific change in smooth muscle reactivity.

(b) Sodium content of arterial smooth muscle - Apart from vascular hypertrophy, other mechanisms have been put forward to explain pressor responsiveness to vasoconstrictor agents.

The classic study of Tobian and Binion (1952) demonstrated increased amounts of sodium and water in renal arteries from hypertensive subjects in comparison with post-mortem segments from normotensive subjects. This was confirmed in a later study in arteries taken from 1-kidney 1-clip hypertensive rats (Tobian and Binion, 1961). From the evidence, Tobian (1974) put forward the proposal that the increase in peripheral resistance in hypertension may be due to "water logging" of the vessel wall; the vascular wall, expanded by the excess fluid within it, encroaches on the lumen to decrease its diameter. This also impairs the capacity of the arterial wall to dilate when the arterial pressure has been elevated.

However, it is known that some of the thickening seen in arterioles is due to accumulation of collagen which results in enhanced non-osmotic binding of sodium (Jonsson, Lundgren, and Wennergren, 1975).
This may be a secondary rather than a primary effect since arterial wall sodium is only increased in that part of the aorta exposed to high pressure in dogs with coarctation (Hollander, Kransch, Farmelant, and Madoff, 1968). Furthermore, hindlimb perfusion experiments in chronic 1-kidney 1-clip hypertensive rats has demonstrated that the contribution of the vascular wall "water-logging" to increased structural arterial resistance is minor (Simon, 1980).

(c) Role of cations in vascular smooth muscle - Abnormalities in the intracellular ionic content may alter the contractile nature of the vascular smooth muscle. Calcium is an essential component of excitation-contraction coupling in the vascular wall. Thus on a theoretical assumption, increased calcium concentration gradient in the arteriole would cause a greater vasoconstriction. Blaustein (1977) has postulated that an increased intracellular sodium content of vascular smooth muscle may increase the free calcium within the smooth muscle cell via a sodium-calcium exchange process causing enhanced responsiveness.

An abnormality of membrane sodium transport has been exhibited in the vascular smooth muscle of spontaneously hypertensive rats (Jones, 1973). It has been suggested that the depolarisation of the vascular smooth muscle which results from altered membrane permeability might enhance responsiveness to vasoconstrictor stimuli (Jones, 1973).

3.4 PURPOSE OF THE EXPERIMENTAL STUDIES

Methodological principals:

Changes in vascular reactivity to angiotensin II and noradrenaline which occur in the various sections of the cardiovascular system show
not only qualitative but also quantitative differences in different models of hypertension. Therefore, the choice of the preparation for study becomes crucial. Experiments on the resistance vessels offer great advantages in both relevance and precision. We have chosen an isolated hindlimb perfusion system perfused with the animal's own blood. The reasons for this approach are:

- any changes in the blood flow are amplified to the fourth power according to Poiseuille's law, greatly improving the measurement of sensitivity and amplifying changes in average muscle length;

- resistance measurements reflect precisely those microvessels responsible for flow resistance;

- the necessary measurements of pressure and flow can be made precisely.

- undesirable factors such as the effects of resetting of baroreceptor reflexes, changes in cardiac output or tachyphylaxis to angiotensin II observed in artificial media were avoided.

Most previous studies used either whole animal or an isolated bed perfused with an artificial medium. The present studies were designed to explore, using an isolated hindlimb preparation of rat, the underlying mechanism for hyperreactivity to vasoactive stimuli in various models of hypertension. In particular, to establish:

(i) How far increased pressor responsiveness in vivo is mediated by changes common to both angiotensin II and noradrenaline?

(ii) To what extent is the responsiveness to exogenously administered angiotensin II explained by endogenous activity of the renin-angiotensin system?
(iii) Could structural adaptations, per se, perhaps be altered by the nature of the experimental manoeuvres?

(iv) How rapidly can the structural adaptation be brought about, is it reversible and is a different pattern observed in hypertension of different duration?
CHAPTER - 4

METHODS AND MATERIALS
METHODS AND MATERIALS

4.1 Experimental model

The rat was chosen as the experimental model for the present study as the majority of studies have been carried out in this species so that comparison with previous work can be achieved without crossing the species barrier. Other reasons for the popularity of this species as an experimental model include relatively low cost and ease of handling and maintenance. Adult female rats were used in preference to males since they gain less weight with age. This is a crucial factor when conducting long-term experiments and when comparisons between groups have to be made.

4.2 Induction of renal hypertension

Experimental hypertension was induced by the application of a constricting clip to the left renal artery. Although the left or the right renal artery could be clipped, the left renal artery is generally preferred because it is more easily accessible with a larger renal pedicle. The right kidney is either left untouched and left in situ if the Goldblatt 2-kidney 1-clip model is to be studied or removed if the Goldblatt 1-kidney 1-clip model is to be studied.

(a) Goldblatt 2-kidney 1-clip hypertension: A constricting clip is placed on the left renal artery and contralateral kidney is untouched and left in situ.

(b) Goldblatt 1-kidney 1-clip hypertension: In this model, the constricting clip is placed on the left renal artery but the contralateral kidney is excised.
4.3 Preparation of the constricting clip

Clips were made from annealed silver ribbon of 2.29mm width and 0.13mm thickness. Each clip was made from a 15mm length of silver strip. The strip was doubled on itself around a steel feeler gauge of known thickness to produce a precise gap between the two arms of the clip. The size of the gap needed depend largely on the age and sex of the rats used and the degree of hypertension required. The ideal gap to obtain partial renal ischaemia to induce hypertension was found to be 0.20mm. In the control study, a loose clip was made with an internal diameter of 0.50mm which was too wide to constrict the renal artery. The clip was designed to have one arm longer (10mm) than the other (5mm) to facilitate clipping and unclipping procedures.

4.4 Surgical procedures

All the operations described below were performed under light ether anaesthesia. Since the operations were only of short duration (approximately 10 minutes) and provided that a relatively aseptic technique was practised, past experience had shown that it was not necessary to give prophylactic antibiotic treatment. All animals recovered rapidly post-operatively and did not appear to show any sign of wound infections.

4.5 Left renal artery stenosis

The anaesthetised animals were shaved over the left flank overlying the kidney. An incision was made in the loin just below the lower border of the rib cage. The overlying muscle layers were cut to expose the perirenal fat. This together with the adrenal gland and the associated blood vessels were carefully freed away from the left
kidney. The renal artery was then gently cleaned near its origin from the abdominal aorta and separated from the renal vein sufficiently to facilitate the application of the constricting clip. The artery was placed on the long arm of the clip and teased into the gap between the two arms so that the short arm of the clip was above the left renal artery and open ends of the clip faced away from the spleen.

After clipping of the left renal artery, the kidney was covered with perirenal fat. The abdominal musculature was closed with silk sutures and the skin closed with metal surgical clips (Michel Clips, Thackray Limited, London, U.K.).

4.6 Right nephrectomy

The right kidney is removed in models such as Goldblatt 1-kidney 1-clip and mineralocorticoid hypertension. The right kidney was exposed through a right loin incision. The kidney and the renal vessels were carefully separated from the perirenal fat and right adrenal gland. The renal vessels together with the ureter were then clamped with artery forceps near the hilum of the kidney. A silk ligature was tied below the clamp and the kidney excised from the pedicle distal to the clamp. The clamp was then removed and the wound closed as described above.

4.7 Surgical correction of hypertension

In Goldblatt 2-kidney 1-clip hypertension, reversal of renovascular hypertension was accomplished either by removal of the constricting clip applied to the left renal artery or the ischaemic kidney.

(a) Removal of the constricting clip - The left flank was reopened through the same loin incision made during the clipping procedure (see above). The clip was relocated and was invariably found to be enveloped
in thin film of tough fibrous tissue. This had to be gently cleaned away near the opening of the clip. The two arms of the clip were forced apart and the clip was gently withdrawn from the artery without much difficulty. The kidney was then placed back in its original position and the wound closed as described above.

(b) Removal of the ischaemic kidney - The left kidney was severed from its pedicle, leaving the constricting silver clip in situ. The left kidney was removed in an identical fashion to that described for right nephrectomy (see above).

4.8 Indirect measurement of blood pressure by a light plethysmographic method

After clipping of the left renal artery, hypertensive animals were selected for study when the indirect blood pressure level was greater than 150mmHg as measured by the plethysmographic method. Blood pressure was measured three weeks after renal artery constriction and thereafter on a weekly basis for another two weeks. Animals which had failed to develop hypertension i.e. blood pressure <150mmHg were rejected.

Indirect measurement of blood pressure by a light plethysmographic method employed here has been fully described by Swales and Tange (1970). The general arrangement of the apparatus is shown in Plate 4A. In brief, this method is able to detect small changes in light translucency during systole and diastole when a rat's tail is positioned on a photoelectric sensor (a light transducer). The photoelectric sensor, consisting of a photocell, picks up the signal which is relayed to a control box containing an amplifier and a frequency filter. The control box is, in turn, connected to an oscilloscope. The impulses from the control box can be tuned to the
heart rate of the rat under study (between 350-500 beats per minute) and monitored visually on an oscilloscope screen.

(a) Method: The rat is lightly anaesthetised with ether and maintained under light anaesthesia. The rat's tail is passed through a specially designed inflatable cuff, attached to an ordinary mercury sphygmomanometer, and is positioned near the base of the tail. The photoelectric sensor is also placed on the tail immediately behind the cuff and retained in position by a spring-loaded clamp. The light source is switched on and the pulse appears on the oscilloscope screen. If there is no pulse, the tail of the animal can be rotated slightly until a trace is produced. The tuning on the control box can be altered to obtain maximum amplitude for each beat. The sphygmomanometer cuff is gently inflated to occlude the blood flow of the tail vessels until the pulse disappears completely to give a straight line on the oscilloscope screen. The cuff is then deflated slowly until a pulse just reappears and the blood pressure value is noted from the sphygmomanometer. This can be done as the animal is regaining consciousness by removal of the nose beaker containing ether soaked cotton wool since ether is known to lower blood pressure through its vasodilatory action. This technique enables rapid and reproducible measurements of blood pressure to be made at normal room temperature (20°C - 24°C).

In theory, the above method only measures systolic blood pressure. However, in practice the indirect method correlates well with direct mean arterial pressure (Swales and Tange, 1970). The apparent discrepancy can be attributed to the fact that when the animal is regaining consciousness, the residual effect of ether still persists thereby yielding a slightly low blood pressure value which approximates more closely to the mean arterial blood pressure than the systolic
PLATE 4A: Indirect method of measuring pressure by light plethysmography.
blood pressure.

(b) Accuracy of the method: The reliability of the technique is largely due to the incorporation of a frequency filter and the measurement of blood pressure without pre-warming of the animal. Previous indirect methods relied on warming the animal's body in order to obtain a measurable signal, thereby, yielding misleading results. But, even marginally raising the body temperature causes an elevation of blood pressure. This problem has been overcome here by the use of a highly sensitive photoelectric cell which is able to detect even the smallest changes in optical density produced by the pulse wave at the rat's normal body temperature.

(c) Incidence of hypertension in the animals: More than 90% of the animals prepared as Goldblatt 1-kidney 1-clip models became hypertensive by the end of the fourth week after clipping of the left artery. However, if those animals which died before this time, the vast majority due to malignant hypertension, are also included this figure rises to over 95%.

In the case of the Goldblatt 2-kidney 1-clip animals, over 85% of the rats were hypertensive at 3 weeks after clipping of the left renal artery. In addition, 5% of the animals that were clipped died before the fourth week, again probably as a consequence of the development of malignant hypertension. Thus, the success rate can be reasonably assumed to be over 90% in the Goldblatt 2-kidney 1-clip hypertensive model.

4.9 Measurement of plasma renin concentration (PRC)

PRC reflects the amount of the circulatory enzyme renin and its
measurement serves as an useful index for the activity of the renin-angiotensin system.

Thus, a PRC assay measures the rate of generation of angiotensin I by renin under conditions which prevent the enzymatic degradation of angiotensin I and its subsequent conversion to angiotensin II. The angiotensin I formed is then measured by radioimmunoassay.

4.9.1 Basic principles of renin assay:

The plasma collected from the rat contains an enzyme renin which acts on renin substrate to produce the decapeptide hormone angiotensin I. The plasma also contains angiotensinases I and II and converting enzyme both of which primarily hydrolyse angiotensin I to peptide fragments and angiotensin II respectively (Figure 2.1).

Using carefully controlled in vitro incubation conditions, the rate of angiotensin I generation reflects the renin enzyme activity. Angiotensinases I is most effective at low pH and can be effectively inhibited by phenylmethylsulphonyl fluoride (PMSF). Angiotensinases II have a pH optimum of around pH 7.4 and since they require divalent cations for activity, they can be inhibited by suitable chelators such as EDTA. The use of suitable buffers overcomes the problem of pH instability during incubation; tris maleate (pK 6.5) has been found to be ideally suited to rat renin incubation mixture (Figure 4.1).

Although several methods are now available, a modification of the radioimmunoassay technique, described by Sealey and Laragh (1977) for measuring plasma renin activity in human plasma remains a reliable technique and was utilised for the study to measure renin concentration in rat plasma.
FIGURE 4.1: Selective positions in the degradation pathway of renin substrate at which EDTA, PMSF and tris maleate operate.
The term PRC should not be confused with plasma renin activity (PRA). PRC is the renin activity in the presence of endogenous substrate only. PRC was measured for the experiments in preference to PRA since it measures the absolute amount of renin in any given sample. PRA measurement, on the other hand, reflects the quantity of renin substrate as well as renin content, since substrate is the rate limiting step at high concentration of renin. Renin levels in the rat, particularly in view of the mode of blood sampling, are much higher than in man and thus the effect of substrate becomes much more important. In cases where the circulating blood volume is small as in small animals (e.g. in the rat about 12-15ml), blood sampling is limited to only 1-2ml for effective determination of PRC.

4.9.2 Plasma collection and storage:

The rat was lightly anaesthetised with ether and approximately 5mm of the tail end was cut. About 0.5ml of blood flowing freely from the tail vein was collected into a chilled test tube (LP3 tube) containing 0.1ml of a 10% solution of dipotassium ethylene diamine tetracetate (K2 EDTA). The presence of EDTA has a dual purpose. It not only acts as an anticoagulant but also inhibits the action of converting enzyme preventing the generation of angiotensin II. Furthermore, it inhibits certain angiotensinases preventing the direct degradation of angiotensin I to peptide fragments (see above). The blood sample was then immediately spun at 4°C in a pre-cooled refrigerated centrifuge at 2800 r.p.m. The plasma was separated from the tube and frozen at -20°C prior to renin assay.

4.9.3 The renin assay

Firstly, the general principles of the assay will be discussed.
Basically there are two main stages in the procedure - incubation of the plasma sample for the generation of angiotensin I and the measurement of angiotensin I by radioimmunoassay.

(a) Incubation: The plasma sample was thawed at room temperature and 100µl of the sample was incubated at 37°C in a shaking water bath with 400µl of the substrate plasma. The substrate plasma was obtained from a pool of normal female Wistar rats which had been previously bilaterally nephrectomised for 24 hours. Angiotensinase and angiotensin converting enzyme inhibitors were added to the renin substrate and the pH adjusted to 6.5. The inhibitor phenylmethylsulphonyl fluoride (PMSF) which together with EDTA ensured total inhibition of angiotensinases. The buffer tris maleate ensures that the incubation pH stays constant at pH 6.5 for the conversion of renin substrate by renin to angiotensin I.

Incubation of normal and high renin samples was carried out for 15 and 30 minutes respectively, removing the tubes onto ice after completion to stop the reaction. High renin samples were diluted with assay buffer following the incubation stage. Low renin samples were incubated for longer periods of 1 and 3 hours in order to increase the sensitivity of the assay. The lower limits of detection were 2.5ng and 0.42ng angiotensin I generated/ml/hour for 15 and 30 minutes incubation time respectively. The assay tubes were left overnight at 4°C for at least 16 to 20 hours before the radioimmunoassay procedure. This prolonged incubation period improves the sensitivity by generating more angiotensin I and improves the accuracy of the assay.

(b) Radioimmunoassay: The amount of angiotensin I generated during the incubation stage was measured by radioimmunoassay. Thus, the variable amount of unlabelled angiotensin I formed in the rat plasma
sample competes with a fixed amount of labelled angiotensin I ($^{125}$I-angiotensin I; New England Nuclear Chemicals GmbH, Southampton, U.K.) for a limited and constant number of antibody binding sites of angiotensin I antiserum prepared from rabbits (Becton Dickinson U.K. Limited, Wembley, Middx., U.K.). Angiotensin I standards (Beckman Bioproducts, Geneva, Switzerland) were used as controls. The amount of angiotensin I that is bound to the antibody was separated from the free angiotensin I by the addition of dextran-coated charcoal (Dextran t70; Pharmacia, Hounslow, Middx., U.K., - Charcoal Novit SXL; Analytical Supplies Ltd., Little Eaton, Derby, U.K.) which preferentially adsorbs the latter. The free angiotensin I is, therefore, found in the charcoal pellet sediment at the bottom of the test tube after centrifugation at 4°C. The supernatant contains the antibody-bound angiotensin I.

