STUDIES IN EXPERIMENTAL HYPERTENSION AND THE RENIN-ANGIOTENSIN SYSTEM.

by

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SUMMARY.

Blood pressure, angiotensin II and renin content were measured in unanaesthetized two-kidney, one clip hypertensive rats at 1 and 2 days, at weekly intervals up to 10 weeks and at 15 and 20 weeks after clipping. Blood pressure was elevated at 1-2 days and remained at this level until 2 weeks when there was a further progressive rise reaching a plateau at 5-6 weeks after which time hypertension was sustained up to 20 weeks. Compared with values in sham-operated rats angiotensin II and renin were initially increased at 1-2 days but were then supressed between 2 and 4 weeks to levels similar to those found in the sham-operated rats. Between 5 and 20 weeks angiotensin II and renin increased again to high levels. There was a significant correlation between angiotensin II and blood pressure in acute rats 1-2 days after clipping ($p<0.05$) and in chronic rats 8-20 weeks after clipping ($p<0.001$). There was no difference in the slope of the regression lines but the regression line for the chronic rats was shifted upwards in a parallel manner.

The acute hypotensive response (-20.3 +/- SD 24.9 mmHg) in 26 rats given converting enzyme inhibitor was related to the basal renin and angiotensin II levels and followed the slope of the angiotensin II/blood pressure regression line for all chronic rats. Only one out of 26 rats had its blood pressure reduced to normal levels. In 12 rats at 4 weeks after clipping when blood pressure was

elevated but angiotensin II was supressed, there was only a small fall in blood pressure (-7.1 +/- SD 7.2 mmHg). This also followed the angiotensin II/blood pressure regression line for the chronic rats but at the lower end. Blood pressure again was not reduced to normal.

These results suggest that there is no change in the net vascular responsiveness to endogenous angiotensin II at any stage in this experimental model and that the acute effect of angiotensin II on blood pressure is determined solely by its position in the same dose response curve. Also with the exception of 1-2 days after clipping the acute effect of angiotensin II plays only a minor role in the hypertension and that some other mechanism is of greater importance and begins to have an effect as early as 2 weeks after clipping.

In an attempt to elucidate this other mechanism the effect on blood pressure of acute and chronic suppression of angiotensin II was studied in chronic hypertensive two-kidney, one clip rats. Conscious chronically catheterized rats were given a bolus injection of captopril (2.5 mg/kg) followed by a chronic infusion of either dextrose or captopril (1mg/kg/h) lasting 5 days. Blood pressure was measured continuously by a computer technique. Following the acute injection of captopril, arterial blood pressure fell from 165.1 +/- SD 19.4 mmHg to a minimum of 137.6 +/- SD 23.3 mmHg after 15 minutes. Twelve hours after starting the chronic infusion of captopril, blood pressure fell to a minimum of 112.5 +/-

SD 19.4 mmHg. This was significantly lower than after the acute injection of captopril. Blood pressure remained lower throughout the 5 day infusion ranging on the 5th day from 122.1 +/- SD 23.4 to 136.0 +/- SD 30.2 mmHg. In contrast, in the rats given dextrose chronically blood pressure continued to rise, ranging on the 5th day from 163.6 +/- SD 23.8 to 180.4 +/- SD 22.5 mmHg. Both the fall in blood pressure after acute captopril and that after chronic captopril were related to pre-treatment levels of plasma renin concentration.

These results suggest that in the two-kidney, one clip hypertensive rat, angiotensin II, in addition to its acute vasoconstrictor property, might contribute to the hypertension through a secondary effect.

CHAPTER 1

REVIEW OF THE LITERATURE.

1.1 Introduction.

The most common form of unilateral renal disease responsible for the development of elevated blood pressure in man is stenosis or occlusion of a renal artery. In addition to patient investigation, the use of animal models of renal artery stenosis has allowed further study of this disease. In particular the extension of Goldblatt's dog model (1934) to the rat (Wilson & Byrom,1939) resulted in an animal model which mimicked to a remarkable extent the disease as seen in man. As will be seen from work presented in this thesis, this similarity between human renal artery stenosis with hypertension and the Goldblatt two-kidney, one clip rat model extends to the relationship between changes in blood pressure and the renin-angiotensin system; a major causal factor implicated in this disease. Such similarities emphasise the particular relevance to man of studies carried out in this model. The involvement of the renin-angiotensin system in renovascular hypertension has been extensively studied and while much is understood, the story is not yet complete. Today there is still disagreement, particularly relating to the long term

involvement of the renin-angiotensin system in the disease.

1.2 The renin-angiotensin system, historical background.

The association between kidney disease and raised arterial pressure was first noted by Richard Bright as early as 1836. Over 60 years later, influenced by these observations, Tigerstedt and Bergman (1898) began to investigate the possible endocrine function of the kidney. They found that crude saline extracts of rabbit kidneys injected into other rabbits produced a pressor response. They named this active extract renin. The importance of their observaton was disputed for over 30 years until in 1934 Goldblatt and his colleagues carried out their classical experiments in which they produced a reproducible model of persistant hypertension in dogs by the constriction of both renal arteries with specially designed silver clamps. The availability of this model stimulated considerable research into the possible humoral or neural mechanisms that might cause renal hypertension. Page (1935) and Goldblatt, Gross and Hanzal (1937) showed that neural factors were not involved in the development of the hypertension. These findings pointed to a humoral mechanism being responsible and at the end of the 1930's Tigerstedt and Bergman's original work was confirmed by Harrison, Blalock and Mason (1936), Prinzmetal & Friedman

(1936), Landis, Montgomery and Sparkman (1938) and Pickering & Prinzmetal (1938). The first biological assay for renin was described in 1938 (Pickering & Prinzmetal) in which a stable preparation of renin was made as a standard and extracts from tissues could be compared with this using the blood pressure responses of unanaesthetised rabbits.

After the rediscovery of renin it became apparent that renin itself did not act directly on the vascular system. Kohlstaedt, Helmer and Page (1938) found that renin was not pressor in its own right, but that it became pressor on incubation with a substance or substances in the plasma which they named 'renin activator'. In 1940 Page & Helmer in their search for a product resulting from the interaction of renin and renin activator discovered a new pressor substance which they named 'angiotonin'. Independently, and at the same time, Braun-Menendez et al. (1940) extracted a pressor substance from the venous blood of ischaemic kidneys which was shown to have different properties from renin. They named this substance 'hypertensin' and concluded that the production of hypertensin by renin was enzymatic, the substrate for this reaction being a blood protein which they named 'hypertensinogen'. It was soon recognised that angiotonin and hypertensin were the same substance and that renin activator and hypertensinogen were synonymous, but it was not until 1958 (Braun-Menendez & Page) that the two terms, angiotonin and hypertensin, were blended to give

angiotensin.

The first step towards the isolation and structural characterisation of angiotensin was made by Skeggs et al. (1954) when they demonstrated that two forms of angiotensin could be produced from horse plasma (designated by them as hypertensin I and II). They described the purification of hypertensin I. Peart (1956) isolated a pure angiotensin I from ox serum which contained eight different amino acids whereas Skeggs et al. (1955) showed that their product was a decapeptide containing nine different amino acids. Eventually the difference was reconciled when the structures of ox angiotensin and horse angiotensin were elucidated by Elliot & Peart (1956,1957), Lentz et al. (1956) and Skeggs et al. (1956). The important difference lay in position five in which ox has valine and horse has isoleucine. The amino acid sequences of both angiotensin I decapeptide and angiotensin II octapeptide were subsequently confirmed by synthesis (Schwarz, Bumpus & Page,1957; Rittel et al.,1957; Schwyzer et al.,1958a,1958b).

1.3 Components of the renin-angiotensin system.

The renin-angiotensin system is a biochemical cascade (Figure 1.1). Renin substrate or angiotensinogen,

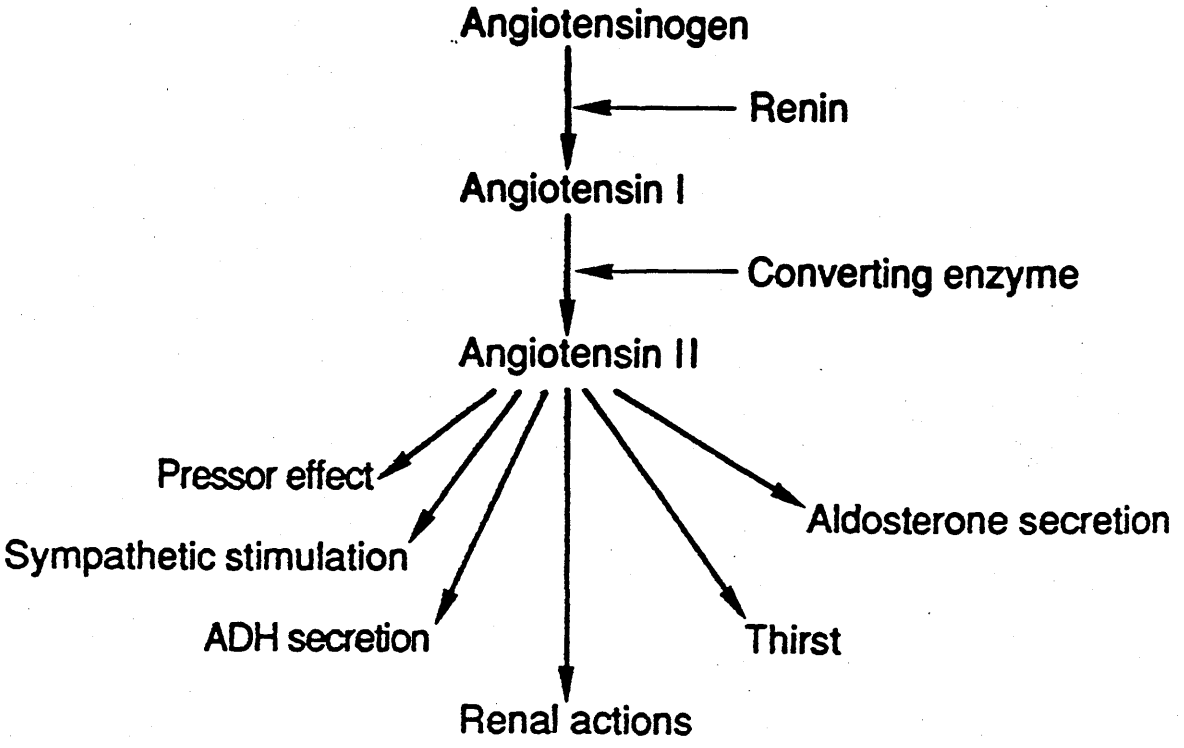


Figure 1.1 Outline of the renin-angiotensin system and some of the main actions of angiotensin II.

a glycoprotein, is secreted by the liver into the circulation where it is converted to the decapeptide angiotensin I by the action of the renal enzyme renin. Angiotensin I is in turn converted to the active component of the system, the octapeptide angiotensin II. Among other properties, angiotensin II acts directly on smooth muscle of vascular tissue to raise blood pressure.

1.3:1 Angiotensinogen.

Angiotensinogen is an α 2 globulin synthesised in the liver (Nasjletti & Masson,1971) and released into the blood stream. While the liver appears to be its main site of synthesis, renin substrate has also been detected in the brain (Ganten et al.,1971), vasculature (Desjardins-Giasson et al.,1981) and recently mRNA angiotensinase sequences have been detected in many non-liver sites (Dzau et al.,1986; Ohkubo et al.,1986). Early attempts to purify pig angiotensinogen (Skeggs et al.,1963) showed the existance of multiple forms, all of which were susceptible to conversion by pig renin, and all had a molecular weight around 57,000 daltons. A similar molecular weight was found for human angiotensinogen by Printz et al. (1977). Although renin will cleave a number of synthetic peptide substrates, angiotensinogen is the only naturally occurring renin substrate. The minimum peptide sequence required for substrate activity is the

6-13 octapeptide (Skeggs et al.,1957). Renin specifically cleaves the bond between amino acid residues at positions 10 and 11. As can be seen from Figure 1.2 there are some species differences in the amino acids at these positions and in other positions in the N-terminal sequence. The differences are reflected in the species specificity of the renin substrate reactions. Human angiotensinogen is cleaved only by human renin, whereas other mammalian angiotensinogens are cleaved by both human and animal renins. These differences have proved to be crucial in the development of effective species specific renin inhibitors.

1.3:2 Renin.

(i) Properties:

Renal renin is a member of the aspartic proteinase group of enzymes which include pepsin, chymosin, cathepsin D and various microbial proteinases. It is synthesised and stored in, and released from granular cells of the juxtaglomerular apparatus (Goormaghtigh,1939). While the kidney is the main source of circulating renin, renin isoenzymes have been found in other tissues such as the uterus and placenta (Bing & Faarup,1966; Symonds, Stanley & Skinner,1968), amniotic fluid (Brown et al.,1964), adrenal gland (Naruse et al.,1983), salivary gland (Werle,1968) and brain (Inagami et al.,1982; Ganten et al.,1971;1983). Renin is also present in the vasculature

	1	2	3	4	5	6	7	8	9	10	Renin ↓ 11	12	13	14
Horse	asp	arg	val	tyr	ile	his	pro	phe	his	leu	leu	val	tyr	ser
Human	asp	arg	val	tyr	ile	his	pro	phe	his	leu	val	ile	his	thr
Rat	asp	arg	val	tyr	ile	his	pro	phe	his	leu	leu	tyr	tyr	ser

Figure 1.2 Comparison of the N-terminal sequences of equine, human and rat angiotensinogens.

(Thurston et al.,1979; Loudon et al.,1982;1983). More recently renin mRNA expression has been identified in a range of extra renal sites (Samani et al.,1987; Samani, Brammar & Swales,1988). The precise function of these extrarenal renins and in particular whether brain renin is important in the regulation of blood pressure is far from clear (Reid,1977; Ganong,1977), however it is possible that angiotensin II generated locally within resistance vessels might act to modulate both vascular tone and the pressor response to injected angiotensin II (Swales,1979;1983a&b; Dzau,1986; Swales & Heagerty,1987). This will be discussed in more detail later (see 5.4:1).

Inagami and Murakami (1977) were first to isolate renin from pig kidneys in a pure and stable form. They showed it to have a molecular weight of 36,400. Rat renin in the form of three isoenzymes was purified in 1978 by Matoba, Murakami and Inagami with molecular weights 35,000, 36,000 and 37,000. Using naturally occurring substrates the pH optimum was between 5.5 and 6.5 and the average half life in normal rats was 11.5 minutes. Human renin was eventually purified in 1980 by Yokosawa et al. and shown to have a molecular weight of 40,000. More recently the purification of renin has been greatly simplified using a new transition state analogue of renin substrate (H-77) as an affinity ligand (McIntyre et al.,1983).

Renin has been described in an inactive form which can be activated by acidification to pH 3.3, by trypsin,

by cooling to -5 C or by kallikrein (Day & Leutcher,1975; Atlas, Sealey & Laragh,1978; Sealey et al.,1978; Leckie,1981). The physiological importance of inactive renin is uncertain and the concept that it may be a precursor of renin has yet to be proved (Leckie,1981).

(ii) Control of renin release:

There are five main interacting mechanisms governing the release of renin from the juxtaglomerular apparatus:

1. Intrarenal baroreceptors which are thought to respond to changes of stretch in the wall of the afferent arteriole.
2. The detection of filtered sodium by the macula densa (chemoreceptor).
3. The autonomic nervous system and circulating catecholamines.
4. Other hormones (angiotensin II and vasopressin).
5. Plasma electrolytes (potassium).

Calcium is recognised as an important intracellular link between stimulus and effect in many hormonal systems (Rubin,1970) and is present in both secretory and smooth muscle cells. In 1977 Peart suggested that calcium plays a central role in controlling renin secretion, and Baumbach and Leyssac (1977) demonstratsd that intracellular rather than extracellular calcium was important as regards renin release. This has

led to the view that cytosolic calcium is an intracellular signal which regulates renin secretion through the baroreceptor, macula densa and neurohormonal pathways. Factors which physiologically stimulate renin secretion do so by lowering cytosolic calcium and those which inhibit secretion do so by raising calcium. This inverse relationship is in contrast to most similar secretory systems where there is a direct relationship between intracellular calcium and secretion. (For a review see Fray, Park & Valentine,1987).

1. The intrarenal baroreceptor: The constriction of the renal arteries of dogs by Goldblatt et al. (1934) first indicated that reduced renal perfusion pressure could be a stimulus for renin release. In 1959 Tobian, Tomboullan and Janecek observed reduced juxtaglomerular cell renal perfusion pressure and postulated a renal baroreceptor which responded to changes in perfusion pressure. In 1964 Skinner, McCubbin and Page were first to demonstrate increased renin release in response to decreased perfusion pressure following graded aortic constriction. It was proposed by Tobian (1962) that this baroreceptor or 'stretch' receptor was situated on the afferent arteriole, the amount of stretch being determined by the perfusion pressure and intrarenal tissue pressure. However a recent analysis of the strech receptor hypothesis shows that the receptor responds most consistently to changes in cytosolic calcium (Fray, Lush & Park,1986). High renal

perfusion pressure increases calcium permeability and promotes inflow of calcium thus inhibiting renin secretion.

2. The macula densa: Brown et al. (1963) were the first to demonstrate the link between sodium intake and changes in renin concentration, and Vander & Miller (1964) were the first to study in more detail the relationship between tubular sodium load and renin secretion. Churchill, Churchill and McDonald (1978), using micropuncture techniques, have demonstrated a clear inverse relationship between renin secretion and sodium load. It is now generally believed that the macula densa receptor (or chemoreceptor) operates by detecting changes in distal tubule fluid composition, either as a consequence of the rate of delivery of sodium to the distal tubule, or by the rate of transport of sodium by the macula densa. It has been suggested that intracellular calcium may be the final signal through which the macula densa communicates with the juxtaglomerular cell and thus the renin secretory process (Fray et al.,1987), increased sodium load being linked with an increase in calcium influx to juxtaglomerular cells.

3. Autonomic nervous system and catecholamines: The granular juxtaglomerular cells are innervated by sympathetic fibres (Nilsson,1965; Biava & West,1966; Wagermark, Ungerstedt & Ljungqvist,1968) and electrical

stimulation of the nerves releases renin into the circulation (Vander,1965). Renal nerve stimulated renin release is blocked by the action of propranalol (Assaykeen & Ganong, 1971) indicating that this mechanism probably operates via β-receptors.

Scornick and Paladini (1964) were the first to demonstrate increased renin release in response to infused noradrenaline. In 1970, Ueda et al. confirmed this observation and showed that it was accompanied by a reduced renal blood flow, glomerular filtration and sodium excretion. They also showed that the renin response could be blocked by propanalol, suggesting a β-receptor mediated mechanism.

β-adrenergic agonists have been shown to stimulate renin release by a process involving cyclic AMP. Cyclic AMP lowers cytosolic calcium by increasing calcium efflux and by sequestering calcium into intracellular storage sites (Churchill,1985) and thus calcium appears to be the final intracellular signal for the neurohormonal pathway of renin secretion. ∝-adrenergic agonists raise cytosolic calcium by increasing calcium influx and thus inhibit renin secretion (Hackenthal, Schwertschlag & Taugner,1983).

4. Other hormones: Angiotensin II has an inhibitory effect on renin secretion (Vander & Geelhoed,1965) and is thought to operate through two negative feedback loops. The first of these is the 'short loop' (Keeton, Pettinger

& Campbell,1976) in which angiotensin II acts directly on the juxtaglomerular cells via renal receptors similar to those in the vasculature (Naftilan & Oparil,1978). Evidence to support a renal receptor mediated effect comes from the observation that the angiotensin receptor antagonist Sar1-Ala8-angiotensin II, saralasin, blocked the effect. The 'long loop' operates more slowly via stimulation of aldosterone with sodium retention and consequent expansion of extracellular fluid volume which in turn suppresses renin release via the macula densa and baroreceptor mechanisms.

Vasopressin also inhibits renin release (Bunag, Page & McCubbin,1967). This effect may operate by a direct mechanism or indirectly via haemodynamic changes. Vandongen (1975) using the isolated perfused rat kidney was unable to demonstrate an effect of vasopressin on renin release while Konrads et al. (1978) concluded that its ability to suppress renin was independent of its vasoconstrictor effect. In this respect Morton, Garcia del Rio and Hughes (1982) demonstrated a clear suppressent effect on plasma renin concentration in the conscious rat at vasopressin infusion rates which had no detectable effect on systemic blood pressure.

Again calcium is implicated, calcium influx appears to be the inhibitory signal by which angiotensin II and vasopressin control renin secretion, both peptides promoting calcium influx primarily through voltage sensitive channels (Buhrle, Nobiling & Taugner,1985).

5. Other electrolytes: Potassium infused at very low concentrations inhibits renin release. The mechanism of this is not clear but Schneider et al. (1972) have suggested that it is an indirect action in which potassium inhibits sodium reabsorption in the ascending limb of the loop of Henle thereby increasing the sodium load at the macula densa which in turn suppresses renin release.

1.3:3 Angiotensin I

The decapeptide angiotensin I is formed by the action of renin on renin substrate (Figure 1.3). It has been suggested that angiotensin I, at high concentrations, may act on the adrenal medulla to release catecholamines (Peach, Bumpus & Khairallah,1971) and on the kidney to alter blood flow (Itskovitz & McGiff,1974). It is possible, however, that the effect is due to angiotensin II formed by localised conversion of angiotensin I (Leckie et al.,1972; Peach,1977). Certainly, at more physiological concentrations it is probable that angiotensin I possesses little or no biological action.

1.3:4 Angiotensin Converting enzyme.

Angiotensin converting enzyme is a non specific peptidyl dipeptide hydrolase and cleaves bonds of a wide variety of amino acids (Yang, Erdos & Levin,1971). It

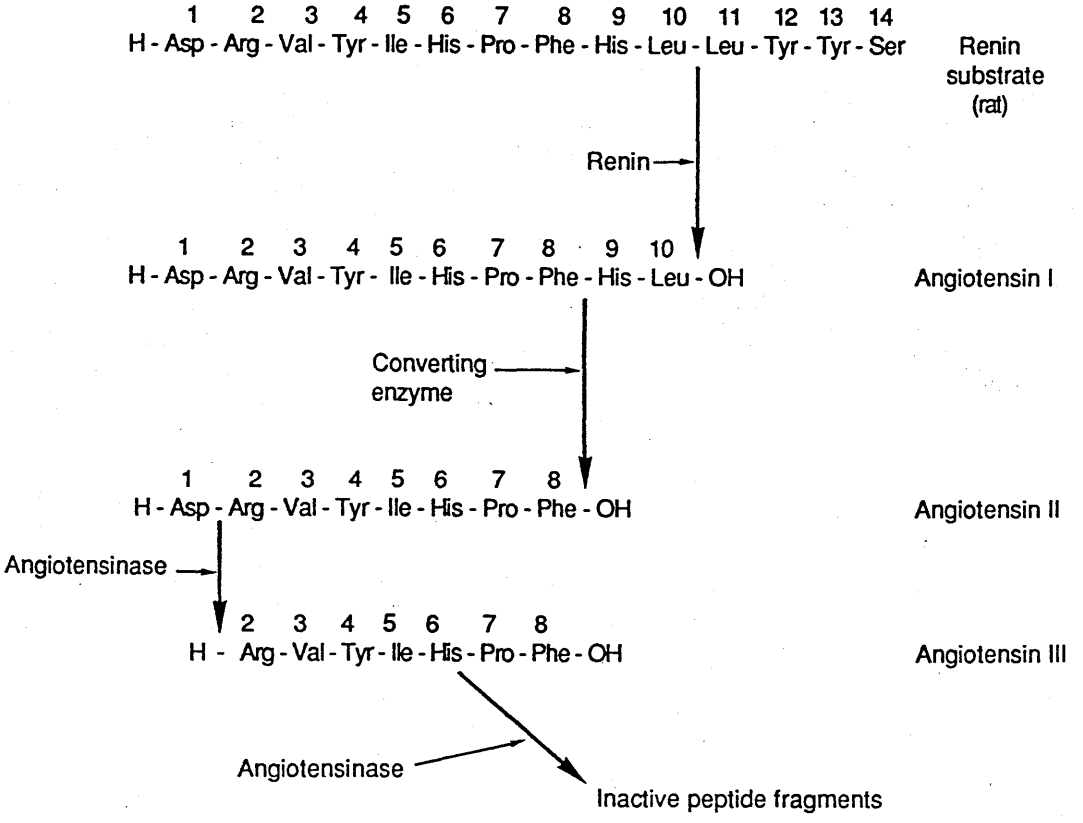


Figure 1.3 Formation and metabolism of the angiotensins.

converts angiotensin I to angiotensin II by removing the C-terminal histidyl leucine dipeptide (Figure 1.3) and inactivates bradykinin to peptide fragments. It is now accepted that converting enzyme and kininase II, which degrades bradykinin, are the same enzyme (Yang, Erdos & Levin,1970). Consequently the same enzyme both activates a hypertensive mechanism and inactivates a hypotensive mechanism. In 1968, Ng & Vane demonstrated in the dog that conversion of angiotensin I to angiotensin II in plasma was very slow. In contrast, passage of angiotensin I through the lungs resulted in large amounts of angiotensin II being formed. The lung has since been shown to contain large amounts of converting enzyme localised in the vascular endothelium of the pulmonary arteries (Aiken & Vane,1970; Cushman & Cheung,1972), and is the main site for the formation of angiotensin II. Converting enzyme is also present in other tissues including the kidney (Oshima, Gesce & Erdos,1974), rat brain (Yang & Neff,1972), rat testes (Cushman & Cheung,1971a) and peripheral vascular tissue (Aiken & Vane,1972; Ryan et al.,1976a; Takada et al.,1982; Miyazaki et al.,1984). The converting enzyme-angiotensin I reaction has a Km of 5x10-5M (Cushman & Cheung,1972) indicating that it is probably not a rate limiting step in the formation of angiotensin II *in vivo* as the circulating concentration of angiotensin I is much lower, in the region of 5x10-11M. (For a review of converting enzyme see Erdos,1975).

1.3:5 Angiotensin II.

(i) Biological properties:

Angiotensin II is recognised as the main effector hormone of the renin angiotensin system and has a number of important physiological and pharmacological properties. The most important are:

1. It has the ability to raise blood pressure both acutely and by a slow chronic effect.
2. It affects the central nervous system to raise blood pressure, promote drinking, and stimulate vasopressin secretion.
3. It interacts with the sympathetic nervous system at several points.
4. It stimulates the secretion of aldosterone in the adrenal cortex.
5. It has a distinct intrarenal function regulating renal haemodynamics.

1. Pressor actions: Angiotensin II is one of the most potent vasoconstrictor substances known (Oparil & Haber,1974). It has selectivity for arterial vessels, being less potent as a venoconstricter (Folkow, Johansson & Mellander,1961). The characteristic response following intravenous injection is a prompt rise in blood pressure reaching a maximum at about one minute and with a duration of response of approximately 5 minutes. If doses are

large tachyphylaxis may occur (Bock & Gross,1961). The blood pressure responses to angiotensin II are partly influenced by the prevailing sodium status, there being an inverse relationship between dietary sodium intake and pressor response. Recent studies indicate that endogenous angiotensin II has the ability to regulate the number of its own receptors (Gunther, Gimbrone & Alexander,1980; Aguilera & Catt,1981; McQueen & Semple,1987). High endogenous angiotensin II levels (as seen during low salt status) down-regulate the number of receptors resulting in a diminished pressor response. Conversely there is up-regulation of receptor numbers during high salt intake.

