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Nancy Lapp Kanagy

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Major professor

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HUMORAL FACTORS IN SODIUM-DEPENDENT HYPERTENSION: CHARACTERIZATION IN REDUCED RENAL MASS RATS

By

Nancy Lapp Kanagy

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ABSTRACT

HUMORAL FACTORS IN SODIUM-DEPENDENT HYPERTENSION: CHARACTERIZATION IN REDUCED RENAL MASS RATS

By

Nancy Lapp Kanagy

There is a subset of the human population whose blood pressure is sensitive to changes in dietary sodium. In these people, there is a direct positive correlation between dietary sodium intake and their mean arterial pressure (MAP) so that high sodium intake leads to hypertension. The differences between sodium sensitive and sodium resistant individuals have not been clearly defined. However, both clinical and animal studies suggest that alterations in humoral substances are at least partly responsible. Two circulating hormones that appear to be involved in this pathogenic response are angiotensin II (ANG II) and aldosterone, both products of the renin angiotensin system. The reduced renal mass (RRM) rat is an animal model that has been used to study the mechanisms of sodium dependent hypertension (SDH). This model of hypertension is very similar to the clinical condition of chronic renal failure, a syndrome which almost invariably leads to SDH. As in human SDH, MAP in RRM rats is very sensitive to the effects of dietary sodium. Treatment with angiotensin converting enzyme inhibitors (CEI), however, prevents a sodium-induced rise

in MAP implicating ANG II in the development of SDH in RRM. Because of the lack of specificity of CEI, we used a more specific pharmacological tool and found that the specific ANG II antagonist, losartan also prevents RRM hypertension in rats. Further, we found that RRM rats are more sensitive than intact rats to the hypertensive effects of circulating ANG II. Together these findings suggest that an increased sensitivity to ANG II in RRM rats contributes to their development of SDH.

This dissertation is dedicated to my family and my husband who encouraged me to work to my fullest potential and who have always believed in my abilities.

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TABLE OF CONTENTS

GENERAL INTRODUCTION 1

I. Introduction 2

A. Overview of the renin-angiotensin system 2

B. Role of sodium in the development of hypertension 4

1. Epidemiological evidence 4

2. Sodium intake intervention studies 7

C. Characteristics of sodium-sensitive hypertension 9

D. Potential mechanisms of sodium-sensitive hypertension 14

1. Primary alteration of renal function 17

2. Hormonal influences 21

a. hormone influences at the kidney 21

b. extrarenal hormonal influences 26

3. Alteration of the sympathetic nervous system 34

a. renal sympathetic nervous system actions 34

b. extrarenal sympathetic nervous system actions . . 36

E. Animal models of hypertension 37

1. Genetic models 38

2. Hormone infusion models 40

a. mineralocorticoid infusion 40

b. ANG II infusion 42

3. Reduced renal mass model of sodium-dependent hypertension 44

a. physical and metabolic changes 44

b. whole animal hemodynamic changes 48

c. hormonal changes 52

1) angiotensin II 52

2) arginine vasopressin 54

3) aldosterone 55

4) natriuretic factors 57

d. sympathetic nervous system 59

II. Methods and Materials 64

A. General experimental protocols 64

1. Surgical procedures 64

a. catheterization 64

b. subcutaneous osmotic minipumps 65

c. renal reduction 65

2. Hemodynamic measurements 66

3. Analytical methods 67

a. sodium and potassium 67

b. plasma osmolality 68

c. blood urea nitrogen 68

d. plasma aldosterone concentration 68

e. plasma immunoreactive angiotensin II 69

f. fluid homeostasis 70

4. Statistical methods 70

B. Specific protocols 70

1. Aldosterone infusion 71

2. PAC during chronic infusion of angiotensin II 72

3. Aldosterone in reduced renal mass hypertension 75

4. Angiotensin II antagonism in reduced renal mass hypertension 76

a. chronic antagonist treatment 76

b. acute antagonist treatment 78

5. Angiotensin II infusion in reduced renal mass rats 78

III. Results 80

A. Plasma aldosterone 80

B. Chronic infusion of angiotensin II 86

C. Plasma aldosterone in reduced renal mass hypertension 92

D. Angiotensin II antagonism in reduced renal mass hypertension 95

1. Chronic angiotensin II antagonist treatment 95

2. Acute antagonist treatment 104

E. Angiotensin II infusion in reduced renal mass rats 107

IV. Discussion 114

A. Plasma aldosterone during angiotensin II infusion 116

B. Plasma aldosterone in reduced renal mass hypertension 125

C. Endogenous angiotensin II in reduced renal mass hypertension 129

1. Chronic losartan in RRM rats 129

2. Acute losartan treatment in hypertensive RRM rats 136

D. Changes in sensitivity to chronic angiotensin II in reduced renal mass rats 137

LIST OF TABLES

Table 1: Plasma aldosterone following angiotensin II infusion 81

LIST OF FIGURES

Figure 1: Effect of aldosterone infusion on blood pressure, heart rate and plasma aldosterone concentration. 82

Figure 2: Water intake and urine output in intact rats receiving aldosterone 84

Figure 3: Effect of aldosterone infusion on sodium and potassium excretion in intact rats 85

Figure 4: Blood pressure, heart rate and plasma aldosterone in intact rats receiving angiotensin II 87

Figure 5: Urine output, water intake, urinary sodium excretion and urinary potassium excretion in rats receiving ANG II infusion . . . 88

Figure 6: Plasma sodium, potassium and osmolality during angiotensin II infusion in rats on high sodium intake 90

Figure 7: Blood pressure, heart rate and plasma aldosterone concentration in rats receiving angiotensin II on normal sodium . . 91

Figure 8: Blood pressure, plasma aldosterone and heart rate in intact and RRM rats during changes in sodium intake 93

Figure 9: Blood urea nitrogen in intact and RRM rats during changes in sodium intake 94

Figure 10: Water intake, urine output and water balance in intact and

RRM rats during changes in sodium intake 96

Figure 11: Urinary electrolyte excretion and sodium balance in RRM and intact rats during changes in sodium intake. 97

Figure 12. Effect of losartan treatment on blood pressure and heart rate in RRM and intact rats during changes in sodium intake. . . . 98

Figure 13. Correlation between blood pressure and BUN in RRM with or without losartan treatment 99

Figure 14: Effect of losartan treatment on water intake, urine output and water balance in RRM and intact rats during different sodium intakes. 101

Figure 15: Effect of losartan treatment on urinary sodium excretion and sodium balance in RRM and intact rats during different sodium intakes. 102

Figure 16: Effect of losartan treatment on blood urea nitrogen in intact and RRM rats during changes in sodium intake. 103

Figure 17: Acute blood pressure response to ANG II infusion in intact and RRM rats with and without losartan blockade. 105

Figure 18: Blood pressure and heart rate response to acute losartan in hypertensive RRM rats on high sodium intake 106

Figure 19: Blood pressure and heart rate during angiotensin II infusion

in RRM and intact rats on normal sodium intake. 108

Figure 20: Effect of angiotensin II infusion on water intake and urine output in intact and RRM rats on normal sodium intake. 109

Figure 21: Effect of angiotensin II infusion of urinary electrolyte excretion in RRM and intact rats on normal sodium intake. 111

Figure 22: Plasma levels of immunoreactive angiotensin II in RRM and intact rats receiving angiotensin II infusion. 112

Figure 23: Effect of angiotensin II infusion on BUN in RRM and intact rats. 113

LIST OF ABBREVIATIONS

ACE - angiotensin converting enzyme

ANOVA - analysis of variance

ANG II - angiotensin II

ANP - atrial natriuretic peptide

AP - area postrema

AVP - arginine vasopressin

BV- blood volume

CEI - angiotensin converting enzyme inhibitor

CO - cardiac output

CI - cardiac index

DOCA - deoxycorticosterone

GFR - glomerular filtration rate

HR - heart rate

iv - intravenous

JG - juxtaglomerular

MAP - mean arterial pressure

mRNA - messenger ribonucleic acid

Na^+/K^+-ATPase - sodium potassium adenine triphosphate

transport enzyme

NTS - nucleus of the tractus solitarius

OLF - ouabain-like factor

PAC - plasma aldosterone concentration

PRA - plasma renin activity

RAS - renin angiotensin system

RRM - reduced renal mass

SAD - sinoaortic denervation

SEM - standard error of the mean

SDH - sodium dependent hypertension

SHR - spontaneously hypertensive rat

SNA - sympathetic nervous system activity

SNGFR - single nephron glomerular filtration rate

SNS - sympathetic nervous system

TPR - total peripheral resistance

UKV - urinary potassium excretion

UNaV - urinary sodium excretion

UO - urine output

WI - water intake

GENERAL INTRODUCTION

The role of sodium chloride (i.e. salt) in the development of hypertension has been the topic of research for a decade and is covered extensively in the literature. The first section of the Introduction is a review of some of the more recent work in this area.

The second section of the Introduction reviews common characteristics of sodium-dependent hypertension (SDH) and the possible mechanisms they suggest to explain the genesis of this form of the disease. This, too, is a very extensive literature and the review covers only major advances providing an overview of the specific humoral factors that may affect blood pressure in SDH.

The final section describes the animal models of SDH. It includes a discussion of the mechanisms which may lead to the development of hypertension in these models and examines the evidence that humoral factors are involved in the sodium-induced rise in blood pressure of reduced renal mass rats.

The experiments which are described following the Introduction were designed to clarify the role of specific humoral factors in reduced renal mass hypertension and to apply those findings to the general condition of SDH.

I. Introduction

A. Overview of the renin-angiotensin system

The renin-angiotensin system (RAS) affects sodium homeostasis and blood pressure in many ways. Thus a discussion of sodium-dependent hypertension demands an understanding of the physiology of the RAS synthesis cascade. The precursor molecule in the RAS is angiotensinogen, a circulating polypeptide synthesized mainly in the liver. Production of angiotensinogen increases during sodium deprivation and is inhibited by the end product angiotensin (ANG) II. Angiotensinogen is released into the bloodstream from the liver and is cleaved by renin to form ANG I, an inactive decapeptide. Renin, the rate limiting enzyme, is produced primarily in the kidneys by specialized smooth muscle cells called the juxtaglomerular (JG) cells located in the afferent arterioles at the entrance to the glomerulus. Renin is released from the kidney into the circulation where it acts to produce ANG I. ANG I is further cleaved by angiotensin-converting enzyme (ACE) to form the octapeptide ANG II also known as angiotensin (1-8). ACE is a non-specific zinc-metaloproteinase found in the vascular endothelial cells of many tissues including the lungs, brain and kidneys and degrades kinins in addition to its action on ANG I. The peripheral cells with the highest ACE activity are the endothelial cells of the bronchial arterioles where the majority

of circulating ANG II is produced and released into the systemic circulation.

In addition to ANG II, there are several other peptide products of the RAS with demonstrated physiological actions. These are designated angiotensin (2-8) or ANG III, angiotensin (1-7), and angiotensin (3-8) (Ferrario *et al.* 1991). Circulating ANG II and ANG III bind to the same membrane-bound receptors to induce similar actions. Angiotensin (1-7) produces very different actions, perhaps through a separate receptor (Diz and Pirro 1991). There are two known ANG receptors, designated as AT_1 and AT_2 (Wong *et al.* 1990). ANG II and ANG III both act on membrane-bound AT_1 receptors causing constriction of vascular smooth muscle cells, the release of aldosterone from adrenal zona glomerulosa cells, and the absorption of sodium at the renal proximal tubule cells. The intracellular response to AT_1 receptor activation is mediated through a membrane bound guanine nucleotide binding protein. The so-called G-protein stimulates inositol triphosphate dependent calcium release in vascular smooth muscle cells and adenylate cyclase generated cyclic AMP accumulation in renal proximal tubular cells. The sensitivity of the adrenal AT_1 receptors appears to increase during sodium depletion, a time of high ANG II levels, and decrease during sodium excess (Aguilera and Catt 1978). The increased sensitivity reinforces ANG II stimulated aldosterone release to contribute to restoration of sodium

balance. Tachyphylaxis of the vascular and renal responses occurs during chronic elevations in circulating ANG II and there is a decrease in the number of ANG II receptors during chronic sodium restriction and ANG II excess (Schiffrin *et al.* 1983). In addition to the peripheral effects, both ANG II and ANG III act centrally through AT_1 receptors to stimulate drinking and to cause the release of arginine vasopressin (AVP) from hypothalamic nuclei. The AT_2 receptor has not been shown to mediate any action of the ANG peptides and does not interact with guanine nucleotide binding proteins although it is located in many tissues where ANG II acts such as the kidney, adrenal gland and brain (Botarri *et al.* 1991). Future studies are needed to determine what role, if any, these receptors play in the systemic response to ANG peptides.

In addition to the well characterized circulating RAS, it is speculated that there are independent tissue RASs. ANG II produced by the tissue RAS is proposed to act in an autocrine or paracrine fashion and independent of circulating levels of renin or ANG II. In support of this proposal, mRNA coding for angiotensinogen, renin and ACE has recently been found in a multitude of tissues including the kidney (Dzau *et al.* 1988), the adrenal (Kohara *et al.* 1992) and the brain (Kohara *et al.* 1992). Furthermore, it has become increasingly clear that ANG II is not the only active component of

the RAS and other products of angiotensinogen appear to play important physiological roles. Thus, circulating levels of ANG II may not be an accurate measure of the activity of the RAS and activation of RAS may be present even in the face of normal or suppressed plasma levels of the peptide.

B. Role of sodium in the development of hypertension

1. Epidemiological evidence

A direct relationship between salt intake and blood pressure has been postulated for many years but has been difficult to prove. A landmark study by Dahl combined the observations of many populations consuming a wide range of sodium intakes, including primitive cultures with very low sodium intakes and industrial societies with relatively high sodium intakes. He found a significant tendency for blood pressure to be slightly higher in populations with a higher intake of sodium (Dahl 1960). However, not all subsequent studies have supported his findings and a direct relationship between salt intake and blood pressure is neither clearly defined nor universally accepted.

The mechanisms through which salt intake can influence blood pressure are undoubtedly related to the influence of sodium on fluid homeostasis. Sodium is the major determinant of the osmolality of blood and extracellular fluid. Dietary sodium intake, primarily in the form of sodium chloride increases the amount of sodium in the body. If renal excretory

mechanisms do not completely eliminate the sodium ingested, it accumulates to increase total body sodium content. When the amount of sodium in the body changes, water volume also changes to maintain sodium concentration and osmolality constant within a very narrow range. Sodium retention therefore leads to body fluid volume expansion. This volume expansion can in turn expand vascular volume causing increased venous return, elevated cardiac output (CO) and a rise in blood pressure. Additionally, volume expansion can result in edema of the vascular wall, thereby contributing to increased vascular resistance. In both instances, there is an increase in blood pressure.

In a recent review by Elliot of the accumulated epidemiological evidence for an influence of dietary sodium on blood pressure, data from sixteen independent observational studies were combined using meta analysis (Elliot 1991). The data was derived from populations unscreened for hypertension and covered a wide range of sodium intakes (Elliot 1991). Although it was criticized for including populations with very different cultural and physical attributes which may affect the relationship between sodium and blood pressure, this study did find a significant positive correlation between sodium intake and blood pressure.

Another large body of data was gathered in a huge international project

known as INTERSALT (Stammler *et al.* 1991). This study recorded blood pressure and sodium intake in 10,049 patients at 52 separate clinics around the world and was the most comprehensive epidemiological examination of sodium intake's effect on blood pressure ever performed. INTERSALT data revealed a significant positive correlation between the average sodium intake and the average blood pressure of the adult population within a geographical region. The incidence of hypertension and the tendency for blood pressure to increase with age also correlated positively with sodium intake. In this study, a change of 100 mmol/day in sodium intake caused a 3.54 mm Hg change in arterial blood pressure (Stammler *et al.*1991). The authors suggest that essential hypertension may not be a metabolic disorder or a disease as is commonly perceived, but is instead a normal response to the modern diet with its sodium intake far in excess of what is needed for health or survival. Indeed, in the few regions of the world where sodium intake is less than 60 mmol/day hypertension does not exist! However, excessive sodium intake alone cannot be the sole contributor to the development of all essential hypertension since there is a wide variation in blood pressure between individuals at any given sodium intake. High sodium intake is therefore a risk factor for the development of hypertension in the same way that obesity and smoking are

risk factors. It is, however, a very pervasive risk factor in industrialized countries where sodium intake is typically in excess of 200 mmol/day.

2. Sodium intake intervention studies

A weakness of epidemiological studies such as those cited above is that there are many differences between populations with divergent sodium intakes in addition to disparate dietary sodium. Intervention trials performed within a single population can control those confounding variables. These studies alter sodium intake within a group of individuals matched for age, weight and other variables. The corresponding changes in blood pressure quantify the dependence of blood pressure on sodium intake within individuals rather than within an entire population. Intervention studies focus on the ability of dietary modification to alter blood pressure and evaluate the role of sodium in the development and management of hypertension.

A consistent observation in sodium intervention studies has been that individuals within a population respond to changes in sodium intake with both qualitatively and quantitatively different blood pressure responses (Sakaguchi *et al.* 1988, Weinberger *et al.*1986, Weinberger 1991b, Mascioli *et al.*1991) so that even within a fairly homogeneous population some people show an increase in blood pressure to a sodium load while others show a

decrease. The average affect in most populations is a slight decrease (Sullivan 1991). Thus, within the general population there is a subset of persons whose blood pressure varies in direct correlation to sodium intake and another subset whose blood pressure is very resistant to sodium-induced alterations (Mascioli *et al.* 1991).

The trait of sodium-sensitive blood pressure appears to be a heritable one (Weinberger 1991a, Weinberger *et al.*1987) and occurs more frequently in hypertensive than normotensive individuals (Weinberger 1991a, Sullivan 1991, Sakaguchi *et al.* 1988). In fact, it is estimated that anywhere from 20 to 80% of hypertensive patients experience a fall in blood pressure with dietary sodium restriction while the frequency of sodium sensitivity in normotensive individuals is between 5 to 20% (Cutler *et al.*1991).

Sodium intervention trials have thus defined a subset of essential hypertensive patients with "sodium-sensitive hypertension", defined as "chronically elevated blood pressure directly correlated to sodium intake". This can be evaluated clinically by measuring the blood pressure response to sodium loading and/or to sodium restriction (Muntzel and Darke 1992).

C. Characteristics of sodium-sensitive hypertension

Within the general population, it is not possible to accurately identify

individuals with sodium sensitive blood pressure except through their response to changes in sodium intake. It would be useful clinically to identify sodium sensitive individuals through non-invasive observations and experimentally to identify a set of characteristics which clarify the cause of sodium-sensitivity. Many factors have been proposed to this end. Age, race, insulin resistance, obesity, a positive family history for hypertension, activity of the renin-angiotensin-aldosterone system, and circulating levels of catecholamines are some of the factors proposed.

Indications that sodium sensitivity increases with age came from epidemiological studies. As discussed previously, the INTERSALT study found that blood pressure increases with age in populations consuming a high sodium intake. In this study, the statistical association of blood pressure with sodium intake was stronger in older compared with younger patients (Stammler *et al.*1991). Some intervention studies have also shown an increased incidence of sodium-sensitivity in older patients versus younger patients (Weinberger *et al.* 1986, Zemel and Sowers 1988, Luft *et al.* 1991), but this association has not held true in every investigation (Umeda *et al.* 1988). In older patients as in younger patients, higher blood pressure increases the probability of sodium sensitivity so that in studies of only hypertensives, there is a much better correlation of sodium-sensitivity with

age (Umeda *et al.*1988, Zemel and Sowers 1988). Older individuals have also been shown to come into sodium balance more slowly than younger individuals, an effect perhaps of decreased renal function or of increased levels of naturally occurring antinatriuretic factors. This tendency may contribute to their increased susceptibility to sodium-induced hypertension (Luft *et al.*1991).

Racial differences have also been noted in the prevalence of sodium-sensitive blood pressure. American blacks have a greater incidence of sodium-sensitivity than either African blacks or American whites (Luft *et al.*1991). It has been suggested that this apparent genetic alteration is the result of natural selection brought about by the history of slavery which created living conditions where the ability to conserve sodium was an advantage (Wilson and Grim 1991). Similar to other groups at increased risk for sodium-sensitivity, American blacks tend to excrete a sodium load more slowly and are more responsive to diuretics than whites (Luft *et al.*1991, Sowers *et al.*1988). Furthermore, black hypertensives are particularly responsive to diuretic therapy. This is generally accompanied by lower plasma renin activity and increased levels of a circulating Na^+/K^+-ATPase inhibitor, indications of volume expansion and sodium retention (Sowers *et al.* 1988).

A third trait associated with sodium-sensitive hypertension is insulin resistance. Patients and animal models of insulin-resistant diabetes exhibit a greater incidence of sodium-induced alterations in blood pressure and slower excretion of a sodium load (Rocchini *et al.*1989, Finch *et al.*1990). A related characteristic is obesity. Obesity is frequently a complicating factor in both hypertension and diabetes and there is some evidence that it is independently associated with increased sodium-sensitivity of blood pressure and exaggerated sodium retention (Rocchini *et al.*1989).

A positive family history for hypertension also increases the incidence of sodium sensitivity (Weinberger *et al.* 1986, Weinberger 1991a). The tendency for this trait to follow family lines suggests a genetic abnormality may be responsible for the altered response to sodium and for an overall increased tendency to develop hypertension.

In addition to physical characteristics, certain hormonal alterations are common in sodium-sensitive individuals. The first is an altered response of the renin-angiotensin-aldosterone system (RAS) during changes in sodium intake. In normal individuals, increased sodium intake leads to activation of cardiopulmonary baroreceptors and concomitant decreases in sympathetic activity, renin secretion, plasma ANG II concentration, and aldosterone release (Creager *et al.* 1991). Among sodium-sensitive patients, there are two

subgroups that exhibit abnormal RAS responses to changes in sodium intake. One subgroup, termed "non-modulators", fails to alter adrenal responsiveness to ANG II during low sodium intake so that plasma levels of aldosterone do not change during changes in sodium intake. A second characteristic of this subgroup is failure of the renal blood flow to increase during high sodium intake in response to lower ANG II levels. These changes could contribute to sodium-sensitivity. The second subgroup with abnormal RAS responses are "low-renin" hypertensives, a trait more common among black than white hypertensives (Williams 1991, Keane *et al.* 1991). These patients have low plasma renin levels and normal aldosterone levels. However, they exhibit excessive aldosterone secretion in response to ANG II. When exposed to high sodium, therefore, these patients have suppressed renin and ANG II but abnormally high aldosterone that may contribute to the sodium-sensitive hypertension (reviewed Williams 1991, Sullivan 1991).

Additionally, observations of elevated plasma levels of other vasoactive hormones have been made. These include: arginine vasopressin (AVP), a potent neurohypophyseal vasoconstrictive and antidiuretic hormone (Mitch and Wilcox 1981); atrial natriuretic peptide (ANP), a vasodilating and natriuretic cardiac-secreted peptide (Sowers *et al.*1988); endogenous ouabain-like factor (OLF), a newly discovered natriuretic and perhaps vasoconstricting

compound (Keane *et al.*1991); and insulin, an antinatriuretic and sympathostimulatory compound in addition to its role in glucose homeostasis (Tuck 1991). Finally, elevated plasma levels of catecholamines have also been measured in sodium-sensitive patients suggesting that the expected changes in sympathetic activity during alterations in sodium intake do not occur in sodium-sensitive patients (Nishio *et al.*1988).

There is not sufficient evidence to implicate any specific factor as the sole cause of sodium sensitivity of blood pressure. In fact, sodium-sensitive blood pressure appears to be a heterogenous condition with multiple causes. The characteristics can, however, be used as guidelines in the experimental search for mechanisms which mediate this condition.

D. Potential mechanisms of sodium-sensitive hypertension

Mean arterial pressure (MAP) can be elevated in one of two ways, through increased CO or increased total peripheral resistance (TPR). Under most conditions, an increase in either of the two variables causes a reflex decrease in the other variable; therefore blood pressure does not change. However, in cases of sustained hypertension, the appropriate compensation does not occur resulting in a persistent increase in one or both of these variables. In almost all cases of hypertension including SDH, TPR is

increased.

One way TPR can be increased is through physical alterations of the vascular bed. Remodeling of the vascular structure that reduces the number of resistance vessels decreases the capacity of the vasculature and increases the resistance to the same volume of blood. Similarly, decreased compliance of blood vessels prevents volume induced distension of resistance vessels during small changes in CO and increases blood pressure. Alternatively, extrinsic neural or hormonal stimulation can cause vasoconstriction of structurally normal resistance vessels leading to increased TPR. Increased sympathetic nervous system activity (SNA) stimulates α_1-adrenergic receptors on small arterioles while vasoactive hormones such as ANG II or AVP act directly on vascular receptors to stimulate vascular smooth muscle contraction. The evidence for direct neural and hormonal vasoconstriction in SDH is discussed below.

TPR may also be increased independent of structural changes to blood vessels by tissue "autoregulation" in response to an initial elevation in CO. The theory of autoregulation was first proposed by Borst and Borst-de-Geus (Borst and Borst-de-Geus 1963) to explain the observed elevation of TPR under conditions expected to produce elevated CO. According to this theory, elevated CO increases the blood flow to peripheral vascular beds causing

overperfusion of peripheral tissues. Intrinsic autoregulatory control systems cause vasoconstriction of the overperfused beds normalizing tissue blood flow. In cases of chronically elevated CO, sustained autoregulatory vasoconstriction in overperfused beds should lead to a sustained elevation in TPR.

