SODIUM BALANCE AND VASCULAR RESPONSIVENESS IN THE RAT

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BY

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the University of Manitoba in partial fulfillment of the requirements
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THIS THESIS IS DEDICATED TO
MY LOVING PARENTS AND BELOVED
WIFE, WHOSE LOVE, UNDERSTANDING
AND SELF-SACRIFICE ENABLED ME
TO COMPLETE THIS THESIS.

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Sodium Balance and Vascular Responsiveness in the Rat

by Donald D. Smyth

ABSTRACT

Vascular responsiveness is clearly an important factor in the regulation of total peripheral resistance and hence, blood pressure. Data from recent studies indicate that responsiveness of the vasculature to certain vasoactive agents may be altered by changes in dietary sodium intake. We, therefore, studied the effect of sodium balance on the vascular responsiveness for angiotensin II (AII) and norepinephrine (NE) and attempted to delineate the mechanisms responsible for any observed changes in responsiveness.

The pressor response for AII was attenuated in rats on a low salt intake when compared to animals on a high salt intake. The suppression of endogenous AII formation with captopril greatly enhanced the response for AII in the low salt group while only modestly increasing the response in the high salt group. This suggested that an elevated endogenous level of AII in the low salt rats may account for the attenuated response. In contrast, the response for NE was unaffected by salt intake and captopril, suggesting that the salt induced alteration in vascular reactivity was not a non-specific event. As well, nitroprusside-induced hypotension or acute saline volume expansion (3% body weight) failed to alter the response for AII in the low salt rats. Thus, the hypotensive action of captopril or the lower plasma volume of the low salt group did not contribute to changes in AII pressor responsiveness in vivo.

In the isolated kidney preparation perfused with Krebs-Henseleit, an enhanced renovascular response to AII was observed in kidneys from

rats previously on a high salt intake as compared to a low salt intake. Conversely, the response for NE was greater in the low salt group, although this difference was observed only at perfusion pressure changes greater than 100 mm Hg. These results suggested that the altered vascular reactivity *in vitro* may contribute to changes in pressor responsiveness *in vivo* in different states of sodium balance.

We also assessed the role of the adrenergic system and its interaction with the renin-angiotensin system. Alpha-adrenergic blockade with phenoxybenzamine significantly reduced the pressor response to AII in both salt groups to a similar degree. Thus, α-adrenergic receptor activation was required for the full pressor effect of AII *in vivo*. However, this interaction does not appear to be sensitive to salt intake. The failure of pentolinium to alter the response for AII suggested this was a post-ganglionic phenomenon. β-adrenergic blockade with oral propranolol (one or seven days) produced a dose related increase in the response to AII only in low salt, but not high salt intake rats. The elevated response to AII was possibly due to a decrease in endogenous AII levels following β-blockade.

The role of endogenous prostaglandins was also assessed. The pressor response for AII, but not NE, was enhanced following meclofenamate or indomethacin treatment in rats previously on a low salt intake. The addition of captopril further increased the AII pressor response. The high salt group was unaffected by these treatments. However, in rats treated with captopril, indomethacin failed to potentiate the response for AII. It is possible that indomethacin potentiated the response for AII by decreasing endogenous AII levels since no potentiation was observed when AII levels were suppressed, either by captopril or a high salt intake.

As well, pentolinium or phenoxybenzamine also abolished the potentiating action of indomethacin. Thus, it appears that an intact adrenergic nervous system is also necessary for prostaglandins to be effective. Finally, the hypotensive action of captopril in low salt intake rats was decreased in the presence of indomethacin, suggesting prostaglandins play a role in mediating this response.

In summary, our data provide considerable evidence to support the thesis that the dietary salt sensitive changes in the pressor response for angiotensin II was determined by the level of activity and interaction of the renin-angiotensin system, adrenergic nervous system and the prostaglandin system. A direct relationship was observed between the pressor response for exogenous AII and the endogenous level of angiotensin. As well, parallel changes in renovascular sensitivity *in vitro* and pressor responsiveness *in vivo* for AII were observed. An intact α-adrenergic system was required to observe the full pressor action of angiotensin, however, this did not occur in a salt sensitive fashion. Finally, alterations in sodium intake also affected the role played by prostaglandins, since antagonism of the pressor response to AII was only observed in sodium depleted rats. Whether endogenous prostaglandins decrease the response to AII by increasing endogenous AII levels or antagonizing the α-adrenergic system is unclear.

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AI	- angiotensin I
AII	- angiotensin II
C_{Cr}	- creatinine clearance
DOCA	- desoxycorticosterone acetate
$\%FE_{Na}$	- per cent fractional excretion of sodium
NE	- norepinephrine
P_{Na}	- plasma sodium
POB	- phenoxybenzamine
RVR-gm	- renal vascular resistance to flow per gram of kidney weight
U_{Cr}	- urinary creatinine
UV_K	- urinary potassium excretion rate
U_{Na}	- urinary sodium
UV_{Na}	- urinary sodium excretion rate
$\dot{V}$	- urinary volume

SECTION I

REVIEW OF LITERATURE

A. Introduction

Since the description of the circulation by Harvey in 1628 and the demonstration of a considerable hydrostatic pressure in the arterial system of the horse by Reverend Stephen Hale in 1711, the regulation of blood pressure has been the subject of intensive investigation. The later appreciation of essential hypertension as a common disease with serious complications indicated further the need of research in this area.

In man, arterial blood pressure is normally maintained and regulated within narrow limits. The nature and quantitative aspects of the mechanisms which regulate blood pressure have been recently reviewed by Guyton (1977, 1980). In general, the acute or short-term regulation is achieved mainly through neural "buffering" processes. The long-term regulation of blood pressure is dependent on other factors, many of which are not neurally mediated.

Although these regulatory mechanisms determine blood pressure, blood pressure is, in essence, the product of cardiac output and total peripheral resistance. Thus, deviations of arterial blood pressure from the normal levels must be due to inadequate or inappropriate control of the regulation of cardiac output and/or peripheral vascular resistance. In this section I wish to review the roles of cardiac output and peripheral vascular resistance in the long-term regulation of blood pressure. The contribution of vascular reactivity to peripheral vascular resistance and its relationship to the state of sodium balance is also discussed.

1. Cardiac Output and Long-term Regulation of Blood Pressure

If total peripheral resistance were to remain fairly constant, any change in cardiac output would result in a proportional change in arterial blood pressure. Hypotension, which occurs in low-output states of heart failure, inspite of a markedly elevated total peripheral resistance, is an example of the failure of blood pressure regulation due to a primary defect in the heart. Similarly, an elevated blood pressure may also be attributed to high states of cardiac output such as seen in hyperthyroidism and anemia. Thus, deviations of blood pressure from the "normal" level may be related to a "cardiac" factor.

The cardiogenic contribution in other forms of hypertension is unclear. The initial elevation of blood pressure has been correlated with increases in cardiac output early in the development of renal hypertension (Ledingham and Cohen, 1963), salt-induced hypertension (Coleman and Guyton, 1969), spontaneous hypertension in rats (Pfeffer and Frohlich, 1973) and essential hypertension in man (Frohlich, 1977).

Hawthorne et al. (1974) suggested that the increased cardiac output in early stages of renal hypertension in the dog may be due to an enhanced contractility of the heart. Similar findings have been reported in renal hypertension in the rat where removal of the cardiac sympathetic nerve supply failed to prevent the observed increase in cardiac output (Chessar et al., 1972). Alternatively, the increased cardiac output has been related to elevations in blood volume (Borst, 1963).

Ferrario et al. (1970) found in the dog that wrapping one kidney in cellophane and removing the contralateral kidney resulted in an increased blood pressure associated with an elevated cardiac output.

However, the absence of a change in blood volume suggested to these authors that the observed elevation in cardiac output may be attributed to a constriction of the capacitance vessels. Overbeck (1972) proposed that a reduced venous compliance may be present in the early stages of renal hypertension in dogs.

In early essential hypertension in man, especially in labile hypertension, cardiac output has been usually found to be elevated. Initially, it was anticipated that β-adrenergic blockade would define the role of cardiac output in this type of hypertension. However, the acute fall in cardiac output following propranolol was not associated with a concomitant fall in blood pressure (Tarazi and Dustan, 1972). In fact, the eventual decrease in blood pressure was related to a fall in peripheral resistance. The negative inotropism of β-blockers, however, is not the only proposed antihypertensive mechanism of these agents (Buckingham and Hamilton, 1979; Scriabine, 1979). As well, these agents are effective in lowering blood pressure in hypertensive subjects without altering cardiac output (Tarazi and Dustan, 1972).

Similarly, in spontaneously hypertensive rats, β-blockade failed to prevent the development of hypertension, although initial increases in cardiac output were prevented by propranolol (Pfeffer et al., 1974).

Thus, it appears that an increase in cardiac output early in the course of hypertension is a common phenomenon in many forms of clinical and experimental hypertension. However, the available data suggest that this increase in cardiac output probably does not play a primary pathogenetic role in the eventual development of hypertension.

This should not be interpreted to mean that the heart does not

participate in the overall long term regulation of blood pressure. A primary increase in peripheral vascular resistance and the resulting elevation of blood pressure will place a demand on the heart, via the regulatory mechanisms, to reduce the cardiac output. However, the metabolic requirements for a certain blood supply may exceed the need to maintain a lower blood pressure over the long term. As a result, in hypertensive states, the "normal" level of cardiac output may, in fact, be elevated for that level of blood pressure. In this sense, it is conceivable that cardiac factors contribute to the maintenance of hypertension.

2. Peripheral Vascular Resistance and Blood Pressure

An increase in peripheral vascular resistance may be due to either structural or functional factors. Structural alterations in the vasculature due to atherosclerosis or muscular hypertrophy can result in luminal narrowing and thus, a passive increase in resistance to flow (Furuyama, 1962), even at maximal vasodilation. In addition, Folkow et al. (1970) showed that under these conditions, a similar degree of vascular smooth muscle shortening will have a greater effect on the resistance to flow than found in the normal vasculature. Similarly, functional alterations of the vasculature, such that the vasoconstrictor response for any given degree of stimulation is greater, will result in a greater effect on the peripheral resistance. Thus, a passive-structural or an active-functional alteration of the vasculature can play a pathogenetic role in the development and the maintenance of elevated blood pressures in hypertension.

Vasoconstriction due to an enhanced vascular tone clearly

contributes to an increased peripheral vascular resistance. This may be due to an enhanced neural activity, humoral activity and/or vascular responsiveness. An increased level of activity of the sympathetic nervous system would be expected to increase blood pressure through vasoconstriction. In fact, hyperactivity of the sympathetic nervous system has been implicated as an important factor in the development of some models of experimental hypertension (Laverty and Smirk, 1961; Haeusler et al., 1972) since antagonism of this system prevents the elevation of blood pressure. However, not all models of experimental hypertension require an intact nervous system, as in one kidney renal hypertension (Douglas et al., 1974). As well, the increased nervous activity may not be the cause of the maintained elevated blood pressures. Touw et al. (1980) found that blockade of sympathetic nerve transmission with hexamethonium produced a similar fall in blood pressure in normotensive and hypertensive rats. This suggested that the increased peripheral resistance in the hypertensive rats was not due to an enhanced neurogenic tone, although, this neurogenic tone may be important in the initial stages of hypertension.

The absolute level of vasoactive humoral substances are also determinants of vascular resistance. In phaeochromocytoma, blood pressure is elevated due to the excessive release of catecholamines. In two kidney Goldblatt hypertension, the elevation of blood pressure has been attributed to enhanced activity of the renin-angiotensin system (Gross et al., 1964). Similarly, a decreased release of vasodilators, which are known to be released in response to vasoconstriction in certain vascular beds, (Aiken and Vane, 1973) would add to the elevation of vascular resistance.

Finally, the degree of end-organ sensitivity will determine vascular resistance for any given degree of neurohumoral agonism. In some forms of hypertension, an increase in vascular reactivity has been demonstrated (Haeusler and Haefely, 1970; Kubo, 1979). As a result, "normal" levels of endogenous humoral substances would have a greater effect on peripheral resistance. Thus, changes in vascular tone, whether due to changes in neurohumoral agonist activity or end-organ responsiveness, are potential candidates in the long term control of blood pressure.

