ARTERIAL WALL RENIN-LIKE ACTIVITY AND BLOOD PRESSURE REGULATION IN THE RAT

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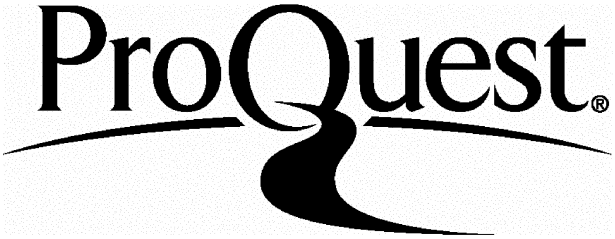
A thesis submitted for the degree of

Doctor of Philosophy

to the University of Leicester

March 1985



Acknowledgements

I would like to thank Professor J.D. Swales for the privilege of working in his laboratory and Dr. R.F. Bing for acting as my supervisor. I would also like to thank all the other members of the Department of Medicine for their help and support. My thanks are due to Miss Denise Huckerby for typing the manuscript and to Miss Frances Rankine for preparing the figures. Finally, I am indebted to the British Heart Foundation for financial support.

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CHAPTER ONE

General Introduction

Historical Background

In 1898 Tigerstedt and Bergman demonstrated pressor activity in saline extracts of the cortex of rabbit kidney. The substance responsible was water soluble, non-dialysable and heat labile; that is it had the properties of a protein. They gave it the name renin.

Forty years later Goldblatt published the results of a series of experiments describing a model of renovascular hypertension produced in the dog (Goldblatt, Lynch, Hanzal and Summerville, 1934). He placed metal clamps on the renal arteries of these animals so that the blood flow reaching the kidneys was reduced, although the vessels were not completely occluded. This gave rise to hypertension. Similar experimental ischaemia of other organs did not produce hypertension. He found that neural factors were not involved. Tying off the renal vein from the constricted kidney prevented the development of hypertension. He therefore suggested that the kidney produced a humoral factor which was responsible for the rise in blood pressure.

The involvement of a pressor agent was confirmed by Houssay and Fasciolo (1937). They produced hypertension by grafting an ischaemic kidney into the neck of a nephrectomised dog. Transplantation of a normal kidney did not give rise to an increase in blood pressure. This pressor agent produced by the ischaemic kidney was capable of causing vasoconstriction in the perfused hind limb of the toad (Houssay and Taquini, 1938). However, *in vitro* the pressor agent had no effect.

At the same time Page and Helmer (1940) and Braun-Menendez, Fasciolo, Leloir and Muñoz (1939) separately demonstrated that the renin produced by the kidney had no direct pressor activity. Both

showed that renin cleaved a substrate molecule present in the circulation, generating a pressor agent named angiotonin and hypertensin by the two groups respectively. In vitro studies using aortic strips showed that the product of the renin reaction was itself inactive until exposed to plasma (Helmer, 1955). A further distinction was subsequently made between this substance, a decapeptide called hypertensin I, and hypertensin II to which the former was rapidly converted by a factor present in the plasma (Lentz, Skeggs, Woods, Kahn and Shumway, 1956). The conversion factor was shown by Skeggs, Kahn and Shumway (1956) to be an enzyme which they called hypertensin converting enzyme. Conversion involved the removal of two amino acids from hypertensin I (Lentz et al, 1956). The major active form of hypertensin was therefore an octapeptide (Peart, 1956). These two substances have subsequently become commonly known as angiotensin I and angiotensin II.

The "renin-angiotensin" system therefore involves a series of reactions resulting in the production of an extremely potent pressor agent, angiotensin II. The sequence is initiated by renin.

Components of the Renin-Angiotensin System

Renin

The main source of renin is the kidney although it has also been demonstrated in the uterus, brain, adrenals and arterial tissue of a number of species. Extra-renal renin will be discussed in detail in a later section. Significant quantities are also present in the circulation.

In the kidney renin is produced by the juxtaglomerular apparatus. This consists of specialised smooth muscle cells of the afferent arteriole which show cytoplasmic granulation, and the macula densa, a modified portion of the wall of the distal tubule lying immediately

adjacent to the junction of the afferent and efferent arterioles.

A number of factors are instrumental in controlling the release of renin from the kidneys. They have been reviewed by Davis and Freeman (1976) and Zanchetti, Stella, Leonetti and Mancia (1981).

In general the two important stimuli which result in changes in renin secretion are changes in the renal artery perfusion pressure and changes in the extracellular fluid volume. This latter effect itself produces a change in the perfusion pressure of the kidney but also results in a change in the composition of the renal tubular fluid.

Changes in the perfusion pressure are detected by stretch receptors present in the afferent arteriole of the juxtaglomerular apparatus. These receptors also respond to renal sympathetic nerve activity.

Changes in the composition of the renal tubular fluid are detected by the macula densa which acts as a chemoreceptor. Evidence suggests that the concentration of both the sodium and the chloride ion may be important in this respect (Zanchetti et al, 1981). The macula densa is thought to respond either to the rate of delivery of the ions to the distal tubule or the rate of transport of these ions by the macula densa.

The release of renin from the kidney is also under the control of the central nervous system. The activity of the sympathetic nerve supply to the kidneys is determined by the response of baroreceptors at a number of sites within the circulation. The renal nerves act directly on the renin containing cells of the juxtaglomerular apparatus and also alter the tension in the afferent arteriolar wall. By constriction of the afferent and efferent

arterioles of the glomerulus they influence glomerular filtration rate. This in turn alters the composition of the renal tubular fluid and hence results in the release of renin by stimulation of the chemoreceptors. Catecholamines have also been shown to stimulate renin secretion by an intrarenal effect which is independent of changes in renal perfusion pressure. The beta-adrenoreceptors mediating this action are thought to be an integral component of renin producing cells (Vandongen and Greenwood, 1975).

In addition to these factors a number of humoral agents are involved in the control of renin secretion. Both angiotensin II and vasopressin inhibit renin secretion. A number of prostaglandins have been shown to stimulate renin release, apparently acting by a mechanism which is independent of renal vasodilatation (Data, Gerber, Crump, Frölich, Hollifield and Nies, 1978; Gerber, Keller and Nies, 1979). However, prostaglandins formed in the renal cortex also participate in the control of renal blood flow and glomerular filtration rate and are thus indirectly involved in the control of the secretion of renin (Weber and Siess, 1980).

Suzuki, Franco-Saenz, Tan and Mulrow (1980) have demonstrated that kallikrein can stimulate renin release from the rat kidney. They have suggested that this occurs via a process of activation of inactive renin within the kidney resulting in an increase in the secretion of active renin.

Despite its early identification, the biochemical characterisation of renin proved difficult, mainly because of its instability during purification. However, the recent application of affinity chromatography techniques has allowed the preparation of relatively pure renin (Matoba, Murakami and Inagami, 1978; Yokosawa, Holladay, Inagami, Haas and Murakami, 1980).

Renin has been purified from a number of sources including pig (Corvol, Devaux, Ito, Sicard, Ducloux and Menard, 1977; Inagami and Murakami, 1977), rat (Matoba et al, 1978), dog (Dzau, Slater and Haber, 1979) and human (Galén, Devaux, Guyenne, Menard and Corvol, 1979; Yokosawa et al, 1980; Slater and Strout, 1981) but the low concentration of renin in both animal and human kidneys has prevented its exact sequence from being determined. However, renin is produced in sufficiently large quantities by the submaxillary gland of the mouse for the sequencing procedure to be carried out. This has been done both by a protein sequencing technique (Misono, Chang and Inagami, 1982) and by cloning of the mouse renin gene (Panthier, Foote, Chambraud, Strosberg, Corvol and Rougeon, 1982). Mouse submaxillary gland renin consists of two polypeptide chains, A and B, linked by a disulphide bridge, the A chain being much the larger of the two. The physicochemical and enzymatic characteristics of the enzyme have been shown to be similar to renal renin and it is therefore concluded that their structures are also comparable. This subject has been reviewed recently by Corvol, Panthier, Soubrier, Menard and Rougeon (1983).

Different molecular weights have been obtained for fully active renin, ranging from 36,400 to 40,000 in hog kidney and 39,400 to 43,000 for human renin of renal origin (Peach, 1977). These discrepancies may be due to differences in the purification techniques used although renin is known to exist as a number of isoenzymes (Ondetti and Cushman, 1982). Three different renins with molecular weights of 35, 36 and 37,000 have been isolated from rat plasma (Matoba et al, 1978). The difference in molecular weight of these isoenzymes is probably due to different degrees of glycosylation of the protein molecule as represented by multiple iso-

electric points (Barrett, Eggena, Krall and Sambhi, 1981; Chang, Kisaragi, Okamoto and Inagami, 1981).

Renin is classified as an acid protease on the basis of the amino-acid composition of its active site and of its inhibition characteristics. Studies on the inhibition of mouse submaxillary gland renin have suggested that renin is similar to other acid proteases (Misono and Inagami, 1980). Hog renal renin has also been found to have similar inhibition characteristics (Inagami, Misono and Michelakis, 1974). A high degree of homology has been found between the amino-acid sequence of submaxillary gland renin and other acid proteases. This is especially true of the residues which make up the catalytic site (Inagami et al, 1974; Misono and Inagami, 1980; Panthier et al, 1982).

Although these properties justify the inclusion of renin in the category of acid proteases, it has a broad pH optimum which lies in the neutral rather than in the acid range. The pH optimum is species specific and varies with the source of substrate used. The pH optimum of purified human renin was measured by Yokosawa et al (1980) as 6.0. Rat renal renin has been shown to be optimally active within the range 6.0 to 6.5 (Matoba et al, 1978) and hog renin has a broader pH optimum between 5.5 and 7.0 (Inagami and Murakami, 1977). All these enzymes thus have a similar pH range of optimal activity.

Renin is an unusual protease in being highly specific, acting on only the one substrate. It cleaves the substrate molecule at the leucyl-leucine peptide bond between the tenth and eleventh amino-acid residues releasing the N-terminal decapeptide angiotensin I. Its activity is also species specific and there is marked variation between the reaction characteristics of renin with substrates from

different sources. Matoba et al (1978) found that the pH optimum of purified rat renin varied with the type of substrate used. With rat plasma substrate the range was 6.0 to 6.5 and with hog substrate, 5.5 to 6.5. In contrast, hydrolysis of a synthetic substrate was maximal between 4.0 and 4.5.

Mouse submaxillary gland renin has been shown to be synthesised as a single-chain precursor polypeptide, prorenin (Poulsen, Vuust, Lykkegaard, Nielsen and Lund, 1979). Furthermore, a prorenin precursor, pre-prorenin, from mouse submaxillary gland, has been characterised and its sequence determined (Panthier et al, 1982). The molecule is a single polypeptide which is cleaved by membrane-bound processing enzymes removing a peptide signal to produce prorenin. Prorenin itself has been shown to be converted to an active form by further cleavage at two sites (Panthier et al, 1982).

Inactive Renin

That renin might be synthesised or stored as a precursor was first suggested by the finding that renin activity of amniotic fluid could be increased by dialysis at pH 3.3 followed by returning the pH to 7.4 (Lumbers, 1971). It was subsequently found that about seventy percent of the renin present in human plasma was present as an inactive form (Atlas, Laragh, Sealey and Hesson, 1980) and that activation could also be induced by the addition of exogenous proteases such as trypsin or pepsin (Shulkes, Gibson and Skinner, 1978; Sealey, Atlas, Laragh, Oza and Ryan, 1979) or by incubation of the plasma at $-5^{o}C$ for four days, that is, by cryoactivation (Sealey, Moon, Laragh and Alderman, 1976).

The significance of inactive renin remains unclear. It does not appear to be a true renin precursor since the prorenin identified

by Poulsen et al (1979) is not present in the plasma. It is converted to enzymatically active renin inside the cell. A number of studies have indicated that active renin may be associated with other moieties. Boyd (1974) isolated a high molecular weight renin (60,000) from hog kidney which underwent a decrease in molecular weight and a two and a half fold increase in activity on acid treatment. This was associated with the loss of a renin binding protein. Totally inactive renin with a higher molecular weight than active renin has been found in the human kidney (Atlas, Sealey, Dharmgrongartama, Hesson and Laragh, 1981; Chang et al, 1981; Kawamura, Akabane, Ito, Ogino, Yutani, Go and Ikeda, 1982). Leckie (1981) has suggested that inactive and partially active forms of renin consist of an association between renin and renin binding proteins which may modify renin activity. This suggestion fits the observation that renin in storage granules in dog kidney is present in the low molecular weight active form while a renin binding protein is present in the cytoplasm (Kawamura, Ikemoto, Funakawa and Yamamoto, 1979). This has also been demonstrated in the rat (Sagnella, Price and Peart, 1980) and the pig (Sagnella, Caldwell and Peart, 1980). Although more recently Kawamura et al (1982) have found that about twenty-five percent of the renin in storage granules in the human kidney is present in an inactive form.

Atlas et al (1981) and Chang et al (1981) have found that both active and inactive renin from human kidneys have lower molecular weights compared to the respective plasma enzymes. This finding favours the theory of association of renin with a binding protein on secretion. Alternatively, Chang et al (1981) have suggested that secretion produces a change in molecular conformation,

altering the Stokes radius and hence the apparent molecular weight.

Inactive renin in plasma has been found to have the same (Shulkes et al, 1978) or larger (Hsueh, Luetscher, Carlson and Grislis, 1980; Atlas et al, 1981; Chang et al, 1981) molecular weight than active renin. However, when the larger molecular weight inactive renin is activated *in vitro* the molecular weight does not change (Hsueh et al, 1980; Chang et al, 1981). This suggests that these mechanisms of activation may be different from the *in vivo* process. Nevertheless, models of *in vivo* activation have been based on the proposed mechanisms observed *in vitro*.

Acid activation depends on the presence of endogenous serine proteases (Atlas, Sealey and Laragh, 1978; Derkx, Tan-Tjiong and Schalekamp, 1978; Leckie, 1978) and takes place in two stages. The first is a reversible process possibly involving some degree of unfolding of the inactive molecule induced by the acid environment. This either exposes the active site (Hsueh, Carlson and Israel-Hagman, 1981) or results in dissociation from a binding protein (Leckie, 1981). These processes are both reversible but during dialysis to pH 7.4 over twenty-four hours the change is rendered irreversible, possibly by the action of serine proteases. The acid phase destroys the naturally occuring inhibitors of these enzymes. Protease action could either render exposure of the active site permanent by removal of a portion of the molecule (Hsueh et al, 1981) or inactivate the dissociated binding protein (Leckie, 1981).

Chemical modification is thought to be the mechanism by which exogenous proteases activate renin (Chang et al, 1981). Cryo-activation results in less activation but is also thought to be dependent on serine protease activity (Atlas et al, 1981).

Although these *in vitro* studies may help in the understanding of the molecular mechanisms involved in renin activation, the significance and mechanisms of the process *in vivo* are not known. However, models involving naturally occurring systems have been proposed. Sealey, Atlas, Laragh, Oza and Ryan (1978) found that urinary kallikrein could convert inactive plasma renin to its active form *in vitro*. However, this enzyme was thought unlikely to be important *in vivo*. Subsequently, both Sealey, Atlas, Laragh, Silverberg and Kaplan (1979a) and Derkx, Tan-Tjiong, Man In'T Veld, Schalekamp and Schalekamp (1979) showed that plasma and tissue kallikrein systems were involved in the activation of renin. Furthermore, activation was shown to be initiated by the conversion of prekallikrein to kallikrein (Sealey et al, 1979a). This reaction is dependent on the presence of Hageman factor (Factor XII), a substance also involved in coagulation and fibrinolysis and which is activated in contact with negatively charged surfaces (Ogston and Bennett, 1978). Sealey, Atlas, Laragh, Silverberg and Kaplan (1979b) have also suggested that the production of plasmin from plasminogen by kallikrein may be involved. This links the activation of the renin-angiotensin system with other surface mediated reactions known to occur *in vivo* (Derkx, Bouma, Tan-Tjiong and Schalekamp, 1979).

Schalekamp and Derkx (1981) have suggested that a balance may exist between the renin-angiotensin and kallikrein-kinin systems with their opposite effects on vascular smooth muscle, possibly involving control of renin activation. They propose that kallikrein and plasmin could be formed on the surface of blood vessel walls, where they may be less susceptible to circulating

inhibitors. Activation of renin could then take place at this site. Corvol, Galen, Devaux and Menard (1983) have shown that prorenin is bound to blood vessel walls.

Renin Substrate

Renin substrate, which is also referred to as angiotensinogen, is synthesised by the liver which secretes it into the circulation (Nasjletti and Masson, 1972). A constant level of substrate is maintained despite variations in plasma renin concentration (Blair-West, 1976). The precise mechanisms which regulate substrate levels are not clearly understood but overall synthesis appears to be dependent on glucocorticoid levels. Production is also related to blood oestrogen levels. It increases after bilateral nephrectomy and adrenalectomy (Nasjletti and Masson, 1972) and is decreased by liver disease or damage (Peach, 1977).

There is marked species variation in the molecular weights of renin substrate, ranging from 57,000 in the hog (Skeggs, Lentz, Hochstrasser and Kahn, 1963) to 110,000 in human plasma (Eggena, Chu, Barrett and Sambhi, 1976).

Skeggs, Kahn, Lentz and Shumway (1957) sequenced the N-terminal tetradecapeptide of equine angiotensinogen. They showed that the minimum sequence required for significant cleavage by renin was the octapaptide of residues six to thirteen (Skeggs, Lentz, Kahn and Hochstrasser, 1968). This section of the molecule accounts for a large part of the binding affinity between renin and its substrate.

The overall amino-acid composition of renin substrate from different species is essentially the same (Peach, 1977). However, there is species variation in the susceptibility of the substrate to hydrolysis by heterologous renin (Peach, 1977). The kinetics

of the reactions between the two will thus depend on the sources of both renin and its substrate. The most striking example is that of human renin and substrate. Although human renin can cleave substrates from other species, human substrate can only be cleaved by renin from primates. Tewksbury, Dart and Travis (1981) have shown that the bond between positions ten and eleven in human substrate is leucyl-valine rather than leucyl-leucine. Less dramatic differences in the interspecies reaction kinetics between renin and substrate may also be due to less crucial amino-acid substitutions (Gordon and Sachin, 1975).

At the pH optimum of the renin substrate reaction the concentration of substrate present in plasma would influence the reaction velocity. However, at pH 7.4, the pH of plasma, enough substrate is present to sustain a maximum reaction rate (Favre and Vallotton, 1973). It is therefore important to ensure that substrate is present in excess when measuring renin concentration in vitro at its pH optimum rather than at 7.4. This is discussed in Chapter three.

Although renin is specific only for renin substrate, the substrate molecule itself can be cleaved by a number of other proteases. These reactions can also produce angiotensin I. The significance of non-specific generation of angiotensin I is also discussed in more detail in Chapter three.

Angiotensin Converting Enzyme

As part of the renin-angiotensin system, angiotensin converting enzyme generates the octapeptide angiotensin II from the decapeptide angiotensin I. The enzyme is a non-specific dipeptidyl-carboxypeptidase and is capable of removing carboxy-terminal dipeptides from a variety of substrates. In addition to its action

in the production of angiotensin II it also inactivates bradykinin (Yang, Erdos and Levin, 1970) thus providing a link between the kallikrein-kinin and renin-angiotensin systems.

Converting enzyme is a metalloenzyme containing zinc (Das and Soffer, 1975; Fernley, 1977). It also requires the chloride ion for activity. This acts as an allosteric modifier of the enzyme, lowering the Km. The degree to which the reaction involving the enzyme depends on the chloride ion varies with the substrate. The reaction involving bradykinin requires less than that with angiotensin I (Peach, 1977).

Converting enzyme was initially discovered in plasma (Skeggs et al, 1956). However, it was subsequently demonstrated by Ng and Vane (1967) that the lungs were a more important site for the conversion of angiotensin I to angiotensin II. Since then angiotensin converting enzyme has been found in significant quantities in many other tissues (Aiken and Vane, 1970; Cushman and Cheung, 1971a). It has been shown to be localised within the vascular endothelium of these tissues (Caldwell, Seegal, Hsu, Das and Soffer, 1976; Wigger and Stalcup, 1978).

Angiotensin converting enzyme has been purified from the tissues of a number of species. It is a glycoprotein with a molecular weight of between 130,000 to 160,000 (Ondetti and Cushman, 1982) although there may be some degree of polymerization in vivo (Ondetti and Cushman, 1982; Peach, 1977). The pH optimum of the enzyme is in the neutral range (Bakhle, 1974; Cushman and Cheung, 1971b).

Angiotensin I

Angiotensin I is the decapeptide derived from the action of renin on renin substrate.

It has been reported that angiotensin I may act directly upon the adrenal medulla and central nervous system, but the importance of such activity is obscured by the rapid conversion of angiotensin I to angiotensin II which itself has such action. No specific receptor sites for angiotensin I have been discovered. It does bind to angiotensin II receptors but only when present in high concentrations, greater than those which have been measured physiologically. Due to the absence of any definite intrinsic activity, angiotensin I is classed as a pro-hormone. The evidence in favour of such a classification has been reviewed by Peach (1977).

Angiotensin II

The octapeptide angiotensin II is the principal active component of the renin-angiotensin system. It has a number of actions on a variety of systems within the body.

Angiotensin II has a number of actions on the cardiovascular system. These have been reviewed by Peach (1977). Intravenous injection of angiotensin II results in an immediate pressor response through its direct action on the smooth muscles of resistance vessels. It produces reflex bradycardia when the baroreceptors are intact but also has a positive chronotropic effect through its action on the central nervous system. It has a positive inotrophic action through direct interaction of angiotensin II with receptors on the surface of cardiac cells. Angiotensin II decreases cardiac output by reflex bradycardia following the rise in peripheral resistance and elevation of the blood pressure. In addition, when applied in high concentrations directly to the large arteries, angiotensin II produces a widening of the gap between the lining endothelial cells (Giese, 1973). This action is thought to be

mediated by local prostaglandin synthesis. Folkow (1971) has shown that angiotensin II also alters the wall to lumen ratio of the resistance vessels.

Angiotensin II influences the action of the nervous system. It causes a pressor response when administered into the cerebral arterial circulation (Ferrario, Dickinson and McCubbin, 1970). The central effects are mediated mainly by increased efferent sympathetic activity producing increases in both peripheral resistance and cardiac output. There is also a direct interaction between angiotensin II and the sympathetic nervous system resulting in the facilitation of adrenergic neurotransmission (Zimmerman, 1981). An increase in the biosynthesis (Roth, 1972) and release (Hughes and Roth, 1971) of noradrenaline from the sympathetic nerve endings occurs but the mechanism by which this change is produced remains uncertain. Angiotensin II has been shown to affect the neural membrane, possibly by altering calcium flux (Zimmerman, 1981).

The administration of angiotensin II directly into the central nervous system stimulates a dipsogenic response. A specific angiotensin II receptor in the brain has been implicated in this response. The effect is thought to be mediated by dopaminergic pathways (Fitzsimons and Setler, 1975).

Angiotensin II also appears to stimulate the release of antidiuretic hormone although intravenous administration does not increase plasma ADH. Stimulation of the release of ADH is thought to be mediated by the action of angiotensin II on the central nervous system (Severs and Daniels-Severs, 1973).

The kidney is a major target organ of angiotensin II where it plays an important role in modifying the process of excretion of water and electrolytes. It has a number of direct actions,

producing a decrease in total renal blood flow and glomerular filtration rate and an increase in the filtration fraction which is due to efferent arteriolar vasoconstriction. The effects of angiotensin II on the kidney have been found to vary with the dose of angiotensin II administered (Navar and Langford, 1974).

Through its action on the kidney angiotensin II plays a role in the control of sodium, potassium and water homeostasis. This has been found to occur as a result of direct stimulation of sodium reabsorption (Johnson and Malvin, 1977). It acts by facilitation of transmembrane electrolyte transport, resulting in sodium retention and potassium excretion. Similarly, this action has been demonstrated in smooth muscle cells where sodium is moved into, and potassium moved out of the cells (Friedman and Friedman, 1964).

The presence of locally formed angiotensin II within the kidney suggests that local generation of angiotensin II may be important in the regulation of renal function (Mendelsohn, 1979). Thus the response of the kidney need not depend only on changes in the level of circulating angiotensin II. Changes in the amounts of other components of the renin-angiotensin system within the kidney may therefore determine the renal actions of angiotensin II. It has also been suggested that the local kallikrein-kinin system and prostaglandins may be important in regulating the production of renal angiotensin II (Brown, Leckie, Lever, McIntyre, Morton, Semple and Robertson, 1983).

Angiotensin II directly stimulates the secretion of aldosterone by the adrenal cortex (Davis , 1974). It influences the rate of conversion of cholesterol to pregnenolone which is the

first step in the biosynthetic pathway of aldosterone (Peach, 1977). Indirectly, by lowering hepatic blood flow, an increase in circulating angiotensin II results in a decrease in the clearance of aldosterone from the plasma by the liver (Brown et al, 1983). The action of angiotensin II on aldosterone secretion is considerably modified by changes in sodium balance. It is thought to be a major factor involved in the control of aldosterone in response to changes in sodium balance (Boyd and Peart, 1971).

Angiotensin II also stimulates the adrenal medulla to release catecholamines but there is little evidence that this effect has any significant role in the pressor response to angiotensin II (Peach, 1977).

As well as an immediate pressor response, angiotensin II has a much slower action on blood pressure (Dickinson and Lawrence, 1963; Cowley and McCaa, 1976; Brown, Casals-Stenzel, Gofford, Lever and Morton, 1981). This effect is revealed by infusions of acutely sub-pressor doses of angiotensin for days or weeks. The effect is thought to result from a combination of the influences of angiotensin II which are additional to its acute vasoconstriction. This effect is discussed in more detail on page 25.

The characteristics of the responses to angiotensin II suggest that its action is receptor mediated. It has been demonstrated that angiotensin II is bound by a number of tissues including resistance vessels (Gunther, Gimbrone and Alexander, 1980; McQueen, Murray and Semple, 1983), the aorta (Le Morvan and Palaic, 1975), the uterus (Rouzaire-Dubois, Devynck, Chevillotte and Meyer, 1975), the adrenal zona glomerulosa (Catt, Aguilera, Capponi, Fujita, Schirar and Fakunding, 1979) and other tissues (Lin and Goodfriend,

1970). Macromolecules which bind angiotensin II and which are thought to be angiotensin II receptors *in vivo* have been solubilised and purified from the adrenal glomerulosa and myometrium (Devynck, Pernollet, Meyer, Fermandjian, Fromageot and Bumpus, 1974; Capponi and Catt, 1980). Several investigators have shown that these receptors are present in a relatively high concentration in the cell membrane (Devynck et al, 1974; Catt et al, 1979; Capponi and Catt, 1980). However, there is some evidence that angiotensin II enters cells and some binding sites have been found on intracellular organelles (Robertson and Khairallah, 1971; Goodfriend, Fyhrquist, Gutmann, Knych, Hollemans, Allmann, Kent and Cooper, 1972). There is evidence that intracellular receptors for angiotensin II are present in the membranes of microsomal vesicles *in vitro* (Baudouin, Meyer, Fermandjian and Morgat, 1972) and angiotensin II has been found to have multiple effects on the metabolism of mitochondria *in vitro* (Goodfriend et al, 1972).

Although angiotensin II exerts its effect through specific binding sites the details of the subsequent cellular events remain obscure (Goodfriend, Fyhrquist and Allmann, 1974). The process is thought to involve changes in levels of intracellular (D'Auriac, Baudouin and Meyer, 1972; Fakunding, Chow and Catt, 1979) and extracellular (Freer, 1975) calcium, the metabolism of phospholipids and the phosphorylation of proteins (Billah and Michell, 1979; Garrison, Borland, Florio and Twible, 1979; Anderson, Gimbrone and Alexander, 1981). Prostaglandin synthesis may also be involved (Danon, Chang, Sweetman, Nies and Oates, 1975; Campbell, Gomez-Sanchez, Adams, Schmitz and Itskovitz, 1979).

Homogenates of every tissue that have been surveyed contain an abundance of enzymes which are capable of inactivating angiotensin

II (Ryan, 1974) but the precise contribution of much of this activity to the degradation of angiotensin II *in vivo* is not known. However, the liver and kidneys are thought to be important sites of angiotensin II catabolism and the lungs and cardiac and vascular tissue appear also to be involved, although to a lesser extent (Ledingham and Leary, 1974).

One of the products of angiotensin II breakdown is the heptapeptide des-Asp^1-angiotensin II or angiotensin III.

Angiotensin III

Angiotensin III is itself biologically active and has similar actions to angiotensin II as a vasoconstrictor peptide although its potency is considerably reduced. This may be due in part to its more rapid metabolism. However, its potency has been found to be similar to angiotensin II in the release of aldosterone although there are species dependent differences (Goodfriend, 1983). It is also equally potent in suppressing renin release from the kidney (Freeman, Davis and Lohmeier, 1975) and is very potent in stimulating the release of prostaglandins from perfused vessels *in vitro* (Blumberg, Denny, Nishikawa, Pure, Marshall and Needleman, 1976).

The des-Asp^1 derivative of angiotensin I has similar properties to angiotensin III. There is now evidence that these peptides may have physiological roles similar to but separate from angiotensin II itself (Goodfriend, 1983). The NH_2-terminal heptapeptide and all the other peptide fragments which result from angiotensin II degradation are biologically inactive.

Renin in Hypertension

Bright in 1836 described an association between renal disease and left ventricular hypertrophy with raised systemic arterial

pressure. It was for some time assumed that hypertension was invariably due to renal ischaemia.

In 1896 Allbutt made a distinction between essential and renal hypertension. Since then the role of the kidney in raising blood pressure has been the subject of intense investigation. In particular, attention has been focused on the renin-angiotensin system.

True renin-dependent hypertension is only seen in patients with renin secreting tumours and possibly in patients with terminal renal failure and renovascular hypertension. Hypertension in patients with Cushings syndrome is only partially renin dependent. There is little evidence that the renin-angiotensin system plays any part in essential hypertension (Swales, 1979a).

The main focus of attention has therefore been renovascular hypertension, both clinically and in Goldblatt animal models of hypertension.

Clinically, renovascular hypertension develops in those patients in whom the blood supply to one or both kidneys has been reduced by local vascular disease. The two most common causes are atheroma and fibromuscular dysplasia of the renal artery. This subgroup of hypertensive patients may be the natural equivalent of the Goldblatt experimental model of hypertension.

The model of Goldblatt hypertension involves the production of hypertension by placing a constricting clip on the renal artery with consequent renal ischaemia and an associated rise in blood pressure. The contralateral kidney can either be removed (1-kidney 1 clip hypertension) or left untouched (2-kidney 1 clip hypertension). The mechanisms by which hypertension results in these two models are quite different.

The renin-angiotensin system does not appear to be involved in the generation of hypertension in the Goldblatt 1-kidney 1 clip model. Renal renin, juxtaglomerular granulation and plasma renin levels have all been shown to be normal during both the development and maintenance of hypertension (Regoli, Brunner, Peters and Gross, 1962; Heptinstall, 1965; Miksche, Miksche and Gross, 1970). On the other hand renin does appear to play a part in the Goldblatt 2-kidney 1 clip model although to what extent remains undetermined.