The elevation in CO necessary to stimulate autoregulation can be achieved in several ways. First, chronically expanded blood volume (BV) elevates CO by overfilling the vasculature. Expanded BV due to inadequate excretion and accumulation of water and sodium can be caused by a primary renal defect; increased renal SNA leading to tubular sodium reabsorption and the release of anti-natriuretic hormones (ANG II, aldosterone, eg.); oversecretion of antinatriuretic hormones independent of SNA; or a combination of the above. CO can also be elevated independent of BV expansion by increased SNA to the heart causing increased strength and frequency of contraction. This inotropic and chronotropic stimulation delivers increased blood flow to the tissues resulting in autoregulatory vasoconstriction. The primary stimuli that can lead to autoregulation induced elevated TPR are therefore a primary renal defect, increased circulating antinatriuretic hormones, or elevated SNA. The experimental evidence for the participation of autoregulation in SDH is discussed below.

In summary, the mechanisms causing hypertension in any given condition are complex and interrelated. Unraveling the mechanisms responsible for sodium sensitivity of blood pressure is important, however, to efficiently and effectively treat the hypertension which is a frequent result of this condition. In SDH, increased sodium intake must cause an increase in either CO or TPR to raise blood pressure. Normally, when sodium intake is high enough to alter body sodium content, cardiopulmonary receptors are stimulated to decrease SNA (lowering TPR and renal sodium retention), the renin-angiotensin system is suppressed (Holtzman *et al.* 1989). Any increase in CO by expanded blood volume is offset by a concurrent decrease in TPR and blood pressure does not change. However, in SDH arterial pressure does change so there must be either an insufficient suppression of the SNA, the RAS or a markedly exaggerated increase in CO. One way CO could be elevated is through excessive renal sodium and water retention.

1. Primary alteration of renal function

A primary role for renal function in the pathogenesis of essential hypertension, especially sodium-sensitive hypertension, has been postulated for several decades. Renal function and blood pressure are inexorably linked through their common function of fluid homeostasis: the ability of the kidneys to excrete water and sodium is dependent on renal perfusion pressure

(systemic pressure), while perfusion pressure in turn is dependent on body fluid volume, CO and thus blood pressure. A decrease in renal excretory rate can thus increase body fluid volume and raise blood pressure. The elevated blood pressure then restores renal excretion of water and sodium back to initial levels. This relationship is frequently described as "pressure natriuresis" and some investigators attribute the genesis of all hypertension to an abnormal pressure natriuresis relationship (deWardener 1990 parts I & II, Guyton *et al.* 1988).

According to this theory, any condition leading to impaired renal excretion of sodium and water will cause increased blood volume and ultimately, elevated blood pressure. Higher arterial pressure is seen as a requirement to achieve fluid homeostasis in the face of impaired sodium excretion by the kidney. Studies have shown that changes in renal function do in fact, profoundly influence blood pressure (reviewed in deWardener 1990 parts I & II). A problem with this theory, however, is that it suggests that hypertension should be associated with increased CO (due to expanded blood volume); yet almost all hypertension, including sodium-sensitive hypertension, is caused by increased vascular resistance (Sullivan 1991, Muntzel 1992). To resolve this apparent contradiction, it has been proposed that elevated CO rapidly brings about whole body autoregulation. As

described above, overperfusion of all vascular beds causes autoregulatory vasoconstriction, increased total peripheral vascular resistance and a return of CO to normal (Cowley 1990). This sequence of events could link reduced renal sodium excretory capacity to a condition of chronic hypertension characterized by increased TPR (Guyton 1990), without the need to invoke any hormonal or neural abnormalities as etiologic factors. This theory requires a residual increase in CO to maintain the elevated TPR but studies in SDH have not revealed expanded plasma volume (Sullivan *et al.*1987) and persons genetically predisposed to hypertension do not have increased body sodium compared to normal subjects (Berretta-Piccoli 1990). This has prevented definitive proof that decreases in renal function *per se* are the primary cause of clinical hypertension. Furthermore, renal damage can also be a consequence rather than an initiating factor of hypertension (Obatomi *et al.* 1992).

Chronically elevated blood pressure leads to damage of glomerular mesangial cells and to reduced glomerular filtration (Anderson *et al.*1985, Jackson and Johnston 1988). This is a primary symptom of renal damage in clinical hypertension (Blythe and Maddux 1991). Damage to the glomerulus leads to tissue hypertrophy and eventual necrosis (Ylitalo and Gross 1979) which in turn increases the filtration load on the remaining glomeruli and a

vicious cycle of progressive renal deterioration leading to malignant hypertension (Kincaid-Smith 1991). If renal arterioles are able to control renal perfusion pressure at the glomerulus or if pharmacological agents are used to selectively decrease glomerular perfusion pressure, damage to glomeruli is prevented (Hostetter *et al.* 1981). In clinical hypertension exhibiting renal damage, the amount of overt renal damage is directly proportional to the duration and severity of the elevated blood pressure, and it is difficult to determine if the renal damage or the hypertension was the initiating condition (Blythe and Maddux 1991, Obatomi *et al.* 1992).

The possible importance of the kidney in human hypertension has been highlighted by studies of renal transplant recipients. In these studies, recipients of kidneys from patients with no family history of hypertension had a decreased incidence of hypertension compared to transplant recipients with kidneys from donors with a positive family history for hypertension (Guido *et al.*1985, Strandgaard and Hansen 1986). The protective effect of "normotensive kidneys" was only seen, however, when the recipient was also normotensive prior to the transplant surgery, so that in patients already predisposed to hypertension, a kidney from a normotensive donor did not alter the progression of the disease. It is possible, however, that in this latter case the existing hypertension rapidly damaged otherwise normal kidneys,

leading to impaired renal function.

In some sodium-sensitive hypertensives there is evidence of decreased renal excretory capacity as evidenced by acute excessive sodium retention when compared to sodium-resistant patients (Sullivan 1991, Wu *et al.*1990). This could reflect an inherent (perhaps genetic in nature) defect in sodium handling by the kidneys of these patients, or be the result of inappropriate hormonal or neural influences on the kidney, as will be discussed in the following section. Even though the majority of sodium-sensitive patients do not have a clearly defined renal defect or gross sodium retention, most researchers have sought to characterize sodium-sensitive hypertension in terms of an intrinsic renal abnormality (Hall and Hungerford 1982). This characterization seems unjustified in light of the multiple mechanisms in addition to intrinsic renal abnormalities which also appear to be involved in SDH.

2. Hormonal influences

a. hormone influences at the kidney

In the absence of structural or functional renal abnormalities, altered renal function may still initiate SDH. Abnormal levels of circulating hormones which affect renal function can act on a normal kidney to produce hypertension.

First, excessive secretion of mineralocorticoids or infusion of exogenous mineralocorticoids such as aldosterone act at the kidney to cause transient sodium and water retention and produce a gradual elevation of arterial pressure (Pan and Young 1982, Garwitz and Jones 1982). Although excessive levels of mineralocorticoids are not typically found in the plasma of sodium-sensitive hypertensives, the renin-angiotensin-aldosterone system responds abnormally to changes in sodium intake in certain sodium sensitive patients (Williams 1991).

The low-renin sodium-sensitive hypertensives described earlier provide the best clinical example of how aldosterone acting at the kidney could contribute to sodium sensitivity in apparent essential hypertension. In these patients, adrenal secretion of aldosterone is abnormally sensitive to ANG II so that blood levels of the mineralocorticoid are higher than normal for any given level of ANG II (Williams 1991, Fraser and Padfield 1989). Studies in animals given aldosterone infusion have shown that the mineralocorticoid acts in the distal tubule of the kidney to cause sodium and water retention (reviewed Brown *et al.*1979). In addition, excess circulating aldosterone is associated with increased glomerular filtration rate (GFR) and filtered sodium load (Pan and Young 1982). These effects are accompanied by an initial transient sodium retention and, when accompanied by high sodium intake, a

sustained increase in arterial pressure (Pan and Young 1982, Garwitz and Jones 1982). In this way, chronic excess aldosterone could cause renally-mediated SDH without apparent volume expansion. The absence of excessive sodium and fluid retention during aldosterone excess is due to the sodium escape phenomenon that has been described in cases of continued aldosterone excess.

Evidence from angiotensin converting enzyme inhibitors (CEI) studies suggests that ANG II can alter renal function in some cases of SDH. In the group of sodium-sensitive patients termed non-modulators, CEI have been shown to be very effective antihypertensive agents (Dluhy *et al.* 1989). Also, in hypertensive patients with chronic renal failure, CEI are very effective at lowering blood pressure, reversing proteinuria and protecting against further degradation of renal function (Brenner *et al.* 1985).

Within the kidney, ANG II may affect MAP by stimulating increased sodium reabsorption and decreased renal blood flow (Mattson *et al.* 1991). First, ANG II causes vasoconstriction of renal arterioles to decrease renal blood flow and sodium delivery to the glomerulus. This is accompanied by an ANG II receptor mediated decrease in the glomerular ultrafiltration coefficient and a concomitant decrease in GFR (Navar *et al.* 1991). In the face of elevated systemic pressure, preglomerular afferent renal arterioles

exhibit autoregulatory vasoconstriction further decreasing renal blood flow (reviewed Carmines and Fleming 1990). In the proximal distal tubule, ANG II stimulates Na^+/H^+ exchange to directly increase sodium reabsorption and enhances the sensitivity of tubuloglomerular feedback (Cogan 1990, reviewed Ichikawa and Harris 1991). In the face of elevated ANG II delivery to the kidney, these intrarenal actions of ANG II during sodium loading should combine to produce sodium retention and volume expansion leading to increased CO, autoregulatory vasoconstriction and elevated blood pressure. These actions at the kidney are independent of any intrinsic renal defect. It has been proposed that increased activity of an entirely intrarenal RAS could produce hypertension in the face of normal plasma levels of ANG II such as those found in low-renin essential hypertension. The possible contribution of an intrarenal RAS to SDH remains undetermined but the actions of circulating ANG II are not confined to the kidney. Therefore the vascular and neural actions of the peptide during chronic elevations in plasma ANG II may be more important in the maintenance of hypertension than the renal effects and will be discussed in the next section.

Another circulating peptide that affects renal function is AVP, a hormone produced and released in the anterior pituitary in response to changes in plasma osmolality and by stimulation of the sympathetic nervous

system (Simon-Oppermann and Gunther 1990, Berecek and Swords 1990, Simon *et al.* 1989). AVP acts on V_2 receptors in the cortical collecting ducts of the kidney to increase sodium and water reabsorption (Ando *et al.* 1989, Jeffries *et al.*1991). Alterations in AVP have been noted in some cases of SDH (Mitch and Maddux 1982) and systemic administration of the peptide causes vasoconstriction, antidiuresis and antinatriuresis (Liard *et al.* 1987). AVP differs from ANG II, however, in its effects in the renal vasculature. Systemic infusion of AVP does not alter either renal blood flow or GFR suggesting that it does not constrict renal arterioles (reviewed Carmines and Fleming 1991). Recent reports in isolated blood vessels show that AVP may induce postglomerular vasoconstriction which contributes to the reabsorption of water at the vasa recta but which does not decrease total renal blood flow (Edwards *et al.* 1989). Consequently, the primary renal effect of AVP is water, and to a lesser extent, sodium reabsorption. The lack of a strong anti-natriuretic effect may be why chronic infusion of AVP only produces sustained hypertension when renal perfusion pressure is servocontrolled to decrease renal blood flow (Hall *et al.*1987, Liard 1987). For this reason it is unlikely that alterations in AVP actions on the kidney are the stimulus for SDH.

Another alteration that could induce renally-mediated hypertension is

decreased delivery of natriuretic hormones to the kidney. However, no decrease in atrial natriuretic peptide, a potent natriuretic peptide released from the cardiac atria, has been found in SDH. In fact, some animal models of SDH actually show a slight increase in the peptide (Brandt *et al.*1987). The other primary natriuretic substance is an endogenous analog of the plant product, ouabain. This factor has been found to be elevated rather than reduced in the plasma of some essential hypertensive patients and to increase during sodium loading (Haddy 1990, Graves *et al.*1989). Therefore the renal effect would be to promote natriuresis and thus reduce blood pressure. A contribution of this ouabain like factor (OLF) would therefore not be through altered renal sodium handling but through a non-renal mechanism.

b. extrarenal hormonal influences

In addition to the renal actions outlined above, circulating ANG II affects blood pressure through direct vasoconstriction of vascular smooth muscle cells. This vasoconstrictor action is dependent on AT_1 type receptors and is responsible for the acute increase in blood pressure following intravenous ANG II administration (reviewed Timmermans *et al.*1991). Studies in both humans and laboratory animals have shown that during chronic administration of ANG II, there is an apparent decrease in both the number of vascular receptors and the vasoconstrictor response (Pawloski

1990, Bruner and Fink 1986). Therefore the chronic condition of sodium-sensitive hypertension is unlikely to be mediated through direct vasoconstriction by excess circulating ANG II.

An additional peripheral effect of circulating ANG II is enhanced norepinephrine release from peripheral adrenergic terminals. This has been demonstrated after acute direct administration of the peptide (Trachte 1988, Ellis and Burnstock 1989) and after chronic ANG II treatment (Randall and Zimmerman 1990). Chronic treatment with CEI has also been shown to decrease norepinephrine release by sympathetic nerve stimulation (SNS)--an effect reversed by ANG II replacement (Randall and Zimmerman 1990). These studies suggest enhancement of SNA by ANG II could contribute to chronic increases in TPR.

Circulating ANG II also acts at the adrenal medulla where it stimulates epinephrine and norepinephrine release (Feuerstein *et al.*1977) to augment sympathetically mediated vasoconstriction. These direct and indirect vasoconstricting actions of circulating ANG II may contribute to the increased blood pressure of some sodium-sensitive hypertension. The possible contribution of these actions have not been investigated because plasma levels of ANG II are not elevated in sodium-sensitive hypertension and the antihypertensive mechanism of CEI action in non-modulators and in

hypertensive patients with chronic renal failure has not been determined. If the sensitivity to circulating ANG II is increased in these conditions, ANG II interaction with the peripheral components of the SNA mediated vasoconstriction could be responsible for the chronic elevation in TPR.

Within the central nervous system, ANG II alters efferent sympathetic activity and therefore vascular tone. In the brain, specific ANG II binding has been demonstrated in brainstem nuclei known to control cardiovascular function (Gehlert *et al.*1991, Wamsley *et al.*1990) and central administration of ANG II stimulates the release of norepinephrine and modulates the function of the baroreflex (Xiong and Marshall 1990, Kannan *et al.*1991). These actions are all within the blood brain barrier and support a role for centrally produced ANG II. This role is further substantiated by the finding that direct infusion of ANG II into the lateral cerebral ventricles produces sustained SDH (Fink *et al.* 1983, Bruner and Fink 1986).

However, intracerebroventricular administration of the ANG II antagonist saralasin does not block the development of hypertension in animals made hypertensive by chronic iv infusion of ANG II (Bruner and Fink 1985). In contrast, lesion of the area postrema (AP) a circumventricular organ with a high number of ANG II receptors (Ferguson 1990), prevents development of hypertension dependent on circulating ANG II (Fink *et*

*al.*1987, Matsukawa and Ried 1990). The hypertension produced by circulating ANG II therefore may be mediated through this brain structure outside the blood brain barrier, independent of the separate actions of ANG II within the brain.

Finally, ANG II is a potent secretagogue of aldosterone acutely (Williams 1991) but the effect of chronic ANG II excess on aldosterone release is not clear. In dogs (Cowley and McCaa 1976) and sheep (Blair-West *et al.*1963), a chronic ANG II infusion did not maintain elevated PAC whereas in humans (Oelkers and Schoneshofer 1983) and rats (Hauger *et al.*1978) chronic elevations have been reported. The involvement of ANG II in the alterations in PAC in some sodium-sensitive patients has not been investigated and the effect of chronic excess ANG II on PAC during elevated sodium intake is an important area that remains to be investigated.

Alternatively, the elevated PAC observed in some sodium-sensitive hypertensive patients could be independent of the RAS and alone responsible for the hypertension. Aldosterone excess was one of the first clinical observations shown to produce SDH. Aldosterone acts on cytosolic receptors stimulating increased mRNA transcription and subsequent protein synthesis. The exact proteins induced are unknown but the direct effect is enhanced Na^+/K^+-ATPase synthesis and increased number of active Na^+ channels

leading to increased renal sodium reabsorption accompanied by water retention and potassium excretion (Horisberger and Rossier 1992). In the gastrointestinal tract, aldosterone causes sodium reabsorption and enhances fecal potassium loss. Excess aldosterone is characterized by expanded total body fluid volume, plasma hypokalemia, and systemic alkalosis. Increased sodium intake exacerbates and accelerates the development of this condition (Williams 1991, Mitch and Wilcox 1981).

The mineralocorticoid, deoxycorticosterone (DOCA), has been widely used experimentally to establish a model of sodium-dependent, volume-expanded hypertension. In this model, increased sodium intake causes sodium and water retention and a gradual rise in blood pressure (Pan and Young 1982). However, CO, a relative measure of expanded blood volume, is not consistently elevated and the driving force of the hypertension appears to be elevated peripheral resistance (Pan and Young 1982). Some have suggested that mineralocorticoids act directly on vascular smooth muscle to cause increased reactivity and enhance vasoconstriction (Garwitz and Jones 1982). It has also been suggested that autoregulation caused by increased blood flow may be the mediator of the elevated TPR in DOCA-salt hypertension (Hall *et al.*1987). A recent study has reported a direct receptor-mediated blunting of baroreceptor discharge by circulating aldosterone (Wang

*et al.*1992).

Other investigators have suggested that aldosterone acts within the central nervous system to alter the release of the vasoactive hormone AVP (Janiak and Brody 1988) and the efferent activity of the sympathetic nervous system (Gomez-Sanchez 1986). All of these actions may combine during aldosterone excess to contribute to the resultant hypertension and be a part of the etiology of sodium-sensitive essential hypertension (Williams 1991).

In addition to the abnormal renin-angiotensin-aldosterone response, extrarenal actions of other humoral factors have been implicated in sodium-sensitive hypertension. One of these is OLF, a blood-borne factor which inhibits ATP dependent sodium-potassium exchange (Na^+/K^+-ATPase) and which may be over-secreted in sodium-sensitive individuals. This factor is known to elicit natriuresis, among other actions, and is often called the natriuretic hormone. Evidence that the factor is involved in hypertension is substantial but mostly indirect. The plasma of some volume-expanded hypertensive patients inhibited Na^+/K^+-pump activity *in vitro* (Keane *et al.* 1991, Nishio *et al.* 1988, Poston *et al.* 1981) and pump inhibition in experimental animals induced natriuresis and vasoconstriction *in vivo* (Pamnani *et al.*1991). Plasma from patients with pregnancy-induced hypertension (Kaminski and Rechsberger 1991), insulin-induced hypertension

(Graves *et al.*1989), as well as sodium-sensitive essential hypertension (Keane *et al.*1991) has the ability to inhibit the Na^+/K^+ exchanger. In animal studies, increased activity of a pump inhibitor has been demonstrated in volume expanded dogs (Gruber 1980), and in reduced renal mass rats (Hout *et al.* 1983, Shima *et al.* 1988, Nakagawa *et al.*1990). Platelets from hypertensive individuals have inhibited ATP sensitive Na^+/K^+ transport and elevated levels of intracellular calcium, presumably as a result of increased Na^+/Ca^{+2} exchange in response to decreased Na^+/K^+ exchange (Poston *et al.* 1981, Keane *et al.*1991).

A candidate molecule for this factor has recently been isolated from plasma and identified as a structural twin to the plant-derived cardiac glycoside, ouabain (Ludens *et al.*.1991, Bova *et al.*1991). Preliminary assays of this cholesterol-derived steroid show that the factor is elevated during volume expansion (Nishio *et al.*1988, Kaminski *et al.*1991 and Keane et el. 1991), increases contractility in isolated human resistance arteries (Bova *et al.* 1991, Woolfson *et al.* 1990) and raises blood pressure in rats (Pamnani *et al.*1991). Contradictory evidence has also been published in the rat, where chronic infusion of ouabain did not elevate blood pressure (personal observation and Yasujima *et al.* 1986).

The way in which such an inhibitor could theoretically contribute to

elevated TPR in sodium-sensitive hypertension is outlined below. Inhibition of the Na^+/K^+-ATPase elevates intracellular sodium levels stimulating sodium-calcium exchange. This in turn increases intracellular calcium concentration as sodium is exchanged for calcium. The electrical gradient across the cell membrane is altered and the resultant decreased polarization in the membrane potential leads to hyper-reactivity of the cell. Additionally, the increased calcium content in smooth muscle cells causes increased force of contraction (see Haddy 1990 for review).

In addition to effects on vascular smooth muscle cells, Na^+/K^+-ATPase inhibitors affect contractility of cardiac myocytes and Na^+/K^+ exchange in renal cortical collecting tubules (Pedrinelli *et al.* 1989, Haddy 1990). Chronic inhibition of renal Na^+/K^+ exchange would promote natriuresis and diuresis to prevent volume expansion but this would be offset by the chronic actions at the heart to increase CO. Inappropriate expression of OLF in sodium-sensitive individuals may thus contribute to the development of elevated TPR directly and indirectly in SDH (Keane *et al.* 1991, Graves *et al.* 1989, Kaminski and Rechsberger 1991, Nishio *et al.* 1988).

Another circulating hormone that has nonrenal cardiovascular actions and may contribute to the development of sodium-sensitive hypertension is the neurohypophysial peptide, AVP. Elevated plasma levels of this

vasoconstrictor have been measured in some sodium-sensitive hypertensives (Mitch and Wilcox 1981). In addition to its renal effects discussed earlier, circulating AVP acts on V_1 receptors on vascular smooth muscle to cause vasoconstriction, in the central nervous system to increase sympathetic outflow (Berecek and Swords 1990) and at unidentified peripheral sites to alter baroreflex function (Pullan *et al.*1980). Acutely, intravenous administration of AVP causes a rapid increase in arterial pressure and a significant decrease in water and sodium excretion (Pawloski *et al.*1989). Although it has been reported that infusion of AVP into baroreceptor denervated rabbits causes a sustained elevation in blood pressure (Ryuzaki *et al.*1991), this is not the case in intact animals. In intact animals, prolonged infusion of vasopressin for several days or weeks does not cause a sustained rise in arterial pressure, even in the face of high sodium intake (Pawloski *et al.* 1989, Liard 1987). Additionally, vasopressin antagonists fail to lower blood pressure in models of SDH (Pawloski *et al.*1989, Liard 1987) making it difficult to contend that elevated circulating levels of this hormone contribute to the chronic increase in peripheral resistance of SDH.

3. Alteration of the sympathetic nervous system

a. renal sympathetic nervous system actions

Altered activity of the sympathetic nervous system independent of

circulating hormones may contribute to sodium-sensitive hypertension. Evidence of increased SNA in the periphery during SDH includes elevation of plasma catecholamines (Campese *et al.* 1982, Takeshita *et al.* 1982, Nishio *et al.* 1988), forearm vascular resistance (Fujita *et al.* 1980, Sullivan *et al.* 1987) and TPR (Mark *et al.* 1975). Additional support is found in the abnormal hypotensive response to clonidine blockade (Dichtchenkenian *et al.* 1989) and the measurement of abnormally high norepinephrine levels in some sodium-sensitive patients during high sodium intake (Gill *et al.*1988). Normally, high sodium intake stimulates the cardiopulmonary baroreflex which decreases sympathetic outflow. However, this response appears to be altered in some sodium sensitive patients and the vascular response to norepinephrine infusion during high sodium intake is elevated compared to non-sodium sensitive patients (Sakaguchi *et al.* 1988, Creager *et al.* 1991, Trimarco *et al.* 1991) suggesting that high sodium intake alters the post-synaptic response to this catecholamine. Inappropriate SNA may contribute to SDH through both renal and extrarenal actions. Indeed, there is evidence of elevated total SNA (Anderson *et al.* 1989) and of selective renal SNA (reviewed DiBona 1992) in some essential hypertensives.

The way increased renal SNA elevates blood pressure appears to be through at least three separate mechanisms. First, selective stimulation of

efferent renal nerves causes sodium retention and vasoconstriction of renal arterioles (Osborn *et al.* 1983). Secondly, direct application of norepinephrine into the kidneys stimulates renin release and increases plasma renin activity (Osborn 1987, Greenburg *et al.* 1991). The release of renin is mediated through stimulation of β_1-adrenergic receptors on the granular cells of the juxtaglomerular apparatus of the kidney and leads to increased plasma concentration of ANG II (Osborn *et al.* 1983, Inagami *et al.* 1990). Circulating ANG II, as described above, can cause hypertension.

A third renal effect of SNA is antinatriuresis independent of decreases in renal blood flow and renin secretion. This antinatriuresis appears to be an intrarenal response to α_1 receptor stimulation (Osborn *et al.* 1983). Finally, stimulation of efferent renal nerves decreases renal blood flow and therefore sodium delivery to the glomerulus. The combined renal effect of SNA is therefore decreased sodium excretion, increased renin release, decreased renal blood flow and elevated systemic pressure. Increased sympathetic stimulation at the kidney in SDH could therefore contribute to hypertension but should be accompanied by sodium retention and elevated plasma levels of renin. These are not seen in SDH so it is unlikely that purely renal effects of SNA are responsible for the chronic elevation in blood pressure seen in SDH.

b. extrarenal sympathetic nervous system actions

The sympathetic nervous system innervates all tissues known to affect blood pressure. In addition to the kidney, this includes the adrenal medulla and cortex, arterial blood vessels, and the heart. The primary effects of the SNS on systemic blood pressure include vasoconstriction of resistance vessels through α_1 vascular receptors, elevated CO through β_1 cardiac receptors, and release of ANG II. As described above, a sympathetically mediated elevation in TPR or CO increases MAP. While circulating catecholamines affect many physiological functions, the systemic cardiovascular effect is increased heart rate and MAP so that elevated SNA is almost invariably associated with elevated MAP (reviewed by DiBona 1992).

E. Animal models of hypertension

Because of the inherent ethical limitations of intervention in clinical studies, animal models have been developed to more precisely examine the ways alterations in renal function, hormone levels, the nervous system and sodium intake interact to affect blood pressure.