However, these mechanisms to elevate peripheral resistance are not totally independent of each other. The elevation of endogenous humoral substances is generally associated with a decrease in the vascular response to these agonists. Conversely, low endogenous levels are associated with a general enhanced vascular response for the agonist (Rankin et al., 1981; Thurston and Laragh, 1975). Thus, primary alteration of one variable could potentially induce secondary compensatory response in others. The failure to maintain a normal blood pressure over the long term implies the involvement of more than the failure of only one simple control mechanism.

B. Salt and Hypertension

Although the etiology of essential hypertension is unclear, it is generally recognized that a certain relationship exists between sodium metabolism and blood pressure. The pathogenetic role of dietary salt intake in hypertension has been extensively reviewed recently (Tobian, 1977; Dahl, 1977; Freis, 1979; Morgan et al., 1978). Several lines of indirect evidence suggest that long term increases in salt intake may alter the long term regulation of blood pressure, resulting in a higher incidence of hypertension. The supporting evidence is mostly derived from: a) epidemiological studies, b) salt-induced hypertension in experimental animals and c) effects of salt restriction.

1. Epidemiological Studies

That salt intake may play a vital role in the development of hypertension was first noted by Ambard and Beaujard in the early 1900's. These authors proposed that the elevation of blood pressure was due to an inability of the body to adapt to an excess of dietary salt (Freis, 1979). This prophetic work was not accepted until almost 50 years later. Kempner (1948) proposed a rice-fruit-sugar diet for the treatment of hypertension and advocated that a salt restricted diet was not necessary. When it was later revealed that the extremely low sodium content of the rice diet was the most significant factor in the reduction of elevated blood pressure (Murphy, 1950; Watkin et al., 1950), the importance of dietary salt became more widely accepted. This led to a number of epidemiologic studies to verify if salt intake in various societies did correlate to the incidence of hypertension.

In western societies blood pressure is known to increase with age, reaching hypertensive levels in some. It has been observed in some

groups, however, that the blood pressure of individuals did not increase with age. Page et al. (1974) studied six Solomon Island communities utilizing a total of 1,390 adult subjects. The six communities varied in levels of unacculturated and exposure to western civilization. They found the communities with a higher degree of exposure to western cultural influences demonstrated an increase in blood pressure with age, whereas the more unaccultured groups did not. The increase in blood pressure with age was found to correlate best with dietary salt intake, particularly in the imported tinned meat and fish.

Maddocks (1967) studied two groups of people in New Guinea. The two groups consisted of the coastal urban community and people from the inland rural areas. The rural group showed no trend of blood pressure elevation with age, while the coastal people did. The increase in the latter group was correlated to a high level of urinary sodium excretion. Similar results were also reported by Prior et al. (1968) who studied two communities in the Cook Islands. In this study it was found that the community that had the lowest level of urinary salt excretion also had the lowest incidence of hypertension. The group ingesting large amounts of salt had a much greater incidence of hypertension. Finally, Tobian (1977) cited a study which reported that in northern Japan, the farmers ingest extremely large amounts of salt. At least 84% of the villagers have elevated blood pressures. The most common cause of death in these people is stroke.

Although genetic and other factors have not been taken into consideration, it appears that there is substantial epidemiological information suggesting that an increase in dietary salt intake is associated with an increased incidence of hypertension.

2. Salt-Induced Hypertension in Experimental Animals

The first study illustrating the pathogenetic role of salt ingestion in the development of high blood pressure was the classic experiment of Meneely et al. (1953, 1963). Rats were fed diets supplemented with different quantities of salt in the drinking water, which ranged from 0.01 to 9.8% sodium chloride. In all animals given 9.8% sodium chloride, severe hypertension developed after nine months. Animals fed 2-5% had a mild form of hypertension. However, no change in blood pressure with age was observed in animals on a low salt intake.

In man, it is clear that not all individuals ingesting large quantities of dietary salt develop hypertension. It appears that some individuals have an apparent resistance whereas others are susceptible to the adverse effects of high sodium intake.

After studying this problem extensively for 25 years, Dahl (1972; 1977) came to a similar conclusion. In Sprague-Dawley rats, the response to high levels of salt intake (8% of total dietary intake by weight) ranged from no effect to an extreme elevation of blood pressure (Dahl, 1960; Dahl and Heine, 1961; Dahl, 1961). In fact, only 25% developed blood pressures in the hypertensive range. This suggested to the authors that the susceptibility to salt may involve an additional genetic factor. In order to verify this theory, Dahl et al. (1962) attempted to separate and breed a salt sensitive and salt resistant strain within their population of rats. By the third generation of selective breeding, they had two distinct strains. The resistant strain was not affected by increases in dietary salt. However, the sensitive strain responded with a gradual elevation of blood pressure when dietary sodium was increased. In these sensitive rats, the blood pressure remained near normal if

sodium chloride intake was restricted in the diet from birth. Interestingly, these salt-sensitive and salt-resistant strains of rats showed similar responses as previously mentioned when they were subjected to other experimental hypertensive stresses such as renal artery constriction and desoxycorticosterone treatment (Dahl et al., 1963).

The ability of desoxycorticosterone (DOC) treatment to elevate blood pressure in normal rats is related directly to the level of dietary sodium intake as well (de Champlain, 1973). Similarly, in rats fed a sodium deficient diet, DOC treatment fails to elevate blood pressure. Thus, this model is dietary salt sensitive. As well, this has been associated with sodium and water retention as the pathogenic factors (Gavras et al., 1975).

Thus, these experiments strongly suggest a pathogenic role of salt in the development of hypertension in genetically susceptible animals. It also appears that certain genetic characteristics may also be protective in more than one form of experimental hypertension.

3. Effect of Dietary Salt Restriction

If excessive salt intake results in hypertension, it is reasonable to expect that dietary salt restriction should lower blood pressure in hypertension. This was first suggested in the early 1900's by Ambard and Beaujard who proposed that blood pressure in hypertensive patients could be decreased by a salt restricted diet. However, they erroneously concluded that blood pressure lowering and its elevation was due to chloride rather than sodium. Later, the success of sodium restriction in the treatment of hypertension was reported by Kempner (1948) and Watkins et al. (1950). However, a strict low sodium diet is difficult to follow. Thus, with the discovery of effective antihypertensive diuretic agents,

moderate sodium restriction has been advocated as an adjunct to drug therapy in the management of hypertension. Nevertheless, the effectiveness of dietary sodium restriction in blood pressure lowering in many hypertensives is widely accepted today (Parijs et al., 1973; Morgan et al., 1978).

The evidence for the effectiveness of sodium retention in lowering blood pressure in experimental animals has been less straight forward. In the salt sensitive strain of rats, Dahl (1961) noted that salt restriction was not uniformly effective in reversing hypertension once it had been established. It is generally believed that prolonged hypertension, regardless of its mode of induction, results in a number of secondary lesions which may serve to maintain the elevated pressures (Meneely et al., 1953).

Although this is an over-simplification of the actions of diuretic agents, it is interesting to note that these agents which increased the renal excretion of salt and water are also effective in the treatment of hypertension (Morgan et al., 1978). That these agents may act by causing sodium depletion is supported by the finding that salt ingestion can abolish the antihypertensive effects of some diuretics (Winer, 1961). Of course, these findings do not mean that antihypertensive agents which are also natriuretic may not have other mechanisms of action.

In summary, it appears that a relationship exists between dietary sodium intake and the long term regulation of blood pressure. The association of the development of hypertension with salt intake as reported in numerous epidemiological studies is indirect but persuasive evidence that salt is important in starting a process eventually ending in hypertension. As well, the ability of a high salt intake in inducing

experimental hypertension, and the effectiveness of dietary salt restriction with or without concomittant drug therapy lends further support to the pathogenic role of sodium in hypertension. Of course, other genetic and environmental factors must also be considered, but the connection still exists.

That an excessive intake of salt can cause hypertension is by no means a modern observation. A significant role has already been assigned to sodium by some astute clinician as recorded in the Nei Ching, The Yellow Emperor's Classic of Internal Medicine, 2500 B.C.

> "Hence if too much salt is used in food, the pulse hardens."

4. Pathogenetic Mechanisms

A state of positive sodium balance exists when the amount ingested exceeds excretion over a considerable period of time. Since the kidney is the major organ responsible for the regulation of salt and water balance, early investigators hypothesized that primary or secondary renal damage or dysfunction with the resulting chronic volume excess caused the hypertension. Alternatively, others suggested that excessive salt intake may alter the function of other physiological processes, such as sympathetic nervous system hyperactivity, leading to an increase in blood pressure. These possibilities are discussed below:

a) Extracellular Fluid Volume Expansion and Renal Response

Tobian et al. (1974) studied the effect of salt intake in three groups of salt sensitive rats and how the extracellular fluid volume was altered when the animals became hypertensive. Two groups of animals

were fed a high salt diet. However, one group was given a diuretic to prevent expansion of the extracellular fluid (ECF) volume. Another group was fed a low salt chow. In these three groups, only the high salt intake group without the diuretic became hypertensive. Extracellular fluid volume was estimated by the determination of inulin space. The high salt group had an inulin space which was significantly greater than the other two groups. The high salt group given a diuretic had an inulin space similar to the low salt group, suggesting that an expanded ECF was necessary for the development of elevated blood pressures. These results also demonstrated that the increased renal sodium excretion in the high salt groups did not damage the kidneys and thereby increase blood pressure.

In another experiment, Ledingham (1953) noted that an increase in ECF volume preceded the elevation of blood pressure in experimental renovascular hypertension. However, once the blood pressure was elevated, the ECF volume returned towards normal. Later, the same authors found that the increased ECF caused an increase in cardiac output, thereby elevating blood pressure (Ledingham et al., 1963). When the ECF volume was decreased due to a pressure diuresis, cardiac output fell, but blood pressure remained elevated. These results suggested that the initial elevation of blood pressure was due to an expansion of the ECF volume resulting in an increased cardiac output. However, blood pressure was maintained by an elevation of the peripheral resistance.

A similar sequence of events has been shown in other forms of hypertension which are thought to be due to sodium retention. Borst (1963) demonstrated that aldosteronism secondary to ingesting large

quantities of licorice would result in a state of sodium excess with an elevated central venous pressure. He suggested that an increase in the ECF volume led to an increase in cardiac output and blood pressure. The resulting increased blood pressure caused a pressure diuresis and thus, the excretion of the excess ECF volume. He further postulated that the reason some people become hypertensive is that these individuals require a higher blood pressure to excrete the excess salt and water.

Tobian et al. (1974) also observed a similar phenomenon. In the isolated perfused kidney they found that for any given level of renal perfusion pressure, the kidneys from salt resistant rats excreted more sodium and water than the kidneys from salt sensitive rats. This difference in ability to excrete salt may be the underlying mechanism which determines whether an animal is salt sensitive.

The above evidence suggests that the initial reaction to excessive sodium intake is an increase in ECF volume. The eventual development of an elevated blood pressure probably depends on an inadequate or inappropriate renal response. This probable cause was further elucidated by the studies of Dahl et al. (1974) and Bianchi et al. (1974a). Dahl and his associates used their inbred salt sensitive and salt resistant rats. Bianchi's group studied a strain of rats which became spontaneously hypertensive at an early age and a similar strain which remained normotensive. To elucidate whether the kidney was the site which determined the level of blood pressure between groups, these investigators transplanted kidneys from one strain to another. Thus, animals known to develop hypertension were transplanted with kidneys from the animals which were known to be less susceptible to the development of hypertension and vice versa. Both investigators found that the

source of the donor kidney determined the final blood pressure. For example, spontaneously hypertensive rats (SHR) had lower blood pressures when given a kidney from a normotensive rat. Likewise, the normotensive rat became hypertensive following the transplant of a kidney from an SHR.