The mechanism by which angiotensin II acts on vascular smooth muscle to produce vasoconstriction is believed to be an interaction of angiotensin with its membrane bound receptor which in turn produces a rise in intracellular ionized calcium (Daniels & Kwan,1981) producing the contractile response. The interaction of angiotensin with its receptor brings about a rise in intracellular calcium by increasing the permeability of the membrane to calcium through depolarisation and/or an effect on receptor operated channels (ROC's) and also by releasing bound calcium within the cell by activation of the second messenger inositol triphosphate (Bolton,1979; Berridge,1984; Berridge & Irvine,1984; Brock et al.,1985; Capponi, Lew & Vallotton,1985; Nabika et al,1985).

As well as a rapid effect on blood pressure, angiotensin II also has a slow pressor property raising

arterial pressure gradually when infused at low doses for a prolonged period of time. This effect has been demonstrated in the rabbit (Dickinson & Lawrence,1963), dog (McCubbin et al.,1965; Bean et al.,1979), rat (Brown et al.,1981; Brown, Clark & Lever,1983) and man (Ames et al.,1965). The mechanism of this phenomenon is unclear and some possibilities for it will be discussed in detail later (5.4.3). These include action of angiotensin II on the nervous system, effect of salt and water excretion, structural changes in the blood vessel wall and vascular renin.

2. Central nervous system: An increase in blood pressure induced by centrally administered angiotensin has been demonstrated in the dog (Lowe & Scroop,1969), cat (Severs et al.,1966) and the rat (Fink et al.,1980).

Angiotensin II administered into the central nervous system stimulates drinking in several species (Epstein, Fitzsimons & Rolls,1970; Fitzsimons, Kucharczyk & Richards,1978). The subfornical region appears most sensitive to this response with as little as 0.1ng angiotensin II eliciting a drinking response (Simpson & Routtenberg, 1973). Recent studies in man in which angiotensin was infused into the circulation (Phillips et al.,1985) suggest that this response is more pharmacological than physiological as it was observed only at very high plasma concentrations.

It has been claimed that angiotensin II stimulates

the secretion of arginine vasopressin from the posterior pituitary (Bonjour & Malvin,1970; Mouw et al.,1971; Keil, Summy-Long & Severs,1975) This however has not been confirmed by other workers (Claybaugh, Share & Shimuzu,1972; Shade & Share,1975) and so there is controversy as to whether this relatioship is of physiological importance. Following angiotensin II infusion in man, Padfield & Morton (1977) found increased plasma vasopressin but only at supraphysiological levels of angiotensin. At more physiological levels, found during sod ium depletion, there was no increase in plasma vasopressin (Morton et al.,1985). Similarly, in the dog, infusion of physiological levels of angiotensin II had no effect on the long term control of vasopressin secretion (Cowley, Switzer & Skelton,1981).

3. Sympathetic nervous system: There is now good evidence that some of the effects of angiotensin II result from an interaction with the sympathetic nervous system (McCubbin & Page,1963; Kaneko et al.,1966). The precise mechanism is uncertain but it is believed that there is both a postsynaptic and a presynapyic effect. The former is thought to involve an increase in the sensitivity of the effector to noradrenaline (Zimmerman,1978), while the latter is thought to involve an augmentation of the outflow of noradrenaline following nerve stimulation (Zimmerman & Whitmore,1967), either by inhibiting neuronal uptake of noradrenaline (Khairallah,1972) or by increasing

the amount of transmitter released (Starke,1977).

4. Aldosterone stimulation: The possibility that renin might stimulate the synthesis and release of aldosterone by the adrenal cortex was suggested by Gross in 1958. In 1961 Davis et al. demonstrated this effect and at about the same time it was shown that angiotensin II was the effector hormone (Laragh et al.,1960; Biron et al.,1961). There is now little doubt that angiotensin II stimulates the synthesis of aldosterone and is probably the major factor controlling its secretion in response to alteration in sodium balance (Oelkers et al.,1974; Nicholls et al.,1978).

5. Renal effects: Angiotensin II infused at low doses results in retention of sodium and water (Malvin & Vander,1967; Waugh,1972),and it has been proposed that this may be due to angiotensin II exerting a direct stimulatory effect on tubular reabsorption (Johnson & Malvin,1977). Following the detection of angiotensin II within the rat kidney, it has been proposed (Lever & Peart,1962; Mendelsohn,1979) that there is an intrarenal renin system with angiotensin II acting as a local hormone regulating renal haemodynamics. (For review see Levens, Peach & Carey,1981).

(ii) Destruction of angiotensin II:

The circulating half-life of angiotensin II is less than 30 seconds. Although degradative enzymes (angiotensinases) are present in blood, angiotensin II is catabolised mainly during passage through tissue vascular beds including the spleen, limbs, kidney and brain (Hodge, Ng & Vane,1967; Ng & Vane,1968; Bakhle, Reynard & Vane 1969; Bailie & Oparil,1977) Three main groups of enzymes have been shown to hydrolyse angiotensin II:

(a) Aminopeptidases or angiotensinase 'A': These degrade the peptide at the N-terminal bond. This is thought to be the main degradative enzyme (Ryan,1974). There are now known to be two aminopeptidases; angiotensinase A1 which is specific for Asn1 angiotensin II, and angiotensinase A2 which is specific for Asp1 angiotensin II.
Aminopeptidases have a requirement for calcium.

(b) Endopeptidases or angiotensinase 'B': These hydrolyse bonds within the peptide and were discovered by Regoli, Riniker and Brunner (1963).

(c) Carboxypeptidases or angiotensinase 'C': These hydrolyse the C-terminal peptide bond and are probably the least important of the angiotensinases.

1.3:6 Angiotensin III.

This is a heptapeptide (des Asp1 angiotensin II) formed by the action of aminopeptidase A1 on angiotensin II. An alternative pathway to its formation has been described in the rat (Garcia del Rio, Smellie &

Morton,1981) in which aminopeptidase A1 hydrolyses the N-terminal aspartic acid of angiotensin I to form des Asp1 angiotensin I which is then a substrate for converting enzyme to form angiotensin III without prior formation of angiotensin II. Angiotensin III possesses some residual pressor activity (Kono et al.,1975; Carey et al.,1979) and was at first thought to be an important steroidogenic hormone (Blair-West et al.,1971; Campbell, Brooks & Pettinger,1974; Campbell, Schmitz & Itskovitz,1977; Freeman et al.,1977a). However with the exception of the rat (Semple & Morton,1976) the concentration of angiotensin III in plasma is small relative to the octapeptide (Semple et al.,1976; Carravagi et al.,1976).

1.4 Inhibitors of the renin-angiotensin system.

1.4:1 Structural analogues.

Analogues of angiotensin II which compete for the angiotensin II receptor were one of the first groups of inhibitors of the renin-angiotensin system to be developed. Their conception followed the recognition by Bumpus & Smeby (1968) of the initial importance of position 8 (phenylalanine) for the biological activity of angiotensin II. In 1970, Khairallah, Toth and Bumpus observed that Ala8-angiotensin II blocked the contractile response of angiotensin II on the isolated guinea pig

ileum. Shortly afterwards Ile8-angiotensin II was synthesised (Bumpus,1971). However both these analogues suffered from poor *in vivo* activity. This was eventually improved by substituting sarcosine at position 1 (Pals et al.,1971) which protected the analogue from degradation by aminopeptidase A, creating a more effective inhibitor and increasing its half life (Bumpus,1977). However all inhibitory analogues suffer from the disadvantage, to a greater or lesser extent, of residual agonist activity. This disadvantage has severely limited the use of such inhibitors as investigative tools to study the renin angiotensin system. The three most effective inhibitors, listed in order of increasing agonist activity are Sar1,Ile8-angiotensinII, Sar1,Ala8-angiotensin II, Sar1,Thr8-angiotensin II (Bumpus,1977).

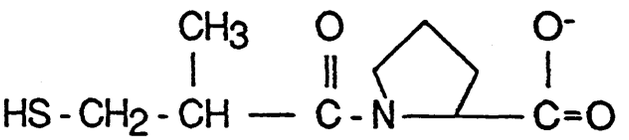
1.4:2 Angiotensin converting enzyme inhibitors.

Before 1968, the only known inhibitors of angiotensin converting enzyme were nonspecific metal chelators such as EDTA. However the key discovery that led to the first potent and specific inhibitor of this enzyme was published three years earlier. In 1965 Ferreira showed that an ethanol extract of the Brazilian arrowhead viper *Bothrops jararaca* , potentiated the vasodilating effects of bradykinin. In 1966, Ferreira was able to show that the activity of the bradykinin potentiating factor (BPF) was due to its inhibition of the

enzymatic degradation of bradykinin by kininases. Soon after, Bakhle (1968) reported that the bradykinin potentiating factor (BPF) was also a potent inhibitor of angiotensin converting enzyme, inhibiting the formation of angiotensin II. It was subsequently shown that converting enzyme and kininase II were the same enzyme (Yang et al.,1970). Up to fifteen active peptides were isolated, characterised and eventually synthesised (Ferreira, Bartlet & Greene,1970; Ondetti et al.,1971). Two of the most potent were a pentapeptide (BPF5a) and a nonapeptide (SQ 20881, Figure 1.4). BPF5a has a short duration of activity *in vivo* being rapidly broken down by peptidases, including converting enzyme. SQ 20881 (Teprotide) has a much longer half life (1 to 2 hours) and while it is an invaluable investigative tool, and shown to be effective for the treatment of human hypertension, it suffers from the disadvantage that it is active only by parenteral administration. To overcome this, orally active inhibitors of converting enzyme were designed and synthesised (Cushman et al.,1977; Ondetti, Rubin & Cushman,1977). The design of these orally active compounds was based on the known active site of carboxypeptidase A, a zinc metallopeptidase thought to be similar to converting enzyme. Derivatives of proline were chosen as this amino acid is present at the C-terminal position of all the venom peptide inhibitors. Of these derivatives, 2-D-methyl-3-mercaptopropanoyl-L-proline (SQ 14225, captopril, Figure 1.4) was found to be the most

Glu- Trp - Pro - Arg - Pro - Gln - Ile - Pro - Pro

SQ 20881 Teprotide



SQ 14225 Captopril

Figure 1.4 Teprotide and captopril.

potent both in vitro and in vivo . Since the development of captopril other orally active inhibitors have been synthesised, all of which are based on the origional proline model.

One potential disadvantage of captopril as a research tool for the investigation of the renin-angiotensin system in its regulation of both normal blood pressure and hypertension, concerns its specificity. Any hypotensive effect it elicits might, in part, result from a potentiation of the vasodilator effect of bradykinin. However direct evidence for the involvement of kinins in the hypotensive effect of captopril is scant, principally due to the difficulty of measuring kinin blood levels. While McCaa, Hall and McCaa (1978) reported increased levels after captopril, Millar & Johnston (1979) could find no change. More recent studies have failed to detect bradykinin in normal blood (Nielsen et al.,1982) suggesting that it is possibly a tissue hormone rather than a circulating hormone. Similarly, substantive evidence that other extra-renin mechanisms are involved is scant. To date, most of its blood pressure lowering effects can be explained on the basis of its interaction with the renin angiotensin system, the function for which it was specifically designed (Cushman et al.,1981).

1.4:3 Renin inhibitors.

Another approach used to inhibit the actions of

the renin angiotensin system has been to synthesise peptides which inhibit directly the activity of renin itself. In general three methods have been used, all of which involve modifying the N-terminal fragment of angiotensinogen. The first of these, used by Burton, Poulsen and Haber (1975), was to substitute various naturally occurring amino acids at different positions in the peptide sequence, particularly at positions 10 and 11 which flank the peptide bond hydrolysed by renin. These initial inhibitors had a ki of 3 to 25 uM. However they proved ineffective *in vivo* due to poor solubility at physiological pH. Haber (1980) described the decapeptide Pro5,Phe10,Phe11,Lys14 angiotensinogen as an effective inhibitor of renin *in vivo* (ki=2uM). The second method has been to substitute the unusual amino acid statine at position 10 (Boger et al.,1983). Inhibition with ki values as low as 27 nM have been obtained by this technique. Probably the most effective inhibitors have been obtained by the method of Szelke et al. (1982a) in which the Leu10,Val11 peptide bond is replaced with a reduced isostere, that is -CO-NH- is replaced with -CH2-NH-. In this way potent inhibitors of both dog (ki=20nM) and human (ki=10nM) renin have been obtained which are both highly active *in vivo* (Szelke et al.,1982b; Webb et al.,1983). At present renin inhibitors suffer from one disadvantage; like angiotensin II inhibitory analogues and the early peptide inhibitors of converting enzyme, they are active only by parenteral

administration. However as in the case of converting enzyme inhibitors, orally active non-peptide renin inhibitors will certainly be developed.

1.5 Experimental renovascular hypertension, historical background.

The experimental production of renal hypertension is generally considered to date from the definitive studies of Goldblatt and colleagues in 1934. By constriction of both renal arteries of the dog they were able to produce, for the first time, a sustained elevation of arterial pressure. In contrast, constriction of one renal artery produced only a temporary rise in pressure. It is from this original work that the two most common models of experimental renal hypertension have been derived. The first of these is the two-kidney, one clip model (2K1C) in which one renal artery (usually the left) is constricted by means of a silver clip, while the contralateral kidney is untouched. The second is the one-kidney, one clip model (1K1C) in which one renal artery is constricted and the contralateral kidney is removed. Interestingly, there appears to be some species difference in the susceptibility of different animals to the development of hypertension by these procedures. The dog, as demonstrated by Goldblatt, and the rabbit require either bilateral artery constriction, or unilateral

constriction with contralateral nephrectomy to produce permanent hypertension. In contrast, the rat (Wilson & Byrom,1939) like man, may be rendered hypertensive by unilateral constriction only (2K1C).

1.6 Role of renin in renovascular hypertension, acute and chronic phases.

These observations rekindled interest in renin and its possible involvement in the development of renal hypertension of the type described. At the time methods for the measurement of circulating renin were not generally available and investigators had to rely on indirect techniques. These involved removal of the clip producing the constriction, removal of the kidney whose artery had been clipped, and in the two-kidney, one clip model, removal of the contralateral untouched kidney. Using these techniques, Pickering (1945) was the first to suggest that there may be two phases in renal hypertension. He demonstrated that removal of a single clipped kidney in rabbits (1K1C) produced a fall in blood pressure with hypertension of one weeks duration but not of eight weeks duration. The two phases were called respectively the acute and chronic phases of renal hypertension. In addition to this, Byrom & Dodson (1949) showed that by removing the clip from the renal artery of rats with one-kidney, one clip hypertension blood pressure

returned to normal regardless of whether they were in the acute or chronic stage.

Two conclusions were drawn from these studies. Firstly in the one-kidney, one clip model, hypertension during the acute stage results from an intrarenal mechanism, possibly involving the direct vasoconstrictor effect of renin, but that during the chronic stage hypertension is maintained by an extrarenal mechanism not involving renin and which exists as a consequence of the inadequate renal function of the clipped kidney. Secondly, the cardiovascular changes which occur to produce the hypertension in the chronic stage are not irreversible.

The effects of such manoeuvres in rats with two-kidney, one clip hypertension showed some important differences compared to the one-kidney, one clip model. While removal of the clip in the acute phase resulted in the blood pressure returning to normal (Floyer,1954) this was not always the case during the chronic stage (Wilson & Byrom,1941; Floyer,1951). Also, subsequent removal of the clipped kidney resulted in this residual hypertension being sustained at an even higher level. However, if the untouched kidney was removed the residual hypertension disappeared and the pressure returned to normal. The interpretation of these observations was that, as in the case of the one-kidney, one clip model, renin from the kidney was responsible for the hypertension during the acute phase. During the chronic phase however, renin was

not involved directly and hypertension was maintained by an extrarenal mechanism, partly because of an inability of the clipped kidney to control blood pressure but also by a contributing effect of the opposite untouched kidney. Wilson & Byrom (1939) clearly showed that in two-kidney, one clip hypertension prolonged exposure to the hypertension resulted in the appearance of vascular lesions in the untouched kidney and when this occured removal of the ischaemic kidney no longer returned the blood pressure to normal. It was concluded that the damaged untouched kidney contributes to the hypertension producing a further rise in pressure which results in further progressive damage to the kidney (Byrom & Dodson,1949). This situation was described by Wilson & Byrom (1939) as the vicious circle of hypertension. However, Floyer (1954,1957) claimed that such persistant hypertension can occur in the presence of an apparently normal untouched kidney and before the appearance of hypertensive vascular damage. He suggested that renin released from the clipped kidney, while insufficient to have a direct vasoconstrictor effect, might have, through angiotensin II, a localised effect on the opposite kidney and inhibit the function concerned with maintaining normal blood pressure. This observation and its possible explanation has particular relevance to the work presented in this thesis.

With the advent of reliable methods for the measurement of renin in plasma it became possible to

examine directly its involvement in renal hypertension. In one-kidney, one clip hypertension renin levels are raised acutely and then fall to normal within a few days (Lever & Robertson,1964; Brown et al.,1966; Bianchi, Tilde Tenconi & Lucca,1970; Miksche, Miksche & Gross,1970; Liard et al.,1974). In two-kidney, one clip hypertension the initial rise in renin is followed either by a return to normal levels or the presence of a mildly elevated level: in the presence of severe or malignant hypertension the level is usually elevated (Brown et al.,1966; Blair-West et al.,1968; Bianchi et al.,1972; Leenen, DeJong & DeWied,1973; Leenen et al.,1975; Liard et al.,1974; Mohring et al.,1975; Carretero & Gulati,1978). The renin content of the clipped kidney is raised and that of the contralateral kidney lowered (Miksche et al.,1970; Mohring et al.,1975).

The rise in blood pressure during the acute phase is almost certainly due to the increase in circulating renin and to the direct action of generated angiotensin II on the peripheral resistance vessels. The relationship between changes in pressure and changes in circulating renin and angiotensin II was similar to that seen when renin and angiotensin II were infused (Bianchi et al.,1970; Cowley, Miller & Guyton,1971; Gutmann et al.,1973; Caravaggi et al.,1976). Also treatment with angiotensin converting enzyme inhibitors or angiotensin receptor antagonists during the acute phase effectively prevented the rise in blood pressure (Pals et al.,1971;

Miller et al.,1972,1975; Thurston & Swales,1974a; Coleman & Guyton,1975; Freeman et al.,1977b; Masaki et al.,1977).

During the chronic phase of one-kidney, one clip hypertension renin is normal and acute blockade of the renin-angiotensin system fails to lower blood pressure (Krieger et al.,1971; Bumpus et al.,1973; Romero, Mak & Hoobler,1974) unless sodium depletion is induced (Gavras et al.,1973). In chronic two-kidney, one clip hypertension renin has been reported as being normal or slightly elevated and, if in the malignant phase, raised, and acute blockade is only partially sucessful in lowering blood pressure (Brunner et al.,1971; Krieger et al.,1971; Pals et al.,1971; Bumpus et al.,1973; Romero et al.,1974; Thurston & Swales,1974a; Carretero & Gulati,1978) unless again sodium depletion is induced (Gavras et al.,1975).

These latter studies appeared to confirm the original conclusions of Floyer (1951,1957) that the acute phase of renal hypertension was renal in origin, acting through renin, and that the chronic phase was extrarenal, not involving renin.

1.7 Autoregulatory theory of chronic renovascular hypertension.

In view of the failure of the acute effects of the renin-angiotensin system satisfactorily to account for chronic renal hypertension the hypothesis of a volume

expanded mechanism was advanced (Ledingham,1975). This is based on the same homeostatic mechanism as that advanced to explain the hypertension seen in partially or completely nephrectomised sodium-loaded animals (Ledingham,1971). The postulated steps are shown in Figure 1.5. Application of a clip to the renal artery results in an increase in renin release into the circulation. This in turn produces an increase in angiotensin II which has a direct vasoconstrictor effect and raises blood pressure acutely. Angiotensin II also stimulates sodium retention via aldosterone and also possibly by a direct intrarenal effect. This results in an expansion of extracellular fluid and plasma volume. This increases cardiac output and leads to tissue overperfusion. In response there is local autoregulatory vasoconstriction which results in a chronic rise in arterial pressure. There will then be a tendency to overcome the renal retention of salt and water with the eventual restitution of volume homeostasis. Both cardiac output and renin will return towards normal. All of this is brought about at the expense of a sustained elevation of arterial pressure. As propounded by Guyton et al. (1972,1975) the fundamental change in the process is a resetting upwards of the pressure threshold for renal sodium and water excretion. In this view of events the renin-angiotensin system is important only during the acute phase when it has a direct action in raising blood pressure, and as a pre-requisite for the development of

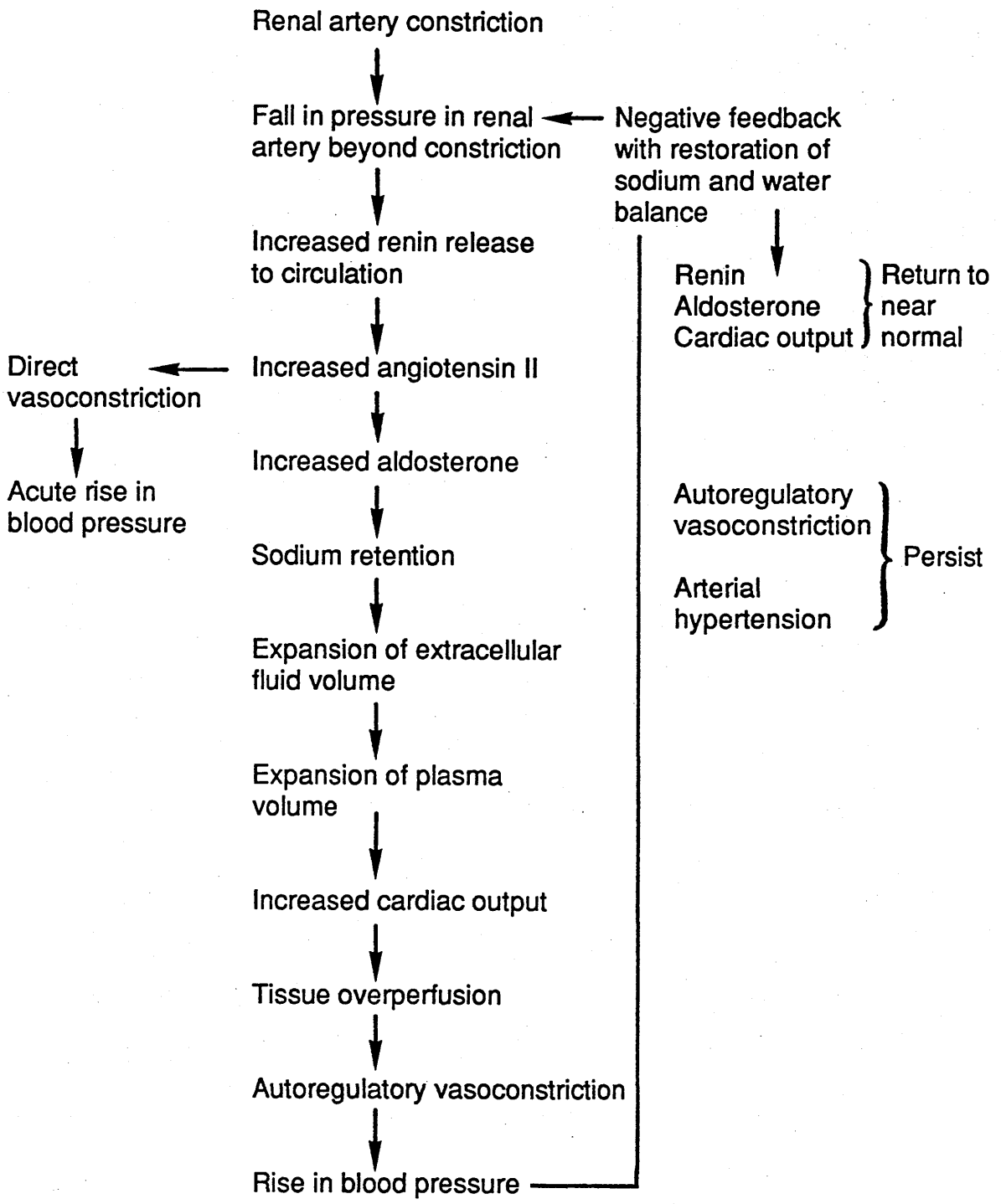


Figure 1.5 Postulated sequence of events during the development of hypertension after renal artery constriction.

the hypertension in the chronic stage, but that during this time its direct vasoconsrictor action has no direct role in maintaining the hypertension.

Experimental evidence would seem to support such a mechanism in one-kidney, one clip hypertension. As mentioned, renin is elevated acutely then returns to normal. Aldosterone has been shown to be initially, transiently elevated (Blair-West et al.,1968; Liard et al.,1974). There is a higher than normal exchangable sodium (Tobian, Coffee & McCrea,1969; McAreavey et al.,1984) and there is initially a positive sodium balance (Bianchi et al.,1970; Swales et al.,1972; Liard et al.,1974). Cardiac output is also raised initially then falls (Bianchi et al.,1970).

Some of the evidence from studies of two-kidney, one clip hypertension also fit with a volume expanded mechanism but there may be important differences. In particular it is by no means certain that there is overt sodium retention in this model. Only two studies found a positive sodium balance during the early phase (Leenen & De Jong,1975; Mohring et al.,1975) while most found either a negative sodium balance (Blair-West et al.,1968; Swales et al.,1972) or no change (Bianchi et al.,1972; Tobian et al.,1969; McAreavey, Brown & Robertson,1982; McAreavey et al.,1984). Also the extent to which renin returns towards normal after the the initial increase is uncertain. This uncertainty rises from the variety of methods employed by different investigators to study this model. These

include differences in strain, age and sex of rat, the size of clip used and the site at which it was applied to produce the stenosis, blood sampling procedures, used to measure circulating renin, have also varied and these differences have often made it difficult to compare results obtained from different laboratories and may go some way to explaining some of the contradictory findings published in the literature. In view of these differences and uncertainties and taking into account the original suggestion of Kolff & Page (1955) and Floyer (1957) that renin released from the clipped kidney, though insufficient to affect blood pressure directly, might nontheless have a functional effect on the opposite untouched kidney and thereby raise blood pressure by a second mechanism, it is conceivable that there may be a subtle but important difference in the route by which volume homeostasic hypertension is reached in the two-kidney, one clip model.