The first animal model of chronic hypertension was developed by Goldblatt in 1934. By partially constricting the renal artery and removing the opposite kidney, dogs were made to develop persistent hypertension that was exacerbated by elevated sodium intake (Goldblatt *et al.* 1934). Blood and

plasma from animals with Goldblatt hypertension cross-circulated into anephric normal animals caused a sustained hypertension through a circulating hormone subsequently identified as ANG II (Goldblatt 1968). This model showed clearly for the first time that alterations in renal function can cause chronic hypertension, in this case via a predominantly hormonal mechanism.

Infusion of extracts from normal kidneys also elevated blood pressure in rats. The substance in the renal extracts that increased pressure is renin, an enzyme responsible for the formation of angiotensin II (reviewed Osborn 1991). Both of these early models revealed the important interaction between circulating hormones and renal function in the genesis of SDH. Later models of hypertension with specific correlates in clinical SDH have attempted to further unravel the mechanisms of human SDH. Included among these models are genetically selected animal strains which spontaneously develop hypertension, surgical alterations of renal function which lead to sodium dependent hypertension and hormone infusion models which artificially increase vasoactive and/or antinatriuretic hormones to elevate blood pressure.

1. Genetic models

Since the majority of human hypertension, including SDH, is not secondary to a specific physiological alteration but appears to be an heritable

condition of unknown etiology, animal strains which also have a genetic predisposition to hypertension have been developed and used extensively in hypertension research.

In 1962, Dahl and coworkers developed a genetic model of SDH. They used selective breeding to develop two inbred strains of rats with markedly different responses to increased sodium intake. On a diet of moderately elevated sodium intake one strain developed a rapid increase in blood pressure (Dahl-S) while the other showed complete resistance to sodium-induced hypertension (Dahl-R) (Dahl 1960). In this model the kidney is again implicated. When the kidney from a Dahl-S rat is transplanted into a nephrectomized Dahl-R rat, the resistant rat develops SDH. This is not the case when the Dahl-R rat receives a kidney from another Dahl-R rat. However, Dahl-S rats receiving Dahl-R kidneys also develop hypertension, suggesting that, while the kidney is important for the development of hypertension in this strain, extrarenal factors also play a role (Morgan *et al.*1990). In addition, cross-circulation of blood from a hypertensive Dahl-S rat to a normotensive Dahl-R rat causes increased blood pressure (Dahl 1969). This suggests that a circulating factor which is either released from the kidney or which causes irreversible changes in the kidney, confers sodium-sensitivity of blood pressure in this model. Interestingly, however, Dahl-S

rats do not retain more sodium than Dahl-R rats when sodium intake is increased (Greene *et al.* 1990) showing that volume expansion is not the primary mechanism maintaining hypertension in Dahl-S rats.

Another genetic model of hypertension, the spontaneously hypertensive rat (SHR), is also sodium-sensitive and hypertension can again be transferred by transplanting a kidney (Rettig *et al.* 1990, Rettig *et al.* 1991). In these rats, a high sodium intake enhances the renal SNA and antinatriuresis during environmental stress suggesting that the apparent renal defect may be an altered response to sympathetic stimulation (Koepke and DiBona 1985).

In genetic models of hypertension it is assumed that all genetic alterations contribute to hypertension development. However, the inbreeding necessary to develop such strains produces many strain-specific characteristics that may be entirely independent of the hypertension. An alternative approach in hypertension research has been to mimic hormonal alterations observed in human SDH. By examining a specific alteration in isolation it can be determined if such alterations indeed can initiate and maintain hypertension.

2. Hormone infusion models

a. mineralocorticoid infusion

A widely studied model of SDH is mineralocorticoid excess

accompanied by high sodium intake. Deoxycorticosterone (DOCA), an endogenous adrenal steroid, has been used more extensively than aldosterone because of its solubility in water. However, findings with the two hormones have been very similar and correlate well with observations in the human condition of hyperaldosteronism. This rare form of hypertension is characterized by elevated plasma levels of aldosterone, sodium retention, hypokalemia, and chronically elevated TPR. In addition, there is increased vascular smooth muscle reactivity to norepinephrine and metabolic alkalosis (Fraser and Pudfield 1988).

The mechanisms responsible for mineralocorticoid hypertension have not been clearly defined although many have been suggested. Excessive sodium retention leading to blood volume expansion is a logical choice, particularly since exogenous infusion of mineralocorticoids caused sodium retention (Pan and Young 1982, Cox 1986). However, the increase in blood pressure did not occur simultaneously with the sodium retention and blood pressure continued to rise even after sodium balance was achieved (Pan and Young 1982). It is possible that the initial volume expansion triggered an autoregulatory increase in TPR as described above. This is in agreement with the observation that the sustained phase of aldosterone-salt hypertension is dependent on elevated vascular resistance (Hall *et al.* 1987). Alternatively,

the elevated vascular resistance may be due to direct actions of the steroid on vascular smooth muscle. Isolated blood vessels from DOCA-salt rats have an increased response to pharmacological vasoconstrictors, apparently due to altered ionic transport in vascular smooth muscle cells (Worcel and Moura 1987, Smith *et al.* 1988, Garwitz and Jones 1982) leading to the chronic vasoconstriction and elevated TPR. However, administration of DOCA without elevated sodium intake does not elevate MAP or increase vascular reactivity in spite of altered sodium transport in vascular smooth muscle cells (Cox 1986). In all cases, the hypertension following mineralocorticoid infusion is sodium dependent and mediated chronically by elevated peripheral vascular resistance. The contributions of volume-induced autoregulation, direct vascular effects and centrally mediated elevated SNA to the hypertension remain undefined.

b. ANG II infusion

Another widely used experimental model of SDH is ANG II infusion. As described earlier, exogenous administration of low doses of ANG II in combination with a moderate elevation in sodium intake produces a rapidly reversible chronic elevation in blood pressure. Numerous investigators have shown that ANG II-sodium hypertension is initially dependent on direct vasoconstriction, but the chronic effect on MAP is due to actions at non-

vascular receptors (Brown *et al.*1981, Pawloski 1990, Cox and Bishop 1991). As discussed previously, ANG II has many non-vascular sites of action which could contribute to hypertension including renal, adrenal and brain. It has also been suggested that a trophic effect of ANG II on resistance vessels may contribute to decreased compliance and hypertension (Simon 1992). More evidence is available, however, to support the contention that the increased TPR is due to either elevated SNA dependent on altered baroreflex, or autoregulatory vasoconstriction dependent on decreased renal sodium clearance. The evidence for a centrally mediated rise in SNA includes the lesion studies cited earlier and the observation that the drop in MAP in response to pharmacological ganglionic blockade during the chronic phase of the hypertension is greater than during the initial phase (Pawloski 1990). The evidence of enhanced renal sodium retention has depended largely on *in vitro* studies of ANG II affects on renal sodium handling. A recent study by Krieger and Cowley found that sodium induced fluid volume expansion must be present for ANG II to chronically elevate MAP (Krieger and Cowley 1990). However, in a similar study it was found that while volume expansion was necessary for ANG II induced hypertension, volume expansion alone did not increase MAP. Therefore volume expansion alone cannot initiate hypertension and the mechanisms of ANG II hypertension are not yet clear.

The possible role of increased aldosterone secretion during elevated circulating ANG II is also not resolved. The conflicting evidence from different species suggests that rats and humans respond similarly with an increase in PAC during chronic ANG II excess but the contribution of this elevation in aldosterone to hypertension requires further investigation.

Finally, a role for ANG II in experimental models of hypertension not marked by elevated plasma levels of the peptide is suggested by the ability of CEI to lower blood pressure in SHR (Okumishi *et al.* 1991) and reduced renal mass animals (Olson *et al.* 1982). The role of the peptide is in these models needs further evaluation, perhaps through the use of the new specific nonpeptide antagonists of ANG II. Furthermore, similarities between different models of hypertension that depend on ANG II to maintain the elevated MAP should clarify ways the peptide chronically increases MAP.

One animal model of SDH where ANG II appears to play a role is the reduced renal mass model. This model directly correlates to the human condition of chronic renal failure, a condition frequently associated with hypertension (Blythe 1991) and has been used extensively in research of the mechanisms in the progressive renal degeneration characteristic of chronic renal failure. In addition, RRM-salt hypertension provides a method of investigating the initiation of a form of SDH instead of observing only an

already established hypertensive condition.

3. Reduced renal mass model of sodium-dependent hypertension

a. physical and metabolic changes

In 1932 Chanutin and Ferris first introduced the model of reduced renal mass (RRM) hypertension. In this model one kidney and both poles of the other kidney are removed resulting in 66-75% ablation of the total renal mass. The remaining renal tissue undergoes functional and anatomical adaptation to maintain sufficient excretory function so that dialysis is not necessary. The animals remain healthy and normotensive on normal or low sodium intake for weeks to months. When the animals are placed on a high sodium intake, however, hypertension rapidly develops (Ylitalo 1976) making this a good model of SDH.

A longitudinal study of the evolution of RRM hypertension in rats (Koletsky and Goodsitt 1960) documented the renal and hemodynamic changes that occur over a ten month period following 75% renal ablation. When RRM rats were given tap water to drink and normal laboratory rat chow *ad libitum*, they remained healthy and gained weight throughout the observation period. While on tap water, only 16% became hypertensive within the first week and only 56% were hypertensive by the end of the ten month period. Blood urea nitrogen (BUN) was essentially normal

immediately following ablation then rose steadily to terminal values of 46-105 mg%. In the same study, a second group of RRM rats were given 1% sodium chloride in their drinking water. They failed to gain weight and had a much shorter lifespan. One hundred per cent of these rats were hypertensive within one week and blood pressures were much higher than in the water drinking rats. BUN was similar in the two groups (49 - 108 mg%) suggesting that all rats had similar reductions in renal mass before the divergence in sodium intake. Vascular, renal and cardiac lesions appeared in both groups but were much more prevalent in the saline drinking rats. The lesions typically appeared coincident with or following the development of the hypertension and were proportional to the severity of the hypertension. Morphological examination of the kidney stump after several months of RRM-salt hypertension revealed normal glomeruli; diseased, sclerotic and non-functioning glomeruli; and hypertrophic glomeruli. Over time, the proportion of sclerotic glomeruli increased and it was hypothesized that the increased blood pressure at the glomerulus of the hypertrophic nephrons damaged the glomerular membranes causing the sclerosis (Hostetter 1981, Brenner 1985, Kaufman 1975). When glomerular perfusion pressure was controlled, the hypertrophy and resultant tissue damage was prevented (Meyer 1988) supporting the conclusion that elevated perfusion pressure was

responsible for the initiation of the lesions. Further evidence of the detrimental effect of elevated systemic pressure was documented through the use of antihypertensive treatment in this model: lowering blood pressure with either calcium channel blockers (Brunner 1989) or CEI (Brenner 1985, Jackson 1988) prevented the vascular lesions. However, CEI were especially effective at preventing renal damage and were efficacious at lower doses (Tolins 1990, Anderson 1985).

Dramatic hypertrophy of the renal stump occurs due to rapid structural and metabolic changes and at least partially offsets the loss of renal tissue. Hypertensive RRM rats have a compensatory increase in single nephron glomerular filtration rate (SNGFR) proportional to the initial decrease in renal mass, removing 75% of total renal tissue causes a 150% increase in SNGFR. Renal blood flow increases even more while filtration fraction decreases slightly (Kaufman *et al.* 1975). In spite of the increased SNGFR, excretion of urea and other filtered waste products decreases causing a gradual build up of urea in the plasma. The elevated plasma level of urea increases plasma osmolality, stimulates AVP release and induces polydipsia and polyuria (Jackson 1988).

In addition to decreased excretory capacity (ie. fewer nephrons), there is also a decrease in the number of renal secretory cells. In the kidney, the

primary endocrine cells are the granular cells of the JG apparatus which synthesize and secrete renin (Gomez *et al.* 1990). In RRM rats, increased renin production by JG cells and elevated renin content per nephron has been measured (Rosenberg *et al.* 1991) explaining the previously observed normal PRA following extensive renal ablation (Anderson *et al.* 1985, Bouby *et al.* 1990) and supporting the hypothesis that the RAS is stimulated in RRM hypertension in spite of a decreased number of renin synthesizing cells.

Muirhead and others have proposed that renomedullary interstitial cells also secrete a circulating hormone, medullipin I, which is activated in the liver to form medullipin II. Medullipin II is purported to be a vasodilator that suppresses sympathetic tone, causes natriuresis and suppresses the central nervous system (Muirhead 1991). Increased renal perfusion pressure apparently stimulates secretion of medullipin I and formation of medullipin II which is proposed to act in concert with ANG II as a double feedback system to control blood pressure and renal sodium excretion (Muirhead *et al.* 1991). RRM decreases the number of renal interstitial cells, and decreased plasma levels of medullipin II may contribute to the vasoconstriction observed in RRM-salt hypertension.

b. whole animal hemodynamic changes

The comparison of hemodynamic variables between studies is

somewhat difficult. In the majority of studies of RRM hypertension, MAP is recorded at weekly intervals or only once after months of hypertension. Since RRM-salt hypertension is a progressive disease, the duration of the hypertension is an important variable and the time course of the events that follow ablation is important to deduce their cause. Several recent studies measured MAP acutely, recording the animals for days or weeks instead of months of sodium loading. Since acute and chronic measurements have not been made in the same animals or under the exact same conditions it is difficult to discern which acute changes lead to the chronic condition of elevated TPR. However, by combining observations from several studies, three stages of hypertension can be recognized. The first is a short transition phase with rapidly rising MAP accompanied by sodium and volume expansion (Lombard *et al.* 1989). This is followed by a long stable phase with little change in MAP, little or no volume expansion but increased body sodium. Finally, if the study is carried out long enough, there is a terminal phase of uremia, gross edema and very high MAP that ends with total renal failure and death (Koletsky and Goodsitt 1960).

Following renal reduction, the first phase of hypertension is initiated by increased sodium intake and was only detected when MAP was recorded continuously over the first hours or days of sodium and volume loading. The

initial rise in MAP is accompanied by expanded sodium space (Hall *et al.* 1992) and elevated cardiac index (CI), a measure of CO normalized to body weight (Cowley 1980, Lombard *et al.* 1989). In three studies which measured the early volume expansion, plasma volume returned to normal within 24 hours. TPR rose more slowly but remained elevated. MAP also increased in this initial phase and appeared to parallel the TPR response. In several studies, high salt intake increased sodium space and CI to a similar degree in intact and RRM rats (Ando *et al.* 1990, Lombard *et al.* 1989). In this initial phase, PRA and PAC were both depressed by the high sodium intake and sodium balance was achieved by the second day of sodium infusion (Hall *et al.* 1992).

The initial period appears to be an equilibration period in both intact and RRM rats. The rapid increase in plasma volume by intravenous infusion of sodium increased sodium excretion but plasma sodium remained slightly elevated until sodium balance was again achieved. The role that volume expansion plays in the increased pressure is unclear. The authors in the studies cited above suggest that the elevated CO caused overperfusion leading to autoregulatory vasoconstriction. While it is possible that autoregulation caused the increase in TPR, it is difficult to understand why equivalent overperfusion did not elevate MAP in the control animals and why MAP

continued to rise at a time when CO was declining. More studies of this initial period in RRM-salt hypertension need to be conducted to clarify the role sodium retention and volume expansion play.

The second, chronic phase of RRM-salt hypertension is characterized by elevated but relatively stable MAP, slowly rising BUN, increased SNGFR, and elevated proteinuria. The length of this phase is inversely correlated to the sodium intake and the degree of initial renal reduction. The mechanisms maintaining elevated MAP in this phase may be identical to those in the initial phase or may be different. Both the rapid sodium induced rise in blood pressure and the chronic condition of hypertension were prevented by administration of CEI (Meyer *et al.* 1985, Brooks *et al.* 1989) and by administration of [Sar^1,Ala^8]ANG II (saralasin), a non-specific ANG II antagonist (Pelayo *et al.* 1990). However, established RRM-salt hypertension was not reversed by treatment with saralasin so that the involvement of ANG II in the sodium-induced hypertension has not been resolved. It has been contended that the antihypertensive effect of ANG II blockade was a non-specific vasodilator effect that reversed the autoregulatory vasoconstriction. The role of ANG II in this model of hypertension therefore remains unresolved.

In addition to a slow increase in systemic blood pressure, RRM rats

also develop continued renal deterioration during the stable phase of hypertension (Koletsky and Goodsitt 1969, Ylitalo and Gross 1979). ANG II blockade prevented this progressive decrease in renal function (Meyer *et al.* 1985). Based on these observations, intrarenal actions of ANG II may have caused the adaptive changes in renal function (ie. hyperfiltration with elevated SNGFR and increased glomerular perfusion pressure) and systemic hypertension secondary to these renal changes. However, saralasin blockade of ANG II did not alter SNGFR or glomerular pressure in spite of decreased proteinuria and normalization of MAP (Pelayo *et al.* 1990). Therefore, the mechanisms responsible for the renal changes and the hemodynamic changes during this phase appear to be different.

c. hormonal changes

1) angiotensin II

Plasma levels of ANG II and plasma renin activity (PRA) in RRM rats are consistently suppressed (Anderson *et al.* 1985, Ylitalo *et al.*1976). In spite of this, and as noted earlier, administration of CEI prevented the sodium-induced increase in blood pressure in RRM rats and reversed established hypertension as outlined above (Brunner *et al.* 1989). CEI were uniquely able to prevent both the hypertension and the renal damage that typically develops in RRM rats and in patients with chronic renal failure

(Frohlich 1989). The effects of CEI were not due to their vasodilator properties since treatment with calcium channel blockers also lowered blood pressure but did not prevent deterioration of renal function in RRM rats (Jackson and Johnston 1988, Tolins and Raij 1989, Brunner *et al.* 1989). This implies that lowering MAP is not sufficient to alter the fundamental disease process but merely alleviates one symptom. Preventing the formation of ANG II, however, prevented the initiation of the hypertension and slowed the progression of the disease, implying that ANG II was an important contributor to the initiation of RRM-salt hypertension and chronic renal failure in humans..

It is difficult to conclude that increased activity of the RAS is responsible for the pathogenesis of RRM-salt hypertension, since neither PRA (Ylitalo *et al.*1979, Bouby *et al.*1990, Anderson *et al.*1985) nor circulating levels of ANG II (Kuczera *et al.*1991) are elevated. It has been suggested that CEI therapy acts instead through a non-angiotensin mechanism. One proposed mechanism is increased activity of the renomedullary vasodilator medullipin II which depends on kallikrein-kinin synthesis (Karlstrom *et al.*1990). Increased levels of this vasodilator were found in renal extracts from CEI treated rats and demedullation deceased the effectiveness of CEI therapy, suggesting that decreased degradation of this vasodilator may

mediate the protective effects of CEI therapy. Another proposed mechanism is inhibition of an endogenous vascular RAS. (Kuczera *et al.*1991). While endogenous formation of ANG II in isolated perfused hind-limb in intact and RRM rats was similar, ANG II formation during exogenous renin infusion was slightly greater in RRM rats. The authors concluded that RRM rats had increased activity of the vascular renin-angiotensin system and that CEI lowered MAP through actions on this vascular system.

The effect of circulating ANG II on sodium excretion and blood pressure in hypertensive RRM dogs is apparently enhanced (Hall *et al.* 1992). RRM dogs treated with CEI then exposed to increased sodium intake showed a modest increase in MAP. When renal perfusion pressure in these dogs was servo-controlled through an elegant computer system blood pressure rose slightly more. In RRM dogs with controlled perfusion pressure, replacement of endogenous levels of ANG II by iv infusion at 3 ng/min, caused blood pressure to rise steeply (Hall *et al.* 1992). This study showed that decreased circulating ANG II during RRM sodium-loading is very important to control blood pressure and sodium excretion. Even very small alterations in circulating ANG II concentrations therefore, would be expected to have a very profound effect on both blood pressure and sodium excretion in this model of hypertension.

The existing evidence reviewed here and previously suggests that ANG II is importantly involved in the pathogenesis of RRM-salt hypertension. But the exact role of the peptide, the mechanisms of action, and the time course of its involvement remain to be clarified.

2) arginine vasopressin

During the chronic phase of renal insufficiency hypertension, an osmotically induced elevation in AVP has been documented in both clinical cases of chronic renal failure (Mitch and Wilcox 1981) and RRM hypertension (Bouby 1990). In RRM rabbits, plasma levels of AVP were twofold higher compared to intact rabbits. This increase was significantly correlated with an increase in plasma osmolality (Bouby 1990). The remnant kidney appeared relatively insensitive to AVP and polyuria was exhibited even in the presence of elevated AVP (Bouby 1990). The effect of elevated AVP on blood pressure in RRM animals was addressed indirectly by giving RRM rats a hypo-osmotic water load to decrease plasma AVP levels. The hypovolemic animals had a slower rise in blood pressure but the change in plasma AVP was not significant. Other investigations in rats discussed previously have shown that chronically elevated plasma AVP concentrations do not cause hypertension and AVP antagonists do not lower blood pressure (Pawloski 1990). Finally, treatment with an AVP antagonist in hypertensive

RRM rats lowered blood pressure after an acute volume expansion but only produced a minimal decrease during the chronic phase of hypertension (Gavras 1982). Therefore it is unlikely that the modest elevations of plasma AVP in this model contribute to the sodium-induced hypertension.

3) aldosterone

Another hormone implicated in RRM-salt hypertension is the mineralocorticoid, aldosterone. Plasma levels of aldosterone in RRM rats drinking saline are elevated compared to sham rats drinking saline and RRM rats drinking water (Chi *et al.*1986). RRM rabbits drinking saline (Vehaskari and Herndon 1991) and humans with chronic renal failure (Mitch and Wilcox 1982) also have elevated levels of aldosterone. Since it has been shown that chronically elevated PAC causes hypertension (Pan and Young 1982, Cowley and McCaa 1976, Garwitz and Jones 1982), especially in combination with high sodium intake, it is possible that high levels of the mineralocorticoid may contribute to RRM-salt hypertension.

Elevated PAC in RRM rats was measured following seven weeks of hypertension (Chi *et al.* 1986). Since the decrease in renal clearance of potassium causes RRM rats to develop elevated plasma levels of potassium (Chi *et al.* 1986) which stimulates aldosterone release (reviewed Williams 1991), it is possible that the observed increase in PAC was a result of the

hyperkalemia and not a cause of the hypertension. If RRM-salt hypertension is due in part to mineralocorticoid excess, the time course of the increased PAC should precede or parallel the development of the hypertension. Furthermore, if aldosterone excess is contributing to RRM-salt hypertension and is not stimulated by the changes in plasma potassium due to decreased glomerular filtration, the electrolyte imbalances and increased reactivity of the vasculature seen in mineralocorticoid hypertension (Worcel and Moura 1987) should also be present in RRM rats. This has not been investigated, however, and the role of aldosterone in early stages of RRM-salt hypertension has not been defined.

4) natriuretic factors

Atrial natriuretic peptide (ANP) is released in response to atrial stretch and volume loading is a potent stimulus for the release of ANP (Lang *et al.* 1985). The volume expansion in hypertensive RRM rats (Chi *et al.* 1986) leads, as expected, to elevated plasma levels of this peptide. Jackson measured the plasma levels of the peptide during the development of sodium-induced hypertension in RRM rats and found that the plasma levels rose progressively following the first week of sodium-induced hypertension while atrial content fell suggesting increased secretion rather than production was responsible for the change (Jackson *et al.*1988). Other investigators have also

found an increase in ANP during sodium-induced hypertension in RRM but the changes were very slight and followed rather than accompanied the development of the hypertension (Brandt *et al.*1989, Smith *et al.* 1988).

Because of the alterations in ANP levels during RRM hypertension, ANP may modulate the hypertensive response to increased sodium intake in these animals. ANP has been shown to affect blood pressure by direct vasodilation (Winquist *et al.*1984), direct natriuresis (DeBold *et al.*1981) and by down-regulating the renin-angiotensin system (Maack *et al.*1984) to cumulatively promote diuresis, natriuresis and vasodilation (Jackson *et al.* 1988). However, the two-fold increases in ANP measured in RRM rats are not sufficient to effect blood pressure and it is unlikely that this hormone exerts cardiovascular control during RRM-salt hypertension.

RRM alone (Hall *et al.*1992) and with sodium-loading (Hout *et al.*1983, Ylitalo *et al.*1979, Hall *et al.*1992) is associated with extracellular fluid volume expansion and increased body sodium space. The volume expansion and sodium retention may release the endogenous ouabain-like factor (OLF) discussed above. Hout *et al.* performed an extensive examination of the activity of the Na^+/K^+ pump in hypertensive RRM rats and found that the RRM rats had decreased pump activity in vascular smooth muscle and in cardiac microsomes. In addition, boiled plasma extracts caused

in vitro inhibition of Na^+/K^+ pump when applied to tail arteries of normal animals. Specific hypothalamic lesions in RRM rats prevented both the development of sodium-induced hypertension and an increase in circulating pump inhibitor (Hout *et al.* 1983). Others have also found increased circulating levels of a Na^+/K^+ pump inhibitor that cross-reacts with a digitalis antibody and which increases parallel to the rise in blood pressure (Nakagawa *et al.*1990, Wauquier *et al.*1986).

A direct study of the contribution of this endogenous inhibitor to hypertensive states has been impossible because there is no known antagonist. However, a recently developed assay has shown that other forms of hypertension associated with volume expansion do have increased levels of OLF (Kaminski and Rechsberger 1991). Contrary to what would be expected if OLF can influence blood pressure, infusion of plant ouabain into rats did not consistently cause hypertension (Yasujima *et al.* 1986). Therefore it is still unclear if the natural compound would be able to contribute to SDH.

d. sympathetic nervous system

There have been several reports of elevated SNA in RRM-salt hypertension. Initially it was observed that RRM rats on high sodium intake had elevated plasma levels of norepinephrine (NE) and responded to epinephrine synthesis blockade with a fall in MAP. Moreover, when the

inhibitor used was unable to cross the blood brain barrier, there was no decrease in MAP (Dipette et al 1982). It has also been reported that lesion of the anteroventral third ventricle (AV3V) prevented a sodium-induced rise in MAP in RRM rats (Hout *et al.* 1983). These observations suggest that sodium-loading during RRM may cause increased epinephrine synthesis within the CNS leading to elevated sympathetic outflow and the observed hypertension (Gavras 1982).