It should be noted that in the SHR's blood pressure is known to be already elevated at one month of age (Bianchi et al., 1974b). Thus, in Bianchi's experiments, the animals had increased blood pressures when they were used as donors in the transplants. These kidneys may have been "damaged" by the elevated blood pressures. It is still uncertain whether in the SH strain, the renal defect is the cause of or a result of the elevated blood pressures. However, in Dahl's experiments, both strains had comparable blood pressures at the time of transplant. Thus, the salt sensitive form of hypertension appears to be partially renal in origin. In the SHR, this remains uncertain.

A rise in ECF volume does not always precede elevation in blood pressure. In one kidney Goldblatt hypertension or early two kidney Goldblatt hypertension, sodium retention accounts for some of the increase in elevated blood pressure (Tobian et al., 1969). However, even if the elevation in ECF volume is blocked by diuretics or a low salt diet, the animal still becomes hypertensive, presumably due to the increased activity of the renin-angiotensin system (Tobian, 1977).

The proposed sequence of events that leads to an elevation of blood pressure in salt-induced situations, are as follows. A primary event such as excessive salt or impaired excretion secondary to conditions such as aldosteronism leads to an expansion of the ECF. As a result, the venous filling pressure, cardiac output and, therefore,

blood pressure are increased. The resulting elevated blood pressure causes a pressure diuresis and natriuresis restoring the ECF volume back towards normal. However, in some cases, a renal perfusion pressure greater than normal is required to excrete the excess salt and water. This apparent, and perhaps genetically determined defect in renal sodium handling results in a chronic elevation of blood pressure. It is also possible that an associated defect in the sensing of ECF volume may be involved. These mechanisms have been extensively studied and reviewed by Guyton (1980). Clearly, additional mechanisms involving the long term regulation of blood pressure must also be involved. Why peripheral resistance and blood pressure remain elevated at higher levels when ECF volume returns to normal is at present poorly understood.

b) Neural, Humoral and Vascular Mechanisms

It is evident that not all animals ingesting excessive amounts of salt will develop hypertension. It is also clear that a chronically increased extracellular fluid volume does not always lead to long term elevations in blood pressure. Clinical disorders associated with chronic volume excess such as congestive heart failure and salt and water retention due to chronic liver disease, for examples, do not generally cause hypertension in spite of marked reductions in renal sodium excretion. These facts imply that certain mechanisms must operate or others be rendered inactive to avoid the development of hypertension. These mechanisms, neural, humoral or otherwise, are component parts of the system which regulates blood pressure as discussed earlier in this review. Thus, the ability of a high sodium intake in inducing hypertension in a susceptible animal suggests the involvement of the

dysfunction of some of the components of this regulatory system.

The work of Fink et al. (1977) suggested that central neural mechanisms are involved in the pathogenesis of salt-induced hypertension. Two groups of rats were studied. Both groups received desoxycorticosterone (DOC) treatments as well as 1% saline substituted for drinking water. The control group developed hypertension with systolic blood pressures near 170 mm Hg. The experimental group, which had anterior forebrain lesions in the area of the anteroventral third ventricle region, did not develop hypertension. Fluid intake, body weights and plasma volumes were similar in both groups. These authors and others (Brody et al., 1978) suggested that this region of the central nervous system plays a role in the pathogenesis of salt-induced hypertension.

Similar observations have been reported with central chemical sympathectomies. Intraventricular administration of 6-hydroxydopamine prevented the development of DOC and saline induced hypertension (Hausler et al., 1972). More importantly, Brody et al. (1978) showed that ablation of the anteroventral region of the third ventricle did not lower blood pressure in the spontaneously hypertensive rat, although the effects of the ablation may have been more localized than the infusion of drugs intraventricularly. Similarly, peripheral chemical sympathectomy with 6-hydroxydopamine delays the rise in blood pressure in DOC-induced hypertension (Meuller and Thoenen, 1970). Thus, collectively, these studies suggest that certain neural and probably sympathetic mechanisms are involved in the pathogenesis of salt-induced hypertension.

However, Haeusler et al. (1972) noted that intraventricular 6-hydroxydopamine did not lower blood pressure in established DOC and saline-induced hypertension. Thus, it is possible that central neural

mechanisms are not involved in maintaining an elevated blood pressure over the long term. In addition, it is likely that these neural mechanisms are not specific for salt-induced hypertension. The inability of neural ablation to lower blood pressure in the SH rats may be related to the fact that the hypertension is already established and not because of the etiology difference. More studies involving other models of experimental hypertension will have to be performed to clarify this point.

A renal humoral mechanism has been proposed for the pathogenesis of salt-induced hypertension. Tobian et al. (1969) studied hypertension in the rat induced by unilateral nephrectomy associated with a high sodium intake. They proposed that the kidney produces an antihypertensive material, the lack of which allows hypertension to develop. The removal of one kidney presumably reduced the capacity of the animal to synthesize this substance and at the same time amplified the effect of the high salt intake. They also found that as hypertension developed, these proposed antihypertensive granules were significantly reduced. Tissue extracts obtained from this region have been shown to have a blood pressure lowering action similar to that of prostaglandins (Muirhead et al., 1973). An additional antihypertensive substance has also been isolated, however, its identification is uncertain (Muirhead et al., 1977). It was also noted in later experiments that plasma flow to the medullary region of the kidney was decreased in post-salt hypertension (Ganguli and Tobian, 1974). Similar results were also found in the salt-sensitive Dahl rats (Tobian, 1977). Unfortunately, these experiments only established the association, but not the cause effect relationship of these "antihypertensive" renal medullary substances to the development of hypertension

in their experimental model. Further, it does not establish whether these substances play a role specifically in salt-related hypertension or that similar findings are also observed in other forms of high blood pressure. Finally, one cannot dismiss the possibility that these changes are the consequence of a high renal sodium excretory rate and have nothing to do with the pathogenesis of hypertension.

Another mechanism which can explain how salt induces hypertension involves functional and structural changes in the peripheral vasculature. Tobian et al. (1952) found that the sodium and water content of arteries obtained from hypertensive patients and rats was elevated when compared to normotensive controls. He hypothesized that this would result in a swelling of arteriolar walls and a narrower lumen, leading to an increase in total peripheral resistance. Folkow et al. (1973) showed that an increased wall to lumen ratio can result in an exaggerated increase in vascular resistance for the same degree of smooth muscle shortening. Thus, arteriolar swelling may have contributed to the maintenance of the hypertensive state. However, there is no evidence to suggest that the increase in sodium content in the vasculature is related to salt-induced hypertension. In particular, it has yet to be shown that it is due to excessive salt intake.

Vascular reactivity has also been implicated as an important mechanism in the pathogenesis of hypertension. The pressor response to vasoconstrictor agents has been shown to be greater when plasma volume is normal or expanded than when plasma volume is reduced either by a diuretic (Wanko et al., 1958) or by a low sodium diet (Reid and Laragh, 1965). Similarly, it has been observed that there is a general increase in the pressor response to most vasoconstrictor agents in rats with

established hypertension. The relationships between vascular reactivity and hypertension or sodium intake is complex. The pathogenic role of these mechanisms is discussed in the following sections.

In summary, although it is clear that excessive sodium intake plays a pathogenic role in the development of hypertension, or aggravates it, there is no convincing evidence indicating the involvement of specific neural/humoral or vascular mechanisms unique to this form of hypertension. The issue involving vascular reactivity is discussed in the next section.

C. Vascular Reactivity

1. Hypertension and Enhanced Vascular Reactivity

Vascular reactivity may be defined as the ability of vascular smooth muscle to respond to a stimulus. Early studies which attempted to determine vascular reactivity *in vivo* usually consisted of measuring blood pressure changes in response to the intravenous administration of a vasoconstrictor. An exaggerated response was thought to reflect hyperreactivity of the vasculature. However, in many situations, the observed increases in blood pressure may have been due to an additional effect on cardiac output. Thus, investigations with epinephrine as the test agonist (Goldenberg *et al.*, 1948) and similar studies gave little insight to the actual vascular response. It is not surprising that these earlier studies which attempted to demonstrate a difference in vascular reactivity between hypertensive and normotensive subjects were usually unsuccessful.

In 1955, Doyle and Black were able to demonstrate a slight increase in the pressor response for angiotensin and norepinephrine in hypertensive patients and rats. However, following the administration of hexamethonium, this difference was accentuated. Similar observations were also made by Kubo in spontaneously hypertensive rats (Kubo, 1979). These data suggest that vascular reactivity was enhanced in hypertension. As well, interruption of cardiovascular compensatory mechanisms was desirable in separating reflex changes in cardiac output from the peripheral vascular responses.

However, it soon became evident that other homeostatic regulatory mechanisms and changes in blood pressure per se may influence the outcome of the pressor response *in vivo*. Many investigators turned to

studying the vasoconstrictor responses in regional vascular beds at constant perfusion pressures instead. This meant that the observed changes in resistance would be used as an index of changes in the vascular reactivity following the application of the stimulus. Using this approach, numerous authors were successful in demonstrating hyperreactivity to norepinephrine in the vasculature of the forearm and skin of hypertensive subjects (Doyle and Fraser, 1961a; Barany, 1963). However, the strength of this approach is also its weakness. Hypertensive subjects who had an enhanced response in certain isolated vascular beds such as the digit, did not always show an enhanced pressor response to the same agonist (Barany, 1963). As well, not all vascular beds show this enhanced reactivity in hypertensive subjects (Mendlowitz, 1973). In spite of their limitations, there is now a large body of evidence indicating that vascular hyperreactivity occurs in many forms of hypertension in man and animals (Goldenberg et al., 1948; Doyle and Black, 1955; Mendlowitz and Naftchi, 1958; Doyle and Fraser, 1961a; Barany, 1963; Mendlowitz et al., 1965; Okamoto et al., 1966; Haeusler and Haefely, 1970; Kubo, 1979).

Two major questions arose from these studies. What was the cause of the increased reactivity, and is this a major factor in the development of hypertension? One theory suggests that this alteration was due to a structural change in the geometry of the blood vessel. Muscular hypertrophy developed as a direct result of the elevated blood pressures. Alternatively, or concomitantly, the increased reactivity may be due to a functional change in the vascular smooth muscle. The vasculature is simply more responsive for reasons other than structural alterations. This latter mechanism qualifies as a candidate both as a

cause for the development and the maintenance of hypertension.

A generalized hypertrophy of vascular smooth muscle had been observed in many types of chronic hypertension (Heyer and Keeton, 1941; Folkow et al., 1958; Furuyama, 1962). Folkow et al. (1958) deduced theoretically and demonstrated experimentally that vascular smooth muscle hypertrophy can increase reactivity. He proposed that vessels with an increased wall to lumen ratio will show a greater increase in resistance for the same degree of shortening. He further suggested that it is independent of how the increase in wall to lumen ratio was achieved, whether due to medial hypertrophy, encroachment by the surrounding tissue or by an elevated resting tone. Based on these arguments, these authors concluded that a common baseline level of tone had to be achieved before a valid comparison of vascular reactivity could be made in hypertensive and normotensive subjects.

In their study, forearm blood flow was measured by plethysmography and perfusion pressure recorded. Maximal dilation of the vessels in the forearm was induced by periods of ischaemia, increasing workloads on this tissue and by using a warm water bath. They found that hypertensives had an elevated resistance even at maximal dilation. As well the dose-response curve for norepinephrine was steeper and the maximal response was greater in the hypertensives. They concluded that the structural change of the vasculature due to the hypertrophy was such that it encroached on the lumen. The histometrical findings of Furuyama (1962) were in accord with these results. A true medial hypertrophy was observed in arteries obtained from human subjects. The increased wall/lumen ratio meant the caliber of the vessels was decreased even at maximal dilation. These and similar

results led Folkow to propose later that the increased response observed in hypertensive subjects to vasoconstrictor agents was due to the hypertrophy of the vascular smooth muscle and not due to an actual increase in reactivity (Folkow et al., 1970; Folkow, 1971).

The use of vascular strips in vitro in the study of reactivity is a useful approach to separate geometric from functional factors. Hallback et al. (1971) found that the reactivity of helical aortic strips to norepinephrine was similar between hypertensive and normotensive rats. Similarly, others using aortic strips have found either no change (Clineschmidt et al., 1970) or an actual decrease (Spector et al., 1969) in the response to norepinephrine when the tissue was obtained from hypertensive rats. This data suggest that the observed hyperractivity in the intact vessel is due to structural alterations since the aortic strips are functionally similar. However, it may be argued that in the hypertrophied tissue, the amount of agonist per unit of tissue is less. Therefore, for the responses to be similar the hypertrophy tissue would have to have a greater responsiveness.