In this model hypertension develops gradually over a period of days or weeks. This early phase is associated with elevated plasma renin levels, especially when hypertension is severe. However, when hypertension is less severe, plasma renin levels may be normal. An initial rise in plasma renin has been observed in the rat (Miksche et al 1970; Möhring, Möhring, Naumann, Philippi, Homsy, Orth, Dauda, Kazada and Gross, 1975) and an increase has also been noted in the early stages of hypertension in the sheep (Blair-West, Coghlan, Denton, Orchard, Scoggins and Wright, 1968), dog (Masaki, Ferrario, Bumpus, Bravo and Khosla, 1977) and rabbit (Lohmeier and Davis, 1976).

Plasma renin can remain elevated for as long as ten weeks after clipping in the rat (Miksche et al, 1970) but with increasing duration of hypertension, plasma renin levels decline. By thirty six weeks after clipping in the rat the levels were returned completely to normal despite the persistence of elevated blood pressure (Sen, Smeby, Bumpus and Turcotte, 1979).

The use of specific inhibitors of the renin-angiotensin system has confirmed these findings. One in particular which has been used extensively is Sarcosine^{1}-alanine^{8}-angiotensin II (Saralasin). This is an analog antagonist of angiotensin II. It has the same

basic structure as angiotensin II but with sarcosine substituted in amino-acid position number one and alanine in position number eight. It inhibits the action of angiotensin II by binding competitively with angiotensin II receptors. The properties and actions of saralasin together with a number of other inhibitors of the renin-angiotensin system are discussed in more detail in Chapter five.

Infusion of the angiotensin antagonist saralasin produced no fall in blood pressure in the Goldblatt 1-kidney 1 clip model in the rat (Brunner, Kirshman, Sealey and Laragh, 1971). In Goldblatt 2-kidney 1 clip hypertension it produced a fall only in rats with hypertension of a few weeks duration (Thurston and Swales, 1974; MacDonald, Boyd and Peart, 1975) although the blood pressure did not return completely to pre-experimental levels.

In man , renin also seems to be involved in the development of renovascular hypertension but, as with the animal models, the extent of its role is unclear. Plasma renin levels are high in a majority of cases of surgically correctable hypertension (Bath, Gunnells and Robinson, 1968; Vaughan, Buhler, Laragh, Sealey, Baer and Bard, 1974) but the fall in circulating renin levels after correction is often unrelated to the return of blood pressure to within the normal range (Swales, 1979a).

Mackay, Boyle, Brown, Cumming, Forrest, Graham, Lever, Robertson and Semple (1983) have made a study of the effectiveness of specific tests to predict the outcome of surgical correction of hypertension associated with renal artery stenosis. The most successful prognostic test was found to be the renal vein renin ratio. This is the ratio of the renin concentration in venous plasma from the affected and unaffected kidneys. A high ratio

suggests a favourable outcome but some patients with a lower ratio also benefited from the operation. Neither the measurement of the concentration of renin in peripheral plasma nor the response to an infusion of saralasin were found to be of any use in predicting the outcome of surgery.

Hypertension induced by renal ischaemia does not appear to be either invariably or completely dependent on the renin-angiotensin system. A number of hypotheses have been put forward to explain this.

Sodium and Hypertension

Guyton, Coleman, Cowley, Scheel, Manning and Norman (1972) proposed that sodium balance is central to the maintenance of blood pressure. However, conflicting results have been obtained from studies of the state of sodium balance in hypertension. In the Goldblatt 1-kidney 1 clip model sodium retention or an increase in the level of exchangeable sodium has been observed (Tobian, Coffee and McCrea, 1969; Swales, Thurston, Quieroz and Medina, 1972; Albertini, Binia, Otsuka and Carretero, 1979). However, Doyle, Duffy and MacDonald (1976) found no difference in the level of exchangeable sodium between hypertensive and normal control rats. Recently McAreavey, Brown, Murray and Robertson (1984) have shown that although an increase in exchangeable sodium was sustained throughout the period of renal artery constriction, the development of hypertension was progressive and the two were not correlated.

In the Goldblatt 2-kidney 1 clip model the results of a number of studies are again conflicting. A negative sodium balance has been reported during the development of hypertension in this model (Swales et al, 1972). However, Möhring et al (1975)

observed initial sodium retention followed by a negative balance only during the later stages of malignant hypertension. In benign hypertension sodium balance normalised. Doyle et al (1976) found an increase in the level of exchangeable sodium. This was also demonstrated by Albertini et al (1979) but only in severely hypertensive rats. Tobian et al (1969) found no change in this model and this finding has been confirmed by McAreavey, Brown and Robertson (1982).

Möhring et al (1975) have suggested that there is a shift of sodium from the intracellular to the extracellular fluid and that this accounts for the rise in blood pressure despite net sodium excretion. McAreavey et al (1982) have also suggested that changes in the distribution of sodium may be more important than changes in sodium balance in the development of hypertension.

The idea that sodium levels may still be important despite sodium loss has been explained in terms of "inappropriately" high sodium status for the level of blood pressure. This theory was extended by Gavras, Brunner, Thurston and Laragh (1975). They showed that in the Goldblatt 2-kidney 1 clip model the blood pressure no longer responded to angiotensin II blockade five weeks after clipping. However, the response at this time was restored by salt depletion.

Laragh (1973) proposed that blood pressure levels are maintained partly by a volume mechanism and partly by the renin-angiotensin system, in a reciprocal relationship. With sodium status "inappropriately" high the renin-angiotensin system could still be maintaining a blood pressure elevation despite plasma renin levels which are apparently normal. Leenen and Dejong (1975) also suggested that sodium retention supplements the action of the

renin-angiotensin system in raising the blood pressure in the Goldblatt 2-kidney 1 clip model of hypertension. However, Gavras et al (1975) found that a combination of salt restriction and renin-angiotensin blockade failed to restore the blood pressure to normal in this model.

More recent investigations into the role of both sodium balance and circulating renin have lead to the conclusion that, either singly or in combination, these two factors are not wholly responsible for the changes in blood pressure which occur in experimental models of hypertension (Godfrey, Kumar, Bing, Swales and Thurston, 1985; McAreavey et al, 1984). A number of other factors may be involved. Changes in the activity of the renin-angiotensin system other than those determined simply by measuring the levels of circulating renin may be significant. In addition, the renin-angiotensin system may act by mechanisms which are distinct from its acute vasoconstrictor action.

The Slow Pressor Effect of Angiotensin II

Of particular interest in this respect is the slow pressor effect of angiotensin II. It has been shown that sustained, long term infusions of acutely sub-pressor doses of angiotensin II produce a gradual rise in blood pressure. This has been demonstrated in the rabbit (Dickinson and Lawrence, 1963), dog (Cowley and McCaa, 1976) and the rat (Brown et al, 1981).

Infusions of angiotensin II at these low doses produce no direct pressor response but the blood pressure rises gradually to a level which can be produced immediately by much larger doses. The exact mechanisms involved are not known. Increased dietary sodium can accelerate this response to angiotensin II (Cowley and McCaa, 1976) and the effect can be prevented by dietary salt restric-

tion. This rise in blood pressure is not prevented by adrenalectomy (Dickinson and Yu, 1967) and is not seen with chronic infusions of aldosterone (Lohmeier, Cowley, DeClue and Guyton, 1978). When sub-pressor doses of angiotensin II were administered during long term saline infusion the blood pressure rose without natriuresis. Plasma aldosterone levels were unchanged suggesting that the blood pressure rise was due to a direct anti-natriuretic renal action of angiotensin II (DeClue, Guyton, Cowley, Coleman, Norman and McCaa, 1978).

Brown et al (1981) found that sodium retention did not occur during the development of the response. It has therefore been suggested that the blood pressure is raised through the actions of angiotensin II on the central nervous system. Experiments have shown that the progressive rise in the blood pressure can be blocked by adrenergic neurone blocking agents (Dickinson and Yu, 1967). However, angiotensin II acts at many levels in the central and peripheral nervous system and so far no particular site of action has been demonstrated for the slow pressor effect (Brown et al, 1981).

Another mechanism suggested is through an enhancement of the normal response to angiotensin II. It has been shown that structural changes occur in the resistance vessel walls in response to a rise in the blood pressure which then renders them more responsive to vasoactive peptides such as angiotensin II (Folkow, 1971). This effect may explain why the response is maintained by subpressor doses of angiotensin II. However, since hypertension is produced as a result of the initial pressure load in resistance vessels, it is necessarily secondary to an initial rise in blood pressure.

Other actions of angiotensin II which may be involved in the

slow pressor effect are an increase in cardiac output and baroreceptor resetting (Cowley and DeClue, 1976) and enhanced aldosterone secretion (Oelkers, Schöneshöfer, Schultze, Brown, Fraser, Morton, Lever and Robertson, 1975). An alteration in tissue compliance, which alters body fluid distribution, has been postulated in Goldblatt hypertension (Lucas and Floyer, 1974). Theoretically this effect could also contribute to the pressor sensitivity to angiotensin.

Angiotensin II Receptors

The response to angiotensin II may be altered by changes at the level of its interaction with angiotensin II receptors. This could occur either by modification of the formation of the angiotensin II receptor complex or by affecting the response produced by the complex.

It has been shown that the response to angiotensin II is modified by salt balance, being enhanced by salt loading and impaired by salt depletion (Slack and Ledingham, 1976; Bing, Russell, Swales and Thurston, 1982). This effect is not secondary to changes in plasma volume or cardiac output but involves interaction with the receptor complex (Strewler, Hinrichs, Guiod and Hollenberg, 1972). The response to angiotensin II is also increased after bilateral nephrectomy (Gabelman and Rondell, 1966).

Changes both in the number of receptors present and the affinity of the receptors for angiotensin II may account for these observations.

Chevillotte, Devynck, Fyhrquist, Meyer, Rouzaire-Dubois and Worcel (1975) found that there was an increase in the number of specific angiotensin II binding sites in the rabbit uterus nineteen and twenty-two hours after bilateral nephrectomy. However, this

does not coincide with the observed maximum response to angiotensin II which occurs six hours after removal of the kidneys. They also reported an increase in receptor number in the rat uterus and adrenals after salt depletion (Devynck, Pernollet, MacDonald, Matthews, Raisman and Meyer, 1978). However, salt loading also resulted in an increase in receptor number in the uterus but produced no change in the binding capacity of the adrenal gland.

More recently Gunther et al (1980) have demonstrated a significant decrease in receptor number in a preparation of mesenteric artery from salt depleted rats. The opposite effect was produced by inhibiting angiotensin II production with captopril.

Aguilera and Catt (1981) have also shown that changes in salt balance affect the number of vascular angiotensin II receptors. This is thought to be as a direct result of changes in the levels of circulating angiotensin II. The effects are different in the various target organs. Salt depletion raises the receptor number in the adrenals with a simultaneous decrease in the number in smooth muscle (Catt et al, 1979). Salt loading produces the opposite effect. Goodfriend (1983) has suggested that since the molecular structures of the angiotensin II receptors from the various target organs are almost identical, regulation of receptor number may involve a receptor regulator within the receptor complex.

It has also been claimed that the affinity of receptors for angiotensin II alters with changes in sodium balance and following nephrectomy (Brunner, Chang, Wallach, Sealey and Laragh, 1972). However, this conclusion is based on the assumption that the volume of angiotensin II antiserum required to block the pressor response to angiotensin II is a measure of specific receptor affinity. Swales, Tange and Thurston (1975) have shown that this

is not the case since the angiotensin II acts at sites not immediately accessible to the antibody. Nevertheless the affinity of the receptors has also been shown to be susceptible to regulation. Cations can directly regulate the response to angiotensin II by influencing the receptors. Potassium has been shown *in vitro* to affect the binding of angiotensin II to receptors (Fredlund, Saltman, Kondo, Douglas and Catt, 1977). Calcium and magnesium have also been shown to have an effect (Blanc, Sraer, Sraer, Baud and Ardaillou, 1978). In addition, guanyl nucleotides decrease receptor affinity *in vitro* (Glossmann, Baukal and Catt, 1974) and some fatty acids and steroids have also been found to reduce angiotensin II binding affinity (Goodfriend, 1983).

Thurston and Laragh (1975) have proposed the hypothesis of "prior receptor occupancy" to explain this apparent change in receptor affinity. When the levels of endogenous angiotensin II are high, as they are during salt depletion, then there may be fewer free receptors available to respond to circulating exogenous angiotensin II. Conversely, during salt loading or after bilateral nephrectomy (Swales et al, 1975), endogenous angiotensin II levels are low and more receptors may be available to bind and respond to exogenous angiotensin II. This may account for the greater response under these experimental conditions.

The results obtained from antibody blocking experiments have led to the conclusion that the important site of angiotensin II generation is the tissues rather than the circulation. This is discussed in more detail in a later section.

Other Renal Pressor Substances

A number of these substances have been described and it has been suggested that they may play a role in renovascular

hypertension. Renal pressor substances include nephrotensin (Grollman and Krishnamurty, 1973), corticotensin (Fasciolo, Risler and Totel, 1972), renopressin (Skeggs, Kahn, Levine, Dorer and Lentz, 1977; Morris and Roper, 1982) and tonin (Boucher, Garcia, Gutkowska, Demassieux and Genest, 1978).

However, there is little evidence to support the suggestion that they have any significant hypertensive properties. The possible exception is tonin. This protease is able to hydrolyse both renin substrate and angiotensin I to form angiotensin II. Rabbit tonin antiserum given to Goldblatt 1-kidney 1 clip hypertensive rats produced a lowering in blood pressure to normal. However, this was not observed in the 2-kidney 1 clip model (Garcia, Boucher, Gutkowska, Kondo, Demassieux and Genest, 1978).

It has also been argued that hypertension might result from a reduction of the activity of a renal vasodepressor mechanism. Such systems include renomedullary lipids (Muirhead, 1983), prostaglandins (Dunn and Hood, 1977), vasodepressor factor (Rosenthal, Paddock and Hollander, 1973) and the kallikrein-kinin system (Levinsky, 1979; Carretero and Scicli, 1981).

Studies with the converting enzyme inhibitor, captopril, suggest that the kallikrein-kinin system may have an important role in hypertension (Thurston and Swales, 1978). Captopril, given to Goldblatt 2-kidney 1 clip hypertensive rats previously treated with saralasin produces an additional and significant fall in blood pressure. Since the breakdown of bradykinin is also prevented by converting enzyme inhibition, it has been suggested that this fall in blood pressure is due to potentiation of the action bradykinin, which is consistent with the view that the kallikrein-kinin system is involved in the development of hypertension in this model.

Interactions between the kallikrein-kinin system, prostaglandins and the renin-angiotensin system have been demonstrated. Changes in the balance of these factors may contribute to hypertension (Cinotti, 1983; Levinsky, 1979). These systems and the interactions between them are discussed in more detail in Chapter six.

All of the clinical and most of the laboratory experimental work described above has relied on measurement of the renin levels in the plasma as an indicator of the function of the renin-angiotensin system. But renin may act as a local tissue hormone outside the circulation. Such activity may not have a simple relationship to the level of renin in the plasma.

Extra-Renal Renin-Like Activity

The biochemical definition of renin activity derives from the ability of the enzyme to generate angiotensin I from renin substrate. This definition was applied to renin activity derived from the kidney, either in renal homogenates or in the plasma. However, the ability to generate angiotensin has been demonstrated in other tissues besides the kidneys. This activity has been called iso-renin by Ganten, Schelling, Vecsei and Ganten (1976) although not enough is known of its properties to justify its classification as a true isomer of renal renin. Probably the most accurate description is that of a "renin-like" substance (Gross, Schaechtelin, Ziegler and Berger, 1964; Bing and Faarup, 1965) since it is identified by virtue of its renin-like activity rather than by its physical properties.

Renin-like activity has been measured in a number of tissues where it is thought that the presence of renin may have physio-

logical significance.

Renin-like activity has been measured in the uterus of a number of species including human (Skinner, Lumbers and Symonds, 1968; Johnson, 1980), rabbit (Bing and Faarup, 1966; Ferris, Gorden and Mulrow, 1967; Ryan and Johnson, 1969) and dog (Hodari, Carretero and Hodgkinson, 1969; Carretero and Houle, 1970). In the pregnant rabbit uterus it is present in higher concentrations than in the kidney although in the rat there is very little activity (Bing and Faarup, 1966). Renin in this organ is thought to play a role in the local control of uterine blood flow (Ferris, Stein and Kauffman, 1972).

The significance of the renin-like activity present in the adrenal gland has also been investigated (Ryan, 1973; Ganten, Ganten Kubo, Granger, Nowaczynski, Boucher and Genest, 1974; Doi, Atarashi, Franco-Saenz and Mulrow, 1984).

Circulating angiotensin II is important in the control of steroid release from the adrenal gland and local generation of angiotensin II by renin within the tissue may be important in this respect. Doi et al (1984) found a positive correlation between adrenal renin-like activity and aldosterone concentrations in a number of experimental situations. They concluded that adrenal renin may act as a local hormone, involved in the regulation of aldosterone production.

Although renin cannot cross the blood-brain barrier, renin-like activity has been measured in the brain of the rat (Fischer-Ferraro, Nahmod, Goldstein and Finkielman, 1971; Dzau, Brenner, Emmett and Haber, 1980), dog (Fischer-Ferraro et al, 1971; Ganten, Marquez-Julio, Granger, Hayduk, Karsunky, Boucher and Genest, 1971) and human (Daul, Heath and Garey, 1975; Menard, Galen, Devaux,

Kopp, Auzan and Coryol, 1980). Renin has been found to be distributed extensively throughout many areas of the brain (Inagami, Celio, Clemens, Lau, Takii, Kasselberg and Hirose, 1980).

Because of the number of physiological response known to be stimulated by angiotensin II within the brain (Schölkens, Jung, Rascher, Schömig and Ganten, 1980) the significance of a brain renin-angiotensin system which generates angiotensin II locally has received considerable attention.

Renin-like activity in the blood vessel walls of a number of tissues has been described (Gould, Skeggs and Kahn, 1964; Genest, Simard, Rosenthal and Boucher, 1969; Rosenthal, Boucher, Rojo-Ortega and Genest, 1969; Hayduk, Brecht, Vladutiu, Simard, Rojo-Ortega, Belleau, Boucher and Genest, 1970). It is usually measured in the larger vessels such as the aorta, but its presence in smaller resistance vessels may be more important. In hog arteries the renin is localised in the adventitia of the vessels (Gould et al, 1964) and it has been measured in the capillaries of the rabbit uterus (Ganten et al, 1976). In the rat, renin-like activity was found to increase as the diameter of the arteries decreased (Genest et al, 1969).

Properties of Extra-renal Renin-like Activity

Although the tissues described contain enzymes which are capable of generating angiotensin I it cannot be assumed that this is due to the presence of true renin. Skeggs, Levine, Lentz, Kahn and Dorer (1977) gave the name "pseudorenin" to enzyme activity present in all tissues and found in particularly high concentrations in salivary gland, thymus and spleen of the rat. This enzyme(s) has the ability to generate angiotensin I but has a pH optimum of 4.5 although its activity extends into

the neutral range. However, it cannot cleave renin substrate in plasma, since it is inhibited by a naturally occuring plasma inhibitor. Its activity can only be measured by using purified renin substrate or the synthetic substrate tetradecapeptide.

Pepsin has been shown to have the ability to hydrolyse renin substrate to produce angiotensin 1 (Franze de Fernandez, Paladini and Delius, 1965). This action may also be a property of cathepsin D which has a similar substrate specificity to pepsin (Reid, 1977). However, these enzymes are only active at a pH below 6.0. The activity of these enzymes can therefore be distinguished from that of true renin. Thus it is important to define the properties of renin-like activity within any tissue before proposing a specific physiological function for the enzyme activity.

Day and Reid (1976) suggested that the renin-like activity present in brain cannot be distinguished from cathepsin D. This was supported by Hackenthal, Hackenthal and Hilgenfeldt (1978). They isolated renin-like activity from rat brain and compared its properties to those of cathepsin D isolated from bovine spleen. They concluded that the two were closely related and were probably the same enzyme. On the other hand, Hirose, Yokosawa and Inagami (1978) separated a renin-like enzyme from cathepsin D and showed that it was completely inhibited by antibodies raised to renal renin. A number of other studies have also demonstrated that some, at least, of the angiotensin generating activity in vascular tissue is similar to renal renin.

Fordis, Megorden, Ropchak and Keiser (1983) removed non-specific proteolytic activity, including cathepsin D, from aortic homogenates of normal rats by chromatography using bovine haemo-

globin. They then demonstrated the presence of residual renin-like activity which could be inhibited by anti-mouse renin antibody known to cross-react with the rat plasma renin enzyme.

In aortic tissue from rats a pH profile reveals two peaks of angiotensin generating activity (Thurston, Swales, Bing, Hurst and Marks, 1979; Barrett et al, 1981; Rosenthal, Pfeifle, Michailov, Pschorr, Jacob and Dahlheim, 1984). One peak is in the neutral range and the other, larger peak, occurs at below pH 6.0. This activity was measured using plasma from rats after bilateral nephrectomy as a source of substrate thus ruling out the possibility that any of the activity was due to pseudorenin. The biphasic nature of the curve indicates that there is more than one type of enzyme present. The activity below pH 6.0 is due to non-specific protease activity but the peak in the neutral range represents renin-like activity (Thurston et al, 1979). These authors have shown that the vascular wall renin-like activity measured at pH 6.5 is reduced after removal of the kidneys. At the same time the activity measured at pH 5.3 remains unchanged. Similarly, Fordis et al (1983) have shown that the level of non-specific proteolytic activity in aortic tissue remains the same after bilateral nephrectomy whereas the renin-like activity disappears.

Studies of the physical properties of extra-renal renin-like activity have demonstrated many similarities with renal renin.

Renin-like activity in homogenates of arterial tissue from the dog and the rat has an identical Km, velocity constant and activation energy to renal renin (Basso and Taquini, 1971; Barrett, Eggena and Sambhi, 1978) as well as a similar molecular weight and pH profile (Barrett et al, 1981). In addition, Barrett

et al (1978) have demonstrated that homogenates of aorta from spontaneously hypertensive rats produce a pressor response *in vivo*.

Components of the Renin-angiotensin System Outside the Kidney and Circulation

All the components of the renin-angiotensin system have been detected in a variety of tissues other than in the kidney and the circulation. Thus, in addition to renin-like activity, angiotensin converting enzyme and renin substrate are also present.

Although angiotensin-converting enzyme was first measured in the circulation, higher concentrations were found to be present in the lungs (Ng and Vane, 1967). It was subsequently demonstrated that the lung is a major site for the conversion of angiotensin I to angiotensin II (Vane, 1974). However, the studies of Kreye and Gross (1971) have indicated that this activity occurs as rapidly in other tissues. Cushman and Cheung (1971 a) have demonstrated the presence of angiotensin converting enzyme in twenty-five different tissues of the rat.

In the lung, angiotensin converting enzyme has been shown to be located in the vascular endothelium of the pulmonary arteries (Caldwell et al, 1976; Wigger and Stalcup, 1978) and it is thought that this enzyme is present within the vasculature of a variety of tissues. Thus, segments of microvessels or capillaries from brain (Gimbrone, Majeau, Atkinson, Sadler and Cruise, 1979; Brecher, Tercyak and Chobanian, 1981), adrenal cortex (Del Vecchio, Ryan, Chung and Ryan, 1980) and retina (Ward, Stewart, Hammon, Reynolds and Igic, 1979) make angiotensin converting enzyme in tissue culture. Angiotensin converting enzyme has also been demonstrated in vascular tissue itself and vascular

endothelial cells in culture release large amounts of the enzyme into the medium.

Renin substrate has been measured in the human uterus (Johnson, 1980), arterial tissue (Desjardins-Giasson, Gutkowska, Garcia and Genest, 1981), adrenal gland and brain (Ganten et al, 1974).

Angiotensin II binding sites are present in a number of tissues. This would be expected in any target organ for angiotensin II. However, since the components required for generation of angiotensin II are present in these tissues, it has been suggested that the angiotensin II is generated locally rather than simply being taken up from the circulation (Disalvo and Montefusco, 1971; Aiken and Vane, 1972; Collier and Robinson, 1974).

Generation of Angiotensin II Outside the Circulation

Observations on the effects of blockade of the renin-angiotensin system with specific inhibitors have produced indirect evidence in support of the view that generation of angiotensin II occurs outside the circulation.

It has been found that specific antiserum raised to both angiotensin I (Oates, Stokes, Storey, Glover and Snow, 1974) and angiotensin II (Hedwall, 1968; Bing and Poulsen, 1970; Oates et al, 1974) was relatively ineffective in lowering the blood pressure in renal hypertension. Furthermore, the amount of antibody required to block the effect of a standard dose of administered angiotensin II was found to be dependent on the level of circulating endogenous renin. However, the relationship between the two was not one of simple proportion. When plasma renin levels were low as, for example, they are after salt loading or bilateral nephrectomy, the amount of antibody required was much more than after salt depletion

when circulating renin levels were high (Brunner et al, 1972). These latter authors suggested that the affinity of vascular receptors for circulating angiotensin II was conditioned by the state of salt balance and that this relationship was abnormal in hypertension. The effect was confirmed by Swales and Thurston (1973) who, in addition, found that an infusion of renin markedly decreased the amount of antibody required to block the effect of exogenous angiotensin II.

These authors also studied the pressor responsiveness to a standard dose of angiotensin II in salt loaded and salt depleted rats (Swales et al, 1975). The response was greater in the former situation, but after removal of the kidneys the pressor response in both groups was the same. A similar effect to that of nephrectomy was obtained by administration of angiotensin converting enzyme inhibitor suggesting that it is the renin-angiotensin system rather than sodium balance which determines the responsiveness.

The results of the studies cited above cannot be explained by the variations in the levels of circulating angiotensin II. Swales et al (1975) concluded that endogenous angiotensin II was generated in such a way as to be protected from the antibody.

Experiments performed by Oates and Stokes (1974) support the theory that angiotensin II is generated within vascular tissue. Angiotensin II antibody was less effective in blocking intra-arterial than intravenously injected angiotensin I. This indicates that circulating angiotensin I gains access to arterial tissue and is converted to angiotensin II at this site.

The hypothesis that a response to administered angiotensin II is dependent on prior receptor occupancy was put forward by Thurston and Laragh (1975). This hypothesis explains why the

pressor response to a standard dose of exogenous angiotensin II is less when plasma renin levels are high (Swales et al, 1975). Administration of converting enzyme inhibitor, which prevents generation of endogenous angiotensin II, increases the response to exogenous angiotensin II. When levels of renin are low, endogenous angiotensin II levels are correspondingly low and thus the number of receptors available to bind exogenous angiotensin II will be greater. Conversely, when plasma renin and endogenous angiotensin II levels are high more receptors are already occupied. The degree to which antibody is effective in blocking exogenous angiotensin II administration therefore depends on the amount of this angiotensin II which can be bound to receptors within the tissues (Swales, 1979b). The binding of angiotensin II to these receptors "protects" it from the antibody.

Antibody raised against angiotensin II is ineffective in lowering the blood pressure where it has been raised by endogenous angiotensin II. However, such antiserum protects against hypertension produced by injected angiotensin II (Hedwall, 1968). If endogenous angiotensin II were generated within the tissue, close to the receptor sites rather than in the circulation, then immediate binding to the receptor would protect it from the antibody.

The same effect has been demonstrated using aminopeptidase, an enzyme which rapidly degrades angiotensin II. This enzyme is more effective at lowering blood pressure which has been raised by injected angiotensin II than it is when renin is used (Daum, Uehleke and Klaus, 1966). Angiotensin II injected into the circulation is degraded by the enzyme. On the other hand renin, it has been suggested, enters the tissue and generates angiotensin

II at the receptor site. Once the latter is bound to the receptor, it cannot be degraded by the aminopeptidase.

A comparison of the effects of injected renin and angiotensin II in the presence of angiotensin II antibody produced a comparable result (Swales, 1976). Thus the response to angiotensin II depends on "prior receptor occupancy" rather than on a physical change in the affinity of specific receptors for angiotensin II as was originally proposed by Brunner et al in 1972.

The effects obtained using angiotensin II antiserum or aminopeptidase are in marked contrast to the results obtained by using the competitive antagonist of angiotensin II, saralasin. This inhibitor is more effective in reducing blood pressure which has been elevated by the renin-angiotensin system (Bing and Nielson, 1973; Thurston and Swales, 1974) since it can gain access to the angiotensin II receptors within the tissue.

Recently, direct evidence has been obtained which demonstrates that local generation of angiotensin II takes place within the tissue rather than in the circulation and that this process influences vascular tone (Oliver and Sciacca, 1984). These authors used an experimental model from which all circulating renin and angiotensin converting enzyme had been removed. Infusion of synthetic renin substrate into the isolated perfused hind quarters of the rat resulted in an increase in the perfusion pressure. This increase was identical to that produced by infusions of equimolar amounts of angiotensin I or angiotensin II and was abolished by either the converting enzyme inhibitor, captopril, or by specific peptide inhibitors of renin.

The evidence outlined above suggests that some, at least, of the angiotensin generating activity in vascular tissue is due to an enzyme which closely resembles renal renin. In making this assump-

tion it is necessary to specify that generation should be measured at the pH optimum of renin. This is discussed further in Chapter three. However, since this condition was fulfilled in the present study, the activity measured here in aortic tissue has been referred to as "renin" in the following chapters.

Vascular Wall Renin Activity and Blood Pressure Control

Support for the idea that the action of a renin-angiotensin system outside the circulation might be important in the control of blood pressure has come from a number of studies. In particular, such a system within vascular tissue may be physiologically significant.

It has been proposed that the response to exogenous angiotensin II depends on the degree to which vascular angiotensin II receptors are already occupied by endogenous angiotensin II and on the total number of receptors present. A reduction in the source of renin will increase the response to injected angiotensin II by increasing the number of free receptors.

The major source of renin can be removed by bilateral nephrectomy. However, it has been found that when this is done the increase in response to exogenous angiotensin II is not related to the disappearance of circulating renin. Swales et al (1975) showed that the response changed gradually over a period of six hours after removal of the kidneys, even though the half life of renin in the plasma is less than half an hour (De Vito, Koninckx, Cabrera and Nolly, 1977). Similarly, the amount of angiotensin II antiserum required to block the response to exogenous angiotensin after bilateral nephrectomy increased over the same time scale (Swales et al, 1975). This suggests that angiotensin II is still being generated even though plasma renin is no longer present. Thurston

and Swales (1977) showed that the response to angiotensin converting enzyme inhibitor in Goldblatt 2-kidney 1 clip hypertensive rats after bilateral nephrectomy also followed the same pattern. The blood pressure continued to respond to inhibition for up to six hours, long after circulating renin had disappeared. Thus the blood pressure elevation was determined by the renin-angiotensin system but not by circulating renin.

Further support for this conclusion came from a study carried out by Bing and Nielsen (1973). They showed that the blood pressure response to a single injection of renin in rats after bilateral nephrectomy persisted for up to six hours even though injected renin had been cleared from the circulation within one hour. They suggested that the blood pressure response could be maintained by an accumulation of renin in the walls of the arterioles.

Thurston, Hurst, Bing and Swales (1978) showed that the change in the renin content of the aortic wall after bilateral nephrectomy of Goldblatt 2-kidney 1 clip hypertensive rats was consistent with this assumption. Both plasma and aortic wall renin concentrations were elevated in the intact rat, but after bilateral nephrectomy there was a marked divergence in their respective rates of decline. Plasma renin had disappeared within one hour whereas renin in the aortic wall persisted for up to twenty-four hours.