A gamma spectrometer is used to count the bound labelled angiotensin I either in the decanted supernatant or in the charcoal pellets. Decantation of the supernatant into another tube for individual sample is obviously a laborious and tedious task and, therefore, the charcoal pellets are counted by simple removal of the supernatant. The larger the amount of unlabelled antigen the smaller the amount of antibody-bound radioactivity left in the supernatant.

A calibration curve was drawn by plotting the logit of the bound counts versus the amount of angiotensin in each standard.

$$\text{Logit} = \frac{B}{Bo} \quad \text{where,}$$

$$\frac{1 - B}{Bo}$$

$Bo = \text{bound counts in the presence of no added angiotensin I, and}$

$B = \text{bound counts for angiotensin I standards and unknowns.}$
The level of angiotensin I in each plasma sample was thus derived by interpolation. Standard curves were performed in duplicate and the cross-reaction binding of the antibody with non-specific substances in the plasma was measured with each assay. The renin concentration of the plasma sample was expressed as nanograms of angiotensin I generated /ml/hour.

It was found that out of the twelve samples of the same plasma that were measured by the above method, the intra-assay variability was 8.4% while the inter-assay variability was 17.5%.

**HINDQUARTER PERFUSION STUDIES**

The technique of hindquarter perfusion described below involves autoperfusion of neurogenically intact isolated hindquarters of the rat. Blood perfusion ensured that the vascular muscle and other tissues remained in a fully physiological state over a prolonged period of time. Intact nervous system ensured a fully functional nervous supply to the vasculature. Peak vascular responses to vasoactive agents were measured using a modified technique originally described by Brody et al (Brody, Shaffer, and Dixon, 1963).

4.10 Animal preparation

The animals were weighed one day before the perfusion study when blood was collected for PRC determination. The animals were anaesthetised with an intraperitoneal injection of 60mg/kg of pentobarbitone sodium (Sagatal, May and Baker Limited, Dagenham, U.K.). This was selected after consulting the extensive work of Green (1979). Deep level of surgical anaesthesia was obtained with this dose by leaving the animal for approximately 15 minutes before beginning the
surgical manoeuvres. Once narcosis had been established, the animal was reweighed and shaved with a hair clipper in the abdominal region and the anterior side of the cervical region. The animal was then placed with the ventral side uppermost on a bench and secured in a spread-eagle position by applying adhesive tape to the fore- and hind-feet. A rectal thermometer was inserted and attached in position to the tail with the adhesive tape. Body (core) temperature was maintained at 34 ± 2°C throughout the experiment with a heating lamp.

4.11 Tracheostomy

Endotracheal intubation was necessary to maintain a patent respiratory tract and thus avoid accumulation of respiratory fluid.

A midline incision was made in the cervical region on the anterior aspect of the neck. A longitudinal incision was made in the sterno-hyoid muscle which was separated by blunt dissection on either side of the trachea. The thyroid glands together with the connective tissue were separated by blunt dissection. Approximately 10mm length of the trachea, 5mm away from the thyroid glands, was cleared. An incision was made in the muscle layer between the cartilage rings and intubation was performed with about 30mm length of polythene tubing, PE240 (Portex Limited, Hythe, Kent, U.K.). The tube had an internal diameter of 1.67mm and an external diameter of 2.42mm and was secured in position with a ligature.

4.12 Cannulation of the left carotid artery

The left common carotid artery was located in the plane of sterno-hyoid muscle and the sterno-clydomastoid group of muscle. About 10-20mm length of the artery was carefully cleared from the facia of the carotid sheath by blunt dissection.
Three silk ligatures were placed under the artery. The distal ligature was tied off to occlude the blood supply superior to it. The blood flow was stopped by applying tension at the proximal and distal ligatures with artery forceps acting as weights. A small incision was made in the artery nearer to the distal end with special iridectomy scissors and the bevelled end of about 370mm length of non-sterile PE50 catheter (Portex Limited, Hythe, Kent, U.K.) with an internal diameter of 0.58mm and an external diameter 0.96mm was introduced into the lumen of the artery. The catheter had been filled with dextrose (50gm/litre; Travenol Laboratories Limited, Thetford, Norfolk, U.K.) containing 10i.u/ml heparin sodium (Weddel Pharmaceuticals Limited, London, U.K.) before cannulation. The tension on the proximal end of the artery was eased slightly and the catheter was gently inserted for 10mm. Patency of the cannulation was checked by observing the back flow of blood drawn down the cannula. Tension was then reapplied to prevent the catheter sliding out of the artery by the force of the arterial pressure. The catheter was then secured in place by first tying the middle, the distal and finally the proximal ligatures firmly round the artery.

4.13 Direct measurement of blood pressure

The PE50 catheter in the carotid artery to record the direct blood pressure was connected to a Statham 23gb strain gauge transducer (Stagg Instruments, Henley on Thames, U.K.) via a plastic tubing metal adaptor. The transducer was in turn linked to a Grass polygraph recorder (Grass Instruments Company, Quincy, Massachusetts, U.S.A.) with a graph output. The transducer was regularly calibrated with an ordinary mercury sphygmomanometer and checked with an internal calibration each time before recording the arterial blood pressure. A
continuous recording of the systolic and diastolic blood pressures together with pulse rate, could therefore be obtained. The mean arterial blood pressure was calculated from the diastolic blood pressure plus one third of the pulse pressure.

4.14 Cannulation of the left jugular vein

The left external jugular vein was superficially identified, cleaned and cannulated in a similar fashion to carotid artery cannulation. A non-sterile standard 270mm length of PE25 catheter was used which had an internal diameter of 0.40mm and an external diameter of 0.80mm. The catheter was filled with dextrose (Travenol Laboratories Limited, Thetford, Norfolk. U.K.) containing 10 i.u./ml heparin before cannulation. The catheter was connected to a plastic tubing metal adaptor attached to a syringe containing dextrose and was used for systemic intravenous injections. Thus a fixed amount of 60μl of dextrose was injected into the animal each time when 250 μg of captopril was given.

Following tracheostomy, left carotid artery and jugular vein cannulations, the wound in the cervical region was covered with a folded cotton gauze (swab) moistened with 0.9% isotonic saline to prevent dryness.

4.15 Cannulation of the aorta

A laparotomy was performed as shown in Figure 4.2. The aorta was approached from the abdominal mid-dorsal line incision. The skin, muscle and the peritoneum were retracted and the intestines were deflected to the right side of the animal with cotton swab moistened with 0.9% isotonic saline. The aorta was freed and cleaned from the
PLATE 4B: Abdominal aorta prior to cannulation.
surrounding connective tissue and fat by applying gentle pressure and teasing with cotton swab sticks.

One silk ligature was placed around the aorta immediately distal to the left renal artery. Another ligature was similarly placed just proximal to the bifurcation of the common iliac arteries from the abdominal aorta. Traction was applied on the aorta with the aid of these two ligatures in order temporarily to occlude the blood flow to the hindlimbs during the cannulation procedure. Two additional ligatures were placed within 3mm of each other below the renal arteries to tie the proximal cannula in the lower aorta above the bifurcation. One further ligature was placed in the middle to tie off the aorta as shown in Plate 4B. Extreme care has to be taken at this point in the procedure since the aorta and the posterior vena cava are virtually attached together in the abdominal region, the vein lying dorsal to the artery. It was found that delicate blunt dissection with a small curved watchmaker's forceps provides a small opening for the ligature. No attempt was made at any point to free the aorta entirely from the vena cava; dissection was performed only in the discrete areas where the ties were to be placed.

Following intravenous injection of 100-130 i.u./kg heparin sodium, the aorta was centrally ligated and doubly cannulated with an extra-corporeal circuit as depicted in Figure 4.2. The tubing had been filled with heparinised 0.9% isotonic saline prior to insertion into the animal.

4.16 The extra-corporeal circuit

The external circuit was constructed of silastic auto-analyser tubing ("Accurate Flow" - Manifold pump tubing; Sterilin Instruments...
FIGURE 4.2: Schematic representation of hindquarter perfusion study.
Limited, Teddington, Middx., U.K.) which had a flow rate of 1.20 ml/minute with an internal diameter of 1.42 mm. Two 30 mm lengths of polyethylene tubing, PE100, with an internal diameter of 1.0 mm and an external diameter of 2.0 mm, slightly tapered and bent at right angles towards the end (to facilitate the cannulation of the aorta), were inserted into the ends of the silastic tubing. A three-way stopcock (Vygon, France) was interposed into the perfusion circuit between the pump and the perfused hindquarters to measure the perfusion pressure and for injection of the drugs (Figure 4.2). One end of the stopcock was connected to a Statham 23gb strain gauge transducer (Stagg Instruments, Henley on Thames, U.K.) for the registration of the perfusion pressure. A steel needle of gauge 81 (24 x 0.5 mm) attached to a 250 mm length of PE10 tubing was fixed into the 3-way stopcock for micro-injection of drugs to study the dose responses. The approximate length of the whole external circuit was about 500 mm which represented a volume of less than 0.9 ml. This length of tubing was standardized for all the perfusion experiments in order to minimize the errors.

4.17 Perfusion of the hindquarters

With the silastic tubing inserted into the roller (motor) pump (Gilson, Anachem Limited, Luton, Beds., U.K.) and following double cannulation of the aorta, blood was diverted from the upper cannula (below the renal arteries) by the roller pump, propelling blood into the hindquarters via the caudal cannula. Since flow through the tubing is maintained constant, changes in perfusion pressure permit the estimation of changes in arterial vascular resistance, as the perfusion pressure and vascular resistance are directly related. Changes in perfusion pressure are used to calculate alterations in the vascular resistance. This technique has the great advantage that it reveals even
minor shifts of smooth muscle activity in precisely those microvessels which determine resistance to flow because resistance changes amplify the average muscle length changes to at least the fourth power according to Poiseuille-Hagen's law.

The circuit had been previously calibrated at different flow both after cannulating the aorta and independent of cannulations. The roller pump delivers fluid at a constant rate irrespective of the inflow or outflow pressures. The blood flow to the hindquarters was adjusted so that the perfusion pressure closely approximated to the existing mean arterial blood pressure. An equilibration period of approximately 30 minutes was allowed in the preparation before recording the dose-response curves.

4.18 Calibration of the perfusion pump

The pump perfusing the hindlimb vasculature of the rat was calibrated without being connected to the animal. The entire extracorporeal circuit was calibrated at various flow settings using water instead of blood as the perfusate. The purpose was to establish flow rate at a given flow. The results indicate a linear relationship such that at maximal flow setting of 1000, the flow rate was 4.15ml/min (Figure 4.3). Since all the perfusion experiments were carried out at maximal flow, the value for flow rate of 4.15ml/min was used to calculate hindlimb vascular resistance.

4.19 Determination of dose-responses

Two routes of drug administration are available in this preparation. Intravenous injections can be made through the catheter in the left jugular vein. This enables one to study whole body vascular responses.
FIGURE 4.3: Relationship between flow rate and dial setting on perfusion pump.
Intra-arterial injection of drugs into the hindquarters were made via a steel needle fixed into the three-way stopcock just above the caudal cannula. All the drugs were administered into the perfusion circuit immediately distal to the perfusion pump to allow adequate mixing and dilution before they entered the hindlimb vasculature.

The drugs were administered in volumes of 5-50μl with a 250μl microsyringe (The L-S Starrett Company, Athol, Mass., U.S.A., Number 263-M) attached to the PE10 tubing leading to the three-way stopcock. Vasoconstrictor agents tested in this study included noradrenaline (Sigma, Poole, England), synthetic angiotensin II amide (Beckman Bioproducts, Geneva, Switzerland; [Asp-Ileu] angiotensin II, 0.96mmols peptide per milligram), and angiotensin I (Beckman Bioproducts, Geneva, Switzerland; [Asp-Ileu] angiotensin I, 0.77mmols peptide per milligram). All the agents were administered as solutions made up in dextrose solution (50g/litre) containing heparin (10 i.u./ml). Concentrated solutions of angiotensin I, angiotensin II, and noradrenaline were prepared in dextrose and stored at -20°C.

The two vasoconstrictors, angiotensin II and noradrenaline, were used to determine whether in various forms of hypertension, or due to various dietary treatments, specific changes in vascular reactivity occur to one or to the other hormone since both pressor substances have different modes of action.

In order to obtain sub-threshold and threshold values for the two vasoactive agents, angiotensin II and noradrenaline, it was necessary to have two solutions of different concentration. Angiotensin II was made up as 1ng/ml and 5ng/ml and bolus intra-arterial injections were made in the order of 1ng, 2ng, 4ng, 8ng, 10ng, 25ng, 50ng, 100ng, 150ng and 200ng. Each new dose of the drug was injected after the previous
base line had been established.

The microsyringe was thoroughly washed out with dextrose each time before the change of concentration of the drugs. The PE10 tubing, attached to the three-way tap in the extra-corporeal circuit and connected to the micro-syringe, was also thoroughly flushed with the drug before the start of the dose responses. After the pressor response was complete and with the microsyringe detached, the PE10 tubing was once again flushed with a very small amount of dextrose to avoid any contamination of previous injection.

Noradrenaline was made up as 1ng/ml and 20ng/ml to give doses of 1ng, 2ng, 4ng, 8ng, 10ng, 100ng, 200ng, 600ng, 800ng and 1000ng.

The angiotensin-converting enzyme inhibitor, captopril (SQ 14,225; E.R.Squibb and Sons Limited, Princeton, New Jersey, U.S.A) was used as an inhibitor of the renin-angiotensin system. A single dose of 250µg dissolved in dextrose was administered intravenously and the preparation left to run for 10-15 minutes. To assess the degree of inhibition of converting enzyme by the test dose of captopril, pressor responses to angiotensin I of 50ng and 100ng (5ng/ml) were carried out both before and after the injection of captopril.

Pressor responses to angiotensin II and noradrenaline were measured before and after the administration of captopril in each perfusion study. The whole length of the experiment lasted for the maximum of three and a half hours. The perfusion pressure was checked at the beginning and at the end of each perfusion study to exclude the possibility of ‘drifting’ which may be due to the effect of anaesthetic wearing off. Additional anaesthetic was not given at any stage of the experiment.
After completion of full dose responses, all the cannulae were removed. The animal was exsanguinated and killed by cervical dislocation.

**EXPERIMENTAL STUDIES**

All experiments were carried out on white female rats of Wistar strain obtained from the same supplier (Bantin and Kingman, Grimston, Aldbrough, Hull, U.K.). Rats of weight 180-310g were used throughout.

All the rats used for the study were housed in an animal laboratory with a temperature and humidity controlled environment with a 12-hour automatic lighting cycle. Unless otherwise indicated, all the animals were maintained on a regime of standard pellet diet (sodium content 0.12mmol/g, potassium 0.290mmol/g; Labsure Economy R&M, Poole, Dorset, U.K.) and tap water ad libitum.

This study comprised of four separate series of experiments. All the groups described below composed of eight animals.

4.20 Influence of dietary sodium and the kidney in normotensive rats

Study I consisted of four groups of animals 1-4.

Group 1 - Normal salt diet:

Rats weighing 180-250g were housed in groups of four per cage under normal laboratory conditions. They were maintained on a standard pellet food diet (sodium content 0.12mmol/g, potassium 0.290mmol/g; Labsure Economy R&M, Poole, Dorset, U.K.) and tap water ad libitum.

Plasma for PRC was taken under light ether anaesthesia a day before the perfusion study (see above). Food, but not water, was withheld
overnight.

Group 2 - Low salt diet:

Normal rats were given powdered diet (sodium content 0.013mmol/g, potassium 0.18mmol/g; Edosol Cow and Gate Baby Foods, Trowbridge, Wilts., U.K.) made into a thick paste with de-ionised water. Concentrated vitamin drops were also added to the diet to supplement the necessary intake of vitamins. The animals were given de-ionised water to drink in order to minimise sodium intake. The animals were maintained on this regime for two weeks before the experiments. Plasma for PRC was taken as for Group 1.

Group 3 - High salt diet:

Normal rats were allowed free access to standard pellet diet (sodium content 0.12mmol/g, potassium content 0.20mmol/g; Labsure Economy R&M, Poole, Dorset, U.K.) and 2% saline (2g sodium chloride/100ml distilled water - sodium content 321.67mmol/l) to drink. The animals were kept on this treatment for two weeks before the perfusion studies. Plasma for PRC was taken as for Group 1.