An alternative role for the SNS is increased release of NE mediated by a circulating factor such as ANG II aldosterone acting either in the CNS or at peripheral sites. Exogenous application of NE to isolated blood vessels produced a greater response in RRM vessels compared to vessels from intact rats (Chi *et al.* 1986, Shima *et al.* 1988) while plasma from RRM-salt rats increased NE overflow during electrical stimulation (Shima *et al.* 1988). Furthermore, in situ recording of membrane potentials in resistance vessels in the mesenteric vascular bed found lower potentials (ie. depolarization) in hypertensive RRM rats compared to sodium loaded intact rats (Stekiel *et al.* 1991). The basis of the increased reactivity of the blood vessels may be elevated levels of the Na^+/K^+-ATPase inhibitor, OLF, or through another circulating factor that interacts with the SNS such as ANG II. The identity of this factor is not known but the in situ depolarization to ouabain application

was identical between RRM and sham rats suggesting that there is not endogenous blockade of these receptors in RRM hypertension (Stekiel *et al.* 1991).

In spite of the evidence of both humoral and neurological changes in RRM-salt hypertension, the mechanisms responsible for the condition are not clearly defined. There appear to be multiple factors altered during the sodium-induced rise in MAP in RRM animals and the possible interactions between circulating factors and the SNS make interpretation of data difficult. Finally, the mechanisms responsible for the initial rise in pressure and the sustained hypertension may not be the same. Therefore additional experiments which closely examine alterations in hormone levels throughout the development of the hypertension are needed to define which changes initiate the hypertensive response and which changes occur in response to the hypertension.

Statement of Purpose

Sodium sensitive hypertension (SDH) is defined by the blood pressure response to sodium intake. It is not defined by a homogeneous set of individuals, but is a condition common to several different forms of hypertension. In spite of the different etiologies of the condition, there are several common characteristics of SDH. The purpose of the experiments described here was to characterize one of these features, alterations in humoral factors, and to determine what changes in humoral factors occur in one form of SDH, RRM-salt hypertension.

Humoral factors known to influence both blood pressure and sodium homeostasis have been implicated in previous studies of SDH and in RRM-hypertension in particular. Angiotensin II, aldosterone and an endogenous ouabain-like factor have all been suggested as contributors to this hypertension and these hormones were the ones investigated.

Initially, we sought to establish the parameters under which each of these factors initiates the development of SDH and to characterize the changes that occur as the increase in blood pressure develops. Because there are interactions between these hormones and between the hormones and the sympathetic nervous system, it was necessary to determine how the hormones

alone affect blood pressure and sodium homeostasis.

After characterization in normal rats, the RRM model was used to determine if any of these hormones might be playing a role in the development of the sodium-induced increase in arterial pressure. The RRM model was chosen because of its relevance to the human condition of chronic renal failure, a condition associated with a high incidence of SDH, and because it is not associated with any genetic alterations in blood pressure control that might be independent of the sodium-induced hypertension. Furthermore, characteristics of this model have been shown to apply directly to human hypertension associated with chronic renal failure and presumably the more general condition of SDH .

II. Materials and Methods

A. General experimental protocols

1. Surgical procedures

a. catheterization

Catheters were constructed of polyethylene (Tygon^{R}) tubing attached to silicon (Silastin^{R}) tubing. Arterial and venous catheters were surgically implanted under pentobarbital anesthesia (40 mg/kg plus 0.4 mg atropine sulfate). One silicon-rubber/polyvinyl catheter was placed into the abdominal aorta via the external iliac artery. This catheter was used to record arterial pressure and heart rate and to draw blood samples from conscious, freely-moving animals. Another silicon-rubber/polyvinyl catheter was inserted into posterior vena cava via the external iliac vein. This catheter was used to infuse drugs and sodium. Both catheters were tunneled subcutaneously to the top of the head where they were exteriorized and fed through a stainless steel spring to protect the tubing from the rat. One end of the metal spring was attached to the skull with dental acrylic and jewelers screws, while the other end was attached to a plastic swivel mounted above the cage allowing the rat to move freely within the cage. The venous line was attached to a syringe-type Harvard infusion pump (Boston, MA) via the swivel to allow continuous

24 hour infusions. Arterial lines were filled with a heparin-sucrose solution and occluded when not in use. After the animal awoke following surgery, it was placed in a metabolism cage with the swivel suspended over the cage. This allowed the rats freedom of movement within the cage and free access to the venous and arterial catheters from outside of the cage. Rats were allowed at least three days recovery after surgery before measurements were begun.

b. subcutaneous osmotic minipumps

Alzet osmotic minipumps (Alza, CA) were filled with a sterile solution of the drug to be administered. The concentration of the drug was calculated on the basis that the pumps deliver either 1.0 or 2.0 µl/hr over a 24 hour period. Rats were anesthetized with methohexital (40 mg/kg ip or 25 mg/kg iv) and a small incision was made in the fold of skin just behind the head. The pumps were slipped under the skin to deliver drugs subcutaneously. The incision was closed with silk sutures and the animals were given 20,000 units penicillin G and 25 mg streptomycin, 0.1 ml Combiotic^{R} ip (Pfizer New York, NY) to prevent infection.

c. renal reduction

Rats were pretreated with atropine sulfate (0.40 mg ip) and

anesthetized with sodium pentobarbital (40 mg/kg; ip) for all surgical procedures. Surgical renal reduction was performed in two stages. Initially, a left mid-flank incision was made and the left kidney exteriorized. The renal artery was temporarily occluded and both poles of the kidney (2/3 of the mass) excised with a scalpel. The clamp was removed and bleeding controlled with Thrombostat (Parke-Davis, Ann Arbor, MI) and pressure before the kidney stump was returned to the peritoneum. The incision was sutured and the rat allowed one week recovery after which the right kidney was exposed, the right renal artery, vein and ureter ligated with silk, and the entire kidney removed. Rats were allowed one to two weeks recovery to return to pre-surgery weight after the second procedure before catheterization surgery.

2. Hemodynamic measurements

Blood pressure was measured by connecting the exposed arterial line to a Gould P50 Statham pressure transducer (Oxnard, CA) attached to a Grass polygraph (Quincy, MA). The signal was fed into a Stiemke digital output device (New Orleans, LA) which displays the average mean arterial blood pressure on a digital display. Heart rate was read directly from the tracing on the recording chart. For animals with sinoaortic denervation, the pressure

signal was fed into an Apple computer which sampled blood pressure and heart rate every 30 seconds over a twenty minute period. The mean values and standard deviations were calculated by a program written by Dr. Gregory Fink and displayed on the screen.

All blood samples were taken following daily blood pressure recording. Blood was drawn into syringes containing either 0.05 ml 3M EDTA or 0.05 ml 10,000 IU/ml heparin from the exposed arterial line. Samples were immediately spun in 4°C refrigerated centrifuge, the plasma drawn off and frozen at -70°C until assays were performed. In animals with repeated blood samples, red cells were returned suspended in physiological saline equal to the volume of the sample. In other animals, saline equal to the volume of the sample was given. Blood samples were not drawn more frequently than every 4 days in any of the protocols.

3. Analytical methods

a. sodium and potassium

Sodium and potassium concentrations in urine and plasma were measured with an Instrumentation Laboratory IL943 flame photometer (Lexington, MA). Within assay variability was 4%. The appropriate standards were run daily before analysis so that between assay variability was

less than 10%. Electrolyte excretion was calculated by multiplying urine concentration times 24 hour urine volume. All plasma samples from each rat were analyzed on the same day to control for interassay variability.

b. plasma osmolality

Plasma osmolality was measured by freezing point depression using a Precision Systems, Inc. Model 5004 μ Osmette (Natick, MA). Three determinations were made for each sample and the mean of the three values recorded. Appropriate standards were run daily before each determination and interassay variability was 2-3%.

c. blood urea nitrogen

Blood urea nitrogen (BUN) was determined by a colorometric assay using a prepared assay kit, No. 640, from Sigma Chemical (St. Louis, MO). All samples from each rat were run in the same assay and a new standard curve was calculated for each assay. Standard curves between assays were virtually identical so that interassay variability was negligible.

d. plasma aldosterone concentration

Plasma aldosterone concentration was measured with a radioimmunoassay kit (Coat-a-Count) from Diagnostic Products (Los Angeles, CA). 1 ml of blood was drawn using heparin treated syringes to obtain 500 μl of plasma. Two 200 μl plasma samples were assayed and the average of

the two values recorded. The sensitivity limit of the assay is 16 pg/ml. The intraassay coefficient of variation averages 5% and interassay coefficient of variation is 8%. All samples from one animal were run in the same assay to eliminate interassay variability.

e. plasma immunoreactive angiotensin II

1 ml of blood was drawn into an EDTA treated syringe. 400 µl of plasma was extracted twice with 100 % ethanol, the supernatants combined and dried under a stream of air. The residue was stored in the freezer until assayed. At the time of the assay, samples were reconstituted with 200 µl assay buffer solution (0.30% bovine serum albumin, 0.50M Trizma base, 0.20% neomycin sulphate, pH 7.4) and the concentration determined by radioimmunoassay. Recovery of angiotensin II was 95% after extraction. ^{125}I angiotensin II was purchased from New England Nuclear (Boston MA) and the antibody was purchased from Arnel laboratories (New York, NY). The crossreactivity of the antibody has recently been evaluated by Navar *et al*. They found that angiotensin III crossreacts 100% with the antibody while angiotensin I was only detected in concentrations >100 fold higher than angiotensin II {Navar 1992). The sensitivity of the assay allowed measurement down to about 20 pg/ml. The intraassay coefficient of variation was approximately 4% and the interassay coefficient of variation was 9%.

All samples from one animal were run in the same assay.

f. fluid homeostasis

Daily water intake was determined from a calibrated drinking tube and daily urine output was determined by collecting 24 hour urine samples in a calibrated urine cup. Water balance was calculated by subtracting urine volume from the sum of water intake volume plus 5 ml/day iv infusion volume.

4. Statistical methods

A mixed-design analysis of variance (ANOVA) blocked for between animal variation was used to evaluate within groups differences in daily measurements. A one-way ANOVA was used to evaluate differences between groups at individual time points. The Student-Newman-Keuls' test of significance was used for comparisons between groups and least significant difference t-test was used to test for the significance of changes over time. Paired t-tests were used to evaluate differences when only two measurements were made. A p-value of less than 0.05 was considered statistically significant.

B. Specific protocols

1. Aldosterone infusion

Chronic elevation of aldosterone causes increased blood pressure in uninephrectomized rats on high sodium intake and aldosterone excess may play a role in other forms of SDH. It has been reported that aldosterone is elevated in hypertensive RRM rats(Chi *et al.* 1986) and that infusion of ANG II causes increases in plasma aldosterone concentration (PAC) (Cowley and McCaa 1976). The first study was performed to establish what level of PAC will alone cause hypertension during a moderately elevated sodium intake to determine the relevance of any increases in PAC seen in later protocols. To accomplish this, the following experiment was performed.

Arterial and venous catheters were surgically implanted in fifteen rats as described above. Immediately following surgery, intravenous infusion delivering six meq/day sodium was begun. Animals were allowed access *ad libitum* to sodium deficient rat chow and tap water. Beginning three days after surgery, daily determinations of MAP, HR, WI, UO, urinary potassium excretion and urinary sodium excretion were made. After three days of control measurements, the rats were briefly anesthetized with sodium methohexital, Brevital^{R} (Lilly, Indianapolis, IN) and a small incision was made at the back of the neck. An osmotic minipump, Alzet^{R} model 2002 (Alza, CA), filled to deliver one of three doses of aldosterone was positioned

subcutaneously and the incision sutured. The minipump was replaced after twelve days with a new model 2001 Alzet^{R} minipump filled with aldosterone. The second pump was removed four days later following the daily MAP and HR measurements. All pumps were prepared according the manufacturer's directions to deliver either 0.10, 0.25, or 0.50 μg/hr of aldosterone. PAC was monitored by drawing 1.2 ml arterial blood samples into heparin containing syringes approximately every four days in the morning just after recording MAP and HR. PAC was determined as described under analytical methods. Plasma samples were also used to determine plasma osmolality, potassium and sodium.

2. PAC during chronic infusion of angiotensin II

This study was performed to determine the involvement of aldosterone during chronic infusion of ANG II. First, infusion of ANG II over hours to days causes increased secretion of aldosterone (Aguilera and Catt 1978, Hauger at al. 1978, Komer and Muller 1979). Second, chronic elevations in PAC cause an increase in arterial pressure both in clinical and animal studies (Garwitz and Jones 1982, Oelkers and Schoneshofer 1983) as demonstrated in the previous study. Finally, chronic exposure of the adrenal gland to ANG II

causes an increase in adrenal ANG II receptor number and increased sensitivity of receptors to ANG II both in rats and humans (Aguelera *et al.* 1980, Naruse *et al.* 1987). This last point suggests that chronically elevated plasma levels of ANG II in these species could lead to progressively increasing plasma levels of aldosterone. The aims of the present study were to: 1) measure changes in PAC occurring in response to chronic low dose infusions of ANG II in rats under conditions where the peptide does (high sodium intake) or does not (normal sodium intake) cause hypertension; and 2) determine from the above results whether changes in PAC could make an independent contribution to the development of hypertension caused by chronic low dose ANG II infusion. These findings would be expected to apply to any situation of prolonged elevation of plasma levels of ANG II. To test these hypotheses, the following two experiments were performed.

Chronic ANG II infusion 1: Fifteen rats had venous and arterial catheters surgically implanted. Ten rats were randomly placed in an ANG II group and five in a control group. Following surgery, rats were allowed only sodium deficient rat chow and received 6 meq/day sodium iv infused continuously over twenty-four hours. The rats were maintained on high sodium intake for the remainder of the protocol. The protocol included three control days of sodium infusion alone, sixteen ANG II plus sodium infusion

days and four recovery days when again, only sodium was infused. In the ANG II group, ANG II was added daily to the infusate during the ANG II infusion period in concentrations to produce a constant infusion rate of 10 ng/min. The control rats continued to receive only the 6 meq/day sodium solution during this period. WI, UO, urinary sodium excretion (UNaV), and urinary potassium (UKV) excretion were measured daily. MAP, and HR were recorded every other day and plasma sodium, plasma potassium, and PAC were measured every fourth day from blood samples drawn into syringes containing EDTA immediately after the daily MAP and HR recording. One sample was taken on the second day of the control period, four during the ANG II infusion period and one on the last day of recovery. Care was taken to draw samples at approximately the same time each day to control for diurnal variation in aldosterone secretion (Quinn and Williams 1988).

Chronic ANG II infusion 2: Catheters were surgically implanted in ten animals as described above and divided into one group of five control animals and one group of five ANG II animals. Following surgery, animals were allowed only sodium deficient rat chow and were placed on a continuous 2 meq/day sodium iv infusion. The experimental protocol consisted of three control days, twenty-eight ANG II infusion days and four

recovery days. The ANG II group received 10 ng/min ANG II added to the saline infusion during the ANG II infusion period. WI, UO, urinary sodium and urinary potassium excretion were measured daily. MAP and HR were measured every other day and plasma sodium, plasma potassium and PAC measured every fourth day from blood samples taken as described above, beginning on day two of the control period.

3. Aldosterone in reduced renal mass hypertension

Reduced renal mass rats have been shown to have elevated plasma levels of aldosterone during the established phase of sodium-induced hypertension. It is possible that this increase causes at least a portion of the blood pressure elevation. If the increase in PAC is of sufficient magnitude and occurs during the initiation of the hypertension would support the hypothesis that RRM-salt hypertension is dependent on the elevated PAC. This hypothesis was tested with the following experiment.

Nineteen rats underwent renal reduction and catheterization as described above. 5 additional intact control rats were catheterized. After recovery from surgery, all animals were given normal sodium intake (2 meq/day) for 5 days, high sodium intake (6 meq/day) for 10 days followed by 5 more days normal sodium intake. MAP, HR, WI, UO, UNaV, UKV were

recorded daily. Blood samples were drawn into heparinized syringes every 4 days to measure BUN, plasma sodium, plasma potassium, plasma osmolality and PAC.

4. Angiotensin II antagonism in reduced renal mass hypertension

Plasma levels in RRM-sodium hypertension in rats in normal or even slightly suppressed. However treatment with CEI prevents the development of hypertension and can reverse the hypertension once it is established. This may be due to an action of CEI independent of the renin-angiotensin system or may be due to actions of normal circulating levels of ANG II. If it is due to ANG II actions, treatment with the specific ANG II antagonist losartan should mimic both the chronic and acute CEI results. If the antagonist results are different from the CEI results, it is unlikely that circulating ANG II is involved.

a. chronic antagonist treatment

4 groups of rats were used. Rats in groups 1 and 2 underwent 2-stage renal reduction followed by recovery before catheterization. All rats were catheterized and, following surgical recovery on normal sodium intake,

maintained for 5 days on normal sodium followed by 7 days of high sodium. Group 1 (n = 9) received daily bolus intravenous injections of 3 mg/kg losartan during high sodium intake. Group 2 RRM rats (n = 7) received vehicle during the high sodium period. Group 3 sham operated rats (n = 8) received losartan and group 4 sham controls (n = 8) received vehicle. The RRM groups had approximately 80% renal ablation while the sham groups had no reduction. Losartan injections were given once daily as an intravenous bolus in dextrose (3 mg/ml) after the daily recording of MAP and HR beginning on day 5 of normal sodium intake. Losartan treatment began simultaneously with the change from normal to high sodium. MAP, HR, WI, UO, UNaV, and UKV were measured daily in all groups. Plasma samples were drawn into heparinized syringes on day 1 of normal sodium to measure initial BUN and on day 7 of high sodium to measure final BUN. Graded 8 minute infusions of ANG II (3, 10 and 30 ng/min) were given on day 3 of normal sodium and on day 5 of high sodium to measure the acute pressor response to ANG II. In animals receiving losartan, the ANG II infusions were given before the daily losartan bolus. After a stabilization period of 20 minutes, a baseline MAP was recorded and the 3 ng/min infusion was started. After 8 minutes, the MAP and HR were recorded and the change in MAP and HR calculated. The infusion rate was increased to 10 ng/min and after 8

minutes, the change in MAP at the new rate was calculated from the initial baseline value. After the third step increase, the change in AP and HR were again calculated and the infusion was stopped.

b. acute antagonist treatment

Six RRM rats were catheterized and maintained on high sodium intake (6 meq/day). After 3 days recovery from surgery, MAP, HR, WI, UO, UNAV and UKV were recorded daily. On day 5 of high sodium, rats were given a bolus of 3 mg/kg losartan intravenously. MAP and HR were recorded 5, 15, 30, 60, 120, and 360 minutes after the losartan challenge. 5 days after the first challenge, a second challenge was given and measurements were again made at 5, 15, 30, 60, 120, and 360 minutes . MAP and HR were recorded daily on the 3 days following the second losartan challenge. UNAV and water balance were calculated daily.

5. Angiotensin II infusion in reduced renal mass rats

Another test of the involvement of ANG II in RRM-sodium hypertension was to examine the sensitivity of normotensive RRM rats to elevations in circulating ANG II. It is possible that renal reduction increases the response to ANG II so that compared to intact rats, plasma levels of the

peptide appear normal. If RRM rats are more responsive to ANG II hypertension, then they will become hypertensive at plasma levels that do not produce hypertension in intact rats just as they would become normotensive to antagonist treatment even though plasma levels are not elevated.

Thirty-eight rats underwent renal reduction followed by catheterization as described above. Eleven animals were randomly chosen as controls and the other 27 animals received 10 ng/min ANG II. An additional control group of 7 rats with both kidneys intact received 10 ng/min ANG II infusion. Following catheterization surgery, animals were allowed only sodium deficient rat chow and received 2 meq Na/day in 5 ml water by continuous iv infusion. The experimental protocol consisted of 3 control days, 10 ANG II infusion days and 4 recovery days. The RRM control group received sodium infusion during the ANG II infusion period. MAP, HR, WI, UO, UNaV and UKV were measured daily in all three groups. Plasma sodium concentration, plasma potassium concentration, plasma osmolality and plasma ANG II concentration were determined from blood samples taken as described above. Samples were taken once during control, twice during ANG II infusion and once during recovery.

III. Results

A. Plasma aldosterone concentration in intact rats

Acute ANG II Infusion- The data in Table 1 is from previous work in our laboratory and illustrates the MAP and PAC of rats receiving one hour infusions of ANG II at 10, 30 and 60 ng/min; the infusion rate of 10 ng/min produced plasma levels of ANG II similar to those measured during renoprival hypertension (Kuczera 1991) was used for the remainder of the studies. The 10 ng/min infusion of ANG II caused MAP to increase significantly from 105.6 ± 2.5 to 128.8 ± 5.1 mm Hg while PAC increased significantly from 73.5 ± 23.8 to 168.6 ± 36.5 pg/ml. Higher infusion rates of ANG II caused higher PACs and correspondingly greater pressor responses.

Chronic Aldosterone Infusion- The MAP, PAC and HR of the three groups of rats receiving subcutaneous aldosterone infusions are shown in Figure 1. Only the group receiving the highest rate of aldosterone (0.50 μg/hr) had a sustained hypertension during the aldosterone infusion. The MAP in this group tended to increase slowly over time, reaching statistical significance on day 10 and the highest MAP level on day 16 of the infusion.

PAC in the rats receiving the highest aldosterone infusion rate reached

	10 ng/min		30 ng/min		60 ng/min	
	MAP	PAC	MAP	PAC	MAP	PAC
pre-infusion	105.6 ± 2.5	73.5 ± 23.8	101.2 ± 1.5	81.2 ± 74.9	101.7 ± 4.6	32.3 ± 9.6
post-infusion	* 128.8 ± 5.1	* 168.6 ± 36.5	* 137.6 ± 6.1	* 486.6 ± 125.6	* 147.2 ± 6.2	* 762.0 ± 198.9

Table 1: Plasma aldosterone and blood pressure following angiotensin II infusion. Mean arterial pressure (MAP) is listed as mm Hg and plasma aldosterone concentration (PAC) is listed as pg/ml in rats receiving ANG II infused iv at either 10, 30, or 60 ng/min. MAP was recorded and blood samples drawn prior to ANG II infusion and 60 minutes after the infusion was started. MAP and PAC were both significantly elevated at all three infusion rates ($p \leq 0.05$). Values are the mean of 7 (10 ng/min) or 5 (30 and 60 ng/min) animals ± standard error of the mean (SEM).

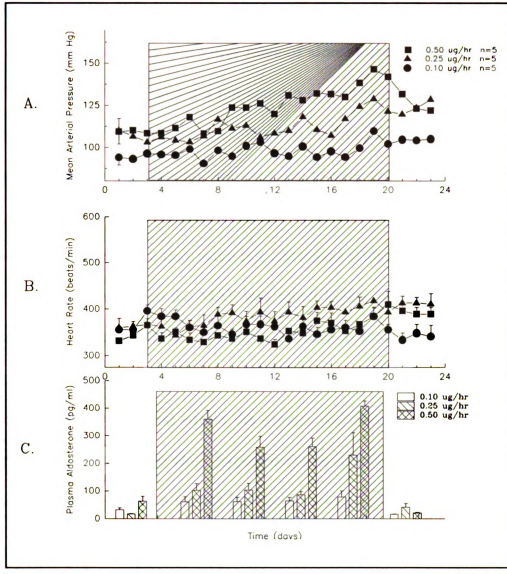

Figure 1: Effect of aldosterone infusion on blood pressure, heart rate and plasma aldosterone concentration. A. shows mean arterial pressure, B. shows plasma aldosterone and C. shows heart rate in 3 groups of rats receiving subcutaneous aldosterone infusion at 0.10 (circles and open bars), 0.25 (triangles and hatched bars) or 0.50 (squares and cross-hatched bars) μg/hr. The first 3 days were control (no aldosterone), the 16 days in the shaded area were aldosterone treatment and the final 4 days were recovery. Each point represents the mean of five rats. Statistically significant differences between control and infusion periods within a group for $p \leq 0.05$ are marked with *. Error bars represent standard error of the mean (SEM).

five to eight times the control level and was significantly increased by day four of the infusion. The two lower rates of aldosterone infusion caused a smaller increase in PAC and did not cause a sustained increase in MAP over the time course of this experiment. The PAC on day 16 during the 0.25 μg/hr infusion was significantly elevated due to very high PAC's in 2 of the 5 rats. The high levels could have been due to an error in preparing the replacement pumps since they were not consistent with the data from the other infusion rates. Also the MAP was much higher in these two animals. These results indicate that the minimum increase in PAC required to show a statistically measurable elevation from control levels was 45 to 100 pg/ml (depending on the variation within the group); and that a PAC of at least 250-300 pg/ml was required to elicit hypertension in rats on a 6 meq/day sodium intake and that an infusion rate of 0.50 μg/hr was required to produce this PAC. WI and UO were unaltered by the two lower infusion rates but were significantly elevated in the group receiving 0.50 μg/hr (Figure 2). There was no difference in water balance in any of the groups. The 0.50 μg/hr group also had a significant decrease in urinary sodium excretion (Figure 3). There were no other differences in the electrolyte excretion in the three groups.