Indirect evidence thus suggests that there is a role in blood pressure control for the renin which is present in the walls of blood vessels. This was investigated directly by Garst, Koletsky, Wisenbaugh, Hadady and Matthews (1979). An infusion of renin raised the blood pressure in normal rats and was associated with a rise in arterial wall renin levels. However, these authors did not isolate the changes in vascular wall renin levels from those of

circulating renin.

Vascular wall renin levels have been shown to alter in experimental situations known to effect the renin-angiotensin system. Changes in dietary sodium, early Goldblatt 2-kidney 1 clip hypertension (Thurston et al, 1978), dehydration and bilateral adrenalectomy (Rosenthal et al, 1969) are all associated with a change in vascular wall renin levels which parallel a similar change in the levels of plasma renin. Some recent reports have suggested that in spontaneously hypertensive rats (Barrett et al, 1978; Garst et al, 1979; Asaad and Antonaccio, 1982) and in chronic Goldblatt 2-kidney 1 clip hypertensive rats (Garst et al, 1979) there may be a divergence of the two. Nevertheless, in all these examples the continuing presence of plasma renin complicates any study of vascular wall renin. It is only after bilateral nephrectomy, when circulating renin disappears much more rapidly, that the vascular wall component can be studied in isolation.

Vascular Wall Renin: Local Synthesis or Uptake from the Plasma?

The renin present in vascular tissue does appear to be more relevant in blood pressure control than circulating renin in a number of experimental situations. However, there is uncertainty as to the source of this extra-renal renin. Renin of renal origin could be specifically taken up from, or passively diffuse out of, the circulation into vascular tissue. Alternatively, renin could be synthesised separately by the tissue itself.

The arguement has centred around the amount of renin which can be measured in vascular tissue after bilateral nephrectomy. If the renin were of renal origin then removal of the kidneys should result in a gradual, though eventually complete, disappearance of renin from the tissue. This has been observed by a number of authors.

The level of renin present in the arterial wall of Goldblatt 2-kidney 1 clip hypertensive rats (Thurston et al, 1979) and normal rats (Rosenthal et al, 1969) fell after bilateral nephrectomy although a small proportion of renin activity persisted for up to twenty-four hours. On the other hand, some authors have found that renin activity remains unchanged after bilateral nephrectomy. This was observed by Basso and Taquini (1971) in dogs where renin activity persisted in vascular tissue when measured seventy-two hours after removal of the kidneys. Similarly, Barrett et al (1978 and 1981) found no change in the level of arterial wall renin of rats after bilateral nephrectomy.

In studying the persistence of renin activity in the aortic wall after bilateral nephrectomy, differences in the pH at which the measurements are made may explain these apparently incompatible results. Basso and Taquini (1971) and Barrett et al (1978 and 1981) measured renin activity at a physiological pH. Thurston et al (1979), on the other hand, made their measurements at the pH optimum of rat renin, 6.5. They also demonstrated that although the level of activity at this pH fell after bilateral nephrectomy, the level of activity at pH 5.3, measured in the same animals, remained unchanged. This indicates that non-specific generation of angiotensin is not affected by removal of the kidneys.

A background level of non-specific generation of angiotensin I is almost certain to occur at all pH's. However, at the pH optimum of renin the proportion of this activity which is due to renin activity will be greatest. At a physiological pH the proportion of renin activity will be relatively less and any changes in the amount of renin present may be correspondingly more difficult to detect.

That renin might be synthesised locally has however, been supported by the finding that renin activity in the mesenteric arterial wall of dogs after bilateral nephrectomy was stimulated and released into the circulation by haemorrhage (Ganten, Hayduk, Brecht, Boucher and Genest, 1970). Splanchnic renin was also found to be increased after haemorrhage, being greater in the portal vein than in arterial blood.

Studies using cells in tissue culture have demonstrated the ability of cells other than those of the kidney to synthesise an enzyme which resembles renin. A renin system has been studied in mouse fibroblasts. These cells contain a significant amount of cellular bound renin activity and production of this enzyme was suppressed by the addition of angiotensin II to the culture medium and increased by the addition of saralasin (Ganten, Schelling, Flügel and Fischer, 1975). Renin synthesis has also been demonstrated in cultures of canine aortic smooth muscle cells (Re, Fallon, Dzau, Quay and Haber, 1982).

Recently, Barrett et al (1981) have characterised renin from the plasma, kidney and aortic tissue of rats. The enzymes from all three tissues showed heterogeneity of their isoelectric points, possibly due to small structural differences. The authors found that one form was common to all three tissues but that not all forms present in the plasma were found in aortic tissue. One was also present in aortic tissue but not in the plasma. They suggested that the latter form was synthesised by the aortic wall itself. Similarly, Rosenthal et al (1984) reported that the renin activity present in aortic homogenates from rats had a different isoelectric point to that obtained for the plasma and renal enzymes. However, Barrett et al (1981) also showed

that there was a form of renin which was common to all three tissues. Uptake by arterial tissue of a circulating enzyme originating in the kidney cannot therefore be discounted as a source of renin in the vascular compartment. Indeed, Garst et al (1979) showed that renin infusion increased the amount of renin present in arterial tissue indicating that circulating renin does enter vascular tissue.

However, in contrast to these studies, Fordis et al (1983) concluded that almost all the renin present in aortic tissue was due to contamination by plasma renin. Thorough perfusion of the tissue before homogenisation, together with removal of non-specific proteolytic activity by inhibition and chromatography, resulted in loss of all renin activity in the tissue. The problem of contamination of aortic samples with plasma renin, as opposed to uptake of renin from the plasma into the tissue, is discussed in Chapter three.

Although the origin of the renin activity within the blood vessel wall remains uncertain, it would be surprising if circulating renin were unable to enter the tissue. Renin is, after all, a globular protein whose molecular weight is similar to that of albumen. Like albumen it can therefore be expected to gain ready access to the extravascular compartment of tissues supplied by continuous capillaries. In Chapter four of this thesis evidence is presented that circulating renin does indeed gain access to the walls of blood vessels. This conclusion does not however, rule out the possibility that local synthesis also takes place.

Summary

The discovery of renin and its action in promoting the production of the pressor agent angiotensin II lead initially to the

assumption that this enzyme must have a key role in both the physiology and the pathology of arterial blood pressure. However, the significance of the renin-angiotensin system is not nearly so simple as it originally appeared.

In both clinical and laboratory studies it has become apparent that circulating renin levels do not bear a direct relationship to the level of the blood pressure. The involvement of a number of other systems in the pathogenesis of hypertension have been considered. However, experimental studies on the blood pressure response to exogenous renin or angiotensin, injected into the circulation under a variety of conditions, have lead to the concept that angiotensin may be generated outside the circulation. Thus it has been suggested that the level of renin within the blood vessel walls, rather than in the circulation, is a more relevant indicator of the action of the renin-angiotensin system.

Other studies have confirmed the presence of renin-like activity outside the kidney and circulation which has been demonstrated to have properties in common with renal renin. This activity does indeed appear to play an important physiological role in a variety of homeostatic mechanisms including the control of blood pressure.

CHAPTER TWO

The Aims of this Study and an Outline of the Thesis

The Aims of This Study

The work described in this thesis was carried out in order to examine further the significance of vascular wall renin activity in the rat.

The experiments were designed to determine whether the activity measured in this tissue could be derived from uptake of renal renin from the plasma. In addition, a comparison was made of the respective roles of circulating and vascular wall renin in the control of blood pressure.

A single injection of renal renin was given. The distribution of this exogenous renin between the vascular tissue and the plasma was determined at various times after the injection. The blood pressure response to the renin injection was also measured at these times.

In order to avoid the confusing effects of endogenous renin the effect of the injection was observed in the first set of experiments in rats after bilateral nephrectomy. A further set of experiments was later carried out to determine the effect of renin injection in the presence of the kidneys. In these experiments renin injections were therefore given to rats whose kidneys had not been removed.

The relationship between the renin activity measured in both plasma and the vascular wall and the pressor response resulting from the renin injection was determined. The nature of the pressor response itself was investigated by the use of the inhibitors of the renin-angiotensin system saralasin and captopril.

An Outline of the Thesis

The biochemical techniques involved in the measurement of the renin concentrations in both plasma and aortic samples are described

and discussed in Chapter three. This chapter also includes a description of the method of preparation of the rat renal renin used for the injections.

Chapter four is concerned with the effects of an injection of exogenous renin on the blood pressure response and on the distribution of the injected renin between plasma and the aortic wall. These experiments were conducted in rats which had previously undergone bilateral nephrectomy. The details of the setting up of the animal models used, together with the techniques involved in administration of renin, measurement of the blood pressure and collection of plasma and aortic samples are described. The results obtained in the injected, nephrectomised groups were compared to normal control rats where no renin was administered. The possibility that the pressor response might produce a change in the permeability of vascular tissue was also investigated.

The nature of the pressor response produced by a single renin injection in rats after bilateral nephrectomy was further investigated using the renin-angiotensin system inhibitors saralasin and captopril. These experiments are described in Chapter five.

Rats after bilateral nephrectomy were studied in order to avoid the complications that arise when the kidneys are present. In Chapter six the results obtained using nephrectomised rats were compared to the effect of a similar injection into rats whose kidneys were left in situ. In addition to normal rats, injections were given to rats after chemical renal medullectomy. This group was included in order to observe the possible role of the renal medulla in determining the pressor response to injected renin.

CHAPTER THREE

The Biochemical Methods Used in this Study

Introduction

This chapter describes the measurement of renin concentrations in both the plasma and homogenates of aortic tissue of the rat. A simple method for preparation of the rat renal renin used for the injections is also described.

The renin assay

Renin was originally measured by bioassay and the definition of its activity was based on this method. Thus, one unit of renin activity was defined as that quantity of renin which, when injected intravenously into an unanaesthetised trained dog, raised the direct, mean, systolic (femoral) blood pressure by thirty mmHg in about two minutes (Goldblatt, Katz, Lewis and Richardson, 1943).

However, the bioassay was found to be impractical because in order to obtain a measurable response, such large quantities of renin were required. The assay was thus relatively insensitive. The sensitivity of the assay was initially improved by the addition of an extra step in which angiotensin I was generated *in vitro* by the renin before injection into the experimental animal (Helmer and Judson, 1963). The development of a radioimmunoassay for angiotensin I finally replaced the need for the bioassay altogether.

Despite the fact that the techniques for measuring renin have changed completely, the term "Goldblatt unit of renin activity" is still employed. The relationship between Goldblatt units and the rate of generation of angiotensin I by renin has been determined by Haas, Lewis, Scipione and Koshy (1979). The rate of generation of angiotensin I is expressed in nanograms per ml of plasma per hour of incubation and is given as:

$$1 \text{ ngAI/ml/h} = \frac{1 \text{ Goldblatt unit}}{3.4 \times 10^4}$$

As with any assay, the standardistation of measurement of renin activity is a problem. The rate at which angiotensin I is generated varies with the in vitro conditions under which the generation is carried out and with the species from which the renin and its substrate are obtained. The conversion factor worked out by Haas et al (1979) is an average calculated from the results obtained from a study of seven different species. It can therefore only be used to give approximate conversions.

The rate of generation of angiotensin I by renin can be measured under a variety of experimental conditions. It can be measured under "physiological" conditions that is, at pH 7.4, using only endogenous substrate. The result obtained by this method is then expressed as the renin activity. Alternatively, the conditions of the reaction can be standardised by adjusting the pH of the incubation medium to the optimum for renin and by the addition of excess substrate. The results are then expressed as the renin concentration.

The reaction between rat plasma renin and its substrate is optimal at pH 6.5 (Matoba et al, 1978; Thurston et al, 1979).

Sealey, Laragh, Gerten-Banes and Aceto (1974) found that in human plasma the pH tended to become unstable when incubated above pH 6.0. This can be prevented by the addition of a suitable buffer. Tris Maleate, with a pKa of 6.5 has been used for the assay of renin in rat tissues.

The choice of substrate used in the measurement of human plasma renin concentrations is problematic. If endogenous plasma substrate is used then the results can only be expressed in terms of renin activity since conditions of excess substrate may not necessarily apply. If the plasma renin concentration is required,

extra substrate must be added to satisfy this condition. The kinetics of the reaction will then be affected by the type of substrate used since human renin substrate is not commercially available.

This problem is avoided in the rat since rat renin substrate is present in high concentrations in the plasma of rats twenty-four hours after bilateral nephrectomy (Peach, 1977). At the same time the endogenous renin levels in the plasma are very low. Conditions of excess substrate can be fulfilled by incubating a mixture of the sample to be analysed with nephrectomised rat plasma in a ratio of four to one.

When direct measurement of renin was replaced by a step involving the generation of angiotensin I, it was at first found that the concentration of renin was not directly related to the amount of angiotensin I formed. This is due to the presence of angiotensinases which degrade the angiotensin as it is generated. In order to avoid this problem, renin can be purified (Haas, Goldblatt, Gipson and Lewis, 1966) or the angiotensin I can be absorbed onto dowex as it is formed (Boucher, Veyrat, De Champlain and Genest, 1964). However, the method most commonly used involves the addition of angiotensinase inhibitors to the incubation medium.

Angiotensin I is degraded by a number of enzymes. Specifically it is converted to angiotensin II by angiotensin converting enzyme, but it is also hydrolysed by a number of other non-specific aminopeptidases.

Aminopeptidase A degrades angiotensin I to des-Asp^1-angiotensin I and has a pH optimum in the neutral range. In addition, endopeptidase activity, which also has a pH optimum in the neutral range, may also contribute to angiotensin I breakdown

(Khairallah, Bumpus, Page and Smeby, 1963). The significance of these enzymes in vivo is not known (Peach, 1977) but prevention of their activity in vitro during measurement of renin activity is nevertheless important.

Angiotensinase activity with an acid pH optimum can be inhibited by fluorophosphates such as disopropyfluorophosphate (DFP) (Pickens, Bumpus, Lloyd, Smeby and Page, 1965). Other angiotensinases, including converting enzyme, with a neutral pH optimum, require divalent metal ions for activity. They are therefore inhibited by chelating agents such as dimercaprol (BAL) and 8-hydroxyquinoline (8OHQ). In addition, ethylenediamine-tetraacetate (EDTA), which is usually used as an anti-coagulant when collecting blood for measurement of renin activity, is a powerful chelating agent.

A number of combinations of these inhibitors have been used. Haber, Koerner, Page, Kliman and Purnode (1969) used EDTA, BAL and 8OHQ while Sealey et al (1974) and Gould, Skeggs and Kahn (1966) used EDTA and DFP. Kodish and Katz (1974) found that DFP could be substituted by phenylmethylsulphonylfluoride (PMSF) which has the advantage of being less dangerous to handle.

Oparil, Koerner and Haber (1974) have shown that the effectiveness of all these combinations varies markedly with the pH of incubation. Initially, renin activity was measured at a physiological pH (Gould et al, 1966; Haber et al, 1969). More recently, generation has been allowed to proceed at the pH optimum for renin, that is between 5.0 and 6.0 in human plasma. Oparil et al (1974) showed that a combination of BAL, 8OHQ and EDTA resulted in preservation of ninety percent of the angiotensin I generated at pH 7.4 but was much less effective at

pH 5.5. On the other hand, DFP and EDTA were only ten percent effective at pH 7.4.

The conditions described above apply to the measurement of renin in human tissues. Tikkanen, Fyhrquist and Puutula-Räsänen (1980) studied the efficacy of these inhibitors in rat plasma. They reported that at the high pH optimum of rat renin, that is pH 6.5, 8OHQ was superior to both PMSF and DFP. A combination of 8OHQ and EDTA resulted in close to one hundred percent preservation of the angiotensin I generated at this pH. They also recommended that for complete enzyme inhibition a minimum final concentration of 8OHQ in the incubation medium of 5mM should be used. In addition, 8OHQ has bacteriostatic properties which may be important during prolonged incubation periods (Fleming, Stewart and Hutchinson, 1974).

In this study, a combination of EDTA, PMSF and 8OHQ was used to measure renin activity both in plasma and aortic homogenates.

The plasma renin concentration measured in the present study was therefore expressed in terms of the rate of generation of angiotensin I from rat substrate under standard conditions of optimum pH, excess substrate and at a temperature at 37° C, and using the angiotensinase inhibitors EDTA, PMSF and 8OHQ.

The measurement of the concentration of renin within the aortic wall is considered in a later section.

Radioimmunoassay Of Angiotensin I

Radioimmunological techniques have now been developed which have replaced the bioassay as the standard method for measuring the amount of angiotensin I generated by renin. The technique described in this thesis is based on the method of Sealey et al (1974).

A known amount of ^{125}I-labelled angiotensin I competes with a known (standard) or unknown (sample) amount of cold angiotensin I

for a fixed and limited number of specific binding sites. After incubation the remaining free angiotensin I is separated from the reaction mixture and the activity of the ^{125}I-labelled angiotensin I in this fraction is determined. Thus the concentration of cold angiotensin I in each sample is directly proportional to the quantity of unbound labelled angiotensin I.

Using this method, picogram amounts of angiotensin I can be detected. This is considerably more sensitive than the bioassay. One Goldblatt unit of renin activity is equivalent to approximately 3.4×10^7 picograms of angiotensin I (Haas et al, 1979).

Angiotensin I antibody cross-reacts with angiotensin I and des-Asp^1-angiotensin I. The proportion of the latter in rat plasma is about twenty percent (Del Rio, Smellie and Morton, 1981). However, the principle of the renin assay is the generation of a large amount of angiotensin I. The concentration of des-Asp^1-angiotensin I, on the other hand, will remain relatively unchanged because of the presence of angiotensinase inhibitors. The proportion of this latter component will therefore become insignificant.

Measurement Of The Aortic Wall Renin Concentration

This involves an initial extraction to release the renin from the tissue. Following this, the principle of measurement of the renin concentration in homogenates of aortic tissue is the same as that described for measurement of the plasma renin concentration. However, some adjustment needs to be made for the very different proportions of renin present in plasma and aortic tissue.

When measuring the plasma renin concentration, generation for a period of thirty minutes is sufficient to produce measurable quantities of angiotensin I. However, the renin concentration in the rat aortic wall is less than half a percent of

that present in normal rat plasma. In order to generate enough angiotensin I it is necessary to prolong the incubation stage for up to twenty-four hours.

The effectiveness of the combination of inhibitors used in this study over the extended incubation period was determined by measuring the recovery of a known amount of angiotensin I added to the incubation medium. The slope of the line representing the rate of generation of angiotensin I over a twenty-four hour period was also observed.

Since the renin concentration in plasma is proportionately so much higher, very small amounts of contaminating plasma in the aortic homogenates will contribute significantly to the generation of angiotensin I during a twenty-four hour incubation period.

In order to assess the degree of contamination, the distribution of injected ^{125}I-labelled albumen between plasma and the aorta was measured after a period of equilibration.

Preparation of The Rat Renin Used For Injection

The renin used in this study was purified from normal rat kidneys using a modification of the method described by Haas et al (1966). Hog renin is now commercially available and renin from this species has been used for studies in the rat (Bing and Nielsen, 1973; Basso, Kurnjek and Taquini, 1977). But because there is some degree of interspecies variation in the kinetics of the renin reaction (Peach, 1977), the use of rat renin for these studies was obviously preferable. The procedure followed to extract the renin was relatively simple and sufficient quantities of a semi-purified preparation of rat renin were therefore easily obtained.

Materials and Methods

In this chapter, the biochemical techniques used in this study are described. Details of the methods for collecting the plasma and aortic samples on which these measurements were made are described in Chapter four.

Preparation of Rat Renin Substrate

Bilateral nephrectomy was performed through flank incisions on 250 g female Wistar rats under ether anaesthesia. Groups of six to ten rats were prepared at a time. The animals were allowed to recover and left with free access to water, but not food, for twenty-four hours. After this time, they were reanaesthetised and the lower portion of the aorta was exposed down to the bifurcation through an abdominal incision. As much blood as possible (usually 7 to 10 ml) was withdrawn into a syringe containing 500 μl of a 10 percent solution of potassium EDTA. The blood was immediately transferred to containers on ice and then spun at 2,000 g for seven minutes at 4^{o} C as soon as possible. The plasma so obtained was pooled and stored at -20^{o} C in 10 ml aliquots.

To 10 ml of thawed plasma wereadded 500 μl of 1M Tris Maleate (final concentration, 50 mM), 500 μl of a saturated ethanolic solution of PMSF and 500 μl of an aqueous solution of 8OHQ (sulphate form) (final concentration 10 mM). The pH of the solution was adjusted to 6.5 with 2M NaOH and the mixture spun at 2,000 g for seven minutes to remove the precipitated protein produced by the addition of ethanol.

Measurement of Plasma Renin Concentration

100 μl of plasma were mixed with 400 μl of substrate plasma. The mixture was divided into three aliquots of approximately 150 μl.

One of these was kept on ice throughout. This was the blank which gave a measure of the angiotensin I already present in the sample before incubation. The remaining two were incubated at 37° C in a shaking water bath for fifteen or thirty minutes.

Samples with a very low renin content were incubated for one or three hours. Those with a high renin concentration were diluted with saline before mixing with substrate plasma.

The reaction was stopped by transferring the tubes to ice. The incubates were then either assayed immediately or stored at -20° C until required.

The angiotensin I generated during the incubation was measured by radioimmunoassay.

Measurement of Aortic Wall Renin Concentration

Extraction of Renin Activity From Aortic Tissue

Each aorta was frozen and thawed four times and washed four times with saline. It was then blotted dry, weighed and transferred to a 5 ml conical glass homogeniser on ice. The tissue was homogenised for ten seconds using a motor driven pestle. Ice cold saline was then added (10 µl/mg wet weight) and the homogenisation continued for a further sixty seconds. The entire homogenate was frozen and thawed once more and spun at 36,000 g for thirty minutes at 4° C. The supernatants were either assayed immediately or stored at -20° C until required.

Assay of Aortic Wall Renin Activity

125 µl of supernatant were mixed with 500 µl of substrate plasma. This mixture was divided into four aliquots of approximately 150 µl each. One was immediately frozen to serve as a blank while the others were incubated for six, sixteen or twenty-four hours in a shaking water bath at 37° C. The reaction was stopped by

freezing the samples to -20° C. The angiotensin I generated was measured by radioimmunoassay.

During these long incubations the substrate plasma itself generates a significant proportion of the total angiotensin I produced. For this reason an additional blank containing 125 µl of saline mixed with 500 µl of substrate plasma was treated in the same way.

The base-line generation of angiotensin I in each pool of substrate plasma used was measured for each separate incubation. This was because it was not practical to prepare a plasma pool of sufficient size for all the experiments and there was some variation between batches.

Radioimmunoassay of Angiotensin I

Lyophilised rabbit angiotensin I antiserum was obtained from Becton Dickinson (Wembley, U.K.). A dilution of 1:25,000 in assay buffer (100 mM Tris Maleate, pH 7.4 containing 0.1 percent radioimmunoassay grade bovine serum albumen) was made which gave a range of binding between 40 and 70 percent under the assay conditions used.

^{125}I-labelled angiotensin I (New England Nuclear Chemicals, Southampton, U.K.) was also diluted in assay buffer to give 0.2 µCi/ml, equivalent to 10,000 cpm/50 µl.

The reagents and the reaction mixture were kept cold at all times.

25 µl of incubate were diluted with 200 µl of assay buffer and mixed with 200 µl of antibody and 50 µl of ^{125}I-labelled angiotensin I. Two standard curves were included in each assay consisting of eight tubes containing a range of between 3.125 and 400 pg of angiotensin I (Beckman, Geneva, Switzerland). The standard curves

were constructed by serial dilution in assay buffer. Each tube contained 200 µl of the appropriate standard.

The total activity (To) and the total binding (Bo) were also determined in duplicate for each assay. To is the total of unbound (free) counts measured in the absence of both unlabelled angiotensin I and antibody. Bo is the total of free counts measured in the absence of unlabelled angiotensin I alone. Non-specific binding was less than 5 percent.

The reaction mixute was incubated at 4^{o} C overnight (for a minimum of sixteen or a maximum of twenty-four hours). After this time the remaining free angiotensin I was separated from the reaction mixture by absorption onto dextran coated charcoal. This was made by suspending 1.25 g Dextran T70 (Pharmacia Fine Chemicals, Uppsala, Sweden) and 1.50 g Charcoal Norit SX1 (Analytical Supplies, Derby, U.K.) in 500 ml distilled water. It was precooled before use and stirred continuously during addition to the incubated samples. 1 ml was added to each tube, thoroughly mixed and immediately spun at 2,000 g for seven minutes at 4^{o} C. The supernatant was discarded and the activity in the pellet counted using an automatic gamma counter (LKB-WALLAC 80,000). The counting time was adjusted to give 10,000 cpm in the To tube. This was usually between fifty-five and seventy-five seconds. All the tubes were then counted for this length of time.

A standard curve was constructed using an Olivetti desk top computer which converted the counts of the meaned duplicates (B) into logit values based on the value of Bo. A straight line plot ($r > 0.990$) was then obtained of the logit of free counts versus pg of angiotensin I in the standards:

$$\text{Logit} = \frac{B/Bo}{1-B/Bo}$$

The counts in the sample tubes were then read off this standard curve and converted into pg of angiotensin 1.

Renin concentrations were expressed in ng angiotensin 1/ml/hour for plasma and ng angiotensin 1/100 mg (wet weight of the aorta)/hour for aortic homogenates.

Measurement of Plasma Contamination of Aortic Homogenates

A group of rats, eighteen hours after catheterisation and bilateral nephrectomy (following the procedure outlined in detail in Chapter four), received a bolus injection of 100 µl of ^{125}I-labelled albumen (2.25 µCi/ml; The Radiochemical Centre, Amersham, U.K.). After thirty minutes a blood sample was collected. The animal was immediately killed and the aorta removed (as described in Chapter four). The vessel was cleaned, split lengthwise and washed thoroughly four times in saline.

Samples of 100 µl of plasma were counted for five minutes and the aorta itself was counted for ten minutes. Contamination of the aorta by plasma not removed by the washing procedure was expressed as µl of plasma/100 mg of aortic tissue.

Recovery of Angiotensin 1

100 µl of a solution of angiotensin 1 (10 ng/ml) made up in assay buffer were mixed with 400 µl of substrate plasma. This gave a final concentration of angiotensin 1 in the 25 µl of incubation mixture used for the radioimmunoassay of 50 pg/25 µl. The mixture was then incubated at 37^{o} C for twenty-four hours and assayed as described previously.

Rate of Generation of Angiotensin 1 over Twenty-four Hours

The slope of the rate of generation of angiotensin 1 in the presence and absence of 8OHQ as an additional inhibitor was determined using aortic homogenates from normal rats. The

procedure was identical to that outlined above for measuring the aortic renin concentration. The results were expressed in ng angiotensin/100 mg and plotted against the time of incubation.

pH Curve of Aortic Wall Renin-Like Activity

10 ml of plasma from nephrectomised rats were made up containing inhibitors and buffer as described and divided into twelve aliquots. The pH of each of these aliquots was adjusted with 1M HCl and 2M NaOH to give a range of pH's between 5.3 and 7.5.

400 µl of substrate plasma at each pH were mixed with 100 µl of either aortic homogenate or saline. The mixture was divided into three aliquots of approximately 150 µl. Two of these were incubated for sixteen hours and the third was immediately frozen at -20° C as an angiotensin 1 blank.

The angiotensin 1 generated was measured by radioimmunoassay.

Preparation of Rat Renin

Rat kidneys, obtained from normal female Wistar rats, were stored at -70° C before use. Renin was extracted from approximately 10 g of chopped kidney cortex at a time, using the following method.

The tissue was frozen and thawed four times and homogenised on ice in 10 ml of distilled water using a polytron homogeniser (Kinematica, Switzerland). The resulting homogenate was diluted to 50 ml with distilled water and stirred at room temperature for fifteen minutes. The residue was removed by spinning at 2,000 g for fifteen minutes. The supernatant was cooled on ice and the pH adjusted to 2.3 with 5M H_2SO_4. Ice cold ethanol (5 ml) was then added and the mixture was incubated for one hour on ice without stirring. The precipitate was removed by spinning at 2,000g for fifteen minutes and the pH of the supernatant was adjusted to 4.3 with 5M KOH. This solution was dialysed against two

litres of distilled water at 4^{o} C. After dialysis the pH was again adjusted to 2.8 with 1M H_2SO_4.

Further purification was obtained by salt precipitation. The supernatant was stirred for ten minutes on ice with the addition of NaCl (final concentration, 0.8M) followed by $(NH_4)_2$ SO_4 (final concentration, 1M) in two separate steps. After each addition the precipitate was removed by spinning at 2,000 g for fifteen minutes. Finally, a concentration of 2.3M $(NH_4)_2$ SO_4 was used to precipitate the fraction containing the renin activity. This precipitate was collected by spinning at 20,000 g for fifteen minutes. The precipitate obtained was resuspended in 3 ml 0.9 percent NaCl and dialysed for twenty-four hours at 4^{o} C against two litres of distilled water. The dialysate was stored in 100 µl aliquots which were thawed and diluted as required and used immediately. Before injection each 100 µl was diluted with 300 µl of heparinised dextrose. This was sufficient for the injection of two rats. After dialysis one sample was reserved to determine the total renin concentration of the preparation.

Assay of the Renin Activity in the Renin Preparation

The renin activity in the renin preparation was determined, as described for plasma and aortic homogenates, by measuring the rate of generation of angiotensin I from renin substrate at pH 6.5. In order to do this the concentrated solution obtained was first diluted 1×10^{4} in ice cold saline. 100 µl of the diluted solution were then mixed with 400 µl of substrate plasma, divided into three 150 µl aliquots and incubated for zero, fifteen and thirty minutes. The angiotensin I generated was measured by radioimmunoassay, and the result converted to Goldblatt units.

Results

Measurement of Plasma Renin Concentration

Plasma renin concentration was expressed in ng angiotensin I generated/ml of plasma/hour of incubation at 37° C (ngAI/ml/h).

The minimum level of detection of the radioimmunoassay for any sample was 6.25 pg angiotensin I in 25 µl of incubate. Samples were routinely incubated for fifteen and thirty minutes. During this time enough angiotensin I was usually generated for easy detection, that is, within the range 6.25 to 400 pg angiotensin I/25 µl.

For samples with very low renin concentrations, for example plasma from rats after bilateral nephrectomy, generation was allowed to proceed for three hours. The lowest renin concentration detected after this time was therefore equivalent to 0.41 ng AI/ml/h. Any sample incubated for three hours which contained less than this amount was given a value of 0.41 ng AI/ml/h.

For the measurement of plasma renin concentration the intra-assay variation was 8.4 percent and the inter-assay variation was 17.5 percent. These values were obtained by carrying out plasma renin concentration measurements on twelve samples of the same plasma.

Measurement of Aortic Wall Renin Concentration

The aortic wall renin concentration was expressed in ng angiotensin I generated/100 mg aortic tissue (wet weight)/hour of incubation at 37° C (ngAI/100 mg/h).