Group 4 - Bilaterally nephrectomized rats:

In this group, normal rats were subjected to bilateral nephrectomy and studied after 24 hours. Extreme care was taken not to disturb the adrenal glands. The rats were allowed free access to water but deprived of food in the post-operative period. All the animals appeared to be in good state of health at 24-hours. Plasma for PRC was taken one hour before the perfusion experiment.
4.21 Mineralocorticoid and 1-kidney 1-clip Goldblatt hypertension

Study II consisted of three groups of animals 5-7.

Group 5 - Deoxycorticosterone (DOC) + 1% saline (DOC-salt):

DOC-salt hypertension was induced in normal rats that had undergone right nephrectomy and 12.5mg of deoxycorticosterone pivalate in saline as vehicle (Percorten 'Crystules', Ciba Laboratories, Horsham, Sussex, U.K.) was injected subcutaneously twice weekly. Rats were maintained on a standard pellet diet and saline (1g sodium chloride / 100ml water) to drink.

Indirect tail blood pressures were measured twice weekly. Hypertensive animals with blood pressure of >150mmHg were studied 1 to 2 weeks after the establishment of hypertension. PRC was taken as for Group 1.

Group 6 - Control for DOC-salt group:

Control animals for DOC-salt hypertension (Group 5) were treated in an identical manner except that same volume of 1% saline as DOC was substituted for subcutaneous injections. Control animals, irrespective of their indirect blood pressure measurements, were paired with the DOC-salt hypertensive rats for the duration of their treatment.

Group 7 - Early 1-kidney 1-clip Goldblatt hypertension:

1-kidney 1-clip renovascular hypertension was produced by applying a constricting silver clip (0.2mm internal diameter) to the left renal artery, the right kidney was excised. Indirect tail blood pressures were measured three weeks after clipping and thereafter on a weekly basis. Hypertensive animals were selected on the basis of blood
pressure >150mmHg within 6 weeks of clipping. PRC was taken as for Group 1.

4.22 Early and chronic renovascular hypertension

Study III consisted of 4 groups of animals 8-11.

Hypertension was induced in animals by the application of a constricting silver clip to the left renal artery as described in a previous section of this chapter. Early phase hypertension was defined as hypertension of less than four weeks duration after clipping and the chronic phase as hypertension of greater than 16 weeks duration.

Group 8 - Early 2-kidney 1-clip Goldblatt hypertension:

Hypertension was produced in this model by the application of a silver constricting clip to the left renal artery leaving the right kidney undisturbed. Indirect blood pressure measurements were performed as previously described. Hypertensive animals were used between the 5th and the 6th week after left renal ischaemia. Plasma for PRC was taken as for Group 1.

Group 9 - ‘Loose’ clip control (Early):

A ‘loose’ silver clip with an internal diameter of 0.50mm was placed on the left renal artery in the manner described earlier. These animals were matched with the early Goldblatt 2-kidney 1-clip hypertensive animals of Group 8 to serve as controls so that both groups were of approximately the same age and weight.

Group 10 - Chronic 2-kidney 1-clip Goldblatt hypertension:

Hypertension was induced in rats as described earlier. Once
hypertension with indirect blood pressure of >150mmHg had been established, the animals were left for a total period of 16 weeks after left renal artery constriction. Perfusion studies were carried out after this period. Plasma for PRC was collected as for Group 1.

Group II - 'Loose' clip control (Chronic):

A 'loose' silver clip with an internal diameter of 0.50mm instead of a constricting clip on the left renal artery served as control for Group 10. The animals were exactly matched for age post clipping for perfusion experiments. PRC was taken as for Group 1.

4.23 Surgical reversal of renovascular hypertension

Study IV consisted of 12 groups of animals 12-23.

Groups 12 & 14 (Early) and 18 & 20 (Chronic) - 24-hours unclip or nephrectomy of the ischaemic kidney in 2-kidney 1-clip Goldblatt hypertension:

Blood for PRC measurement was collected for 2-kidney 1-clip Goldblatt hypertensive rats before removal of the constricting clip or the ischaemic kidney. Rats were then allowed to recover consciousness. After a 24-hour period, the rats were again lightly anaesthetised with ether and a tail vein sample of blood was collected for PRC. The rats were allowed to recover from the anaesthetic over a period of one hour before beginning the perfusion experiments.

The above protocol was observed for both early and chronic phase hypertensive rats.

Groups 13 & 15 (Early) and 19 & 21 (Chronic) - 24-hours unclip or nephrectomy - 'loose' clip control:
Procedures for control animals were carried out in an identical manner to their respective experimental groups described in the preceding section.

Groups 16 & 22 - 60 days unclip in 2-kidney 1-clip Goldblatt hypertension:

Blood for PRC measurement was taken from 2-kidney 1-clip Goldblatt hypertensive animals before removal of the constricting clip. After the clip had been removed, the rats were allowed to recover consciousness again and left for 60 days after the surgery. On day 61, the rats were lightly anaesthetised with ether for PRC and allowed to recover from the anaesthetic for one hour before the perfusion studies.

The above protocol was observed for both early and chronic phase hypertensive rats.

Group 17 & 23 - 60 days unclip in 'loose' clip controls:

Control animals were matched for age and treated in exactly the same manner as their experimental counterparts (i.e. early and chronic hypertensive rats - Groups 16 & 22) described in the last section.

4.24 Statistical analysis

All the results are expressed as mean values ± SEM (standard error of the mean). Analysis of variance was used to make multiple comparisons. One way analysis of variance was used for comparison of the dose-response data between treatments. A paired sample Student 't' test was used to compare results obtained before and after treatment in the same animal. Other statistical comparisons were made using a two-sample 't' test for independent samples. The plasma renin concentration
(PRC) was transformed into logarithms before comparisons were made since PRC is logarithmically and not normally distributed. However, in the text, the results of the PRC are expressed as arithmetical means ± SEM. Probability values (P) smaller than 0.05 were considered to be significant.
CHAPTER - 5

RESULTS
5.1 INFLUENCE OF DIETARY SODIUM AND THE KIDNEY ON VASCULAR REACTIVITY IN NORMOTENSIVE ANIMALS

5.1.1 Blood pressure and renin

There was no significant difference in direct mean blood pressure of the salt restricted or salt loaded rats and rats maintained on a normal salt intake (Table-1). Twenty-four hours after bilateral nephrectomy, blood pressure had fallen significantly (p <0.001) compared with normal controls. At the end of the dietary treatment, body weight was identical in the salt restricted and normal diet rats but fell significantly in the group receiving a high salt intake (p <0.001). Plasma renin concentration (PRC) changed reciprocally with sodium balance, rising with salt depletion and falling with salt loading (p <0.01, Table-1). Removal of both kidneys resulted in a fall of plasma renin to an almost undetectable level. Basal hindlimb vascular resistance was also reciprocally related with sodium balance - high in the salt restricted and low in the salt loaded rats compared with normal controls. Twenty-four hours after bilateral nephrectomy, vascular resistance in the hindlimb fell significantly compared with normal rats.

5.1.2 Effect of captopril on hindlimb perfusion pressure

Administration of captopril (250μg i.v.) resulted in an immediate fall in perfusion pressure of the hindlimbs (Table-2). This reduction in perfusion pressure is reciprocally related with sodium balance - greater in salt restricted (p <0.001) and lower in salt loaded rats (p <0.001) compared with rats maintained on normal salt diet. The perfusion pressure was similarly correlated with PRC (Figure-5.1,
TABLE 1: Direct mean blood pressure, body weight, plasma renin concentration (PRC) and basal hindlimb vascular resistance of rats maintained on different salt intakes and 24-hour after bilateral nephrectomy.

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>n</th>
<th>Mean blood pressure (mmHg)</th>
<th>Body weight (gms)</th>
<th>PRC (ngAI/ml/hr)</th>
<th>Basal hindlimb vascular resistance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Low salt diet</td>
<td>8</td>
<td>135 ± 11.6</td>
<td>210 ± 11.6</td>
<td>207.0 ± 16.7**</td>
<td>33.4 ± 2.4*</td>
</tr>
<tr>
<td>1</td>
<td>Normal salt diet</td>
<td>8</td>
<td>126 ± 12.0</td>
<td>221 ± 8.2</td>
<td>79.5 ± 18.8</td>
<td>25.3 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>High salt diet</td>
<td>8</td>
<td>137 ± 8.7</td>
<td>190 ± 4.6**</td>
<td>9.03 ± 2.4**</td>
<td>20.2 ± 1.3*</td>
</tr>
<tr>
<td>4</td>
<td>24-hour bilateral</td>
<td>8</td>
<td>77 ± 6.5**</td>
<td>228 ± 7.0</td>
<td>0.9 ± 0.2***</td>
<td>12.8 ± 1.8***</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01  
*** p<0.001  
compared with normal salt diet animals.
TABLE 2: Fall in perfusion pressure after captopril.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dietary Treatment</th>
<th>Mean ± SEM (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8</td>
<td>Low salt diet</td>
<td>32 ± 1.7*</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>Normal salt diet</td>
<td>19 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>High salt diet</td>
<td>11 ± 1.6*</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>24-hour bilateral nephrectomy</td>
<td>4 ± 1.1*</td>
</tr>
</tbody>
</table>

* p<0.001 compared with normal salt animals.
FIGURE 5.1: Relationship of the fall in hindlimb perfusion pressure after captopril (250µg i.v.) with plasma renin concentration (PRC) in rats given low salt (◇), normal salt (○), high salt (△) diets and 24 hours after bilateral nephrectomy (□).
Low salt diet animals with raised PRC had a significantly greater fall in perfusion pressure whereas high salt diet animals with low PRC showed a relatively small fall in perfusion pressure. The smallest fall occurred in animals 24-hours after bilateral nephrectomy.

5.1.3 Conversion of angiotensin I to angiotensin II

The in vivo conversion of angiotensin I to angiotensin II can be quantitatively calculated from the angiotensin II pressor response curves. For each dose of angiotensin I tested, the dose of angiotensin II required to produce an equivalent increase in hindlimb perfusion pressure can be determined from the dose-response curves of angiotensin II prior to and after administration of the converting enzyme inhibitor, captopril (Figure-5.4). A calculated example for an increase in perfusion pressure by 50ng dose of angiotensin I in animals given normal salt diet is shown below.

Calculation of angiotensin II generation

Molecular weight of angiotensin I = 1296.7
Molecular weight of angiotensin II = 1046.0

In equivalent doses

\[ \frac{50 \text{ng angiotensin I}}{1296.7} = \frac{1046 \times 50}{1296.7} \]

\[ = 40 \text{ng angiotensin II}. \]

50ng angiotensin I \( \equiv 40 \text{ng angiotensin II} \) caused a 27mmHg increase in the hindlimb perfusion pressure.

19ng angiotensin II caused a 27mmHg pressor response.

\[ \therefore \text{conversion of angiotensin I to angiotensin II at equipressor dose} \]
Similar calculation for 100ng angiotensin I gave a value of 92% conversion to angiotensin II. Following blockade of the converting enzyme with captopril, the percentage conversion of 50ng and 100ng angiotensin I was again calculated. Thus after captopril, the percentage conversion of 50ng angiotensin I to angiotensin II was 2.5%. This would permit calculation of the percentage inhibition by captopril of conversion to angiotensin II.

Calculation of percentage inhibition by captopril in the hindlimb

Percentage conversion of 50ng angiotensin I to angiotensin II

Before captopril = 47.5%
After captopril = 2.5%

.: percentage inhibition of angiotensin I converted to angiotensin II

\[ \frac{47.5 - 2.5}{47.5} \times 100 = 95\% \]

Similarly, calculation for 100ng angiotensin I resulted in 96% inhibition by captopril.

Calculations were made for other groups in the manner described above (Table-3).

5.1.4 Increase in hindlimb perfusion pressure by angiotensin I

Two doses of angiotensin I i.e. 50ng and 100ng were injected into the extra-corporeal circuit to evaluate the degree of converting enzyme
TABLE 3: Calculated percentage conversion of angiotensin I (AI) to angiotensin II (AII) before and after converting enzyme blockade and inhibition of AI to AII in blood-perfused hindlimb vasculature of rats given low, normal and high salt and 24-hours after bilateral nephrectomy (AI dose = mean values, n=8 in each).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary treatment</th>
<th>AI dose (ng)</th>
<th>Equivalent pressor dose of AII* (ng)</th>
<th>Percentage conversion of AI**</th>
<th>Percentage inhibition of conversion to AII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>before captopril</td>
<td>after captopril</td>
<td>before captopril</td>
</tr>
<tr>
<td>2</td>
<td>Low salt diet</td>
<td>50</td>
<td>18</td>
<td>1.5</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>64</td>
<td>2.8</td>
<td>80.0</td>
</tr>
<tr>
<td>1</td>
<td>Normal salt diet</td>
<td>50</td>
<td>19</td>
<td>1.0</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>74</td>
<td>2.8</td>
<td>92.5</td>
</tr>
<tr>
<td>3</td>
<td>High salt diet</td>
<td>50</td>
<td>33</td>
<td>2.0</td>
<td>82.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>75</td>
<td>5.8</td>
<td>93.8</td>
</tr>
<tr>
<td>4</td>
<td>24-hour bilateral nephrectomy</td>
<td>50</td>
<td>12</td>
<td>1.0</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>80</td>
<td>2.8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* estimated from AII dose-response curves.
** AII ng/AI ng x 1.25 x 100.
FIGURE 5.2: Effect of angiotensin I on hindlimb perfusion pressure before and after inhibition of angiotensin converting enzyme with captopril (250μg i.v.). Arrows indicate points of injection.
FIGURE 5.3: Increase in perfusion pressure by angiotensin I (mean ± SEM, n=8) in low salt (LS), normal salt (NS), high salt (HS) and 24 hours after bilateral nephrectomy (BNX) rats before (A) and after (B) converting enzyme inhibitor, captopril.
blockade by the administration of captopril (Figures 5.2 and 5.3). In all the animals, intra-arterial injection of angiotensin I resulted in a dose dependent rise in hindlimb perfusion pressure (Figures 5.2 and 5.3).

The increase in perfusion pressure was reciprocally related to PRC. Angiotensin I pressor response was greater in animals maintained on high salt diet than those on low salt diet (Figure 5.3).

Intravenous injection of captopril markedly blunted the angiotensin I pressor response (Figures 5.2 and 5.3). Following blockade of the converting enzyme, the angiotensin I pressor responses were not significantly different from each other at either 50ng or 100ng angiotensin I (Figure 5.3).

5.1.5 Angiotensin II pressor reactivity

Pressor responses to angiotensin II were obtained in hindlimb of rats maintained on low salt, normal salt and high salt diets and 24-hours after bilateral nephrectomy (Figure 5.4). The salt restricted animals were less responsive to angiotensin II than animals maintained on normal salt diet (p <0.001, i.e. the dose response curve shifted to the right). The pressor response was enhanced in salt loaded animals compared with normal salt controls (p <0.005, i.e. the dose response curve shifted to the left). Animals after bilateral nephrectomy were less responsive to angiotensin II than intact animals (p <0.005).

Blockade of the converting enzyme activity with captopril significantly enhanced the pressor responses to angiotensin II in all the intact animals receiving the salt (p <0.001, Figure 5.4B). The pressor response was unaltered in animals 24-hours after bilateral nephrectomy. After captopril the dose response curve of the low salt
FIGURE 5.4: Angiotensin II pressor responses (mean,n=8) in low salt, normal salt, high salt and 24 hours after bilateral nephrectomy rats before (A) and after (B) converting enzyme inhibitor, captopril.

**KEY**

- = Low salt diet
- = Normal salt diet
= High salt diet
= 24 hours bilateral nephrectomy
diet group approached that of the normal salt animals such that there was no significant difference between the two curves. Animals on high salt diet remained significantly more responsive compared with those on normal salt diet (p < 0.001) and the dose response curves shifted further towards the left.

5.1.6 Noradrenaline pressor responses

Pressor responses to noradrenaline were obtained in hindlimb of rats maintained on low salt diet, normal salt and high salt diets and 24-hours after bilateral nephrectomy (Figure 5.5). Pressor responses were not significantly different in rats maintained either on low salt and normal salt diets or in animals after bilateral nephrectomy. Animals on high salt diet showed enhanced pressor response compared with normal salt controls (p < 0.005, i.e. the dose response curve shifted to the left).