1.8 A possible second effect of angiotensin II on blood pressure in two-kidney, one clip hypertension.

The possibility that renin, through angiotensin II, might contribute to the raised blood pressure in renovascular hypertension by a second effect was also suggested by Brown et al. (1976). They proposed that between the acute and chronic phases there was a second or

intermediate phase. This intermediate phase was characterised by blood pressure being inappropriately high for prevailing plasma levels of renin and angiotensin II. It was proposed that hypertension during this time was maintained principally by a second effect of angiotensin II on blood pressure. It was suggested that this might be a slowly developing effect which is brought about by plasma angiotensin II concentrations below those which are acutely vasoconstrictor (Dickinson & Lawrence,1963; McCubbin et al.,1965). Studies to investigate the possible involvement of such an effect in renovascular hypertension have been controversial.. There is good evidence that chronic suppression of angiotensin II by continuous administration of converting enzyme inhibitors teprotide and captopril before and up to 24 weeks after application of the clip effectively prevents the subsequent development of the hypertension (Freeman et al.,1979; DeForrest et al.,1982). However, the ability of chronic angiotensin II blockade to lower blood pressure after the hypertension has developed is less certain. Using two-kidney, one clip hypertensive rats, Riegger et al. (1977) claimed that chronic infusion of both saralasin and teprotide produced an additional significant fall in blood pressure and Bengis & Coleman (1979) found arterial pressure was restored to normal after 1 week of converting enzyme inhibitor administration. However, Bing et al. (1981) and Russell et al. (1982) were unable to find any additional effect on blood pressure following the chronic

infusion of either saralasin or captopril.

1.9. The present study.

The purpose of the present study initially was to establish a standard and reproducible model of two-kidney, one clip hypertension in the rat for use in our laboratory. Using this model I proceeded to examine more precisely the long-term evolution of two-kidney, one clip renovascular hypertension in relation to the changes which take place in the renin-angiotensin system. Once this had been achieved, the importance or otherwise of renin and angiotensin in the development and maintenance of the hypertension at different stages was investigated. The approach to this latter part of the study was to assess not only the the contribution made by the direct acute vasoconstrictor effect of the renin system but also to examine whether renin, through angiotensin II, exerted a second, additional effect on the hypertension. This was done by comparing the effect on blood pressure of acutely administered inhibitors of converting enzyme with that found following their long-term chronic administration.

The whole study was performed in the rat; a small, cheap and robust experimental animal which can easily be rendered hypertensive in a relatively quantitate way by the application of a silver clip to one renal artery, the contralateral kidney remaining untouched. Techniques were

used whereby measurements of blood pressure, plasma renin concentration and plasma angiotensin II could be made in the conscious and unrestrained animal, thus eliminating the effects of anaesthesia and stress.

CHAPTER 2.

MATERIALS AND METHODS.

2.1 Rats.

For all of the studies to be described male Sprague Dawley rats were used. These weighed 150-180g and were supplied by Olac 1976 Ltd (Bicester, Oxfordshire). They ate a standard diet (41B Oxoid) and drank water *ad libitum* throughout.

2.2 Preparation of renal hypertensive animals.

Rats were made hypertensive by constriction of the left renal artery using a silver clip with a 0.2mm internal diameter, the right kidney being untouched (two-kidney, one clip hypertension).

2.2:1 Construction of renal artery clip.

Clips were constructed from silver ribbon 0.38mm by 1.0mm (Johnsson and Matthey Metals Ltd., 43 Hatton Garden, London). A 10mm length of silver was cut from the strip and the edges were filed until smooth. This length was bent round a feeler gauge of appropriate

thickness (0.2mm) to make one short (4mm) and one long (6mm) leg. The end of the longer leg was then bent upwards at an angle of 45 degrees to be used for holding during application of the clip to the artery. Since this may have opened the clip slightly it was then compressed again over the feeler gauge, this time using pliers. The clips were sterilised in a chlorhexidine/alcohol solution and soaked in sterile saline before implantation.

2.2:2 Clipping of renal artery.

Rats were fully anaesthetised with ether which was maintained by swab and drop bottle. The abdomen was shaved and a 3-5cm incision was made in the midline through the skin and the abdominal wall ending at the xiphoid cartilage. The exposed gut was displaced to the left where it was covered with a saline soaked gauze swab. The incision was held open at the right side by rubber covered artery forceps and a cotton bud was placed under the spleen, pancreas and liver in order to hold them away from the area of the left renal artery. This artery was chosen to be clipped as it is more accessible than the right. The left renal artery was exposed and cleared of connective tissue and the clip gently placed across the artery just below its junction with the aorta. If the kidney blanched the clip was immediatly removed and the artery was cleared further before the clip was re-applied. The gut was then replaced and the abdominal muscle was

sutured with surgical silk. The skin was closed either with sutures or with 12mm wound clips.

A sham operation was performed on some animals. The surgical procedure for this was exactly as above except that no clip was applied to the artery. In both these operations the right kidney was untouched.

2.3 Catheterisation.

Indwelling arterial and venous catheters were required for blood pressure measurement, blood sampling and for intravenous injections and infusions. For short-term experiments a carotid artery catheter was implanted, and for long-term experiments requiring continuous blood pressure recording and continuous infusions, catheters were inserted into the abdominal aorta and the inferior vena cava (IVC).

2.3:1 Carotid artery catheters.

(i) Construction of carotid artery catheters.

The form of the carotid catheter is illustrated in Figure 2.1. The construction of the carotid catheter and the materials used are detailed in Appendix 1.

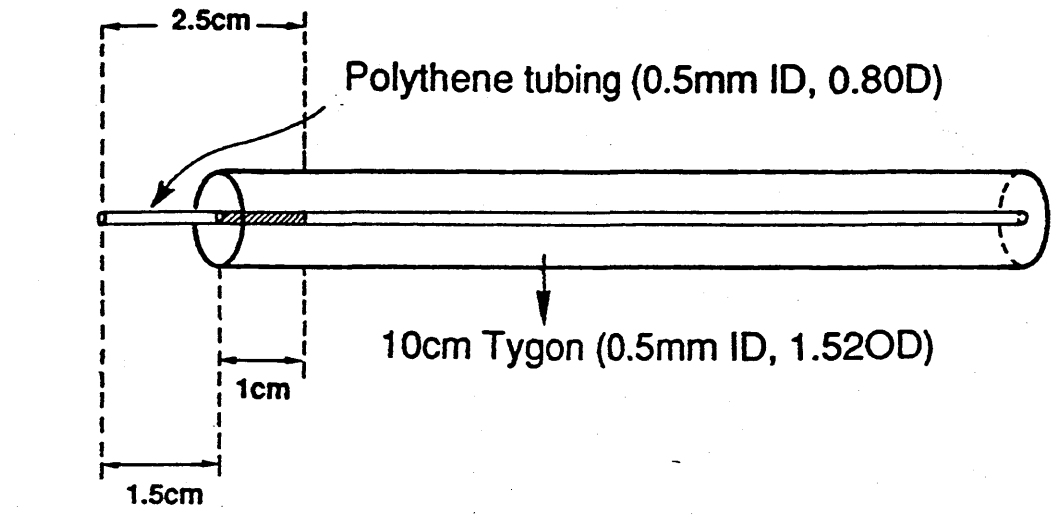


Figure 2.1 Carotid artery catheter
Materials used and construction are detailed in Appendix 1.

(ii) Implantation of a carotid artery catheter.

The catheter was filled with heparin (5000 units/ml) and the end was plugged. The rat was anaesthetised with ether and then shaved at the front and the back of the neck. A 1-2cm longitudinal incision was made at the front of the neck and the left carotid artery was freed from the surrounding muscle and nerves by blunt dissection. Two lengths of surgical silk were passed beneath the artery. The most distal thread from the heart was tied in a knot and gentle traction was exerted on this tie by artery forceps. The second thread was tied in a loose single knot. An aneurism clip was placed as far down the exposed part of the carotid artery as possible in order to stop the blood flow. Using small sharp scissors an incision was made in the side of the carotid artery between the clip and the tie. The catheter was inserted and moved up until its tip lay against the clip. The second tie was knotted more tightly, the clip was removed and the catheter was pushed gently into the artery until the whole of the polythene tip lay within the vessel. The second thread was then tied tightly in a second knot around the catheter. One more thread was sewn into the underlying muscle and tied securely around the catheter. Using a hollow needle, the catheter was then passed subcutaneously to the back of the neck where it was secured by a suture and was cut to a length which made it impossible for the rat to be able to chew it. The front

of the neck was sutured.

On the two days following the operation blood was drawn back into a syringe and the dead space of the catheter was filled with fresh heparin (5000 units/ml) in order to maintain patency.

2.3:2 Aortic and vena caval (IVC) catheters.

(i) Construction of aortic catheter.

This method was modified from the technique of Weeks & Jones (1960) (Browning, Ledingham & Pelling,1970; Brown,1981). These catheters consisted of four pieces of polythene, A, B, C & D, welded together by heating. Figure 2.2 shows an aortic catheter. The construction of these catheters and the materials used are detailed in Appendix 2.

(ii) Construction of the IVC catheter.

The IVC catheter also consisted of four pieces of polythene sealed together by heating. The materials used are identical to those in the aortic catheter construction with one exception; for part A, 19cm of polythene was used instead of 17cm as used for the aortic catheter. A constructed IVC catheter is shown in Figure 2.3. and their construction is detailed in Appendix 3.

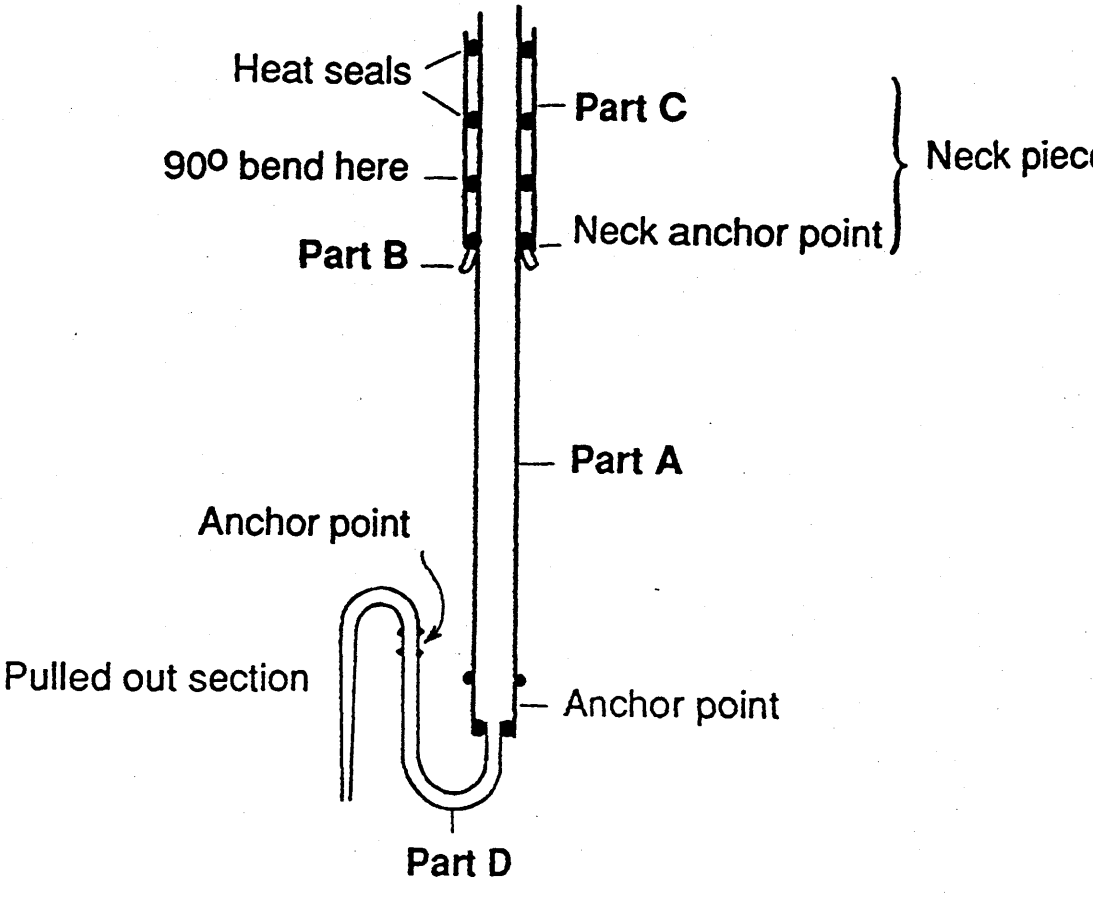


Figure 2.2 Aortic catheter
Materials used and construction are detailed in Appendix 2.

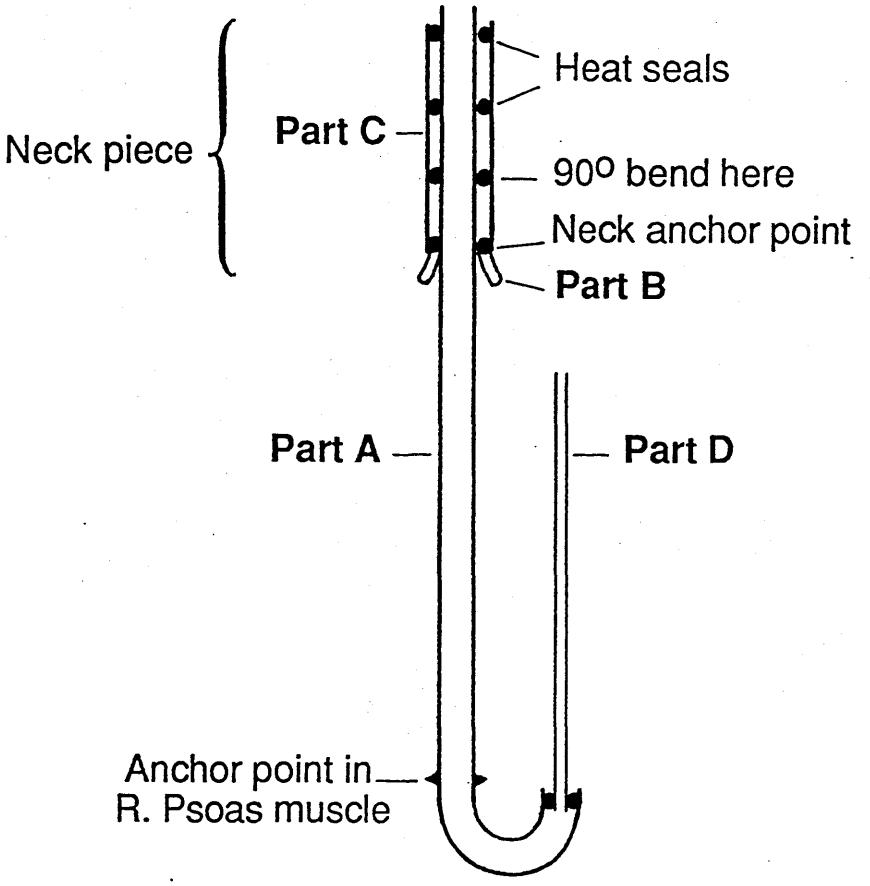


Figure 2.3 Vena caval catheter
Materials used and construction are detailed in Appendix 3.

(iii) Testing the catheter.

Both types of catheter, once constructed, were tested under pressure for leakage. This was done by sealing the end of the catheter and then forcing saline into the catheter through a syringe with a filed number 23 gauge hypodermic needle inserted into the top of the catheter. If any leakage occured the catheter was discarded.

(iv) Implantation of aortic and IVC catheters.

Catheters were sterilised overnight in a chlorhexidine solution. Before implantation they were soaked in a sterile saline solution. Rats were anaesthetised with Saffan (Glaxovet Ltd, Harefield, Oxbridge, England) which was injected intraperitoneally (4ml/kg) and the abdomen and the back of the neck were shaved and cleaned with the chlorhexidine solution. An incision was made along the midline from the pubis to the xiphisternum through the skin and the body wall to expose the gut. The gut was reflected to the left (rat's right) and covered with sterile saline soaked gauze. The aorta and IVC were carefully exposed with the fingers from just below the left renal vein to the area of the iliac bifurcation. Any fat and connective tissue was removed with cotton buds. The left and right psoas muscles were

also exposed and cleaned and the left lumbar vein and artery were freed from the body wall.

The aortic catheter was positioned approximately as shown in Figure 2.4 in order to estimate where it should enter the psoas muscle. A sharpened guide needle was passed through the psoas muscle at this point to the subcutaneous tissue of the back. The needle was then turned and passed subcutaneously to emerge through the skin at the back of the neck between the ears. The neck piece of the catheter, with a length of surgical silk tied to the anchor point before the S-bend, was fitted onto the end of the guide needle. The guide needle, with catheter attached, was then pulled carefully through to the back of the neck, care being taken not to pull any intra-abdominal structures (eg ureter) into the muscles of the posterior abdominal wall. At this time the IVC catheter was also positioned. The correct entry point in the right psoas muscle was identified and the guide needle plus catheter, with a length of surgical silk attached to the lower anchor point, was passed through the muscle and subcutaneously to the back of the neck and through the hole made for the aortic catheter. Both catheters were placed in position so that they lay flat on the posterior body wall.

The aortic catheter was secured by sutures to the left psoas muscle using the silk already attached. The top of the S-bend was passed under the freed left lumbar vessels so that the final straight part of the catheter

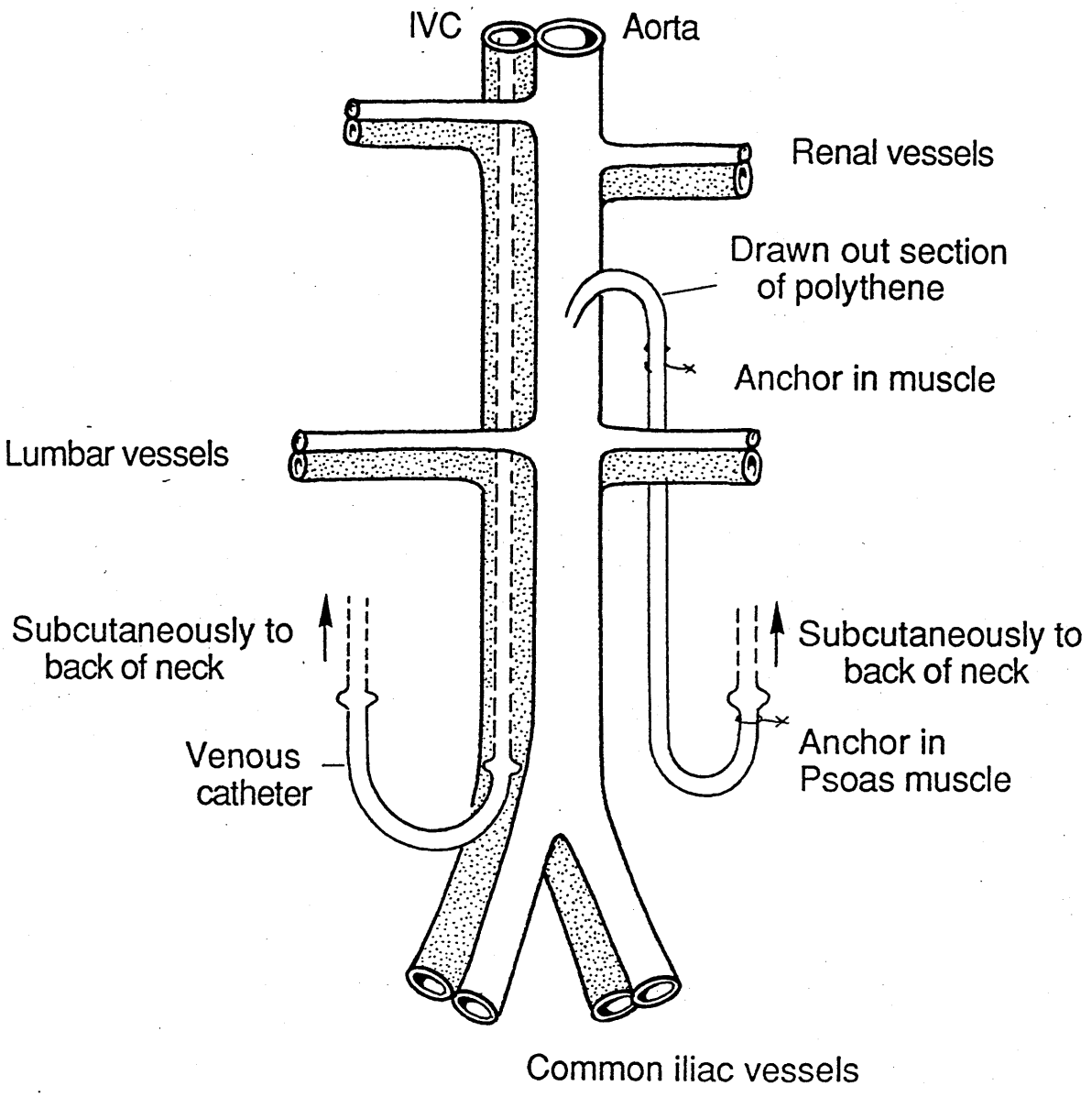


Figure 2.4 Position of aortic and vena caval catheters.

was lying on top of the aorta and parallel to it (Fig 2.4). The anchor point on the S-bend was then secured to the underlying muscle with surgical silk. The catheter tip was trimmed to a bevel of about 45 degrees at a point 5-10mm above the aortic bifurcation. A 1ml syringe with a filed down number 23 gauge hypodermic needle was filled with saline and attached to a 30cm length of polythene tubing (ID 0.58mm, OD 0.96mm). This was connected to the end of the catheter by 2cm of needle tubing (number 23 gauge). The catheter, now filled with saline, was ready to be inserted.

The aorta was occluded with the finger above the level of the renal arteries. Using a number 25 gauge sterile hypodermic needle a hole was made in the aorta approximately 5mm below the level of the left renal vessels. The needle used to pierce the aorta had previously been bent to a right angle about 3mm from the tip so that there was less possibility of the needle piercing the posterior wall of the aorta. The needle was removed and the free end of the catheter inserted through the hole into the aorta and gently fed down the vessel. The occlusion on the aorta was released slowly and if any bleeding occured through the site of insertion pressure was maintained until a clot had formed. In practice there was little bleeding. A small ammount of saline was squirted into the catheter to clear any blood out of it. The neck end of the catheter was then clamped with artery forceps, the length of polythene tubing with syringe

attached was removed, and the catheter was plugged with a 1cm length of stainless steel wire (0.7mm diameter) which had been filed smooth at the ends to prevent damage to the catheter.

The syringe and polythene tubing were then connected to the IVC catheter. The tip of this catheter was trimmed straight across to 5cm. The catheter was gently pulled back through the muscle from the neck until the tip was lying just above the bifurcation of the IVC. The tip of the catheter was then used to pierce a hole in the IVC at its bifurcation and the catheter was pulled gently at the back of the neck until the whole of the tip lay inside the IVC and the A-D seal (see Appendix 3) lay against the hole in the bifurcation. The silk attached to the anchor point on part A was sutured into the muscle and a small ammoumt of saline was injected through the catheter in order to remove any blood. The syringe and tubing were removed and the catheter was plugged. The gut was then replaced, the abdominal muscle sutured and the skin closed with 12mm wound clips.

At the back of the neck, the skin was incised longitudinally from the point of exteriorisation of the catheters until the neck anchor points on the catheters were exposed, usually 15 to 20mm. Sutures were tied around these anchor points and sewn into the muscles of the neck in order to prevent the catheters being pulled out. Second sutures were tied around the 90 degree bends in the catheters and these too were sewn into the

musculature. The skin was sutured and the rats were given ampicillin (170ug) subcutaneously. On the day after implantation blood was drawn up the arterial catheter and the dead space was filled with 0.04ml sodium heparin (1000 units/ml) which was replaced twice daily during the experiment to maintain patency. The venous catheter was filled with 0.04ml saline containing sodium heparin (150 units/ml).

2.4 Experimental cages.

Before the catheters were implanted rats were housed in groups of four in plastic cages with aluminium lids in an animal house of constant temperature (18-22C) and lighting (12 hour light/darkness cycle). Following catheterisation they were housed individually in these cages for a period of recovery.

2.4:1 Short-term studies.

During the short-term experiments, in which carotid artery catheters had been implanted, rats were placed in cylindrical polycarbonate cages which were 32.5cm high and 21.5cm in diameter. These cages were fitted with a lid which had an 8cm hole cut in the centre. The floor of the cage was covered with a layer of sawdust. A 45cm extension of polythene tubing (ID 0.58mm, OD

0.96mm) filled with saline was connected to the carotid catheter with a 2cm length of filed 23 gauge needle tubing. This extension was protected by a 30cm length of polythene tubing (200/490/060 Portex ID) which fitted snugly over the end of the carotid catheter. This protective tubing containing the catheter was held upright by means of a fine nylon line connected to a horizontally free running swivel which in turn was attached to another length of line which was fed over a vertically free running pulley wheel fixed above the cage and by means of a small lead weight tied to the end of the line (see Figure 2.5). This swivel and weighted line allowed free movement of the rat in all directions and prevented tangling of the extended catheter, which in turn had been connected by another length of polythene (2.5m, ID 0.58mm, OD 0.96mm) to the blood pressure recorder (see Figure 2.5). The maximum ammount of time the rats spent in these cages never exceeded 3 hours.

2.4:2 Long-term studies.