As for the plasma renin concentration measurement, the minimum level of detection in the radioimmunoassay was 6.25 pg angiotensin I in 25 µl of incubate, since the procedure for the radioimmunoassay was the same for both measurements. However, for

the measurement of the aortic wall renin concentration, generation was allowed to proceed for up to twenty-four hours and at least six hours compared to fifteen and thirty minutes for plasma. The lowest measurable renin concentration was therefore 0.05 ngAI/100 mg/h. Any aortic wall renin concentration which was lower than this after twenty-four hours incubation was given a value of 0.05 ngAI/100 mg/h.

For the measurement of aortic wall renin concentration, intra-assay variation was 9.7 percent and the inter-assay variation 21.8 percent when measured on a group of ten samples of pooled aortic homogenate.

Recovery of Angiotensin I

The recovery of angiotensin I added to the incubation mixture was 94.1 $\pm$ 2.4 percent ($n = 12$) after a twenty-four hour incubation period.

Linear Generation of Angiotensin I Over Twenty-four Hours in The Presence of 8OHQ

The rate of generation of angiotensin I in the presence of the inhibitors 8OHQ and PMSF compared to PMSF alone is illustrated in Figure 1.

In the presence of PMSF alone, generation of angiotensin I was linear for only six hours whereas in combination with 8OHQ, generation was linear over the full twenty-four hour period.

pH Profiles of Aortic Homogenates

pH curves were obtained for aortic homogenates from normal rats and also from homogenates of aortae from bilaterally nephrectomised rats three hours after renin injection. The results of these measurements are illustrated in Figures 2 and 3 respectively.

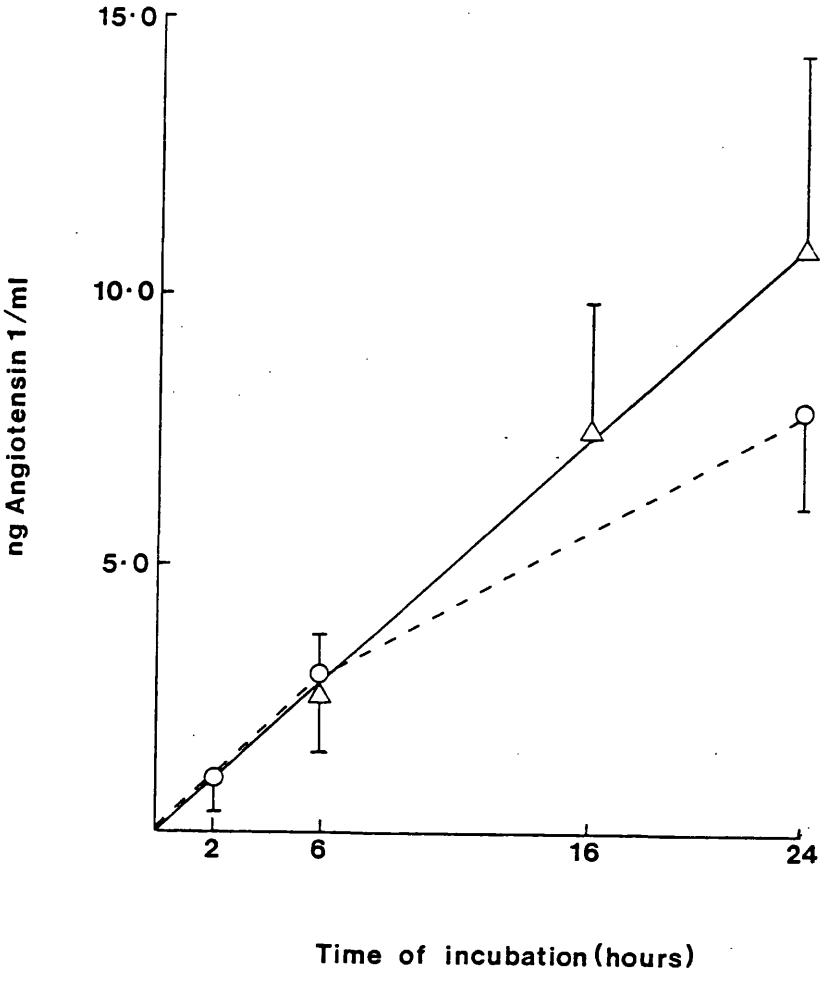

Figure 1. Generation of angiotensin I from rat plasma renin substrate by aortic homogenates at 37° C over a period of twenty-four hours in the presence (△) and absence (○) of the angiotensinase inhibitor 8-hydroxyquinoline.

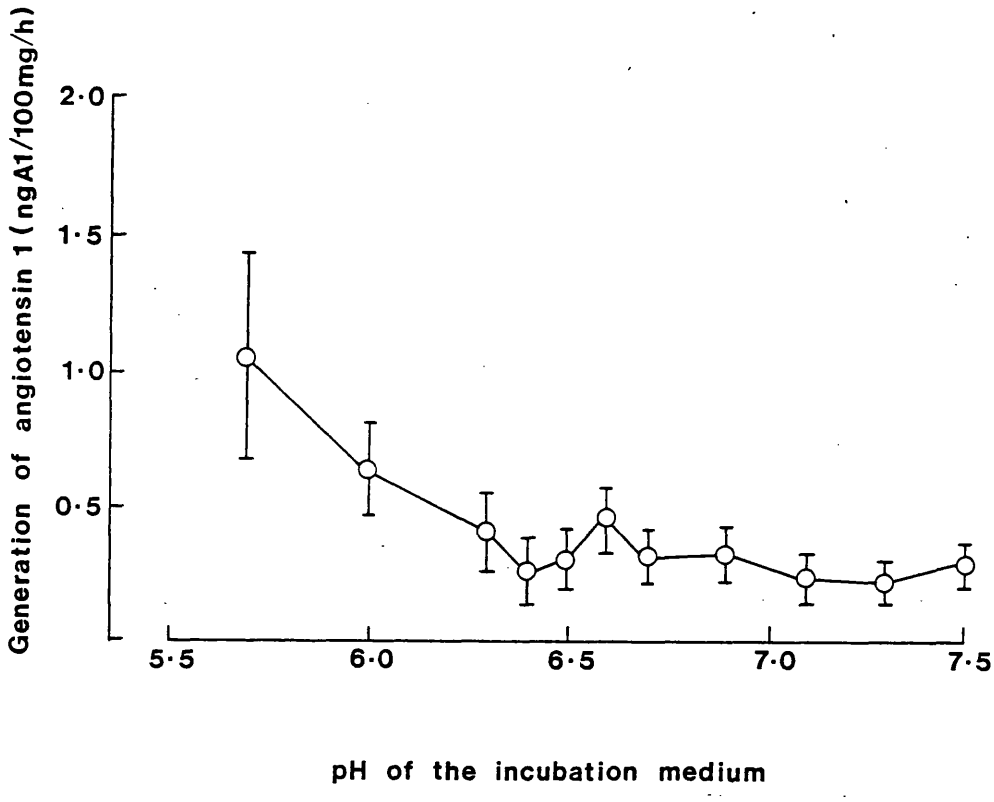

Figure 2. The pH profile of the rate of generation of angiotensin I from rat renin substrate at 37° C. Measurements made on pooled aortic homogenates from normal rats.

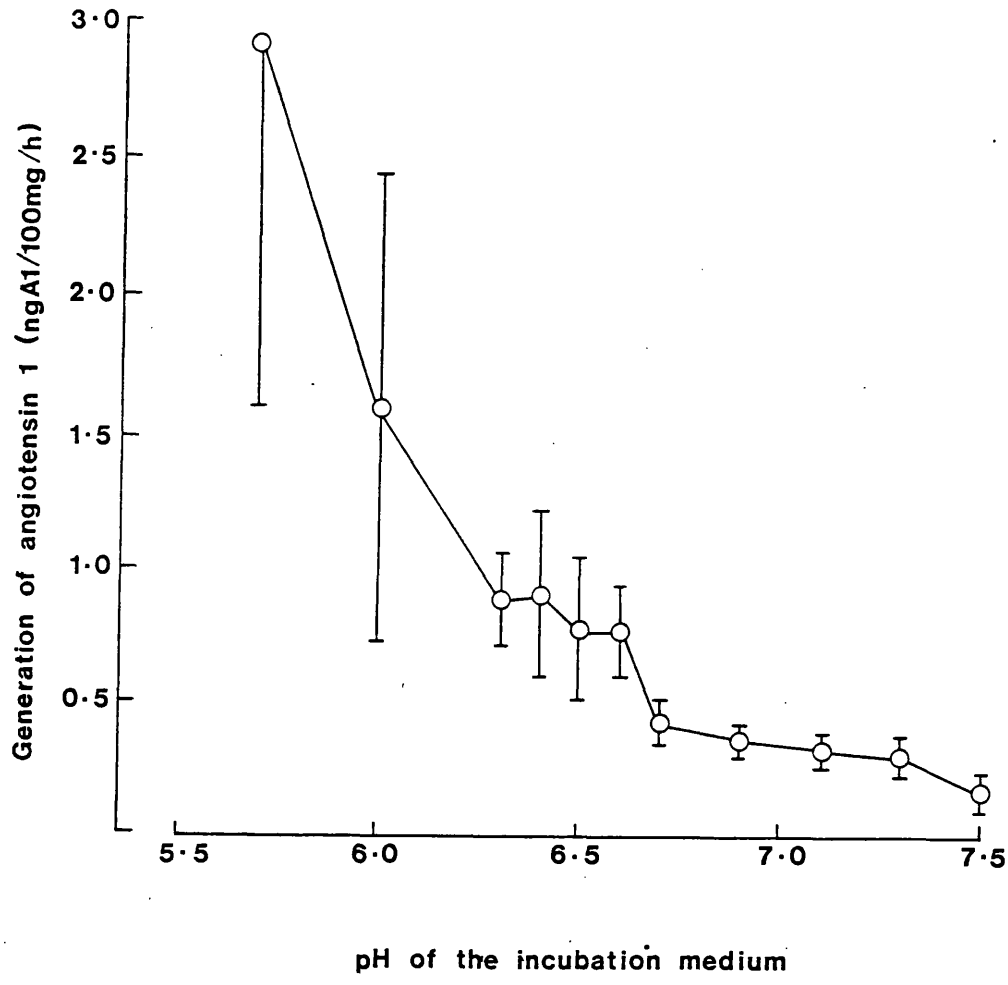

Figure 3. The pH profile of the rate of generation of angiotensin I from rat renin substrate at 37° C. Measurements made on pooled aortic homogenates from nephrectomised rats three hours after a single injection of 0.6 Goldblatt units of rat renin.

Both profiles were biphasic, with maximal activity at an acid pH below 5.5 and a second, smaller peak in the neutral range between 6.4 and 6.6.

Plasma Contamination of Aortic Homogenates

The average value for the amount of contaminating plasma in aortic homogenates was 2.29 $\pm$ 0.39 μl of plasma/100 mg of aortic tissue. This was an average of the contamination of ten different aortic homogenates.

The aortic wall renin concentration was expressed per 100 mg of aortic tissue. To correct for plasma contamination the activity present in 2.29 μl of plasma was calculated from the individual values of plasma renin concentration. The value was then subtracted from the corresponding aortic renin concentration measured in the same rat.

The Renin Preparation

The activity of the renin preparation used for the injections was measured following the same procedure described for plasma and aortic wall renin concentrations. This was expressed in ng angiotensin I generated/ml of the renin preparation/hour of incubation at 37° C.

The preparation had a renin concentration equivalent to 800×10^3 ngAI/ml/h. The specific activity was 192.3×10^3 ng AI/h/mg protein.

The concentration of 800×10^3 ngAI/ml/h was equivalent to 24 Goldblatt units/ml when converted according to the formula

$$1 \text{ ngAI/ml/h} = \frac{1 \text{ Goldblatt unit}}{3.4 \times 10^4}$$

described by Haas et al (1979).

Discussion

This chapter has been concerned with the validation of the method used for the measurement of renin-like activity in the aortic wall and with the preparation of the renal renin used for the injections in the experiments described in the following chapters. In particular, consideration was given to the justification of applying the procedure for assaying renin in the plasma to the measurement of renin-like activity in the aorta.

Renin was first discovered as a substance obtained from the kidney and has, until recently, been considered exclusively as a renal enzyme. Thus the definition of renin in terms of its physical characteristics was based on studies of the properties of renal renin. On the other hand, scientific study of the physiological significance of renin activity has previously been confined, for the most part, to its measurement as a constituent of the plasma. It is only recently that renin activity has been measured in other tissues such as the walls of blood vessels. It is of particular interest whether or not the activity measured at this site is identical to renal and plasma renin and whether it has any physiological role.

The method used in this study to measure renin activity in the blood vessel wall was based on that described by Thurston et al (1979). This was a modification of the method devised to measure renin activity in the plasma (Sealey et al, 1974). There are a number of problems associated with applying an assay designed for one tissue to another.

The first and most important consideration is whether the activity measured in the two tissues by the assay is the same. The principle of the renin assay is the measurement of the rate

of generation of angiotensin I from renin substrate. A number of enzymes other than renin have been shown to be capable of doing this (Franze de Fernandez et al, 1965; Reid, 1977). However, when generation is measured under specific conditions, the renin-like activity measured in the blood vessel wall has been shown to have many properties in common with renal and plasma renin. These include a similar pH optimum, isoelectric point, K_m, velocity constant, activation energy and molecular weight (Barrett et al, 1978 and 1981). It has therefore been concluded that a substance resembling the renin found in the kidney and plasma is present in the blood vessel wall.

Since this renin-like activity is similar to the renin present in the plasma, it is probably justifiable to use the standard renin assay to measure its concentration within the blood vessel wall. However, there are a number of modifications which must be made to the original procedure before it can be applied to measuring renin at this site. In the model described in this thesis the modifications included a lengthening of the period of incubation during which the generation of angiotensin I occurred and a change in the pH at which this generation was allowed to proceed. These changes in turn resulted in the necessity of changing the inhibitors required to prevent angiotensin I degredation. It was therefore important to determine to what extent the assay procedure could be modified while still giving accurate results.

A major modification to the original plasma renin assay which was used in this study was a change in the pH at which the generation of angiotensin I took place. The renin assay described by Sealey et al (1974) was designed to measure renin activity in

human plasma. The pH optimum of human plasma renin is 6.0 whereas in rat plasma, maximum generation of angiotensin I occurs at a pH of 6.5. Homogenates of rat aortae have also been shown to have a peak of angiotensin I generating activity at pH 6.5. However, there is also a second peak of angiotensin I generating activity below pH 6.0. This biphasic pH curve has been demonstrated by Thurston et al (1979) and Barrett et al (1981). The shape of the curve is thought to represent two distinct populations of angiotensin I generating activity, the activity of non-specific proteases being confined to the acid range, that is below pH 6.0, while the peak of activity at pH 6.5 is due to the activity of an enzyme which resembles renin.

The shape of the curve was confirmed in the present study, as illustrated in figure 2. This figure shows the pH profile of angiotensin I generating activity by aortic tissue obtained from normal rats. A similar pH profile was obtained using aortic homogenates from nephrectomised rats three hours after the injection of semi-purified renal renin. This is illustrated in figure 3. The pH profile was similar in shape to that obtained from normal rats but the smaller peak of pH 6.5 was less clearly defined. In addition the peak of activity below pH 6.0 was much larger. This may be a reflection of the fact that the renin used for the injections was relatively impure. The nature of the method followed for the preparation of rat renal renin meant that only a semi-purified renin was obtained. The preparation would therefore be expected to contain non-specific protease activity as well as renin. Injection of this preparation into the plasma therefore appeared to result in the entry not only of renin, but of a large amount of non-specific protease activity into the

aortic wall. Despite this the overall shape of the profiles obtained from both normal and injected rat aortic homogenates was similar and it was concluded that it was the activity measured at pH 6.5 which represented renin activity. In the experiments described in the following chapters, renin activity was therefore measured at this pH.

The other modification to the plasma renin assay was an increase in the length of time during which the generation of angiotensin I was allowed to proceed. The level of renin activity measured in aortic homogenates was found to be less than half a percent of the renin level in the plasma. The limits of the angiotensin I radioimmunoassay are fixed. In order to increase the sensitivity of the assay the length of time over which the angiotensin I is generated must be increased. The level of renin usually present in the plasma generates sufficient quantities of angiotensin I for detection by the radioimmunoassay within thirty minutes. However, because the level of renin activity in the aortic homogenates is so much lower, generation must be allowed to proceed for between six and twenty-four hours for the level of angiotensin I produced to fall within the correct range. Since this is a considerable increase in the incubation period it was important to ensure that the generation of angiotensin I remained linear during this time. Linearity depends in turn upon the efficiency with which the breakdown of angiotensin I by angiotensinases is prevented during incubation. The method upon which the assay used here was based used PMSF alone as an angiotensinase inhibitor (Thurston et al, 1979). In the present study it was found that PMSF alone provided adequate inhibition during an incubation period of up to six hours. This is illustrated in

figure 1. However, the generation of angiotensin I did not remain linear during more prolonged incubation. Tikkanen et al (1980) showed that the most effective angiotensinase inhibitor for measuring rat plasma renin activity was 8OHQ. It was found in the present study that addition of this substance to the incubation mixture extended the period of linear generation for up to twenty-four hours, as illustrated in figure 1.

As well as the prevention of angiotensin degradation during the prolonged incubation period, linear generation also depends on the maintenance of conditions of excess renin substrate. It has been shown that, in the method followed here for the assay of the plasma renin concentration, the composition of the incubation mixture ensures conditions of excess substrate during incubation. Since the amount of angiotensin I generated by the renin-like activity in the aortic homogenates did not exceed that generated by plasma renin, even though the incubation period was considerably extended, it was assumed that the substrate concentration effectively did not change over the twenty-four hours. This was confirmed by the fact that linear generation was observed. However, during incubation over this period of time account must be taken of the contribution of the low level of renin activity in the substrate plasma. The generation of angiotensin I produced by this renin is negligible during short incubations. But during the prolonged periods employed when measuring aortic wall renin concentrations this renin generated significant quantities of angiotensin I. For this reason a substrate blank was included for each measurement of the aortic wall renin concentration.

It was therefore concluded from the results described above that modification of the established method for measuring plasma

renin activity was feasible and that after such modifications it could be used to assay aortic wall renin activity provided that conditions which allow linear generation of angiotensin I were fulfilled.

Aortic wall renin levels were therefore estimated by measuring the rate of generation of angiotensin I in the presence of the inhibitors 8OHQ and PMSF and of excess substrate. The pH of the incubation mixture was 6.5 and the temperature 37^{o} C. All the aortic samples obtained from experiments described in the following chapters were analysed under these conditions. The results obtained were measurements of the concentration of renin at this site as they were in the plasma.

Because the incubation period of the assay has to be extended to detect aortic wall renin activity, the contamination of aortic homogenates by small quantities of plasma renin can make a significant contribution to the measured activity. Indeed, Fordis et al (1983) have suggested that all the renin activity present in the aortic wall is due to contamination by plasma. A set of experiments was therefore carried out in this study to assess the contribution of this plasma contamination to the measured values.

Garst et al (1979) estimated plasma contamination of aortic homogenates by measuring the spectral absorbance of haemoglobin at 415nm using the blood from each individual rat as a standard. However, this results in an overestimation of the contamination because some of the peak is due to non-haemoglobin material. In addition, some red cells and clots adhere to the tissue and sometimes cannot be removed by the washing process. These problems are overcome by using ^{125}I-radiolabelled albumen although this method has its own drawbacks.

The results obtained in this study relied on the assumption that there was no significant uptake of the albumen by the aortic tissue during the thirty minutes after the injection of albumen. This time interval was allowed in order to ensure that the bolus injection of albumen was evenly distributed throughout the circulation. The assumption was probably not strictly true and some of the injected albumen could have entered the extracellular fluid compartment of the aortic tissue during this time. This would therefore result in an overestimation of the degree of contamination. However, for determining the degree to which plasma contamination contributes to the measured results it was considered that it was better to make an over rather than an underestimate.

In order to calculate the degree of plasma contamination a separate set of experiments was carried out. The resulting correction factor was thus an average value rather than an individual value for each separate rat. Statistical analysis was therefore not applied to the corrected results.

Although the existence of inactive forms of renin is well documented, their physiological significance is uncertain. The possibility that an inactive form of renin may have contributed to the results obtained from the experiments described in this thesis needs to be considered. However, it is unlikely that this form of renin was important since the presence of inactive renin in the rat is anyway open to doubt.

Most of the investigations on the significance of inactive renin have focused on human inactive renin since a large proportion of human plasma renin is present in this form, but inactive renin has also been found in a number of other species. These include the pig (Bailie, Derkx and Schalekamp, 1979), rabbit (Leckie, 1973)

and sheep (Lush, Munday and Noble, 1980).

The presence of inactive renin in the rat is controversial. A number of studies have failed to detect any inactive renin in this animal. Nakane, Nakane, Corvol and Menard (1979) showed that the rat kidney secretes only active renin. They also found that there was no change in the plasma renin concentration after acid treatment of plasma from unanaesthetised rats (Nakane, Nakane, Corvol and Menard, 1980). De Keijzer, Provoost and Derkx (1982) did not detect any activation of rat plasma renin after treatment with either acid or trypsin. Prolonged incubation of rat plasma at 4^{o} C also produced no change in the plasma renin concentration. However, Inagami, Hirose, Murakami and Matoba (1977) showed that rat and hog kidneys contain a high molecular weight renin which was converted to a lower molecular weight form by renal proteases. This conversion occurred more readily in the rat. Sagnella, Price and Peart (1980) found that low molecular weight renin in the rat kidney was membrane bound but could associate with a soluble factor to form a high molecular weight form of renin. Suzuki et al (1980) showed that kallikrein stimulates renin release from the rat kidney. Since it is thought that kallikrein may be involved in the process of renin activation, these authors have proposed that kallikrein activates inactive renin within the kidney. However, there is no direct evidence that this process occurs. Barrett, Eggena and Sambhi (1980) have demonstrated trypsin activation of rat plasma renin after prior stimulation of renin levels by ether anaesthesia. They reported that the process occurs very rapidly and may thus be very difficult to detect.

Recently Doi, Franco-Saenz and Mulrow (1984) have measured active and inactive renin in rat plasma after bilateral nephrectomy.

They showed that whilst the level of active renin declined rapidly immediately after bilateral nephrectomy, the level of inactive renin increased almost ten-fold. The amount of inactive renin measured in the plasma reached a maximum at twenty hours after bilateral nephrectomy. This was in contrast to the findings of Barrett, Eggena, Sowers and Sambhi (1982) who found that active and inactive renin both declined at the same rate after bilateral nephrectomy.

It should be noted, however, that although Doi et al (1984) found that the level of inactive renin increased markedly after bilateral nephrectomy, conversion of inactive to active renin did not take place *in vivo* in peripheral blood. Inactive renin was only detected after incubation of the plasma with trypsin *in vitro*.

It therefore seems unlikely that activation of inactive renin contributed to the results obtained in this study since none of the experimental procedures used for the measurement of renin activity would have resulted in *in vitro* activation of endogenous inactive renin. This probably applies equally to the renin present in the aortic wall although as yet the presence of inactive renin at this site has not been demonstrated.

This thesis was therefore confined to considering the effects of exogenous renin in its active form, measured both in the plasma and the aortic wall, in the experimental models described in the following chapters.

CHAPTER FOUR

The Effects of Renin Injection After Bilateral Nephrectomy: Measurements of Blood Pressure and of the Distribution of Renin Between the Plasma and the Wall of the Aorta

Introduction

In this chapter the effect of injected exogenous renin on the blood pressure was observed in rats after bilateral nephrectomy. The relationship between this response and the levels of exogenous renin measured in the plasma and the wall of the aorta was investigated.

Vascular Wall Renin Activity and the Pressor Response to Exogenous Renin

Basso et al (1977) observed the effect of a single injection of purified hog renin in Goldblatt 1-kidney 1 clip hypertensive rats which they compared to the response in sham operated rats. They found that in both groups the pressor response was prolonged compared to the blood pressure response to injected renin in normal rats. In the hypertensive rats the response persisted for longer than one hour but although the half-life of the circulating exogenous renin was doubled in the hypertensive rats it was still only twelve minutes. There was thus a dissociation between renin levels in the plasma and the pressor response. But although circulating renin had returned to within normal levels, the authors demonstrated an increase in renin activity in the aortic tissue of the hypertensive rats one hour after injection.

Garst et al (1979) also demonstrated an increase in the renin activity present in aortic tissue from normal rats after a thirty minute infusion of rat renin. This was associated with a marked pressor response induced by the administration of the exogenous renin.

Although these experiments demonstrate an association between vascular wall renin activity and the blood pressure response, the precise relationship is unclear. The results are complicated

by the presence of the kidneys which, as well as being a source of endogenous renin, may lower the blood pressure by mechanisms other than the renin-angiotensin system. This is discussed in Chapter six.

Vascular Wall Renin Activity After Bilateral Nephrectomy

By using the model of the rat after bilateral nephrectomy the effects of the kidney on blood pressure control are removed. After bilateral nephrectomy there is a marked divergence between circulating and vascular wall renin levels (Thurston et al, 1979), a situation which may therefore be exploited in this model in the study of the significance of the renin present in the two tissues.

Both Schaechtelin, Regoli and Gross (1964) and Bing and Nielsen (1973) demonstrated a prolonged pressor response to renin injection after bilateral nephrectomy. The blood pressure was found to be elevated for up to six hours after the initial administration of renin. Although the half-life of circulating renin is increased after bilateral nephrectomy (Peters-Haefeli, 1971) for as long as one hour, there is still a marked dissociation between the plasma renin levels and the blood pressure response. Bing and Nielsen (1973) suggested that renin activity within the blood vessel walls might be more relevant than circulating renin in maintaining the blood pressure. This conclusion was supported by the finding that the blood pressure response to converting enzyme inhibition persisted for up to six hours after bilateral nephrectomy in Goldblatt 2-kidney 1 clip hypertensive rats (Thurston and Swales, 1977). This suggests that the blood pressure is still maintained by the renin-angiotensin system. However, the response to converting enzyme inhibition was found to be related to the renin activity measured in aortic tissue rather

than to the levels of renin in the plasma (Thurston et al, 1978).

The model of the rat after bilateral nephrectomy can also be used to demonstrate the possible origin of renin activity in the vascular wall. The possibilities are either that it is taken up from the plasma or that it is synthesised locally. Central to this argument is the amount of renin activity which persists in the vessel wall after the removal of the kidneys.

There is some dispute about the amount of renin remaining in the vascular wall at various times after bilateral nephrectomy. Barrett et al (1981) found that the renin activity present in the aortic tissue of rats remained unchanged up to thirty hours after bilateral nephrectomy. Conversely, Thurston et al (1979) showed that by twenty-four hours after bilateral nephrectomy both plasma renin and aortic wall renin concentrations were very low. The discrepancy between these two observations may reflect the differences in the techniques used in the measurement of the aortic wall renin activity by the two groups. In particular the importance of the pH at which the generation of angiotensin I is allowed to proceed is underlined. Barrett et al (1981) made their measurements at pH 7.4 whereas Thurston et al (1979) used a pH of 6.5, the pH optimum of rat renin. In contrast to the findings described above, Fordis et al (1983), measuring renin activity at pH 6.0, found that the activity measured in the aortic wall disappeared at the same rate as that measured in the plasma. In the experiments described in this thesis, renin was measured following the method of Thurston et al (1979). Renin activity was thus measured at pH 6.5.

Despite the doubts surrounding the amount of endogenous

renin activity remaining after bilateral nephrectomy, this model is the most suitable for studying the distribution of injected renin between the circulation and the blood vessel walls. The divergence in the levels of renin activity in these two compartments observed after removal of the kidneys allows their respective roles in blood pressure control to be assessed.

The experiments described in this chapter were carried out over a period of two days for each rat. The animals were conscious and blood pressure was monitored continuously. Plasma and aortic wall renin concentrations were determined at a number of times after a single injection of partially purified rat renin.

All the animals were operated on on the afternoon of the first day and were injected, and samples collected, at one, three, six or nine hours after injection on the second day. Animals were injected at different times, depending on the time between this and collection of the samples. The samples were therefore always collected twenty-four hours after bilateral nephrectomy.

Vascular Permeability After Renin Injection

High concentrations of angiotensin II have been shown to alter the permeability of the walls of large blood vessels (Giese, 1973; Robertson and Khairallah, 1972). Angiotensin II appears to act directly to produce a widening of the gap between the lining endothelial cells. Widening of interendothelial spaces has been demonstrated in the aorta and in coronary, mesenteric and peripheral arteries (Robertson and Khairallah, 1973; Wiener and Giacomelli, 1973; Giacomelli, Anversa and Wiener, 1976). Peach (1977) has suggested that the effect is produced by peptide-induced contractions of the arterial endothelial cells, the angiotensin II acting via stimulation of local prostaglandin synthesis. When this

occurs the endothelial lining becomes more permeable to high molecular weight materials (Giacomelli et al, 1976).

It is therefore possible that the angiotensin II generated by the large quantity of injected renin might alter the permeability of the aorta. This might increase the amount of renin measured in the tissue after injection. In order to determine the contribution of a change in permeability to the results obtained, uptake of radiolabelled albumen by the aorta was determined in the presence of the pressor response. This was compared to the uptake of albumen when no exogenous renin was administered. The amount of exogenous renin present in the aorta was also determined when the pressor response had been inhibited by captopril. This is discussed in Chapter five.

Materials and Methods

White female Wistar rats weighing 180-200g were used throughout. All operations were performed under ether anaesthesia.

Insertion Of The Catheters

Polyethylene catheters (Portex Ltd., Hythe, U.K.) were inserted into the left carotid artery and jugular vein for continuous blood pressure monitoring and intravenous injection respectively. Catheterisation was performed through an incision on the anterior surface of the neck. The carotid catheter (p50) had internal and external diameters of 0.58 mm and 0.96 mm respectively. The jugular catheter (p10) had dimensions of 0.28 mm and 0.61 mm respectively.

The catheters were inserted into the respective vessels and each was tied in place with three ties. The two catheters were then tied together in the midline to secure their position and brought through to the exterior between the scapula, the p50 passing to the right and the p10 to the left of the trachea, between the muscle layers.

The catheters were protected by a stainless steel spring, 40 cm in length. At one end of the spring was a metal flange containing four equidistant holes. Through these the flange was sutured to the rat at the site of the exit of the catheters between the scapula. A linen jacket around the rat further secured the flange in place. At the other end the spring was attached to a metal rod, 60 cm in length and pivoted at its mid-point. The combined weight of the rat and the spring was counterbalanced by an adjustable aluminium weight. The metal rod was fixed at a height of 60 cm above the bench to a retort stand. This was placed next to the perspex cage (30 x 30 x 39 cm) in which the rat was

housed. This arrangement allowed the rat freedom of movement within the cage whilst at the same time protecting the catheters from damage.

Nephrectomy

Nephrectomy was performed through flank incisions. Through these the kidney was isolated and cleaned of perinephric fat. The renal vessels were exposed and clamped as near to the kidney as possible. A ligature was then applied below the clamp and the kidney cut away. The clamp was then removed and the incision closed.