The failure to find an elevated reactivity in aortic tissue strips from hypertensive rats may be due to the tissue studied. Large vessels such as the aorta may not be a suitable vascular tissue to represent the responsiveness of the peripheral vasculature. Hermsmeyer (1976) studied the response to norepinephrine in smaller tail artery strips from SHR and normotensive rats (Kyoto Wistar). The threshold responses were similar but the maximal response was only elevated in the SHR. The dose response curve for the hypertensive rats was shifted to the left of the normotensive rats suggesting an increased reactivity in this tissue. These authors also found that the increased contractile

response was associated with a greater depolorization in the tissue from the SHR's. Thus, these studies support both functional and structural alterations as the mechanism of increased reactivity. Collis and Vanhoutte (1977) arrived at a similar conclusion with the isolated perfused kidney preparation. They found an elevated renovascular pressor response to angiotensin II, barium chloride, 5-hydroxytryptamine and norepinephrine in kidneys obtained from spontaneously hypertensive rats as compared to the normotensive strains. However, the extent of the shift of the dose response curve to the left depended on the agonist used. If a structural difference was the sole factor determining the degree of enhancement, the responses should have been similar for all agonists. These authors suggested that structural changes alone cannot explain the enhanced renovascular reactivity. However, in spite of the elegance of Folkow's experiments and the logic of his arguments, his theory would demand that hypertensive vessels would be hyperreactive to whatever stimulus is being applied. This has not been found to be the case. In addition, in vitro studies showing increased vascular reactivity suggests additional factors other than geometric ones may also be involved in the enhanced reactivity in hypertension.

Data from other in vitro studies support this view. Hinke (1966) studied the artificially perfused ventral tail artery from desoxycorticosterone hypertensive rats and normotensive rats. They found the arteries from hypertensive rats performed more work than those from normotensive animals when contracting against a similar perfusion pressure. In this preparation, relaxation in response to a calcium-free medium was slower in the hypertensive preparation. As well, less

calcium was required to re-establish the contraction in the hypertensive preparation. Although structural alterations are known to occur, this data clearly suggested a functional alteration in the handling of calcium had occurred as well. Shibata et al. (1973), using aortic strips in a normal Ringer's medium, found that low concentrations of cobalt chloride, manganese chloride, lanthanum chloride and strontium chloride all produced contractions in tissue obtained from pre-hypertensive and hypertensive rats (SHR). The normotensive controls (Wistar) were unaffected, although the aortic strips from the SHR showed a lower contractility to norepinephrine and potassium. Janz (1979) also demonstrated a number of differences in the vascular response to other cations between the SHR and control Kyoto Wistar rat (WKY). Thus, these data demonstrated unequivocally functional differences in the vasculature between hypertensive and normotensive tissues.

The evidence supporting that structural and functional changes in vascular smooth muscle can result in increased reactivity in hypertension has been reviewed. However, it is uncertain whether these alterations play an important role in the pathogenesis of hypertension. In a series of experiments, Folkow and his co-workers established that the observed hypertrophy could be the result, rather than the cause, of the high blood pressure. The structural changes in systemic blood vessels were noted during the induction of hypertension (Folkow et al., 1973b; Lundgren, 1974) and following the lowering of regional blood pressure in the SHR by aortic ligation (Folkow et al., 1973a). Similar studies were done in the cat by ligation of arteries to the hind limb (Folkow and Sivertsson, 1968). A rapid hypertrophy was observed following a sustained rise in blood pressure. Conversely, a rapid regression

of the hypertrophy occurred when blood pressure was lowered. Pharmacological intervention to lower blood pressure early in the SHR resulted in similar changes (Folkow et al., 1973b). However, once the hypertension was well established (6 months) the reversal of the hypertrophy was less dramatic (Weiss, 1974). These results indicate that the structural changes observed were due to a reactive hypertrophy following the elevation of blood pressure. However, primary structural changes such as atherosclerosis do occur in patients with hypertension. Thus, it appears that the elevated reactivity due to structural alterations can play a role in the development of blood pressure, as well as in the maintenance of the hypertension.

Finally, studying ^{14}C-lysine incorporation into vascular proteins in prehypertensive SHR, Yamabe and Lovenberg (1974) noted that there was a slight increase in vascular smooth muscle growth when compared to the control rats. This indicated that a slight hypertrophy may preceed the elevation in blood pressure. Whether this modest increase in tissue mass is sufficient to enhance reactivity in vivo is uncertain. However, Lais and Brody (1978) studied the vasoconstrictor responsiveness to norepinephrine, and barium chloride in the isolated perfused hindquarters of the young SHR at a time when the blood pressure rise was barely detectable. They found an elevated resistance to flow, as well as a hyperresponsiveness to the vasoconstricting agents in the SHR, when compared to the normotensive controls (Wistar Kyoto). These authors implied both an altered structure due to the elevated flow resistance, as well as a functional elevation of reactivity. Doyle and Fraser (1961a,b) studied the pressor response to norepinephrine in normotensive children whose parents were either both hypertensive,

or both normotensive. Interestingly, the children of hypertensive parents showed a greater response to norepinephrine. Recently, Hollenberg et al. (1981) noted that the renal vasoconstrictor response to mental stress was greater in normotensive men that had hypertensive relatives than those who did not.

In a previous section, we discussed how some forms of hypertension may be genetically determined. It is interesting that these subjects showed an enhanced vascular responsiveness which may be the genetic marker which will eventually lead to an elevation of blood pressure.

It is difficult to separate either structural or functional factors in hypertension. However, it appears that both, by contributing to an enhanced vascular reactivity, may play an important role in the pathogenesis and the maintenance of hypertension.

2. Sodium Balance and Enhanced Vascular Reactivity

Early clinical observations and the pioneering experimental work of Dahl and his associates have implicated salt in the pathogenesis of hypertension, although the pathophysiological mechanisms responsible are still unclear. Nevertheless, over the past 20 years these findings have led to a large number of studies concerning salt-induced changes in vascular reactivity and the regulation of blood pressure.

In 1965, Reid and Laragh proposed that increased ingestion of salt may elevate blood pressure by enhancing vascular responsiveness to endogenous vasoconstrictor substances. These authors found that normal rats fed a diet high in salt, with 1% saline for drinking water, had an enhanced pressor response to angiotensin as compared to animals on a low

salt intake. Blood pressures were similar in both groups. Recent studies have further established the relationship between sodium intake and vascular responsiveness both in vivo and in vitro. It is well documented that following a high salt intake, the pressor response to exogenous angiotensin II is enhanced in normal man (Hollenberg et al., 1974), in the rabbit (Strewler et al., 1972) and in the rat (Reid and Laragh, 1975). Renal vascular reactivity is also augmented under similar conditions (Hollenberg et al., 1972; Oliver and Cannon, 1978). In vitro studies with aortic strips and isolated perfused vascular beds leads to the same conclusion. The sodium depleted state, on the other hand, resulted in attenuation of the response to angiotensin II. These alterations in vascular responsiveness have also been observed in DOCA and saline-induced hypertension in the rat (Berecek et al., 1980).

The effect of changes in sodium balance on vascular responsiveness to norepinephrine have been less consistent. The pressor response for norepinephrine has been observed to be enhanced following a high salt intake (Yoshimura et al., 1980; Thurston and Laragh, 1975). However, the renovascular response to norepinephrine following a low salt intake has been reported to be either unaltered (Oliver and Cannon, 1978) or increased (Hollenberg et al., 1972). Similarly, Strewler et al. (1972) found that a sodium restricted diet enhanced the response of aortic strips and the isolated perfused hindlimb to norepinephrine in the rabbit. Thus, depending on the experimental model chosen, and the vascular bed studied, the effect of changes in sodium balance on vascular responsiveness to norepinephrine is not uniform. In normal man, it has been recently shown that a high salt intake enhances the pressor response to norepinephrine (Rankin et al., 1981). In the

isolated perfused kidney obtained from DOCA-induced hypertensive rats, Berecek et al. (1980) demonstrated an enhanced vascular response not only to norepinephrine, but vasopressin as well.

There is considerable evidence that a high salt intake enhances vascular responsiveness for angiotensin and possibly for norepinephrine and vasopressin. A number of possible explanations for these dietary salt induced changes in the apparent vascular responsiveness exist. These are:

a) changes in the endogenous agonist level;

b) specific changes in receptor characteristics;

c) non-specific post-receptor changes;

d) interactions between vasoactive systems.

a) Changes in the Endogenous Agonist Level

In states of sodium depletion, the level of activity of the renin-angiotensin system is elevated (Brown et al., 1964; Coughlan et al., 1972; Churchill et al., 1973) coinciding with an attenuated pressor response for angiotensin II. These observations led a number of investigators to propose that alterations in sodium balance will affect the response for angiotensin by a secondary change in the plasma levels of angiotensin (Hollenberg et al., 1972; Swales et al., 1975; Thurston and Laragh, 1975; Cowley and Lohmeier, 1978; Oliver and Cannon, 1978). Elevated endogenous levels of an agonist will result in a large number of the available receptor sites being occupied. Thus, fewer receptor sites will be available to interact with the exogenous agonist.

Experimental support for this hypothesis has been derived from studies which demonstrated an enhanced response for exogenous angiotensin following acute or chronic suppression of endogenous AII levels.

Thurston and Laragh (1975) observed an enhanced response in salt restricted rats following angiotensin converting enzyme inhibition with teprotide. However, the response to norepinephrine was also enhanced suggesting a non-specific increase in reactivity, perhaps due to a lowering of the baseline blood pressure by teprotide. These authors also observed an enhanced response to AII following bilateral nephrectomy. However, the response to AII continued to increase gradually, even after the plasma angiotensin II levels would have been maximally suppressed (Oates et al., 1974), suggesting other factors were also involved. Similarly, other studies have failed to differentiate whether the observed changes in response were due to changes in the endogenous AII levels, or due to some other factors, such as increased blood volume or an actual alteration of vascular sensitivity (Hollenberg et al., 1972; Swales et al., 1975; Oliver and Cannon, 1978).

In the sodium depleted state, the plasma levels of norepinephrine are also elevated (Luft et al., 1979; Nichols et al., 1980). The vasoconstrictor response to norepinephrine has been reported to be enhanced in states of salt excess and attenuated by salt restriction (Yoshimura et al., 1980: Rankin et al., 1981). However, the response in certain vascular beds was the opposite of these systemic pressor responses (Hollenberg et al., 1972; Strewler et al., 1972). Thus, it is possible that in sodium depleted animals the vascular sensitivity for norepinephrine was enhanced (Strewler et al., 1972), however, the elevated endogenous adrenergic activity or the reduced plasma volume may have masked this sensitivity. The net result would be an attenuated pressor response to norepinephrine in vivo. This is supported by the findings of Touw et al. (1980), who reported an enhanced response for norepinephrine following

hexamethonium treatment in the rat. These experiments, though, were performed in rats on a normal sodium diet. It would be interesting to speculate that this enhanced response may have been due to a suppression of the endogenous norepinephrine levels.

Thus, unless a non-specific enhancement of vascular reactivity has occurred, the endogenous levels of an agonist could be an important determinant of the vascular responsiveness for that agonist *in vivo*.

b) Specific Changes in Receptor Characteristics

A change in receptor characteristics (affinity and/or number) would be expected to change the response to the specific agonist.

Strewler *et al*. (1972) avoided the potential effects of endogenous levels of agonist by studying isolated aortic strips. These authors found an enhanced response for AII in strips obtained from rabbits previously on a high salt intake, as compared to a low salt intake. However, the reverse was found for norepinephrine. The isoperfused rabbit hindlimb produced similar results. Similarly, Williams *et al*. (1976) found that a lower dose of saralasin, a competitive angiotensin II antagonist, was required to block the effect of angiotensin II in aortic strips from rabbits previously on a high salt intake. This greater effectiveness of saralasin at lower doses was suggested to be due to an increased affinity of the AII receptor.