For these experiments, aortic and IVC catheters were implanted. After a period of recovery in their normal cages rats were transferred to modified metabolic cages (Techniplast (R), Forth-Tech Services Ltd. Midlothian, Scotland) (Figure 2.5). These cages allowed the rats free access to food and water, but as a metabolic balance study was not being carried out, the metal grid on

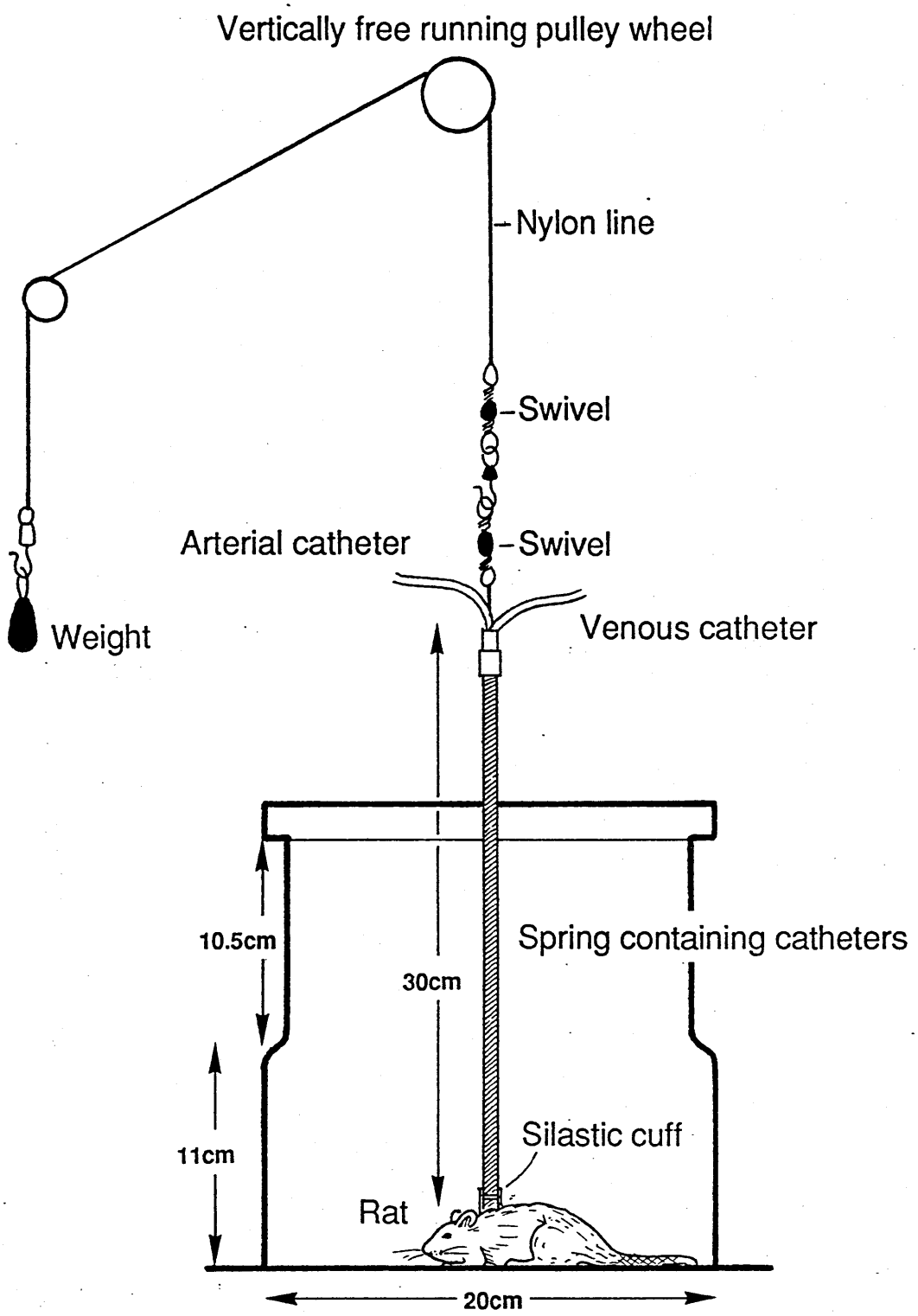


Figure 2.5 Experimental cage for long-term studies. The same nylon line and swivel system was used in the short-term studies using a carotid catheter.

the floor of the cage was covered with a metal plate upon which a layer of shavings was spread. A 10cm length of perspex cylinder was added to the top of the standard cage to raise its height to 26cm. This allowed the rat to stand on its hind legs and just reach the upper rim. The cage was closed by a perspex lid with a 5cm hole in its centre.

Polythene tubing extensions (45cm) were connected to the aortic and IVC catheters as described for the carotid artery catheter. These were filled with 130ul of sodium heparin (1000 units/ml) and heparin/saline (150 units/ml) respectively and when not in use for blood pressure measurements or intravenous infusions were plugged with stainless steel plugs. The extensions were protected by a 30cm long flexible but tightly wound steel spring (0.2cm ID). A 3cm length of silastic tubing was fixed over the end of the spring and fitted snugly over the connections to lie flush with the neck of the rat and serve to protect the catheters at a potentially vulnerable point. The other end of the spring was held upright by the same swivel and nylon line system as described for the carotid artery catheter.

2.5 Blood pressure measurement.

2.5:1 Measurement of arterial pressure.

Arterial catheters were connected to Elcomatic EM 751 transducers via a length of polythene tubing (2.5m, ID 0.58mm, OD 0.96mm) and filed number 23 gauge needle tubing. The transducer was connected to an EM720 amplifier and pen recorder (Elcomatic, Kirktonfield Road, Neilston, Glasgow G78 3PL) which was calibrated weekly against a standard mercury manometer. In the first experiment, where blood pressure recording lasted no longer than 2.5 hours, blood pressures were calculated by hand from the recorder chart as the average of 5 minute interval readings of the mean pressure (diastolic plus one third of the pulse pressure) for the last 1 hour of recording. In Experiment 2, where blood pressure was recorded continuously for 9 days an Apple IIe microcomputer was connected via an A1-02 analogue/digital interface (Interactive Structures, Bala Cynwyd, USA) to the voltage output of two Elcomatic recorder amplifiers. A pressure range of 0-250 mmHg produced a voltage range from the amplifier of 0 to 2.5 volts. This was converted by the interface to a digital range of 0 to 128. Before the start of and every 2 days during the experiment each of the 4 amplifier chart recorders and the equivalent computer channels were calibrated simultaneously over the range 0 to 250mmHg using an external pressure standard given by a mercury

manometer. A linear relationship between standard pressure and digital output was obtained for each channel and a channel calibration factor calculated. The calibration factor for each channel was entered in the basic programme as a variable and was used to convert digital output to millimeters of mercury pressure before storage on the disk.

A programme was written in basic which allowed arterial pressure to be sampled and stored at a minimum time interval of one minute. Each rat was sampled sequentially and the mean pressure on each occasion was calculated by the programme to be the mean of 200 separate digital measurements taken over a maximum period of 2 seconds. Each of these 200 measurements would be taken at random over approximately 10 pulse waves. Thus the mean value multiplied by the calibration factor would be an accurate estimate of the mean pressure; defined as the diastolic plus half the pulse pressure. After sampling all four rats, the time of sampling, the rats' numbers and their mean arterial pressures were stored in random access files on disk. The programme was also able to identify incorrect pressures due to the blocking of catheters. To do this, the pulse pressure from the 200 separate measurements was calculated and if this was less than a stated value then the mean blood pressure recorded was indicated as having been obtained from a damped pulse. The programme also allowed temporary interruption of blood pressure monitoring which allowed catheters to be cleared

and the system to be recalibrated and at any time all or part of the stored data to be produced as a hard copy. A mean value of any number of sequentially stored pressure measurements for any rat could also be calculated and this could be stored in a separate file on disk.

2.5:2 Measurement of heart rate.

Heart rate was measured during the recording of arterial pressure by speeding up the trace until individual pulse waves were visible and countable. The number of pulse waves in a 5 second interval were counted and this was multiplied by 12 to give the heart rate in beats per minute.

2.5:3 Measurement of systolic blood pressure.

Systolic blood pressure was measured by tail plethysmography using a W & W 8005 recorder (W and W Electronic Instruments, CH-4002 Basel, Munchenstein, Switzerland). The rat was warmed in an incubator at 37C for 15-20 minutes to ensure vasodilation. When it was removed it was lightly restrained by wrapping in a towel with its tail protruding and the pressure cuff was pushed over the tail up to the root followed by the pulse detector. Six readings were taken within 2 minutes and the blood pressure was calculated as the mean of the last 4 readings.

2.6 Continuous intravenous infusion.

Continuous intravenous infusion was given by an infusion pump (Dascon IP 300, Uden, The Netherlands) which had a variable flow rate. For all the experiments described the infusion rate was 1.0ml/24 hours. The pump was modified to take a 5ml syringe and was recalibrated for this. The syringe was connected by means of a filed number 23 gauge hypodermic needle to a length of tygon tubing (ID 0.5mm, OD 1.52mm, Norton Plastics, Akron , Ohio). This in turn was connected to the end of the venous catheter extension via a 2cm length of number 23 gauge needle tubing.

2.6:1 Captopril.

Captopril was supplied by Squibb Europe Inc. (Hounslow, Middlesex) in powder form which was stored in an air tight, moisture free container at -20C. When required as infusate it was dissolved in 5% dextrose containing heparin (150 units/ml).

2.7 Injections.

During the short term experiments injections of angiotensin converting enzyme nonapeptide (teprotide) were given via the carotid catheter.

2.7:1 Teprotide.

The teprotide was supplied by Beckman, (High Wycombe, Buckinghamshire). It was dissolved in saline (154mmol/l) at a concentration of 1mg teprotide /kg rat in 0.1ml saline. The catheter was disconnected from the transducer and the blood was drawn back to the top of the extension line. The material was then injected slowly down this line followed by 0.25ml saline. The catheter was then reconnected to the blood pressure transducer.

In the long term experiments, in which aortic and IVC catheters had been implanted, the rats received bolus injections of angiotensin I and of captopril.

2.7:2 Angiotensin I.

Angiotensin I was supplied by Cambridge Research Biochemicals Limited (Harston, Cambridgeshire) and was dissolved in saline at a concentration of 100µg/ml saline. This was frozen in aliquots and stored at -20C until used. When required for injection this was thawed and diluted to a concentration of 100ng/0.1ml saline. Injections of 0.1ml angiotensin I solution were used as a test of the effectiveness of the angiotensin converting inhibitor (captopril) to inhibit angiotensin converting enzyme activity and hence block the formation of angiotensin II.

2.7:3 Captopril.

Captopril for injections was diluted in 0.9% saline at a concentration of 2.5mg/kg rat in 0.1ml saline.

Before infusions were started the venous catheter was used as the route for injections. The material was injected straight into the venous line followed by 0.25ml heparin saline (150 units/ml). As an immediate pressor response is seen after injection with angiotensin I this proved useful as a test of the patency of the venous catheter as it is not always possible to draw blood back up the catheter even though it lies inside the vein.

Once the rats were being infused, injections were given via the aortic catheter. Blood was drawn to the end of the aortic catheter extension line, as for the carotid catheters, and after the injection followed by saline, the catheter and line were filled with 0.17ml of fresh heparin (1000 units/ml) and reconnected to the transducer.

2.8 Blood sampling.

For angiotensin II and renin measurements 0.75ml blood was drawn rapidly from the arterial line into an ice cold syringe containing 0.075ml of an aqueous solution of 0.1M ethylenediaminetetra-acetic acid (disodium salt) and 0.05M o-phenanthroline and were immediatly chilled in ice. This solution completely inhibits rat plasma converting

enzyme and angiotensinases and is used with arterial blood in a ratio of 1:10. Blood samples were centrifuged as soon as possible at 5°C and 1400g for 20 minutes and the plasma was stored at -20°C until extracted.

For angiotensin converting enzyme estimation 0.1ml of arterial blood was drawn rapidly into a heparinised syringe. All converting enzyme assays were performed less than 5 hours after the samples were taken as it has been shown that angiotensin converting enzyme activity increases with storage time in plasma samples containing captopril (Roulston & MacGregor,1980).

0.05ml of blood was drawn into a heparinised capillary tube for haematocrit measurement.

At the end of experiments rats were killed by either an intravenous or intra-arterial injection of pentobarbitone sodium (Sagatal, May & Baker Ltd, Dagenham).

2.8:1 Measurement of plasma angiotensin II.

Angiotensin II immunoactive material was extracted from plasma by the addition of sodium chloride solution (154mmol/l: saline; 5ml), followed by mixing for 1 hour in batches with Dowex resin (50-100 mesh, H+, 400mg; Bio-Rad, Richmond, CA, USA) at 4°C. The plasma supernatant was sucked off and the Dowex, containing the extracted angiotensin II was washed with two volumes of distilled

water (5ml) followed by two volumes of methyl alcohol (5ml). Elution of angiotensin II was achieved by mixing the Dowex with 10% methanolic ammonia (2ml) for 30 minutes. The tubes were centrifuged and the 2ml supernatants were removed. These extracts were then dried in a water bath at 37C under a stream of air. The dried extracts were stored at -20°C until assayed, when they were dissolved in 0.05M Tris pH 7.5. For the radioimmunoassay, 0.1ml of extract were incubated with 0.1ml angiotensin II antiserum (9/P) at a dilution of 1:20,000, and 50µl of 125I-labelled angiotensin II (2pg). Duplicate standard curves from 0 to 50 pg were set up. Incubations were carried out for 18 hours at 5°C. The separation of antibody-bound from free labelled angiotensin II was achieved using dextran coated charcoal. One ml of the charcoal suspension was added to each tube, mixed rapidly and centrifuged at 1000g for 7 minutes at 5°C. The supernatant, containing the bound labelled angiotensin II, was aspirated with a water pump and discarded. The radioactivity of the free label adsorbed to the charcoal pellet was then counted in an automatic gamma-ray counter. The interassay coefficient of variation for the assay is 13.6% and it is capable of detecting as little as 0.5 fmol of angiotensin II; that is 7 fmol/ml of plasma for a 0.25ml sample corrected for a recovery of 84% and a 1:3 dilution. For further details see Dusterdieck & McElwee (1971) and Garcia Del Rio et al. (1981).

2.8:2 Measurement of plasma renin concentration.

The method for the measurement of plasma renin concentration was a modification of the antibody trapping method of Poulsen & Jorgensen (1974) as published for human blood by Millar et al. (1980). The enzyme incubation and antibody trapping procedure was as follows. Duplicate rat plasma samples (3µl) were incubated at 0°C and at 37°C for 5, 30 or 60 minutes, depending on the renin content, with a mixture (14µl) containing angiotensin I antiserum (981/7) diluted 1:80 in 3M Tris/HCl pH 7.0, and rat renin substrate in a ratio of 1:6. The enzyme incubation was stopped by rapidly cooling the samples in ice-water. The angiotensin I generated and trapped was estimated in the same tube by radioimmunoassay as follows. Cold 0.05M Tris/HCl, pH 7.5 (0.3ml) was added to each sample, followed by 10 pg of 125I-labelled angiotensin I (in 0.05ml of Tris, pH 7.5). This gave a final radioimmunoassay antibody dilution of 1:14680 in a final volume of 0.367ml. The samples were incubated at 5C for 18 hours, after which time the bound and free labelled angiotensin I were separated as previously described by the addition of plasma coated charcoal (Millar et al.,1980). Duplicate standard curves (1.6-200pg) were subjected to exactly the same procedures as the unknown plasma samples.

Rat renin substrate was prepared by bleeding rats which had been bi-nephrectomised 24 hours previously and

which had been given a subcutaneous injection of long acting polyestradiol phosphate (Estradurin, Lunbeck, Luton, UK) 10 days before nephrectomy (Nasjletti, Matsunaga & Masson,1969). This substrate preparation was shown to be completely free of renin and sufficient was added to the enzyme incubation to ensure zero-order kinetics.

Estimation of the above modifications showed that the pH of the enzyme incubation was stable at 7.0-7.2 for up to 24 hours at 37°C. Also, the initial trapping dilution of the angiotensin I antibody (1:80) was shown to be well in excess of that required over the range of the standard curve used. The limit of detection of the assay was as little as 4.0 fmol of angiotensin I; that is a plasma renin concentration of as low as 1-1.5 pmol of angiotensin I/ml/hour for a 3µl plasma sample incubated for 1 hour. The between assay coefficient of variation was +/- 14.6%

2.8:3 Measurement of plasma angiotensin converting enzyme.

Plasma angiotensin converting enzyme was measured by a slight modification of the spectrophotometric method of Cushman & Cheung (1971b) with a hippuryl-histidyl-leucine substrate (Sigma, Poole, Dorset) and 0.025ml plasma samples. For each plasma sample duplicate blank and test incubations were set up in glass

test tubes as follows. 0.075ml of saline (154mM) was added to 0.025ml of plasma and this was incubated at 37C for 60 minutes with 100µl of 12.5mM hippuryl-histidyl-leucine in 400mM potassium phosphate, pH 8.3, and 600mM sodium chloride. Blank tubes also contained 0.25ml 1M HCl at this stage. The reaction in the test incubations was stopped after 1 hour by the addition of 250µl 1M HCl. Ethyl acetate (1.5ml) was then added to all tubes and the samples were extracted by vortexing for 15 seconds. After centrifugation for 10 minutes at 1400g, 1ml of the supernatant was removed into a glass tube and dried in a water bath at 37°C under an air stream. The dried extract was dissolved in 1ml of distilled water by mixing vigorously. The optical density of the samples was then read on a spectrophotometer at a wavelength of 228nm and the concentration of angiotensin converting enzyme in µmoles/ml/h was calculated from a standard curve of hippuric acid.

2.9 Statistical methods.

Mean values were tested for significant differences by Students t-test for paired or unpaired observations. Correlations between two variables were tested by linear regression analysis and the slopes of the regression lines were compared using a common slope regression test (f-test) based on analysis of variance. A

one-tailed Wilcoxon signed-ranks test for matched pairs was used in the second experiment to compare mean blood pressures after chronic captopril infusion with the lowest blood pressure seen after an acute captopril injection. All values quoted throughout are mean +/- standard deviation.

CHAPTER 3

CHANGES IN RENIN AND ANGIOTENSIN II DURING THE DEVELOPMENT OF TWO-KIDNEY, ONE CLIP HYPERTENSION IN THE RAT AND THE EFFECT OF ACUTE ADMINISTRATION OF CONVERTING ENZYME INHIBITOR.

3.1 Introduction.

The model of the two-kidney, one clip Goldblatt rat was first demonstrated by Wilson and Byrom in 1939. Since this time the involvement of renin and angiotensin in this model has been extensively studied. From studies using a number of different species it has been suggested that immediatly after the application of the clip plasma renin rises (Brown et al.,1966; Blair-West et al.,1968; Bianchi et al.,1972; Liard et al.,1974; Carretero & Gulati,1978) and is probably responsible for the hypertension which develops during this acute phase (Bianchi et al.,1968; Pals et al.,1971; Thurston & Swales,1974a; Coleman & Guyton,1975; Miller et al.,1975; Caravaggi et al.,1976; Freeman et al.,1977b; Masaki et al.,1977; Carretero & Gulati,1978). However the involvement of the renin angiotensin system in the development and maintenance of the hypertension after this acute phase is less certain. Much of this uncertainty may be due to the different methods used by different

investigators. These include using rats of different strain, sex and age, and different sizes of clips or ligatures placed at different sites along the renal artery, both of which may influence the rate of development and the severity of the hypertension (Leenen & Myers,1984). Various methods of blood sampling have also been used, for example sampling from indwelling catheters in conscious rats (Riegger et al.,1977; Otsuka et al.,1979), decapitation (Leenen & DeJong,1975; Ten Berg, Leenen & DeJong,1979), and sampling under ether anaesthesia (Mohring et al.,1975; Sen et al.,1979; Thurston, Bing & Swales,1980), ether being a well known stimulus of renin secretion (Leenen & DeJong,1975). These differences in technique make the comparison of results between laboratories difficult.

The purpose here was to set up a reproducible experimental model in our laboratory and using this to follow the changes which took place in the renin-angiotensin system and to determine the importance of such changes in the regulation of the hypertension which develops.

3.2 EXPERIMENT 1(a).

A study of the changes in blood pressure, angiotensin II and plasma renin concentration during the developement of two kidney one-clip hypertension in the rat.

3.2:1 Experimental design.

Groups of up to 30 rats were operated on at any one time. These were either clipped or sham-operated (2.2:2) in pairs alternately. Rats were selected at random and a carotid artery catheter was inserted, as previously described (2.3:1), at one of the following times: 1 and 2 days after clipping, at weekly intervals up to 10 weeks and at 15 weeks and 20 weeks. The 1 and 2 day rats had their catheter implanted at the same time as the renal artery was clipped. On the second day after implantation of the catheter rats were placed in cylindrical polycarbonate cages and connected via their carotid catheter and the pressure transducer to the blood pressure recorder. The rats were allowed to settle for 30 minutes after which their blood pressure was recorded directly and continuously for 1.5 hours. At the end of this time heart rate was measured and blood rapidly removed via the carotid catheter for the measurement of angiotensin II, plasma renin concentration and packed cell volume (0.85ml total). Rats were then either studied further (see 3.3) or killed by an intra-arterial injection of pentobarbitone sodium. Blood pressure was calculated

as the average of 5 minute interval readings of the mean pressure (diastolic plus one third of the pulse pressure) for the last one hour of recording. All values quoted are mean +/- standard deviation.

3.2:2 Results.

(i) Number of rats studied.

A total of 199 rats were clipped but of these 40 either died or were killed by intraperitoneally administered pentobarbitone sodium because they appeared unwell. These rats all died or were killed between 4 and 20 weeks after clipping, the majority (58%) dying between 6 and 9 weeks, and 90% having died by 13 weeks. The remaining 159 clipped rats and the 134 rats which had been sham-operated were randomly allocated into groups as shown in Table 3.1. Each group contained between 7 and 11 rats. As the results from the 1 day and 2 day rats were similar these were combined to give one group of 21 rats. The 4 week clipped group was chosen to be studied further with the administration of converting enzyme inhibitor and so extra rats were included. The results from 9 rats were not included in the analysis, 3 of these were from the 14 rats in the 15 week group and 6 were from the 14 rats in the 20 week group. The reason for their exclusion was that since 90% of the rats that died did so within 13 weeks of clipping, it was difficult to obtain rats with

Group	Clipped rats No.	Sham operated rats No.
1-2 days	21	21
1 week	10	9
2 weeks	9	10
3 weeks	9	8
4 weeks	21	7
5 weeks	10	10
6 weeks	11	9
7 weeks	9	10
8 weeks	11	11
9 weeks	10	9
10 weeks	10	10
15 weeks	11(14)	10
20 weeks	8(14)	10
	150(159)	134

Table 3.1 Groups of rats studied.

severe hypertension at 15 and 20 weeks as a large proportion of the rats which survived to this time were only mildly or moderately hypertensive. Therefore because these rats were only moderately hypertensive compared to the rest of the rats in their groups they were excluded. This allowed a comparison of angiotensin II and plasma renin concentrations in the 15 and 20 week late chronic rats with those found for the 8 to 10 week early chronic rats where the severity of the hypertension was similar for both. In all other groups the results from all rats studied in each group were included in the analysis whether the rats were normotensive, mildly hypertensive or severely hypertensive.

(ii) Changes in blood pressure.

Figure 3.1(i) shows the blood pressures of the clipped and sham-operated rats up to 20 weeks after operation. There were no detectable differences between the blood pressures of any groups of sham-operated rats, the mean blood pressure for all sham-operated rats being 107 +/- 12.3 mmHg, n= 132. For the clipped rats blood pressure was elevated at 1 and 2 days after clipping compared to the pressures in the equivalent sham-operated group. Pressures in the clipped rats remained at this elevated level for up to 2 weeks after which there was a progressive rise in pressure which reached a plateau at 5 to 6 weeks. From 5-6 weeks to 20 weeks after clipping

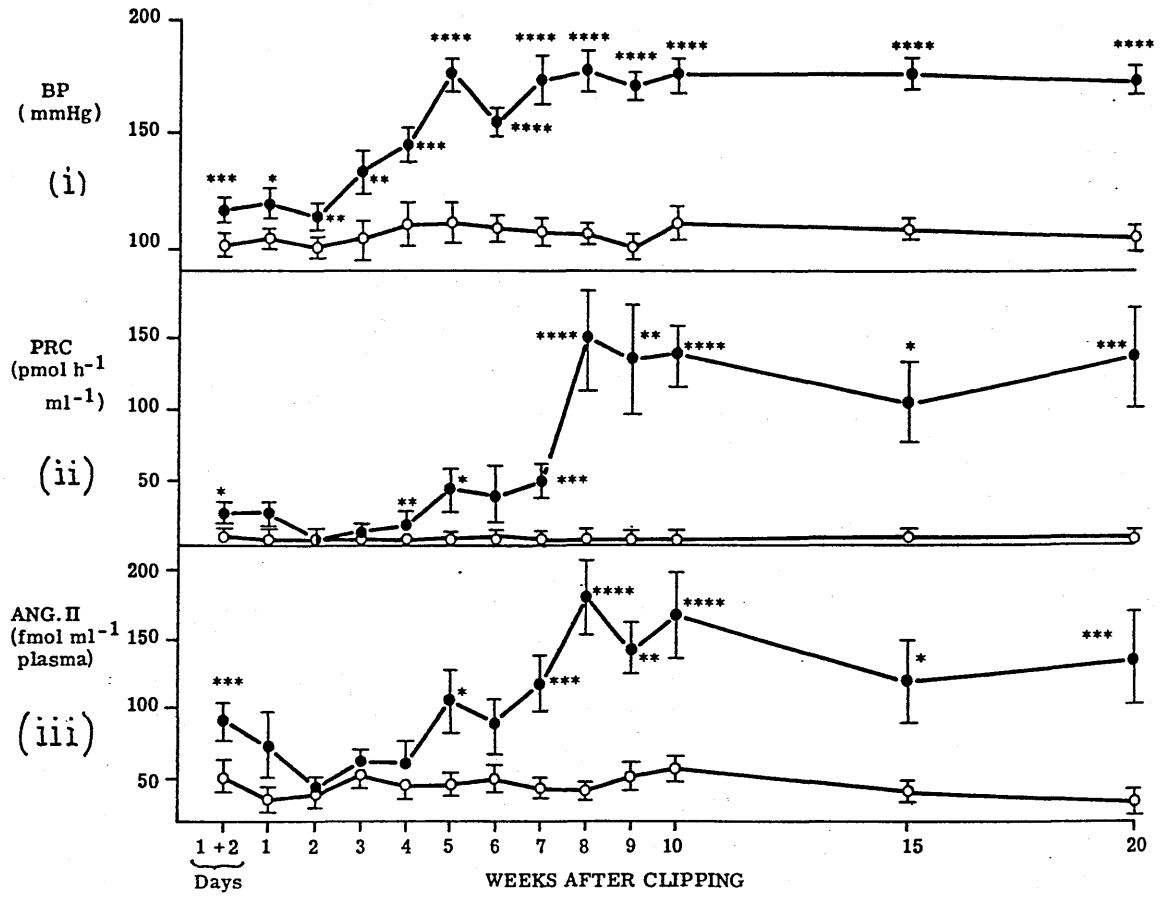


Figure 3.1 Changes in blood pressure (BP) (i), plasma renin concentration (PRC) (ii) and angiotensin II (ANG.II) (iii) in two-kidney, one clip hypertensive (●) and sham-operated rats (O) 1-2 days, at weekly intervals up to 10 weeks and at 15 and 20 weeks after operation. Bars show +/- SEM. Significant differences (unpaired t-test) are related to the equivalent group of sham-operated rats: **** $p<0.001$; *** $p<0.01$: ** $p<0.02$; * $p<0.05$.

this hypertension was sustained with average mean pressures ranging between 150-179 mmHg. If the 9 rats from the 15 and 20 week groups which were excluded from this analysis had been included , then a reduced blood pressure at 15 and 20 weeks of 167 +/- 27.3 and 144 +/- 28.6 mmHg respectively would be seen (instead of 176.9 +/- 20.6 and 173.1 +/- 12.6)

In a separate experiment systolic blood pressure was measured sequentially by tail plethysmography in 14 rats, 8 clipped and 6 sham-operated at various stages after clipping. The results from the clipped rats in this longitudional study are shown in Figure 3.2. This sequential rise in pressure was very similar to that found for the individual groups of rats in the main cross sectional study. The mean systolic pressure for the 6 sham-operated rats was 143 +/- 8.2 mmHg.

(iii) Changes in angiotensin II and plasma renin concentration.