Measurement Of Blood Pressure

The catheter in the carotid artery was connected to a statham P23 gb strain gauge transducer (Stagg Instruments, Henley-on-Thames, U.K.) via a metal tubing adapter (Clay Adams, New Jersey, U.S.A.) and a plastic three-way tap (Vygon, Cirencester, U.K.). A continuous recording of blood pressure was made using a Grass polygraph recorder (Grass Instruments Co., Quincy, Mass., U.S.A.) to which the transducer was linked. A continuous recording of both systolic and diastolic blood pressures was thus obtained. The patency of the arterial line was maintained by a slow infusion of dextrose (50g/litre) containing 5 percent heparin (10 iu/ml). The solution was infused through a side arm at a rate of 0.125 ml/h using a Braun perfusor pump (Melsungen, W. Germany). Because the infusion of dextrose may damp the true blood pressure reading, comparisons were made between the mean arterial pressures.

Collection Of Samples

Plasma

In the majority of cases, blood for measurement of plasma renin concentration was collected directly into a pre-cooled tube

from the carotid catheter. Where this was not possible (for example, the normal uncannulated controls) blood was obtained by cardiac puncture using a pre-cooled plastic syringe after cervical dislocation. By either method the blood (approximate volume, 1 ml) was mixed with 100 µl of a 10 percent solution of potassium EDTA as it was collected. The blood was immediately spun at 2,000g for seven minutes at 4° C and the plasma so obtained was then stored at -20° C before assay.

Aorta

Either before or immediately after collecting the blood sample the animals were stunned by a blow to the head and killed by cervical dislocation. The thoracic and abdominal contents were exposed through a mid-line ventral incision. The aorta was then exposed and clamped just above the bifurcation. The entire length of the vessel was dissected free up to and including the ascending aorta. It was then cut lengthwise, cleaned of adhering fat and connective tissue and stored at -20° C before use.

Experimental Protocol

The catheters were inserted in all rats on the afternoon of the first day. Bilateral nephrectomy, if performed, was carried out at the same time. The animals were then placed in a perspex cage and allowed to recover overnight for a minimum of fifteen hours. Once in the cage the animals had free access to water but not food.

Continuous blood pressure recording was started on the morning of the second day. At various times after the start of the recording, when a steady base-line recording had been obtained, renin injections were given. Animals received their injections at different times so that they were all killed twenty-four hours

after bilateral nephrectomy. Aortic wall and plasma renin concentrations were measured in four separate groups of rats either one, three, six or nine hours after renin injection. In the group of rats killed one hour after injection an extra blood sample was obtained ten minutes after injection. In order to do this the carotid catheter was cut and then rejoined with a short length of tubing after collection of 1 ml of plasma. The volume was replaced with 1 ml of dextrose.

Controls

Bilateral nephrectomy control rats received an injection of dextrose instead of renin. Continuous blood pressure was monitored in one group over the nine hour period of day two. In another group aortic wall and plasma renin concentrations were measured at time zero on day two, that is, fifteen hours after bilateral nephrectomy.

Normal control rats were treated in a similar way. Continuous blood pressure was monitored over the nine hours of day two in one group. Aortic wall and plasma renin concentrations were also measured twenty-four hours after cannulation.

Aortic wall and plasma renin concentrations were also measured in rats which had not been cannulated.

Uptake Of ^{125}I-Labelled Albumen With and Without The Pressor Response

Rats were cannulated and nephrectomised in pairs on the afternoon of the first day. On the second day both received an injection of 100 μl of ^{125}I-labelled albumen followed immediately by an injection of renin in one rat and dextrose in the other. A blood sample was collected and the aorta removed from each rat three hours after the injection.

The aorta was thoroughly washed four times in saline, split lengthwise and counted for ten minutes. Samples of 100 μl plasma

were counted for five minutes. The activity measured in the aorta was corrected for plasma contamination using the average value of 2.29 µl of plasma, obtained as described in Chapter three. The number of counts in the aorta was thus corrected for the counts due to 2.29 µl of the corresponding plasma sample and finally expressed as cpm/100 mg of aortic tissue.

Results

All results are expressed as the arithmetic mean value ± the standard error of the mean.

Statistical comparisons of blood pressure were made using either paired or unpaired students 't' tests as appropriate. However, neither plasma nor aortic renin concentrations are normally distributed and statistical comparisons involving these values were made using the Wilcoxon test for two independent samples. Values of plasma and aortic renin concentrations were also transformed to logarithms for comparison with the corresponding pressor responses.

Regression analysis was performed by the method of least squares using logarithmic values of plasma and aortic renin concentrations.

The mean direct arterial blood pressure was calculated from the diastolic pressure plus one third of the pulse pressure (that is, the difference between the systolic and diastolic pressures) obtained from the continuous trace.

Normal Controls

The base-line blood pressure was measured continuously in normal cannulated rats over the nine hour experimental period of day two. The blood pressure did not change significantly during this time, as is shown in figure 4.

The initial blood pressure at time zero was 116.1 ± 2.5 mmHg ($n = 8$).

The plasma renin concentration for normal rats killed by stunning was 66.0 ± 14.3 ngAI/ml/h ($n = 10$). The aortic wall renin concentration in the same group of rats was 0.23 ± 0.05 ngAI/100mg/h ($n = 10$). The plasma renin concentration measured in a

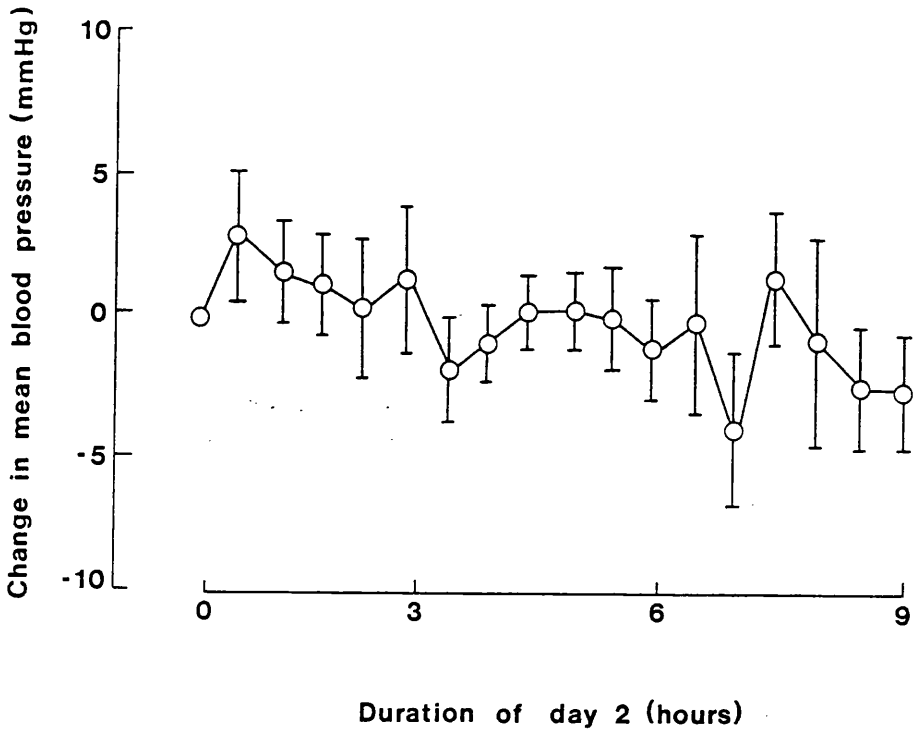

Figure 4. The change in mean blood pressure during the nine hour experimental period of day two in a single group of normal rats. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

separate group of normal rats twenty-four hours after cannulation was 21.4 ± 3.0 ngAI/ml/h (n = 10). This was significantly lower than that obtained from the previous group in which blood was obtained by cardiac puncture after stunning rather than from conscious rats through the carotid cannula ($p < 0.01$). The difference between these two values may reflect the differences in the methods of collection of the samples. However, the aortic wall renin concentration for this second control group was 0.20 ± 0.05 ngAI/100mg/h (n = 10) which was not significantly different from that obtained for the first group.

Bilateral Nephrectomy Controls

As with the normal controls, the blood pressure measured in a single group of eight nephrectomised rats over the nine hour experimental period of day two did not show any consistent or significant change. This is illustrated in figure 5.

The initial blood pressure at time zero was 120.7 ± 3.3 mmHg (n = 8). For this group, time zero was equivalent to fifteen hours after bilateral nephrectomy.

In a separate group of rats eighteen hours after bilateral nephrectomy, the plasma renin concentration was 4.9 ± 1.7 ngAI/ml/h (n = 10). This was significantly lower than normal when compared to both the intact ($p < 0.001$) and cannulated ($p < 0.01$) normal rats.

The aortic wall renin concentration in this group was 0.13 ± 0.04 ngAI/100mg/h (n = 10). Although this figure was lower than those obtained for the two normal groups (0.23 ± 0.05 and 0.20 ± 0.05) the difference was not significant.

Renin Injection After Bilateral Nephrectomy

Blood Pressure

The effect of a single injection of 0.6 Goldblatt units of

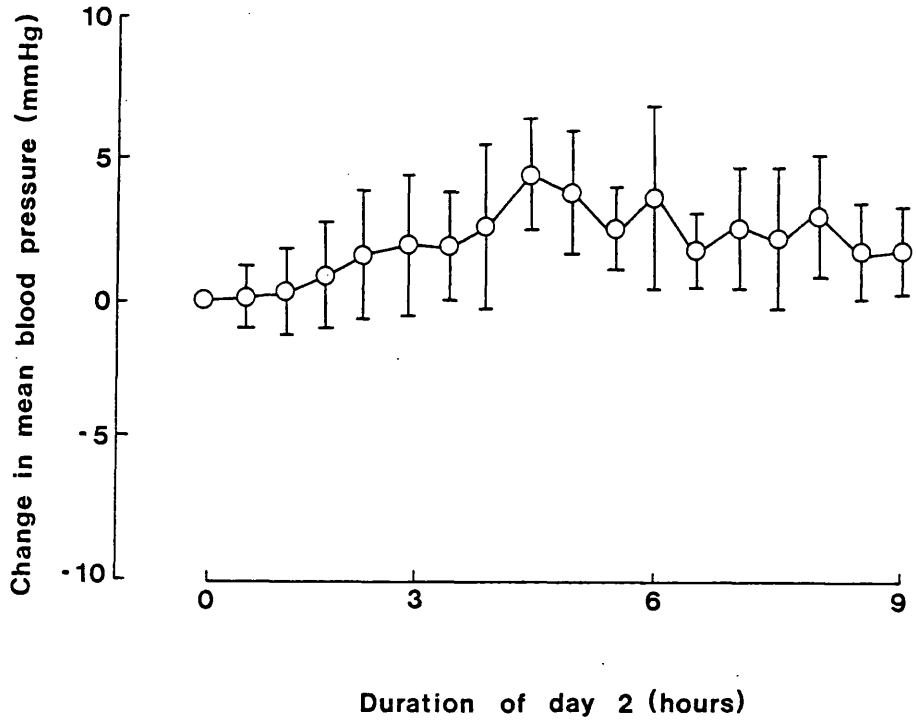

Figure 5. The change in mean blood pressure during the nine hour experimental period of day two in a single group of nephrectomised rats. The experimental period began fifteen hours after bilateral nephrectomy. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

rat renin, given at time zero, to rats fifteen hours after bilateral nephrectomy, is shown in figure 6. The blood pressure was measured continuously over nine hours after the renin injection.

The injection produced an immediate rise in blood pressure, reaching a peak at between one and five minutes after injection. The maximum pressor response was +44.7 $\pm$ 3.3 mmHg ($n = 10$). After this time the blood pressure fell, reaching a plateau of elevation between +20 and +30 mmHg which was maintained for up to five hours after injection. The curve appeared to be biphasic in nature with a second, smaller peak between two and four hours after injection reaching a maximum of +27.0 $\pm$ 2.4 mmHg at three hours. The blood pressure returned slowly to the baseline (that is, pre-injection) level over the next six hours, remaining significantly elevated at six hours after injection at +11.1 $\pm$ 3.5 mmHg ($p < 0.05$). The blood pressure had returned to a level not significantly different from the pre-injection value by nine hours (+3.1 $\pm$ 3.6 mmHg).

In addition to the continuous blood pressure recording obtained from this single group of rats over the entire nine hour period, the blood pressure was also measured in four separate groups of rats at one ($n = 9$), three ($n = 10$), six ($n = 10$) and nine ($n = 9$) hours after renin injection. These readings were taken to coincide with simultaneous measurements of plasma and aortic wall renin concentrations.

The results of the measurements made in these four separate groups are shown in figure 7.

The blood pressure response was expressed as the change in blood pressure compared to the individual pre-injection values. The results for the separate groups were +24.8 $\pm$ 8.2 (one hour),

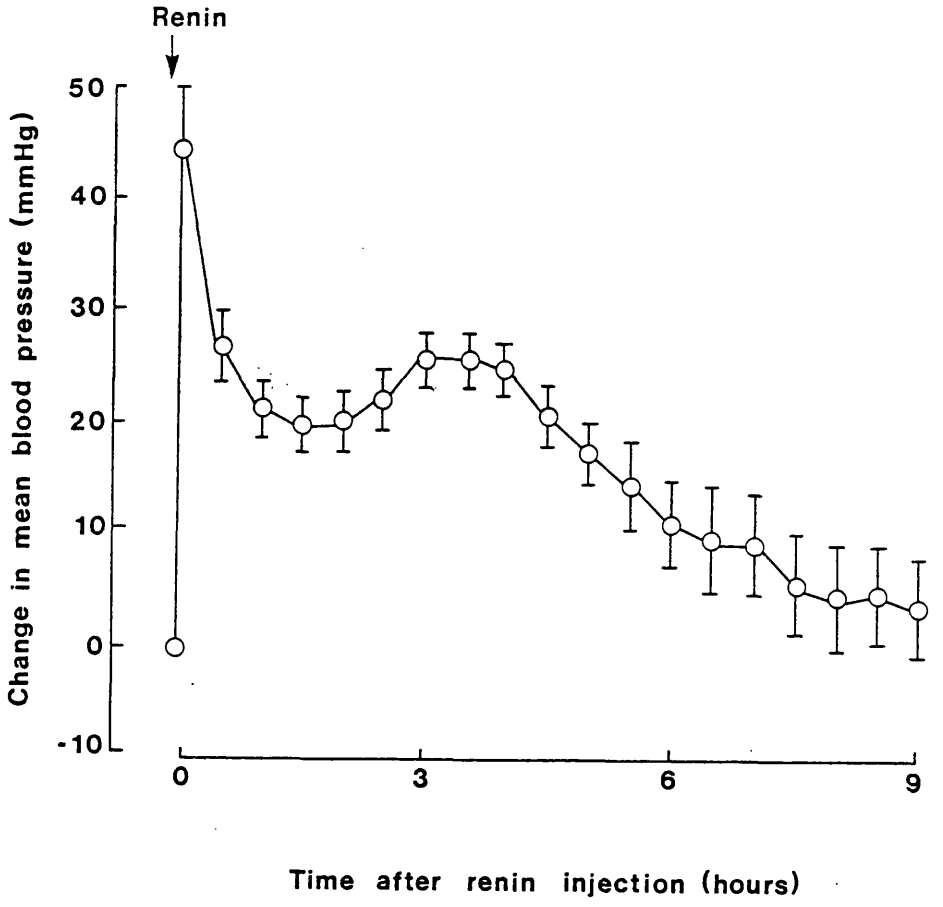

Figure 6. The change in mean blood pressure during the nine hour experimental period of day two in a single group of nephrectomised rats after an injection of 0.6 Goldblatt units of rat renin given at time zero. The injections were given fifteen hours after bilateral nephrectomy. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

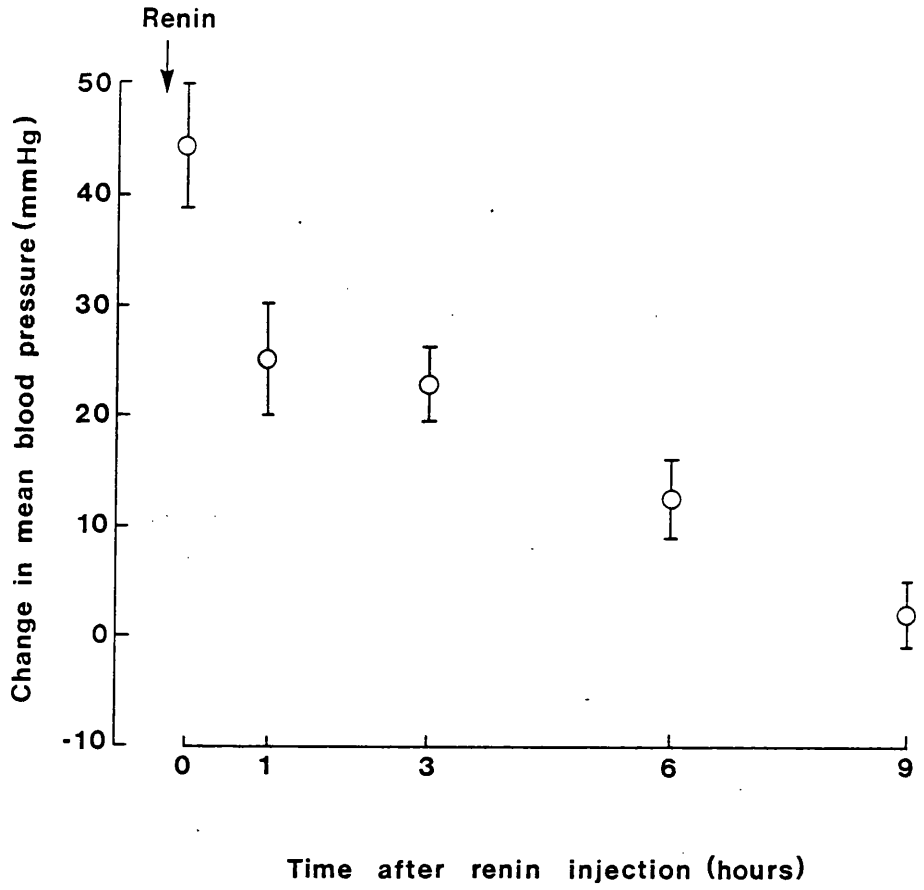

Figure 7. The change in mean blood pressure during the nine hour experimental period of day two in nephrectomised rats after an injection of 0.6 Goldblatt units of rat renin given at time zero. Measurements were made on separate groups of rats at ten minutes and one, three, six and nine hours after renin injection to coincide with measurements of plasma and aortic wall renin concentrations made at the same times. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

+23.1 ± 2.6 (three hours), +12.7 ± 2.6 (six hours) and +1.5 ± 3.4 (nine hours) mmHg. The blood pressure elevation was significantly higher than the pre-injection level for each group at one, three and six hours ($p < 0.01$, 0.01 and 0.05 respectively). By nine hours the blood pressure was not significantly raised compared to pre-injection levels.

Using these results the half life of the blood pressure response was calculated at 4.67 hours.

The blood pressure response to the renin injection was obtained by comparing the readings of blood pressure taken before and after the renin injection was given in each individual animal. However, since the aortic wall renin concentration can only be measured once in each rat, the levels of renin after injection in both the plasma and aortic wall were compared to a separate control group.

Plasma Renin Concentration

The level of circulating renin was initially very high being 1,115.2 ± 159.4 ngAI/ml/h ten minutes after the injection. By one hour after the renin injection the level had fallen to 297.1 ± 22.0 ngAI/ml/h and after three hours the level was 46.4 ± 7.7 ngAI/ml/h. At this time the value was still significantly elevated compared to the cannulated normal control group ($p < 0.01$). However, by six hours the plasma renin concentration was 19.4 ± 6.0 ngAI/ml/h which was not significantly different from the level measured in cannulated normal rats. At nine hours after injection circulating renin had returned to the pre-injection level of 4.9 ± 0.8 ngAI/ml/h which was significantly lower than the cannulated normal control group ($p < 0.01$).

The plasma renin concentration after renin injection is illustrated in figure 8. The half life of circulating exogenous

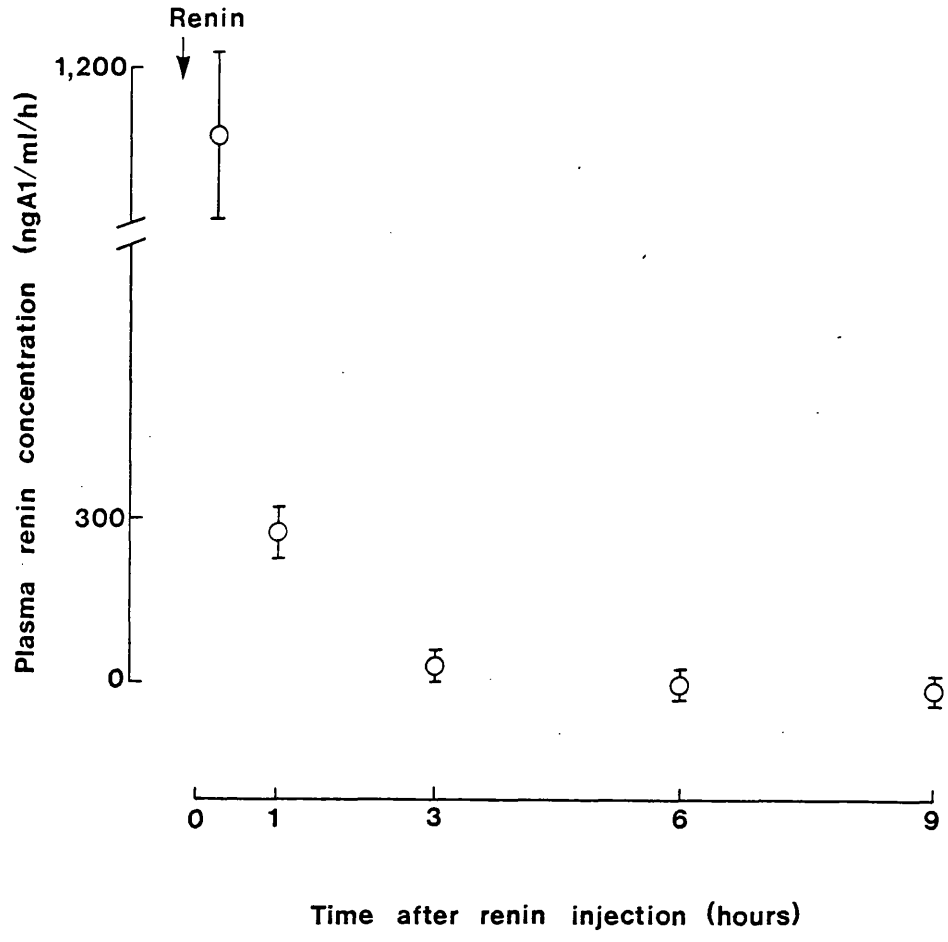

Figure 8. The plasma renin concentration measured in separate groups of nephrectomised rats after an injection of 0.6 Goldblatt units of rat renin. The measurements were made at ten minutes and one, three, six and nine hours after renin injection. The plasma renin concentration is expressed as the rate of generation of angiotensin I from rat renin substrate/ml of plasma/hour of incubation at 37° C.

renin was calculated as one hour.

The values obtained for the individual plasma renin concentrations were transformed into logarithms and plotted against the corresponding pressor response to exogenous renin in each rat. There was a significant positive correlation between these two measurements ($r = 0.671$, $p < 0.001$). This is illustrated in figure 9. However, the relationship is not statistically valid. This point is discussed on page 106.

Aortic Wall Renin Concentration

In contrast to the rate of disappearance of renin from the plasma, the aortic wall renin concentration measured in the same group of rats remained significantly elevated compared to the cannulated normal control group for up to six hours after renin injection. This is shown in figure 10. The values obtained at one, three and six hours were 1.48 ± 0.29, 0.61 ± 0.15 and 0.65 ± 0.21 ngAI/100mg/h respectively ($p < 0.01$, 0.01 and 0.05 compared to normal controls). By nine hours after injection the aortic wall renin concentration was 0.31 ± 0.13 ngAI/100mg/h. This was not significantly different from the normal control group.

Using the average correction factor of 2.29 μl of plasma/100mg of aortic tissue to correct each value of aortic wall renin concentration for contaminating plasma, the corrected values were 0.87, 0.51, 0.60 and 0.29 ngAI/100mg/h at one, three, six and nine hours respectively. Values for the standard error of the mean were not given because although corrections were made for each individual aortic sample using the corresponding plasma renin concentration, the corrections were obtained using an average correction factor. The values are plotted out in figure 11.

A relatively large proportion (47 percent) of the renin present

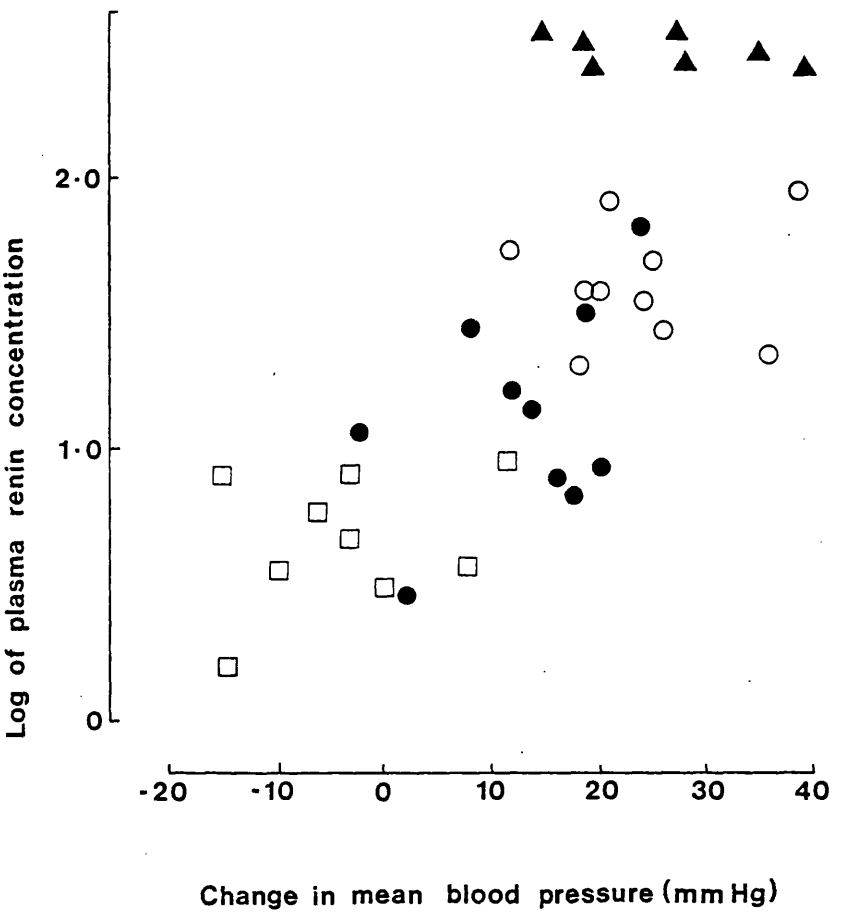

Figure 9. The relationship between the log of the plasma renin concentration and the corresponding pressor response, measured in nephrectomised rats, to an injection of 0.6 Goldblatt units of rat renin. The values given are those obtained for each individual rat from measurements made at one (▲), three (○), six (●) and nine (□) hours after renin injection.

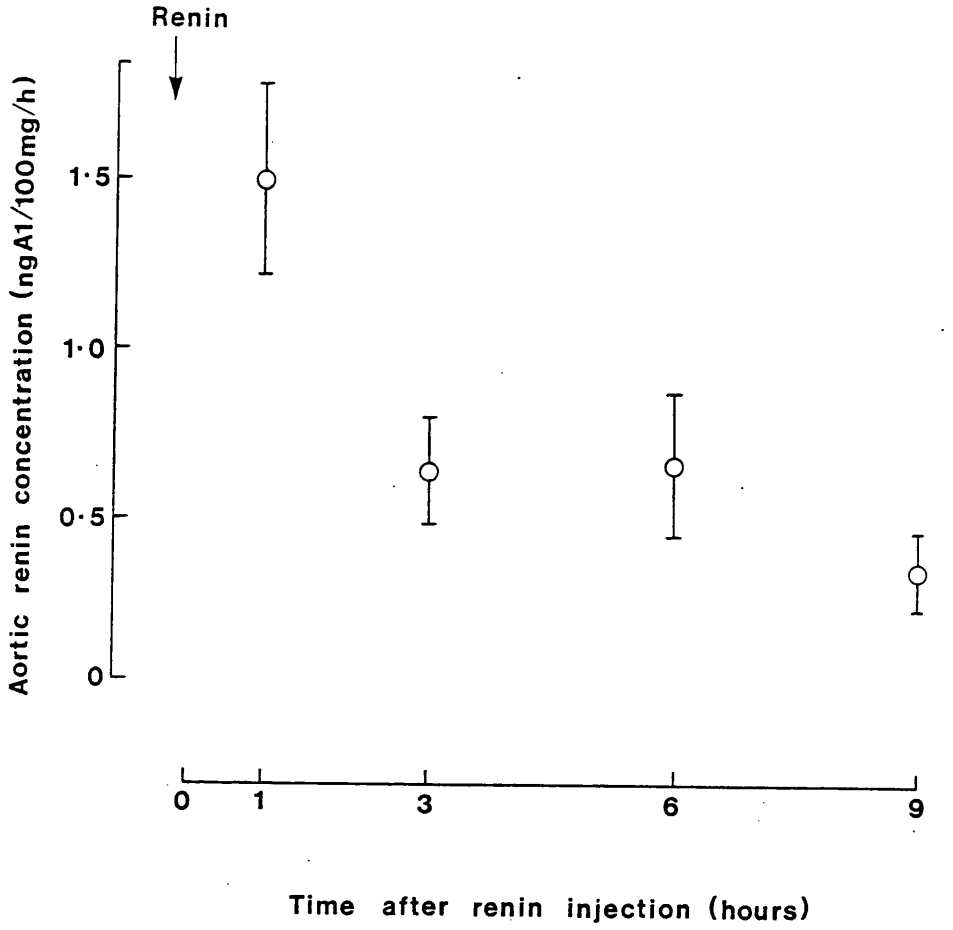

Figure 10. The aortic wall renin concentration measured in separate groups of nephrectomised rats after an injection of 0.6 Goldblatt units of rat renin. The measurements were made at one, three, six and nine hours after renin injection. The aortic wall renin concentration is expressed as the rate of generation of angiotensin I from rat renin substrate/100 mg of aortic tissue/hour of incubation at 37° C.