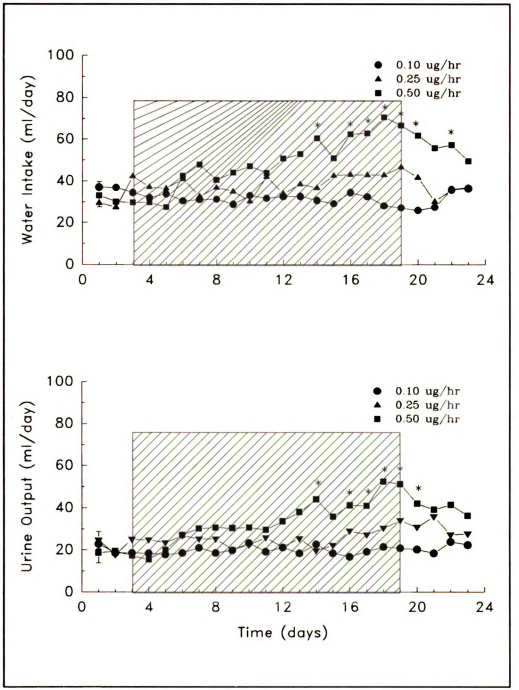

Figure 2: Water intake and urine output in intact rats receiving aldosterone subcutaneously either 0.10, 0.25, or 0.50 μg/hr aldosterone subcutaneously. The aldosterone infusion period is indicated by the hatched area. Statistically significant differences for $p \leq 0.05$ are indicated by *. Error bars represent the SEM. All points represent the mean of measurements made in 5 rats.

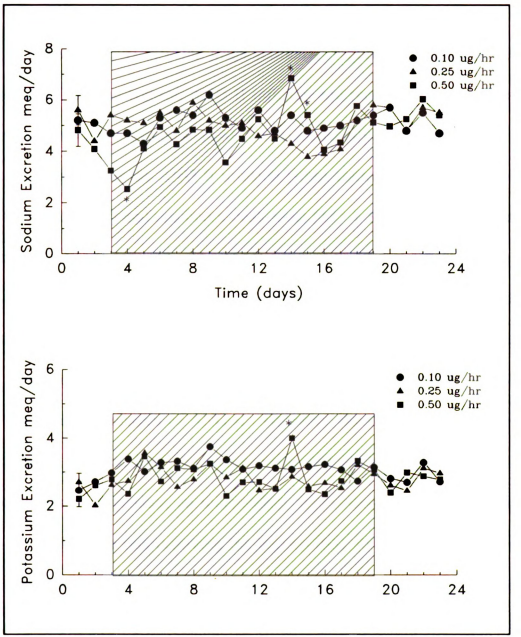

Figure 3: Effect of aldosterone infusion on sodium and potassium excretion in intact rats receiving 0.10, 0.25, or 0.50 μg/hr aldosterone infusion. The aldosterone infusion period is in the shaded area bracketed by 3 control and 4 recovery days. The solid line in the top figure represents the daily sodium intake. Significant differences within groups are marked with a * for $p < 0.05$. Each point represents the mean of 5 rats and error bars are SEM.

B. Chronic infusion of angiotensin II in intact rats

Chronic ANG II Infusion (high sodium)- Figure 4 illustrates the MAP, HR and PAC for the control, ANG II infusion and recovery periods of rats receiving 6 meq/day sodium. MAP was significantly increased above controlby day two of the ANG II infusion (from control values of 97 ± 4 and 101 ± 4 to 130 ± 7) and reached a peak level (155 ± 9 mm Hg) on the eighth day of the ANG II infusion. The MAP returned to control levels when the ANG II infusion was stopped. No change in HR occurred during ANG II infusion. There was an upward trend in PAC from a control value of 32.1 ± 10.1 pg/ml to a high of 109 ± 39.5 pg/ml on day eight of ANG II and 96 ± 35.2 pg/ml on day sixteen of ANG II. However, the latter PACs were not significantly different from the control value and did not reach the level found necessary to produce an independent hypertensive response (250-300 pg/ml). Unlike the rats receiving the highest dose of aldosterone, urinary electrolyte excretion was not altered by the ANG II infusion. Water balance, sodium excretion and potassium excretion were measured daily and did not differ between control and infusion periods or between the saline infused and the ANG II infused groups. Urinary electrolyte excretion and water balance (Figure 5) was identical within both groups throughout the protocol. Although others have reported sodium and water retention in rats chronically

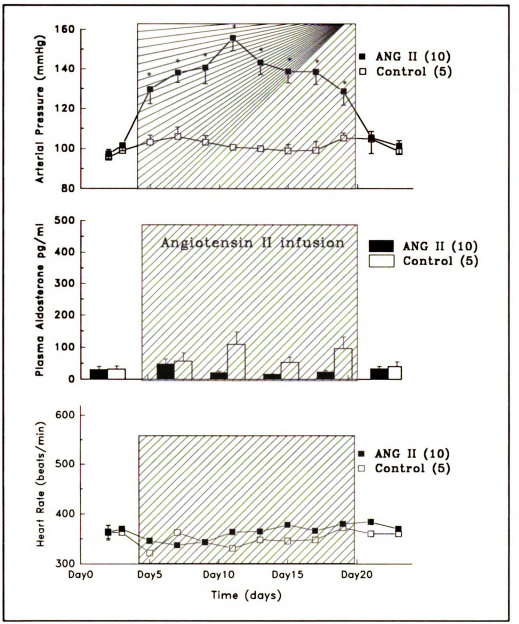

Figure 4: Blood pressure, heart rate and plasma aldosterone in intact rats receiving angiotensin II . Control rats are shown in open squares and bars while ANG II rats are depicted by solid squares and bars. The ANG II infusion period (10 ng/min iv) is inside the shaded area. Each point represents the mean of 10 (ANG II) or 5 (control) rats. Statistically significant differences between control and infusion periods for $p \leq 0.05$ are marked with *. Bars represent SEM.

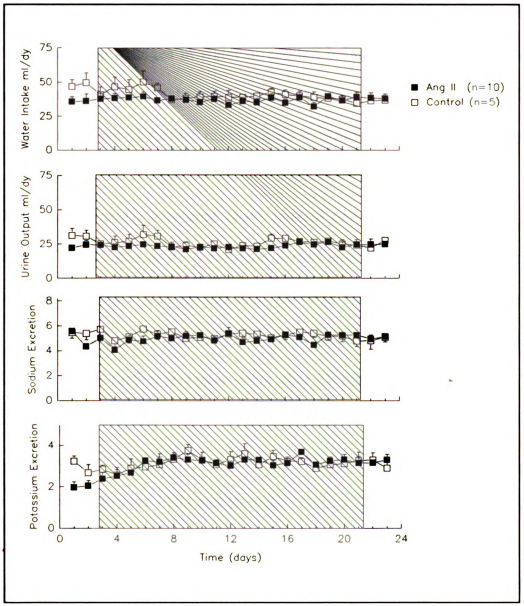

Figure 5: Urine output, water intake, urinary sodium excretion and urinary potassium excretion in rats receiving ANG II infusion. Control rats are depicted by the open squares and ANG II rats by the solid squares. All rats were on a fixed 6 meq/day sodium intake with sodium free chow and water *ad libitum*. ANG II infusion period (10 ng/min) is inside the shaded area. Points represent the mean of 10 (ANG II) or 5 (control) rats. There were no statistically significant differences between control and infusion periods for $p \leq 0.05$. Bars represent SEM..

receiving ANG II, no such retention was observed here and the hypertension developed without changes in water or sodium balance. Furthermore, plasma potassium and sodium levels and plasma osmolality were also unchanged by the infusion (Figure 6) further revealing no evidence of volume expansion.

Chronic ANG II Infusion (normal sodium)- As shown in Figure 7, MAP was significantly increased above control only on days four and eighteen of the 28 day ANG II infusion period in the rats receiving 2 meq sodium/day. Figure 7 also reveals that, although the control level for PAC was higher in the normal sodium group than in the high sodium group, ANG II did not cause a further increase--even after 28 days of continuous 10 ng/min ANG II infusion. The HR was unchanged from control in either group of animals. The water and electrolyte data showed no differences between the two groups (data not shown). Plasma osmolality, plasma sodium and plasma potassium were also unchanged by ANG II infusion .

In summary, aldosterone infusion elevates plasma levels of aldosterone (PAC) and MAP in a dose-dependent manner so that a 3-fold elevation in PAC caused a significant elevation in blood pressure during high salt intake. Infusion of ANG II acutely elevates plasma levels of aldosterone to hypertensive levels and elevates MAP in a dose-dependent relationship. However, infusion of 10 ng/min ANG II for 2 to 28 days

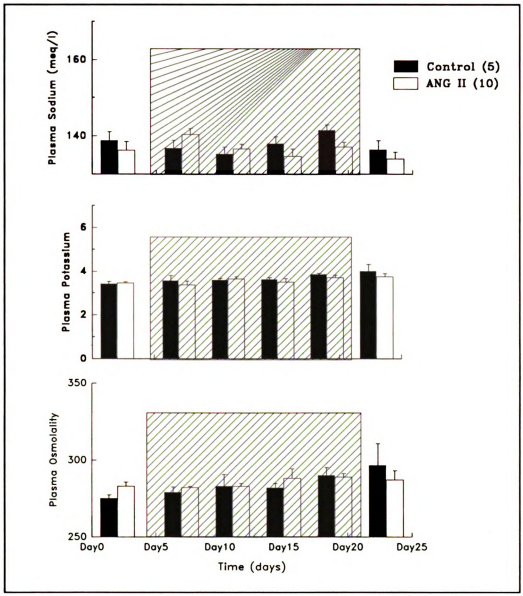

Figure 6: Plasma sodium, potassium and osmolality during angiotensin II infusion in rats on high sodium intake. Control rats are depicted by the solid bars and ANG II rats by the open bars The ANG II infusion period (10 ng/min iv) is inside the shaded area. Bars represent the mean of 10 (ANG II) or 5 (control) rats. Blood samples were drawn approximately every 4 days and blood volume replaced with physiological saline. Statistically significant differences between control and infusion periods for $p \leq 0.05$ are marked with *. Eror bars represent SEM.

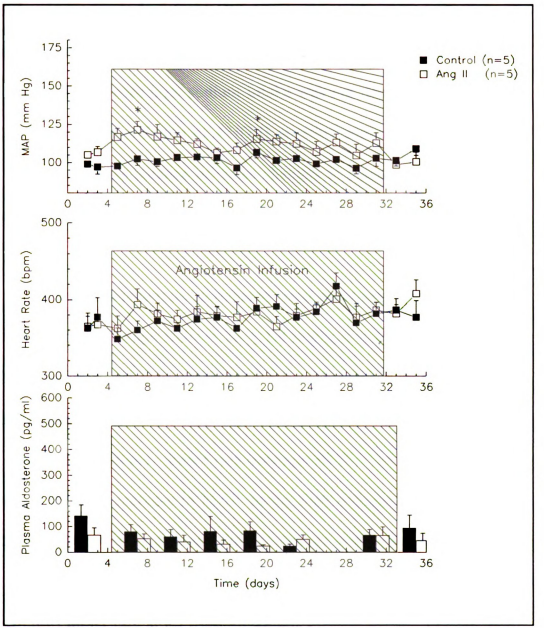

Figure 7: Blood pressure, heart rate and plasma aldosterone concentration in rats receiving angiotensin II on normal sodium. Control rats are depicted in open squares and bars while ANG II rats are depicted by the solid squares and bars during a fixed 2 meq/day sodium intake. The ANG II infusion period (10 ng/min iv) is in the shaded area. Points represent the mean of 5 rats. Statistically significant differences between control and infusion periods for $p \leq 0.05$ are marked with *. Error bars represent SEM.

not cause a sustained elevation in PAC in rats on either high sodium intake or normal sodium intake. Therefore, the elevated MAP during the ANG II infusion combined with high sodium intake was not dependent on elevated PAC. Furthermore, infusion of ANG II at either sodium intake does not alter sodium or water balance or cause changes in plasma electrolyte concentrations such as would be expected during PAC excess.

C. Plasma aldosterone in reduced renal mass hypertension

RRM rats (average BUN = 28 $\pm$ 1.8 mg%; n = 19) consistently exhibited a significant increase in MAP when changed from normal to high sodium intake. MAP returned toward control values after rats were returned to normal sodium intake. Intact rats (average BUN = 14.4 $\pm$ 1.1 mg%; n = 5) did not show an increase in MAP during high sodium intake. PAC was not different between groups and did not change when the sodium intake increased (Figure 8). BUN was significantly higher in the RRM rats compared to intact rats at all time points but did not change significantly withingroups. There was a tendency for BUN to increase in the RRM rats over time (Figure 9). There was a significant positive correlation (r = 0.43) between the mean BUN value and MAP on day 10 of high sodium in RRM rats. In RRM rats WI and UO were both significantly higher throughout the

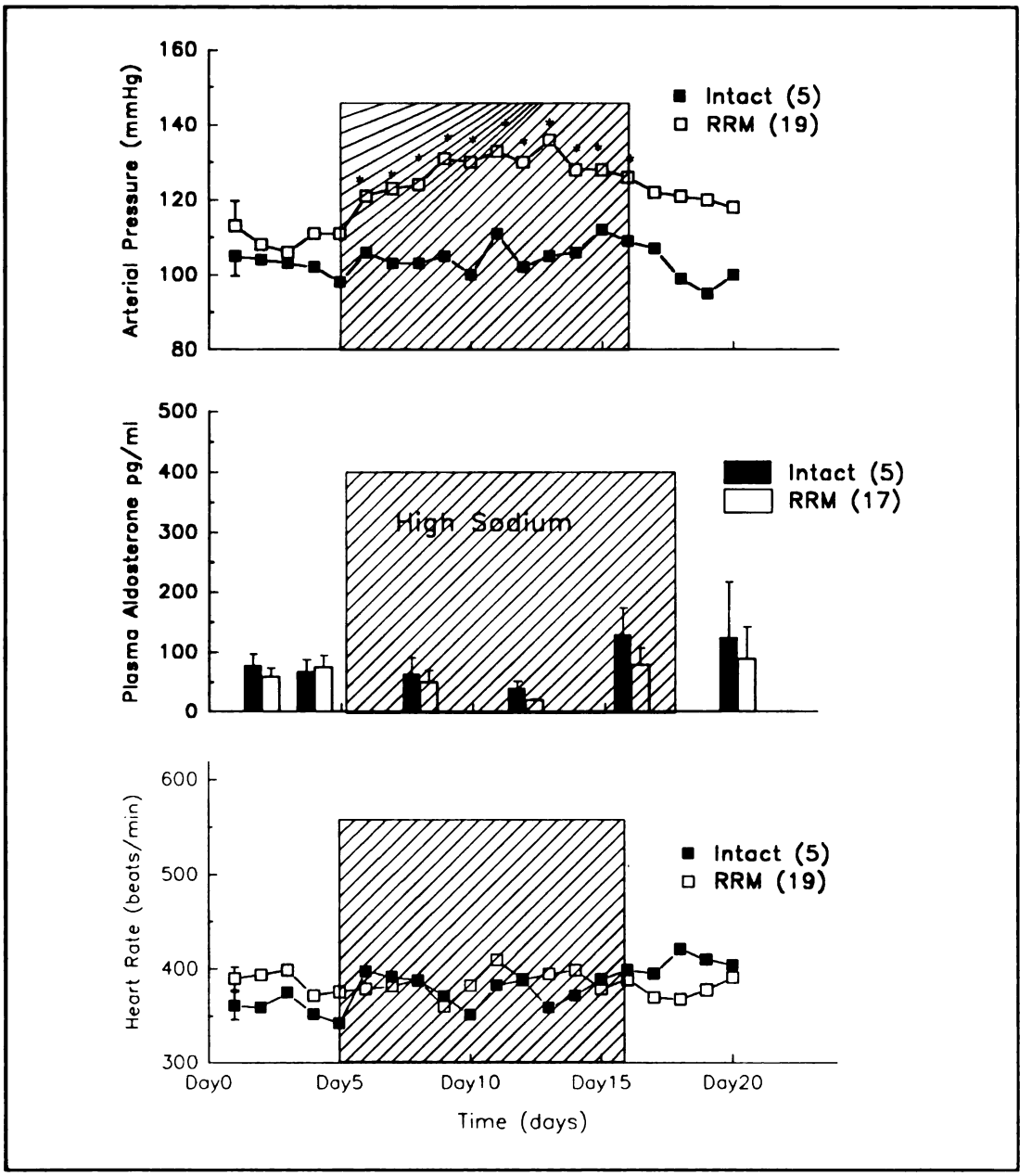

Figure 8: Blood pressure, plasma aldosterone and heart rate in intact and RRM rats during changes in sodium intake. Intact--2 kidney--rats are depicted in the solid circles and bars and while RRM --5/6 nephrectomy rats are depicted in open circles and bars during a normal sodium intake period (2 meq/day) a high sodium intake period (6 meq/day) and another normal sodium intake period. The high sodium period is inside the hatched box. Points represent the mean of 19 (RRM) or 5 (intact) rats. Statistically significant differences between control and infusion periods for $p \leq 0.05$ are marked with *. Bars represent SEM.

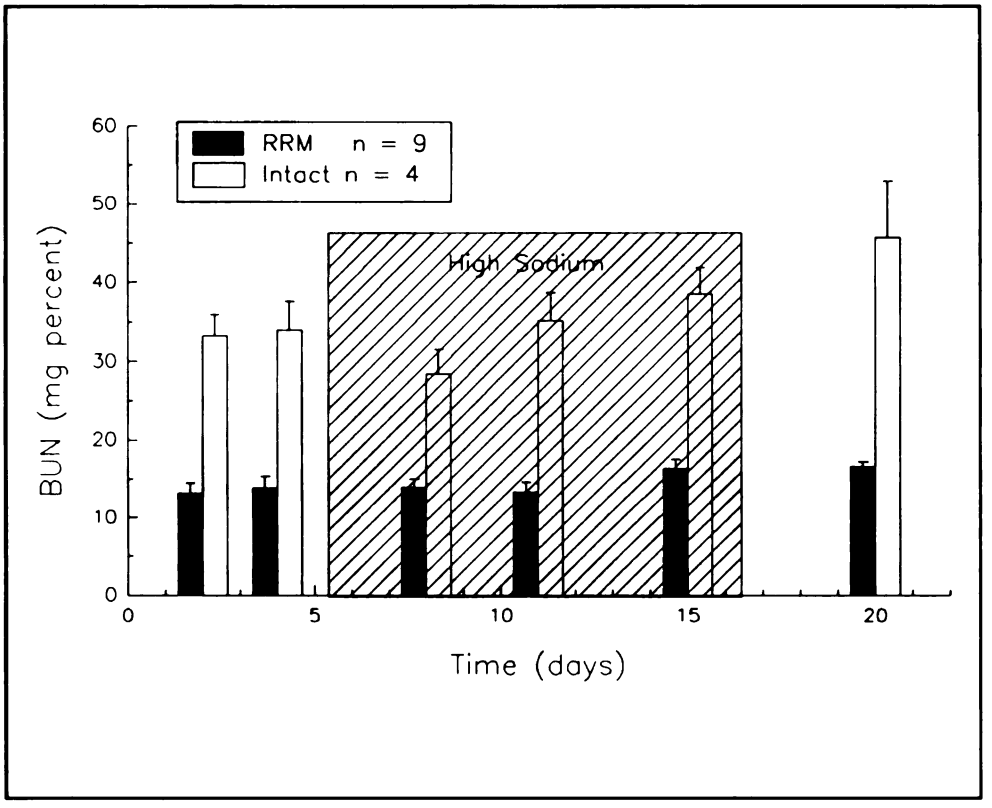

Figure 9: Blood urea nitrogen in intact and RRM rats during changes in sodium intake. Intact rats are depicted by the open bars and RRM rats by the solid bars during a normal sodium intake period (2 meq/day), a high sodium intake period (6 meq/day) and another normal sodium intake period. The high sodium period is inside the hatched box. Points represent the mean of 9 (RRM) or 4 (intact) rats. There were no statistically significant differences between control and infusion periods for $p \leq 0.05$. Bars represent SEM.

protocol but did not change when sodium intake was increased. Water balance, the difference between water intake and urine output, was not different between the two groups and was not altered by the change in sodium intake (Figure 10). UNaV increased in all groups during highsodium intake and, although there was apparent sodium retention in all groups during high sodium intake, the differences were not statistically significant because of the large within groups variance. Potassium excretion was not different between any of the groups (Figure 11). Plasma electrolytes did not change in any of the groups (data not shown).

D. Angiotensin II antagonism in reduced renal mass hypertension

1. Chronic angiotensin II antagonist treatment

Control RRM rats (average initial BUN = 34.7 $\pm$ 3.5 mg%; n = 7) had a significant increase in MAP when changed from normal to high sodium intake. Losartan treated RRM rats (average BUN = 38.4 $\pm$ 2.4 mg%; n = 9) did not show an increase in MAP during high sodium intake. Both treated (average BUN = 15.9 $\pm$ 1.8, n = 7) and control (average BUN = 18.2 $\pm$ 1.8, n = 7) intact rats had no change in MAP. Heart rate did not change in any group over the 12 day experimental period and was not different between groups. These data are shown in Figure 12. There was a significant positive

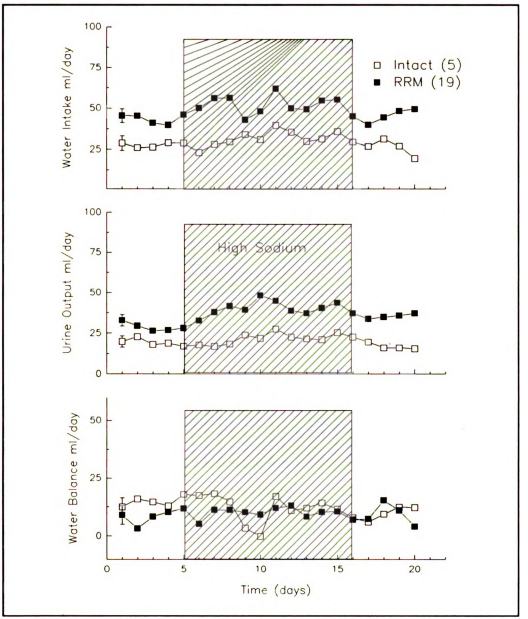

Figure 10: Water intake, urine output and water balance in intact and RRM rats during changes in sodium intake. Intact rats are depicted by the open squares and RRM rats by the solid squares during a normal sodium intake period (2 meq/day), a high sodium intake period (6 meq/day) and another normal sodium intake period. The high sodium period is inside the hatched box. Points represent the mean of 19 (RRM) or 5 (intact) rats. There were no statistically significant within group differences in WI or UO and no statistically significant between group differences in WB. Bars represent SEM.

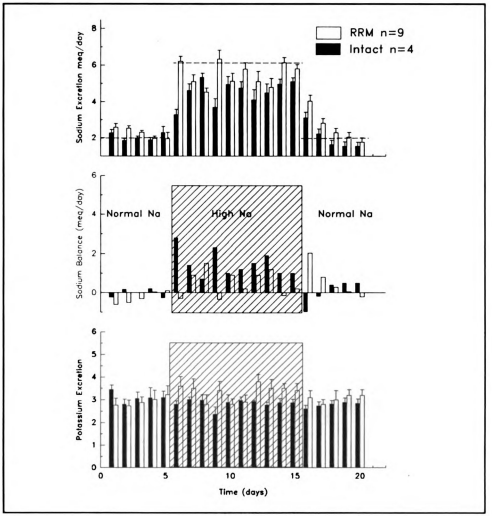

Figure 11: Urinary electrolyte excretion and sodium balance in RRM and intact rats during changes in sodium intake. Daily sodium excretion in the top figure represents the average of 19 RRM rats (open bars) and 5 intact rats (solid bars). The horizontal lines represent the daily sodium intake during normal (2 meq/day) or high (6 meq/day) sodium intake. The middle figure illustrates daily sodium balance, calculated by subtracting urinary sodium excretion from sodium intake, during the same three periods. The hatched box is the high sodium intake period. The bottom figure illustrates the daily urinary potassium excretion in the two groups of rats. There are no error bars on the middle figure because differences were very large. There were no statistically significant differences between groups for any of the variables. Error bars represent SEM.

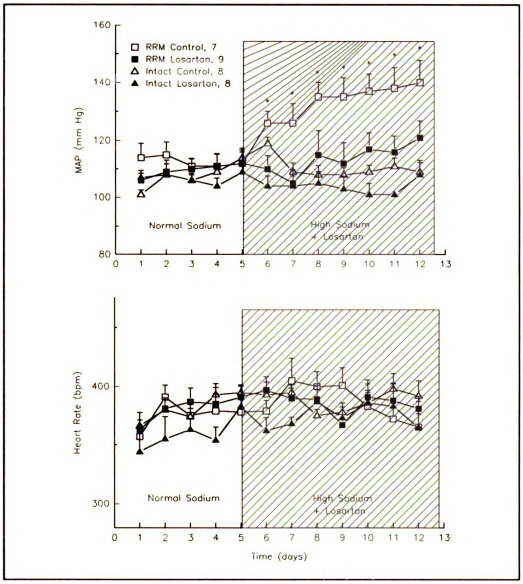

Figure 12. Effect of losartan treatment on blood pressure and heart rate in RRM and intact rats during changes in sodium intake. 4 groups of rats are depicted during normal sodium intake (2 meq/day) and high sodium intake (6 meq/day). The two control groups received no drug treatment. The two losartan groups received 3 mg/kg losartan as an iv bolus once a day following measurement of blood pressure during the high sodium period only. The high sodium (and losartan treatment) period is in the hatched box. Error bars represent SEM. Statistically significant differences within groups for $p \leq 0.05$ are marked with *.