The plasma renin and angiotensin II concentrations for the groups of clipped and sham-operated rats at the different stages up to 20 weeks after operation are shown in Figure 3.1(ii) and (iii). In the clipped group both angiotensin II and plasma renin concentrations were significantly increased at 1-2 days compared with the group of sham-operated rats (89.7 +/- 8.9 and 24.6 +/- 4.9 respectively, compared with 54.3 +/-

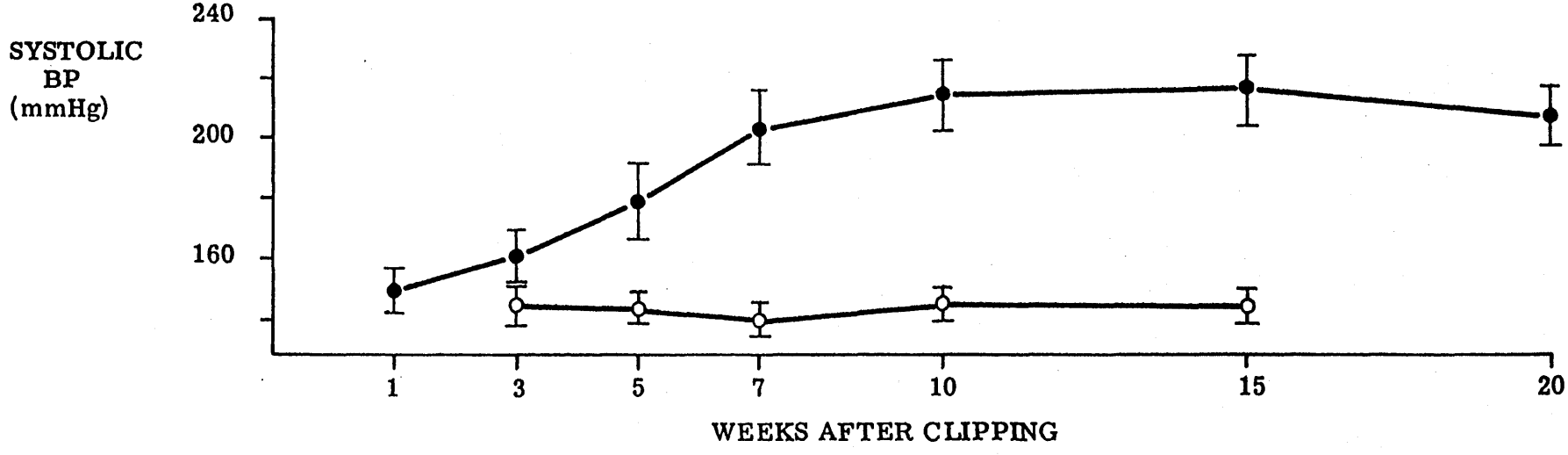


Figure 3.2 Changes in systolic blood pressure sequentially measured in 8 two-kidney, one clip rats (●) and 6 equivalent sham-operated rats (O).

5.5 fmol/ml and 12.3 +/- 2.0 pmol AngI/ml/h). Following this, concentrations of both fell to reach their lowest values at 2 weeks, 43.0 +/- 11.8 fmol/ml for angiotensin II and 6.5 +/- 4.0 pmol AngI/ml/h for plasma renin compared to 40.5 +/- 2.9 and 3.5 +/-0.6 respectively for the sham-operated group. At 2, 3 and 4 weeks angiotensin II levels were significantly lower than at 1-2 days ($p < 0.01$, 0.05 and 0.1 respectively) and were not significantly different from the 2, 3 and 4 week groups of sham-operated rats. Plasma renin levels were also lower at 2, 3 and 4 weeks than at 1-2 days, this difference only being significant at 2 weeks ($p < 0.05$) and only at 2 and 3 weeks was there no significant difference from the equivalent sham-operated groups.

It is interesting to note that at 3 and 4 weeks, when the angiotensin II levels were significantly lower than at 1-2 days, the blood pressure was rising, and at both 3 and 4 weeks was significantly higher than at 1-2 days ($p < 0.05$ and 0.001 respectively).

After 5 weeks both angiotensin II and plasma renin concentrations increased again reaching a maximum at 8 weeks. This increase remained significant up to 20 weeks after clipping, the mean values ranging between 109-176 fmol/ml for angiotensin II and 99-153 pmol AngI/ml/h for plasma renin.

For all clipped rats there was a highly significant correlation between blood pressure and log angiotensin II concentration ($r = 0.68$, $p < 0.001$, $n =$

140) (Figure 3.3), and also between blood pressure and log plasma renin concentration ($r = 0.70$, $p < 0.001$, $n = 145$). In the sham-operated rats there was no detectable relationship between blood pressure and either angiotensin II or plasma renin concentration.

If the 6 rats excluded from the 20 week group had been included then the plasma angiotensin II and plasma renin levels for this group would be lower, 82.7 +/-85.4 fmol/ml instead of 139.6 +/- 106.9 and 69.5 +/- 105.9 pmol AngI/ml/h instead of 146.1 +/- 125.5 respectively.

(iv) Changes in body weight.

The mean body weight at the time of operation was 180 +/- 14.4 g for all clipped rats and 183.9 +/- 17.0 g for all sham-operated rats. After a small decrease in body weight immediatly after clipping both clipped and sham-operated rats gained weight at the same rate from 1 week to 4-5 weeks. After this time the clipped rats did not grow as rapidly as the sham-operated rats and were significantly lighter at 8, 9, 10 and 20 weeks. At 15 weeks although the clipped rats were lighter the difference from the sham-operated rats was not statistically significant. Figure 3.4 (i) shows the changes in body weight.

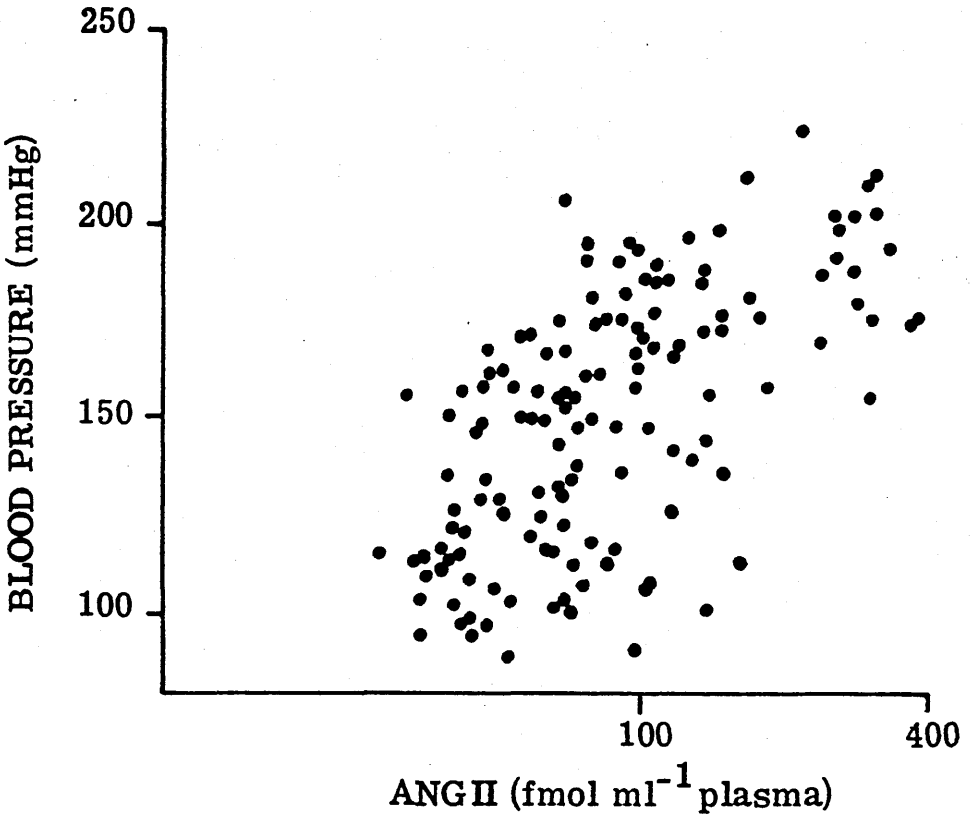


Figure 3.3 Correlation between mean blood pressures and log angiotensin II for all clipped rats.

(v) Changes in packed cell volume.

There was no significant difference in packed cell volume between the clipped rats and the sham-operated rats at any time (Figure 3.4 (ii))

(vi) Changes in heart rate.

There was no significant difference in heart rate between the clipped and sham-operated rats at any time (Figure 3.4 (iii)), however as they grew older both clipped and sham-operated rats showed a significant reduction in heart rate compared with the 1 week values, this first being evident at 6 weeks.

(vii) Angiotensin II / blood pressure regression lines.

Relationships between angiotensin II concentration and blood pressure were tested for 3 groups of clipped rats at the 3 different stages in the development of the hypertension as defined from Figure 3.1. The first of these stages is the acute stage where both the blood pressure and the angiotensin II are elevated. The group of rats representing this stage consisted of the 1-2 day clipped rats. The second stage is the intermediate stage in which blood pressure is rising further but the angiotensin II is suppressed. The 3 and 4 week rats

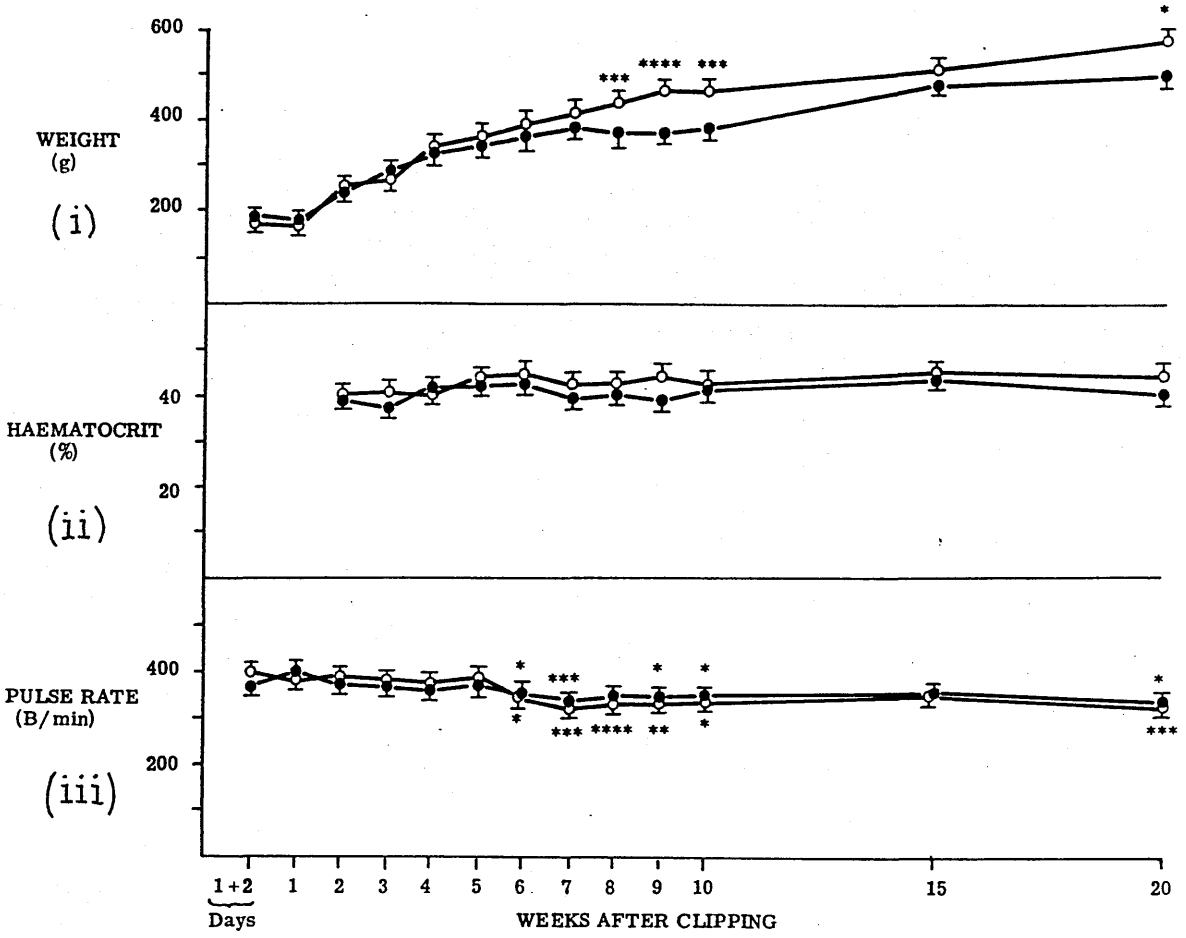


Figure 3.4 Changes in body weight (i), packed cell volume (haematocrit) (ii) and heart rate (iii) in two-kidney, one clip hypertensive (●) and sham-operated (O) rats, 1-2 days, at weekly intervals up to 10 weeks and at 15 and 20 weeks after operation. Bars show +/- SEM. With the exception of heart rate significant differences (unpaired t-test) are related to the equivalent group of sham-operated rats. For heart rate significant differences (paired t-test) for both clipped and sham-operated rats are related to the equivalent values at 1 week after operation: **** $p<0.001$; *** $p<0.01$; ** $p<0.02$; * $p<0.05$.

represented this stage. The final stage is the chronic stage where the hypertension has become established and the angiotensin II is again elevated. This group consisted of the 8 to 20 week rats.

A relationship between the blood pressure and the angiotensin II concentration was found for the 1-2 day rats in the acute phase ($r= 0.48$, $p< 0.05$) and for the 8-20 week rats in the chronic phase ($r= 0.51$, $p< 0.001$), but no relationship was found for the rats in the intermediate phase (3 and 4 week rats). Figure 3.5 shows the regression lines for the acute and chronic phase clipped rats. Although there was no difference in the slope of the regression lines for both groups (a given change in concentration of angiotensin II being associated with the same change in blood pressure for both groups) it can be seen that the regression line for the rats in the chronic phase has been shifted upwards in a parallel fashion. The fact that these lines are parallel suggests that the rats in the chronic phase of the hypertension have the same net vascular responsiveness to the acute effects of endogenous angiotensin II as the rats in the acute phase, 1-2 days after clipping. The upward shift seen in the regression line for the chronic phase rats suggests that some mechanism other than the acute effect of angiotensin II is responsible for the greater part of the hypertension in the chronic phase.

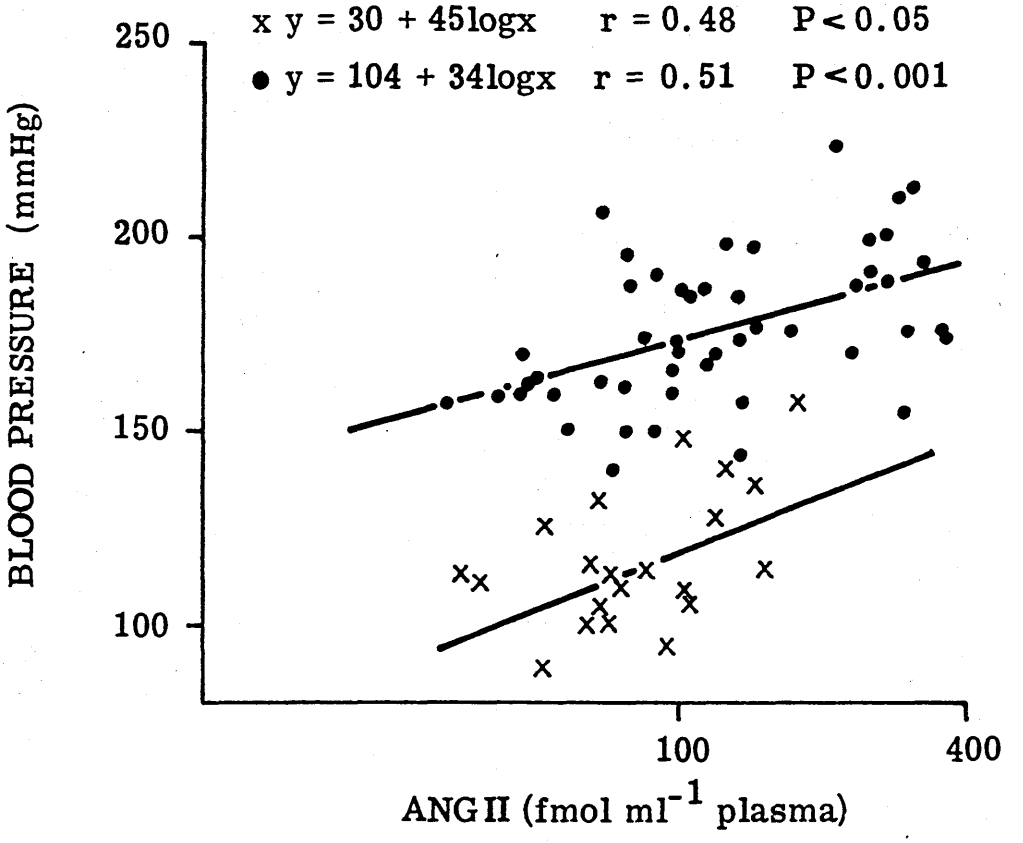


Figure 3.5 Angiotensin II/blood pressure regression lines for acute(1-2 days, X) and chronic (8-20 weeks, ●) hypertensive rats. There was no difference in the slope of the two lines.

3.3 EXPERIMENT 1(b).

Effects of acute converting enzyme inhibition on blood pressure at the intermediate and chronic stages of two-kidney, one-clip hypertension in the rat.

To test the accuracy of the angiotensin II / blood pressure regression line obtained for the chronic hypertensive rats a number of rats ranging from 8 to 20 weeks after clipping were further studied with acute administration of converting enzyme inhibitor. Twelve rats from the 21 in the 4 week group were similarly studied even although these rats showed no detectable angiotensin II / blood pressure relationship.

3.3:1 Experimental design.

Thirty eight rats were studied further. Of these 12 had been clipped 4 weeks previously and the other 26 had been clipped between 8 and 20 weeks previously (Table 3.2). After the blood pressure measurement and blood sampling had been carried out these were allowed to settle for 20 minutes to enable their blood pressure to stabilise if necessary. After this time an injection of angiotensin converting enzyme inhibitor nonapeptide, teprotide (1mg/kg in 0.1ml of sodium chloride (154 mmol/l: saline) was given via the carotid catheter and blood pressure was recorded for a further 5-8 minutes. Following this blood samples were taken for the measurement of angiotensin II,

Number of weeks clipped	Number of rats
4	12
8	6
9	3
10	3
15	7
20	7

Table 3.2 Rats studied further.

plasma renin concentration and angiotensin converting enzyme (0.85ml total). The rats were then killed as described above (3.2:1).

3.3:2 Results.

(i) Blood pressure. (Figure 3.6(i))

Chronic hypertensive rats: There was no difference in the mean basal blood pressure of the 26 chronic hypertensive rats (179.3 +/-17.1 mmHg) as compared with any of the individual groups from 8 to 20 weeks after clipping. After the administration of the converting enzyme inhibitor there was a rapid and significant fall in blood pressure which levelled out between 3 and 5 minutes to a minimum of 150.0 +/- 20.6 mmHg at 5 minutes. Of these 26 rats, 12 were from the 8, 9 and 10 week groups and 14 were from the 15 and 20 week groups. The hypotensive response for the 15 and 20 week late chronic hypertensive rats was smaller (177.3 +/- 16.1 falling to 163.1 +/- 17.3 mmHg) than for the earlier 8, 9 and 10 week groups (181.1 +/- 18.8 falling to 154.2 +/- 17.3 mmHg) but this difference was not significant. With the exception of one rat, none of the 26 rats studied had a blood pressure, after converting enzyme inhibition, within the normal range found for sham-operated rats.

Intermediate 4 week rats: The 12 intermediate 4 week rats

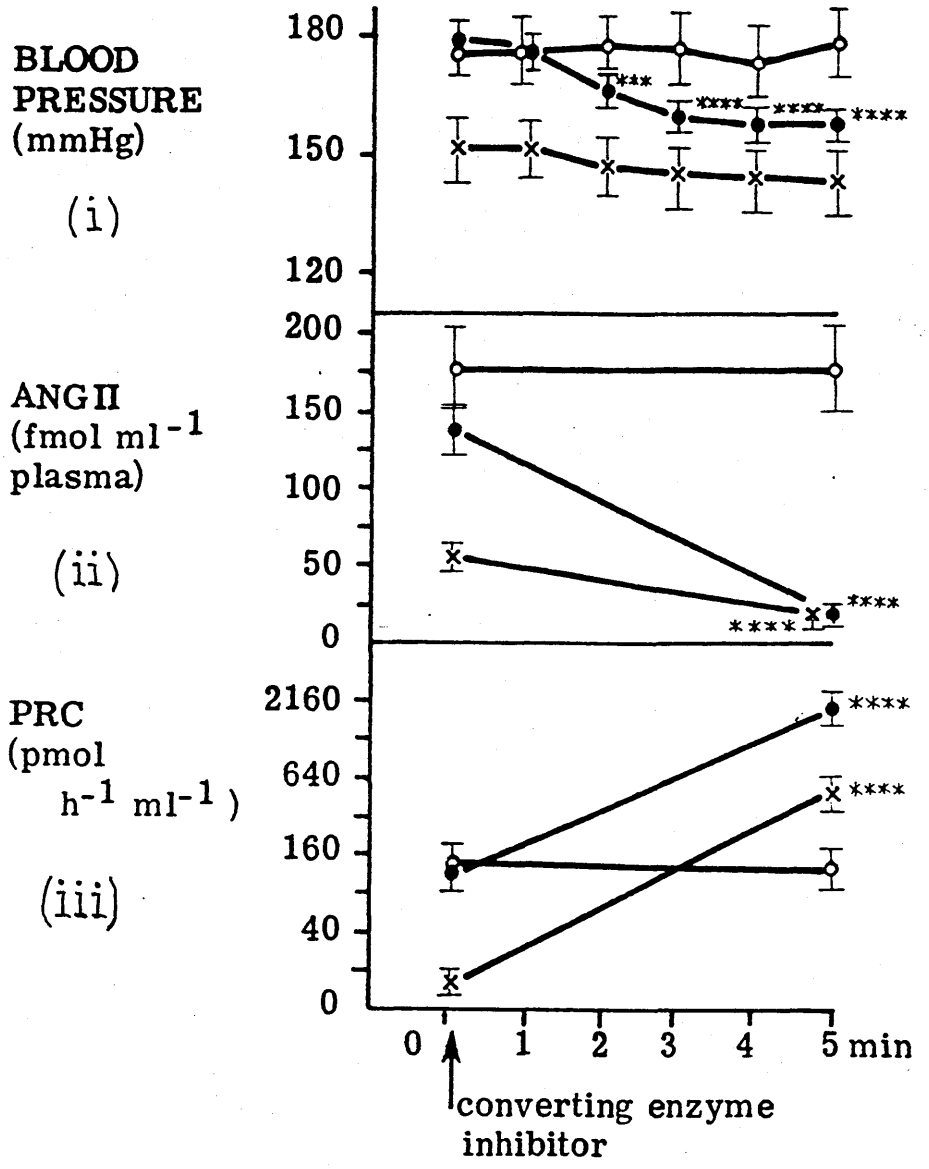


Figure 3.6 Changes in blood pressure (i) angiotensin II (ANGII) (ii) and plasma renin (PRC) (iii) in 26 chronic hypertensive rats (8-20 weeks, ●) and 12 intermediate hypertensive rats (4 weeks, X) given an acute injection of angiotensin converting enzyme inhibitor nonapeptide (1mg/kg/0.1ml), and six chronic hypertensive control rats (O) given saline (0.1ml). Bars show +/- SEM. Significant changes (paired t-test) are compared with basal values: **** $p<0.001$; *** $p<0.01$.

had a basal blood pressure of 151.8 +/- 29.9 mmHg which was not significantly different from that of the 21 rats in the 4 week group as a whole. This basal blood pressure was significantly higher than that of the 1-2 day acute rats (116.7 +/- 3.9 mmHg, $p<0.001$) and significantly lower than that of the 26 chronic rats studied (179.3 +/- 17.1 mmHg, $p<0.001$). Following administration of converting enzyme inhibitor the blood pressure in this group showed a small fall to a minimum of 144.7 +/- 29.8 mmHg after 5 minutes. This fall in blood pressure was not significant.

(ii) Angiotensin II. (Figure 3.6(ii))

Chronic hypertensive rats: For all 26 rats there was a significant fall in angiotensin II levels from a high basal level of 133.6 +/- 97.0 fmol/ml to a concentration at 5 minutes of 22.1 +/- 12.8 fmol/ml, which was well below normal values as found for the equivalent sham-operated rats (44.1 +/- 13.4 fmol/ml).

Intermediate 4 week rats: The basal angiotensin II levels for these 12 rats was 55.0 +/- 5.8 fmol/ml. This was similar to the 4 week group as a whole, but in contrast to the blood pressure it was significantly lower than that found for the 1-2 day acute rats (89.7 +/- 8.9 fmol/ml, $p<0.001$). After converting enzyme inhibition angiotensin II was again supressed significantly, falling to 18.0 +/- 9.1 fmol/ml at 5 minutes.

(iii) Plasma renin concentration. (Figure 3.6(iii))

Chronic hypertensive rats: The high basal levels of plasma renin concentration rose markedly and significantly after converting enzyme inhibition from 128.2 +/- 116.7 to 1936 +/- 1306 pmol AngI/ml/h ($p<0.001$).

Intermediate 4 week rats: The plasma renin concentration for these rats had risen significantly at 5 minutes after administration of converting enzyme inhibitor from 18.7 +/- 13.2 to 550 +/- 377 pmol AngI/ml/h ($p<0.001$).

The fall in blood pressure for the 26 chronic hypertensive rats was significantly related to both the basal angiotensin II concentration ($r= 0.80$, $p<0.001$) (Figure 3.7) and to the basal plasma renin concentration ($r= 0.61$, $p<0.001$). In the 4 week intermediate group there was no detectable correlation between the fall in blood pressure and either basal angiotensin II or basal plasma renin concentrations.

(iv) Control rats.

Six chronic hypertensive rats (9, 10 and 12 weeks) were given an injection of saline instead of converting enzyme inhibitor. These rats showed no changes in blood pressure (Figure 3.6(i)), angiotensin II (Figure 3.6(ii))

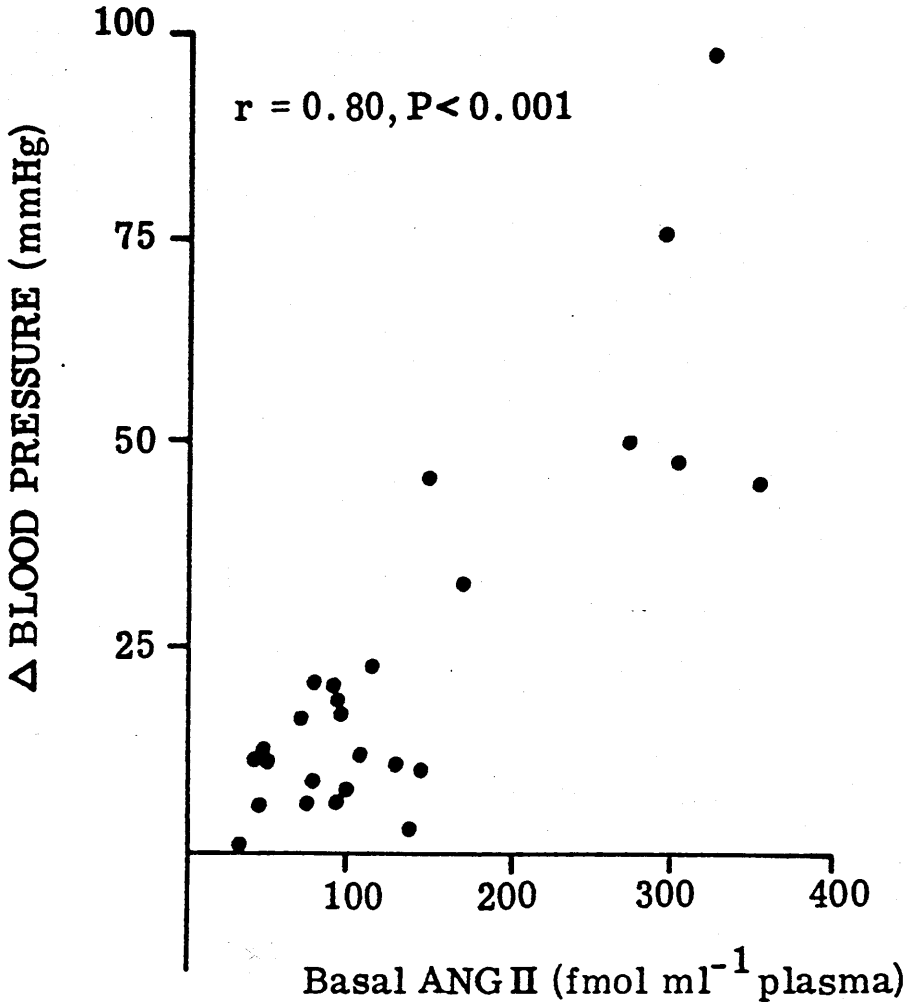


Figure 3.7 Correlation between the fall in blood pressure and basal angiotensin II (ANGII) in chronic hypertensive rats given converting enzyme inhibitor.

or plasma renin concentration (Figure 3.6(iii)).