Administration of the converting enzyme inhibitor, captopril, significantly enhanced the pressor response to noradrenaline in all the animals (p < 0.001, Figure 5.5B). The dose response curves of rats maintained on low salt diet and normal salt diets came closer together and they were not significantly different. Animals receiving high salt diet remained significantly more responsive compared with normal salt diet controls (p < 0.01). The pressor response was significantly less in animals after bilateral nephrectomy compared with intact normal controls (p < 0.005).
FIGURE 5.5: Noradrenaline pressor responses (mean, n=8) in low salt, normal salt, high salt and 24 hours after bilateral nephrectomy rats before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

\[\text{Low salt diet}\]
\[\text{Normal salt diet}\]
\[\text{High salt diet}\]
\[\text{24 hours bilateral nephrectomy}\]
5.2 PRESSOR RESPONSIVENESS TO ANGIOTENSIN II AND NORADRENALINE IN STEROID AND 1-KIDNEY 1-CLIP GOLDBLATT HYPERTENSION

5.2.1 Blood pressure and plasma renin

Direct mean blood pressure of rats given deoxycorticosterone (DOC) was higher than their age-matched control animals but this failed to reach statistical significance (Table-4). Rats with 1-kidney 1-clip Goldblatt hypertension of less than 6 weeks duration had significantly elevated blood pressure compared with either DOC hypertensive rats (p <0.02) or intact normal animals (p <0.001). Body weight was not altered in any of the groups. Plasma renin concentration (PRC) was suppressed in the DOC hypertensive animals compared with controls which did not receive DOC (p <0.001). DOC control animals had significantly low renin compared with normal intact animals (p <0.001). PRC was similar in rats with 1-kidney 1-clip renovascular hypertension and normal controls (p >0.7). Surprisingly, there was no significant difference in basal hindlimb vascular resistance of DOC hypertensive rats and either their controls (p >0.7) or the normal intact animals. The 1-kidney 1-clip Goldblatt hypertensive rats had significantly raised hindlimb vascular resistance compared with either the normotensive intact animals (p <0.005) or the DOC hypertensive rats (p <0.001).

5.2.2 Effect of captopril on hindlimb perfusion pressure

Following administration of captopril (250 μg i.v.) there was a significantly greater fall in perfusion pressure of rats with 1-kidney 1-clip Goldblatt hypertension than in any of the other animals (Table-5). There was no significant change in perfusion pressure between the two hypertensive groups (p >0.1). The fall in perfusion pressure was
TABLE 4: Direct mean blood pressure, body weight, plasma renin concentration (PRC) and basal hindlimb vascular resistance of normotensive, 1-kidney 1-clip Goldblatt hypertensive, DOC-salt hypertensive and DOC-control animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean blood pressure (mmHg)</th>
<th>Body weight (gms)</th>
<th>PRC (ngAI/ml/hr)</th>
<th>Basal hindlimb vascular resistance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normotensive animals</td>
<td>8</td>
<td>126 ± 12.0</td>
<td>221 ± 8.2</td>
<td>79.50 ± 18.8</td>
<td>25.3 ± 1.6</td>
</tr>
<tr>
<td>7 1-kidney 1-clip Goldblatt hypertension</td>
<td>8</td>
<td>194 ± 9.7*</td>
<td>216 ± 4.9</td>
<td>80.75 ± 12.21</td>
<td>34.5 ± 2.2**</td>
</tr>
<tr>
<td>5 DOC-salt hypertension</td>
<td>8</td>
<td>162 ± 6.5</td>
<td>215 ± 3.3</td>
<td>00.47 ± 0.0 †</td>
<td>21.7 ± 1.2</td>
</tr>
<tr>
<td>6 DOC-control animals</td>
<td>8</td>
<td>147 ± 5.6</td>
<td>223 ± 5.1</td>
<td>15.80 ± 6.9*</td>
<td>22.3 ± 1.8</td>
</tr>
</tbody>
</table>

* p<0.001 \} compared with Normotensive animals.

** p<0.005 \} compared with Normotensive animals.

† p<0.001 \} compared with DOC-control animals.
Table 5: Fall in perfusion pressure after captopril.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Description</th>
<th>Mean ± SEM (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Normotensive animals</td>
<td>19 ± 2.4</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>1-kidney 1-clip Goldblatt hypertension</td>
<td>39 ± 8.3*</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>DOC- salt hypertension</td>
<td>25 ± 4.5†</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>DOC-control animals</td>
<td>14 ± 2.1</td>
</tr>
</tbody>
</table>

* p<0.01 compared with Normotensive animals.
† p<0.05 compared with DOC-control animals.
FIGURE 5.6: Increase in perfusion pressure by angiotensin I (mean ± SEM, n=8) in normal (N), 1-kidney 1-clip Goldblatt hypertensive (GI), DOC-salt hypertensive (DS) and DOC control (DC) animals before (A) and after (B) converting enzyme inhibitor, captopril.
TABLE 6: Calculated percentage conversion of angiotensin I (AI) to angiotensin II (AII) before and after converting enzyme blockade and inhibition of AI to AII in blood-perfused hindlimb vasculature of normotensive animals, rats with 1-kidney 1-clip (1-K 1-C) Goldblatt hypertension, DOC-salt hypertension, and DOC-salt control animals (AI dose = mean values, n=8 in each).

<table>
<thead>
<tr>
<th>Group</th>
<th>AI dose (ng)</th>
<th>Equivalent pressor dose of AII* (ng)</th>
<th>Percentage Conversion of AI**</th>
<th>Percentage Inhibition of conversion to AII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before captopril</td>
<td>after captopril</td>
<td>before captopril</td>
</tr>
<tr>
<td>1 Normotensive animals</td>
<td>50</td>
<td>19</td>
<td>1.0</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>74</td>
<td>2.8</td>
<td>92.5</td>
</tr>
<tr>
<td>2 1-K 1-C Goldblatt Hypertension</td>
<td>50</td>
<td>33</td>
<td>1.3</td>
<td>82.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>84</td>
<td>2.4</td>
<td>105.0</td>
</tr>
<tr>
<td>3 DOC-salt Hypertension</td>
<td>50</td>
<td>35</td>
<td>1.0</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>2.8</td>
<td>125.0</td>
</tr>
<tr>
<td>4 DOC-control animals</td>
<td>50</td>
<td>33</td>
<td>2.0</td>
<td>82.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>96</td>
<td>5.8</td>
<td>120.0</td>
</tr>
</tbody>
</table>

* determined from AII pressor response curves.
** AII ng/AI ng x 1.25 x 100.
greater in the DOC-salt hypertensive animals compared with their controls.

5.2.3 Angiotensin I and inhibition of conversion to angiotensin II by captopril

Intra-arterial injection of angiotensin I produced a dose-dependent rise in hindlimb perfusion pressure (Figure 5.6). The pressor responses at 50ng and 100ng were significantly higher in all the animals before blockade of the converting enzyme. Following inhibition of the converting enzyme with captopril, the pressor responses were not statistically different in all the groups at either dose of angiotensin I.

Injection of captopril brought about a net inhibition of about 97% of conversion to angiotensin II in rats with 1-kidney 1-clip Goldblatt hypertension and about 96% in the normotensive intact animals. In the DOC-salt hypertensive animals, captopril caused an average of 97% inhibition compared with 94% in their controls (Table-6).

5.2.4 Angiotensin II pressor reactivity

Pressor responses to angiotensin II were obtained in hindlimb of normotensive intact animals, rats with acute 1-kidney 1-clip Goldblatt hypertension and DOC-salt hypertension and DOC-salt control animals (Figure-5.7). The two groups of hypertensive animals were significantly more responsive to angiotensin II than any of the control animals. The DOC-salt hypertensive animals were hyperresponsive to angiotensin II compared with DOC controls (F=116.3, p <0.001, i.e.the dose-response curve shifted to the left). Rats with 1-kidney 1-clip Goldblatt hypertension also had enhanced constrictor response to angiotensin II.
FIGURE 5.7: Angiotensin II pressor responses (mean, n=8) in normal, 1-kidney 1-clip Goldblatt hypertensive, DOC-salt hypertensive, and DOC control animals before (A) and after (B) converting enzyme inhibitor, captopril.

**KEY**

- = Normal rats
- = 1-kidney 1-clip Goldblatt hypertension
- = DOC-salt hypertension
- = DOC controls
compared with either the normotensive intact animals (p <0.001) or the DOC controls (p <0.005).

Inhibition of the converting enzyme activity with captopril significantly enhanced the sensitivity to angiotensin II in all the groups (p <0.001, Figure 5.7B). The DOC-salt hypertensive rats were significantly more responsive to angiotensin II so that their dose-response curve shifted further towards the left compared with either DOC controls or the normotensive rats (p <0.001). Animals with 1-kidney 1-clip Goldblatt hypertension remained significantly more responsive compared with normal intact animals (p <0.005) or the DOC controls (p <0.001).

5.2.5 Noradrenaline pressor responses

Pressor responses to noradrenaline were obtained in hindlimbs of normotensive intact animals, rats with acute 1-kidney 1-clip Goldblatt hypertension and DOC-salt hypertension and DOC-salt control animals (Figure 5.8). The pattern of responses were similar to that with angiotensin II so that the two hypertensive groups had a greater degree of sensitivity to noradrenaline than either of the control animals. The DOC-salt hypertensive animals were much more responsive so that the dose-response curve was steeper and towards the left than the DOC controls (F=217.5, p <0.001). Animals with 1-kidney 1-clip renovascular hypertension also exhibited enhanced pressor response compared with normotensive intact animals (p <0.005).

Blockade of the converting enzyme with captopril significantly potentiated the dose response curves to noradrenaline in all the animals (p <0.001, Figure 5.8B). The DOC-salt hypertensive animals continued to show exaggerated pressor response compared with DOC
FIGURE 5.8: Noradrenaline pressor responses (mean, n=8) in normal, 1-kidney 1-clip Goldblatt hypertensive, DOC-salt hypertensive and DOC control animals before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

= Normal rats
= 1-kidney 1-clip Goldblatt hypertension
= DOC-salt hypertension
= DOC controls
controls (F=106.1,  \( p < 0.001 \)). Rats with 1-kidney 1-clip Goldblatt hypertension remained more responsive compared with normal intact animals (\( p < 0.02 \)) or the DOC control animals.
5.3 PRESSOR RESPONSE STUDIES IN EARLY AND CHRONIC 2-KIDNEY 1-CLIP GOLDBLATT HYPERTENSION

5.3.1 Blood pressure and renin

The direct blood pressure of rats with early phase hypertension was similar to their 'loose' clip controls ($p > 0.5$). The direct and indirect values of blood pressure were similar in rats with early phase hypertension and animals with established hypertension ($p > 0.6$, Table-7). The direct blood pressure of chronically hypertensive animals was significantly raised compared with their 'loose' clip controls ($p < 0.001$).

Plasma renin concentration (PRC) of animals with early and chronic phase hypertension was statistically similar to their respective controls. PRC was elevated in rats with early phase hypertension compared with chronically hypertensive animals but this failed to reach statistical significance ($p > 0.5$).

Body weight was not significantly different in rats with early phase hypertension and their controls. The chronically hypertensive animals were significantly heavier than those with early phase hypertension ($p < 0.01$) but they were similar in weight compared with their controls.

The basal hindlimb vascular resistance was increased in rats with early phase hypertension compared with their controls ($p < 0.01$). The chronically hypertensive animals had significantly raised vascular resistance compared with their 'loose' clip controls ($p < 0.01$) but it was lower compared to rats with early phase hypertension ($p < 0.05$).
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean Blood Pressure (mmHg)</th>
<th>Body Weight (gns)</th>
<th>FRC (ngAI/ml/hr)</th>
<th>Basal hindlimb vascular resistance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Early 2-K-1-C Goldblatt hypertension</td>
<td>8</td>
<td>176 ± 7.3</td>
<td>210 ± 5.2</td>
<td>266.7 ± 130.2</td>
<td>36.4 ± 2.4</td>
</tr>
<tr>
<td>9 Early 'loose' clip control</td>
<td>8</td>
<td>179 ± 7.1</td>
<td>204 ± 4.9</td>
<td>143.5 ± 37.1</td>
<td>25.4 ± 2.1</td>
</tr>
<tr>
<td>10 Chronic 2-K-1-C Goldblatt</td>
<td>8</td>
<td>176 ± 7.2**</td>
<td>244 ± 7.6</td>
<td>175.5 ± 65.4</td>
<td>29.2 ± 2.1**</td>
</tr>
<tr>
<td>11 Chronic 'loose' clip controls</td>
<td>8</td>
<td>116 ± 6.9</td>
<td>254 ± 6.7</td>
<td>77.4 ± 10.8</td>
<td>20.8 ± 1.5</td>
</tr>
</tbody>
</table>

* p<0.01; ** p<0.001 compared with 'loose' clip controls.
TABLE 8: Fall in hindlimb perfusion pressure after captopril.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean ± SEM (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>8</td>
<td>Early 2-kidney 1-clip hypertension</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>Early 'loose' clip control</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Chronic 2-kidney 1-clip hypertension</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>Chronic 'loose' clip control</td>
</tr>
</tbody>
</table>

* p<0.01 compared with early 'loose' clip controls.
† p<0.05 compared with chronic 2-kidney 1-clip Goldblatt hypertension.
FIGURE 5.9: Increase in perfusion pressure by angiotensin I (mean ± SEM, n=8) in rats with early (E), chronic (C) 2-kidney 1-clip Goldblatt hypertension and their respective 'loose' clip controls (E-L, C-L) before (A) and after (B) converting enzyme inhibitor, captopril.
<table>
<thead>
<tr>
<th>Group</th>
<th>AI dose (ng)</th>
<th>Equivalent pressor dose of AII* (ng)</th>
<th>Percentage conversion of AI**</th>
<th>Percentage inhibition of conversion to AII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before captopril</td>
<td>after captopril</td>
<td>before captopril</td>
</tr>
<tr>
<td>8 Early 2-kidney 1-clip hypertension</td>
<td>50</td>
<td>24</td>
<td>1.0</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>62</td>
<td>2.4</td>
<td>77.5</td>
</tr>
<tr>
<td>9 Early 'loose' clip control</td>
<td>50</td>
<td>31</td>
<td>2.4</td>
<td>77.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>105</td>
<td>7.6</td>
<td>131.3</td>
</tr>
<tr>
<td>10 Chronic 2-kidney hypertension</td>
<td>50</td>
<td>33</td>
<td>2.9</td>
<td>82.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>96</td>
<td>7.4</td>
<td>120.0</td>
</tr>
<tr>
<td>11 Chronic 'loose' clip control</td>
<td>50</td>
<td>25</td>
<td>1.4</td>
<td>62.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>74</td>
<td>4.1</td>
<td>92.5</td>
</tr>
</tbody>
</table>

* determined from AII dose-response curves.
** AII ng/AI ng x 1.25 x 100.
5.3.2 Effect of captopril on hindlimb perfusion pressure

Administration of captopril (250μg i.v.) resulted in a significantly greater fall in perfusion pressure by one-way analysis of variance in rats with early phase hypertension compared with either their 'loose' clip control (p <0.01) or the chronically hypertensive animals (p <0.05, Table-8). The fall in perfusion pressure was similar in rats with established hypertension and their 'loose' clip controls.

5.3.3 Angiotensin I and inhibition of conversion to angiotensin II by captopril

Intra-arterial injection of angiotensin I produced a dose-dependent increase in perfusion pressure in all the animals (Figure-5.9). After blockade of the converting enzyme with captopril, the perfusion pressure fell significantly and was statistically similar at either dose of angiotensin I.

Intravenous injection of captopril resulted in more than 90% inhibition of conversion to angiotensin II in all the groups (Table-9).

5.3.4 Angiotensin II pressor reactivity

Pressor responses to angiotensin II were obtained in rats with early and chronic 2-kidney 1-clip Goldblatt hypertension and their age-matched 'loose' clip controls (Figure 5.10). Rats with early phase hypertension were similar in response to their 'loose' clip controls. The chronically hypertensive animals were significantly more responsive compared with either their 'loose' clip controls (p <0.001) or rats with early phase hypertension (p <0.01, i.e. the dose-response curve had shifted towards the left).
FIGURE 5.10: Angiotensin II pressor responses (mean, n=8) in rats with 2-kidney 1-clip Goldblatt hypertension and their 'loose' clip controls before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

\[\text{Early 2-kidney 1-clip Goldblatt hypertension}\]

\[\text{Early 'loose' clip controls}\]

\[\text{Chronic 2-kidney 1-clip Goldblatt hypertension}\]

\[\text{Chronic 'loose' clip controls}\]
Inhibition of the converting enzyme with captopril significantly potentiated the pressor responses in all the animals (p <0.001, Figure 5.10B). The dose-response curves of rats with early phase hypertension, their 'loose' clip controls and chronically hypertensive animals approached each other and they were not significantly different. The chronically hypertensive animals remained significantly more responsive compared with their 'loose' clip controls (p <0.001).

5.3.5 Noradrenaline pressor responses

Pressor responses to noradrenaline were obtained in rats with early and chronic 2-kidney 1-clip renovascular hypertension and their age-matched 'loose' clip controls (Figure 5.11). The dose-response curves were similar in pattern to those with angiotensin II. Rats with early phase hypertension were not significantly different from their 'loose' clip controls. The response for chronically hypertensive animals was statistically similar to rats with early phase hypertension but they were hyperresponsive compared with the 'loose' clip controls (F=73.5, p <0.001).