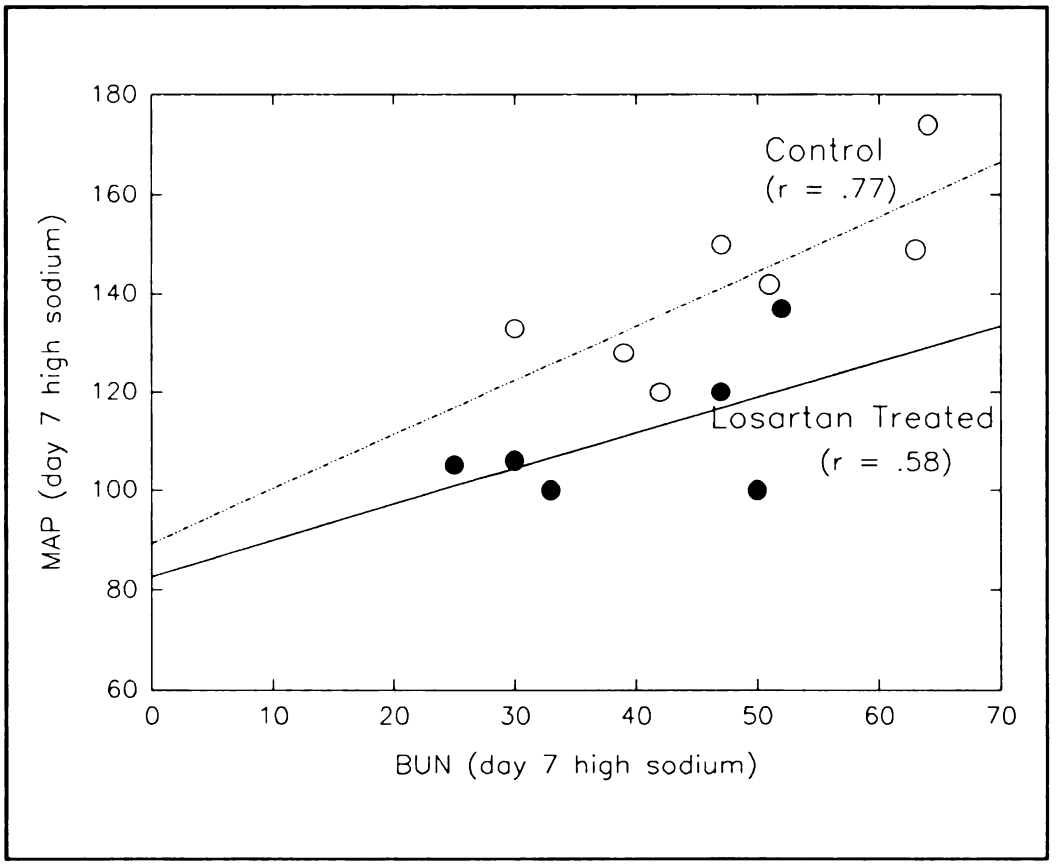

Figure 13. Correlation between blood pressure and BUN in RRM with or without losartan treatment on day 7 of high sodium intake for each of the RRM rats. The lines of regression for the 7 untreated control rats (open circles, broken line) and 6 losartan treated rats (solid circles, solid line) are shown. There is a significant correlation between the two variables in both groups but the lines of regression are different.

correlation (r = 0.77 for control and r = 0.58 for treated) between BUN and MAP on day 7 of high salt in both groups of RRM rats. The scatter plot is shown in Figure 13.

Figure 14 shows water intake and urine output in the 4 groups. The change in sodium intake did not cause a change in water intake or urine output in any of the rats except for the control RRM rats which had an increase in both WI and UO on two of the high sodium days. Water balance (the difference between WI and UO) was unchanged over time in all groups.

Sodium excretion and sodium balance are shown in Figure 15. Urinary sodium excretion was equivalent between groups with sodium retention apparent in all groups during the first 3 days after the increase in sodium intake from 2 to 6 meq/day. There was no difference between groups in the time course or amount of sodium retention. Furthermore, sodium retention did not correlate with MAP. Urinary potassium excretion (data not shown) was not different within or between any of the groups throughout the protocol.

The BUN values of the 4 groups are illustrated in Figure 16. BUN was significantly greater in both RRM groups than in the intact groups during the entire protocol. The initial BUN values were not different between the two intact groups or between the two RRM groups although BUN was

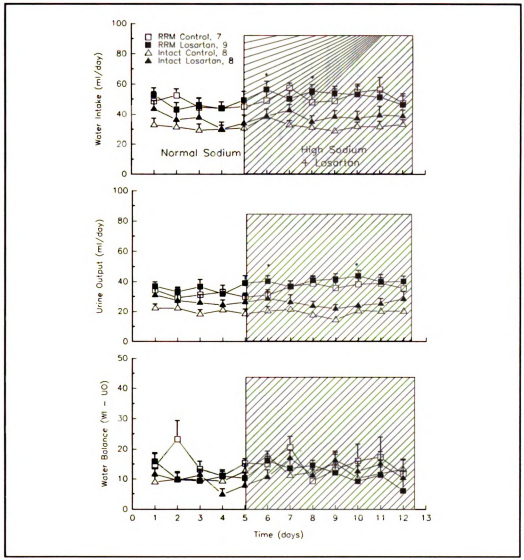

Figure 14: Effect of losartan treatment on water intake, urine output and water balance in RRM and intact rats during different sodium intakes. The two control groups did not receive any drug treatment. The two losartan groups were untreated during the normal sodium period (2 meq/day Na) and received 3 mg/kg/day losartan iv during the high sodium period (6 meq/day) only. Losartan treatment and high sodium intake both started immediately following daily recordings on day 5 of normal sodium, indicated by the shaded area. The losartan bolus was given each day after measurements were made. Error bars represent SEM. Statistically significant differences within group for $p < 0.05$ are marked with a *.

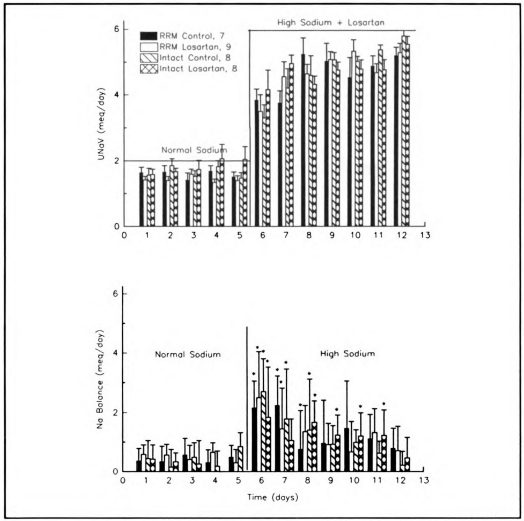

Figure 15: Effect of losartan treatment on urinary sodium excretion and sodium balance in RRM and intact rats during different sodium intakes. The two control groups did not receive any drug treatment. The two losartan groups were untreated during the normal sodium period (2 meq/day Na) and received 3 mg/kg/day losartan iv during the high sodium period (6 meq/day) only. Losartan treatment and high sodium intake (the shaded area) both started immediately following daily recordings on day 5 of normal sodium, indicated by the shaded area. The losartan bolus was given each day following measurement of water intake and urine output. The horizontal lines represent daily water intake. Error bars represent SEM. Statistically significant differences within group for $p < 0.05$ are marked with a *. There were no significant differences between groups.

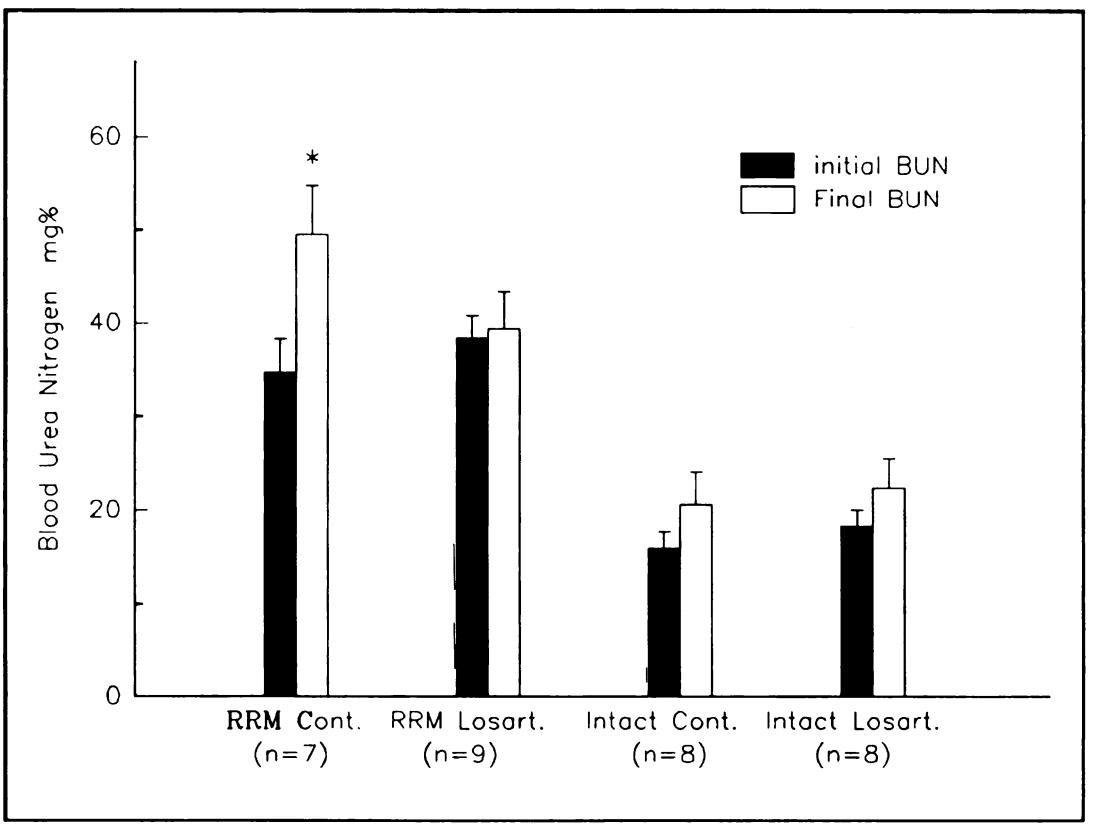

Figure 16: Effect of losartan treatment on blood urea nitrogen in intact and RRM rats during changes in sodium intake. The initial and final BUN in 2 groups of RRM rats and 2 groups of intact rats maintained for 5 days on normal sodium followed by 7 days on high sodium are depicted in the ifgure above. The solid bars represent the mean of each group on the first day of normal sodium intake (2 meq/day Na). The open bars represent the mean of each group on the last day of high sodium intake (6 meq/day). Control groups did not receive any drug treatment. The two losartan groups were untreated during the normal sodium period and received 3 mg/kg/day losartan iv during the high sodium period. Error bars represent SEM. Significant differences within group are marked with a *. Significant differences between intact and RRM groups are marked with +. Differences were considered statistically significant for $p \leq 0.05$.

significantly higher in RRM than intact rats at both time points. Final BUN was significantly elevated above the initial value in control RRM but not in the treated RRM rats. BUN did not change over time in either of the intact groups.

Acute ANG II infusion caused a dose dependent increase in MAP in all rats during the normal sodium period (no antagonist treatment in any group) and in the untreated groups during the high sodium period (Figure 17). Losartan treatment completely prevented a rise in blood pressure to ANG II during the high sodium period. There were no differences between intact and RRM groups on either normal or high sodium intake and the elevated MAP in the untreated RRM rats did not affect the acute response to ANG II.

2. Acute antagonist treatment

Bolus iv injections of 3 mg/kg losartan into RRM rats with a modest sodium-induced hypertension did not significantly lower MAP on the fifth day of high sodium intake. (Figure 18). There was a modest decrease in MAP on the tenth day of high sodium intake but the decrease was only significant at 2 and 6 hours following losartan treatment. Losartan did not alter WI or UO in this group of rats (data not shown).

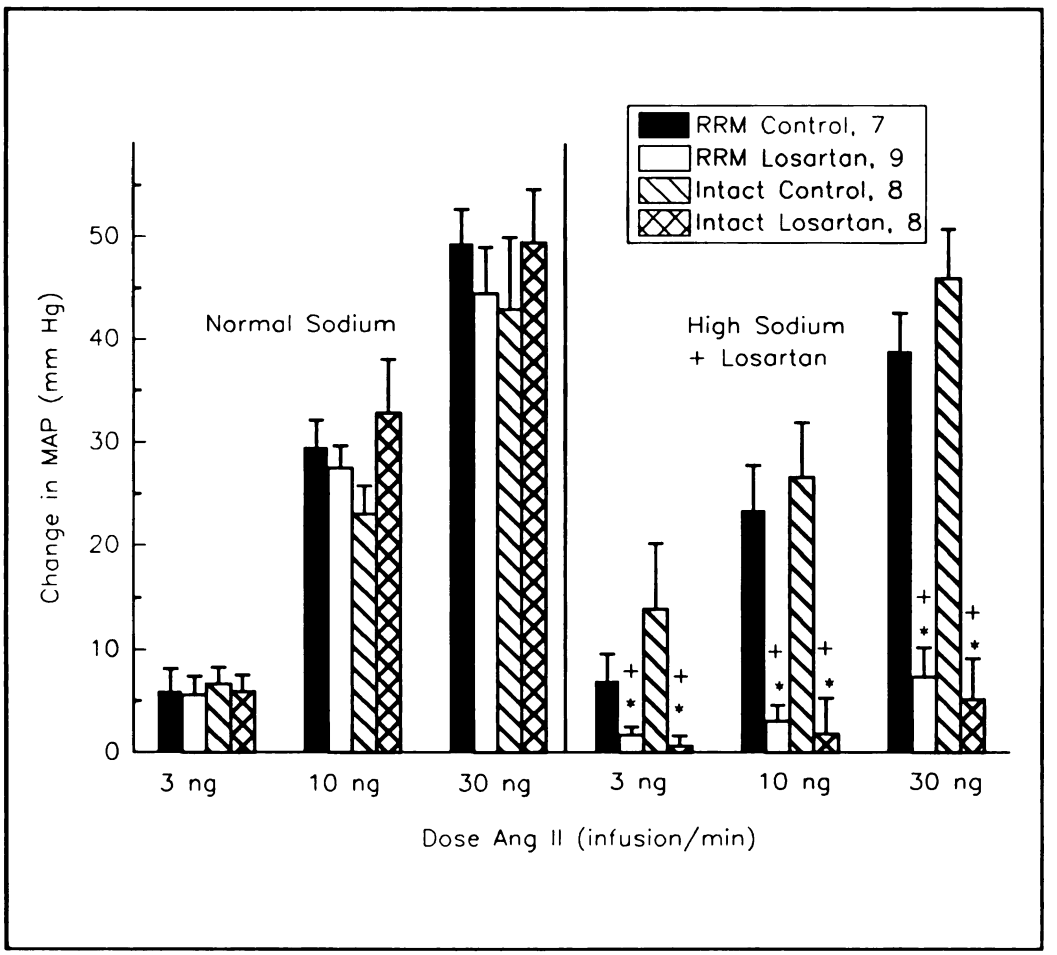

Figure 17: Acute blood pressure response to ANG II infusion in intact and RRM rats with and without losartan blockade. The change in MAP was recorded 5 minute after the start of ANG II infusion at 3, 10 or 30 ng/min iv in each of 4 groups of rats. The bars on the left side of the figure represent the average response of each group while on normal sodium intake (2 meq/day Na). None of the groups were receiving drug treatment during this time. The bars on the right side of the figure represent the average response of each group during high sodium intake (6 meq/day Na). Both losartan groups were receiving a daily iv 3 mg/kg bolus of losartan during high sodium intake. RRM control rats were hypertensive during the high sodium period. Error bars represent SEM. Significant differences within group are marked with a *. Significant differences between losartan and control groups at any time point are marked with +. There were no differences between RRM and intact. Differences were considered statistically significant for $p \leq 0.05$.

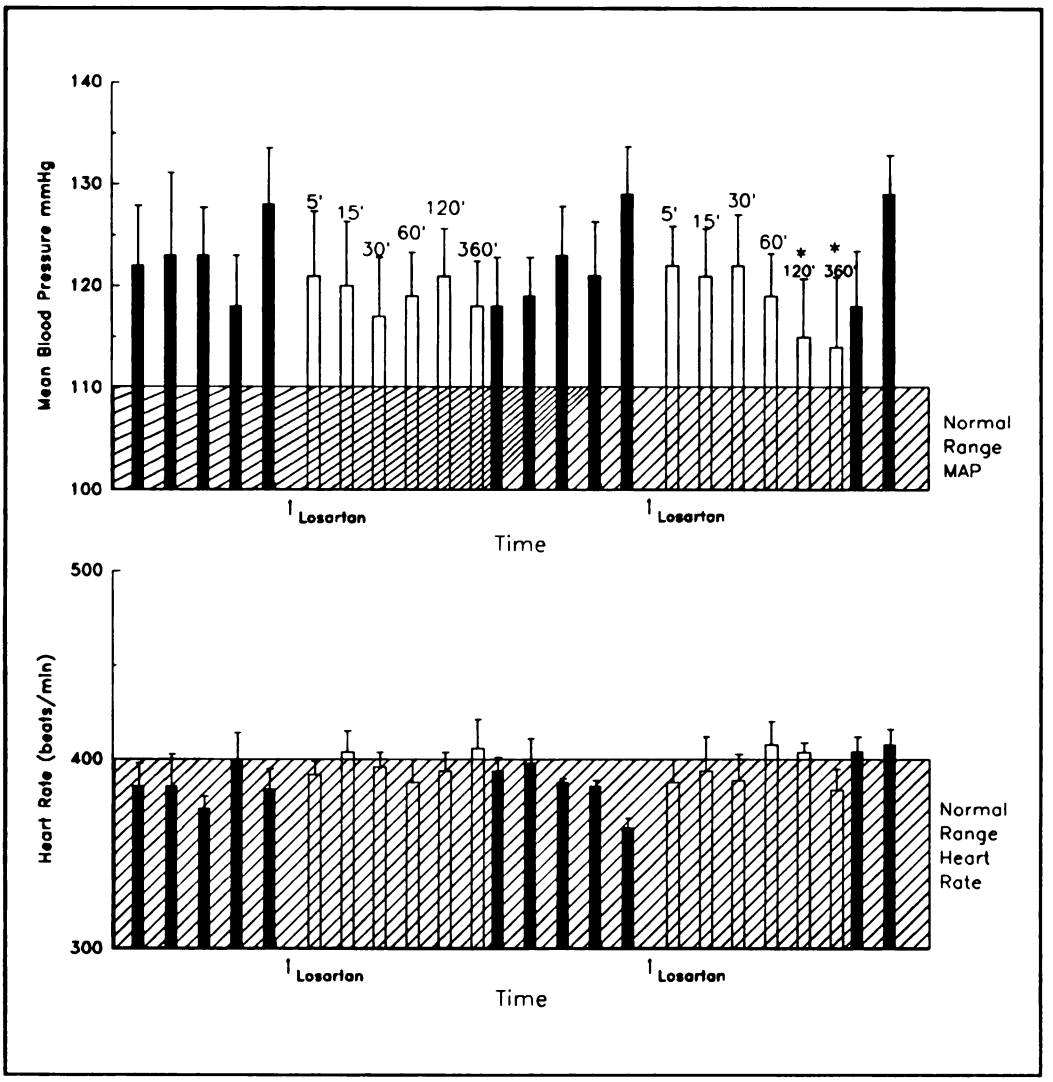

Figure 18: Blood pressure and heart rate response to acute losartan in hypertensive RRM rats on high sodium intake (6 meq/day). MAP before and following an iv bolus of 3 mg/kg losartan is shown. The solid bars are daily measurements. The open bars are measurements following losartan challenge at 5, 15, 30, 60, 120, and 360 minutes. Each bar represents the mean of 7 animals. Error bars are SEM. Losartan was given on day 5 and day 10 of high sodium intake. The only significant differences ($p \leq 0.05$) were at 120 and 360 minutes following the second losartan challenge. MAP was not significantly different from control 24 hours after the second challenge butdid not reach normal levels (the shaded area) at any time. HR did not change throughout and was in the normal range (the shaded area).

In summary, RRM rats switched from normal sodium to high sodium intake developed the expected increase in MAP while RRM rats treated with losartan did not. MAP in intact rats was not affected by the change in sodium intake or by losartan treatment. Furthermore, neither losartan treatment nor renal reduction affected the time required to achieve sodium and water balance following the change in sodium intake. Losartan prevented a sodium-induced increase in BUN in the losartan treated RRM rats. Acute losartan treatment in hypertensive RRM rats did not lower MAP after 5 days on high salt but did cause a small decrease after 10 days of high sodium treatment.

E. Angiotensin II infusion in reduced renal mass rats

Figure 19 shows the daily blood pressure and heart rate in three groups of rats maintained on normal sodium intake: intact rats receiving 10 ng/min ANG II (n = 7), RRM rats receiving 10 ng/min ANG II (n = 27) and control RRM rats (n = 11). Both intact rats and RRM rats receiving ANG II had a significant increase in MAP during the first 4 days of ANG II infusion. However, RRM rats had a larger increase in MAP and their MAP remained elevated throughout the ANG II infusion period. MAP in intact rats returned

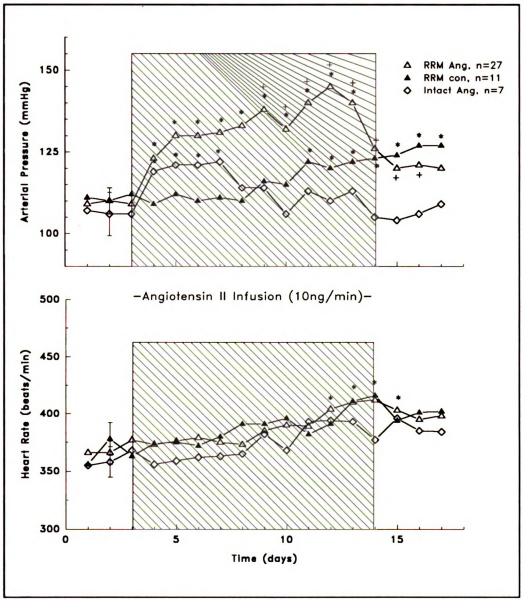

Figure 19: Blood pressure and heart rate during angiotensin II infusion in RRM and intact rats on normal sodium intake. Daily MAP and HR in each of 3 groups of rats: RRM receiving ANG II, intact rats receiving ANG II, and RRM controls. The ANG II infusion period (10 ng/min), indicated by the shaded area, was preceded by 3 control days and followed by 4 recovery days. All rats received a fixed sodium intake of 2 meq/day. Error bars represent SEM. Significant differences within group are marked with a *. Significant differences between RRM and intact at any time point are marked with +. Differences were considered statistically significant for $p \leq 0.05$.

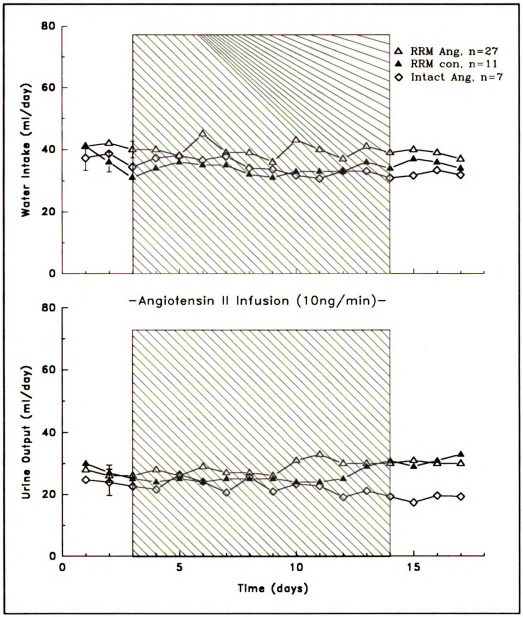

Figure 20: Effect of angiotensin II infusion on water intake and urine output in intact and RRM rats on normal sodium intake. Daily water intake and urine output in each of 3 groups of rats is depicted: RRM receiving ANG II, intact rats receiving ANG II, and RRM controls. The ANG II infusion period (10 ng/min), indicated by the shaded area, was preceded by 3 control days and followed by 4 recovery days. All rats received a fixed sodium intake of 2 meq/day. Error bars represent SEM. There were no differences between groups or within any group.

to control levels by day 5 of ANG II infusion. From day 6 of ANG II infusion through day 3 of recovery, MAP in RRM rats receiving ANG II was significantly higher than in intact rats receiving ANG II. There was a positive correlation ($r = 0.34$) between mean BUN and MAP on day 10 of ANG II infusion in all rats receiving ANG II; however, the correlation was not statistically significant ($p = 0.11$). Control RRM rats exhibited a gradual increase in MAP throughout the protocol. Heart rate tended to increase in all three groups of rats over the 17 day experimental period but was not different between groups.

Figure 20 shows water intake and urine output in the 3 groups of rats. ANG II infusion did not cause a change in water intake or urine output ineither group of rats. Water balance was unchanged over time in all three groups.

Urinary electrolyte excretion is illustrated in Figure 21. Urinary sodium excretion was equivalent in all three groups. Urinary potassium excretion was significantly higher in the intact rats than in the RRM rats throughout the protocol.