Recently, Aquilera and Catt (1980) reported on angiotensin II receptor changes in the mesenteric artery and bladder obtained from rats previously on a high salt or low salt intake. They found a low salt diet decreased, while a high salt diet increased, the receptor number in these tissue. As well, the low salt situation was duplicated by the infusion of angiotensin. Although vascular tissue was studied,

it is uncertain whether this effect found in larger arteries would be representative of the effect which would occur in the peripheral vasculature.

It has been reported that renal tubular α-receptors are elevated in hypertensive (SHR) and normotensive rats following a high salt intake (Sanchez and Pettinger, 1982). Although these results are not indicative of what may occur in vascular tissue, if this were the case, then it may explain why the response to norepinephrine has been reported to be enhanced following a high salt intake (Yoshimura et al., 1980; Rankin et al., 1981). However, this seems unlikely since in isolated aortic strip preparations, the response for norepinephrine has been shown to be decreased in tissue obtained from animals previously on a high salt intake (Strewler et al., 1972).

c) Non-Specific Post-Receptor Changes

Sodium balance may also affect vascular responsiveness by inducing post-receptor changes. Earlier in vitro studies suggested that a high extracellular sodium concentration can cause an increased response to vasoconstrictor agents (Napodano et al., 1962; Heistad et al., 1971). However, these studies must be disregarded as chronic changes in the state of sodium balance do not alter the extracellular fluid sodium concentration substantially (Best et al., 1972).

In desoxycorticosterone acetate (DOCA) and saline-induced hypertension in the rat, a non-specific increase in vascular sensitivity precedes the rise in blood pressure (Berecek et al., 1980). This model was dietary salt sensitive since the rise in blood pressure, as well as the increase in vascular sensitivity was blocked by a sodium restricted diet (de Champlain, 1973; Berecek et al., 1980). This initial increase

in vascular sensitivity is not related to a muscular hypertrophy of the vasculature (Berecek and Bohr, 1977). However, a high salt intake without DOCA had no effect on the pressor response for norepinephrine and vasopressin, but did enhance the response to AII (Berecek et al., 1980). Thus, the non-specific increase in vascular sensitivity in this model is due to the combined action of DOCA and saline and not saline alone.

As well, if the enhanced contractile response was non-specific following a high salt intake, a general enhancement to all vasoconstrictor agents would be expected. Unfortunately, there have been few studies investigating the effect of salt intake on vascular responsiveness to various ions, such as barium and calcium. Indeed, the effect of sodium intake on the observed response to a number of vasoconstrictor agents is quite variable (Hollenberg et al., 1972; Strewler et al., 1972; Oliver and Cannon, 1978). Thus, while changes in salt intake may produce non-specific changes in the vascular contractile processes, the contribution of this mechanism to the long-term regulation of blood pressure is far from established.

d) Interactions Between Vasoactive Systems

A complex interrelationship exists between the various vasoactive systems of the body. The state of sodium balance influences the level of activity of a number of these systems. These include the renin-angiotensin system (Brown et al., 1964; Churchill et al., 1973), the kallikrein-kinin system (Levinsky, 1979; Margolius et al., 1974), the sympathetic nervous system (Luft et al., 1979; Nicholls et al., 1980) and the prostaglandin system (Davila et al., 1978; Oliver et al., 1980). Thus, changes in salt intake may conceivably alter the interrelationship

which exists between these systems and thus alter vascular reactivity. For example, the infusion of a vasoconstrictor into the renal vasculature results in a concomitant release of a prostaglandin-like substance (McGiff et al., 1970a,b; Aikin and Vane, 1973). In this situation, a prostaglandin synthetase inhibitor will potentiate the vasoconstrictor effects of norepinephrine and angiotensin (Aikin and Vane, 1973). Similarly, the infusion of some prostaglandins will attenuate the response to subsequent vasoconstrictor administration in the renal vascular bed (Lonigro et al., 1973).

This action may be of physiological importance since the level of activity of the prostaglandin system has been shown to be elevated in sodium depleted states in association with the increased activity of two vasoconstrictor systems, the renin-angiotensin system and the sympathetic nervous system. This may function to offset the vasoconstrictor activity of these systems since prostaglandins are known to be effective vasodilators in isolated aortic strips (Bunting et al., 1976), in isoperfused vascular beds (Dusting et al., 1978) and in whole animal preparations (Armstrong et al., 1978).

Although prostaglandins have been advocated as determinants of vascular reactivity (McGiff et al., 1976), the results of studies investigating the effect of prostaglandin synthetase inhibitors on pressor responsiveness have been inconsistent. Negus et al. (1976) found an enhanced pressor response for angiotensin II in man following indomethacin treatment. In the conscious rabbit, indomethacin had no significant effect on the pressor response for angiotensin II (Murthy et al., 1978). The discrepancy in results may be due to the species studied. Alternatively, it be be related to the endogenous levels of

prostaglandins at time of blockade. In this regard, it may be related to the level of sodium intake. A slightly elevated intake of sodium would suppress the endogenous level of prostaglandins, thus, prostaglandin synthetase inhibitors would be expected to be ineffective. The converse would hold if a low dietary sodium level were involved.

An interaction between angiotensin and the sympathetic nervous system has been well established. Angiotensin in subpressor and pressor doses potentiates the constrictor effect of nerve stimulation in isolated vascular tissue strips and certain vascular beds (Benelli et al., 1964; Zimmerman and Gomez, 1965; Zimmerman et al., 1972). Recently, Jackson and Campbell (1981) demonstrated an inhibition of this potentiation in the isolated perfused mesenteric bed of the rat following the infusion of prostaglandin E_2. These findings are consistent with the literature since prostaglandins inhibit the release of norepinephrine from adrenergic nerve terminals (Frame and Hedqvist, 1975; Hedqvist, 1976; Hedqvist, 1978), an action which would oppose the mechanism by which AII has been proposed to enhance the response to nerve stimulation (Zimmerman et al., 1972). Thus, changes in sodium intake would alter the level of activity of these systems, the interrelationship between them and conceivably vascular reactivity in vivo.

D. Summary

Blood pressure homeostasis is dependent on the regulation of cardiac output and total peripheral resistance. The failure to maintain blood pressure at "normal" levels can be assumed to be due to the failure of regulatory mechanisms to adjust cardiac output and/or total peripheral resistance appropriately. Similarly, maintained elevations of blood pressure must be due to either or both of these parameters being increased. Although total peripheral resistance is elevated in most forms of clinical and experimental hypertension, cardiac output may also be playing a role in the elevation of blood pressure due to its failure to decrease, and thereby lower blood pressure. To this extent, both parameters may be assumed to be involved in maintaining an elevated blood pressure.

Several lines of evidence indicate that increased salt ingestion is associated with a higher incidence of hypertension. Epidemiological studies as well as a number of models of experimental hypertension suggest that salt may be important in the pathogenesis of hypertension. However, the mechanisms by which increased salt intake may lead to hypertension are still unclear. A number of possible mechanisms have been proposed but none are conclusive. Enhanced vascular reactivity secondary to high salt intake remains a distinct possibility.

In established hypertension, an enhanced vascular reactivity to vasoconstrictor agents has been observed. This may be due to either a structural or functional alteration of the peripheral vasculature. Although the enhanced reactivity may be important in the maintenance of the hypertension, it is still not established if it plays a role in the initial rise in blood pressure.

Changes in sodium balancehave a consistent effect on the pressor response to angiotensin II and this is well documented. It has been proposed that the attenuated response for AII in sodium restricted animals is due to elevated endogenous levels of this agonist. However, the effect of changes in endogenous AII levels has not been satisfactorily separated from other consequences following changes in salt intake. For example, changes in plasma volume or the activity of other vasoactive systems may also have an effect on the AII pressor response.

SECTION II

PURPOSE AND SCOPE OF STUDY

A. Statement of the Problem

Vascular responsiveness to endogenous vasoactive substances is clearly an important factor in the regulation of total peripheral resistance. The contribution of any vasoconstrictor to peripheral resistance would be enhanced, in spite of "normal" plasma levels, if the vascular responsiveness to this agent was increased.

It is now generally acknowledged that the pressor responsiveness for angiotensin II and possibly norepinephrine is dietary salt sensitive. A number of hypotheses have been proposed to explain the enhanced response for AII following dietary sodium excess and the attenuated response following dietary sodium restriction, but none of these are conclusive.

Alterations in sodium balance may change the endogenous levels of certain vasoactive substances, for example, angiotensin II and norepinephrine. Elevation of these endogenous substances following a sodium restricted diet has been proposed as the mechanism by which vascular responsiveness is attenuated. The converse occurs following a high sodium intake. However, it is difficult to dissociate the effect of elevated endogenous agonists from the potential effect of dietary sodium on the vasculature, in relation to the determination of pressor responsiveness.

It has also been demonstrated that the levels of activity of a number of vasoactive systems, such as the renin-angiotensin system, the sympathetic nervous system and the prostaglandin system, are affected by dietary salt intake. Each of these systems may contribute individually to overall pressor responsiveness for AII. However, it is not known whether these interactions with angiotensin are sensitive to changes in sodium balance.

Dietary salt intake is implicated in the pathogenesis of hypertension and since vascular responsiveness is obviously an important factor in the regulation of blood pressure, the elucidation of the mechanisms which alter the response for AII and NE following changes in dietary sodium intake may contribute to our understanding of blood pressure regulation and the significance of sodium balance.

B. Choice of Experimental Model

In this thesis, the effect of sodium balance on vascular responsiveness was studied. We chose to assess vascular responsiveness by measuring blood pressure changes following intravenous infusions of vasoactive substances at incremental infusion rates. There are a number of obvious advantages with this approach. The surgical procedure was simple and no extensive manipulation was required. Once the surgery was completed, the preparation remained stable over the duration of the experiment. As well, results obtained with the constant infusion method were much more reproducible than those with bolus injections. In addition, a large body of data from the literature was available pertaining to studies in the rat on dietary sodium intake, plasma renin activity, plasma volume and hypertensive models etc., which have utilized a similar approach.

With this preparation, a complete dose response curve for AII or NE could be easily obtained which could provide more valuable information than would be obtained from the single dose studies. Finally, the preparation is physiological. The use of pressor responses in vivo in the study of blood pressure regulation has an advantage over studies involving vascular responses in regional beds or isolated organs in that the parameter being measured is the parameter under study.

A few disadvantages were also inherent in this preparation. The observed change in blood pressure may be due to changes in cardiac output as well as total peripheral resistance. Theoretically, it would be erroneous to conclude that any change in blood pressure directly reflects corresponding change in peripheral resistance. However, experiments in our laboratory, as well as others (Touw et al., 1980), have demonstrated

that, for the most part, the rates of infusion used in this study do not affect cardiac output significantly. Thus, changes in the blood pressure response or pressor responsiveness could be used as a qualitative index of the vascular reactivity. It was, however, unknown whether the infusion of the exogenous substances did not some how alter the cardiovascular compensatory reflexes.

The evaluation of vascular sensitivity in high and low states of sodium balance was achieved with the isolated kidney perfused with Krebs-Henseleit solution. The kidney to be perfused can be easily isolated *in situ*. As well, the surgical preparation was such that there was no manipulation of the kidney, and at all times the kidney to be studied was perfused with either blood or perfusate. The isolated kidney as a vascular preparation has an added advantage over tissues obtained from large arteries. It represents a peripheral resistance bed. As well, it allows the elimination of neural and blood-borne factors which may have altered the *in vivo* pressor responses.

Obviously, the renal vascular bed may not be representative of the total vasculature. This is a problem inherent in any isolated organ preparation. It may be questioned whether this *in vitro* preparation is sensitive enough to detect any changes in vascular sensitivity, however, preliminary studies in our laboratory showed it to be sufficiently sensitive with a protein-free perfusate at 20°C. The addition of protein in the perfusate and maintaining the medium at 37°C did not alter the results qualitatively.

These experiments required placing animals on a high or low sodium diet. The Sprague-Dawley rat was chosen because it is convenient to handle and economically feasible. A larger animal such as the dog would be less desirable.