Administration of the converting enzyme inhibitor, captopril, significantly potentiated the pressor responses in all the animals (p <0.001, Figure 5.11B). Like angiotensin II, the dose-response curves of animals with early phase hypertension, their 'loose' clip controls and the chronically hypertensive animals came closer together and they were not significantly different. The chronically hypertensive animals remained significantly more responsive compared with their 'loose' clip controls (F=64.4, p <0.001).
FIGURE 5.11: Noradrenaline pressor responses (mean, n=8) in rats with 2-kidney 1-clip Goldblatt hypertension and their ‘loose’ clip controls before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

♦-♦-♦-♦-♦-♦-♦-♦-♦ = Early 2-kidney 1-clip Goldblatt hypertension

•-•-•-•-•-•-•-•-• = Early ‘loose’ clip controls

▲-▲-▲-▲-▲-▲-▲-▲-▲ = Chronic 2-kidney 1-clip Goldblatt hypertension

■-■-■-■-■-■-■-■-■ = Chronic ‘loose’ clip controls
5.4.1 Blood pressure

Relief of the renal artery constriction caused a significant fall in blood pressure of rats with early phase hypertension at 24 hours (p <0.002, Table-10) and at 60 days (p <0.002, Table-12). Neither of these unclipped groups was significantly different from their age-matched 'loose' clip controls. Removal of the ischaemic kidney also caused a rapid return of the blood pressure to normal within 24 hours (p <0.001, Table-11) and this was not statistically different from the 'loose' clip controls.

There was no significant difference between the pooled indirect and direct blood pressure of rats with early and chronic phase hypertension. Renal artery unclipping in rats with established hypertension caused a significant fall in blood pressure at 24 hours (p <0.005, Table-10) and at 60 days (p <0.05, Table-12) and neither of these values were significantly different from their respective unclipped controls (Tables 10 and 12). Removal of the ischaemic kidney caused a rapid normalisation of blood pressure within 24 hours (p <0.001) and this was not significantly different from the 'loose' clip controls (Table-11).

5.4.2 Plasma renin concentration (PRC)

The pooled initial PRC was significantly elevated in rats with early and chronic phase hypertension compared with their 'loose' clip controls (p <0.001, n=62). Surgical correction of hypertension caused a
TABLE 10: Mean blood pressure, body weight, plasma renin concentration (PRC) and basal hindlimb vascular resistance 24-hours after unclipping in Early and Chronic 2-kidney 1-clip (2-K 1-C) Goldblatt hypertension.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Blood pressure (mmHg)</th>
<th>Body weight (gms)</th>
<th>PRC (ngAI/ml/hr)</th>
<th>Basal hindlimb vascular resistance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Indirect Direct Initial Final</td>
<td></td>
<td>Initial Final</td>
<td></td>
</tr>
<tr>
<td>12 Early 2-K 1-C hypertension</td>
<td>8</td>
<td>173 ± 8.6 135 ± 4.9* 225 ± 6.1 206 ± 5.6†</td>
<td>368.5 ± 251.4 97.8 ± 26.2</td>
<td>25.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>13 Early 'loose' clip controls</td>
<td>8</td>
<td>-------- 136 ± 9.0 227 ± 5.1 209 ± 5.1†</td>
<td>79.6 ± 23.7 122.4 ± 61.9</td>
<td>27.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>18 Chronic 2-K 1-C hypertension</td>
<td>8</td>
<td>181 ± 5.0 149 ± 11.8† 245 ± 6.5 228 ± 6.3</td>
<td>81.7 ± 16.2 89.3 ± 25.7</td>
<td>22.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>19 Chronic 'loose' clip controls</td>
<td>8</td>
<td>-------- 120 ± 7.6 250 ± 8.7 234 ± 7.9</td>
<td>16.7 ± 3.8 29.1 ± 7.4</td>
<td>22.0 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>

† p<0.05  
* p<0.002  
significantly different from the pre-operative values for the same group.

# values before unclipping.
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Blood pressure (mmHg)</th>
<th>Body weight (gms)</th>
<th>PRC (ngAI/ml/hr)</th>
<th>Basal hindlimb vascular resistance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Indirect</td>
<td>Direct</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>14 Early 2-K 1-C hypertension</td>
<td>8</td>
<td>183 ± 6.0</td>
<td>110 ± 10.3*</td>
<td>227 ± 3.8</td>
<td>205 ± 3.5*</td>
</tr>
<tr>
<td>15 Early 'loose' clip controls</td>
<td>8</td>
<td>————</td>
<td>135 ± 5.6</td>
<td>216 ± 5.9</td>
<td>197 ± 5.1†</td>
</tr>
<tr>
<td>20 Chronic 2-K 1-C hypertension</td>
<td>8</td>
<td>164 ± 3.9</td>
<td>113 ± 11.0*</td>
<td>249 ± 8.0</td>
<td>233 ± 7.5</td>
</tr>
<tr>
<td>21 Chronic 'loose' clip controls</td>
<td>8</td>
<td>————</td>
<td>130 ± 8.1</td>
<td>252 ± 4.6</td>
<td>233 ± 5.1</td>
</tr>
</tbody>
</table>

* p<0.001 significantly different from the pre-operative values for the same group.
† p<0.05 values before left nephrectomy.
TABLE 12: Mean blood pressure, body weight, plasma renin concentration (PRC) and basal hindlimb vascular resistance 60-days after unclipping in Early and Chronic 2-kidney 1-clip (2-K 1-C) Goldblatt hypertension.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Blood pressure (mmHg)</th>
<th>Body weight (gms)</th>
<th>PRC (ngAI/ml/hr)</th>
<th>Basal hindlimb vascular resistance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Indirect #</td>
<td>Direct</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Early 2-K 1-C hypertension</td>
<td>8</td>
<td>198 ± 8.4</td>
<td>135 ± 13.6**</td>
<td>195 ± 6.6</td>
<td>227 ± 5.6*</td>
</tr>
<tr>
<td>Early 'loose' clip controls</td>
<td>8</td>
<td>————</td>
<td>145 ± 8.6</td>
<td>201 ± 3.8</td>
<td>215 ± 4.9†</td>
</tr>
<tr>
<td>Chronic 2-K 1-C hypertension</td>
<td>8</td>
<td>190 ± 7.5</td>
<td>156 ± 13.0†</td>
<td>258 ± 10.1</td>
<td>268 ± 14.4</td>
</tr>
<tr>
<td>Chronic 'loose' clip controls</td>
<td>8</td>
<td>————</td>
<td>147 ± 4.3</td>
<td>240 ± 6.4</td>
<td>243 ± 9.2</td>
</tr>
</tbody>
</table>

† p<0.05  
* p<0.01  
** p<0.002  
*** p<0.001  
# values before unclipping.

significantly different from the pre-operative value for the same group.
significant fall in PRC in both early and chronic phase hypertension. PRC was normal 1-day after unclipping (Table-10) but subnormal at 1-day after left nephrectomy (p <0.001, Table-11) and at 60 days after declipping (p <0.001, Table-12). Removal of the ischaemic kidney in both early and chronic phase hypertension caused a sharp fall in PRC compared with their respective 'loose' clip controls (p <0.001, Table-11) and the PRC was significantly lower compared with that of 1-day unclipped hypertensive animals (p <0.001, Tables 10 and 11).

5.4.3 Body weight

Reversal of hypertension caused a significant fall in body weight at 24-hours in rats with early phase hypertension (Tables 10 and 11). There was a significant gain in weight at 60 days after unclipping in the early hypertensive rats (p <0.01) and their 'loose' clip controls (p <0.05, Table-12). The chronically hypertensive animals were heavier than rats with early phase hypertension. There was no significant difference in body weight following reversal of hypertension in the chronic phase and their controls (p >0.1).

5.4.4 Hindlimb vascular resistance

Surgical correction of hypertension caused a significant reduction in vascular resistance of the hindlimb in both early and chronic phase hypertension. The fall was much greater after removal of the kidney at 24 hours in the hypertensive animals (p <0.001, Table-11). After the operation, hindlimb vascular resistance was not significantly different at any stage between the hypertensive animals and their corresponding 'loose' clip controls. Administration of captopril failed to have a significant effect on hindlimb perfusion pressure after reversal of early and chronic renovascular hypertension (p >0.1, Tables 13 and 14).
TABLE 13: Fall in hindlimb perfusion pressure after captopril following reversal of Early 2-kidney 1-clip Goldblatt hypertension.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean ± SEM (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>8</td>
<td>24-hours Unclip</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>'loose' unclip control</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>24-hours left Nephrectomy</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>'loose'clip nephrectomy control</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>60-days Unclip</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>'loose'unclip control</td>
</tr>
</tbody>
</table>

19 ± 7.2
18 ± 5.2
11 ± 1.1
12 ± 2.4
27 ± 4.6
17 ± 4.0
**TABLE 14:** Fall in hindlimb perfusion pressure after captopril following reversal of chronic 2-kidney 1-clip Goldblatt hypertension.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Mean ± SEM (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>8</td>
<td>24-hours Unclip</td>
<td>23 ± 5.2</td>
</tr>
<tr>
<td>19</td>
<td>8</td>
<td>'loose' unclip control</td>
<td>14 ± 1.6</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>24-hours Left Nephrectomy</td>
<td>17 ± 2.7</td>
</tr>
<tr>
<td>21</td>
<td>8</td>
<td>'loose' clip nephrectomy control</td>
<td>18 ± 5.0</td>
</tr>
<tr>
<td>22</td>
<td>8</td>
<td>60-days Unclip</td>
<td>16 ± 3.2</td>
</tr>
<tr>
<td>23</td>
<td>8</td>
<td>'loose' clip control</td>
<td>22 ± 3.2</td>
</tr>
</tbody>
</table>
FIGURE 5.12: Increase in perfusion pressure by angiotensin I (mean ± SEM, n=8) following reversal of early 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

1-U = 1-day unclip
1-N = 1-day nephrectomy
60-U = 60-day unclip
LU = unclip controls
LN = nephrectomy controls
LC = 60-day unclip controls
FIGURE 5.13: Increase in perfusion pressure by angiotensin I (mean ± SEM, n=8) following reversal of chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

1-U = 1-day unclip
1-N = 1-day nephrectomy
60-U = 60-day unclip

LU = unclip controls
LN = nephrectomy controls
LC = 60-day unclip controls
TABLE 15: Calculated percentage conversion of angiotensin I (AI) to angiotensin (AII) before and after converting enzyme blockade and inhibition of AII to AII in blood-perfused hindlimb vasculature of rats following reversal of early 2-kidney 1-clip Goldblatt hypertension (AI dose = mean values, n=8 in each).

<table>
<thead>
<tr>
<th>Group</th>
<th>AI dose (ng)</th>
<th>Equivalent pressor dose of AII* (ng)</th>
<th>Percentage conversion of AI**</th>
<th>Percentage inhibition of conversion to AII</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>before captopril</td>
<td>after captopril</td>
<td>before captopril</td>
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<tr>
<td>12</td>
<td>24-hour unclip</td>
<td>50</td>
<td>56</td>
<td>2.4</td>
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<td></td>
<td></td>
<td>100</td>
<td>150</td>
<td>6.4</td>
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<tr>
<td>13</td>
<td>'Loose' clip control</td>
<td>50</td>
<td>45</td>
<td>1.1</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>320</td>
<td>3.1</td>
</tr>
<tr>
<td>14</td>
<td>24-hour left nephrectomy</td>
<td>50</td>
<td>52</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>400</td>
<td>2.1</td>
</tr>
<tr>
<td>15</td>
<td>'Loose' clip control</td>
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<td>44</td>
<td>2.2</td>
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<td></td>
<td></td>
<td>100</td>
<td>115</td>
<td>10.0</td>
</tr>
<tr>
<td>16</td>
<td>60-days unclip</td>
<td>50</td>
<td>34</td>
<td>1.5</td>
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<td></td>
<td>100</td>
<td>100</td>
<td>4.5</td>
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<tr>
<td>17</td>
<td>'Loose' clip control</td>
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<td>35</td>
<td>1.6</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>105</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* determined from AII dose-response curves.
** AII ng/AI ng x 1.25 x 100.
TABLE 16: Calculated percentage conversion of angiotensin I (AI) to angiotensin II (AII) before and after converting enzyme blockade and inhibition of AI to AII in blood-perfused hindlimb vasculature of rats following reversal of chronic 2-kidney 1-clip Goldblatt hypertension (AI dose = mean values, n=8 in each).

<table>
<thead>
<tr>
<th>Group</th>
<th>AI dose (ng)</th>
<th>Equivalent pressor dose of AII* (ng)</th>
<th>Percentage conversion of AI** before captopril</th>
<th>Percentage conversion of AI** after captopril</th>
<th>Percentage inhibition of conversion to AII</th>
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<tr>
<td></td>
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<tr>
<td>18  24-hour unclip</td>
<td>50</td>
<td>19</td>
<td>1.4</td>
<td>47.5</td>
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<td>100</td>
<td>115</td>
<td>4.0</td>
<td>143.8</td>
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</tr>
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<td>50</td>
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<td>1.1</td>
<td>77.5</td>
<td>2.8</td>
</tr>
<tr>
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<td>100</td>
<td>125</td>
<td>2.2</td>
<td>156.3</td>
<td>2.8</td>
</tr>
<tr>
<td>20  24-hour left nephrectomy</td>
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<td>1.8</td>
<td>82.5</td>
<td>4.5</td>
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<tr>
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<td>100</td>
<td>135</td>
<td>3.4</td>
<td>168.8</td>
<td>4.3</td>
</tr>
<tr>
<td>21  'Loose' clip control</td>
<td>50</td>
<td>40</td>
<td>1.0</td>
<td>100.0</td>
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<td>140</td>
<td>1.5</td>
<td>175.0</td>
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<td>22  60-days unclip</td>
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<td>2.2</td>
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<td>125.0</td>
<td>7.3</td>
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<td>23  'Loose' clip control</td>
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<td>36</td>
<td>2.5</td>
<td>90.0</td>
<td>6.3</td>
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<td></td>
<td>100</td>
<td>200</td>
<td>5.0</td>
<td>250.0</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* determined from AII dose-response curves.
* AII ng/AI ng x 1.25 x 100.
5.4.5 Angiotensin I and inhibition of conversion to angiotensin II by captopril

Injection of two different doses of angiotensin I into the perfusion circuit caused a dose-dependent increase in the pressor response (Figures 5.12 and 5.13). The pressor response to angiotensin I was most marked in rats with early phase hypertension 24-hours after left nephrectomy. Following inhibition of the converting enzyme with captopril, the pressor response to angiotensin I fell significantly at 50ng and 100ng and was similar at either dose in all the animals.

The percent conversion to angiotensin II was found to be greatest at higher dose of angiotensin I before captopril for all the groups (Tables 15 and 16). Following blockade of the converting enzyme, >90% of conversion to angiotensin II was inhibited by captopril (Tables 15 and 16).

5.4.6 Angiotensin II pressor reactivity

Pressor responses to angiotensin II were obtained in hindlimb of rats after surgical correction of early and chronic renovascular hypertension (Figures 5.14, 5.15 and 5.16). Removal of renal artery clip or the ischaemic kidney at 24 hours caused hyperresponsiveness to angiotensin II in rats with both early and chronic 2-kidney 1-clip Goldblatt hypertension compared with their corresponding ‘loose’ clip controls (p <0.001, Figures 5.14 and 5.15). Removal of the constricting clip abolished any differences after 60 days between the previously early and chronic hypertensive rats and their ‘loose’ clip controls (Figure 5.16).

Administration of the converting enzyme inhibitor, captopril,
FIGURE 5.14: Angiotensin II pressor responses (mean, n=8) 24 hours after unclipping in early and chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

- = Early unclip
- - - - - - - = Early controls
- = Chronic unclip
- - - - - - - = Chronic controls
FIGURE 5.15: Angiotensin II pressor responses (mean, n=8) 24 hours after nephrectomy in early and chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

- Early nephrectomy
- Early controls
- Chronic nephrectomy
- Chronic controls
FIGURE 5.16: Angiotensin II pressor responses (mean, n=8) 60 days after unclipping in early and chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

- Early unclip
- Early controls
- Chronic unclip
- Chronic controls
significantly potentiated the responses to angiotensin II in all the groups (p < 0.001, i.e. the dose-response curves shifted towards the left). The pattern of dose-response curves remained as before blockade of the converting enzyme. Rats previously with early and chronic phase hypertension were hyperresponsive to angiotensin II at 24-hours after declipping compared with their 'loose' clip controls (p < 0.001, Figure 5.14). Removal of the ischaemic kidney enhanced the responsiveness to angiotensin II at 24-hours in rats with early phase hypertension (p < 0.001) and chronic phase hypertension (p < 0.05, Figure 5.15) compared with their 'loose' clip controls. The responses were similar at 60 days after unclipping in rats with either early or chronic phase hypertension and their controls (Figure 5.16).