Plasma immunoreactive ANG II concentrations ($[ANG\ II]_{ir}$) in the three groups are depicted in Figure 22. All three groups had similar $[ANG\ II]_{ir}$ during the control period, although the level in the control RRM group

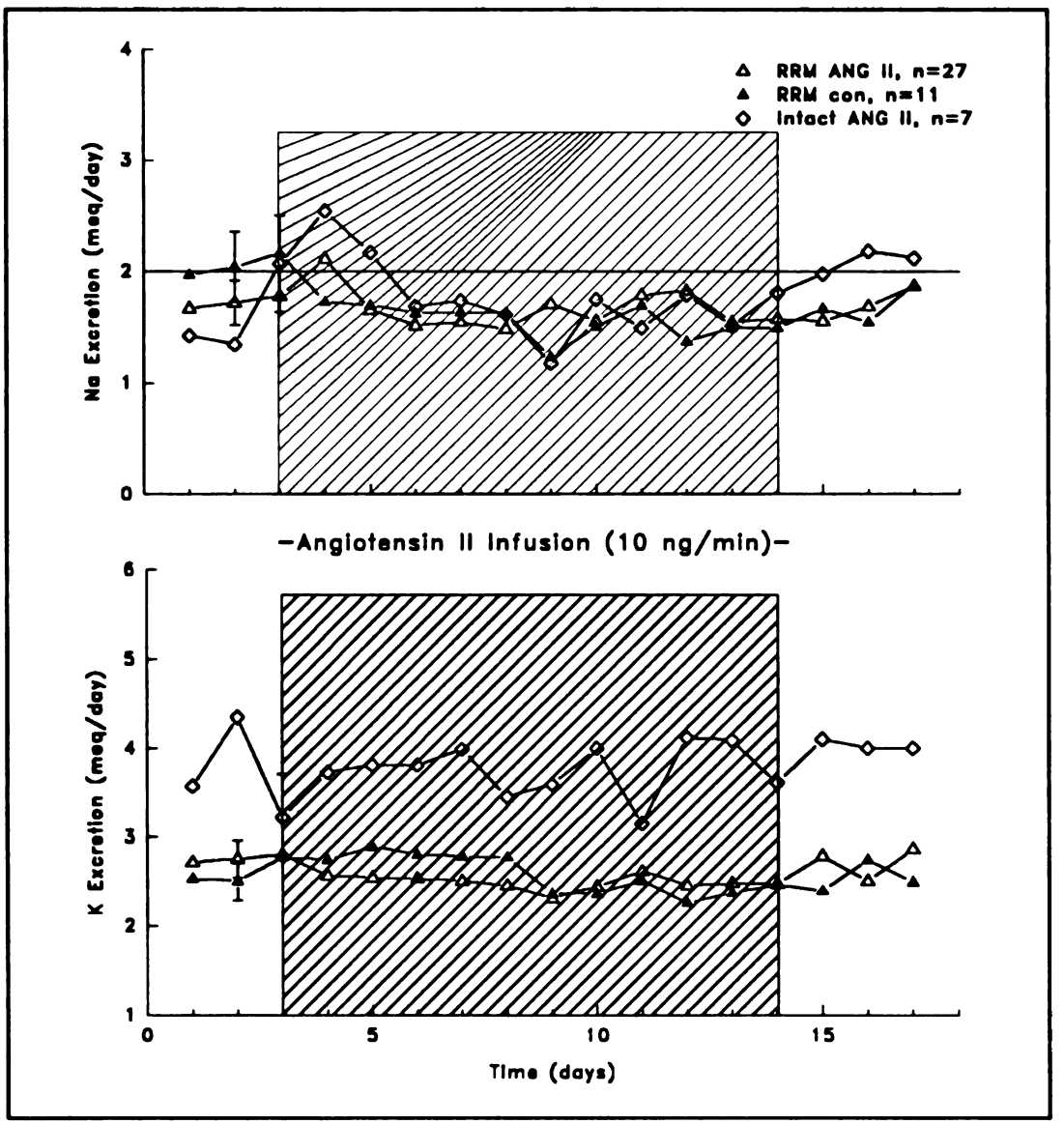

Figure 21: Effect of angiotensin II infusion of urinary electrolyte excretion in RRM and intact rats on normal sodium intake. Daily sodium and potassium excretion in each of 3 groups of rats is depicted: RRM receiving ANG II, intact rats receiving ANG II, and RRM controls. The ANG II infusion period (10 ng/min) is indicated by the shaded area, preceded by 3 control days and 4 recovery days. All rats received a fixed sodium intake of 2 meq/day. Error bars represent SEM. There were no differences in sodium intake either within groups or between groups. Potassium excretion in intact rats was significantly higher than either of the two RRM groups through the protocol but there was no difference between the 2 RRM groups. Differences were considered statistically significant for $p \leq 0.05$.

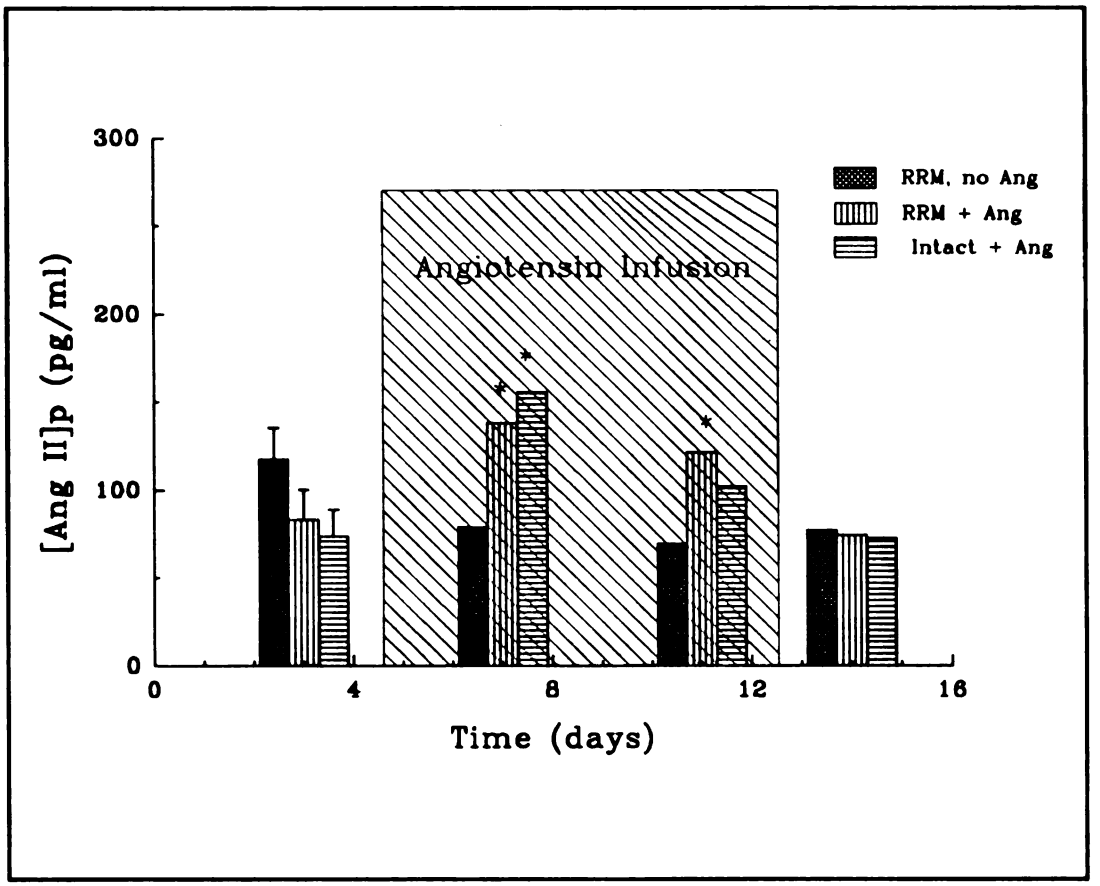

Figure 22: Plasma levels of immunoreactive angiotensin II in RRM and intact rats receiving angiotensin II infusion. Plasma levels of ANG II in each of 3 groups of rats is depicted: intact receiving ANG II, RRM receiving ANG II and RRM control. The 10 day ANG II infusion period (10 ng/min) is indicated by the shaded area, preceded by 3 control days and 4 recovery days. Plasma samples were taken on day 3 control, days 5 and 10 of ANG II infusion and on day 4 of recovery. All rats received a fixed sodium intake of 2 meq/day. Error bars represent SEM. Significant differences within group are marked with a *. Significant differences between RRM and intact at any time point are marked with +. Differences were considered statistically significant for $p \leq 0.05$.

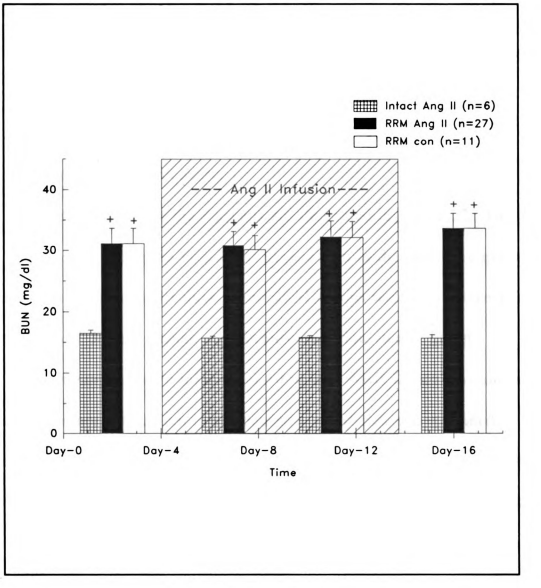

Figure 23: Effect of angiotensin II infusion on BUN in RRM and intact rats. BUN in each of 3 groups of rats is depicted: intact receiving ANG II, RRM receiving ANG II and RRM control. The 10 day ANG II infusion period (10 ng/min) is indicated by the shaded area, preceded by 3 control days and 4 recovery days. Plasma samples were taken on day 3 control, days 5 and 10 of ANG II infusion and on day 4 of recovery. All rats received a fixed sodium intake of 2 meq/day. Error bars represent SEM. Significant differences between RRM and intact at any time point are marked with +. There were no significant differences over time within any of the groups Differences were considered statistically significant for $p \leq 0.05$.

slightly but significantly higher than that of intact rats. ANG II infusion produced similar increases in $[ANG\ II]_{ir}$ in intact and RRM rats. In control RRM rats, $[ANG\ II]_{ir}$ decreased somewhat over the course of the protocol but the changes were not statistically significant and the three groups had equivalent levels during recovery.

The BUN values of the 3 groups are illustrated in Figure 23. BUN was significantly greater in both RRM groups than in the intact group during the entire protocol. BUN values in the RRM groups were not different from each other and did not change significantly over time in any group.

In summary, chronic infusion of 10 ng/min ANG II into normotensive RRM rats on normal sodium intake caused a significant and sustained increase in MAP compared to either intact rats receiving the same dose ANG II or with control RRM rats (no ANG II). Infusion of ANG II into intact rats on normal sodium intake caused an initial increasein MAP but MAP returned to control levels during the last 6 days of the 10 day infusion while MAP remained elevated throughout the infusion in RRM rats. The hypertension in the RRM rats was not accompanied by changes in water or sodium balance and MAP returned to control levels during the recovery period.

IV. Discussion

In the face of different causes, the basic hemodynamic abnormality underlying the development and maintenance of hypertension is, almost without exception, increased peripheral vascular resistance. Two major factors lead to increased vascular resistance--elevated activity of the sympathetic nervous system and altered levels of vasoactive hormones. These two systems interact with each other so that vascular resistance is the product of the combined actions of circulating hormones and sympathetic outflow.

Both clinical and experimental cases of SDH are indeed characterized by elevated TPR. The cause of the increased vasoconstriction is not clearly defined but there are indications that it is due to increases in one or more transferable circulating factors (Posten *et al.* 1981, Nishio *et al.*1988) and increased activity of the sympathetic nervous system (Mark *et al.* 1975, Gill *et al.*1988). Clinical studies of sodium-sensitivity have demonstrated alterations in the activity of the renin-angiotensin system (Weinberger 1991, Sullivan 1991), plasma levels of aldosterone (Weinberger 1991), and plasma levels of an endogenous ouabain-like factor (Nishio *et al.* 1988). These same changes are also seen in animal models of sodium-induced hypertension (Nakagawa *et al.* 1990, Wauquier *et al.* 1986). The focus of this dissertation

is on the changes in humoral factors that occur during the initiation of SDH and how those changes influence resting arterial pressure.

In a review article on the role of the kidney and salt in the aetiology of hypertension, deWardener proposed a theory to explain how alterations in sodium intake could initiate hypertension. The theory proposes that the basic alteration is a renal defect restricting the excretion of sodium. This inability to excrete a sodium load leads to sodium retention during increased sodium intake. Water follows sodium and the animal becomes volume expanded. This triggers so-called "volume controlled mechanisms" in an attempt to re-establish sodium and water balance. The volume controlled mechanisms include elevated sympathetic output, decreased secretion of antinatriuretic hormones such as aldosterone, angiotensin II and vasopressin, and increased secretion of natriuretic factors such as atrial natriuretic peptide and the endogenous Na^+/K^+-ATPase inhibitor. It is the sustained activity of the volume controlled mechanisms that maintain hypertension (deWardener 1990 Parts I & II).

It is difficult to distinguish what the initiating factor is in SDH. In clinical cases, hypertension is in the established phase when it is diagnosed and the observed hormone imbalances (Sullivan 1991) and renal damage (Kincaid-Smith 1991) could be either a cause or a consequence of the

hypertension. In experimental genetic models, alterations in renal function may be extraneous to the genetic alterations that caused the hypertension. The alteration which precipitates the rise in blood pressure can only be defined with certainty in models of hypertension where the initiating event is controlled and defined by the investigator. A useful model for studying SDH with such control is the reduced renal mass(RRM)-salt model of hypertension. In this model there is an obvious decrease in the total capacity of the kidney to excrete excess sodium and water but blood pressure does not rise until sodium intake is elevated. As predicted by deWardener's theory, the initiation of sodium-induced hypertension is associated with a transient sodium and water retention leading to a temporary elevation of CO. After the initial increase in CO, sodium and water balance are re-established and the hypertension is maintained by chronically elevated TPR (Lombard *et al.* 1989). The elevated peripheral resistance is accompanied by increased sympathetic activity, elevated aldosterone and OLF, and altered activity of ANG II. Our studies addressed possible contributions of these circulating hormones in this and other sodium dependent models of hypertension.

A. Plasma aldosterone during angiotensin II infusion

As addressed in the Introduction, rats with established RRM-salt

hypertension have alterations in both the renin-angiotensin system and in PAC (Ylitalo and Gross 1979, Chi and Foreman 1986, Jackson *et al.* 1988). Furthermore, elevations in circulating ANG II affect the secretion rate of aldosterone so that a chronic increase in PAC could be attributed to an increase in circulating ANG II. Our first study examined the possibility that increases in circulating ANG II may increase PAC and that an increase in PAC might contribute to an apparent ANG II induced hypertension. In this study PAC was measured in intact rats receiving chronic ANG II infusion.

The question posed by this study was: What role does aldosterone play in ang-II induced hypertension? A contribution of aldosterone to ANG II-induced hypertension was proposed based on the following observations: 1) acute ANG II infusion in rats caused an increase in plasma levels of aldosterone, 2) elevated PAC in rats lead to sustained hypertension and 3) chronic exposure of rat adrenals to elevated plasma ANG II caused an increase in the number of adrenal ANG II receptors and an accompanying increase in plasma levels of aldosterone.

The ability of ANG II to increase the secretion rate and plasma level of aldosterone in rats has been previously demonstrated by several investigators. For example, a study with rats in 1978 by Aguilera and Catt reported increased plasma ANG II levels and aldosterone secretion in response to

sodium deprivation. The increase in both hormones was prevented by the concomitant administration of angiotensin converting enzyme (ACE) inhibitors (Aguilera and Catt 1978). Since both ANG II and PAC were decreased by the ACE inhibitors, the elevated PAC was apparently caused by elevated ANG II levels. Hauger *et al.* in 1978 found that 50 ng/min ANG II, given to rats on uncontrolled, normal sodium intake produced a sustained elevation in PAC and a significant increase in adrenal ANG II receptor number (Hauger *et al.* 1978). A more recent study by Luft *et al.* measured urinary aldosterone excretion in rats given ANG II (76 ng/min sc) for ten days. Their study found a sustained elevation in urinary aldosterone excretion with a seven-fold elevation in the daily excretion level by day seven (Luft *et al.* 1989).

Table 1 illustrates the increase in PAC we observed following a one hour infusion of 10, 30, and 60 ng/min ANG II. These data support the earlier findings in rats (Hauger *et al.* 1978, Luft *et al.* 1989) and in humans (Oelkers and Schöneshöfer 1974) that ANG II is a potent secretagogue of aldosterone and that a one hour infusion of even a low dose of ANG II produces an increase in PAC.

The second premise of the current study was that elevated PAC alone in intact rats leads to sustained hypertension. Our data (Figure 1) illustrate

that this is a slowly developing hypertension. The animals with sufficiently elevated PACs became hypertensive only after ten days of hormone administration. Mineralocorticoid hypertension can be produced more rapidly by subjecting uninephrectomized rats to a much higher sodium intake (Garwitz and Jones 1982). However, the current study shows that increased PAC in combination with moderate increases in sodium intake also produces hypertension in intact rats. This is consistent with findings in other animals, including primary aldosteronism in humans (Conn and Louis 1955, Darke *et al.* 1977, Pan and Young 1982).

The third observation in support of our hypothesis was first made by Aguilera, *et al.* (Catt *et al.* 1987, Aguilera and Catt 1978). This group reported an increased number of adrenal ANG II receptors and increased aldosterone secretion rates after chronic exposure of isolated adrenals to ANG II. This observation implies that chronically elevated plasma ANG II may lead to progressively higher PAC. This is also suggested by the progressive increase in urinary aldosterone excretion seen in Luft's study (Luft *et al.* 1989). The current study directly examined the relationship between systemic ANG II, PAC and blood pressure during a persistent elevation in plasma ANG II levels produced by iv hormone infusion.

The first chronic ANG II experiment investigated ANG II's ability to

increase PAC and MAP in rats on a moderately high sodium intake (6 meq/day). Both ANG II and aldosterone induce hypertension more rapidly in animals on high sodium intakes (Hall *et al.* 1984, Fink *et al.* 1987, Young *et al.* 1982). We postulated that if aldosterone contributes to the development of ANG II hypertension, increases in PAC of at least five times the control levels should precede the increase in arterial pressure during ANG II administration and that a time course of sixteen days should be sufficient for both the hypertension and the elevation in PAC to be expressed.

Chronic ANG II infusion appeared to cause a slight increase in PAC that lagged behind the increase in arterial pressure but there was no indication of a progressive increase in PAC over time. Also, at no time during ANG II infusion was the PAC significantly higher than control levels, although rapid and substantial increases in MAP occurred. During the ANG II infusion, PAC remained lower than that found necessary to cause a sustained increase in MAP with chronic aldosterone infusion (i.e. 250 - 300 pg/ml) (Figure 2). Hauger's study in rats (Hauger *et al.* 1978) found the increase in PAC to be maintained for at least six days during chronic infusion of 50 ng/min ANG II, and Luft's study in rats (Luft *et al.* 1989) found the elevation in urinary aldosterone to be maintained for nine days with 76 ng/min ANG II. This implies that the high infusion rates used in their study maintain adrenal

stimulation over a period of days. In the current study, the dose of ANG II was 10 ng/min, a dose found previously to cause an acute increase in PAC (Table 1) and a sustained elevation in MAP (Pawloski 1990). The differences in the doses and the resultant aldosterone responses suggest that high doses of ANG II can cause sustained adrenal stimulation, but that a lower dose of ANG II, although acutely stimulatory to the rat adrenal gland, does not produce a significant chronic elevation in PAC. It is evident, in any case, that PAC is not sufficiently elevated in rats receiving 10 ng/min ANG II to contribute to the observed hypertension.

The lack of change in urinary and plasma electrolytes illustrated in Figure 3 supports previous findings that ANG II hypertension in rats is not associated with sodium and water retention, or expanded fluid volume (Fink *et al.* 1987, Brown *et al.* 1979). This lack of change further strengthens the conclusion that aldosterone did not contribute to the development of hypertension in these animals, since aldosterone-induced hypertension is accompanied by polydipsia, hypokalemia and transient sodium retention (Conn and Louis 1955, Darke *et al.* 1979, Pan and Young 1982). No changes in plasma levels of either sodium or potassium occurred, (Figure 4) revealing neither hypernatremia nor hypokalemia occurred during the ANG II infusion.

The second chronic ANG II infusion experiment was performed to investigate the possibility that chronic ANG II stimulation of the adrenal in rats on a normal sodium intake would lead to a progressive increase in PAC, and ultimately an aldosterone-dependent hypertension. This reasoning was based on the known ability of high sodium intake to suppress both PAC and the aldosterone response to ANG II (Holtzman *et al.* 1989).

In animals on normal sodium intake, PAC did not increase significantly or progressively with time of infusion as expected. These data and the blood pressure data are depicted in figure 5 and reveal that administration of ANG II at 10 ng/min in rats on a normal sodium intake did not cause a sustained rise in MAP. The results of these experiments illustrate three points about the relationship between sodium intake, ANG II, PAC and arterial pressure regulation in rats. First, it is apparent that the hypertension produced by low dose ANG II infusion in intact rats is dependent on the level of sodium intake. Although others have reported hypertension development during ANG II infusion in rats on a normal (1 - 2 mEq) daily sodium intake (see Young *et al.* 1982 for references), generally much higher doses of ANG II were employed than used here. Experiments in dogs also support a dose-related dependence of ANG II-induced hypertension on the level of sodium intake (Cowley and McCaa 1976). ANG II infusion in rats, unlike in dogs,

does not cause measurable sodium or water retention as measured in this study and in previous studies (Textor *et al.* 1981).

Second, it is clear that increased PAC is not required for hypertension development during chronic ANG II infusion in rats on increased sodium intake. This finding supports conclusions drawn from earlier experiments in dogs (Cowley and McCaa 1976). Studies involving exogenous aldosterone infusion in rats on increased sodium intake (6 mEq/day) showed that a PAC of 250 - 300 pg/ml was required to increase blood pressure significantly. Yet PAC never achieved this range in rats receiving ANG II on a chronic basis. However, this result does not rule out the possibility that a low blood concentration of aldosterone is necessary to support ANG II-induced hypertension. Adrenal ablation experiments would be necessary to test this point. A synergistic effect of ANG II and aldosterone also is a possibility, but studies conducted in dogs do not support this idea (Cowley *et al.* 1986).

The third point raised by these results is the seeming inability of circulating ANG II to chronically affect aldosterone secretion by the adrenal. The role of ANG II as a chronic modulator of aldosterone secretion in sodium-depleted animals is well established (Aguilera and Catt 1978, Holtzman *et al.* 1989). Sodium depletion also enhances the adrenal response to changes in circulating ANG II concentrations (Cowley and McCaa 1976).

The ability of ANG II to stimulate aldosterone secretion in a potent and prolonged fashion in sodium-depleted animals may be due in part to removal of the inhibitory effect of ANP on ANG II-induced aldosterone release (Chartier *et al.* 1984, Naruse *et al.* 1987. The chronic influence of circulating ANG II on PAC in animals on normal or high sodium intake is more controversial. A number of previous studies in rats (Kömer and Mûller 1979, Marieb and Mulrow 1965), sheep (Blair-West *et al.* 1963) and dogs (Cowley and McCaa 1976) indicated that prolonged infusion of ANG II in animals on normal or high sodium intakes produced only a transient (hours) increase in PAC. Other investigators, however, have reported a sustained elevation in PAC during ANG II infusion in rats (Hauger *et al.* 1978), dogs (Hall and Hungerford 1982), and humans (Oelkers and Schöneshöfer 1983). The reasons for this disparity of results is not clear, but could be related to differences in the dose of ANG II employed, dietary potassium content, or other factors which also influence aldosterone secretion (Quinn and Williams 1988). In the current study, a dose of ANG II was employed which is known to cause hypertension in rats on a 6 meq daily intake of sodium (Fink *et al.* 1987), since ANG II-induced hypertension was the central focus of the work. It is possible that this relatively low dose of ANG II caused chronic increments in PAC below our ability to detect (50 - 100 pg/ml), and that

higher amounts of ANG II would have brought about a measurable and maintained increase in PAC. It should be pointed out, however, that the rate of ANG II administration used here has been shown to increase plasma ANG II concentration to a level equivalent to that observed in benign, two-kidney renovascular hypertension (Mann *et al.* 1980, Brown *et al.* 1981). Thus, the concentrations of ANG II produced in these experiments were presumably within a relevant pathophysiological range.

In summary, long-term intravenous infusion of 10 ng/min ANG II causes sustained hypertension in rats on a 6 meq/day sodium intake, but not in rats on a 2 meq/day sodium intake. In rats on the higher sodium intake, raising PAC to 250 - 300 pg/ml will also cause a slowly-developing hypertension. Administration of ANG II at 10 ng/min does not, however, cause PAC to increase to this level under either of the sodium intake conditions tested. We conclude that, in intact rats, increased PAC is not necessary for chronic ANG II-induced hypertension in rats.

B. Plasma aldosterone in reduced renal mass hypertension

The next experiment addressed the possibility that elevations in PAC play a role in RRM hypertension independent of ANG II. The rationale for the investigation was based on the following two points. First, as established

in the introduction, it has been reported that RRM rats have elevated plasma levels of aldosterone (Chi *et al.* 1986). Second, the previous experiment demonstrated that chronic elevation of PAC in combination with a sodium intake of 6 meq/day will cause hypertension in intact rats. If PAC is sufficiently elevated during RRM hypertension, it would be expected to contribute to the elevated blood pressure.

In the previous reports of elevated aldosterone, the measurements of PAC were made after weeks or months of hypertension (Chi and Foreman 1979). In this stage of established RRM-salt hypertension, plasma levels of potassium are increased. Plasma potassium increases because of the impaired ability of the remnant kidney to secrete potassium. Measurements of plasma potassium in RRM rats reveal elevations that are directly proportional to the degree of renal impairment. This is expected because renal secretion is the primary route of potassium clearance during steady-state conditions (reviewed in Mitch 1982). The increase in plasma potassium of RRM rats presumably stimulates aldosterone secretion since plasma potassium is a potent secretagogue of the mineralocorticoid (Brown *et al.* 1979). Therefore the elevated PAC could either cause the hypertension, if it increased before the blood pressure rose, or it could be a result of the hypertension if it occurs only during the later stages of hypertension.