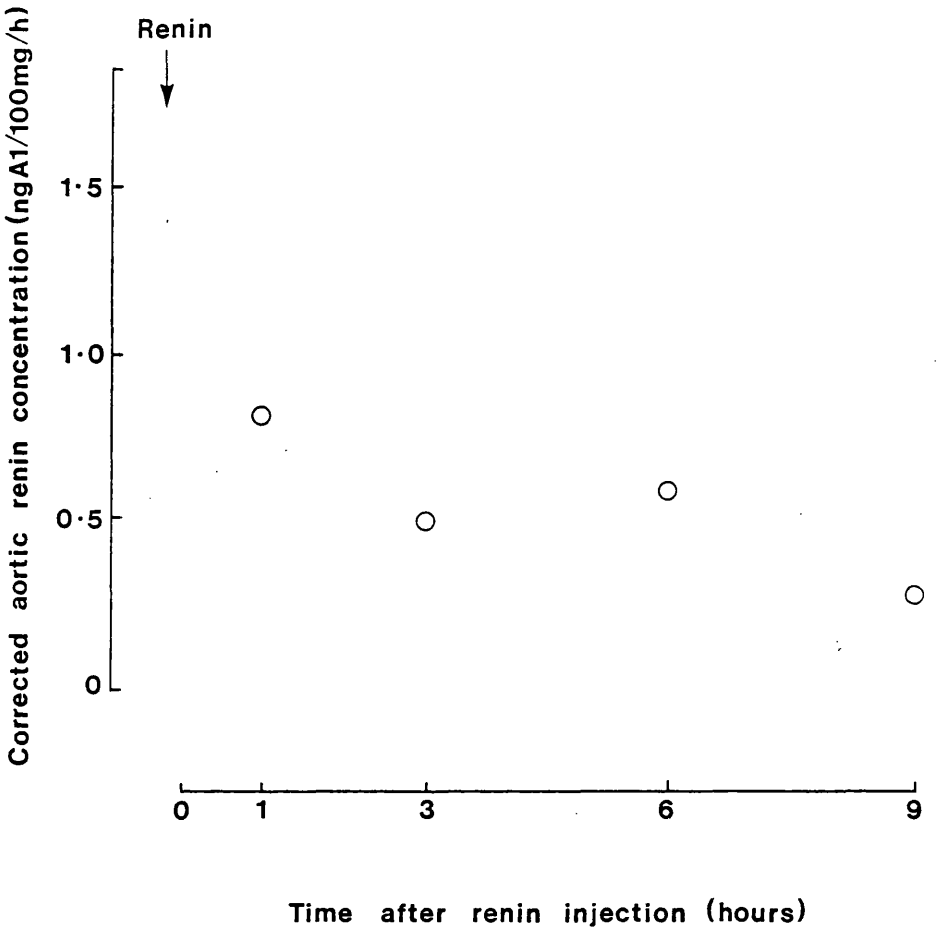

Figure 11. The aortic wall renin concentration, illustrated in figure 10, corrected for plasma contamination.

in aortic homogenates was due to plasma contamination in samples collected one hour after renin injection. This was because the plasma renin concentration was high at this time. At three, six and nine hours after injection, when the plasma renin concentration was lower, the proportion of activity due to plasma in the aortic homogenates was very much less, being 20, 4.2 and 6.6 percent respectively.

The corrected values of the aortic wall renin concentrations were used in the calculation of the half-life of exogenous renin in the vascular tissue. This was 4.83 hours.

The corrected values for the aortic wall renin concentrations in the control rats were also calculated. These were 0.12 ngAI/100mg/h for the group eighteen hours after bilateral nephrectomy, 0.15 ngAI/100mg/h for normal rats twenty-four hours after cannulation and 0.09 ngAI/100mg/h for normal rats which had not been cannulated.

As with the plasma renin concentration measurement, the values obtained for the individual corrected aortic wall renin concentrations were transformed to logarithms and plotted against the corresponding pressor responses to exogenous renin. There was a significant correlation between these two measurements ($r = 0.361$, $p < 0.05$). The results are illustrated in figure 12.

The activity present in the aortic homogenates due to injected ^{125}I-radiolabelled albumen, three hours after the albumen injection, was 58.21 ± 8.18 cpm/100mg of aortic tissue. If a renin injection was given at the same time as the albumen the value was 51.60 ± 11.92 cpm/100mg of aortic tissue. Both of these values are corrected for the counts per minute present in 2.29 µl of plasma for each individual rat. There was no significant difference in the counts present in aortic tissue with or without renin injection.

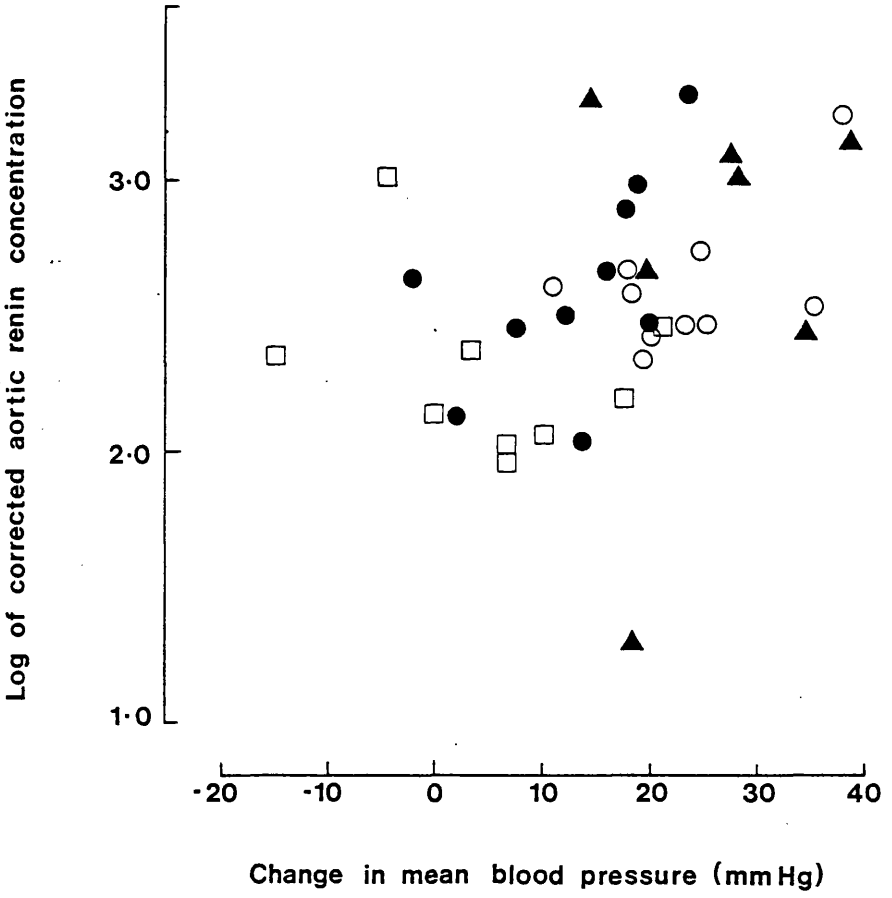

Figure 12. The relationship between the log of the corrected aortic wall renin concentration and the corresponding pressor response, measured in nephrectomised rats, to an injection of 0.6 Goldblatt units of rat renin. The values given are those obtained for each individual rat from measurements made at one (▲), three (○), six (●) and nine (□) hours after renin injection.

Discussion

Although the renin-angiotensin system is a potent pressor system, it has been difficult to establish its role in blood pressure control. Previous studies have used measurements of plasma renin levels as an indicator of the activity of the system. Recently however, it has been suggested that renin present within other tissues, especially within the walls of resistance vessels, may be more relevant to the determination of the blood pressure response in a number of experimental situations (Barrett et al, 1978; Garst et al, 1979; Thurston et al, 1979; Asaad and Antonaccio, 1982).

The present study was designed to allow observations on the separate effects on arterial blood pressure of renin in the circulation and in the blood vessel wall to be made. This was achieved by following the fate of injected renin in rats in which the kidneys had been removed.

The injection of renin in this experimental model produced a temporal dissociation between the blood pressure response and the level of circulating renin. Thus, the main feature of the blood pressure response was a large and significant elevation which lasted for more than six hours as illustrated in figure 6. At the same time it was observed that the levels of circulating renin, initially high following the bolus injection, declined rapidly (figure 8). Although the half-life of circulating renin was increased, as has been observed by others (Peters-Haefli, 1971; Boyd, 1979), the pressor response lasted considerably longer than the plasma renin persisted.

Boyd (1979) has suggested that the blood pressure response to renin in nephrectomised rats is the result of a combination of

factors. He found that as well as an increase in the half-life of renin, both the threshold and the slope of the response to injected angiotensin I were decreased after bilateral nephrectomy. He suggested that the change in the blood pressure response in this situation was the result of both an increase in the responsiveness to angiotensin together with a reduction in the rate of clearance of renin from the plasma. In addition he also postulated that there was a change in the rate of breakdown of both angiotensin I and angiotensin II after bilateral nephrectomy. However, this may not have a significant effect since the half-lives of these two substances are normally very short. Whilst these changes may contribute to the prolongation of the pressor response it is unlikely that they could maintain it for as long as the six hours observed in these experiments.

Bing and Nielsen (1973) suggested that the blood pressure response to exogenous renin could be attributed to the presence of renin within the resistance vessel walls persisting after circulating renin had disappeared. In the present study the level of renin at this site was measured. Renin activity was measured in aortic homogenates of rats at various times after the injection of renal renin. It was found that the activity in the wall of this vessel was significantly increased for six hours after the injection. The levels of renin measured in the aortic tissue after renin injection are illustrated in figure 10. This persistence of renin activity was the same as the duration of the pressor response. The close temporal relationship was thus consistent with the hypothesis that the level of renin present in the blood vessel wall was indeed an important factor in determining the pressor response. However, this conclusion is based on the assumption that the renin activity

measured in the wall of the aorta is correlated with the level of this activity in resistance vessels.

It has been argued that the generation of angiotensin II by vascular wall renin activity is most relevant in the walls of the resistance vessels. In the rabbit uterus the highest concentration of renin activity was present in the walls of the smallest arterioles and venules (Eskildsen, 1972). Since the aorta itself is not considered to be a resistance vessel it may be that renin activity measured in aortic homogenates is not representative of levels within other, smaller vessels. However, it has been shown that, in the hog aortic wall at least, renin-like activity is located primarily within the adventitia and outer media which is the site of the vasa vasorum. If this is so it may be assumed that the renin activity measured in aortic homogenates is related to levels within resistance vessels. In this study the aorta was used because it was the largest vessel and therefore the most easily isolated. This was especially important in an animal as small as the rat. It was felt that in its present form the assay technique employed here could not be used to measure lower concentrations than those present in the aortic wall. To use a smaller, though possibly more relevant vessel, might introduce unacceptable inaccuracies.

The relationship between the renin activity in the aortic wall and the pressor response to the renin injection is shown in figure 12. In this figure the log of the renin concentration is plotted against the change in blood pressure. There was a positive correlation between the two variables. However, there was also a positive correlation between the blood pressure response and the levels of circulating renin as illustrated in figure 9. This observation can be clarified by subdivision of the results according

to the length of time after the renin injection at which the measurements were made. When this was done the distinction between the two relationships became apparent. The initially high level of renin in the plasma immediately after injection fell rapidly, resulting in four distinct groups of readings over the nine hour period. Subdivision of these results, as illustrated in the respective figures, thus showed that the statistical significance of the relationship between the change in blood pressure and the level of circulating renin was not valid. On the other hand, no such distinct groups were obtained by subdividing the results obtained from the measurements of renin activity in the aortic homogenates.

The importance of the renin activity measured in the aortic wall as a determinant of the pressor response was most clearly illustrated by the measurements made at six hours after the renin injection. At this time circulating renin had fallen to a level which was not significantly different from that measured in normal control rats. Despite this there was still a significant elevation of blood pressure. At the same time the level of renin activity present in the aortic wall was significantly higher than that present in the aortae of normal control rats.

Further confirmation of the relationship between the blood pressure response and the aortic wall renin concentration in the model studied was obtained from a calculation of the half-lives of the pressor response and the persistence of renin activity in the aortic wall. These were very similar, being 4.67 and 4.83 respectively, whereas the half-life of circulating renin was only one hour. These half-lives should however, only be regarded as illustrative approximations since there was insufficient data for detailed analysis.

It was a disadvantage of the experimental model used here that it involved several surgical manipulations including removal of both kidneys. This might have altered circulatory responses.

Particular difficulties arise when considering the action of angiotensin II in this model. It has been argued that the level of renin activity in the blood vessel wall is the key determinant of the pressor response to the injected renin. However, such renin acts indirectly via the production of angiotensin II which is the actual pressor substance and the final chemical effector of the system. In the experimental model used here, any change in the number, occupancy or affinity of the angiotensin II receptors would influence the pressor response consequent upon the injection of renin.

It has been suggested that changes in angiotensin II receptor number and affinity occur after bilateral nephrectomy. Chevillotte et al (1975) found an increase in the number of receptors present in the rabbit uterus after bilateral nephrectomy. However, this change was detected long after the maximum response to injected angiotensin II had been found to occur. Brunner et al (1972) showed an apparent change in the affinity of vascular angiotensin II receptors after bilateral nephrectomy. Similarly, Boyd (1979) observed an increase in the sensitivity to angiotensin in this situation. Swales et al (1975) have suggested an alternative mechanism for the apparent increase in sensitivity to administered angiotensin. This theory states that the response to angiotensin II depends on the number of receptors available to bind angiotensin II. This in turn will be determined by the level of endogenous angiotensin II. When it is low, for example after bilateral nephrectomy or salt loading, then the response to injected angiotensin II is greater. Thurston and Laragh (1975) found that the differences in

response to a standard dose of angiotensin II in salt depleted and salt loaded and nephrectomised rats were abolished by an infusion of the converting enzyme inhibitor, teprotide. This result supports the view that a physical alteration of the receptors has not occured in these models.

A change in the sensitivity of the animal after bilateral nephrectomy because of a change in either the number or the affinity of angiotensin II receptors does not appear to be the explanation for the loss in the relationship between circulating renin and the blood pressure observed in the experiments described in this chapter. However, this issue will only finally be clarified by a more precise definition of vascular angiotensin II receptors in this situation.

Alternatively, the initially high concentrations of angiotensin II following the renin injection may result in other physiological responses which bring about an alteration in the response to angiotensin II. This has been shown to occur as a result of prolonged infusions of subpressor doses of angiotensin II (Brown et al, 1981). However, the time scale over which the changes involved in this response take place is a matter of days or weeks rather than hours. It would therefore seem unlikely that this would be relevant in the experiments considered here.

High concentrations of angiotensin II have been shown to alter the permeability of the walls of large blood vessels (Giese, 1973; Robertson and Khairallah, 1972). However, the results obtained in this study suggest that the permeability of the aorta, to renin at least, was not altered by the high levels of circulating angiotensin II.

Although renin activity is present in the walls of blood

vessels, the origin of this activity is controversial. If the renin activity in blood vessel walls were entirely of renal origin, then it should disappear after removal of the kidneys. Several authors have demonstrated a decline in the level of aortic wall renin activity after bilateral nephrectomy (Rosenthal et al, 1969; Thurston et al, 1979). However, others have found that the level of renin activity in vascular tissue remains unchanged, irrespective of whether renal renin is present (Basso and Taquini, 1971; Barrett et al, 1978).

The results obtained in this study demonstrated that renin of renal origin, present in the circulation, can gain access to the wall of the aorta. This supports the view that under normal conditions some, at least, of the renin activity present in the blood vessel wall is derived from the circulation. However, the fact that circulating renin can enter vascular tissue does not rule out the possibility that some of the renin activity in the aortic wall was synthesised by the tissue itself. Of particular interest in this respect was a comparison of the levels of renin activity present in the aortic homogenates of the control groups after correction of the results for plasma contamination.

The aortic wall renin concentration was lower eighteen hours after bilateral nephrectomy than it was in either of the two normal control groups. However, when all the values obtained had been corrected for plasma contamination, this difference disappeared. The initial measurements suggest that the aortic wall renin is of renal origin whereas the corrected results favour the view that bilateral nephrectomy does not influence the level of renin in vascular tissue.

It was not possible to draw firm conclusions from these

particular results. Only one measurement was made after bilateral nephrectomy and the level of renin activity may have declined after this time. In addition, the differences between the values obtained for all these groups, whether corrected or not, were not statistically significant. When the levels of renin were very low, as they were in these groups, the concentrations were at the lower limit of the sensitivity of the renin assay and it was therefore not possible to detect the small differences between them accurately. If there was any local synthesis of renin in the aortic wall, the assay system used here was not sensitive enough to measure such low levels. However, the possibility that renin may have been concentrated at strategic sites should not be excluded.

The results obtained in this study do not support the view of Fordis et al (1983) that the presence of renin-like activity in the aortic wall is entirely due to contamination by plasma renin. It is true that there was a certain degree of contamination, probably due to plasma remaining within the smaller vessels associated with the aortic wall which could not be removed by the washing process. The method used for estimating this proportion of activity has been discussed in Chapter three. However, even when a correction was made for this contamination there was still a significant proportion of renin-like activity which was derived from the aortic tissue itself. These results highlight the importance of distinguishing between plasma contamination and plasma-derived activity when studying the renin activity in vascular tissue. The former is the renin activity which is due to plasma renin which has not been washed away before homogenisation of the samples. The latter is due to the activity of renin which has left the plasma and entered the tissue during the course of the <u>in vivo</u> experiment. When studying

the significance of renin-like activity in the aortic wall it is necessary to take these factors into account.

Although the experiments reported here demonstrated that renin can leave the circulation and enter the wall of the aorta, a number of questions remain to be answered. Firstly, how did the circulating renin gain access to the tissue? Secondly, once inside the tissue, was it free within the extracellular space or could it be bound to specific receptors?

In general, the distribution of a molecular species such as renin between the plasma and the tissue fluid is determined by a number of factors. These include the surface area and the permeability of the vascular endothelium, the concentration gradient between the plasma and the tissue and the size (Stokes radius) of the molecule concerned (Renkin, 1964). If renin is present in the plasma at a very high concentration, as it was immediately after the renin injection, then it would be expected to enter the tissue fluid compartment at a relatively rapid rate. Thus, the simplest and therefore the most likely method of entry into the tissue is by passive diffusion down the steep concentration gradient.

A process of passive diffusion of renin between the plasma and the aortic tissue which was dependent on the concentration gradient, may have contributed to the prolongation of the half-life of renin within the aortic wall.

It was observed in this study that once the renin had entered the aortic tissue it persisted at this site for considerably longer compared to the rate at which it disappeared from the plasma. This marked difference in the observed half-lives indicates that the rate of metabolism of renin in the two compartments was different. This might be expected since circulating renin passes through the liver, an organ which has been shown to be specifically involved

in the degradation of renin (Peach, 1977). On the other hand, the renin present within the tissues was not available for breakdown at this site. In addition, destruction of renin within the blood vessel wall probably occurred at a slower rate than the same process within the liver.

With increasing time after the injection of exogenous renin into the circulation, the rate of passive diffusion of renin out of the circulation and into the tissue will become proportionately less as the concentration gradient becomes less steep. In the model of bilateral nephrectomy used here, as the level of renin in the plasma steadily declined, this concentration gradient tended to disappear and may have reversed. At this time renin from the tissue could then have diffused back into the circulation. However, this would probably only have occurred in the later stages of the experiment.

The persistence of renin within the aortic wall observed in this study thus appeared to be the result of a combination of two factors. Firstly, that there was no net diffusion of renin out of the tissue and back into the circulation until the later stages of the experiment. Secondly, that the renin present in the tissue was broken down at a relatively slow rate compared to the clearance of renin from the plasma.

It is possible that the half-life of renin within the aortic wall was prolonged as a consequence of its being bound to specific receptors. This has been found to be the mechanism of action for a number of hormones and in many respects renin itself can be considered to fit into this category. It has a regulatory function in the maintenance of physiological homeostasis, is synthesised in large amounts by a particular organ, namely the kidney, and is

transported to target organs, such as the heart and blood vessels, in the circulation. However, in other respects it does not fit the definition of a true hormone. It is possibly synthesised in other tissues besides the kidney, and it acts through the production of angiotensin II, itself considered to be a hormone, which is generated within the circulation as well as within the target tissues. Despite this the suggestion that renin might act as a hormone may provide an indication to its mechanism of action within vascular tissue. Thus the possibility that it becomes bound to specific receptors within vascular tissue must be considered. Such a receptor might act as a catalyst, increasing the efficiency with which angiotensin II is generated at particular sites within the tissue or it might allow angiotensin II to be produced inside cells without renin itself entering the cell. Angiotensin converting enzyme has been shown to be bound to the plasma membrane of renal epithelial and vascular endothelial cells and is thought to act as a transmembrane peptidase (Erdös and Gafford, 1983) although to date there is no evidence that renin substrate is present at this site. In addition, angiotensin II binding sites have been detected inside cells (Robertson and Khairallah, 1971; Goodfriend et al, 1972). However, despite these findings the existence of a membrane-bound mechanism for the generation of angiotensin II within the tissues is far from certain.

The precise mechanism of action of renin within the blood vessel wall in the control of blood pressure is not known. However, the results obtained from the experimental model described in this chapter support the view that the activity of a renin-angiotensin system at this site, rather than in the circulation, is more important in the control of blood pressure under both normal and pathological conditions.

CHAPTER FIVE

The Effects of Renin Injection After Bilateral Nephrectomy: The Response to Inhibition by Saralasin and Captopril.

Introduction

The experiments described in this chapter were carried out to assess the role of the renin-angiotensin system in the model described in the preceding chapter. This was achieved by pharmacological blockade using the inhibitors saralasin and captopril.

Inhibitors of the Renin-Angiotensin System

One of the methods for studying the effects of any endogenously produced substance within the body is to observe the effect of the removal of the organ from which it originates. However, a study of the function of the renin-angiotensin system by removal of the major source of renin is complicated by the loss of the many other functions associated with the kidney. The development of inhibitors of the renin-angiotensin system has therefore been of vital importance both in reaching an understanding of the physiological role of this system and in investigating the biochemical properties of its components. In addition, some of the inhibitors have been shown to have therapeutic value in the treatment of hypertension.

A large number of naturally occuring and synthetic inhibitors have been developed which directly inhibit the components of the renin-angiotensin system or interfere with its effects.

Inhibitors of Renin

Inhibitors of renin itself include substrate analogues, protease inhibitors such as pepstatin, phospholipids and renin antibodies.

The substrate analogues have received considerable attention recently. These substances are modified peptides which are based on the octapeptide His^{6}-Tyr^{13} of the N-terminal sequence of renin substrate and they act as competitive inhibitors of renin.

Szelke, Leckie, Tree, Brown, Grant, Hallett, Hughes, Jones and Lever (1982) have shown that reduction of the leucyl^{10}-leucine^{11} peptide bond in the six to thirteen octapeptide sequence of equine renin substrate produced potent and selective inhibitors of canine renin. The most potent of these was known as H77 and was found to be extremely effective as an inhibitor of renin both *in vivo* and *in vitro*. The substrate specificity of renin is illustrated by the fact that these analogues show varying degrees of effectiveness towards renin from different species. Thus H77 can also inhibit rat renin but it is less potent in this animal.

Using the same techniques, an inhibitor of human renin, H142, has also been developed (Szelke, Leckie, Hallett, Jones, Sueiras, Atrash and Lever, 1982). Reduction of the leucyl^{10}-valine^{11} bond of the N-terminal peptide of human angiotensinogen together with the addition of proline and lysine to the N- and C-terminals respectively has resulted in a decapeptide which is the most potent inhibitor of renin so far produced. It is highly specific for renin, being an extremely poor inhibitor of both cathepsin D and renal acid protease activity. H142 has only been administered intravenously (Brown et al, 1983) but it is potentially of great value in the treatment of hypertension.

The problem of non-specific inhibition is encountered with protease inhibitors such as pepstatin. This substance is an acyl-pentapeptide-like compound isolated from cultures of certain species of streptomyces. It is a potent inhibitor of renin both *in vitro* and *in vivo* (Gross, Lazar and Orth, 1972; Miller, Poper, Wilson and De Vito, 1972) but also inhibits other enzymes which cleave renin substrate. In addition to the problem of its general inhibition of acid protease activity it has low solubility *in vivo*.

However, it has been useful in improving techniques of renin purification and has been used to obtain pure renin from human (Yokosawa et al, 1980), hog (Murakami and Inagami, 1975; Corvol et al, 1977), rat (Matoba et al, 1978) and dog (Dzau et al, 1979).

Improvement of the techniques of renin purification have lead to the development of a relatively pure renin which can be used as an antigen in the production of renin-specific antibodies. These have been shown to be effective both in vivo and in vitro as inhibitors of renin activity (Dzau, Kopelman, Barger and Haber, 1980).

In addition to the use of antibodies raised against renin, both passive and active immunisation against angiotensin I and II have been studied. Sustained immunity to angiotensin I (Oates et al, 1974) and angiotensin II (Hedwell, 1968; Bing and Poulsen, 1970; Oates et al, 1974) does not prevent the development or severity of hypertension in the rat. Administration of antibodies to angiotensin II produced a fall in blood pressure but not restoration to normal levels (Brunner et al, 1971; Carretero, Kuk, Piwonska, Houle and Marin-Grez, 1971). Swales (1976) has suggested that the ineffectiveness of immunisation may reflect the inability of the antibodies to gain rapid access to the site of angiotensin generation.

Phospholipids have been used as renin inhibitors. They show a low level of inhibitory activity in vivo but a specific analogue has been found which is capable of lowering blood pressure in renin dependent hypertension and after administration of exogenous renin (Sen, Smeby and Bumpus, 1968). It has also been shown to reduce plasma renin activity in both normotensive and hypertensive rats (Sen, Smeby and Bumpus, 1969). This substance has no inhibitory effect on proteases such as pepsin, papain or trypsin

(Ondetti and Cushman, 1982).

Converting Enzyme Inhibitors

A variety of naturally occuring peptides have been shown to be potent inhibitors of the other enzyme of the renin-angiotensin system, angiotensin converting enzyme (Sander, West and Huggins, 1971). Based on the structure of these oligopeptides, a number of synthetic peptides have been developed. Ondetti, Williams, Sabo, Plusec, Weaver and Kocy (1971) prepared six synthetic peptides possessing inhibitory activity against angiotensin converting enzyme including the nonapeptide known as teprotide (SQ 20881). This has been shown to be a potent inhibitor of angiotensin converting enzyme both in vitro (Bakhle, 1972) and in vivo (Engel, Schaeffer, Gold and Rubin, 1972). A detailed study of the active site of the enzyme and the requirements for its inhibition lead to the preparation of the more potent inhibitor, a peptide known as Captopril (SQ 14225) (Ondetti, Rubin and Cushman, 1977). This peptide has a major advantage over teprotide in being resistant to destruction on oral administration. It has been shown to be a potent antihypertensive agent in vivo in the rat (Ondetti et al, 1977) and has been used as a therapeutic agent (Gavras, Brunner, Turini, Kershaw, Tifft, Cuttelod, Gavras, Vukovich and McKinstry, 1978) as well as being used in animal studies.

Because of the success of captopril the properties of a number of other inhibitors of this type are being investigated. These have been reviewed by Ondetti and Cushman (1982).

Angiotensin II Antagonists

These inhibitors of the renin-angiotensin system act by competitive inhibiton of angiotensin II. Numerous analogues have been synthesised all of which have an alternative to phenylalanine sub-

stituted in position eight of the amino-acid sequence of angiotensin II. The most widely used of these analogue antagonists is saralasin. This molecule has sarcosine substituted in position one and alanine in position eight of the amino-acid sequence of bovine angiotensin II (Sarcosine^{1}-Alanine^{8} - angiotensin II). While replacement of phenylalanine at position eight confers antagonist properties to the molecule, substitution of sarcosine for aspartate at position one potentiates the antagonist properties of the molecule by decreasing its rate of metabolism.

Although most studies have been carried out using saralasin it is less potent than some other analogues, for example sarcosine^{1}-Isoleucine^{8}-angiotensin II (Bumpus and Khosla, 1977). Despite this it has been shown to be very effective in reversing renin dependent hypertension. Thus, it lowers the blood pressure in the early phases of Goldblatt 2-kidney 1 clip hypertension in the rat (Brunner et al, 1971; Bing, Russell, Swales and Thurston, 1981) and after renin injection (Bing and Nielsen, 1973). Such lowering of the blood pressure correlates with the plasma renin concentration (MacDonald et al, 1975; Bing et al, 1981).

Both saralasin and captopril have other actions besides those which directly inhibit the renin-angiotensin system.

As well as being an antagonist of angiotensin II the saralasin molecule has intrinsic agonist properties. Case, Wallace, Keim, Weber, Drayer, White, Sealey and Laragh (1976) suggested that this was why captopril was more effective in lowering blood pressure. But by inhibiting angiotensin converting enzyme, captopril also prevents the breakdown of the vasodepressor substance bradykinin (Ondetti and Cushman, 1982). Thurston and Swales (1978) confirmed that captopril produced a greater lowering of blood pressure than

saralasin when administered to salt depleted rats. Furthermore, captopril produced an additional lowering of blood pressure which had already been reduced by pretreatment with saralasin. Bilateral nephrectomy abolished this additional vasodepressor action of captopril, pointing to the involvement of a renal system in the response. Bradykinin, but not renin infusion was found to restore the response. Thus it was concluded that this effect is due to the bradykinin-potentiating action of converting enzyme inhibition.

Carretero and Scicli (1981) have reported that the acute antihypertensive effect of angiotensin converting enzyme inhibitor in both spontaneously hypertensive rats and in Goldblatt 2-kidney 1 clip hypertensive rats is almost completely blocked by high titre antibodies against kinins. These antibodies have no effect on converting enzyme inhibition in salt depleted normotensive rats. Captopril may therefore be more effective than saralasin because of its additional action on the kallikrein-kinin system.

In addition to its effect on the kallikrein-kinin system, captopril has also been found to interact with prostaglandin metabolism. This may therefore be a further mechanism by which this inhibitor exerts its hypotensive effect since prostaglandins have also been shown to have vasoactive properties. Captopril infusion has been shown to produce an increase in circulating (Vinci, Horowitz, Zusman, Pisano, Catt and Keiser, 1979) and urinary (Abe, Ito, Sato, Haruyama, Sato, Omata, Hiwatari, Sakurai, Imai and Yoshinaga, 1980) prostaglandin E in patients with essential hypertension. Zusman (1984) has shown that in renomedullary interstitial cell culture, captopril stimulates prostaglandin biosynthesis. However, the precise mechanism of interaction is not known. Captopril may stimulate prostaglandin synthesis either directly or through

an increase in the levels of bradykinin (Zusman, 1984).

Although both saralasin and captopril lower blood pressure in experimental hypertension, they do not return it to normal (Thurston and Swales, 1978; Bing et al, 1981). This may be because the reduction in blood pressure resulting from inhibition of the renin-angiotensin system activates other systems which act to compensate for the fall in blood pressure (Thurston and Swales, 1978). Activation of renal mechanisms can be avoided by removal of the kidneys. Administration of inhibitors such as saralasin or captopril will then expose the renin-angiotensin dependent component of blood pressure more precisely.

Bing and Nielsen (1973) showed that saralasin restored the blood pressure to pre-injection levels in nephrectomised rats after renin injection. Thurston and Swales (1978) found no difference between the effects of saralasin and captopril in this model, as described above. It was therefore considered that these two inhibitors would be most useful for investigating the involvement of the renin-angiotensin system in the experimental model described in Chapter four.

Saralasin was given in order to demonstrate the continued involvement of the renin-angiotensin system in the blood pressure response to injected renin throughout the duration of the experiment. However, one drawback to the use of saralasin as an inhibitor of the renin-angiotensin system is that it cross-reacts with the antibody to angiotensin I used in the radioimmunoassay. This prevents the measurement of renin activity when saralasin is used. Only blood pressure measurements could therefore be made in these experiments.

Captopril was used to inhibit both the initial and the

subsequent blood pressure response to renin injection. In this second set of experiments it was therefore possible to determine the effect of the pressor response on the distribution of renin between plasma and the vascular tissue.

The saralasin infusion was given at either three or six hours after the renin injection. Captopril was given immediately before the renin injection followed by a continuous infusion of three hours duration.

Materials and Methods

Saralasin

Two groups of rats were cannulated and bilateral nephectomy was performed as described in Chapter four. On day two both groups received an injection of renin.