C. Experimental Objectives

The experimental objectives are as follows:

1. to determine the effect of changes in sodium balance on the vascular responsiveness for AII and NE *in vivo* and *in vitro* in the rat;

2. to investigate the mechanisms responsible for the dietary salt-induced changes in vascular responsiveness, particularly the interactions between the renin-angiotensin, adrenergic and prostaglandin systems.

SECTION III

GENERAL METHODS

A. Experimental Animals

Male, Long Evans hooded rats weighing 300-450 grams were used. All animals in any study group were generally litter mates. All animals were maintained on a standard Purina rat chow and tap water prior to being placed on either dietary regime outlined below.

1. Low Sodium Intake

Two regimes were used to achieve a low state of sodium balance:

(1) Animals were fed special sodium deficient chow pellets (ICN Pharmaceuticals; sucrose 72.0%, vitamin free casein 18.0%, salt free butter fat 5.0%, sodium free salt mixture 5% and ICN vitamin diet fortification mixture). Tap water was used for drinking. Animals were placed in metabolic cages in groups of four and the diet administered ad libitum for a period of 21-28 days. The weight of each animal was recorded prior to the diet and on the last day of the diet.

(2) In this regime, sodium depletion was exaggerated by the use of the natriuretic drug, furosemide. Animals were fed sodium deficient chow pellets. Tap water was used for drinking. This regime lasted 10 days. On the first two days of the diet, furosemide (5 mg/Kg) in a volume of .30 - .50 ml was administered intraperitoneally. Weights of each animal were recorded on days 1, 2 and 10 of the diet.

2. High Salt Intake

Two regimes were utilized to achieve a high state of sodium balance:

(1) All animals were fed standard Purina rat chow with 0.9% saline substituted as drinking water. Animals were placed in metabolic

cages in groups of four and the diet administered ad libitum for a period of 21-28 days. The weight of each animal was recorded prior to the institution of the diet and on the last day of the diet.

(2) In this regime, dietary salt supplementation was used in conjunction with a salt retaining steroid, desoxycorticosteroid acetate (DOCA). Animals were fed standard Purina rat chow with 0.9% saline substituted as drinking water for a period of 10 days. On days 8, 9 and 10, DOCA (.5 mg/Kg) in a volume of 0.03 - 0.05 ml was administered intraperitoneally. The final injection of DOCA was given at least 24 hours before the start of the experiment. Weight of each animal was recorded on days 1, 8, 9 and 10.

3. Normal Salt Intake

Rats were fed a standard Purina rat chow with tap water as the fluid source. The weights of these animals were recorded only on the day of the experiment.

B. Experimental Design

1. Determination of Renal Function and Electrolyte Excretion

Twenty-four hours prior to the experiment, animals were weighed, placed in separate metabolic cages and food, but not drinking fluid, withheld. A 24 hour urine specimen was collected during this time. At the termination of the experiment, a blood sample was obtained from a carotid catheter as described below. Urine and separated plasma were frozen for subsequent batch analysis. The plasma and urine were analyzed for:

(1) Sodium (Na^+) and Potassium (K^+): standard spectrophotometry using a Beckman model Klina Flame photometer.

(2) Creatinine: colorimentry using a modified Jaffe method (Beckman Creatinine Analyzer Model 2).

From these results, the daily excretion rate of sodium and potassium and the clearances of creatinine, sodium and potassium could be calculated. The methods of calculation are presented in Appendix A.

2. In Vivo Vascular Responsiveness

a) Preparation of Drugs

A summary of drugs used and their preparation is presented in Appendix B. The stock concentration was chosen such that when an effective dose of study drug was administered, the volumes delivered would not become a factor in the blood pressure response. For example, an intravenous angiotensin I bolus injection of .15 ml of stock solution would deliver 200 ng of drug producing an increase in blood pressure of 15-30 mm Hg. A similar volume of saline was shown to have no effect. Larger volumes of indomethacin and phenoxybenzamine were administered (≃ .5 ml), however, this was given intraperitoneally and not intravenously.. Fresh frozen aliquots of drugs were thawed at the start of each experimental week. This drug preparation was kept refrigerated throughout the experiment.

List of drugs used:

Angiotensin I	Peninsula Inc., California
Angiotensin II (Hypertensin^R)	CIBA
Captopril (Capoten^R)	Squibb Ind. - N.J.
Indomethacin (Indocid^R)	Merck, Sharpe and Dohme
Meclofenamate	Warner - Lambert Company
Norepinephrine (Arternol^R)	CALBIOCHEM

Pentobarbital Sodium (Nembutal^{R})	British Drug House
Pentolinium Tartrate (Ansolysen^{R})	Wyeth
Phenoxybenzamine (Dibenzyline^{R})	Smith, Kline and French
Sodium Nitroprusside (Nipride^{R})	Hoffman-LaRoche

b) Surgical Procedures

Following the specified dietary period and completion of urine collection, the experimental animal was anaesthetized with intraperitoneal pentobarbital (≃ 50 mg/Kg). A tracheostomy was performed and the animal was allowed to breathe spontaneously. Mean arterial blood pressure was measured using a Statham pressure transducer (Model P23 Dc), on a Grass polygraph Model V via a polyethylene catheter (PE60) placed in the right carotid artery. The right jugular vein was then cannulated for the administration of additional anesthetic and other drugs. Multiple catheters were used. The specific number and size of the cannulae varied according to the experimental requirements and this information is covered in the appropriate section of Specific Experiments. Infusion of study drugs was achieved with a Harvard variable speed infusion pump. All infusion lines were filled with the appropriate study drug prior to the experiment to eliminate dead space. An overhead lamp was used to help prevent the experimental animal from becoming hypothermic. The preparation was allowed to stabilize for a period of 30 minutes. Additional anesthetic was administered (3 mg/Kg) as required.

c) Experimental Procedure

The general protocol, summarized in Figure 1, was as follows. Following stabilization the pressor responses to increasing incremental

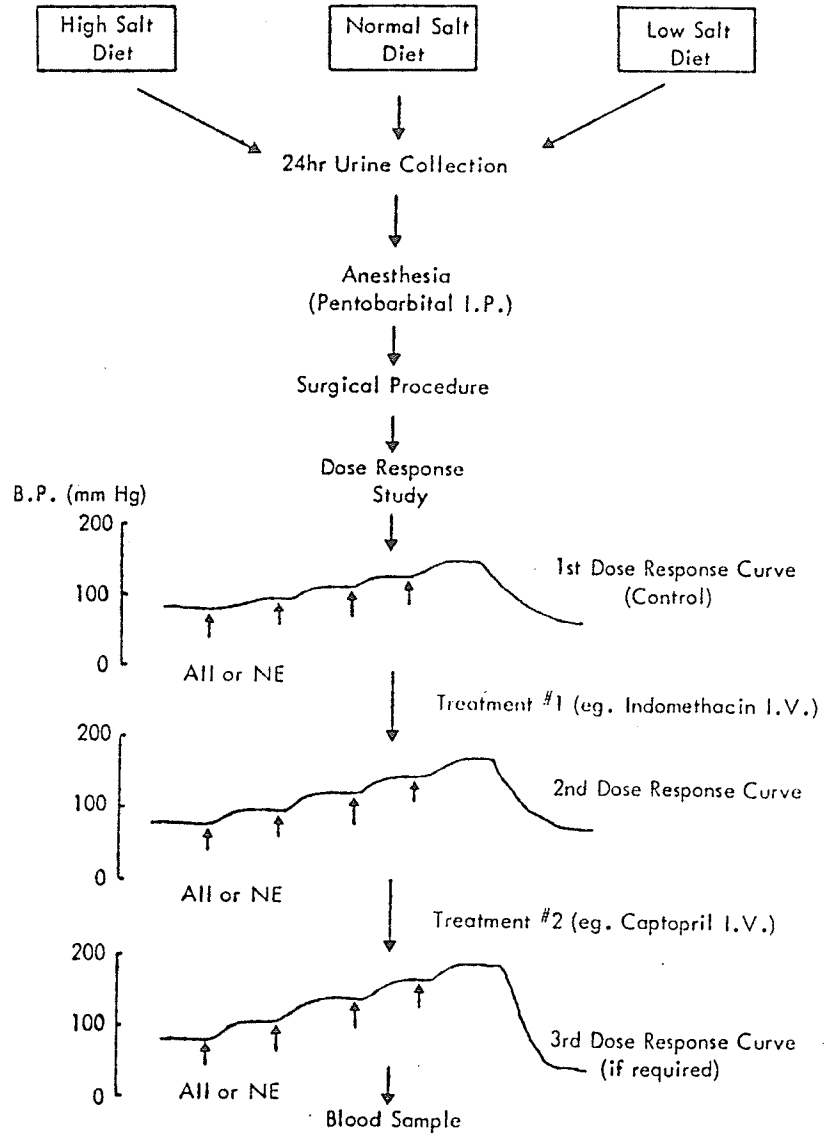

Figure 1: Schematic diagram of experimental protocol. Following the surgical procedure, two or three dose response curves for either angiotensin (AII) or norepinephrine (NE) were done. The first dose response curve represented the control response for that animal. Between each dose response curve done, an experimental treatment was administered. Each dose response curve required 10-15 minutes to complete.

rates of AII (.03, .10, .30, 1.0, 3.0 µg/Kg/min) or NE (.10, .30, 1.0, 3.0, 10.0, 30.0 µg/Kg/min) were obtained. The rate of infusion varied from .0010 to .102 ml/min. A plateau in the blood pressure response was achieved before continuing with the next dose. Except for the lowest infusion rate, the total time of infusion at any speed never exceeded 5 minutes. The total time to complete a dose response curve was 10 to 15 minutes. Generally, only one of the above pressor drugs (AII or NE) was studied in any particular rat. At the end of the infusion, the blood pressure was allowed to return toward control values and reached a steady state before any further manipulation. Two or more of these dose response curves with similar time sequences were done in each animal. Between the dose response studies, at a point when blood pressure was stable, an experimental treatment was administered (see Specific Experiments). Thus, the first dose response curve served as a control response for that animal. At the end of the experiment, a blood sample was taken, and the separated plasma frozen for subsequent determination of sodium, potassium and creatinine. In most cases, the kidneys were removed and weighed. In some studies, the experimental animal was also used for the isolated perfused kidney studies. In these cases, the animal was left intact and given an additional injection of anaesthetic.

The exact experimental procedure varied slightly between studies. These differences are covered under the appropriate sub-heading in the section of Specific Experiments.