(v) Serum converting enzyme.

Serum converting enzyme was measured in the 4 week intermediate rats before (2.5 +/- 0.52 umol hippuric acid /ml/h) and after (0.21 +/- 0.17 umol hippuric acid /ml/h) administration of converting enzyme inhibitor. This confirms a greater than 90% inhibition.

3.4 Commment.

Figure 3.8 shows the angiotensin II / blood pressure regression lines for both acute and chronic rats as determined for Figure 3.5. Superimposed on these are the mean hypotensive responses following converting enzyme inhibition for the 26 chronic rats and the 12 intermediate rats. It can be seen from this that the hypotensive response of the 26 chronic rats follows closely both the slope and the position of the regression line for all the chronic hypertensive rats thus confirming the regression line as a fairly accurate representation of the acute effects of angiotensin II in chronic renal hypertension in rats.

The very small fall in blood pressure after converting enzyme inhibition in the 4 week intermediate rats indicates that the suppressed levels of angiotensin II at this stage do not contribute significantly to the

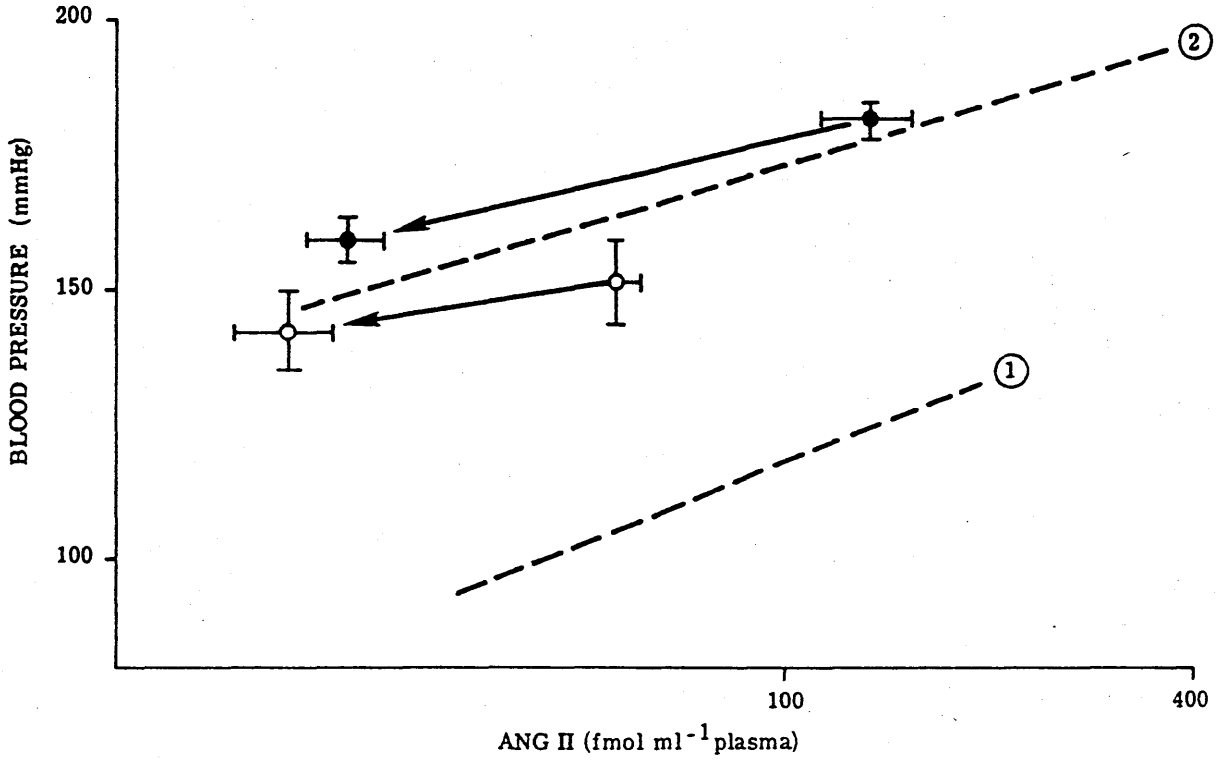


Figure 3.8 Mean angiotensin II and blood pressure values, before and 5 minutes after giving converting enzyme inhibitor, for 26 chronic (8-20 weeks, ●) and 12 intermediate (4 week, O) hypertensive rats, superimposed on the angiotensin II/blood pressure regression lines for acute (1-2 day ①) and all chronic (8-20 weeks ②) hypertensive rats. Bars show +/- SEM.

hypertension. From Figure 3.8 it can be seen that this fall in blood pressure in the 4 week rats follows the slope of the regression lines for both the chronic and acute rats, but is positioned at the lower unresponsive range. This indicates that the net vascular responsiveness during this intermediate phase is the same as for the acute and chronic stages and thus the discrepancy between the rising blood pressure and the low angiotensin II at this intermediate stage cannot be accounted for by an increase in sensitivity. The fact that the line showing the fall in blood pressure for the 4 week rats is positioned upwards from the regression line for the acute rats and is nearer to that for the chronic rats again suggests the onset of some other mechanism other than the acute effect of angiotensin II which must be mainly responsible for the developing hypertension, even at this early stage. One possible explanation for this other mechanism is that angiotensin II may be acting through a slow chronic effect on blood pressure as well as its acute pressor effect. In order to test this the next experiment was devised to compare the effects on blood pressure of captopril administered acutely and chronically to rats with established hypertension (ie rats in the chronic stage).

CHAPTER 4

AN EXAMINATION OF THE SEPARATE EFFECTS OF ACUTE AND CHRONIC INHIBITION OF CONVERTING ENZYME ON BLOOD PRESSURE IN TWO-KIDNEY, ONE CLIP RATS WITH ESTABLISHED HYPERTENSION.

4.1 Introduction.

To summarise so far:

Three overlapping stages in the development of the hypertension in the two-kidney, one clip rat have been identified. The first of these is the acute stage, where blood pressure, angiotensin II and plasma renin concentration are all elevated, the second is the intermediate stage where blood pressure is continuing to rise but both angiotensin II and plasma renin concentration fall towards normal levels and thirdly, the chronic stage where the hypertension has become established and angiotensin II and plasma renin concentration again are rising. The previous experiment has suggested that in the acute stage it is the rise in angiotensin II which is responsible for the hypertension and has suggested that in the intermediate and chronic stages, because the acute administration of converting enzyme failed to return the pressure to normal levels during these stages, some other mechanism is responsible for the greater part of the hypertension. Angiotensin II

has been shown to have a slow pressor effect when infused at a dose below the threshold of the direct pressor effect. This has been demonstrated in rabbits (Dickinson & Lawrence,1963) , dogs (McCubbin et al.,1965; Cowley & McCaa,1976; DeClue et al.,1976; Bean et al.,1979) and rats (Koletsky, Rivera-Velez & Pritchard,1965; Brown et al.,1981,1983) and it may be a possible explanation of the second mechanism involved. One way of testing this is to infuse captopril chronically to rats whose hypertension was already established and to compare the fall in pressure with that occurring following the acute administration of captopril. If the fall in blood pressure with the chronic captopril is greater than with the acute captopril this would provide additional evidence to support the notion that the other mechanism was at least in part due to the secondary slow effect of angiotensin II on blood pressure although there is always the alternative explanation that converting enzyme inhibitors act by kinin potentiation.

4.1:1 Experimental Design.

Table 4.1 shows the protocol for this experiment. Groups of up to 20 rats had a silver clip placed across their left renal artery at any one time, the right kidney being left untouched. Between 5 and 13 weeks after clipping, rats known to be hypertensive (their systolic

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
		Day 1	Day 2	Day 3	Day 4	Day 5
Implant catheters into aorta + IVC Weigh rat	Rats into experimental cages	**10:00** Start continuous BP reading	**09:00-10:00** 1.Blood sample for AII + PRC (0.75ml] 2. AI injection (100ng/0.1ml) **10:00** Captopril injection (2.5mg/kg/0.1ml) **10:15** AI (100ng) **17:00** AI (100ng)	**10:00** 1. AI (100ng) 2. Start chornic captopril/ dextrose infusion (1mg/kg/h) **12:00** AI (100ng)	**10:00** AI (100ng)	**10:00** AI (100ng)
Day 6	Day 7	Day 8	Day 9			
10:00 AI (100ng)	**10:00** AI (100ng)	**09:00-10:00** 1. Blood sample for AII + PRC (0.75ml) 2. AI (100ng) **10:00** Stop infusion **17:00** AI (100ng)	**10:00** AI (100ng)	**09:00-10:00** 1. Blood sample for AII + PRC (0.75ml) 2. AI (100ng) **11:00** 1. Kill rat 2. Weigh		

Table 4.1 Protocol for Experiment 2

pressure having been measured by tail plethysmography) were chosen and catheters were implanted in their abdominal aorta and IVC. After 2 days they were placed in their experimental cages and extensions were fitted to the catheters. At 10.00h on the third day (day 1) the arterial line was connected via the pressure transducer to the blood pressure recorder which in turn was connected to an Apple IIe microcomputer (see 2.5:1). Blood pressure was then recorded continuously throughout the 9 days of the experiment both on the paper chart from the recorder and at 10 minute intervals by means of the computerised data collecting and analysis system. Apart from an initial comparison between blood pressures of 4 rats calculated by hand from the recorder chart and those obtained from the computer (see 4.1:2(ii)), all the long term pressure measurements quoted for this study were obtained using the computer. These pressures were calculated as the 4 hourly mean of measurements taken at 10 minute intervals starting on day 1. Acute changes in pressure following both captopril and angiotensin I injections were calculated from the recorder chart. At 10.00h on day 2, after the 24 hour basal period, blood (0.75ml) was removed for the measurement of angiotensin II and plasma renin concentration. Following this captopril was administered as a bolus injection (2.5mg/kg/0.1ml) via the venous catheter. After a further 24 hour period a chronic infusion was begun. The test rats were infused with captopril (1mg/kg/h in heparin/dextrose, 150

units/ml) and control rats with heparin/dextrose (150 units/ml) at an infusion rate of 1ml/24hours. After 5 days the infusion was stopped and blood (0.75ml) was removed for angiotensin II and plasma renin concentration measurements. Blood pressure was recorded for a further 2 days at the end of which a final blood sample (0.75ml) was taken. As a test of the effectiveness of both acute and chronic captopril treatment to inhibit angiotensin converting enzyme activity and hence block the formation of angiotensin II, angiotensin I (100ng/0.1ml) was injected via the arterial catheter at the end of the basal period, after acute administration of captopril (at 15 minutes, 7 hours and 24 hours), during the chronic infusion of captopril (2, 24, 48, 72, 96 and 120 hours after the start of the infusion) and during the 2 post-infusion days (7, 24 and 48 hours after the end of the infusion). All values quoted in the results are mean +/- standard deviation.

4.1:2 Results.

(i) Number of rats studied.

24 rats were studied all of which were given captopril acutely. Of these, 16 were subsequently infused with captopril (test) and 8 were infused with dextrose (control). No more than 4 rats were studied at any one

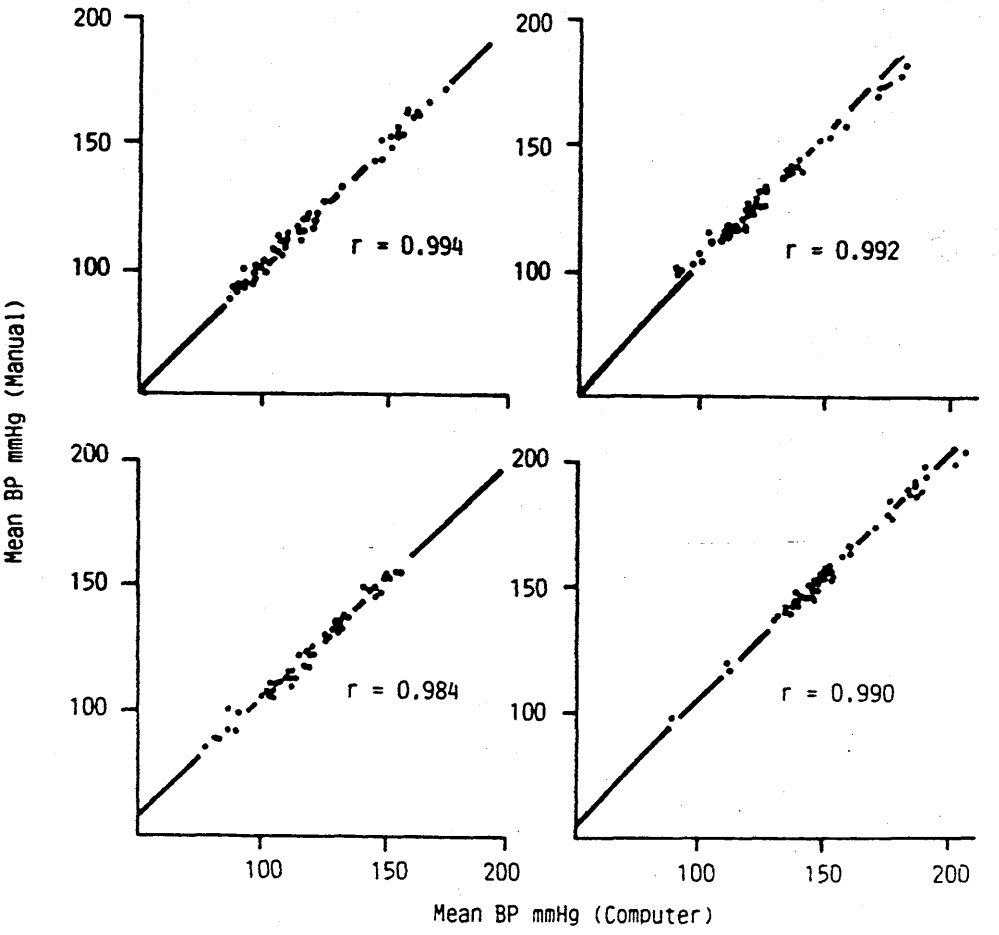


Figure 4.1 Correlations between blood pressures calculated manually from the recorder chart and mean pressure as collected and calculated by computer for 4 rats. Each point is the 4-hourly mean of 10 minute measurements over a 9 day period.

time, at least one of which was always a control.

(ii) Comparison of manual and computer calculated blood pressures.

A comparison between the mean arterial pressures calculated by hand from the recorder chart and the mean arterial pressures calculated by the computer was carried out for 4 rats. Of these, 3 were infused chronically with captopril and one was infused with dextrose. Each rat was connected to a different recorder channel and the comparison was carried out for the full 9 days of the study. Figure 4.1 shows the results of this comparison. There is a highly significant correlation in each case, the only discrepancy being that two of the recorder charts gave slightly higher values than the computer for pressures below 100mmHg. This was confirmed as an innacuracy in the recorder during calibration with the external pressure standard. Thus the computer method was proved to be more accurate and so was used throughout the remainder of the study.

(iii) Changes in blood pressure.

Figure 4.2 shows the continuous blood pressure profile for both groups of rats for the 9 day experiment. Each day is a 24 hour period from 10.00h to 10.00h. Apart from the solid and open triangles, all the points

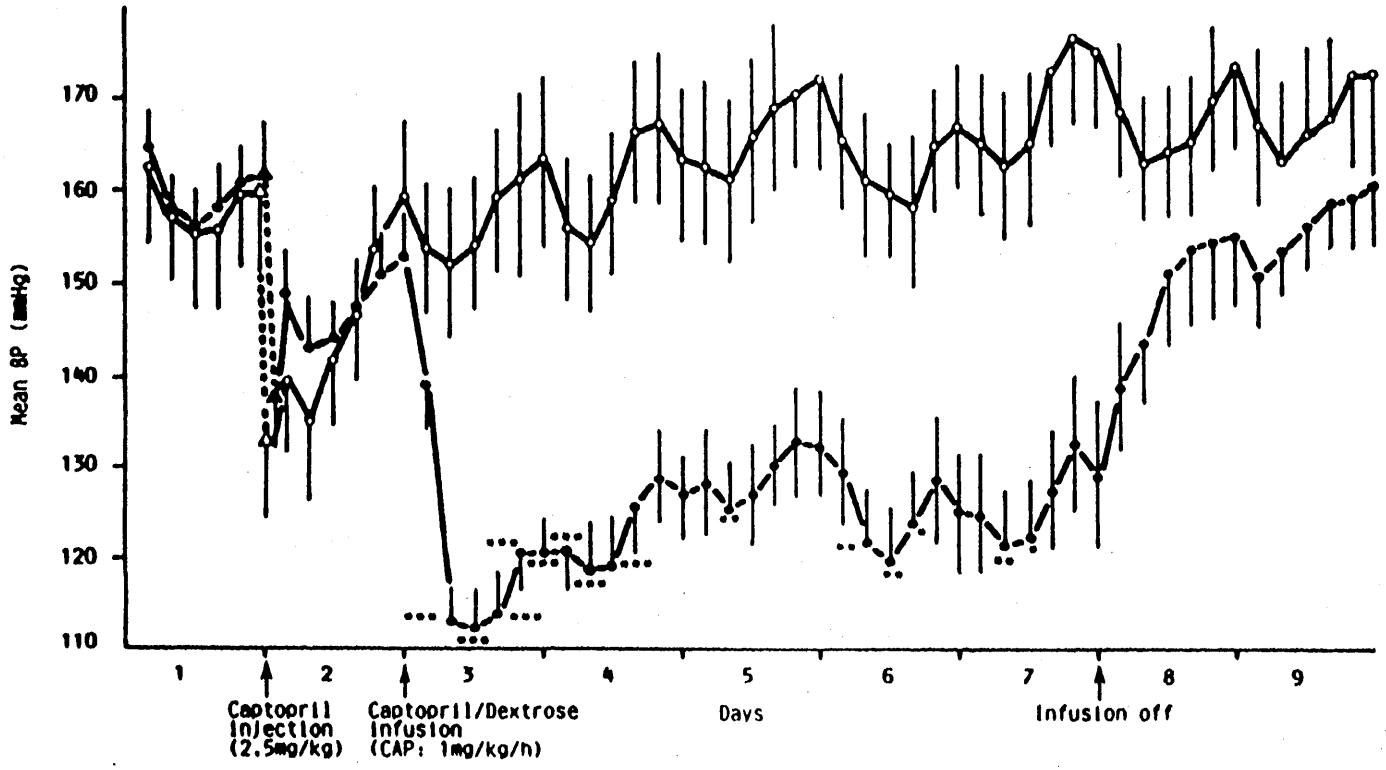


Figure 4.2 Blood pressure profile of rats given an acute injection of captopril followed by a chronic infusion of dextrose (O) and rats given an acute injection of captopril followed by a chronic captopril infusion (●). The circles represent the 4 hourly mean of 10 minute measurements starting at 10.00h each day. The triangles represent the mean of 10, 1 minute measurements before the acute injection of captopril (see Figure 4.3). A one tailed Wilcoxon signed rank test for matched pairs was used to compare mean pressures obtained during the chronic infusion of captopril (●) with the lowest pressure obtained after the acute injection of captopril (▲) *** $p<0.005$; ** $p<0.025$; * $p<0.05$. Bars represent +/- SEM.

represent the 4 hourly mean of pressures taken at 10 minute intervals. The solid and open triangles represent the mean of 10 minute pressure measurements before the acute injection of captopril and the lowest individual pressure measurement after the captopril injection.

Basal daily blood pressure ranges for the test and control animals were similar on day 1 (ranges: 156.6 +/- 18.5 to 166.3 +/- 16.8 and 155.9 +/-25,1 to 163.9 +/- 31.0 mmHg respectively).

(a) Acute captopril injection.

After the injection of captopril on day 2 a rapid fall in blood pressure was seen in both groups of animals (Figure 4.3). Pressure fell to a minimum 10 to 15 minutes after the injection and then began to rise again. The fall in blood pressure was similar for both groups (165.1 +/- 19.4 to 137.6 +/- 23.3 mmHg for the test group and 161.9 +/- 29.2 to 133.0 +/- 24.2 mmHg for the control group). Twenty four hours after the captopril injection blood pressure in both groups had returned towards pre-injection values (Figure 4.2).

(b) Chronic infusion of captopril.

Following the start of the chronic captopril infusion on day 3 pressure in this group fell reaching a minimum at 12 hours of 112.5 +/-19.4 mmHg. During the

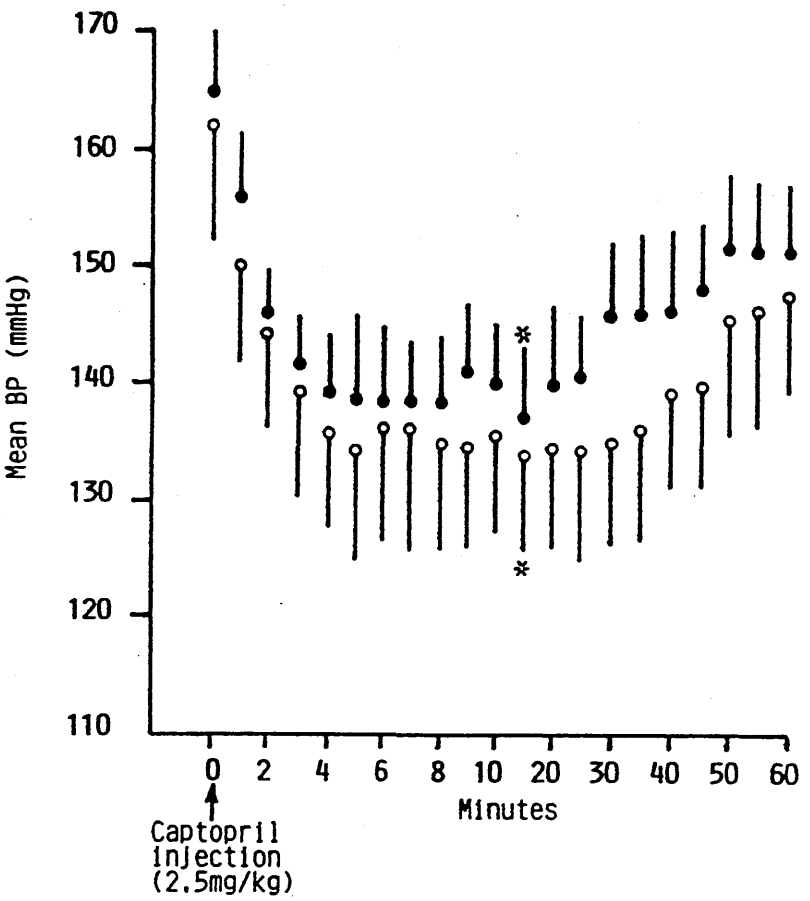


Figure 4.3 The acute fall in mean arterial blood pressure following the injection of captopril in rats subsequently given a chronic infusion of captopril (O) and in rats given a chronic infusion of dextrose (●). The first point of each group represents the mean of 10 one minute measurements before captopril was given. All other points are individual measurements. Bars represent 1 SEM. Asterisk indicates the lowest individual blood pressure for each group.

first 36 hours after the start of the infusion, pressures in this captopril-infused group remained significantly lower than those seen following the acute injection of captopril. After this pressure tended to rise reaching a plateau during the third day of the infusion and while it was always lower than that seen after the acute captopril injection it was only significantly so during the troughs of diurnal variation. In the dextrose-infused control group pressure rose gradually throughout the experiment. During the chronic infusions pressure in the captopril-infused group was always significantly lower ($p<0.001$) than that for the dextrose controls. By day 5 of the infusion for the captopril treated rats daily pressures ranged from 122.1 +/- 23.4 to 136.0 +/- 30.2 mmHg while the range for the dextrose control rats was 163.6 +/- 23.8 to 180.4 +/- 22.5 mmHg. After stopping the captopril infusion pressure started to rise within 4 hours and continued to do so for 2 days after which it was similar to that for the dextrose-infused control group (daily ranges: 151.1 +/- 19.4 to 158.5 +/- 19.6 mmHg for the captopril-infused group and 162.5 +/- 26.6 to 173.7 +/- 31.0 mmHg for the dextrose-infused group) (Figure 4.2).

(iv) Angiotensin I challenge.