Salt-induced hypertension in RRM rats is a progressive condition with three stages. When animals are initially exposed to high sodium intake, there is a rapid rise in blood pressure and a transient sodium retention. Within hours, blood pressure stabilizes and remains relatively stable with only a slight increase over the next few weeks to months (Lombard *et al.* 1989). The duration of the stable period is dependent on sodium intake and on the original amount of renal tissue destroyed. Even during this plateau phase, progressive renal deterioration continues (Meyer and Rennke 1988). Eventually, enough nephrons are destroyed so that the remaining nephrons are unable to maintain renal function. The animal becomes uremic and goes into end-stage renal failure. This is characterized by a rapid rise in blood pressure to very high levels followed by a fall in blood pressure to hypotensive levels just before there is total renal and cardiovascular collapse (Ylitalo and Gross 1979, Kaufman *et al.* 1974).

It is expected that the mechanisms responsible for maintaining blood pressure are different during the different stages of the hypertension. It is the mechanisms responsible for the stable phase in pressure that this study addresses and which are most relevant to the general question of what maintains sodium-induced hypertension.

In the study reported here, the sodium induced rise in MAP was not

associated with changes in PAC (Figure 7). The failure of PAC to increase during the development of the hypertension does not support our hypothesis that elevated PAC contributes to the early stages of hypertension but suggests that elevations in PAC reported previously in RRM rats were due to potassium stimulated secretion after the onset of hypertension. In those animals, it is assumed that chronic renal insufficiency decreased potassium excretion and lead to elevated plasma levels of potassium which stimulated aldosterone release (Brown *et al.* 1979). Plasma levels of potassium were not reported in those previous studies so it is impossible to determine exactly why differences were observed. In the current study, plasma electrolytes were not altered during the alterations in sodium intake. Therefore if potassium stimulation was responsible for the eventual elevation in PAC, elevated PAC would not be expected in these animals. Additionally, there was no apparent deterioration of renal function in the RRM rats in this study as measured by blood urea nitrogen (BUN), which did not increase during the twenty days of the protocol as shown in figure 8. This is indicative that these rats did not progress from the stable phase to end-stage renal failure.

During the initial phase of RRM hypertension, CO is not elevated beyond the first 24 hours of sodium excess. Rather, elevations in TPR cause the increased arterial pressure (Lombard *et al.* 1989). This also supports our

conclusion that aldosterone excess is not responsible for the initial rise in blood pressure in RRM rats, since mineralocorticoid excess is initially associated with sodium and water retention, and increased CO. Furthermore, although the stimulus for the elevated peripheral resistance is not apparent from this study, it does not appear to be dependent on elevated PAC.

C. Endogenous angiotensin II in reduced renal mass hypertension

1. Chronic losartan in RRM rats

As outlined in the Introduction, RRM-salt hypertension in rats can be prevented with CEI (Jackson and Johnston 1988, Meyer *et al.* 1985). Furthermore, it has been shown that CEI are uniquely able to prevent both the hypertension and the deterioration of renal function when compared to other antihypertensive treatments (Tolins and Raij 1990, Brunner *et al.* 1989). In addition, both ANG II-induced hypertension and RRM-salt hypertension are due to increased vasoconstriction. Therefore it is possible that ANG II is responsible for the initial salt-induced rise in blood pressure in RRM rats.

Neither ANG II nor PRA are elevated in RRM animals (Rosenberg *et al.* 1991, Jackson and Johnston 1988. Chi *et al.* 1986). However, there is evidence that chronic angiotensin-dependent hypertension does not require large elevations in plasma levels of the peptide. Brown *et al.* infused ANG II

at the rate of 20 ng/kg/min into rats for seven days. They found that plasma levels were barely elevated at this infusion rate (Brown *et al.* 1981). Blood pressure was not elevated acutely by this dose but rose gradually throughout the week of infusion. Furthermore, plasma levels of ANG II did not change during the infusion so that at the time of maximal blood pressure response, plasma ANG II levels were equal to acutely subpressor levels. Other reports of ANG II-induced hypertension report the same slow pressor response that does not require a large change in plasma levels of the peptide (Cox and Bishop 1991, Pawloski 1990). In addition, CEI and losartan are both able to reverse hypertension in SHR rats, a model that also does not have increased plasma levels of ANG II (Bunkenburg *et al.* 1991, Okunishi *et al.* 1991, Li and Jackson 1987). Additionally, SHR rats appear to be hyper-responsive to the slow-presser effect of ANG II (Li and Jackson 1987) so that CEI apparently protect SHR through an ANG II-dependent mechanism in spite of normal PRA. CEI may act through an analogous ANG II mechanism in RRM rats even though plasma levels of the peptide are not elevated.

Alternatively, it has been shown that CEI affect several cardiovascular control systems in addition to their effects on ANG II formation. CEI treatment increases plasma levels of kinins (Fenoy, *et al.* 1991), increases the synthesis of vasodilatory prostaglandins (Zucker *et al.* 1991) and causes the

release of an endothelium dependent vasodilator (Gilst *et al.* 1988). It is therefore possible that the antihypertensive affect of CEI in RRM rats is independent of alterations in circulating ANG II.

There is some evidence that CEI lower blood pressure in RRM rats by increasing kinin levels (Karlstrom *et al.* 1990). It has also been suggested CEI protection in RRM hypertension is due to inhibition of a local ANG II system rather than effects on circulating levels of the peptide (Jackson and Johnston 1988, Kuczera *et al.* 1991). Therefore the exact mechanism of CEI protection in this model is not clear and this study tested the hypothesis that ANG II is necessary for the initiation of RRM hypertension. This was done by administering an ANG II specific antagonist, losartan, concurrent with high sodium intake.

Losartan is a recently developed antagonist specific for the ANG II AT_1 receptor. The AT_1 and AT_2 receptors are the two known ANG II receptor subtypes with the AT_1 subtype apparently mediating all the cardiovascular (reviewed Timmermans *et al.* 1991), adrenal (Hajnoczky *et al.* 1992) and renal (Edwards *et al.* 1991) actions of ANG II. At concentrations up to 10^{-5} M, losartan does not affect the responses to potassium chloride, norepinephrine, isoproterenol, AVP, bradykinin, acetylcholine, histamine or serotonin (Wong *et al.* 1990a, Wong *et al.*1990b). Also unlike the ANG II

partial agonist saralasin, losartan is a pure antagonist lowering blood pressure in renal hypertension (Wong *et al.* 1991, Bunkenburg *et al.* 1991) with a binding affinity of 1.9 X 10^{-8} M and a pA_2 of 8.48. At the dose of 3 mg/kg iv, losartan lowers blood pressure in SHR, renal hypertensive rats (Wong *et al.* 1990a), and ANG II infused rats (Smits *et al.* 1991) but not in their normotensive controls. Furthermore, although the antagonist crosses the blood brain barrier and inhibits ANG II binding in circumventricular organs, and in select nuclei within the CNS (Song *et al.*1991, Gehlert *et al.* 1990) central administration of losartan does not reverse the hypertension in SHR while peripheral antagonism does (Mangiopane *et al.* 1991). The specific antagonist should therefore selectively block only ANG II dependent changes in blood pressure in RRM rats clarifying the mechanism of CEI antihypertensive effect in rats without elevated plasma levels of ANG II.

In the current study losartan prevented salt-induced hypertension in RRM rats. The same sodium intake caused a significant elevation in blood pressure in untreated RRM rats (illustrated in figure 11). This is analogous to the antihypertensive effect of CEI in RRM rats and supports our hypothesis that the salt-induced rise in blood pressure in this model is dependent on the actions of angiotensin II at AT_1 receptors.

Plasma levels of ANG II were not measured in RRM rats in this study

but previous reports have shown that circulating ANG II levels in RRM rats are no different from the intact rats. In spite of assumed similar plasma levels of ANG II in RRM and control rats, the antagonist lowered blood pressure only in the RRM rats. This suggests that blood pressure in RRM rats is not normally dependent on the actions of circulating ANG II, but that the rise in MAP in RRM rats that follows increased salt intake is dependent on the peptide's action at AT_1 receptors.

In addition, losartan appears to have a renoprotective effect in RRM rats. Previous studies have established that even one week on high salt intake can cause progressive deterioration of renal function in RRM rats (Brandt *et al.* 1989, Ylitalo and Gross 1979) as evidenced by the elevated BUN levels of the untreated rats in this study (figure 15). The losartan treatment prevented an increase in BUN and the treated rats appeared healthier than the untreated rats. This supports the additional earlier finding that CEI afford renal protection in this model (Anderson 1985, Meyer *et al.* 1985, Brooks *et al.* 1989).

The renoprotection by CEI in remnant kidneys produced dramatic decreases renal damage in humans (Sanchez *et al.* 1991) and animal studies (Brenner 1985). The protection was apparently due to a decrease in intrarenal ANG II production resulting in decreased intraglomerular pressure (Raij *et al.*

1989, Meyer 1985, Tolins and Raij 1990). This renoprotection may not contribute to the antihypertensive effect of the drugs. If losartan prevented hypertension by inhibiting renal deterioration, the correlation between MAP and BUN should be identical in treated and untreated animals. That is, losartan should lower blood pressure by improving renal function, not alter blood pressure at a given level of renal function. In the current study, losartan treatment shifted the relationship between MAP and BUN (see figure 12) so that for any given BUN, MAP was lower in the treated animals. This suggests that losartan's antihypertensive effect is independent of its ability to slow renal deterioration.

One possible mechanism by which normal concentrations of circulating ANG II could cause RRM hypertension is if RRM causes an increase in sensitivity of the vascular AT_1 receptors. If the mechanism of action of ANG II in RRM hypertension involves increased sensitivity of vascular receptors, there should be an increased vascular response to a bolus of ANG II during hypertension. As depicted in figure 16, the acute pressor response to ANG II was not different in RRM and intact rats during either normal or high sodium intake. Therefore renal reduction does not cause an increased vascular response to ANG II. Furthermore, the pressor response to ANG II was not altered in either group during elevated sodium intake so that the

increase in blood pressure could not be due to a sodium-induced change in sensitivity of the vascular ANG II receptors. Since the acute response was totally blocked by losartan treatment, the acute pressor response was due only to ANG II actions at AT_1 receptors.

Another effect of ANG II that could contribute to salt-induced hypertension in RRM rats is sodium and water retention at the kidney. It is possible that blockade of renal ANG II receptors by losartan, which have been shown to be of the AT_1 subtype (Herblin *et al.* 1990, Edwards *et al.* 1991), prevented sodium and water retention and the initiation of a volume-dependent hypertension.

The RRM rats in this study did have a higher water intake than the intact rats but they also had a higher urine output so that the water balance was not increased in the RRM animals. Losartan treatment did not affect either water or sodium balance in either the intact or the RRM rats so that the antihypertensive effect was independent of alterations in water and sodium balance (figures 13 and 14).

In summary, salt-induced hypertension and progressive renal deterioration in RRM rats is apparently due to actions of ANG II on AT_1 receptors. The antihypertensive and renoprotective effects appear to be independent and are not accompanied by changes in sensitivity of vascular

ANG II receptors or changes in water and sodium balance.

2. Acute losartan treatment in hypertensive RRM rats

Hypertension produced by angiotensin II infusion is characterized by two phases. Initially, blood pressure rises dose-dependently and appears to be dependent on activation of vascular ANG II receptors since the increase can be acutely reversed by the ANG II antagonist saralasin (Fink *et al.* 1987, Cox and Bishop 1991). This early phase gradually diminishes and a chronic increase in blood pressure dependent on the integrity of the area postrema develops (Fink *et al.* 1987, Cox and Bishop 1991). During the chronic phase, acute vascular receptor blockade only partially reverse the hypertension while ganglionic blockade causes a much greater fall in pressure (Cox and Bishop 1991, Pawloski 1990). Thus it is possible to differentiate between vascular and central ANG II effects on blood pressure.

Although chronic treatment of RRM rats with losartan revealed a role for ANG II in salt-induced hypertension in this model, it is impossible to separate the acute and chronic actions of the treatment. Acute blockade of ANG II receptors in RRM animals with established hypertension should help to differentiate between vascular and centrally mediated actions. If RRM hypertension is entirely dependent on ANG II actions at vascular receptors,

acute blockade of those receptors should cause a rapid reversal of the hypertension. Conversely, if the hypertension is dependent on less accessible receptors, antagonism should only slowly reverse the hypertension.

Acute losartan treatment in RRM rats on high salt intake did not appear to cause either an acute or a delayed decrease in blood pressure. This first challenge was made after 5 days of high salt treatment and blood pressure was only slightly elevated in some of the animals. Therefore the ANG II-dependent mechanisms may not have been operating at this time. A second challenge 5 days later did cause a significant fall in pressure, but only after 2 - 6 hours. These results are only preliminary but further support the previous conclusion that the antihypertensive effect of losartan is due to blockade of non-vascular ANG II receptors.

D. Changes in sensitivity to chronic angiotensin II in reduced renal mass rats

The goal of this investigation was to examine the sensitivity of blood pressure in RRM rats to chronic administration of exogenous ANG II. It was established in the previous experiment that the acute vascular pressor response to ANG II is not increased in RRM rats. Furthermore, ANG II dependent hypertension develops during infusion at doses that are not acutely

pressor and appears to depend on activation of central rather than peripheral pathways (Fink *et al.*1987, Cox and Bishop 1991, Brown *et al.* 1979).

In the Introduction, it was established that circulating levels of ANG II are normal or even suppressed in RRM rats. Therefore, if the chronic hypertension during salt-loading in RRM rats is due to increased activity of the circulating renin-angiotensin system, it would require an increased response to the blood-borne peptide.

In the current experiment, RRM rats on normal sodium intake developed a sustained hypertension throughout the infusion of 10 ng/min ANG II. Intact rats on normal sodium intake receiving the same dose of ANG II had an initial small increase in MAP but MAP returned to control levels and stayed normal during the last 6 days of the 10 day ANG II infusion. Thus this study demonstrated that RRM rats have increased responsiveness to the chronic pressor effect of circulating ANG II.

An obvious explanation for the increased pressor response to ANG II in RRM rats would be decreased clearance of the hormone. The same infusion rate would then produce higher plasma concentrations in RRM compared to intact rats. Measurements of the plasma concentration of immunoreactive ANG II, however, revealed similar levels in intact and RRM throughout the ANG II infusion period. (Figure 22). Thus, RRM rats

developed hypertension at lower circulating levels of the hormone, which are not chronically pressor in intact rats.

Rats with a sufficient impairment in renal function (ie. BUN > 40 mg%) are hypertensive even while on a normal salt intake (Hostetter *et al.* 1981, Hall*et al.* 1985 and unpublished observations). It is possible then that the angiotensin infusion caused a further deterioration in renal function in RRM rats sufficient to produce accelerated renoprival hypertension even while the rats were maintained on a normal salt intake since some investigators have shown that ANG II infusion causes renal damage (Johnson *et al.*1992). This is not supported by our measurements of BUN in RRM rats receiving ANG II since BUN did not increase. Further, the observation that neither water intake nor urine output increased during the development of hypertension lends additional support. However, it is possible that more sensitive measures of renal function might have detected subtle ANG II-induced changes that contributed to the increased sensitivity to the chronic pressor actions of the peptide.

One such change could be altered proximal tubular sodium reabsorption. Previous studies demonstrated that ANG II acutely stimulates Na reabsorption and decreases urinary sodium excretion in both normal (Navar *et al.* 1991) and remnant (Pelayo *et al.* 1990) rat kidneys. Previous

chronic experiments using this dose of ANG II in intact rats have not shown sodium retention (Pawloski 1990), yet the remnant kidneys of RRM rats could respond to chronic infusion of ANG II in a quantitatively different fashion than intact kidneys. An ANG II-induced decrease in urinary sodium excretion in RRM rats could then have produced a "volume-dependent" hypertension (Hall 1986, Hall *et al.* 1984). In the current experiments, however, neither intact nor RRM rats exhibited significant sodium retention during the ANG II infusion period. Water balance also was unchanged by the ANG II infusion in both groups of rats. Therefore, no evidence was found for disparate effects of ANG II on renal sodium and water handling between normal and RRM rats under the conditions of this experiment.

A quite different explanation for the increased chronic pressor responsiveness to ANG II in RRM rats would be an increased sensitivity of a non-renal target tissue for ANG II. Although many such target tissues are known, previous experiments from this laboratory suggest that the brain plays a particularly important role in the rat model of chronic ANG II-induced hypertension utilized here. As discussed earlier, ablation of the area postrema prevented sustained hypertension in rats receiving chronic intravenous infusions of ANG II (Fink *et al.* 1981, Matsukawa and Ried 1990). Physiological changes resulting from a chronic loss of functioning nephrons

in RRM rats might increase the sensitivity of this brainstem cardiovascular control system to ANG II. One such physiological change could be extracellular fluid volume expansion, since it is well established that a high salt intake increases both extracellular fluid volume and the chronic pressor response to ANG II (Krieger *et al.*1989, Ando *et al.* 1990, Cowley and McCaa 1976). High salt intake also potentiates the chronic pressor response to ANG II delivered selectively into the brain via the ventricles (Bruner *et al.* 1985). Further, acute activation of the area postrema-dependent pathway with intravascular infusions of ANG II in dogs was prevented by placing the animals on a low salt intake (Szilagyi and Ferrario 1987). The importance of body fluid volume expansion to the chronic pressor effect of Ang II was convincingly demonstrated in a recent study by Krieger and Cowley. When extracellular fluid accumulation was prevented in dogs on a high salt intake, the chronic hypertensive response to ANG II infusion was completely inhibited (Krieger and Cowley 1990). In the current study and in previous investigations, the acute pressor response to ANG II infusion was identical in both the intact and the RRM rats but the sustained hypertension, the area postrema dependent phase (Szilagyi and Ferrario 1987), was seen only in the RRM rats. This supports the hypothesis that renal reduction sensitizes rats to the slow pressor phase of ANG II-induced hypertension.

Previous studies have shown that RRM rats have significantly increased extracellular fluid volumes (Ylitalo and Gross 1979), even while on normal salt intake. It is probable that volume expansion would be greater in rats with more severe impairment of renal function (higher BUN values) although extracellular fluid volume was not measured in the current experiments. Accordingly, a positive correlation should exist between BUN and the MAP response to ANG II. Such a positive correlation was found here, although the correlation did not reach statistical significance. We hypothesize, therefore, that extracellular fluid volume expansion in RRM rats on a normal salt intake led to the increased chronic pressor responsiveness to ANG II observed in these animals. This enhanced responsiveness may reflect increased sensitivity of the brainstem cardiovascular control system activated by blood-borne ANG II in RRM rats--but this remains to be proven. This hypothesis can be extended to the pathophysiology of salt-induced RRM hypertension. Increased extracellular fluid volume may increase the responsiveness to the chronic pressor effect of ANG II so that normal or even suppressed plasma concentrations of ANG II would be adequate to induce hypertension. The ability of angiotensin converting enzyme inhibitors and angiotensin antagonists to prevent hypertension development in RRM rats could be due then to suppression of circulating ANG II concentrations and

interruption of the activity of this brainstem system. Furthermore, this sensitivity increases with increased volume-expansion so that acute ANG II antagonism should become more effective at reversing RRM-salt hypertension with increased exposure to high salt, as seen in the acute losartan challenge.

V. Summary

Following is a restatement of the main hypotheses tested in this dissertation and a brief summary of the results pertaining to each.

Hypothesis 1: Aldosterone released by elevated plasma levels of angiotensin II contributes to angiotensin II induced hypertension. Aldosterone infusion elevates plasma levels of aldosterone (PAC) and MAP in a dose-dependent manner so that a 3-fold elevation in PAC caused a significant elevation in blood pressure during high salt intake. Infusion of ANG II acutely elevates plasma levels of aldosterone to hypertensive levels and elevates MAP in a dose-dependent relationship. However, infusion of ANG II for 2 to 28 days at the rate of 10 ng/min does not cause a sustained elevation in PAC in rats on either high sodium intake or normal sodium intake. Therefore, the elevated MAP during the ANG II infusion combined with high sodium intake was not dependent on elevated PAC. Furthermore, infusion of ANG II at either sodium intake does not alter sodium or water balance or cause changes in plasma electrolyte concentrations such as would be expected during PAC excess.

Hypothesis 2: Elevations in plasma aldosterone concentration during high

salt intake contributes to reduced renal mass-salt hypertension. When RRM rats with an average BUN > 30 mg % (5/6 reduction in total renal mass) are switched from normal salt intake to high salt intake they develop a significant elevation in MAP within 24 hours. This increase in MAP is accompanied by a reduction in PAC, identical to the reduction seen in intact rats during the same alteration in sodium intake. Additionally, the alteration in sodium intake did not produce different alterations in sodium balance, water balance, plasma electrolytes or plasma osmolality between intact and RRM rats. BUN in the RRM rats increased slightly during high salt intake and was consistently higher than in intact rats.

Hypothesis 3: The initiation of salt-induced hypertension in reduced renal mass rats is dependent on circulating angiotensin II. RRM rats switched from normal sodium to high sodium intake developed the expected increase in MAP while RRM rats treated with losartan throughout the high sodium period did not have a significant increase in MAP. Elevation of sodium intake did not increase MAP in intact control rats or in intact rats treated with losartan. Furthermore, neither losartan treatment nor renal reduction affected the time required to achieve sodium and water balance following the change from normal to high sodium intake. Losartan did prevent a sodium-induced

increase in BUN in the losartan treated RRM rats. Acute losartan treatment in hypertensive RRM rats on high sodium intake did not lower MAP after 5 days on high salt but did cause a small decrease after 10 days of high sodium treatment. The decrease was not significant until 2 hours after losartan challenge. The acute response to ANG II infusion at 10, 30 and 60 ng/min was not different between RRM and intact rats and was not affected by sodium intake. Losartan completely abolished the response to ANG II infusion.

Hypothesis 4: Reduced renal mass rats are more sensitive to the hypertensive effects of circulating ANG II than intact rats. Chronic infusion of 10 ng/min ANG II into normotensive RRM rats on normal sodium intake caused a significant and sustained increase in MAP compared to either intact rats receiving the same dose ANG II or with control RRM rats (no ANG II). Infusion of ANG II into intact rats on normal sodium intake caused an initial increase in MAP but MAP returned to control levels during the last 6 days of the 10 day infusion while MAP remained elevated throughout the infusion in RRM rats. The hypertension in the RRM rats was not accompanied by changes in water or sodium balance and MAP returned to control levels during the recovery period.

VI. Conclusions

The results from these experiments help to answer two different questions. The first is the main one addressed by this dissertation. Namely, what are the mechanisms which initiate and maintain the salt-induced hypertension in RRM rats? The second is the more general question of the mechanism of ANG II-induced hypertension.

Work by other investigators has established that hypertension in RRM rats is dependent on elevated TPR and not on increased CO since changes in MAP parallel the changes TPR but not CO (Lombard *et al.* 1989). The observation in our study that exaggerated sodium retention does not accompany the initiation of RRM-salt hypertension confirms that RRM hypertension is not associated with volume expansion.

Secondly, earlier investigations have also found evidence to suggest that the elevated TPR in RRM appears to be dependent on either increased activity of the SNS as evidenced by the elevated plasma levels of catecholamines or on increased response of VSMC to sympathetic stimulation. The partial depolarization of vascular smooth muscle membranes recorded in hypertensive RRM rats and the elevated constriction of VSMC to both NE and electrical stimulation in the presence of plasma from hypertensive RRM rats further suggests that a humoral factor caused the

depolarization of the VSMC. While our studies do not address the role of the SNS in RRM hypertension, activation of the SNS during hypertension in RRM is consistent with the results in our studies

From our studies, it is concluded that the initiation of RRM-salt hypertension is dependent on the activation of ANG II AT_1 receptors, confirming the earlier findings with CEI. The location of the receptors responsible for this action is not known. Our results suggest that vascular AT_1 receptors are not involved as evidenced by the time course of the acute antihypertensive effect of losartan in RRM-salt rats and by the acute response to an ANG II bolus.

Furthermore, we established that normotensive RRM rats on normal sodium intake have an increased MAP response to circulating ANG II chronically but not acutely. This suggests that the slow pressor response to ANG II is heightened in RRM rats. Together with the evidence that increased sodium intake is associated with an apparent increased response of the SNS, we hypothesize that ANG II may act to augment SNA.

Finally, we can conclude that the elevated PAC observed by other investigators in RRM rats appears to be a result of electrolyte imbalances induced by loss of renal mass, and does not cause the hypertension.

On the more general question of ANG II-dependent hypertension, we

conclude from the first experiment that elevated PAC does not play a role in the hypertension caused by chronic infusion of ANG II. The increased response in RRM rats to circulating ANG II may also indicate the mechanism elevating MAP. First, it has been demonstrated that sodium-loading, which predisposes to ANG II hypertension in intact rats, expands sodium space (Ando *et al.*1990, Krieger and Cowley 1990) and stimulates cardiopulmonary baroreceptors (Andresen *et al.* 1989). RRM should also stimulate cardiopulmonary baroreceptors by expanding body fluid volume

Secondly, desensitization of the cardiopulmonary baroreceptor reflex has been implicated in the slow presser effect of ANG II (Matsukawa and Ried 1990) through the action of ANG II on area postrema (AP) neurons. The AP is necessary for the development of chronic ANG II hypertension (Fink *et al.* 1987), and lesion of the AP prevents ANG II modulation of the baroreflex control of heart rate (Matsukawa and Ried 1990). Electrophysiological connections from the AP to the parabrachial nucleus and the NTS, two regions which appear to control baroreflex responses (Cechetto and Calaresu 1983, Hamilton and Ellenberger 1981) have been established (Shapiro and Miselis 1985, Papas and Ferguson 1990). The AP also receives direct inputs from both the carotid sinus and aortic depressor nerves (Ciriello *et al.* 1981, Davies and Kalia 1981, Wallach and Loewy 1980). Finally,

microinjection of ANG II into the AP selectively stimulates neurons that also respond to changes in blood pressure (Papas *et al.* 1990). These studies suggest that ANG II actions at the AP may be able to alter the baroreflex response to changes in body fluid volume produced by high salt intake. This inhibition of the cardiopulmonary baroreflex may be the common mechanism underlying the increased sensitivity to ANG II in salt loading and RRM hypertension.

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