3. In Vitro Vascular Sensitivity

The isolated kidney perfused at a constant flow rate was used for the assessment of in vitro vascular sensitivity.

a) Preparation of Solutions

i) Perfusion Medium

Krebs-Henseleit was used as the perfusion medium: NaCl, 11.0 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 26.0 mM; Mannitol, 20.0 mM; Dextrose 11.0 mM. Calcium chloride was added only after the other ingredients had been dissolved in distilled water and bubbled with 95% O_2 - 5% CO_2. Creatinine (2 mg%) was added to the solution. The final solution was kept at room temperature and bubbled with 95% O_2 - 5% CO_2.

ii) Angiotensin II

The in vivo stock solution (10^{-4} g/ml) was used to make serial stock solutions, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} gm/ml in distilled water. These solutions were kept refrigerated throughout the experiment.

iii) Norepinephrine

The in vivo experimental stock solution (10^{-3} gm/ml) was used to make the same serial dilutions as above in .01N HCl. These solutions were refrigerated throughout the experiment.

b) Surgical Procedure

Figure 2 represents the in situ isolated perfused kidney preparation diagrammatically. Following anaesthesia, an abdominal cruciate incision was made and the abdominal viscera reflected to the right, exposing the aorta. The infrarenal aorta was cannulated with a PE60 catheter. This catheter was used for continuous pressure monitoring using a pressure transducer as described. The superior mesenteric artery

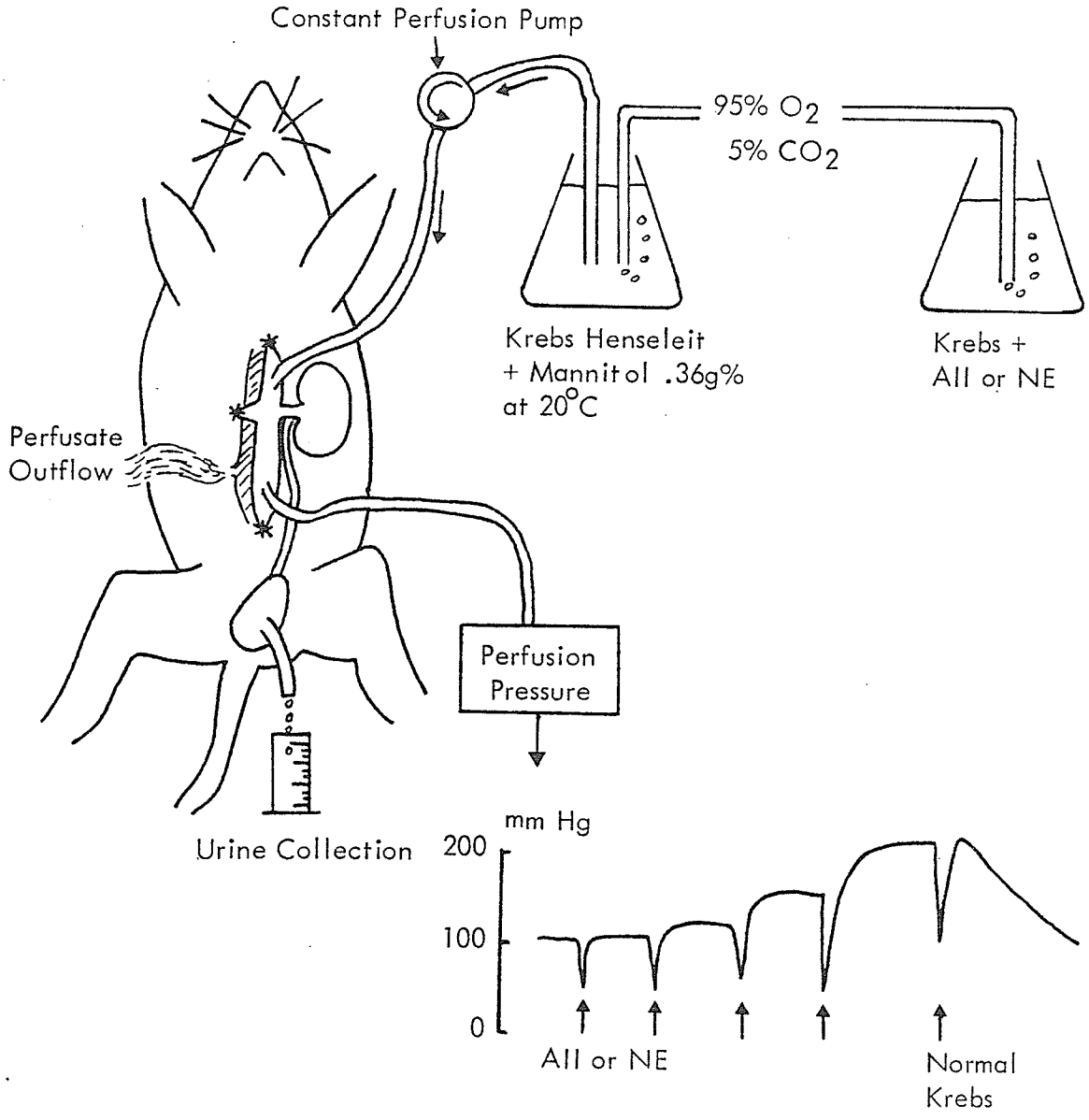

Figure 2: Schematic diagram of the in situ perfusion of the isolated rat kidney. The Krebs-Henseleit perfusate enters the isolated segment of the aorta, including the kidney, via the superior mesenteric artery. The inferior vena cava was severed. Flasks containing increasing concentrations of AII or NE in the perfusate were introduced to the system. Perfusion pressure was measured by a cannula placed in the aorta distal to the renal arteries.

was cannulated in the retrograde direction with a PE20 catheter and connected to a Cole-C Palmer peristaltic perfusion pump. Oxygenated Krebs-Henseleit solution with .36 g% mannitol added was used as the perfusate. Once the perfusion was begun, the aorta above the superior mesenteric artery was ligated, thus isolating the renal circulation. The superior vena cava was then severed to provide an outflow for the perfusate. The right renal artery was ligated, the right kidney removed, and the animal killed. The bladder was cannulated for urine collection. This preparation allowed the isolated perfusion of the left kidney without manipulation of the kidney itself or interruption of flow to this kidney. The effectiveness of isolation could be confirmed at the end of the experiment by the addition of ink to the perfusate. The perfusion rate was adjusted initially to produce a perfusion pressure at the level of the renal artery of about 100 mm Hg. The preparation was allowed to stabilize for 30 minutes. At a constant perfusion rate, any changes in perfusion pressure following the addition of angiotensin II or norepinephrine would reflect a change in renal vascular resistance. Preliminary experiments have shown this preparation to be stable over two hours and the vascular response reproducible at room temperature or 37°C. These experiments were done at room temperature.

c) Experimental Procedure

Following a stabilization period, where the perfusion pressure and rate were unchanged for 15-20 minutes, a timed urine specimen was obtained. Urine volume was measured and frozen with a sample of the perfusate for later determination of the creatinine, sodium and potassium levels. At this time, a dose response curve to AII or NE was done. Erlenmeyer flasks containing a known amount of AII or NE diluted with

perfusate were oxygenated with 95% O_2 - 5% CO_2. The concentrations of AII or NE used varied from 10^{-10} gm/ml to $3x10^{-6}$ gm/ml. The perfusion pump was then stopped. At this point, the source of perfusate was changed to a flask containing the desired concentration of AII or NE and then perfusion restarted. This procedure was repeated for all doses. Perfusion was never interrupted for more than 3 seconds. Perfusion at each drug concentration lasted until the perfusion pressure reached a steady state (≃ 1-2 minutes). The pump speed was left unaltered and the rate of flow determined at the end of the experiment. The perfused kidney was removed and weighed at the termination.

4. Cardiac Output

Cardiac output was measured in vivo with radiolabelled microspheres, by a modified method of Bartrum et al.(1974). Rats maintained on a normal sodium intake were used. Following intraperitoneal pentobarbital anaesthesia (50 mg/Kg) a tracheostomy was performed and the animal was allowed to breathe spontaneously. A polyethylene catheter (PE60) was placed into the left ventricle via the right carotid artery. The position of the tip of the catheter was identified by the typical left ventricular pressure pattern, monitored with a Statham pressure transducer (P23Dc) on a Grass polygraph. The left femoral artery was cannulated (PE10) for collection of the reference blood sample. The right jugular vein was cannulated (PE20) for infusion of a study drug.

Microspheres (3M Company) with a diameter of 15 ± 5μ and labelled with strontium^{85}, cerium^{141} or iodine^{125} were used. The microspheres were suspended in a 64% sucrose solution with a few drops of Tween 80 (a detergent used to prevent aggregation) added. Each cardiac output determination used 60,000 microspheres in a total volume of .2 ml. Prior to injection,

the stock suspension of microspheres (300,000 spheres/ml) was subjected to sonification for no less than 15 minutes to prevent clumping of the spheres.

After completion of surgery and following a stabilization period, 0.2 ml of a radiolabelled microsphere solution (60,000 microspheres) was injected into the catheter placed in the left ventricle. Just prior to, during and following the injection, the animal was allowed to bleed freely from the left femoral catheter. The blood specimen obtained was weighed and the bleeding time accurately recorded. This blood specimen was used as the reference blood sample. The collection period for the reference blood sample varied from 60-90 seconds. The lines were then flushed (.1 - .2 ml saline) and the preparation allowed to stabilize. Following this, a known constant infusion rate of angiotensin, norepinephrine, or saline was administered. When angiotensin or norepinephrine was infused, a plateau in the increase in blood pressure was reached before the second cardiac output determination was made, with a different radiolabelled microsphere. After this, once the preparation was again stable, the infusion rate of the drug being studied was increased. When the blood pressure reached a new steady state, the third cardiac output determination was done with a different radiolabelled microsphere. The order of administration of the three isotopes varied. At the end of the experiment, the kidneys, portions of the lung and ileum were removed and weighed. Standard solutions (1 ml of microspheres), blank tubes, blood samples and weighed tissue samples were counted in a gamma counter (Searle).

Cardiac output was calculated by the equation:

$$C.O. = I_i \times \frac{Q_r}{I_r}$$

Where C.O. = cardiac output (ml/min)

I_i = corrected counts per minute of micrispheres injected into left ventricle.

I_r = corrected counts per minute of reference blood sample.

Q_r = reference blood flow (ml/min).

A computer program was written to calculate cardiac output and blood flow of tissues counted. (Appendix C)

5. Measurement of Plasma Renin Activity

Plasma renin activity was measured by radioimmunoassay in high and low sodium rats in the laboratory of Dr. J. K. McKenzie with a modification of the Haber method (Haber et al., 1969). The renin activity was determined indirectly by measuring the amount of angiotensin I formed in 1 ml of plasma in one hour.

Briefly, angiotensin I antibodies and radiolabelled angiotensin I (I^{125}) were added to plasma that had been previously incubated with three metal binding reagents. These reagents served to inhibit angiotensin I proteolysis by angiotensinases. This mixture was allowed to equilibrate overnight. To separate the bound antibody from the free peptide in the mixture, a second stage antibody was used instead of a dextran coated charcoal. This final step represented the only significant modification from the method of Haber et al. (1969). In a separate set of test tubes, known amounts of angiotensin I were treated in the same fashion as the plasma samples. From this data, a standard curve could be obtained. This allowed the quantitative relationship between the amount of unlabelled angiotensin present and the labelled angiotensin

released from the antibody complex to be assessed in the plasma samples.

C. Statistics

Statistical analysis for comparison of the dose response points was achieved with analysis of variance followed by Duncan's multiple-range test to determine the level of significance between study groups. According to experimental design, data was blocked for analysis when appropriate. Data analyzed in this fashion is presented as the mean ± pooled standard error, unless otherwise stated. Comparison of other parameters was done with the Student's t test and when appropriate, data were paired. This information is presented as mean ± standard error unless otherwise stated. The basis for these comparisons were obtained from "Principles and Procedures of Statistics" (Steel and Torrie, 1960).

SECTION IV

SPECIFIC EXPERIMENTS

A. SODIUM BALANCE AND THE ROLE OF ENDOGENOUS ANGIOTENSIN II IN THE REGULATION OF PRESSOR RESPONSIVENESS IN VIVO AND IN VITRO

1. Effect of Dietary Sodium Intake
2. Effect of Angiotensin Converting Enzyme Inhibition
3. Effect of Nitroprusside-induced Hypotension
4. Effect of Duration of Anesthesia
5. Effect of Sodium Intake on Renovascular Sensitivity In Vitro
6. Effect of Isotonic Saline Volume Expansion
7. Discussion.

1. Effect of Dietary Sodium Intake

The effect of sodium balance on the in vivo vascular responsiveness to angiotensin II and norepinephrine has been previously documented (Reid and Laragh, 1965; Thurston and Laragh, 1975; Slack and Ledingham 1976; Oliver and Cannon, 1978; Brunner et al., 1972 and Hollenberg et al., 1972). In all cases, the pressor response to angiotensin II was enhanced in states of high sodium balance and attenuated during sodium depletion. However, the vasoconstrictor response for norepinephrine was more variable. In states of sodium depletion, either an increased response (Strewler et al., 1972 and Hollenberg et al., 1972) or no change (Oliver and Cannon, 1978) has been observed. The variation in responses reported may have been due to the model studied.