5.4.7 Noradrenaline pressor responses

Pressor responses to noradrenaline were obtained in the hindlimb of rats after reversal of early and chronic renovascular hypertension (Figures 5.17, 5.18 and 5.19). The pattern of dose-response curves were similar to those obtained with angiotensin II. Pressor responses at 24-hours after removal of the renal artery clip or the ischaemic kidney were significantly potentiated in animals with early and chronic phase hypertension compared with their 'loose' clip controls (p < 0.001, Figures 5.17 and 5.18). 60 days after removal of the constricting clip, pressor responses in rats with early phase hypertension were not significantly different from their controls but those with chronic phase hypertension were hypo-responsive compared with their 'loose' clip controls (p < 0.05, Figure 5.19).

Inhibition of the converting enzyme activity with captopril significantly enhanced the pressor responses to noradrenaline in all the groups (p < 0.001). Unclipping potentiated the pressor response at
FIGURE 5.17: Noradrenaline pressor responses (mean, n=8) 24 hours after unclipping in early and chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

**KEY**

- •••• = Early unclip
- ♦♦♦♦ = Early controls
- ▲▲▲▲ = Chronic unclip
- ■■■■ = Chronic controls
FIGURE 5.18: Noradrenaline pressor responses (mean, n=8) 24 hours after nephrectomy in early and chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

**KEY**

- •-• = Early nephrectomy
- ▲-▲ = Early controls
- ▲-▲ = Chronic nephrectomy
- ▼-▼ = Chronic controls
FIGURE 5.19: Noradrenaline pressor responses (mean, n=8) 60 days after unclipping in early and chronic 2-kidney 1-clip Goldblatt hypertension before (A) and after (B) converting enzyme inhibitor, captopril.

KEY

- = Early unclip
♦ = Early controls
▲ = Chronic unclip
■ = Chronic controls
24-hours in rats with early and chronic phase hypertension compared with their controls (p < 0.001, Figure 5.17). The enhanced pressor response was maintained after removal of the ischaemic kidney at 24-hours in early (p < 0.05) and chronic (p < 0.005) phase hypertension. At 60 days after unclipping, the pressor response was unchanged in rats with early phase hypertension compared with their controls but the chronically hypertensive animals were less responsive compared with their 'loose' clip controls (p < 0.05, Figure 5.19).
CHAPTER - 6

DISCUSSION
6.1 INFLUENCE OF DIETARY SODIUM AND THE KIDNEY ON VASCULAR REACTIVITY
IN NORMOTENSIVE ANIMALS

6.1.1 Blood pressure and Sodium balance

The data show a clear association between plasma renin concentration and dietary change with sodium (Table-1). Restriction of salt from the diet was accompanied by a dramatic increase in renin levels. Salt loading was accompanied by a marked suppression of renin release. Such a reciprocal relationship between sodium balance and plasma renin has been well documented (Brown et al, 1964; Samwer et al, 1974; Oliver and Cannon, 1978; Thurston et al, 1979; Bing et al, 1982). These differences in renin were eliminated 24 hours after bilateral nephrectomy. This is consistent with the demonstration that the enzyme has been shown to have a half-life of less than 20 minutes (Schaechtelin et al, 1964; Thurston and Swales, 1977).

The hindlimb vascular resistance was inversely related with dietary sodium. An increase in renin with sodium restriction was shown to raise the hindlimb vascular resistance. In animals with low renin (salt loading and bilateral nephrectomy), the hindlimb resistance was low. However, the autonomic nervous system has been suggested to contribute to peripheral vasoconstriction in salt depletion (Masuo, Ogihara, Kumahara, Yamatodani, and Wada, 1983). Converting enzyme inhibition by captopril produced a fall in hindlimb perfusion pressure which was related to the initial plasma renin concentration (PRC) - greatest where PRC was raised (low salt) and least where PRC was low (Figure 5.1). It should be pointed out, however, that PRC levels were measured the day before the study except in the bilaterally nephrectomised animals where PRC was measured one hour before the dose-response study.
While these values do not reflect absolute renin levels during the study, they do reflect the relationship between PRC in the experimental groups. Renin secretion is also known to be stimulated by ether anaesthesia. However, the relationship between changes in plasma renin in various situations is preserved (Oates, Stokes, and Storey, 1975).

Despite differences in dietary sodium intake and plasma renin levels, the direct mean blood pressure was similar in all the groups. Arterial blood pressure was maintained during sodium restriction by increasing the levels of renin or angiotensin II. Paradoxically, the pressor responsiveness to angiotensin II was diminished during sodium restriction. After sodium loading, where angiotensin II probably contributes little to the maintenance of arterial pressure, there was an enhanced responsiveness to exogenous angiotensin II. The low blood pressure in the bilaterally nephrectomised animals might be the consequence of organic compounds accumulating as a result of uraemia. Decrease in pressor responsiveness has previously been reported in uraemic rats (Swales et al, 1975). After administration of captopril, the perfusion pressure returned to normal (Figure 5.2). This occurred despite continual blockade of the converting enzyme which suggests that blood pressure is maintained by other compensating mechanisms. It is possible that active vasoconstriction may have occurred as a result of sympathetic activity.

6.1.2 Hindlimb perfusion pressure and Conversion to angiotensin II

The pressor response to angiotensin I was reciprocally related to PRC in animals subjected to dietary salt manipulation (Figure 5.3). However, the amount of angiotensin I converted to angiotensin II in plasma is very small (Ng and Vane, 1967). The enzyme has been demonstrated in the vessel wall (Disalvo and Montefusco, 1971; Aiken
and Vane, 1972) which suggests that angiotensin I is most likely to have been converted to angiotensin II in the hindlimb vasculature of rats since a rapid pressor response occurred before angiotensin I could have passed through the pulmonary vasculature.

Using a quantified bioassay approach, most of the injected angiotensin I was demonstrated to be converted to angiotensin II, though, a greater degree of conversion occurred at the higher dose of angiotensin I (Table-3). Similar form of dose-dependent increase with angiotensin I has been reported in dogs (Britton and DiSalvo, 1973) and rats (Spertini, Brunner, Waebner, and Gavras, 1981) though no explanation was offered for this. It is possible that these differences may be related with the prevailing levels of circulating renin.

Angiotensin I elicited pressor responses even after blockade of the converting enzyme which suggests that activity of the enzyme was not completely abolished by the inhibitor. It has been reported that this residual effect of angiotensin I may be due to a small (1-4% relative to angiotensin II) but significant inherent activity of the peptide (Aiken and Vane, 1970). Alternatively, vasoconstriction may have occurred by some conversion to angiotensin II.

6.1.3 Vascular reactivity

This study has demonstrated that the pressor response to exogenous angiotensin II was significantly and selectively impaired by restriction of salt. This was indicated by a rightward shift of the dose-response curve. In contrast, the vascular response to angiotensin II was increased by salt loading (dose-response curve shifted towards the left). When examined across the groups, the pressor responses to angiotensin II appeared to be inversely related to endogenous renin.
levels. These vascular changes with angiotensin II are directionally similar to those reported by several other studies during salt restriction (Kaplan and Silah, 1964; Reid and Laragh, 1965; Barraclough et al., 1967; Strewler, Hinrichs, Guiod, and Hollenberg, 1972; Hollenberg et al., 1972; Samwer et al., 1974; Thurston and Laragh, 1975; Swales et al., 1975; Slack and Ledingham, 1976; Oliver and Cannon, 1978) and salt loading (Kaplan and Silah, 1964; Reid and Laragh, 1965; Barraclough et al., 1967; Thurston and Laragh, 1975; Swales et al., 1975; Slack and Ledingham, 1976; Oliver and Cannon, 1978).

There was no clear relationship between dietary sodium or plasma renin levels and vascular responses to noradrenaline. In contrast to previous studies, sodium restriction failed to show a significant change in vascular response to noradrenaline. Strewler et al. (1972) demonstrated potentiation of response to noradrenaline with salt restriction in rabbits. Similarly, Couture and Regoli (1980a) showed augmented responses to noradrenaline in the whole body of salt restricted rats while in the perfused hindlimb from the same animals, there was a decreased response. However, salt loading enhanced the pressor responsiveness to noradrenaline. Inhibition of converting enzyme with captopril significantly potentiated the pressor responses to noradrenaline in all the animals receiving salt. This increase with captopril appears unlikely to be mediated by bradykinin since responses to noradrenaline were either unaltered (Okuno, Kondo, Konishi, Saruta, and Kato, 1979) or reduced (Marks et al., 1982) after the administration of bradykinin. However, it has been suggested that the differences in vascular reactivity to noradrenaline might be more related to actual differences in catecholamine storage and re-uptake in the tissues during altered sodium balance (DeChamplain et al., 1968). As the
preparation had an intact nervous system, captopril by interfering with the renin-angiotensin system may lead to changes in the concentration of noradrenaline at the prejunctional site of the nerve ending to result in altered vascular reactivity (Clough, Hatton, Keddie, and Collis, 1982). Angiotensin II has been shown to potentiate the release of noradrenaline and increase its pressor responsiveness (Sato and Masuyama, 1971; Malik and Nasjletti, 1976). Thus, blockade of the renin-angiotensin system which eliminates the effect of endogenous angiotensin II, would reduce the concentration of noradrenaline and lead to an increase in the sensitivity to exogenous noradrenaline. This situation would be analogous to denervation which results in supersensitivity to noradrenaline (Trendelenberg, 1966).

The altered pressor responsiveness to angiotensin II with salt before and after inhibition of the converting enzyme could be explained by several proposed mechanisms. These could be -

(i) tachyphylaxis to angiotensin II,

(ii) direct action of sodium on the vessel wall,

(iii) vascular affinity to angiotensin II,

(iv) receptor occupancy, and

(v) "up" and "down" regulation of vascular receptor sites.

(i) Tachyphylaxis to angiotensin II - The hyporesponsiveness with angiotensin II during sodium restriction may perhaps be assumed to represent a form of tachyphylaxis to angiotensin II as a direct result of increased circulating levels. This is not true since tachyphylaxis to angiotensin II was absent in the hindlimb preparation which was perfused with autologous blood and not synthetic media. Moreover, with
repeated dose of angiotensin II, a reproducible pressor response was obtained.

(ii) Direct action of sodium on the vessel wall - The suggestion that perhaps extracellular fluid sodium concentration may have a direct effect on the vessel wall (Strewler et al, 1972) is unlikely, since no major variations of plasma sodium in the extracellular fluid have been reported to occur during dietary restriction of salt (Aguilera and Catt, 1981). Furthermore, in the present studies, the vascular responses to angiotensin II were enhanced immediately after blockade of the converting enzyme and before a change in sodium balance could take place.

(iii) Vascular affinity to angiotensin II - It has been proposed that vascular affinity to angiotensin II (an index for vascular reactivity) is altered with sodium balance (Brunner et al, 1972). Using specific angiotensin II antibody, these investigators demonstrated that vascular affinity for angiotensin II was lowered during sodium restriction while it was increased with sodium loading or bilateral nephrectomy. However, this inverse relationship was not always consistent. For example, the antibody requirement of nephrectomised animals varied according to sodium intake and yet the circulating level of angiotensin II was undetectable in these rats. Furthermore, sodium balance study was carried over a longer period (6 weeks) and only a fixed dose of angiotensin II (50ng) was injected instead of a dose-response study. More recent work, however, suggests that the volume of angiotensin II antibody required to block the pressor response to angiotensin II is not a measure of receptor affinity (Swales et al, 1975).

(iv) Receptor occupancy - According to this hypothesis, when the
hindlimb vascular angiotensin II receptors are occupied by large amounts of endogenous angiotensin II (e.g. salt restriction), this would lead to an impaired response to exogenous angiotensin II. Conversely, when the amount of endogenous angiotensin II at the vascular sites is low (e.g. salt loading), the majority of receptor sites would be free to interact with exogenous angiotensin II, resulting in greater degree of vasoconstriction. There is then, competition for the availability of angiotensin II receptor sites (Kaplan and Silah, 1964; Thurston and Laragh, 1975). This hypothesis requires that the clearance of arterial renin is much slower than the clearance of plasma renin since there is a time lag of six hours for the maximum pressor response to renin and angiotensin II in animals after bilateral nephrectomy (Swales et al, 1975). By contrast, the circulating levels of renin and angiotensin II were shown to fall within an hour after bilateral nephrectomy (Oates, Fretton, and Stokes, 1974). This discrepancy could be explained by the suggestion that perhaps renin-like activity within the peripheral resistance vessel wall (Gould et al, 1964; Thurston et al, 1979) may lead to local generation of angiotensin II in order to maintain vasoconstriction (Swales and Thurston, 1973).

(v) "Up" and "down" regulation of vascular receptor sites - More recently, it has been suggested that perhaps circulating angiotensin II, which can be altered by sodium, has a direct action on its own receptors. Work on rat mesentery has shown that sodium restriction was associated with a decrease in the number of vascular angiotensin II receptors while sodium loading increased the number of angiotensin II receptors (Gunther et al, 1980; Aguilera and Catt, 1981). A similar "up" and "down" regulation of receptors may have also occurred in this study. This hypothesis also explains the hyperresponsiveness to
angiotensin II after captopril in sodium loaded rats. Captopril by decreasing the levels of angiotensin II would rapidly dissociate bound angiotensin from the vascular receptor and create new receptor sites free to respond to exogenous angiotensin II. This interpretation is supported by the evidence of an increase in the number of receptors of mesentery artery from sodium restricted rats given captopril (Gunther et al., 1980). Thus, it is likely that the changes in the vascular reactivity to angiotensin II during altered sodium intake may occur as a result of continuous regulating action of circulating angiotensin II on the homologous receptor sites.

In conclusion, the present study has confirmed previous reports of an inverse relationship between renin release and dietary change with sodium. Sodium restriction increased the renin levels while sodium loading suppressed renin secretion. The magnitude of pressor response by angiotensin I was modulated by the prevailing levels of renin. Acute blockade of converting enzyme activity with captopril markedly reduced the pressor response to test dose of angiotensin I and significantly inhibited the conversion of angiotensin I to angiotensin II. The data indicate that a small degree of conversion to angiotensin II still takes place. The hindlimb vascular reactivity to angiotensin II was directly related with dietary sodium but inversely related with plasma renin. The pressor response to angiotensin II was blunted by sodium restriction and enhanced by sodium loading. Vascular reactivity to noradrenaline was not significantly influenced by sodium restriction but was enhanced by salt loading. Administration of converting enzyme inhibitor, captopril, significantly potentiated the dose-response curves to angiotensin II. This occurred presumably by reducing the level
of circulating angiotensin II which resulted in greater availability of vascular receptor sites free to interact with exogenous angiotensin II. The data are consistent and favour the hypothesis that alteration in the degree of vascular reactivity by exogenous angiotensin II is most likely determined by prior occupancy and "up" and "down" regulation of vascular receptor sites.
6.2 PRESSOR RESPONSIVENESS TO ANGIOTENSIN II AND NORADRENALINE IN
STEROID AND 1-KIDNEY 1-CLIP GOLDBLATT HYPERTENSION

6.2.1 Blood pressure and Plasma renin

The present study confirms other reports that rats with acute deoxycorticosterone (DOC) - salt hypertension have a suppressed plasma renin concentration (PRC) (Goodwin, Knowlton, and Laragh, 1969; Pettinger et al, 1971; Marks et al, 1982; Bing et al, 1982). The data also demonstrate normal PRC in rats with early 1-kidney 1-clip Goldblatt hypertension which is in agreement with other reports (Bumpus et al, 1973; Bengis and Coleman, 1979; Sen et al, 1979) though, some have found an initial transient increase in renin (Brown et al, 1966; Liard and Peters, 1973; Miller, Samuels, Haber, and Barger, 1972).

These and other data suggest that both DOC and salt are necessary for the development of hypertension (Goodwin et al, 1969). However, the results do not implicate increase in the vascular resistance as the causative mechanism for the high blood pressure. Hindlimb vascular resistance was found to be identical and the blood pressure was not significantly different between the DOC-salt hypertensive animals and their controls (Table-4). Alternatively, the total peripheral resistance is elevated but not reflected in the hindlimb resistance i.e. selective vasoconstriction of other vascular beds may have occurred. There is no evidence to suggest this however, but an increase in hindlimb resistance was demonstrated in the hindleg of DOC-salt hypertensive pig (Berecek and Bohr, 1977). The slightly higher blood pressure in the DOC-salt hypertensive animals is probably mediated by an expansion of plasma volume and not the renin-angiotensin system since both sodium and water retention are known to suppress plasma
renin levels. The expansion of plasma volume may lead to an initial increase in the cardiac output (Ledingham and Cohen, 1963). After a period of several days when hypertension is well established, cardiac output returns to normal despite continual rise of blood pressure (Ledingham and Cohen, 1963), indicating that an increase in the total peripheral resistance must have occurred as a manifestation of autoregulation. However, once established, the vascular resistance remains elevated. This is in keeping with the proposed autoregulatory theory which has been described to have a time course measured in days or weeks (Guyton, Coleman, Cowley, Manning, Norman, and Ferguson, 1974).