An infusion of saralasin (Sar^1-Ala^8-angiotensin II; Beckman, Geneva, Switzerland), made up in heparinised dextrose, was then started two and a half or five and a half hours after the renin injection. The infusion was continued for thirty minutes at a rate of 10 μg/kg/min and was administered through the arterial line in place of the dextrose infusion normally given.

Blood pressure was monitored continuously throughout the experimental period.

Captopril

One group of rats was cannulated and bilateral nephrectomy was performed as described in Chapter four. Each rat received a bolus injection of 500 μg of the converting enzyme inhibitor captopril (E.R. Squibb and Sons Inc., Princetown, USA). Fifteen minutes later the renin injection was given followed immediately by an infusion of captopril (8.3 μg/kg/min at a rate of 0.125 ml/h) for three hours after the renin injection. The infusion was administered through the arterial catheter.

Blood pressure was recorded continuously throughout the experimental period and plasma and aortic wall renin concentrations were measured at the end of the three hour period.

Results

Saralasin

Figure 13 illustrates the effect on the pressor response of an infusion of saralasin given either three or six hours after renin injection.

The changes in blood pressure were compared to the base-line pressures of the two groups before renin was given. They were measured immediately before and thirty minutes after the saralasin infusion. The rise in blood pressure following the renin injection was reduced by saralasin. The increment of increased blood pressure fell from +20.6 $\pm$ 3.3 to +4.2 $\pm$ 2.4 mmHg at three hours and from +18.5 $\pm$ 4.9 to +4.9 $\pm$ 4.4 mmHg at six hours after renin injection.

The fall in blood pressure produced by the saralasin infusion was significant at both times ($p < 0.01$ at three hours and $p < 0.05$ at six hours). Although the blood pressure did not return completely to base-line values after half an hour of the saralasin infusion, the difference between these and the pre-injection values was not significant.

Captopril

Administration of captopril also inhibited the pressor response to the renin injection. This is illustrated in figure 14. This figure compares the response to renin over a three hour period after the injection, with and without an additional infusion of captopril. During captopril inhibition there was a small increase in the blood pressure of +7.6 $\pm$ 1.9 mmHg immediately after the renin injection. This change was not, however, statistically significant. The change in blood pressure after three hours of the captopril infusion was +1.0 $\pm$ 2.3 mmHg compared to +23.1 $\pm$ 2.6 mmHg in a separate group of rats where no captopril was given.

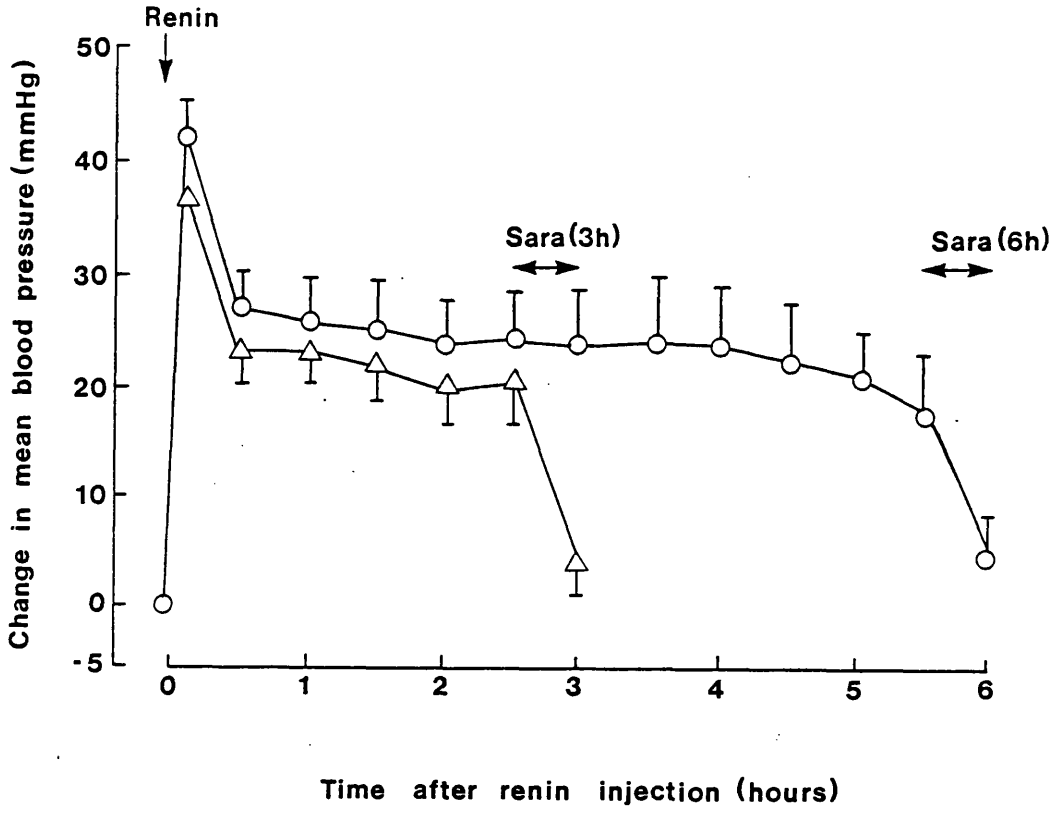

Figure 13. The change in mean blood pressure in two separate groups of nephrectomised rats after renin injection followed by infusions of saralasin. 0.6 Goldblatt units of rat renin were injected at time zero and the infusions of saralasin (10 μg/kg/min) were started either two and a half (△) or five and a half (○) hours after the injection of renin. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

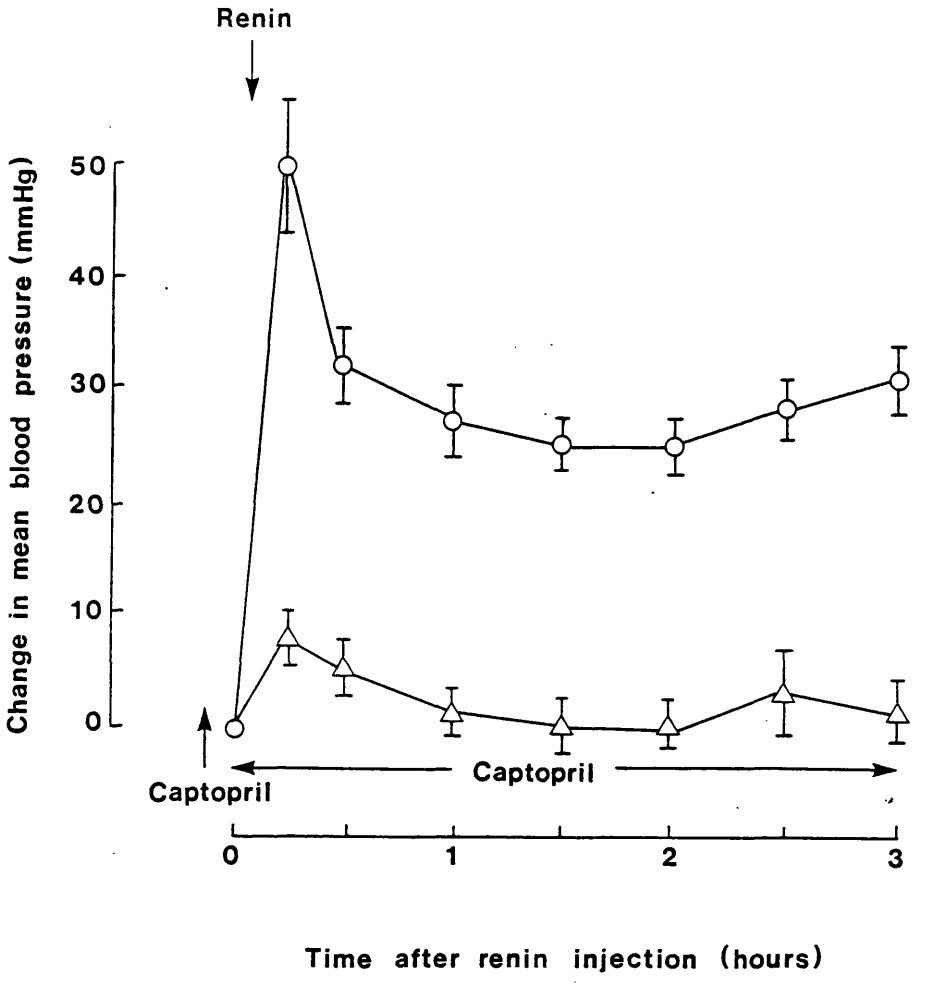

Figure 14. The change in mean blood pressure in two separate groups of nephrectomised rats after an injection of 0.6 Goldblatt units of rat renin with (△) or without (○) an additional infusion of captopril (8.3 μg/kg/min). Blood pressure was measured for a three hour period after the initial injection of renin. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

Plasma and Aortic Wall Renin Concentrations

The plasma renin concentration three hours after renin injection was 47.7 ± 8.6 ngAI/ml/h which was not significantly different from the value obtained when the blood pressure response had not been inhibited by captopril (46.4 ± 7.7 ngAI/ml/h).

The aortic wall renin concentration, measured at the same time, was 0.71 ± 0.32 ngAI/100mg/h. This was higher than in the corresponding group without captopril (0.61 ± 0.15 ngAI/100mg/h) but the difference between these two groups was not significant.

Discussion

The studies outlined in the previous chapter suggest that a renin-angiotensin system within the blood vessel wall may be an important factor in determining the blood pressure response to injected renin. This theory was further tested by a study of the response to renin-angiotensin inhibition in the model described in Chapter four. An initial set of experiments involved producing a rise in the blood pressure of nephrectomised rats by injection of renin. This was then followed by infusions of saralasin starting either three or six hours after the initial renin injection. At both times the blood pressure was restored to pre-injection levels within half an hour.

Saralasin, which acts as a competitive inhibitor of angiotensin II, is a highly specific inhibitor of the renin-angiotensin system. However, it has agonist as well as antagonist properties. Its structure is a modification of the angiotensin II molecule and it can produce a physiological response when bound to angiotensin II receptors. Bing et al (1981) studied the effects of a twelve hour infusion of saralasin in the rat. They found that in normal conscious rats the blood pressure rose slowly over the infusion period. In this study a small pressor response was observed at the start of the saralasin infusion, demonstrating this agonist action. However, since the blood pressure returned to pre-injection levels within half an hour it was concluded that the effect did not make a significant contribution to the overall result.

The observation that the pressor response to injected renin was reversed by saralasin at both three and six hours, as illustrated in figure 13, and that the blood pressure returned to a level not significantly different from pre-injection levels indicates that

the blood pressure response was due entirely to the action of the renin-angiotensin system throughout the experimental period studied.

A comparison with the results obtained in Chapter four shows that at both these times the levels of circulating renin were within, or lower than, the range obtained for normal control rats. Bing et al (1981) showed that when saralasin was administered to normal rats a small pressor response was observed because of the intrinsic agonist properties of the molecule. However, in the experimental model described here, saralasin had a marked vasodepressor effect despite levels of circulating renin which were within the normal range.

The results obtained in this set of experiments therefore support the view that a renin-angiotensin system outside the circulation was responsible for maintaining the blood pressure response. That generation within the resistance vessel wall might be important in this respect was further supported by the fact that the renin concentration in the aortic homogenates was significantly raised at these times.

The second inhibitor used was captopril. This substance is less specific to the renin-angiotensin system since, by inhibiting angiotensin converting enzyme and preventing the inactivation of bradykinin, it potentiates the vasodepressor kallikrein-kinin system. However, it has an advantage over saralasin in that it does not interfere with the renin assay described in Chapter three. This allows measurements of renin levels during blockade of the renin-angiotensin system.

In the experiments described in this chapter, captopril inhibited the pressor response (figure 14) but measurement of the levels of renin in the plasma and aortic wall, three hours after

the renin injection, showed that the pressor response did not alter the partition of renin between these two compartments. Thus, if the pressor response was inhibited by captopril the levels of renin in the plasma and the aortic wall were not significantly different from those obtained when no captopril was given and the pressor response was observed. This confirms the findings of the set of experiments described in Chapter four. In these experiments the uptake of albumen by the blood vessel wall was not affected by the pressor response. There does not, therefore, appear to be any large change in blood vessel wall permeability to molecules such as albumen and renin which, it has been suggested, might occur as a result of the acute rise in the blood pressure following the injection of renin.

A comparison of the effects of saralasin and captopril showed that, at three hours after the renin injection, the blood pressure in those animals which had received captopril was slightly lower than in those in which saralasin was administered. However, the difference was not significant.

Marks, Bing, Thurston, Russell and Swales (1982) found that captopril lowered the blood pressure more than saralasin in hypertensive rats. However, the extent to which the two agents lowered the blood pressure was identical after bilateral nephrectomy. One explanation for this observation is that bilateral nephrectomy results in removal of the renal kallikrein-kinin system so that the additional effect of captopril on this system is abolished. However, there is no experimental evidence that the kallikrein-kinin system is important in blood pressure control. Nevertheless, it is possible that the small difference in blood pressure observed here reflects the action of captopril on a non-renal kallikrein-kinin system.

Such a system has been postulated (Carretero and Scicli, 1981; Schalekamp and Derkx, 1981).

In the experimental model described in this and the preceeding chapter, the effect of an infusion of saralasin demonstrated clearly that the blood pressure response was maintained by the renin-angiotensin system. Inhibition of the response with captopril did not appear to influence the amount of injected renin entering into the tissue of the blood vessels.

CHAPTER SIX

The Effects of Renin Injection in Normal Rats and in Rats After Chemical Renal Medullectomy

Introduction

In addition to comparing the results obtained in Chapter four with normal rats which had not received renin, the effect of a renin injection in rats in which the kidneys were present was also investigated.

That the pressor response to renin is prolonged after bilateral nephrectomy was first pointed out by Tigerstedt and Bergman (1898). Schaechtelin et al (1964) and Bing and Nielsen (1973) found that the pressor response to renin in nephrectomised rats was prolonged for up to six hours. This was confirmed by the results of the experiments described in Chapter four.

Renin Injection In Normal Rats

The duration of the response is considerably reduced when renin is injected into animals where the kidneys are left *in situ*. Basso et al (1977) showed that the response persisted for less than one hour when renin was injected into normal rats. Similarly, Boyd (1979) found that the pressor response was very much shorter when the kidneys remained *in situ*. He showed that there was significant clearance of circulating exogenous renin by the kidney. Peters-Haefeli (1971) found an increase in the half-life of circulating endogenous and exogenous renin after bilateral nephrectomy. This was confirmed by Boyd (1979) who showed that removal of the kidneys resulted in a halving of the clearance rate of exogenous renin. Thus the kidneys play a role in determining the duration of the response to injected renin simply by influencing the rate of clearance of renin. However, other mechanisms are probably involved.

Thurston and Swales (1978) have suggested that the depressor action of inhibitors of the renin-angiotensin system such as saralasin and captopril might stimulate other mechanisms involved

in blood pressure regulation. In the same way, when blood pressure is raised by renin injection, other renal systems besides the renin-angiotensin system, may be activated to counteract the pressor response. A number of mechanisms by which the kidney can lower the blood pressure have been described.

Renal Vasodepressor Systems

It was originally considered that the only mechanism by which the kidney could reduce blood pressure was through the removal of sodium and fluid by natriuresis and diuresis. However, Floyer in 1955 postulated that the kidney could prevent hypertension by inactivating an extra-renal pressor mechanism. When the normal function of the kidney was impaired, such as after renal artery occlusion, Floyer suggested that the blood pressure could be elevated by this system. After unclipping the renal artery the antihypertensive action of the kidney was restored. More recently a number of other renal antihypertensive systems have been described.

Three vasodepressor systems have received most attention. Prostaglandins, present in the renal medulla, the kallikrein-kinin system of the renal cortex and renomedullary lipids.

Prostaglandins

A role for prostaglandins in the control of blood pressure has been suggested. Prostaglandin synthesis has been demonstrated in the kidney, production being greater in the medulla although some takes place in the cortex (Hassid and Dunn, 1980; Dunn, 1981).

Prostaglandins may exert control over blood pressure through a number of actions such as vasoconstriction, vasodilation, natriuresis and inhibition of adrenergic transmission. They may also act as activators of inhibitors of the renin-angiotensin system. The role of prostaglandins in the control of blood pressure has been

reviewed by Dunn and Hood (1977). The action of angiotensin II appears to be closely associated with prostaglandins. Renal vasoconstriction following the administration of angiotensin II is modulated by prostaglandin E_2 from renal medullary interstitial cells (Dunn and Hood, 1977). In the kidney prostaglandins are thought to be involved primarily in the regulation of renal vascular tone. Subsequent renal blood flow modifications may also effect sodium and water excretion (Cinotti, 1983). Renal prostaglandins also influence the rate of production of renin by the kidney (Dunn and Hood, 1977).

In hypertension, an increase in the synthesis of prostaglandins by the renal medulla (Dunn, 1976; Limas and Limas, 1977) and by vascular tissue (Rioux and Regoli, 1975; Pace-Asciak, Carrara, Rangaraj and Nicolaou, 1978) has been demonstrated in spontaneously hypertensive rats. However, this change may be an adaptive one in response to the development of hypertension in this model (Cinotti, 1983).

In humans prostaglandin metabolism has been mainly studied by measuring their levels in the urine. Some authors have demonstrated decreased excretion in essential hypertension (Papanicolaou, Mountokalakis, Safar, Bariety and Milliez, 1976; Weber, Scherer, Held, Seiss and Stoffel, 1979) but others have not detected any changes (Campbell, Holland, Adams and Gomez-Sanchez, 1982; Cinotti, 1983). Again it is difficult to determine whether these alterations represent a primary defect in prostaglandin metabolism which results in hypertension or whether they are a consequence of the hypertension itself.

Technical difficulties in the measurement of prostaglandins have added to the problems of interpreting experimental results.

Responses to administered prostaglandins in experimental animals are highly species specific and even responses in one species may depend on which tissue is studied. For example, different vascular beds in the rat either dilate or constrict in response to prostaglandin administration (Gerber and Nies, 1979).

The Kallikrein-Kinin System

The kallikrein-kinin system may be important in circulatory homeostasis although its exact role remains unclear.

Kinins are potent vasodilator peptides released by enzymes known as kininogenases from precursors known as kininogens. The most widely studied is the kallikrein system in which the enzyme kallikrein releases bradykinin from kallikrein substrate. This system has been reviewed by Levinsky (1979) and Carretero and Scicli (1981). Kinins are rapidly inactivated by kininases, the best known of which is kininase II. This enzyme is the same as angiotensin converting enzyme. The shared role of this enzyme provides a link between the kallikrein-kinin system and the renin-angiotensin system.

The two major kallikreins are plasma and glandular kallikrein. These differ from each other biochemically, immunologically and functionally. Plasma kallikrein is involved in blood clotting and fibrinolysis. Glandular kallikrein, which is present in the kidney, salivary and sweat glands, pancreas and intestine may be involved in the regulation of blood pressure.

In the kidney, all the components of the system are present and kallikrein synthesis has been demonstrated (Scicli, Gandolfi and Carretero, 1978). Most of the kallikrein is present in the cortex, decreasing in concentration from the outer to the inner cortex. Very little is present in the medulla or papilla. Until

recently it was thought that kallikrein produced by the kidney acted as a circulating humoral agent. However, in order to act within the target organs, circulating kinins of renal origin would have to pass through the lungs where the majority would be inactivated. Vane (1969) has shown that eighty percent of a bradykinin infusion was inactivated by the lungs. In addition, circulating kinins are inhibited by naturally occuring inhibitors present in the plasma (Schalekamp and Derkx, 1981). It is now thought that local generation of kinins within the kidney and in other tissues, may be a more important mechanism of action (Carretero and Scicli, 1981).

Infusions of kinins into the renal artery increase blood flow, diuresis and natriuresis within the kidney but the effect of endogenously generated kinins is less clear. The use of inhibitors of the kallikrein-kinin system such as the kallikrein inhibitor aprotonin (Kramer, Moch, Von Sicherer and Düsing, 1979) and antibodies to kinins (Marin-Grez, 1974) suggest that intrarenally released kinins can cause natriuresis, diuresis and the release of prostaglandins.

Studies on the role of the renal kallikrein-kinin system in hypertension involve measuring levels of urinary kallikrein. Excretion of urinary kallikrein is reduced in various models of experimental hypertension (Geller, Margolius, Pisano and Keiser, 1975; Carretero, Scicli, Piwonska and Koch, 1977). This has also been demonstrated in humans (Lechi, Covi, Lechi, Corgnati, Arosio, Zatti and Scuro, 1978; Mersey, Williams, Emanuel, Dluhy, Wong and Moore, 1979) in essential hypertension although some recent studies have failed to show a relationship between hypertension and urinary kallikrein (Holland, Chud and Braunstein, 1980; Cinotti, 1983).

The renal systems described above, together with the renin-angiotensin system, all appear to be involved in the regulation of blood pressure. It is recognised that a balance between the actions of vasoconstrictor and vasodilator systems could control tissue perfusion in the kidney as well as in other tissues. This in turn would indirectly effect the blood pressure (Carretero and Scicli, 1981). Interactions between the systems are therefore likely to be important and many have been described.

The kallikrein-kinin and renin-angiotensin systems are linked by a common enzyme. Angiotensin converting enzyme has been shown to be identical to kininase II which inactivates bradykinin (Yang, Erdos and Levin, 1971). Kallikrein activates inactive renin *in vitro* (Sealey et al, 1979) and it has been proposed that this mechanism of renin activation may be important *in vivo* (Sealey, Atlas and Laragh, 1978). Glandular kallikrein stimulates renin release from super-perfused isolated rat kidney slices (Suzuki et al, 1980) and *in vivo* bradykinin infusion into the renal artery of the dog has been found to stimulate release of renin by the kidney (Flamenbaum, Gagnon and Ramwell, 1979).

Bradykinin infusion has been shown to stimulate prostaglandin release in a number of organs including the heart (Needleman, Key, Denny, Isakson and Marshall, 1975), lungs (Palmer, Piper and Vane, 1973), blood vessels (Terragno, Crowshaw, Terragno and McGiff, 1975) and uterus (Terragno, Terragno, Pacholczyk and McGiff, 1974). It is thought to act by direct stimulation of prostaglandin synthesis. This has been demonstrated in renomedullary interstitial cells (Zusman and Keiser, 1977) and vascular smooth muscle cells (Alexander and Gimbrone, 1976) in tissue culture.

Angiotensin II has been shown to stimulate prostaglandin

synthesis in the kidney (Danon et al, 1975), adrenal gland (Campbell et al, 1979) and in tissue culture (Alexander and Gimbrone, 1976; Zusman and Keiser, 1977).

Prostaglandin infusions have in turn been shown to stimulate renin release (Data et al, 1978; Gerber et al, 1979) and to increase urinary kallikrein excretion in rats (Croxatto, Arriagada, Rojas, Roblero and Rosas, 1978) and dogs (Mills and Obika, 1977) and angiotensin II and prostaglandins have been found to stimulate the release of renal kallikrein (Mills, 1979).

The actions of prostaglandins and kinins in the kidney and their role in blood pressure control are poorly understood. This is in part due to the problems associated with their measurement (Cinotti, 1983). Thus the significance of all these interactions remains uncertain.

Renomedullary Lipids

Muirhead (1983) has reviewed some of the elements of a chemically mediated antihypertensive system within the renal medulla. At the centre of the renal papillae are the renomedullary interstitial cells within which are found lipid containing granules. These granules have been shown to secrete prostaglandins, prostaglandin synthetase inhibitor, proteoglycans and antihypertensive lipids. A number of hypertensive states have been found to be attended by a decrease in the number and volume density of these granules. The latter factor is thought to be more relevant to the function of the granules. An increase in degenerative changes associated with a loss of antihypertensive function has also been observed. The antihypertensive properties of the renal interstitial cells have been observed through the transplantation of either fragments of renal papillae or renomedullary interstitial

cells from monolayer cell cultures. Such transplants have been shown to prevent the development of certain hypertensive states and reverse the existence of others.

Prostaglandins secreted by the renomedullary cells, particularly prostaglandin E_2, are thought to act as local hormones since in the circulation they are rapidly inactivated by the lungs.

Two types of antihypertensive lipids have been implicated as the hormones of the renal medulla. These are alkyl analogues of phosphatidylcholine, of which a number have been studied, and a neutral lipid. They have been shown to be powerful vasodilators capable of causing both acute and prolonged vasodepressor effects. Their primary action is thought to be one of vasodilation of the resistance vessels.

It is therefore likely that the presence of the kidneys will have a profound effect on determining the blood pressure response in the face of the acute change in circulating angiotensin II levels following the injection of exogenous renin.

In this chapter the change in blood pressure in normal rats, after renin injection, was measured together with measurements of the levels of renin in plasma and aortic tissue. The mechanisms involved in this response were then investigated further by comparing the response obtained in these normal rats to that obtained after destruction of the renal medulla.

Renal medullectomy was performed by chemical destruction of the medulla using the compound 2-bromoethylamine hydrobromide. Treatment with this substance has been shown to prevent the reversal of hypertension after unclipping in the Goldblatt 2-kidney 1 clip model of hypertension in the rat (Bing, Russell, Swales, Thurston and Fletcher, 1981) and it produces a rise in blood pressure when

injected into normal rats (Bing, Russell, Thurston, Swales, Godfrey, Lazarus and Jackson, 1983). It has been shown to cause dose dependent medullary damage without affecting the renal cortex (Taverner, Fletcher, Russell, Bing, Jackson, Swales and Thurston, 1983) and is therefore considered to be a useful tool for studying the role of the renal medulla in blood pressure control.

Materials and Methods

All the rats were cannulated on the afternoon of day one according to the procedure described in Chapter four. The renin injections were also given according to the experimental protocol described in Chapter four.

All the rats had free access to food and water at all times.

Normal Rats

Continuous blood pressure was recorded in one group of rats for up to six hours after renin injection. Plasma and aortic wall renin concentrations were measured in two separate groups at one and three hours after renin injection. In the former group an additional blood sample was collected ten minutes after injection.

Chemical Renal Medullectomy

A group of normal rats received an injection of 2-bromoethylamine hydrobromide (0.2 g/kg; Aldrich Chemical Co. Ltd., Gillingham, Dorset, U.K.) intraperitoneally under light ether anaesthesia. After two weeks the rats were placed in metabolic cages and twenty-four hour urine collections were made. Those rats which were polyuric (that is, they produced more than 20 ml of urine during the twenty-four hour period) were subsequently cannulated. Blood pressure was recorded continuously over a six hour period after the renin injection.

Results

Normal Rats

Blood Pressure

The prolonged pressor response to the renin injection observed in rats after bilateral nephrectomy, described in Chapter four, did not occur in the normal rats. The effect of a single injection of 0.6 Goldblatt units of renin on the blood pressure of a single group of eight normal rats is shown in figure 15.

The initial response to the injection of renin was +53.5 ± 2.1 mmHg. This was similar in magnitude to that observed in the group of nephrectomised rats (+44.7 ± 3.3 mmHg). Although there was a difference between these two groups it was not significant.

The plateauing of the response seen in the nephrectomised rats did not occur in the normal group. The blood pressure declined steadily, remaining significantly elevated compared to the base-line value for only one hour after injection. The change in blood pressure at this time was +14.8 ± 2.1 mmHg ($p < 0.01$, compared to pre-injection levels). The blood pressure returned to a level not significantly different from that measured before injection after this time. The half-life of the blood pressure response was 35 minutes.

The plasma and aortic wall renin concentrations were measured in two separate groups of rats at one and three hours after renin injection. An additional blood sample was taken ten minutes after injection from the one hour group.

Plasma Renin Concentration

Circulating renin fell more rapidly than in nephrectomised rats being 497.6 ± 74.3 ngAI/ml/h (compared to 1,115.2 ± 159.4 ngAI/ml/h) when measured ten minutes after renin injection. The

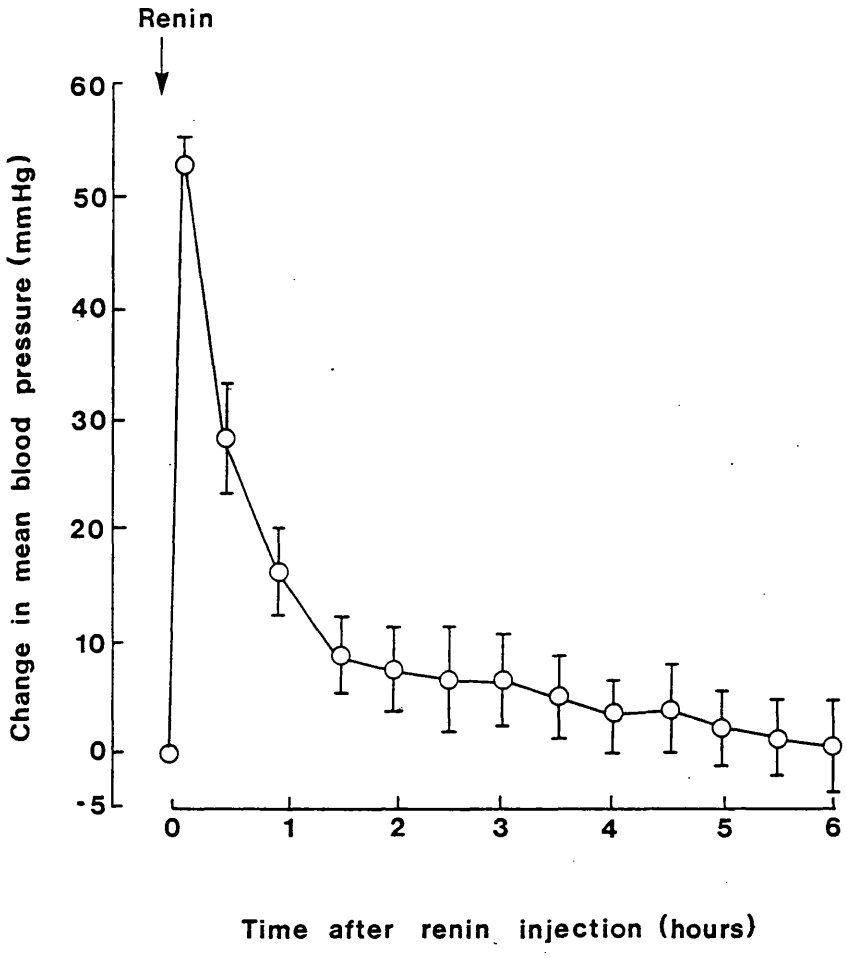

Figure 15. The change in mean blood pressure in a single group of normal rats, over six hours during day two, after an injection of 0.6 Goldblatt units of rat renin given at time zero. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

level had returned to a value not significantly different from normal controls by one hour. The values obtained for the plasma renin concentrations were 35.9 ± 6.7 and 36.8 ± 10.9 ngAI/ml/h at one and three hours after injection respectively. The half-life of circulating renin was 27 minutes. A plot of the logarithms of the values of the individual plasma renin concentrations against the corresponding blood pressure response is illustrated in figure 16. There was no relationship between them ($r = 0.237$).

Aortic Wall Renin Concentration

The measured values obtained for the aortic wall renin concentrations in the normal group were lower at both one and three hours after injection compared to the results obtained for nephrectomised rats. The values were 1.23 ± 0.32 and 1.48 ± 0.29 ngAI/100mg/h respectively at one hour and 0.41 ± 0.10 and 0.61 ± 0.15 ngAI/100mg/h respectively at three hours after the renin injection. However, the differences between the two groups at both times were not statistically significant.