The results are shown in Figure 4.4. Fifteen minutes after the acute captopril injection the pressor

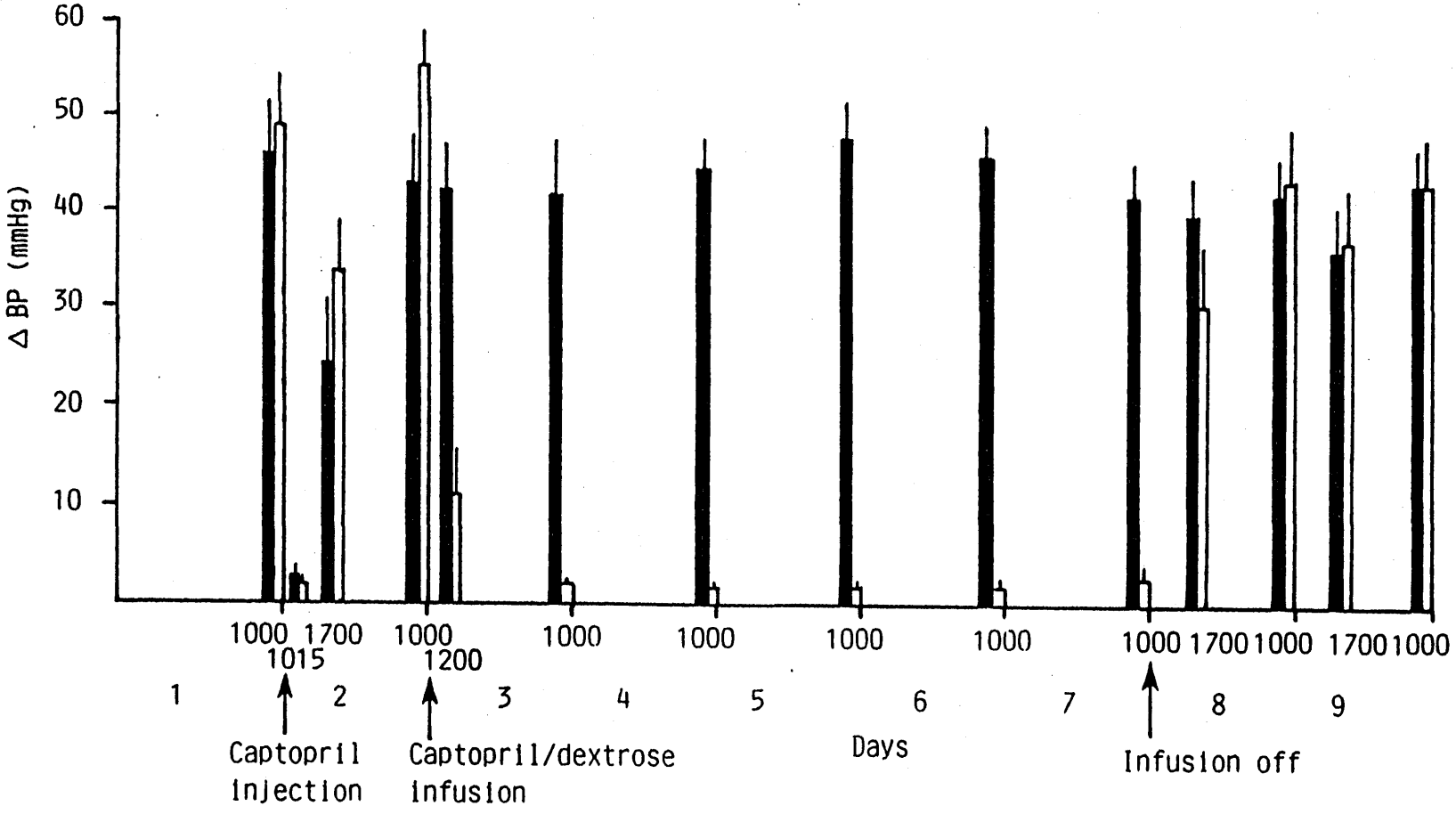


Figure 4.4 Blood pressure response to the administration of 100ng angiotensin I in rats given an acute injection of captopril followed by a chronic dextrose infusion (■) and rats given an acute injection of captopril followed by a chronic infusion of captopril (□). Bars represent 1 SEM.

response to the angiotensin I was less than 5% of the pre-treatment values for both groups of rats. After 7 hours the response had returned to 69% and 53% respectively, and by 24 hours, just before the start of the chronic infusions, the response for both groups had returned to pretreatment values. Two hours after the start of the chronic infusions the pressure response of the captopril group had fallen to 21% of that for the dextrose group which remained unchanged. Throughout the rest of the infusion the pressor response for the dextrose group remained the same while that for the captopril treated group was always less than 5% of that found for the dextrose group. Seven hours after the infusion was stopped the response for the captopril group had returned to 78% of that for the dextrose control group and at 24 and 48 hours there was no difference between the responses of the 2 groups of rats.

(v) Changes in angiotensin II.

The basal angiotensin II value at the end of day 1 for the test animals was 72.6 +/- 52.0 fmol/ml. At the end of the 5th day of the captopril infusion this had fallen to 17.2 +/- 8.0 fmol/ml and two days after stopping the infusion had risen to 57.4 +/- 6.5 fmol/ml. The angiotensin II levels for the dextrose group remained relatively unchanged throughout the experiment, 94.1 +/- 50.1 fmol/ml at the end of day 1, 65.0 +/- 22.9 fmol/ml at

the end of the dextrose infusion and 57.4 +/- 15.5 fmol/ml 2 days after stopping the infusion.

(vi) Changes in plasma renin concentration.

For the test animals the basal plasma renin was 44.6 +/-44.1 pmol AngI/ml/h. This had risen to 332.4 +/-62.9 pmol AngI/ml/h by the end of the 5th day of captopril infusion and 2 days after stopping the infusion it had fallen back to 30.0 +/- 44.9 pmol AngI/ml/h. Plasma renin values for the dextrose-infused group remained unchanged throughout (40.3 +/- 50.1, 32.2 +/- 18.5 and 31.2 +/- 21.0 pmol AngI/ml/h respectively).

(vii) Relationship between blood pressure fall and basal angiotensin II and plasma renin concentration.

(a) Acute captopril injection. The fall in blood pressure was significantly related to the basal angiotensin II and plasma renin concentrations for both groups combined ($r = 0.87$ and 0.82 respectively, $p < 0.001$, $n=24$).

(b) Chronic captopril infusion. Table 4.2 shows the relationship between the fall in blood pressure with increasing duration of captopril, and basal angiotensin II and plasma renin concentration. The correlation remained

Hours of captopril	PRC		Ang.II	
	r	p	r	p
24	0.65	<0.01	0.60	<0.02
48	0.61	<0.02	0.40	NS
72	0.52	<0.05	0.40	NS
96	0.63	<0.01	0.48	NS
120	0.70	<0.01	0.57	<0.05

NS = not significant

Table 4.2 Correlation between the fall in blood pressure with increasing duration of captopril infusion and basal plasma renin concentration (PRC) and angiotensin II (Ang.II).

significant throughout the infusion for plasma renin however for angiotensin II it was either just significant or not significant.

4.2 Comment.

From Figure 4.2 it can be seen that the chronically infused captopril did cause a greater fall in blood pressure in those rats with already established hypertension than did the acute administration of captopril. Although the mechanism involved remains uncertain these results do seem to support the suggestion that a second effect of angiotensin II may be involved. However as can be seen, this fall in blood pressure with the chronic captopril was not entirely sustained throughout the 5 days of the captopril infusion which suggests that there might be a gradual onset of some other compensatory mechanism which possibly has been triggered as a consequence of the chronic suppression of angiotensin II.

CHAPTER 5

GENERAL DISCUSSION.

5.1 Summary of main findings.

From the studies carried out here three main phases can be distinguished in the development and maintenance of hypertension in the two-kidney, one clip rat. These phases are not rigidly defined and considerable overlap between phases seems likely. The first or acute phase is present for 1-2 days after clipping when both blood pressure and angiotensin II are found to be raised. Following this is the second or intermediate phase when blood pressure is continuing to rise but angiotensin II and plasma renin are found to be suppressed. The third chronic stage occurs when hypertension has become established and plasma renin and angiotensin II are again elevated. In this study the chronic stage was established at 5-7 weeks after clipping. During both the acute and chronic stages there was a significant relationship between blood pressure and angiotensin II. In contrast there was no relationship during the intermediate stage. Acute administration of converting enzyme inhibitor to rats in the chronic stage produced a significant fall in blood pressure. However this fall failed to return blood pressure to within the

normal range found for sham operated rats. There was no significant fall in blood pressure after acute converting enzyme inhibitor administration to rats in the intermediate stage. This suggests that an increase of the acute vasoconstrictor effect of angiotensin II was not important in the development and maintenance of the hypertension during these stages and that some other mechanism must be involved. In the second study, chronic administration of converting enzyme inhibitor to rats in the chronic phase with established hypertension resulted in a greater fall in blood pressure than that produced by the acute injection alone suggesting that this other mechanism may be a second effect of angiotensin II.

5.2 Sequence of events in the development of the hypertension.

Figure 5.1 shows the proposed sequence of events for the progressive changes in blood pressure and angiotensin II during the onset, development and establishment of the hypertension. The mean values for angiotensin II and blood pressure at different stages from acute to chronic hypertension are related to the angiotensin II/blood pressure regression lines found for the acute and chronic rats.

Immediatly (1-2 days) after application of the clip blood pressure and angiotensin II rise, moving up the

lower, acute, regression line relating angiotensin II and blood pressure, angiotensin II being mainly responsible for the rise in blood pressure at this time. Two weeks after clipping angiotensin II is suppressed while blood pressure remains elevated, angiotensin II now being again at the lower end of the dose response curve and therefore having little or no acute effect on blood pressure at this stage. At 4 weeks angiotensin II has moved up the dose response curve slightly but is still suppressed compared to the 1-2 day acute stage and so will have only a small effect on blood pressure. In contrast blood pressure has risen considerably at 4 weeks to an extent which cannot be accounted for by the angiotensin II concentration. At 7 weeks blood pressure continues to rise and angiotensin II has also increased. As angiotensin II has moved up the dose response curve its acute effect will also have increased but this effect will still be small in relation to the increase in blood pressure. Thus the acute effect of angiotensin II during the period of 5-7 weeks after clipping varies according to its position on the dose response curve. This acute effect however is overshadowed by, and superimposed on top of, a continuously developing and much more important mechanism whose onset appears to be as early as 2 weeks and which reaches its maximum effect at 5-7 weeks after clipping having shifted the regression line found for the acute rats upwards in a parallel fashion to that position found for the chronic rats.

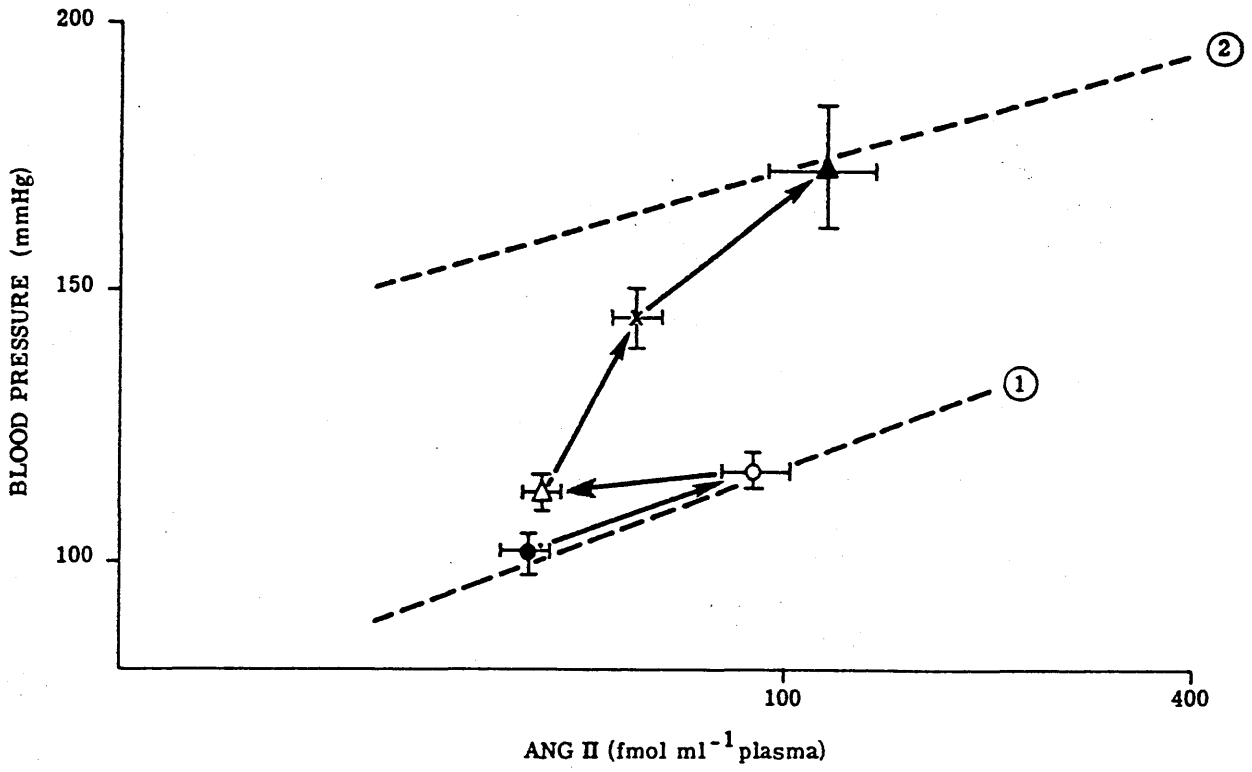


Figure 5.1 Progressive changes in angiotensin II and blood pressure during the onset, development and establishment of hypertension in the two-kidney, one clip hypertensive rat in relation to the upward parallel shift in the angiotensin II/blood pressure regression line from that found for the acute (1-2 day (1)) to that found for the chronic (8-20 weeks (2)) hypertensive rats. Values are the mean +/- SEM for all sham-operated rats (●), 1-2 day clipped rats (O), 2 week clipped rats (Δ), 4 week clipped rats (X) and 7 week clipped rats (▲). (See 3.2 for details)

5.3 Mechanisms at work during the different stages in the hypertension.

5.3:1 Acute stage.

In this study the acute stage was represented by the 1-2 day rats. At this time blood pressure, angiotensin II and plasma renin were all raised compared to the sham operated rats. This observation agrees with the findings of most other workers (Brown et al.,1966; Blair-West et al.,1968; Bianchi et al.,1972; Liard et al.,1974; Carretero and Gulati,1978). Previous studies with angiotensin II inhibitors or antagonists (Pals et al.,1971; Thurston & Swales,1974a; Coleman & Guyton,1975; Miller et al.,1975; Freeman et al.,1977b; Masaki et al.,1977; Carretero and Gulati,1978) and studies involving angiotensin II or renin dose response measurements (Bianchi et al.,1972; Caravaggi et al.,1976) suggest that the raised angiotensin II and plasma renin levels found immediatly after clipping are wholly responsible for the hypertension at this time.

The increased renin and angiotensin II levels are consequent on reduced renal perfusion pressure resulting from application of the clip to the renal artery (Selig et al.,1983). Angiotensin II then has a direct vasoconstrictor effect to raise blood pressure. The

finding here of a significant angiotensin II/blood pressure relationship for the rats 1-2 days after clipping (acute rats) agrees with this, however complete confirmation of this would require angiotensin II infusion studies to verify that the regression lines were superimposible as has been shown in the dog (Caravaggi et al.,1976). Comparison with a previous study of acute angiotensin II infusions in the rat carried out in this department by Brown et al. (1981) suggests that the regression line obtained for the acute animals is a fairly accurate representation of the true effects of endogenous angiotensin II.

5.3:2 Intermediate stage.

The intermediate stage in this study occurs between 2 and 5 weeks after clipping. During this time blood pressure continues to rise but angiotensin II and plasma renin are suppressed and do not start rising again until after 4 weeks. No significant relationship between angiotensin II and blood pressure was found at this time, and acute administration of converting enzyme inhibitor had little effect on blood pressure. It is likely therefore that plasma angiotensin II by its acute vasoconstrictor effect plays little or no part in the hypertension at this stage. These observations pose 3 questions. Firstly why are angiotensin II and plasma renin lower between 2 and 4 weeks, secondly why does blood

pressure continue to rise in the face of the suppressed renin-angiotensin system and thirdly what causes the secondary and progressive rise in angiotensin and renin after 4 weeks.

In addition to the biological mechanisms involved there is the possibility of an artifactual result answering the first question arising from the cross-sectional design of the study. It is possible that the suppressed levels of renin and angiotensin II found between 2 to 4 weeks arises because rats chosen for study at this time are in fact normotensive and would not subsequently develop hypertension. However as the same procedures were used throughout the study and the incidence of hypertension which developed was consistent at about 80% and rats were chosen at random for study in each group then it is likely that rats chosen for the 2 to 4 week groups contained the same proportion of animals which would subsequently become hypertensive. Also in the separate experiment where systolic blood pressure was measured sequentially in a group of rats it was shown that rats which develop hypertension in the chronic stages up to 20 weeks have only slightly elevated blood pressures between 2 and 3 weeks. This is similar to the findings in main cross-sectional study.

A physiological mechanism to explain the suppression of angiotensin II and plasma renin between 2 and 4 weeks is sodium retention. Raised angiotensin II in the acute stage would have a sodium retaining effect via

aldosterone stimulation. Increased sodium retention would in turn have a negative feedback effect to suppress renin and AII. Such a proposal has been put forward by both Ledingham (1975) and Guyton et al. (1975). However evidence for sodium retention is unclear in this renal model. Some studies find a positive sodium balance at this time (Leenen & DeJong,1975; Mohring et al.,1975) while others find no change or a negative balance (Blair-West et al.,1968; Tobian et al.,1969; Bianchi et al.,1972; Swales et al.,1972). In a study carried out in this department by McAreavey et al. (1982) using two-kidney, one clip rats, there was no significant difference in exchangeable sodium between clipped and sham operated rats up to 6 weeks after clipping. Evidence for a sodium retaining effect is therefore inconclusive.

An alternative explanation may be a return to normal of renal blood flow and renal artery pressure in the clipped kidney. In a study performed on dogs with one-kidney, one clip hypertension, (Selig et al.,1983) both renal blood flow and renal artery pressure were found to fall immediatly after application of the clip, however there is evidence that the subsequent increase in systemic blood pressure results in the return of both renal blood flow and renal artery pressure towards values found before clipping. This would in turn also produce a fall in renin and angiotensin II.

One possibility for the continuing rise of blood pressure in spite of the suppressed renin angiotensin

system may be an increase in vascular reactivity to angiotensin II. However the fact that the slopes of the angiotensin II/blood pressure regression lines were the same for both the 1-2 day acute rats and the 8 - 20 week chronic rats, and the observation that the fall in blood pressure in both the chronic and the 4 week intermediate rats after acute converting enzyme inhibitor administration followed the slope of these regression lines indicates that the net vascular responsiveness to angiotensin II remains the same throughout the development and maintenance of the hypertension in this experimental model. This finding agrees with that of Marks et al. (1982) who found that the duration of the hypertension did not alter the vascular responsiveness of exogenously administered angiotensin II and studies in the dog also support this (Brown et al.,1979). Therefore the fact that in the intermediate stage blood pressure is raised while angiotensin II and plasma renin are suppressed cannot be accounted for by an increase in sensitivity to angiotensin II and suggests that some other mechanism must be responsible for the continuing rise in blood pressure in the face of a suppressed renin angiotensin system. Possibilities for this mechanism will be discussed in a later section.

The most likely explanation for the rise in angiotensin II and renin starting at 4 weeks is a progressive loss of sodium as the rats enter the malignant phase of hypertension (Gavras et al.,1975; Mohring et

al.,1975). This is supported by the fact that in experiment 1 the clipped rats were found to be lighter in weight at 5 to 6 weeks than their equivalent sham operated rats, this difference becoming significant at 8 weeks.

5.3:3 Chronic stage.

The chronic stage occurs when the hypertension has become established and both angiotensin II and plasma renin concentration are elevated. In this study this phase is taken as 8 to 20 weeks after clipping. Much of the work previously published suggests that in chronic hypertension plasma renin levels are found to be normal (Koletsky et al.,1967; Oates, Stokes & Storey,1975; Carretero & Gulati,1978; Russell et al.,1982). There are a number of possible reasons for this difference. First, different blood sampling techniques may have been used. Anaesthetic is known to be a stimulus to renin secretion (Leenen & De Jong,1975) and values for renin measurements for samples from normal rats taken under anaesthesia are 10 to 20 times the values found for this study (Koletsky et al.,1967; Oates et al.,1975; Russell et al.,1982). It is therefore possible that any important differences in plasma renin concentrations between clipped and sham operated or normal animals could be obscured by these gross levels of renin.

A second explanation may be that although the rats have been studied at the same chronological time, the

hypertension may in fact be at a different stage in its development. Factors which could contribute to this include differences in the strain and sex of rat used, applying the clip to rats of different weights/ages, using a clip or ligature of different dimensions and applying it to different regions of the renal artery.

Another possible explanation for the low renin values found may be the severity of the hypertension that develops in individual rats during the chronic stage. As previously mentioned (3.2:2 (i)), rats which were found to be only mildly hypertensive in the late chronic stage (15-20 weeks) were excluded from this study in order to allow a comparison with the 8 to 10 week early chronic rats in which the severity of the hypertension was similar for both. In this study a highly significant relationship was found between angiotensin II and blood pressure in the 8 to 20 week (chronic) rats (the lower the blood pressure the lower the angiotensin II and plasma renin concentration) and in fact a number of rats in the chronic stage which did not develop severe hypertension (ie blood pressure above 170mmHg) had relatively normal angiotensin II and plasma renin levels. These findings agree with those of Mohring et al. (1975) and Carretero & Gulati (1978) and also explain the fact that rats can be divided into responders and non-responders after angiotensin II blockade, the angiotensin II/blood pressure regression line suggesting a continuous range of responses. Mohring et al. (1975) have suggested as a reason for this, that

the rats with the lower blood pressure have come into sodium balance (responders) and thus the stimulation of plasma renin and angiotensin II is halted. The rats with the higher blood pressures (non responders) have not come into balance and are continuing to lose sodium and thus stimulate an increase in angiotensin II which causes a further rise in blood pressure which in turn results in further sodium loss. These rats will enter the malignant phase of hypertension via the vicious circle first described by Byrom & Dodson (1949) and will rapidly die. The early death of rats with severe or malignant hypertension and elevated plasma renin and angiotensin II will leave animals with less severe benign hypertension and low plasma renin and angiotensin II levels. This is the most likely explanation for the differences in renin and angiotensin II levels found in various studies of rats in the chronic stage. In the studies reported here it was found that rats with their left renal artery clipped did not grow as rapidly as the equivalent sham operated rats, this difference was seen as early as 6 weeks after clipping and became significant at 8 weeks (see Figure 3.3 (i)). This suggests that perhaps some of these rats were entering the malignant phase of hypertension.

The mechanism sustaining the hypertension during this stage is not clear. As has been discussed earlier (5.2) the influence of the acute effect of angiotensin II on the hypertension at any stage is determined by its plasma concentration and its position on the angiotensin

II/blood pressure dose response curve. The fact that this curve has shifted upwards in a parallel fashion in the chronic stage, and that although acute administration of converting enzyme inhibitor produced a significant fall in blood pressure, this fall failed to reduce the blood pressure to within the range found for the sham operated rats, ie only produced a partial correction of the hypertension, confirms previous observations (Thurston & Swales,1974a; Gavras et al.,1975; Carretero & Gulati,1978; Otsuka et al.,1979) that although the acute effect of circulating angiotensin II has some involvment at this stage, it is only partly responsible for the hypertension and therefore some other mechanism must be involved.

After chronic administration of converting enzyme inhibitor to rats in this chronic stage a greater fall in blood pressure than that seen with acute converting enzyme inhibitor administration occurred. This observation supports the notion that the other mechanism may also operate through angiotensin II. These results are similar to those found by Bengis and Coleman (1979) and the initial fall in blood pressure following the start of the captopril infusion was very similar to that shown by Riegger et al. (1977) who infused saralasin and the converting enzyme inhibitor teprotide to renal hypertension rats. However these observations are in contrast to those of Bing et al. (1981) and Russell et al. (1982) who failed to show any significant effect on blood pressure following a chronic infusion of either saralasin

or captopril. Brown et al. (1983) suggested that the discrepancy between the results of Riegger et al. and those of Bing et al. might be explained by the fact that the study of Riegger et al. (1977) was performed during the day, when blood pressure was in the trough of its diurnal variation, while that of Bing et al. (1981) was performed at night when blood pressure is at its peak and could obscure what otherwise might be a fall in blood pressure. This could also apply to the results of Russell et al. (1982) who also infused overnight. Some evidence to support this comes from the present study which shows that after the initial large fall, the blood pressure during the chronic captopril infusion was significantly lower than that after the acute injection only during the day, ie during the trough of diurnal variation.

The fall in blood pressure caused by chronic captopril administration was not wholly maintained throughout the 5 days of the infusion which suggests the gradual onset of some other compensatory mechanism, possibly as a consequence of the sustained suppression of angiotensin II. This situation is less likely to exist during oral administration of captopril as used by DeForrest et al. (1982) when there is probably daily escape from maximal angiotensin II suppression.

5.4 Possible second mechanisms.

I have shown that the acute effect of angiotensin II is not wholly responsible for the development and maintenance of the hypertension in these two-kidney, one clip rats and that some other mechanism, which appears to be responsible for the greater part of the hypertension, must be involved. The onset of this mechanism seems to occur as early as two weeks after clipping, when blood pressure continues to rise while angiotensin II and plasma renin are suppressed, and reaches its maximum effect at 5-7 weeks. Chronic suppression of angiotensin II in rats with established hypertension by captopril infusion produced a greater fall in blood pressure than that seen after acute converting enzyme inhibitor administration, suggesting that this other mechanism operates through some secondary action of angiotensin II on blood pressure. Although there are a number of possibilities for this other mechanism, two of the most likely which involve the renin-angiotensin system are vascular renin and the slow pressor effect of angiotensin II.

5.4:1 Vascular Renin.

Renin and/or renin like enzymes have been found to be present in many tissues (Ganten et al.1976;1983; Naruse et al.,1983; Dzau et al.,1984; Deschepper et al.,1986; Sealey et al.,1987) including the blood vessel wall

(Thurston & Swales,1974b; Naruse & Inagami,1982; Swales et al,1983a; Swales & Heagerty,1987). Arterial wall renin like activity appears to have two sources. It may be kidney derived renin taken up from plasma (Loudon et al.,1982,1983; Swales et al.,1983a,1983b); changes in arterial renin levels parallel those of plasma renin and infusion of pressor quantities of renin in normal rats results in an increase of arterial renin, and it may be synthesised locally; synthesis of renin has been found in canine smooth muscle aortic cells and bovine endothelial aortic cells in culture (Re et al.,1982; Lilly, Pratt & Alexander,1985) and renin mRNA expression has recently been identified in a range of extra renal sites (Samani et al.,1987,1988). The relative importance of these two processes remains unknown.

Both angiotensin converting enzyme Miyazaki et al.,1984 and renin substrate have been found in the vasculature, angiotensin converting enzyme has been localised to the endothelial cell surface (Ryan et al.1976b; Johnson and Erdos,1977; Hial et al.,1979; Takada et al.,1982) and recently angiotensinogen mRNA sequences have been detected in many non liver sites (Dzau et al.,1986; Ohkubo et al.,1986) suggesting local synthesis. Thus it appears that all the necessary components of the renin-angiotensin system are present and angiotensin II could therefore be generated locally.

It is thought that local generation of angiotensin II within the arteriolar wall by vascular renin may be of

physiological and pathological importance perhaps by exerting local control over vascular vasoconstrictive activity (Dzau,1986; Swales & Heagerty,1987) the vascular angiotensin II levels not being reflected by circulating plasma angiotensin II levels. However demonstration of this *in vivo* is difficult, the problem being distinguishing between plasma and vascular renin effects. In studies where plasma renin levels were acutely reduced by bilateral nephrectomy in early two kidney, one-clip rats (Thurston & Swales,1977; Thurston et al.,1979) arterial renin was found to have a much longer half-life than plasma renin. This slow decline in vascular renin like activity was paralelled by a slow decline in the depressor response to blockade of the renin-angiotensin system suggesting vascular renin is of major importance in maintaining blood pressure. These observations fit with my own finding of a slow decline in blood pressure during chronic infusion of captopril in Experiment 2 and therefore offer a possible additional mechanism to the acute effect of the renin-angiotensin system. An explanation for the greater fall in blood pressure seen after chronically administered captopril than after acute administration could be that the local generation of angiotensin II occurs at a site which may not be so easily accessible to the captopril within the duration of the acute administration as has been suggested by Swales et al. (1983b).