6.2.2 Hindlimb perfusion pressure and Captopril

Increase in the pressor response with angiotensin I was much greater in the DOC-salt hypertensive animals than in any of the other groups. This may be attributed to low endogenous activity of angiotensin II in the animals.

The fall in the hindlimb perfusion pressure with converting enzyme inhibitor, captopril, was much greater in rats with 1-kidney 1-clip Goldblatt hypertension than in the normotensive intact animals. This occurred despite similarities in their PRC values (Table-4). Similarly, a greater depressor response was observed in the DOC-salt hypertensive animals than in their controls even though PRC was much suppressed in the hypertensive group. The fall in the perfusion pressure with captopril was not correlated with renin suggesting that mechanisms other than the renin-angiotensin system are involved. The differences may perhaps be explained by the potentiation of kinins (Murthy, Waldon, and Goldberg, 1978; Marks, Bing, Thurston, and Swales, 1980).
addition, the effects of kinins are believed to be mediated by prostaglandins (McGiff, Malik, and Terragano, 1976) resulting in vasodilatation.

6.2.3 Vascular reactivity

The current study has clearly identified an enhanced pressor responsiveness to both angiotensin II and noradrenaline in the hindlimb vasculature of rats with steroid and renovascular hypertension. Animals with 1-kidney 1-clip Goldblatt hypertension were found to have an increased constrictor response compared with normotensive intact animals. Similarly, vascular hyperresponsiveness was demonstrated in the DOC-salt hypertensive rats compared with their age-matched controls. In both models of hypertension, there was a clear shift towards the left of the dose-response curves with a higher vasoconstrictor effect compared with the control animals. This suggests a greater sensitivity of the hypertensive vasculature to pressor agents tested in this study.

Acute administration of converting enzyme inhibitor, captopril, significantly potentiated the pressor responsiveness to angiotensin II and noradrenaline in both the hypertensive groups and their controls. The dose-response curves shifted further towards the left suggesting that, at least, some form of hypersensitivity of the vessel wall must have taken place. The enhanced sensitivity to angiotensin II may arise as a result of a decrease in endogenous production of angiotensin II and an increase in the availability of angiotensin II receptor sites. Occupancy of the new vascular receptor sites by exogenous angiotensin II would then result in an enhanced response (Swales et al, 1975; Thurston, 1976). The increase in pressor response to angiotensin II, may then be attributed to prior occupancy or an increased availability
or "up" regulation (Gunther et al, 1980) for the vascular receptor sites.

The increase in vascular responses to noradrenaline, on the other hand, is difficult to explain. However, the results are, in part, agreement with those reported in whole body preparations (Marks et al, 1982; Bing et al, 1982). It is suggested that perhaps the presence of blood and the intact sympathetic nervous system of the preparation may modify the interaction between angiotensin II and noradrenaline and cause an apparent hypersensitivity. Inhibition of converting enzyme with captopril significantly potentiated the pressor responses to noradrenaline. As previously discussed, captopril by acting at the prejunctional site (Clough et al, 1982) may perhaps decrease the levels of noradrenaline at the sympathetic nerve terminals and cause a denervation effect (Trendelenberg, 1966).

The hypersensitivity of the vasculature to pressor agents has been suggested to be due to an increase in smooth muscle mass of the blood vessels, as a direct consequence of hypertension (Folkow et al, 1956; Collis and Alps, 1975; Berecik and Bohr, 1977, 1978; Folkow, 1978). To support this view, it would be expected that the response to all agonists would be equivalently affected. Our findings do not support such a proposal since the apparent vasoconstrictor threshold doses of angiotensin II and noradrenaline were different in the hypertensive animals. Further objections are raised by different maximum responses to the two pressor agents. It is possible, that the vessel wall instead of hypertrophy, may be thickened by "water-logging" i.e. an initial fluid retention may lead to an increase in sodium and water content of the vascular smooth muscle cell (Tobian and Binion, 1961). However, it is likely that thickening of the resistance vessels may be due to
deposition of collagen into the interstitial space of the vascular smooth muscle which would lead to an increase in non-osmotic binding of sodium (Jonsson et al., 1975). Thus, it seems possible that different mechanisms, each one contributing to a different degree, may be concerned in producing thickening of the vessel wall.

The main reason why the importance of structural hypertrophy appears to have lost credence to explain increased pressor responsiveness, was the demonstration that a change in vascular reactivity occurred independent of the rise in arterial blood pressure (McQueen, 1956; Collis and Alps, 1975; Berecek and Bohr, 1977, 1978; Collis, 1981). From these latter studies, it seems improbable that vascular hypertrophy could have developed before an increase in blood pressure. It was, therefore, concluded that functional changes occurred within the vasculature of the hypertensive animals. A distinct lowering of the threshold dose to both angiotensin II and noradrenaline was also demonstrated and this was in parallel with alterations in vascular reactivity in 1-kidney Goldblatt hypertensive rabbits (Ichikawa et al., 1978) and in DOC-salt hypertensive rats (Hansen and Bohr, 1975; Berecek et al., 1980) and pigs (Berecek and Bohr, 1978). This parallelism further supports the hypothesis that perhaps alterations in the vascular smooth muscle sensitivity may be the primary vascular defect.

Since structural changes are not considered to be important, perhaps changes in the intracellular ionic content may alter the sensitivity of the vascular smooth muscle. An increase in permeability to sodium and potassium in arterial smooth muscle has been shown in rats with DOC-salt hypertension (Jones and Hart, 1975; Friedman and Nakashima, 1978). This increase in intracellular sodium has been postulated to increase the concentration of active calcium ions in the vascular smooth muscle.
cells, and thereby, increase the responses to vasoconstrictors (Blaustein, 1977). Alternatively, it has been suggested that inhibition of the Na-K ATPase pump in the cell membrane, perhaps by the ubiquitous natriuretic factor (Haddy and Overbeck, 1976) may lead to an increase in intracellular calcium and cause an enhanced responsiveness (Blaustein, 1977), although this view is controversial (Overbeck, 1984).

It has been suggested that the sympathetic nervous system may play a contributory role in vascular reactivity. Measurement of plasma noradrenaline, used as an index to assess the peripheral sympathetic activity, has been shown to be elevated in rats with DOC-salt hypertension (Reid et al, 1975) and 1-kidney 1-clip Goldblatt hypertension (Dargie et al, 1977). However, the role of the sympathetic nervous system in enhanced reactivity has not been fully resolved. This is because one group has suggested that an intact peripheral sympathetic system was not a necessary requirement (Hansen and Bohr, 1975; Berecek and Bohr, 1977) whereas another group has shown renal nerves to be important in the development of DOC-salt hypertension (Katholi, Naftilan, and Oparil, 1980) and 1-kidney 1-clip Goldblatt hypertension in the rat (Katholi et al, 1981).

Recently, factors other than the renin-angiotensin system have also been considered to be involved to explain the hypersensitivity to pressor agents. Sensitising factor (Huang et al, 1978) and or tonin (Boucher et al, 1978) have been tentatively implicated in 1-kidney Goldblatt hypertension but there is less information about these substances and their role therefore remains speculative. A pathogenic role for vasopressin has been suggested (Crofton, Share, Shade, Lee-Kwon, Manning, and Sawyer, 1979). There is, however, evidence that
vascular reactivity to vasopressin is increased in the systemic
circulation (Crofton, Share, Wang, and Shade, 1980), and in isolated
perfused vascular bed of the kidneys (Berecek et al, 1980), mesentery
(Collis, 1981) and hindlimbs (Matsuguchi and Schmid, 1982). Although,
vazopressin was not tested in this study, the hormone like angiotensin
II may participate in the development of DOC-salt hypertension, perhaps
by modulating vascular smooth muscle responses to other
vasoconstrictors, such as the catecholamines (Bartelstone and Nasmyth,
1965).

In conclusion, the present study has confirmed the expected changes
in plasma renin and blood pressure of rats with DOC-salt and 1-kidney
1-clip Goldblatt hypertension. Pressor response to angiotensin I was
found to be increased in face of low prevailing levels of renin and its
conversion to angiotensin II was found to be largely inhibited by the
quantitative bioassay method. Administration of converting enzyme
inhibitor, captopril, lowered perfusion pressure in situations where
the renin-angiotensin system is not responsible for the maintenance of
blood pressure. This may arise as a result of potentiation of
bradykinin. Pressor response studies demonstrated an enhanced vascular
reactivity to angiotensin II and noradrenaline (dose-response curves
shifted to the left) in both models of hypertension. The
hypersensitivity to angiotensin II emphasise the importance of receptor
occupancy and an increase in the number of vascular receptor sites.
Vascular hyperresponsiveness was persistent even after inhibition of
converting enzyme. This increased responsiveness cannot be adequately
explained merely on the basis of an increased wall to lumen ratio of
the hypertensive vessel wall. It is suggested that the increased
sensitivity, perhaps due to additional changes in the ionic permeability, indicate a fundamental defect in the vascular smooth muscle cell membrane of the hypertensive animals.
6.3 PRESSOR RESPONSE STUDIES IN EARLY AND CHRONIC 2-KIDNEY 1-CLIP GOLDBLATT HYPERTENSION

6.3.1 Blood pressure and Plasma renin

The direct and indirect blood pressures were found to be similar regardless of duration of 2-kidney 1-clip Goldblatt hypertension (Table-7). This is despite the fact that both measurements of blood pressure were made under different anaesthetics. General anaesthesia is known to have a profound vasodepressor effect and therefore, the actual blood pressure must be higher than that reported here. It is surprising that the 'loose' clip control animals for early hypertension had slightly elevated blood pressure (>150mmHg) at time of study and there was an associated increase in plasma renin as well (Table-7). It is likely that the renal artery may have inadvertently become constricted by undue formation of collagen and fibrous tissue around the clip in some of the animals.

High blood pressure in both early and chronic groups of hypertensive animals was associated with raised hindlimb vascular resistance. The increase in resistance may arise, either as a result of vasoconstriction and or structural cardiovascular changes (Folkow, 1978), both of which would decrease lumen size of the blood vessels. The hindlimb perfusion pressure fell significantly only in rats with early hypertension after inhibition of the converting enzyme with captopril. This is because of higher levels of plasma renin in these animals.

The increase in plasma renin concentration (PRC) in the early phase of 2-kidney 1-clip Goldblatt hypertension is comparable with the rise
previously reported from this laboratory (Bing, Russell, Swales, and Thurston, 1981; Marks et al, 1982; Russell et al, 1982) and also other centres (Koletsky, Rivera-Valez, Marsh, and Pritchard, 1967; Miksche et al, 1970; Leenen et al, 1973; Oates et al, 1975; Bengis and Coleman, 1979; Eikenberg, Ekas, and Lokhandwala, 1982). This has led to the hypothesis that the early phase of 2-kidney 1-clip Goldblatt hypertension is dependent on the level of circulating renin and hence angiotensin II (Caravaggi, Bianchi, Brown, Lever, Morton, Powell-Jackson, ans Robertson, 1976). This is only partly true since infusions of saralasin and converting enzyme inhibitors partially lower the blood pressure in this model of hypertension (Marks et al, 1980). PRC was 3-4 times above normal levels in these animals. The standard error of the mean was, however, large (Table-7), primarily because one animal had PRC above 1100ng angiotensin I / ml / hour even though the blood pressure appeared to be of non-malignant type. If this value is excluded, then, PRC still remains above the normal range. The elevated PRC could be due to volume depletion in this model combined with reduced perfusion pressure in the ischaemic kidney. Thus plasma renin is known to be considerably elevated within a week after clipping a single renal artery (Morton and Wallace, 1983). In contralateral kidney, on the other hand, renin concentration is decreased to an almost undetectable levels possibly by negative feedback mechanism of angiotensin II.

There did not appear to be a relationship by regression analysis either between indirect or direct mean blood pressure and log PRC in rats with early phase hypertension. This may well be due to large variation in PRC. However, others have demonstrated a significant correlation between blood pressure and PRC in rats with early 2-kidney 1-clip renovascular hypertension (Morton and Wallace, 1983), though, this was not consistently true (Oates et al, 1975). These investigators
found an unusually high PRC in relation to this study and other reports (Bing et al., 1981; Marks et al., 1982) but the stimulatory effect of anaesthesia on renin secretion was ruled out as a possible explanation.

The role of the renin-angiotensin system in the established phase of 2-kidney 1-clip Goldblatt hypertension is controversial (Davis, 1977; Otsuka, Carretero, Albertini, and Binia, 1979). PRC during the chronic phase has been shown to be either elevated (Thurston and Swales, 1974) or normal (Bianchi, Baldoli, Lucca, and Barbin, 1972; Gavras, Brunner, Thurston, and Laragh, 1975; Carretero and Gulati, 1978). In the present study, PRC during the chronic stage was lower than in early phase, but it was still above (56%) the normal value of its 'loose' clip controls (Table-7). This is because two of the animals may have entered malignant phase of hypertension (blood pressure >190mmHg) and they also had elevated PRC (>440ng angiotensin I/ml/hour). This is in agreement with a recent report where a significant positive relationship was found between angiotensin II and blood pressure (Morton and Wallace, 1983). However, no significant relationship was demonstrated either between indirect or direct blood pressure and PRC in this study. Since the raised blood pressure at this stage does not appear to be maintained by the renin-angiotensin system, the secondary development of medial hypertrophy of the blood vessels may be assumed to play a greater role. Even so, the factors responsible for transition from the early renin dependent phase to the chronic phase of 2-kidney 1-clip Goldblatt hypertension are not clear at present.

6.3.2 Vascular reactivity

The present study has demonstrated that pressor responsiveness to angiotensin II and noradrenaline in animals with early 2-kidney 1-clip
Goldblatt hypertension was not significantly different from controls values. In contrast, animals with chronic 2-kidney 1-clip Goldblatt hypertension were markedly hypersensitive to both angiotensin II and noradrenaline as evidenced by the leftward shift in the dose-response relationship relative to their age-matched 'loose' clip controls.

The pressor response studies suggest an inverse relationship between angiotensin II sensitivity and the plasma renin concentration. Thus, in the early phase of 2-kidney 1-clip Goldblatt hypertension, plasma renin was elevated and the sensitivity to angiotensin II was unaltered from controls whereas in the chronically hypertensive rats where endogenous renin was normal, the pressor responsiveness was enhanced. These results are consistent with the earlier observations on dietary salt manipulation and are in keeping with the proposed importance of prior occupancy and availability of receptor sites by the endogenous angiotensin II (Thurston, 1976; Gunther et al, 1980). Thus, in situations such as the early phase of renovascular hypertension, when the renin-angiotensin system has been activated, many of the angiotensin II receptor sites will be occupied by the endogenous hormone and less sites will be made available for the exogenous angiotensin II. This would explain the failure to demonstrate an increased response to angiotensin II. On the other hand, when the renin secretion is not elevated as shown in the chronic phase of hypertension, a large number of unoccupied receptors will be free to react with exogenous angiotensin II and give rise to an enhanced response. The increase in sensitivity to angiotensin II after inhibition of the converting enzyme with captopril in both models of hypertension is also in keeping with the receptor hypothesis. Captopril eliminated the differences in response to angiotensin II such that the dose-response curves of the hypertensive animals came into close
apposition. Captopril by decreasing the formation of endogenous angiotensin II would increase the number of unoccupied receptor sites. Thus more of the receptors would be made available to interact with exogenous angiotensin II and cause an enhanced response. The increase in pressor response after captopril in the early phase of renovascular hypertension suggest that structural changes may have become partially established and there was no evidence for hypersensitivity to pressor agents as such. In the chronically hypertensive rats, on the other hand, the hypersensitivity to pressor agents is most likely to result due to structural vascular change (Folkow, 1978). Increase in sensitivity to pressor agents was demonstrated even 24 hours after surgical reversal of hypertension, when the blood pressure had returned to normal in both the early and chronic phase of renovascular hypertension, further suggesting the presence of structural change (discussed later).