In the normal rats the aortic wall renin concentration measured one hour after injection was significantly increased compared to the normal control rats ($p < 0.01$) but by three hours the level had returned to a value not significantly greater than the control value. This was in contrast to the results obtained after injection in the nephrectomised group. Here the aortic wall renin concentration was significantly elevated for up to six hours after renin injection.

In the normal rats, when the values for the renin concentration in the aortic wall were corrected for plasma contamination the values were 1.15 and 0.32 ngAI/100mg/h at one and three hours after renin injection respectively. The half-life of renin within the aortic wall in the normal rats calculated from these corrected values was 1.38 hours

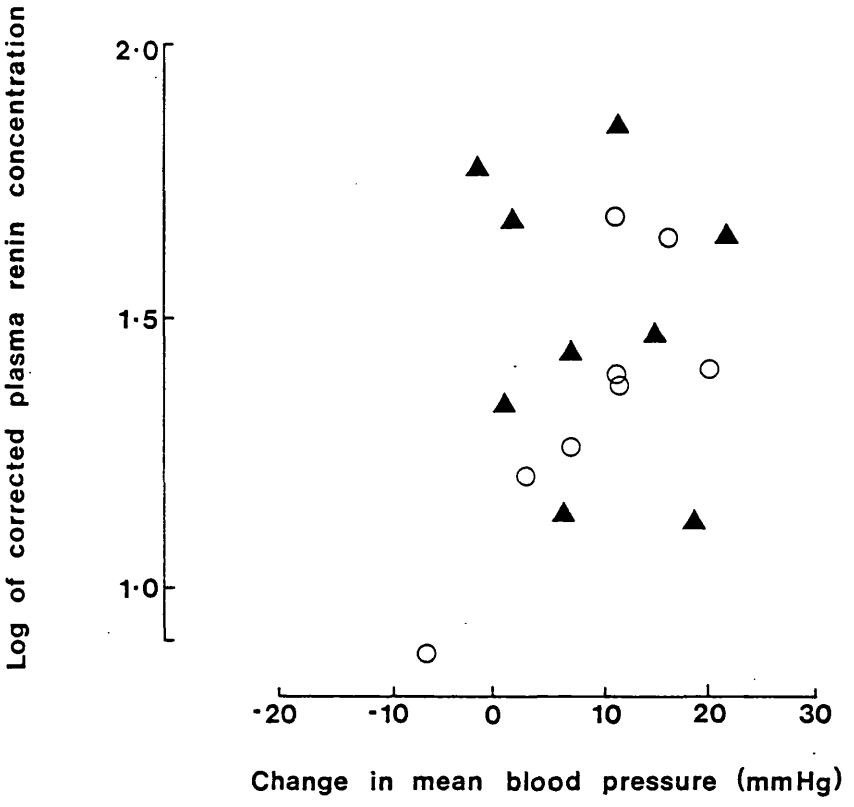

Figure 16. The relationship between the log of the plasma renin concentration and the corresponding pressor response, measured in normal rats, to an injection of 0.6 Goldblatt units of rat renin. The values given are those obtained for each individual rat from measurements made at one (▲) and three (○) hours after renin injection.

The corrected values were transformed to logarithms and plotted against the corresponding pressor responses as illustrated in figure 17. There was no correlation between the two measurements ($r = 0.351$).

Rats After Chemical Renal Medullectomy

The blood pressure response to renin injection in this group of rats was identical to that obtained for the normal rats. The response is illustrated in figure 18. The initial response was $+51.7 \pm 3.2$ mmHg and the blood pressure remained elevated for up to one hour after injection being $+13.7 \pm 5.0$ mmHg at this time ($p < 0.05$ compared to the pre-injection value).

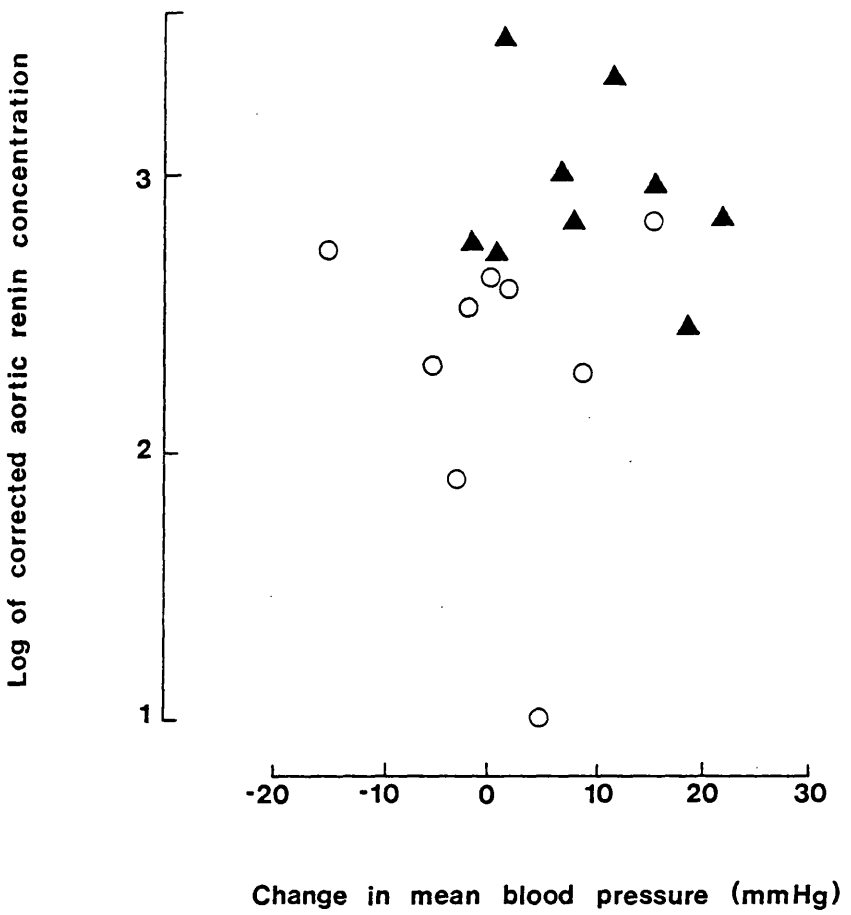

Figure 17. The relationship between the log of the aortic wall renin concentration and the corresponding pressor response, measured in normal rats, to an injection of 0.6 Goldblatt units of rat renin. The values given are those obtained for each individual rat from measurements made at one (▲) and three (○) hours after renin injection.

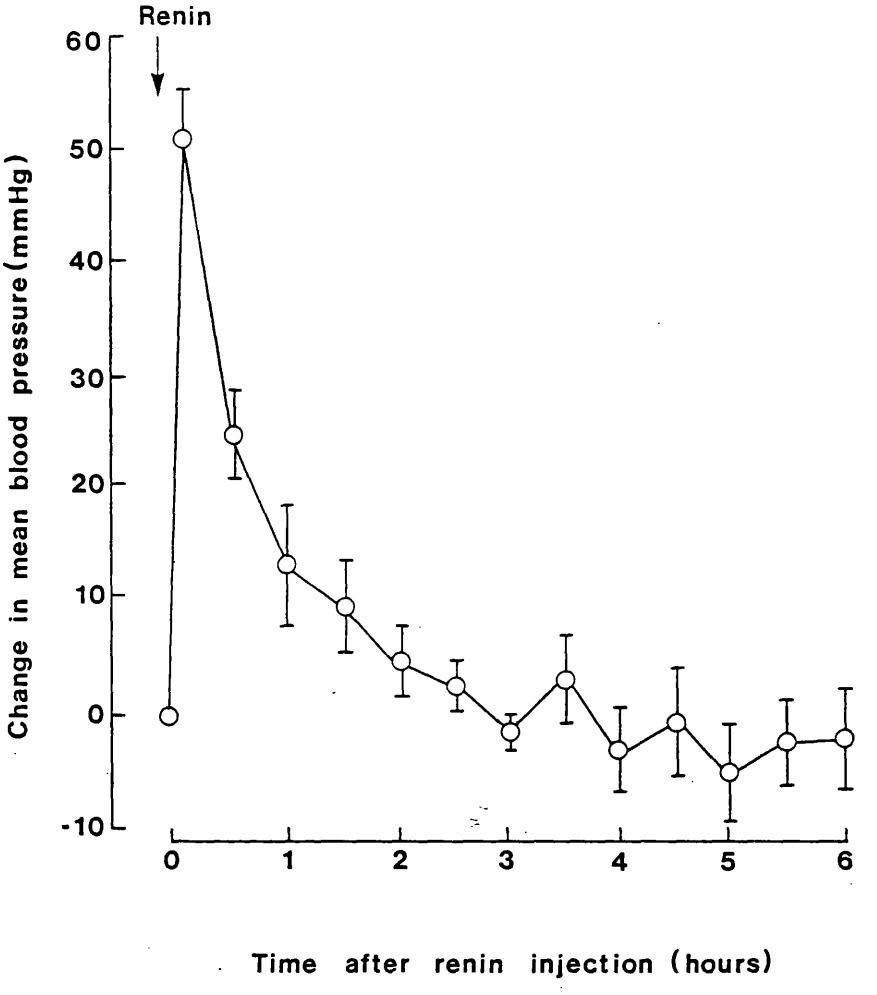

Figure 18. The change in mean blood pressure in a single group of rats after chemical renal medullectomy over six hours of day two, following an injection of 0.6 Goldblatt units of rate renin given at time zero. The change in mean blood pressure is the difference between the absolute blood pressure at any time and that obtained at time zero.

Discussion

For a number of reasons this study has been concerned with the effects of injected renal renin in rats which had undergone bilateral nephrectomy. This experimental model was used in order to isolate the actions of the renin-angiotensin system from other renal systems which are involved in blood pressure control and to ensure that endogenous renal renin was absent from the circulation of the experimental animal.

In the present chapter the effect of renin injection given to rats whose kidneys had not been removed was reported. In the discussion below this group is referred to as the normal group and the results obtained compared to those from nephrectomised rats which have been described in Chapter four.

In the experiments involving normal rats, endogenous renin was present before and presumably during the experiment. In these rats, measurement of plasma renin levels after renin injection did not distinguish between exogenous and endogenous renin.

A comparison of the results obtained from the continuous monitoring of the blood pressure after renin injection in nephrectomised and in normal rats showed that the overall response to the injected renin was different. These blood pressure responses are illustrated in figures 6 and 15 respectively.

The initial pressor response in the two groups was similar although the response in the normal group was slightly greater than that observed in the nephrectomised rats. A general reduction in the pressor responsiveness of rats after bilateral nephrectomy has been observed by Marks et al (1982). In such rats the response to both angiotensin II and noradrenaline was decreased. They concluded that the effect was non-specific and a consequence of renal failure

resulting in an alteration of circulatory responses. This was probably the explanation for the small difference in the initial response to renin injection observed in the present study.

However, although the initial pressor response in the normal rats was similar in magnitude and the blood pressure fell, in the early stages of the experiment, at the same rate as in the nephrectomised rats, a pressor response was not subsequently maintained. Thus, the blood pressure in the normal rats fell steadily after the initial peak and had returned to the pre-injection level in just over one hour. This occurred despite the fact that the renin levels measured in the aortic wall at one hour after the injection of renin were still markedly higher than in the control rats. This suggests that, in the intact rat, other mechanisms are involved in determining the blood pressure response which apparently override the action of the renin-angiotensin system.

The absence of any relationship, in the normal rats, between the blood pressure and the concentration of renin within the aortic wall is illustrated in figure 17. This figure shows a plot of the logarithm of the individual corrected aortic wall renin concentrations against the corresponding pressor responses. A similar plot of the results obtained from the nephrectomised rats, illustrated in figure 12 shows, in contrast, a linear relationship.

The possible role of the kindey in restoring the blood pressure to within the normal range despite the raised levels of renin in the aortic wall is considered in the final section of this discussion.

The half life of renin, both in the plasma and in the aortic wall, was much shorter in the normal rats than it was in the nephrectomised rats. In the plasma the half life was 35 minutes in the normal rats compared to one hour in the nephrectomised rats and

in the aortic wall, 1.38 hours compared to 4.83 hours respectively.

The relatively slow clearance of renin from the plasma of nephrectomised rats, in comparison to its relatively rapid disappearance in normal rats suggests that renin can be cleared from the circulation by the kidneys. However, whilst renin in the plasma may have been cleared by the kidney in the normal rats, the renin present in the tissue of the aortic wall was not available for clearance at this site. Despite this, when the kidneys were present, the half life of renin in the aortic wall was considerably reduced compared to the nephrectomised rats.

In Chapter four the reasons for the prolongation of the half life of renin in the blood vessel wall, compared to that in the plasma, were discussed. It was proposed that renin might enter and leave the tissue of the aortic wall simply by a process of passive diffusion, the rate at which this occurred depending, among other factors, upon the concentration gradient between the plasma and the aortic tissue. It was also suggested that the rate of disappearance of renin from the aortic tissue depended largely on the rate at which renin could diffuse out of the tissue and back into the circulation. After the injection of renin the concentration gradient between the plasma and the tissue would be large and there would be net diffusion of renin into the tissue. Net diffisuon in the reverse direction, namely from the tissue into the circulation, which would result in the disappearance of renin from the aortic tissue, would only occur when the direction of the concentration gradient was reversed. In the nephrectomised rats this reversal probably did not occur until the later stages of the experiment, contributing to the persistence of renin within the tissue.

If the reversal of the concentration gradient occurred earlier

during the course of the experiment in the normal rats, then the rate of disappearance of renin from the aortic tissue of these rats might proceed more rapidly. This would result in a shorter half life than was the case for nephrectomised rats.

In addition to the differences in the half life of renin in the two models, the total amount of renin measured in the blood vessel wall, at both one and three hours after renin injection, was lower in the normal rats than in the respective nephrectomised groups. The explanation for this may follow the same principle. Thus, after renin injection in the normal rats the aortic wall would have been exposed to a high concentration gradient for a shorter period of time because circulating renin was cleared more rapidly. In consequence, less renin may have diffused into the aortic tissue during the initial stages of the experiment. This, combined with a more rapid rate of clearance of renin from the tissue, as described above, may have therefore been responsible for the observed differences in the levels of renin in the aortic tissue of the two models.

The key measurement to justify the suggestions outlined above is that of the actual concentration of renin free within the interstitial fluid of the aortic wall. This fluid cannot be sampled directly to allow a straightforward measurement of the amount of renin in this compartment. However, an estimation of the concentration of renin in this tissue fluid can be made.

The steps taken to reach this estimate are set out in table 1. The values presented as an example are those of the plasma and aortic wall renin concentrations measured at one hour after renin injection in both normal and nephrectomised rats.

If it is assumed that the renin in the aortic wall is present

	Nephrectomised rats	Normal Rats
Aortic wall renin concentration ngAI/100mg/h	1.48	1.23
Aortic wall renin concentration (corrected for plasma contamination) ngAI/100mg/h	0.87	1.15
Aortic wall renin concentration ngAI/g/h	8.7	11.5
Aortic wall renin concentration X 100/50 ngAI/ml/h	17.4	23.0
Plasma renin concentration ngAI/ml/h	297.1	35.5

Table 1: The measured renin concentrations in plasma and in aortic tissue and the estimated renin concentration in the interstitial fluid of the aortic tissue of normal and nephrectomised rats one hour after renin injection.

free within, and restricted to, the interstitial fluid compartment, then the calculation runs as follows. First, the values for the aortic wall renin concentration were corrected for plasma contamination. The basis for this correction has been described in Chapter three although the correction factor is probably too large. This point is discussed below.

Throughout this thesis the plasma renin concentration was expressed in ng angiotensin I generated/ml of plasma/hour whilst the aortic wall renin concentration was expressed in ng angiotensin generated/100 mg aortic tissue/hour. In order to be able to make direct comparisons between the two values, the second step was to multiply the values for the aortic wall renin concentration ten fold on the assumption that one ml of plasma was roughly equivalent in volume to one gram of wet aortic tissue.

Thirdly, the aortic wall renin concentration was further corrected to allow for the proportion of aortic tissue, namely the intracellular compartment, which probably does not contain renin. The proportion of the rat thoracic aorta which is extracellular space has been estimated by Jones (1982) to be 51.8 percent of the total wet weight of the tissue. This volume includes the interstitial fluid and the plasma within the vasa vasorum. The proportion of the sampled aortic tissue which consisted of plasma within the vasa vasorum was taken to be 2.29 percent. This value was an estimate derived from the measurements made to assess plasma contamination of the aortic samples as described in Chapter three.

The interstitial fluid volume of the aortic wall was therefore estimated as the extracellular fluid volume, 51.8 percent, less the plasma volume, 2.29 percent. This gave a value of 49.51 percent. Since this figure was derived from two separate estimates

it seemed reasonable to round this value up to 50 percent, which figure was used in the calculations set out in table 1. It is a considerably larger fraction of the whole than is found, for example, in mammalian striated muscle tissue. However, the above figure is not surprising in view of the microanatomy of the arterial vessel wall in which the layers of elastic tissue of the tunica intima and the tunica media contribute a significant proportion to the thickness of the vessel wall (Bradbury, 1970).

After all these corrections have been applied it can be seen that in normal rats, one hour after renin injection, the aortic wall renin concentration was close to that of the plasma. In contrast to this, the plasma renin concentration in nephrectomised rats, at the same time, was nearly twenty times greater than the renin concentration in the aortic tissue fluid.

Thus, in normal rats, at more than one hour after renin injection, it seems likely that net diffusion of renin out of the aortic tissue and back into the plasma may have occurred. Such a process may therefore have contributed to the relatively rapid decline of the renin concentration in the aortic wall of these rats compared to the nephrectomised rats, as has been suggested above.

The calculations set out in table 1 do not, of course, take account of the possible effect of the binding of renin to specific receptors. The measurement of renin in this study was made on samples of homogenised aortic tissue. Most of the renin in such samples, whether bound or unbound, would probably have contributed to the generation of angiotensin I and thus to the measurement of the renin concentration.

In vivo, only the renin free within the interstitial fluid of the aortic wall could diffuse back into the circulation. If some

of the renin in the aortic wall were bound to receptors, then the reversal of the concentration gradient proposed above might not have occurred at the suggested time during the course of the experiment. The evidence, or lack of it, for specific receptors for renin in the blood vessel wall has been considered in the discussion to Chapter four.

It is interesting to compare the effect of correcting for plasma renin contamination of the aortic samples on the results obtained for both the normal and the nephrectomised rats. Table 1 gives the values, for both groups, of the corrected aortic wall renin concentration, one hour after renin injection. At this time the corrected value for the concentration of renin in the aortic wall was less in the nephrectomised rats than it was in the normal rats. This was observed despite a concentration gradient between the plasma and the aortic tissue which persisted for very much longer in the nephrectomised rats. This was clearly not consistent with the suggestion, as outlined above, that the concentration of renin at this site was largely dependent on the size and duration of the concentration gradient. However, if the correction factor employed were an overestimation, then this might explain the unexpected observation.

In Chapter three it was argued that the correction factor obtained may have been an overestimation of the degree of plasma contamination. The tracer molecular species used was radiolabelled albumen. This molecule is of similar size to renin itself and can diffuse out of the circulation into the interstitial fluid of the aortic wall. During the time allowed, that is, thirty minutes, for the labelled albumen to distribute evenly throughout the circulation it was probable that some of the albumen left the

circulation and entered the tissue fluid of the aortic wall as was the case with injected renin. The estimation of plasma contamination would then have included the albumen within the tissue fluid of the aortic wall as well as the fraction in the vasa vasorum which remained after washing the excised aortic sample.

Since the size of the correction applied to each individual aortic sample depended on the level of renin in the corresponding plasma sample, the effect of such an overcorrection would be considerably larger in those rats where circulating renin levels were very high. This was particularly true of the nephrectomised rats one hour after renin injection. At this time the plasma renin concentration was nearly ten times greater in the nephrectomised rats than it was in the normal rats, making the corresponding correction much larger.

By three hours after renin injection the levels of renin in the plasma of both groups were similar. At this time any error introduced by correcting for plasma contamination was of a similar size in both groups of rats. In addition, the correction factor at this time, depending as it did on the plasma renin levels, was a relatively small proportion of the total renin concentration measured in aortic homogenates.

It should be emphasised that the conclusions drawn in the preceeding discussion have been based on calculations which have relied on a number of approximations. Thus the value obtained by Jones (1982) for the proportion of extracellular fluid present in the aortic tissue may not have been entirely appropriate to the experimental model described in this thesis. It was not determined here whether the experimental methods used in the present study affected the volume of extracellular fluid in the aortic wall. In

addition, not all the renin measured in the aortic tissue may necessarily have been present free within the interstitial fluid compartment of the tissue. Correction for plasma contamination may also have introduced inaccuracies. These two points have been discussed above.

Thus there are certain limitations placed on any conclusions drawn from the calculations outlined in table 1. However, despite this, they appear to provide a useful insight into the possible processes which were responsible for the observed effects of renin injection under the experimental conditions described.

The experiments reported in this chapter have shown that in the presence of the kidneys the prolonged pressor response to renin injection was not maintained. It was therefore concluded that a hypotensive mechanism or system contributed to the decline in the pressor response and that such a mechanism or system depended upon the presence of the kidneys.

The kidney contains several hypotensive systems. These have been outlined in the introduction to this chapter. It is not clear which of these systems was responsible for the decline in the pressor response. It has been shown, however, that the tissue of the renal medulla is of particular importance to the antihypertensive actions of the kidney. For instance, Muirhead (1983) has shown that when renomedullary interstitial cells are removed, either by bilateral nephrectomy or by papillectomy, the development of hypertension in response to salt or volume loading can be prevented by transplanted renomedullary interstitial cells. The action of the renal medulla in lowering the blood pressure in the experimental model described in this thesis was therefore investigated. The renal medulla was selectively destroyed, leaving the

renal cortex and the ureteric structures intact. An identical experimental schedule was then used in these rats to that described for the nephrectomised and normal rats.

It was hoped that this procedure would demonstrate an anti-hypertensive mechanism for the renal medulla. However, the results obtained showed that removal of the renal medulla did not result in a prolonged pressor response to renin injection, as had been demonstrated in nephrectomised rats. On the contrary, the pressor response to injected renin was the same in rats which had undergone chemical renal medullectomy as it was in normal rats. Thus, the initial pressor response to renin injection declined relatively rapidly and, as in normal rats, the blood pressure had returned nearly to the pre-injection level by one hour after renin injection. This is illustrated in figure 18.

It therefore seems that the action of any renal hypertensive system, which was demonstrated by the experiments on rats whose kidneys had not been removed, must reside, at least partly, in those areas of the kidney which were not effected by chemical renal medullectomy. However, this result does not rule out the possibility that the renal medulla has a major role in the anti-hypertensive action of the kidney under physiological conditions. The action of the renal medulla, in the experimental model described here, may have been effectively inhibited by the design of the experiment itself. Muirhead, Pitcock, Brown and Brooks (1981) have shown that renomedullary interstitial cells may be inhibited by high levels of angiotensin II. The injection of a large quantity of renin, resulting initially in high levels of circulating angiotensin II, would thus have been expected to produce the same pressor response whether the renal medulla was present or not.

The experiments carried out in rats which had undergone bilateral nephrectomy allowed a study of the action of the renin-angiotensin system, in isolation from other renal systems, on blood pressure control. These experiments, the results of which were described in Chapter four, demonstrated that renin acts within the blood vessel wall rather than in the circulation. Whilst this conclusion may be important, it is obvious that the renin-angiotensin system cannot and does not act in isolation. This was confirmed by the results obtained from the experiments carried out on normal rats, as described in this chapter.

A comparison of the results obtained from the two groups, that is from normal and nephrectomised rats, lead to a clarification of the mechanisms involved in determining the pressor response to injected renin. In addition, the importance of the concentration gradient of renin between the plasma and the aortic wall in determining the level of renin within the aortic tissue, and its persistence there, was underlined.

A comparison of the results obtained from the normal rats and from rats which had undergone chemical renal medullectomy confirmed that the blood pressure is under the control of a number of renal systems, in addition to the renin-angiotensin system. The precise role of any one of these systems may be difficult to determine in the presence of the others.

CHAPTER SEVEN

Summary and Conclusions

Although the renin-angiotensin system has long been recognised as an important pressor system, its role in blood pressure control and in the development and maintenance of hypertension remains unclear. Measurement of renin levels in the plasma in both clinical and experimental hypertension has been inconclusive. More recently, however, it has been suggested that the level of renin within the tissues rather than in the circulation may be more important in the control of blood pressure. In particular, the relevance of renin within the blood vessel wall has received considerable attention.

The aim of the work carried out in this thesis was to try to define more precisely the role of such a system in blood pressure control.

The kidney has a central and complex role in the control of blood pressure involving a number of interacting systems. The action of renin was therefore separated from the other renal systems, and from endogenous renin, by studying the effect of exogenous renin administered to rats after removal of their kidneys.

Under these conditions the pressor response to injected renin was prolonged but was not apparently related to the level of circulating renin. However, administration of the angiotensin II antagonist saralasin demonstrated that the pressor response to injected renin was maintained by the renin-angiotensin system throughout the course of the experiment.

The prolonged pressor response was found instead to be paralleled by the persistence of renin measured in the tissue of the aortic wall. The half-life of the pressor response was similar to that of the renin within the aortic wall. Both were prolonged in comparison to the half-life of renin in the plasma.

It was therefore concluded that the level of renin in the aortic wall, rather than in the circulation, was a major factor responsible for the pressor response under the experimental conditions described. In reaching this conclusion it was assumed that the distribution and action of renin in the aortic wall was similar to that which occurs in smaller arterial resistance vessels.

Renin, and other components of the renin-angiotensin system, have been detected in many tissues but their origin remains a subject of debate. It is not clear whether they are taken up from the circulation, having been synthesised elsewhere, or whether they are synthesised locally at their site of action.

In the experiments reported in this study the large increase in the level of renin within the aortic wall following the injection of exogenous renin confirms that circulating renin can gain access to the tissue of the aortic wall. It was also concluded that the results obtained in this study were consistent with the passive diffusion of renin from the circulation into the tissue of the aortic wall and, under certain conditions, vice versa.

The results obtained were therefore also compatible with the proposal that the majority of the renin detected in the aortic wall was derived from the circulation. However, this proposal was not proved by the experiments reported in this study. It remains a possibility that some of the renin present at this site was due to local synthesis.

A recent extension to the work described in this thesis has confirmed that renal renin can indeed gain access to vascular tissue (Swales, Abramovici, Beck, Bing, Loudon and Thurston, 1983). In this study injected renin was localised using a polyvalent

rabbit antiserum to mouse submaxillary gland renin. This antiserum cross-reacts poorly with rat renin. The experimental protocol was therefore identical to that described in Chapter four except that the rats were injected with renin prepared from mouse instead of rat kidneys. The pressor response to this injection was similar to that observed after the injection of rat renin. Samples of spleen and aorta were taken three hours after renin injection. These tissues were treated with renin antiserum and examined for the presence of renin by an indirect immunofluorescent technique employing FITC-congugated goat antiserum. Whilst blood clots showed minimal activity, intense fluorescence was found to lie between the elastic fibres of the descending aorta media. Marked activity was also found in the media of the central arteries of the spleen.

When the experiments described in Chapter four were repeated in normal rats, that is, in rats with intact kidneys, it was found that the half-life of injected renin in the tissue of the aortic wall, although prolonged compared to the length of time which renin persisted in the plasma, was considerably shorter than that estimated in nephrectomised rats. From an analysis of the amounts of renin in the aortic wall and the plasma of these two different groups it was concluded that the amount of renin present in the aortic wall, in both experimental groups, was probably dependent on the concentration gradient of renin between the plasma and the tissue of the aortic wall.

Whilst the pressor response in the nephrectomised rats appeared to be dependent on the level of renin in the blood vessel wall after the injection of exogenous renin, this relationship did not exist in the normal rats. It was concluded that one or several antihypertensive mechanisms of the kidney were acting to override

the pressor response to injected renin.

The antihypertensive properties of the renal medulla in this situation were investigated. However, an antihypertensive mechanism dependent on the presence of the renal medulla could not be demonstrated using the experimental technique described in this thesis.

It was therefore concluded that one or more mechanisms, which were associated with that area of the kidney not destroyed by chemical renal medullectomy, were responsible for lowering the blood pressure when it had been raised experimentally under the conditions described. Under physiological conditions such systems may function jointly with the systems present in the renal medulla.

This study achieved its aim which was to demonstrate first, that renin can enter the blood vessel wall from the circulation and secondly, that its action there may be a significant factor in the control of blood pressure. However, it also showed that the role of the renin-angiotensin system cannot be fully understood in terms of its isolated actions. The importance of its interactions with other systems must be considered in order to understand the mechanisms which control blood pressure.

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PUBLICATIONS

Publications Based on the Experiments Described in this Thesis

Changes in blood pressure in relation to vascular and plasma renin after renin injection in rats.

LOUDON, M., BING, R.F., SWALES, J.D. and THURSTON, H.

Clin. Sci. 63, 153s-156s, 1982.

Vascular renin as a determinant of the circulatory response to renin.

LOUDON, M., BING, R.F., SWALES, J.D. and THURSTON, H.

Clin. Exp. Hypertension A4, 2049-2061, 1982.

Arterial wall uptake of renal renin and blood pressure control.

LOUDON, M., BING, R.F., THURSTON, H. and SWALES, J.D.

Hypertension 5, 629-634, 1983.

Arterial wall renin.

SWALES, J.D., ABRAMOVICI, A., BECK, F., BING, R.F., LOUDON, M. and THURSTON, H.

J. Hypertension 1 (suppl. 1), 17-22, 1983.

Renin in the arterial wall.

SWALES, J.D., LOUDON, M., BING, R.F. and THURSTON, H.

Clin. Exp. Hypertension A5, 1127-1136, 198, 1983.

TITLE Arterial wall renin-like activity and blood pressure regulation in the rat

AUTHOR Mary Loudon

ABSTRACT

In this study the importance of the generation of angiotensin II within the blood vessel wall in determining the pressor response to injected renin was investigated.

An injection of renin, given to rats after bilateral nephrectomy, produced a pressor response. The level of circulating renin, although initially elevated, returned to the normal range within three hours. However, the level of renin present in the aortic wall remained significantly elevated for six hours after the injection, as did the pressor response.

Infusions of the angiotensin II antagonist saralasin at three and six hours after the renin injection confirmed that the pressor response was maintained by the renin-angiotensin system.

An injection of renin into normal rats produced the same initial pressor response as was observed in the nephrectomised rats. However, the blood pressure subsequently returned to the pre-injection level after one hour. In the normal rats the pressor response was not related to the level of renin present within the aortic wall.

It was concluded that the activity of the renin present within the blood vessel wall was more relevant to the control of blood pressure than the circulating level. However, when the kidneys were present this local action of the renin-angiotensin system was overriden by renal anti-hypertensive systems. This was not dependent on the presence of the renal medulla since the pressor response after chemical renal medullectomy was the same as that observed in the normal rats.

The increase in the level of renin within the aortic wall after the injection of exogenous renin confirmed that renin can enter the walls of blood vessels from the circulation. It was concluded that this occurred by a process of passive diffusion.