5.4:2 The slow pressor effect of angiotensin II.

Angiotensin II infused over a prolonged period of time at a dose below the threshold of the direct pressor effect has been found to result in a slow rise of blood pressure. This has been demonstrated in the rat (Koletsky et al.,1965; Brown et al.,1981), rabbit (Dickinson & Lawrence,1963), dog (McCubbin et al.,1965; Cowley & McCaa,1976; DeClue et al.,1976; Bean et al.,1979) and man (Ames et al.,1965). The idea that the slow pressor effect might be important in the pathogenisis of hypertension was first suggested by Dickinson and Lawrence (1963). In the experiments described here there are a number of features which would lend support to the notion of a slow effect of angiotensin II on blood pressure:

(1) In the intermediate stage of the hypertension, after 2 weeks, although angiotensin II and plasma renin are suppressed compared to their levels at 1-2 days, they are still raised compared to the levels found for the equivalent sham operated rats. In effect both angiotensin II and plasma renin were always slightly elevated during the intermediate 2-4 week period. Reasons for the fall in angiotensin II and plasma renin at this stage have already been discussed (5.3:2). Therefore while angiotensin II and plasma renin may not have been high enough to influence blood pressure acutely, the prospect of an underlying and increasing chronic effect cannot be ruled out, especially as the slow gradual rise in blood pressure

at 2-5 weeks would fit with the slow pressor effect of angiotensin II. Additional evidence that this slow effect may be involved comes from the observation that chronic suppression of angiotensin II by converting enzyme inhibitor during the early stages of the disease (2-5 weeks after clipping) prevented the development of the hypertension during this period (Wallace & Morton,1984). Similar findings have been reported by Freeman et al. (1979) and DeForrest et al. (1982).

(2) Chronic administration of captopril to rats with established hypertension caused a greater fall in blood pressure than that found with acutely injected converting enzyme inhibitor suggesting a reversal of a slow effect of angiotensin II.

(3) After the chronic captopril infusion was stopped there was a progressive but slow return of the hypertension, which after 2 days had still not reached the same level as that seen in the control animals, further supporting the notion of a slow component operating through the renin angiotensin system.

5.4:3 Possible mechanisms of the slow pressor effect of angiotensin II.

A number of suggestions have been made as to the mechanism of the slow pressor effect of angiotensin II (Brown et al.,1977), some are discussed below.

(1) Action of angiotensin II on the nervous system.

Evidence suggests that the nervous system may be involved in the slow pressor effect: (a) resetting of baroreceptors is partly responsible for the early rise in pressure produced by low dose angiotensin II infusion in the dog (Cowley & DeClue,1976) (b) central administration of angiotensin II modulates baroreceptor response (Fukiyama,1973; Goldstein et al.,1974; Marker, Miles & Scroop,1980) and raises arterial pressure (Lowe & Scroop,1969), (c) drugs that interfere with nervous transmission prevent or attenuate the gradual rise of pressure (McCubbin et al.,1965; Yu & Dickinson,1971; Ferrario, Gildenberg & McCubbin,1972).

(2) An effect on salt and water excretion.

Angiotensin II alters urinary excretion of sodium and water (Brown & Peart,1962) and thus by acting directly or through aldosterone could cause sodium and hence water retention thereby raising blood pressure (Brown et al.,1976). While sodium restriction can prevent the slow rise in pressure in dogs, and dietary salt loading enhances it (Cowley & McCaa,1976), evidence that there is sodium retention in the two-kidney, one clip model is uncertain and controversial. Whether angiotensin II acts by stimulating aldosterone is also unclear. The balance of the evidence suggests that during chronic angiotensin II infusion the increase in aldosterone is not persistant or marked and that the sodium retaining effect of angiotensin II is more likely to be renal than adrenal

(DeClue et al.,1976; Bean et al.,1979; Hall et al.,1979,1984).

(3) Structural changes during the slow effect.

A slow effect on pressure might result from progressive structural alteration to the vessel wall of small resistance vessels in the form of increasing wall thickness and thus wall:lumen ratio. This in turn would raise the resistance to flow at maximum dilatation and result in an increase in responsiveness to vasoconstrictor agents such as angiotensin II (Folkow,1971). This structural alteration (or vascular hypertrophy) may occur in response to raised pressure or may be due to a direct effect of angiotensin II itself. Lever (1986) has suggested that angiotensin II could increase both vascular smooth muscle cell numbers and size leading to a gradual onset of vascular hypertrophy with a resultant slow rise in arterial pressure involving the vascular amplifier first described by Folkow (Folkow, Grimby & Thulesius,1958; Folkow,1978) in which minor but persistant overactivity of a pressor mechanism is amplified by hypertrophy, blood pressure rises slowly and severe hypertension results (Figure 5.2). It has been suggested that one of the ways that angiotensin II might exert this effect could be by acting as a mitogen or trophin for vascular smooth muscle. The pathway by which better known growth factors stimulate growth and protein synthesis in vascular muscle and other cells is illustrated in Figure

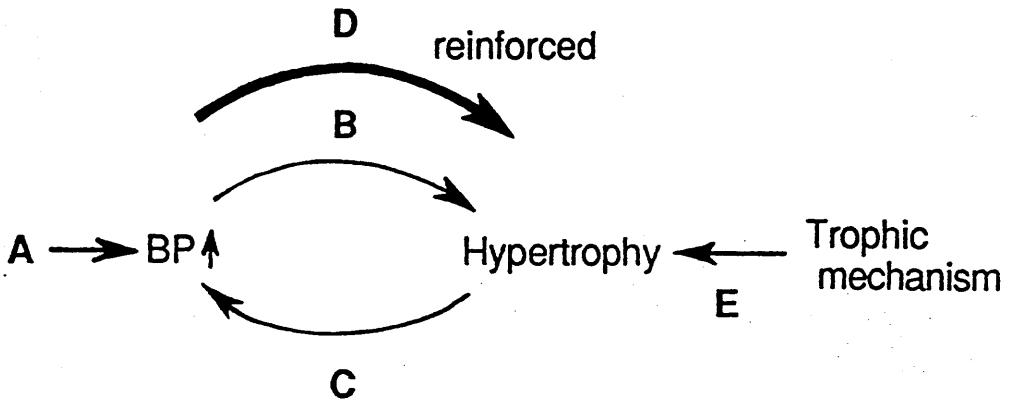


Figure 5.2 Minor overactivity of a pressor mechanism (A) raises blood pressure (BP) slightly, initiating positive feedback (BCB) and a progressive rise in blood pressure. D and E are two additional signals. D: an abnormal or amplified hypertrophic response to pressure. E: increase of an agent causing hypertrophy directly (for example excess growth hormone).

5.3. Like these factors (Moolenaar et al.,1983; L'Allemain et al.,1984; Owen,1984; Pouyssegur,1985) angiotensin increases inositol triphosphate within the cytosol and diacylglycerol within the cell membrane (Williamson et al.,1985; Bingham Smith,1986). As a result of the increase of diacyl glycerol and probably via protein kinase C, angiotensin II activates an amiloride sensitive Na+/H+ exchanger and sodium influx results (Brock, Lewis & Bingham Smith,1982; Bingham Smith & Brock,1983; Berridge & Irvine,1984; Berk et al.,1987). These changes lead to a rise of intracellular pH which is generally accepted as a precursor condition for cell growth (Busa & Nuccitelli,1984; Mahnensmith & Aronson,1985) and could lead to hyperplasia and hypertrophy. Such a process could explain the mitogenic effect of angiotensin II. There is some evidence to suggests that angiotensin II has a trophic or mitogenic action, for example it causes proliferation of vascular smooth muscle in culture (Campbell-Boswell & Robertson,1981; Lyall et al,1988), stimulates ribonucleic acid (RNA) synthesis (Re & Parab,1984) and growth in fibroblasts (Schelling et al.,1979). It is possible that the local generation of angiotensin II by vascular renin (see 5.4:1) as well as circulating angiotensin II could play a part in this.

Although structural alteration would seem plausible as a mechanism for the slow effect of angiotensin II it is doubtful that the fall in blood

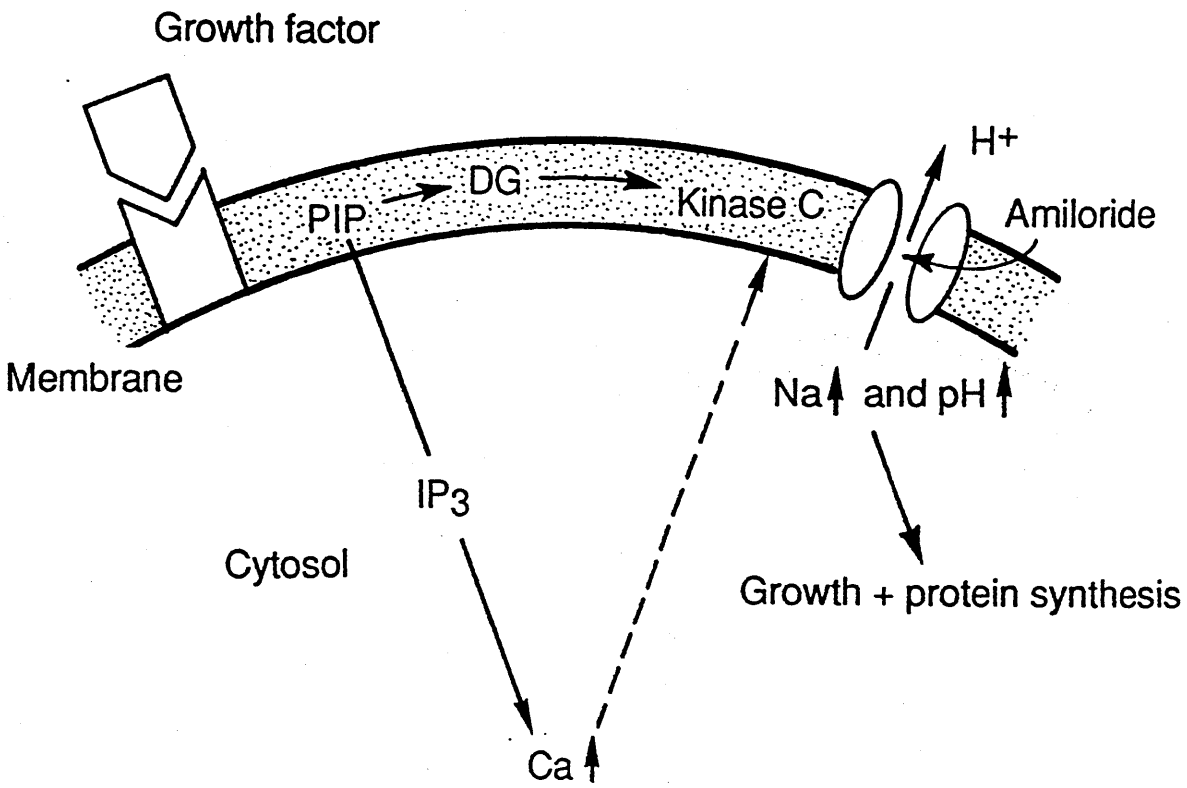


Figure 5.3 Schematic and simplified representation of the main events in a signalling system activated by growth factors. Growth factor occupies a membrane receptor; phosphatidylinositol biphosphate (PIP) is hydrolysed by phosphodiesterase in the membrane releasing inositol triphosphate (IP3) into the cytosol and diacyl glycerol (DG) in the plane of the membrane. DG activates kinase C linked to an amiloride sensitive Na^+/H^+ exchanger whose activity increases. Sodium enters the cell down an electrochemical gradient and protons are extruded resulting in an increased cell alkalinity.

pressure which was found in these studies, 12 hours after the start of captopril infusion to chronic hypertensive rats, could readily be attributed to reversal of structural changes which presumably would take longer to reverse (Lundgren,1974; Weiss,1974; Lundgren & Weiss,1979).

(5) A combination of mechanisms.

It is not clear whether a single mechanism or a combination of mechanisms is responsible for the slowly developing pressor action of angiotensin II. The mechanisms discussed above are not mutually exsclusive: angiotensin II acting through the nervous system could affect salt and water balance and changes of sodium and water could modify the rise in pressure. Also changes in sodium status could affect wall/lumen ratio and hence vascular tone.

However although the evidence for the 'other' mechanism from the experiments carried out here is compatible with a role for the slow pressor effect in the development of chronic renal hypertension this does not establish it and other mechanisms, independant of the renin system, must not be excluded.

It must be pointed out that converting enzyme inhibitors such as captopril are not specific in their action. Converting enzyme inhibitors potentiate the response to exogenously administered bradykinin, a potent

vasodilator (Engel et al.,1972) although there is evidence that captopril does not cause a rise in the plasma concentration of this vasodilator (Johnston et al.,1979).

5.5 Relevance of this study to the disease in man.

This study is clinically relevant because in man hypertension is often associated with unilateral renal disease and the commonest form of this disease is stenosis or occlusion of the renal artery. In the rat, Goldblatt two-kidney, one clip hypertension closely parallels the disease in man, both in the early stages where the rise in pressure immediatly following renal artery constriction is due to the direct pressor effect of the rise of circulating angiotensin II (Brown et al.,1976) and in the later stages where the upward shift of the angiotensin II/ blood pressure regression line in chronic hypertension obtained in the present study is remarkably similar to that seen in patients with chronic renal hypertension (Brown et al.,1979) and confirms the relevance of this experimental model as a tool for the investigation of human renal hypertension.

5.6 Conclusions.

This study has attempted to define more precisely the evolution of the renovascular hypertension in relation to the renin-angiotensin system in the two-kidney, one clip rat. Three main phases have been distinguished in the development and maintenance of the hypertension. While in the first phase the raised blood pressure is the result of the initial rise of circulating renin and hence the direct pressor action of angiotensin II, in the later stages blood pressure is disproportionately high in relation to the plasma angiotensin II levels and some other mechanism must be involved. Vascular renin and/or the slow pressor effect of angiotensin II could at least in part be responsible for this, however other mechanisms, independant of the renin-angiotensin system, cannot be excluded.

APPENDIX 1

CONSTRUCTION OF CAROTID ARTERY CATHETER.

These catheters were constructed from a 10cm length of Tygon polythene, internal diameter 0.5mm, external diameter 1.52mm (Norton Plastics, Akron, Ohio) and a 2.5cm length of polythene tubing, internal diameter 0.5mm, external diameter 0.8mm (800/100/140/100, Portex Ltd Hythe, Kent, England). One centimetre at one end of the Tygon tubing was enlarged by stretching it over a number 21 gauge hypodermic needle which had been filed smooth, then heating it against a hot light bulb, allowing it to cool and then removing the needle. The polythene tubing was then inserted into this widened 1cm and was cut straight across at the tip, a bevelled tip being more likely to either pierce the artery or to lie up against the artery wall thus making blood pressure recording impossible. The tip was then held against a hot light bulb in order to melt away any sharp edges. A 1cm plug was made from stainless steel wire (0.7mm diameter) which was rounded at both ends to prevent damage to the catheters. Figure 1 shows a carotid catheter.

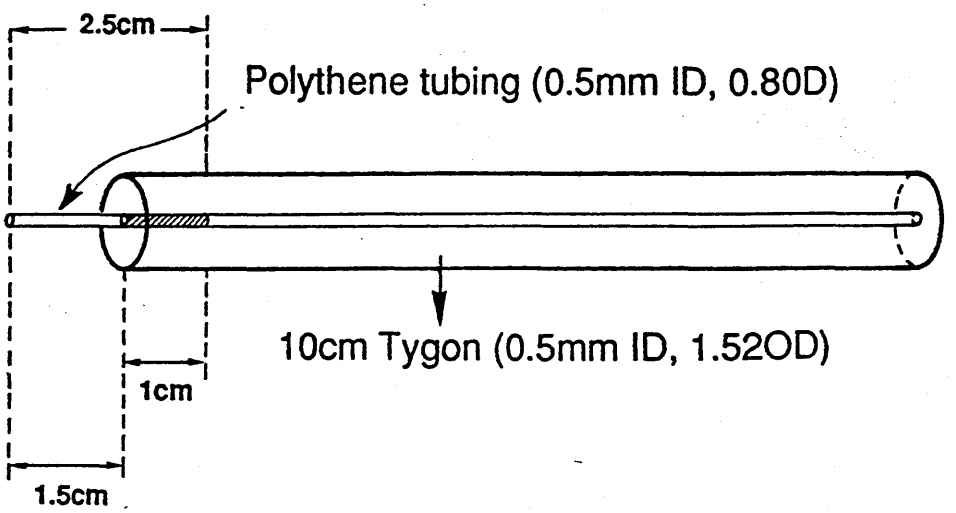


Figure 1 Carotid artery catheter.

APPENDIX 2

CONSTRUCTION OF AORTIC CATHETER.

This method was modified from the technique of Weeks & Jones (1960) (Browning, Ledingham & Pelling,1970). These catheters consisted of four pieces of polythene, A, B, C & D, welded together by heating. Figure 2 shows an aortic catheter.

Parts of the aortic catheter.

Part A: 17cm length of polythene tubing 800/100/200, internal diameter 0.58mm, external diameter 0.96mm (Portex Ltd, Hythe, Kent, England).

Part B: 0.5cm length of polythene tubing 800/100/280, internal diameter 0.86mm, external diameter 1.52mm (Portex Ltd).

Part C: 5cm length of polythene tubing 800/100/320, internal diameter 1.14mm, external diameter 1.5mm (Portex Ltd).

Part D: 10 cm length of polythene tubing PE10, internal diameter 0.28mm, external diameter 0.61mm (Clay Adams, Becton, Dickinson and Company, Parsippany, N.J. 07054).

Preparation of catheter parts before assembly.

Part B: The polythene was heated at one end until it was flared and then it was widened over a length of 18 gauge tinned copper wire which had been heated against a soldering iron. This stretched it sufficiently to fit over part A.

Part C: Approximately 1cm at one end of the polythene tubing was widened over a length of heated 16 gauge copper wire. This expanded it enough to be pushed over parts A and B.

Part D: The PE10 was threaded over stainless steel wire (0.2mm diameter), which had been previously greased with silicone fluid to make the threading easier. The polythene was heated approximately 4cm from the end in a hot air stream. When molten it was removed from the hot air and pulled out firmly to give a thin walled section 3-5cm long. The drawn out tubing was trimmed to give a 2.5cm length of normal walled tubing before the thin walled part.

Assembly of the catheter:

The neck piece. Part B, flared end first, was pushed over part A to about 5cm from the end. The widened part C tubing was then threaded over part A and onto part B.

This join was heat sealed. This seal and the flange of part B formed an anchor point which was used to secure the catheter to the back of the neck with sutures. Further seals between part A and part C at approximately 1cm intervals were made. The final seal was cut through with a scalpel to ensure correct sealing.

Sealing part A to part D: 30cm of the 0.2mm diameter stainless steel wire was inserted inside 20cm of 26 gauge needle tubing so that 5cm of wire protruded at each end. Part D was threaded over the wire and part A threaded over the needle tubing to overlap about 5mm of part D. This overlap was then heat sealed together over a soldering iron. Part A was heated 5-10mm from this seal until pliable and both ends were pushed together to form a ridge which was allowed to harden away from the heat. The seal and the ridge formed another anchor point for the catheter. Two more ridges 0.5cm apart were made as above on part D about 2cm from the join with part A, these formed the third anchor point for the catheter.

The S-bend: Part D was bent on a plastic former and held in place with plasticine. This was dipped into boiling water and then set by cooling rapidly in iced water. The S-bend was such that the A-D junction was on the first bend, the anchor point on part D was just before the second bend and the thin walled part began at the top of the second bend.

The 90 degree bend on the neck piece: A bend was made at the third heat seal on the neck piece. The neck piece was threaded over a length of 26 gauge needle tubing which had been previously been bent to an angle of 90 degrees so that it would protrude from the back of the neck at right angles when the catheter was in position. The needle tubing plus the neck piece was dipped into boiling water and set by cooling in iced water. On removing the needle tubing the 90 degree bend remained in the catheter.

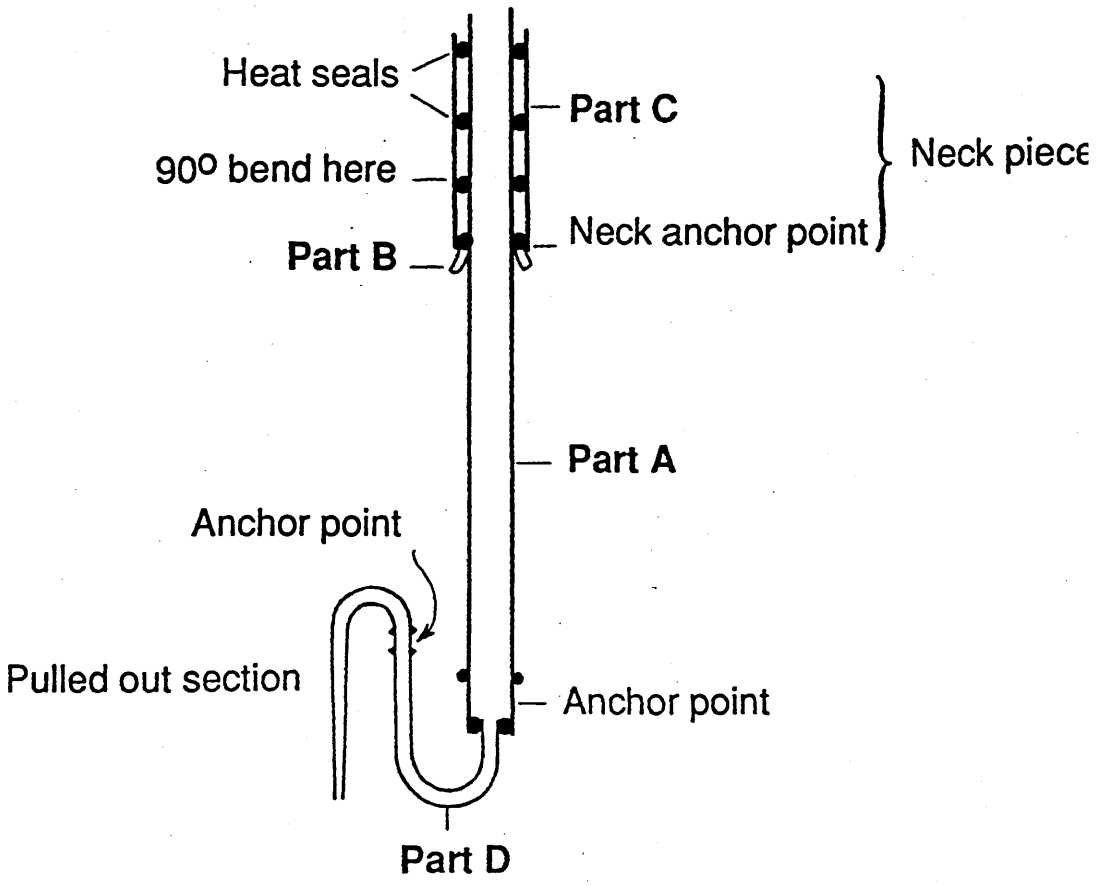


Figure 2 Aortic catheter.

APPENDIX 3.

CONSTRUCTION OF IVC CATHETER.

The IVC catheter consisted of four pieces of polythene sealed together by heating. The materials used are identical to those in the aortic catheter construction (Appendix 2) with one exception; for part A, 19cm of polythene was used instead of 17cm as used for the aortic catheter. A constructed IVC catheter is shown in Figure 3.

Preparation of catheter parts before assembly.

Parts B and C were prepared as for the aortic catheter.

Part D. 10cm of the PE10 polythene was threaded over a length of 0.2mm diameter stainless steel wire. The tubing was clamped at both ends with artery forceps. The polythene and wire were dipped into boiling water and then set by cooling rapidly in iced water. The wire was removed and the polythene was straight.

Assembly of the catheter.

The neck piece was assembled as for the aortic catheter.

Sealing of part A to part D: The straightened length of polythene (part D) was threaded onto the stainless steel wire protruding from the 35 gauge needle tubing. Part A was threaded over the needle tubing to overlap part D by about 5mm. This junction was heat sealed. Two centimeters from this seal on part A a ridge was made as before to form an anchor point.

The U-bend. A U-bend was made in the catheter by bending the part of the catheter distal to the anchor point on part A round the plastic former used for the aortic catheter. This was immersed first in boiling water and then in cold as before.

A 90 degree bend was made at the third seal on the neck piece as in the aortic catheter.

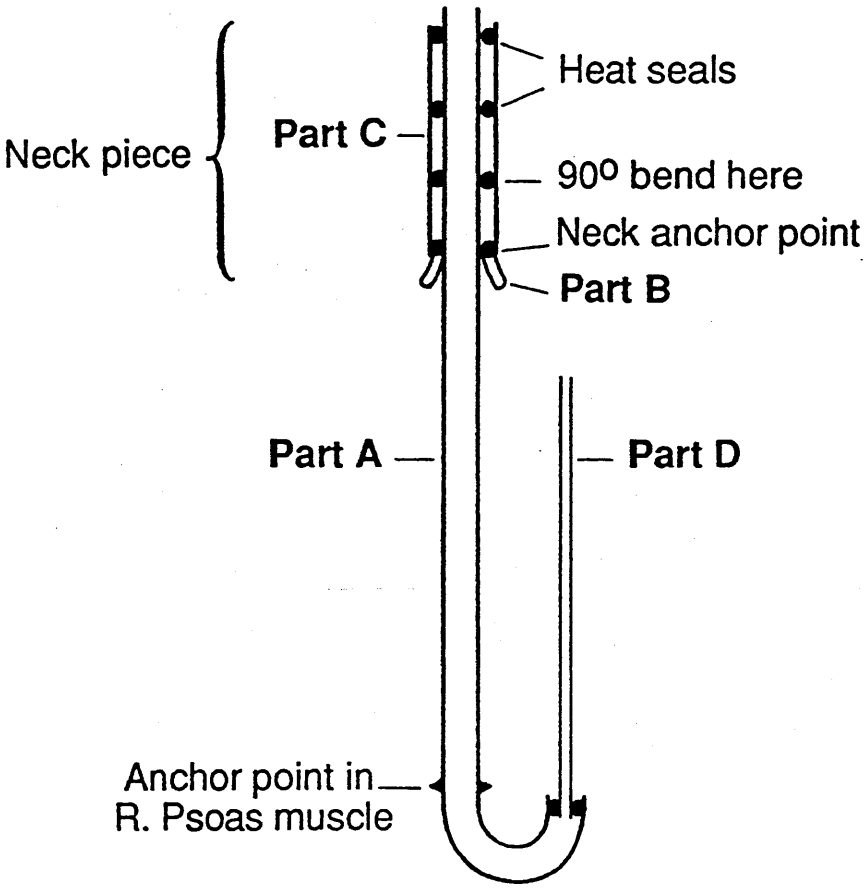


Figure 3 Vena caval (IVC) catheter.

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