In conclusion, elevation of blood pressure in the early phase of 2-kidney 1-clip Goldblatt hypertension was shown to be partly dependent on the level of circulating renin or angiotensin II. Pressor responsiveness to angiotensin II and noradrenaline was not enhanced in these animals. In the chronic phase of 2-kidney 1-clip Goldblatt hypertension, which is only partially dependent on the renin-angiotensin system, vascular sensitivity was greatly potentiated compared with their age-matched controls. This hypersensitivity may be partially attributed to the development of structural hypertrophy which may not have become fully established in the early phase. This is supported by the presence of hypersensitivity to noradrenaline in the chronic phase of hypertension. However, the differences in pressor
response may also be explained by prior occupancy and availability of angiotensin II receptor sites. Inhibition of the converting enzyme with captopril, significantly potentiated the responses and eliminated differences in sensitivity between early and chronic renovascular hypertension. The results suggest that while increased responsiveness does not appear to have developed in the early phase, hyperresponsiveness secondary to vascular hypertrophy was fully developed in the chronic phase of hypertension and this may be important in the maintenance of blood pressure.
6.4 EFFECT OF SURGICAL REVERSAL ON PRESSOR RESPONSIVENESS IN EARLY AND CHRONIC 2-KIDNEY 1-CLIP GOLDBLATT HYPERTENSION

6.4.1 Blood pressure and Plasma renin

It was demonstrated that removal of the constricting clip to the renal artery resulted in an acute fall of blood pressure to normal levels at 24 hours and 60 days in both models of hypertension. This is in agreement with previous reports (Gross, 1971; Thurston et al, 1980; Russell, Bing, Swales, and Thurston, 1983; Bing, Swales, Taverner, and Thurston, 1984) though others have found higher than normal blood pressure after unclipping (Floyer, 1951; Lundgren and Weiss, 1979). Extirpation of the ischaemic kidney alone dramatically lowered the blood pressure to subnormal levels in both groups of hypertensive rats (Table-11). Previous reports have also documented normalisation of blood pressure after removal of the ischaemic kidney in the early phase (Wilson and Byrom, 1941; Koletsky and Rivera-Valez, 1970; Gross, 1971; Thurston et al, 1980; Russell et al, 1983) but blood pressure remained elevated in the chronic phase (Wilson and Byrom, 1941; Koletsky and Rivera-Valez, 1970; Thurston et al, 1980; Russell et al, 1983).

The fall in blood pressure was associated with profound fall in hindlimb vascular resistance which was previously shown to be elevated in the intact hypertensive animals. The reduction in vascular resistance occurred irrespective of the duration of hypertension and whether reversal of hypertension was achieved by removal of the constricting clip or the ischaemic kidney. This fall in resistance occurred despite the presence of structural changes which have been considered to be both reversible and irreversible with time (Lundgren, 1974; Lundgren and Weiss, 1979).
The fall in hindlimb vascular resistance brought about by the surgical reversal of hypertension may be due to single or a combination of mechanisms. It may be speculated that some form of vasopressor substance was eliminated by the kidney since unclipping was as effective as nephrectomy at 24 hours. However, renin does not appear to be the substance though the marked and persistent reduction in blood pressure after the surgical reversal of hypertension still needs to be fully investigated. Alternatively, a vasopressor type of material either released or activated in the renal medulla by the manoeuvre of surgical reversal has been suggested (Muirhead, 1980; Bing et al, 1981; Gothberg, Lundin, and Folkow, 1982).

The data confirm earlier findings of increased levels of plasma renin in the early phase which was drastically reduced by surgical correction of hypertension. PRC was normal at 24 hours after unclipping but it was subnormal both 24 hours after nephrectomy and 60 days after unclipping in both early and chronic models of hypertension. These differences in PRC have been related to greater degree of sodium retention after the operation (Thurston et al, 1980) or increased perfusion pressure suppressing renin secretion by the previously ischaemic kidney.

6.4.2 Vascular reactivity

It was demonstrated that pressor responsiveness to angiotensin II and noradrenaline was significantly enhanced 1 day after unclipping or nephrectomy in both early and chronic renovascular hypertension. This is in agreement with previous reports of an increase in the blood pressure response to infusions of renin, angiotensin II or noradrenaline after the relief of ischaemia by removal of the
constricting clip (Aoki and Mason, 1969; Skulan et al, 1974; TenBerg and DeJong, 1980). These experiments were carried out in whole body and the influence of ressetting of baroreceptor reflexes and the levels of circulating renin need to be taken into account. However, in a recent study, hyperresponsiveness to angiotensin II or noradrenaline was not demonstrated in vivo in unclipped rats when the pressor responses were correlated with endogenous levels of renin (Bing et al, 1984).

The changes in vascular responses could be explained only partially by endogenous levels of renin. Our earlier findings in normal rats have suggested that angiotensin pressor responses are influenced substantially by endogenous levels of renin and there is an inverse relationship between response and PRC. Thus, according to this interpretation, the fall in PRC after unclipping or nephrectomy, would result in decrease in occupancy of angiotensin II receptors (Thurston, 1976) and or an "up" regulation of such receptors (Gunther et al, 1980) so that more receptors sites would be free to interact with exogenous angiotensin II.

However, the structural vascular changes still remain present after the initial reversal of hypertension. Enhanced vascular sensitivity due to partial structural alterations was demonstrated before inhibition of the converting enzyme in the early phase of renovascular hypertension. Structural hypertrophy with consequent non-specific hyperresponsiveness was, however, much more marked in the chronically hypertensive rats. Despite the return of blood pressure to normal, hypersensitivity to pressor agents was still present at 1-day in the chronic and to the same extent in the early phase of renovascular hypertension. With time, the differences in pressor response were shown to disappear over 60 days in both early and chronic 2-kidney 1-clip Goldblatt hypertension.
strongly suggesting regression of structural vascular changes.

Despite the fall of blood pressure to normal or subnormal levels, 24 hours after the surgical correction of renal ischaemia, hypersensitivity to pressor agents was still demonstrated to be present. Clearly, if the fall in blood pressure was entirely dependent on the regression of structural changes, then, a very slow decline would be expected over a period of time. Thus, it would appear that vascular structural changes can be overcome and it has been proposed, therefore, that blood vessels must be in a state of subnormal tone after the reversal of hypertension (Hallback-Nordlander et al, 1979; Gothberg et al, 1982). However, the mechanism responsible for the rapid fall in blood pressure after either declipping or removal of the ischaemic kidney still remains uncertain. The finding that pressor responses were not significantly different 60 days after unclipping does suggest that complete regression of structural vascular changes had occurred.

In conclusion, surgical reversal of hypertension returned arterial blood pressure and plasma renin to normal in rats with early and chronic 2-kidney 1-clip Goldblatt hypertension. The associated fall in hindlimb vascular resistance is believed to be due to subnormal vascular tone. Hypersensitivity to pressor agents was demonstrated 1 day after removal of the constricting clip or the ischaemic kidney. This may be due to structural vascular changes which had developed only partially in the early phase but were fully established in the chronic phase of hypertension. 60 days after surgical correction, the pressor responses had returned to normal, indicating regression of vascular changes had occurred. The results emphasise the importance of endogenous
levels of renin and structural changes in the interpretation of vascular responsiveness to pressor agents during the reversal of renovascular hypertension but also indicate that other factors contribute to the immediate fall in blood pressure.
CONCLUSIONS

(a) Plasma renin concentration was inversely related to dietary sodium intake.

(b) Pressor responsiveness to angiotensin II was reciprocally related to plasma renin and the differences were overcome by blockade of the renin-angiotensin system with captopril.

(c) Pressor reactivity with noradrenaline was not related to plasma renin or dietary sodium.

(d) It is suggested that prior occupancy and "up" and "down" regulation of vascular angiotensin II receptors may explain the differences in sensitivity to angiotensin II in normotensive animals.

(e) Hypersensitivity to pressor agents was demonstrated in rats with early DOC-salt and Goldblatt 1-kidney 1-clip models of hypertension. This was present after inhibition of converting enzyme with captopril which indicates that factors other than endogenous levels of angiotensin II are important. It is possible that changes in the vessel wall may be important for the development of hypertension in these models.

(f) In the early phase of 2-kidney 1-clip Goldblatt hypertension, the vascular reactivity to angiotensin II was not significantly different from controls, while in the chronic phase, the responses were greatly potentiated. Inhibition of converting enzyme with captopril significantly increased the pressor responses but the chronically hypertensive animals remained significantly more responsive than their controls. Thus structural vascular changes leading to increased
vascular reactivity could only be demonstrated in the chronic phase of hypertension.

(g) Renal artery declipping or removal of the ischaemic kidney restored the blood pressure and vascular resistance to normal at 1 day in both the early and chronic phase of 2-kidney 1-clip Goldblatt hypertension. Hypersensitivity to pressor agents was enhanced in the chronic phase but also demonstrated to some extent in rats with early renovascular hypertension. This suggests that structural vascular changes are partially developed in the early phase but fully developed in the chronic phase of hypertension. Since the blood pressure was normal, despite the presence of structural vascular change, the peripheral blood vessels must be in a state of subnormal vascular tone.

(h) 60 days after unclipping the renal artery, the pressor reactivity returned to normal in both early and chronic phase hypertension suggesting that regression of structural vascular changes had occurred.

(i) Studies on renovascular hypertension suggest that structural changes in the vessel wall are a consequence of hypertension and appears to have little importance in the cause of the raised blood pressure.
REFERENCES


AIKEN.JW. and VANE.JR. Inhibition of converting enzyme of the renin angiotensin system in kidneys and hindlegs of dogs. Circ.Res. 30:263-273 (1972)

AMES.RP., BORKOWSKI.AK., SICINSKI.AM., and LARAGH.JH. Prolonged infusions of angiotensin II and norepinephrine and blood pressure, electrolyte balance, and aldosterone and cortisol secretion in normal man and in cirrhosis with ascites. J.Clin.Invest. 44:1171-1186 (1965)


BACKER.M. Essential hypertension : the birth of its concept two hundred years ago. Angiology 4:207-209 (1953)


BARRACLOUGH.MA., JONES.NF., MARSDEN.CD., and BRADFORD.BC. Renal and pressor actions of angiotensin in salt loaded and depleted rabbits. Experientia 23:553-555 (1967)

BARTELSTONE.HJ. and NASMYTH.PA. Vasopressin potentiation of catecholamine actions in dog, cat, and rat aortic strips. Am.J.Physiol. 208:754-762 (1965)


BERECEK.KH. and BOHR.DF. Structural and functional changes in vascular


BROWN.JJ.,DAVIES.DL., LEVER.AF., and ROBERTSON.JIS. Influence of sodium deprivation and loading on the plasma renin in man. J.Physiol. 173:408-419 (1964)


BRODY.MJ., SHAFFER.RA., and DIXON.RL. A method for the study of peripheral vascular responses in the rat. J.Appl.Physiol. 18:645-647 (1963)


Collins, M.G. and Alps, B.J. Vascular reactivity to noradrenaline, potassium chloride, and angiotensin II in the rat perfused mesenteric vasculature preparation, during the development of renal hypertension.
Cardiovasc.Res. 9:118-126 (1975)


COCK.W., GORDON.DB., and PEART.WS. The location of renin in the rabbit kidney. J.Physiol. 135: 46-47 (1957)


COXLEY.AW. Jr. and McCAA.RE. Acute and chronic dose response relationship for angiotensin, aldosterone and arterial pressure at varying levels of sodium intake. Circ.Res. 39:788-797 (1976)


DeCHAMPLAIN.J. The influence of sodium on the sympathetic system in relation to experimental hypertension. In: Hypertension : mechanisms


DeCHAMPLAIN, J., KRAKOFF, L.R., and AXELROD, J. Relationship between sodium intake and norepinephrine storage during the development of experimental hypertension. Circ. Res. 23:479-491 (1968)


ELLIOT, D.F. and PEART, W.S. Amino-acid sequence in a hypertensin.


FLOYER, M.A. The effect of nephrectomy and adrenalectomy upon the blood pressure in hypertensive and normotensive rats. Clin. Sci. 10:405-421 (1951)


FOLKOW, B., HALLBACK, M., LUNDGREN, Y., and WEISS, Y. Renal vascular resistance in spontaneously hypertensive rats.


GOLDBLATT, H. The renal origin of hypertension. Physiol Rev. 27:120-165 (1947)


GOLDBLATT, H., LYNCH, J., HANZEL, R.F., and SUMMerville, W.W. Studies on


GREEN.CJ. Animal anaesthesia. Laboratory animals. London. 1979. p 154-160


GRIESEMAN,SE. The reactivity of the capillary bed of the nailfold to circulating epinephrine and nor-epinephrine in patients with normal blood pressure and with essential hypertension. J.Clin.Invest. 31:782-788 (1952)


GROLLMAN,A., MUIRHEAD,EE., and VANATT,AJ. Role of the kidney in the 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. Am.J.Physiol. 157:21-30 (1949)


Ichikawa, S., Johnson, JA., Fowler, WL., Jr., Payne, VG., Kurz, K., and Keitzer, WF. Pressor responses to norepinephrine in rabbits with 3-day and 30-day renal artery stenosis. The role of angiotensin II. Circ. Res. 43:437-446 (1978)


LARAGH.JH., CASE.DB., ATLAS.SA., and SEALEY.JE. Captopril compared with other antirenin system agents in hypertensive patients: its triphasic effects on blood pressure and its use to identify and treat the renin factor. Hypertension 2:586-593 (1980)


LJARD.JF. and PETERS.G. Role of the retention of water and sodium in two types of experimental renovascular hypertension in the rat.
Pflugers Arch. 344:93-108 (1973)


MALLOV.S. Comparative reactivities of aortic strips from hypertensive and normotensive rats to epinephrine and levarterenol. Circ. Res. 7:196-201 (1959)


MATUBA.T., MURAKAMI.K., and INAGAMI.T. Rat renin: purification and


Morris.BJ. and Roper.KJ. Investigation of the presence of "renopressin" in kidney extracts from rabbits and rats. Hypertension 4:196-204 (1982)


Muirhead.EE., Germain.GS., Armstrong.FB., Brooks.B., Leach.BE., Byers.LW., Pitcock.JA., and Brown.P. Endocrine type antihypertensive function of
renomedullary interstitial cells. Kid.Int. 8:271s-282s (1975)


MURTHY.VS., WALDON.TL., and GOLDBERG.ME. The mechanism of bradykinin potentiation after inhibition of angiotensin-converting enzyme by SQ 14,225 in conscious rabbits. Circ.Res. 43 (suppl I):40-45 (1978)


NOLLA-PANADES.J. Hypertension and increased hind-limb vascular reactivity in experimental coarctation of the aorta. Circ.Res. 12:3-9 (1963)


O'GREN.E., BROWN.LT., and PAGE.EW. The increased sensitivity of arterial muscle in the pre-hypertensive phase of experimental renal hypertension. Am.J.Physiol. 129:560-564 (1940)


OKAMOTO.K., HAZAMA.F., TAKEDA.T., Tabei.R., NOSAKA.M., FUKUSHIMA.M., YAMORI.Y.


PAGE. IH. On the nature of the pressor action of renin. J. Exp. Med. 70:521-542 (1939)


PAGE. IH. and HELMER. CM. A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin-activator. J. Exp. Med. 71:29-42 (1940)


PEACH. MJ. Adrenal medullary stimulation induced by angiotensin I.


PICKERING.GW. The role of the kidney in acute and chronic hypertension following renal artery constriction in the rabbit. Clin.Sci. 5:229-247 (1945)


PREWITT.RL., LEACH.BE., BYERS.IW., BROOKS.B., LANDS.WE., and MUIRHEAD.EE. Antihypertensive polar renomedullary lipid, a semisynthetic vasodilator. Hypertension 1:299-308 (1979)


PRINZMETAL.M. and WILSON.C. The nature of the peripheral resistance in arterial hypertension with special reference to the vasomotor system. J.Cli.Invest. 15:63-83 (1936)

READLEAF.PD. and TOBIAN.L. The question of vascular hyper-responsiveness in hypertension. Circ.Res. 6:185-193 (1958)


ROBERTSON.Al. and KHAIYALLAH.PA. Effects of angiotensin II and some analogues on vascular permeability in the rabbit. Circ.Res. 31:923-931 (1972)

RUSSELL.GI.,BING.RF.,SWALES.JD.,and THURSTON.H. Hemodynamic changes induced by reversal of early and late renovascular hypertension. Am.J.Physiol. 245:734-740 (1983)


SKAGGS.LT. Jr., KAHN.JR., and SHUMWAY.NP. The isolation of hypertensin from the circulating blood of normal dogs with experimental renal hypertension by dialysis in an artificial kidney. Circulation 3:384-389 (1951)


STREMLER.GJ., HINRICH.KJ., GUIOD.LR., and HOLLENBERG.NK. Sodium intake and vascular smooth muscle responsiveness to norepinephrine and angiotensin in the rabbit. Circ. Res. 31:758-766 (1972)


SWALES.JD. Renin-angiotensin system in hypertension. Pharmac.Ther. 7:173-201 (1979)


THURSTON.H.,HURST.BC.,BING.RF.,and SWALES.JD. Role of persistent


Tobian.L. and Binion.JT. Tissue cations and water in arterial hypertension. Circulation 5:754-758 (1952)


Vander. AJ. Inhibition of renin release in the dog by vasopressin and vasotocin. Circ.Res. 23:605-609 (1968)


Weber.PC. and Steiss.W. Influence of renal prostaglandins on renin release. In: The renal papilla and hypertension. A.Mandal. and
WEEKS, JR. and COMPTON, ID. The cardiovascular pharmacology of prostacyclin (PGI2) in the rat. Prostaglandins 17:501-513 (1979)