In this section, the effect of dietary sodium intake on body weight, renal function and vascular responsiveness to angiotensin II and norepinephrine are reported. The purpose of these experiments is to assess the effectiveness of changes in dietary salt intake on vascular responsiveness. These experiments also serve to establish the validity of the experimental model for later studies.

a) Method

The dietary regimes have been described in Section A under General Methods. Five main groups of rats were studied. The first two groups were placed on either a high salt intake only, or a low salt intake only, for a period of three weeks. Because the pressor response to AII was significantly different between these two groups only at one rate of AII infusion (see below), an attempt was made to accentuate this difference by the addition of a salt-retaining steroid, DOCA, to the high salt intake group and the addition of a diuretic, furosemide, to

the low salt intake group. These animals made up the next two groups of animals studied. Finally, a fifth group of animals received a normal dietary regime. In these five groups of rats we compared the body weights, creatinine clearances and the 24 hour urine sodium and potassium excretion. The pressor responses to AII and NE following a high or low salt intake were compared only between animal groups receiving dietary changes or between animal groups receiving one of the supplementary drugs.

b) Results

i) Body Weights

Table 1 shows the effect of the dietary regimes on body weight. There were no significant changes in body weight in all groups following dietary treatment. As well, the final body weights in all groups studied were similar.

The changes in body weight for the groups given the supplementary drug injections are presented as the percent change from the pre-diet weights (Table 2). In the high sodium group, a slight weight gain was noted. The precent body weight after one week on a high dietary sodium intake had increased to 113.1 ± 2.0% of control. However, the low sodium group with added furosemide did not demonstrate the slight increase in body weight observed in the high salt group. In both groups, body weights were decreased on the day of the experiment. This may be due to food being withheld for a 24 hour period for the collection of urine the day prior to the day of the experiment.

ii) In Vivo Renal Function

The effect of sodium balance on creatinine clearance (C_{Cr}), urinary volume, urinary excretion of sodium (UV_{Na}) and potassium (UV_K)

TABLE 1

Effect of Dietary Salt Intake on Body Weight

	N	INITIAL B. Wt. (grams)	B. Wt. AFTER DIET (grams)	P
Normal Salt	5	-	311.4 ± 11.9	-
High Salt Alone	9	296.4 ± 16.9	314.0 ± 24.1	NS
Low Salt Alone	6	291.4 ± 12.3	315.5 ± 25.9	NS
High Salt & DOCA	14	286.8 ± 8.1	312.0 ± 10.0	NS
Low Salt & Furosemide	36	330.4 ± 7.3	315.9 ± 5.5	NS

Values are expressed as means ± S.E.

TABLE 2

Effect of Dietary Salt Intake on Body Weight
in DOCA and Furosemide Supplemented Rats

	Initial Wt. (gm)	% Initial Body Weight				
		Day 1	Day 8	Day 9	Day 10	Exp
High Salt + DOCA (n=14)	286.8 ±8.1	-	113.1 ±2.0	114.6 ±1.7	115.1 ±1.8	109.1 ±3.0
Low Salt + Furosemide (n=36)	330.4 ±7.3	99.1 ±.60	-	-	101.0 ±1.1	96.2 ±1.1

Data are presented as the % of the initial body weight. Days 1, 8, 9 and 10 represent length of time animals had been on dietary regime. Values are expressed as means ± S.E.

are shown in Table 3. Rats placed on a low sodium intake had a lower urinary volume than those with a high sodium intake. In animals on a low sodium diet alone, urine volumes were lower than both of the high salt groups ($P < .01$). The low salt group supplemented with furosemide had a urinary volume that was only significantly less than the high salt group given a DOCA supplement ($P < .05$). Rats on a normal diet had intermediate urine volumes.

Daily sodium excretion rates were used as an index of the state of sodium balance. The low sodium groups excreted .015 ± .003 (n=8) and .010 ± .002 mEq (n=39) of sodium daily. These were significantly lower than either high salt groups at 2.3 ± .36 (n=10) and 1.50 ± .07 (n=14) mEq. The normal salt intake was between these groups at .33 ± .13 mEq/24 hours. There were no significant differences in the daily urinary potassium excretion and the creatinine clearance between the groups.

The percent fractional excretion of sodium (% FE_{Na}) of the low salt intake groups were significantly greater than in the high salt intake groups ($P < .01$).

iii) In Vivo Vascular Responsiveness

Table 4 shows the effect of different dietary regimes on blood pressure. Blood pressure measurements were similar between the animals on a low salt intake (101.7 ± 3.4 mm Hg) and the animals on a low salt intake plus furosemide (107.8 ± 3.1). Rats fed on a high salt diet alone had a mean blood pressure (85.2 ± 5.1 mm Hg) that was significantly less than both low salt groups ($P < 0.5$). Similarly, the high salt plus DOCA group had a mean blood pressure (81.5 ± 3.8 mm Hg) that was significantly less than both low salt groups ($P < .01$) as well as the normal salt group ($P < .01$).

TABLE 3

Effect of Dietary Salt Intake on Renal Function In Vivo

	Normal	High Salt	Low Salt	High Salt + DOCA	Low Salt + Furosemide
Urine Volume (ml/day)	6.8 ±2.2	10.7 ±2.0	3.1 ±1.6	10.2 ±1.2	7.1 ±.7
UV_{Na} (mEq/day)	.33 ±.13	2.3 ±.36	.015 ±.07	1.50 ±.07	.010 ±.002
UV_K (mEq/day)	1.63 ±.24	1.38 ±.21	1.20 ±.17	1.53 ±.13	1.27 ±.08
C_{Cr} (ml/min)	1.4 ±.4	1.6 ±.3	1.3 ±.3	1.3 ±.2	1.4 ±.1
$\%FE_{Na}$	.076 ±.127	.494 ±0.110	.027 ±.083	.664 ±.059	.005 ±.038
N	5	10	8	14	39

Values are expressed as means ± S.E.

TABLE 4

Effect of Dietary Salt Intake on Blood Pressure
in the Anesthetized Rat

	Low Salt	High Salt	Normal	Low Salt + Furosemide	High Salt + DOCA
Blood Pressure	101.8 ±4.4	85.2 ±5.1	104.4 ±9.6	107.8 ±3.1	81.5 ±3.8
N	9	12	5	26	11
P		< 0.05			< 0.01

Values are expressed as means ± S.E.

Figures 3 and 4 show the effect of high and low dietary sodium intake on angiotensin II pressor responsiveness. In the sodium depleted state, the pressor response for angiotensin II at 0.3 μg/Kg/min was lower ($P < .05$).

When the high salt diet was supplemented with DOCA and the low salt diet supplemented with furosemide, the difference was slightly enhanced (Figure 4). The pressor response was significantly greater in the high salt intake plus DOCA group at AII infusion rates of 0.3, 1.0 and 3.0 μg/Kg/min ($P < .05$), when compared to the low salt group plus furosemide group.

Similar experiments were done using norepinephrine. No significant difference in the pressor response to norepinephrine was observed between the high and low salt groups (Figure 5). The mean values, however, were greater in the high salt gorup.

iv) Plasma Renin Activity

In two separate groups of high salt (n=9) and low salt (n=6) rats, a plasma sample was obtained from each animal for subsequent determination of plasma renin activity. The results are shown in Table 5. Animals which had been placed on a low sodium intake for three weeks had a significantly higher plasma renin activity (28.95 ± 3.7 ng AI/ml/hr) than the high salt group (2.18 ± .81 ng AI/ml/hr; $P < .001$).

b) Comments

In this section two distinct dietary groups have been demonstrated. It is reasonable to assume that a steady state in sodium balance was achieved in animals that had been placed on their respective diets for 10 or 21 days. Thus, a high urinary sodium excretion would reflect a high state of sodium balance and a low urinary sodium excretion would reflect a low state of sodium balance. Similarly, the % FE_{Na} was greater

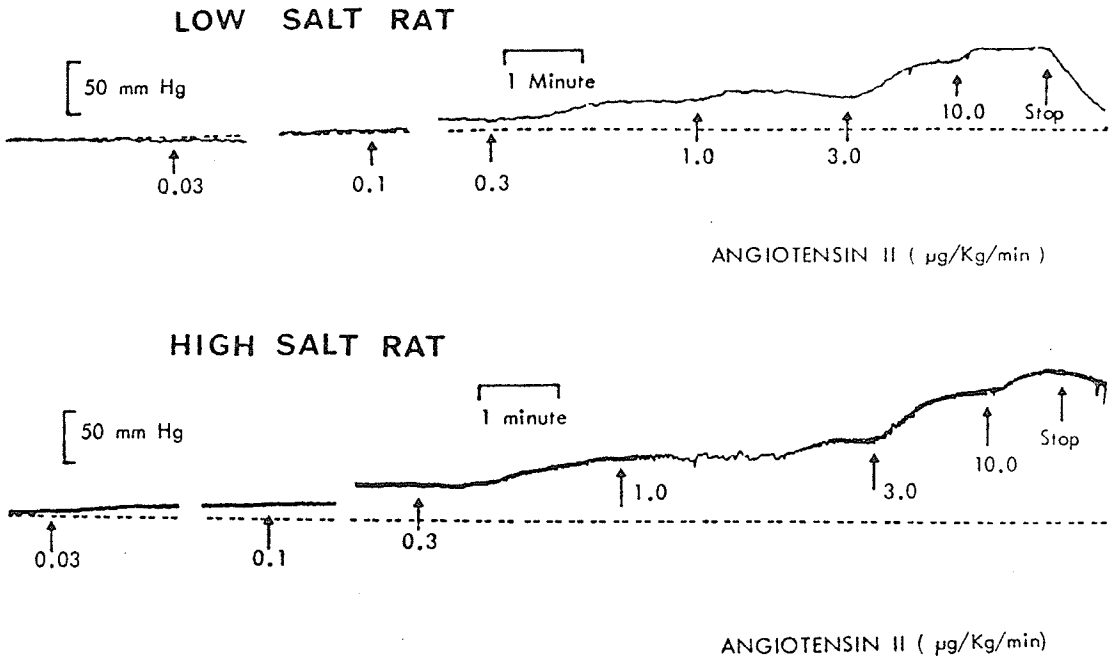

Figure 3: Representative record of blood pressure response to angiotensin II in a high salt and a low salt rat.

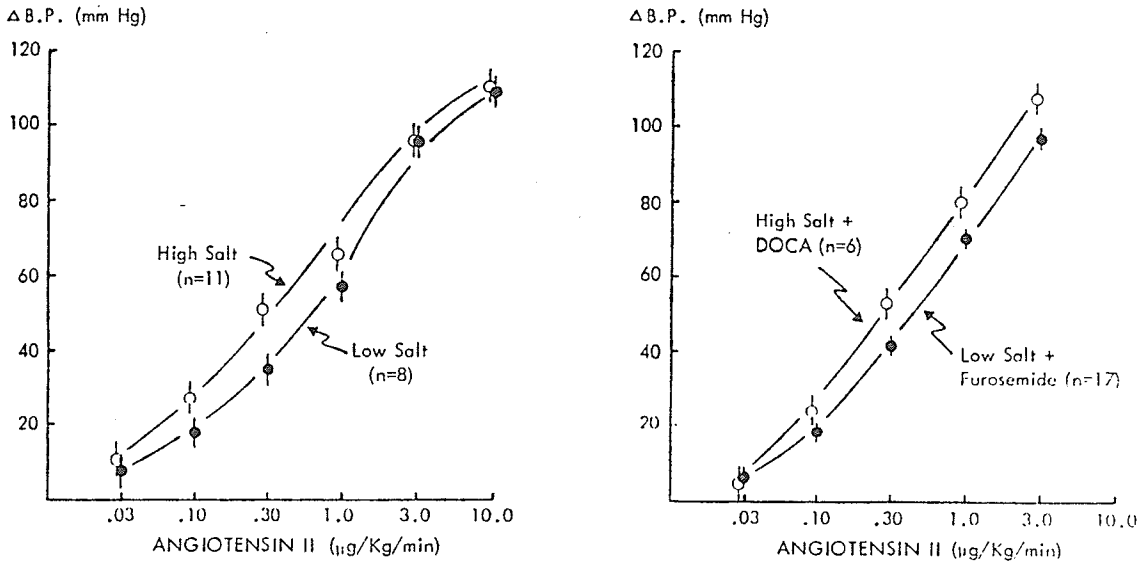

Figure 4: Effect of sodium balance on pressor responsiveness to angiotensin II in the rat. Animals were placed in a state of high or low sodium balance by diet alone (left) or diet supplemented with DOCA or furosemide (right).

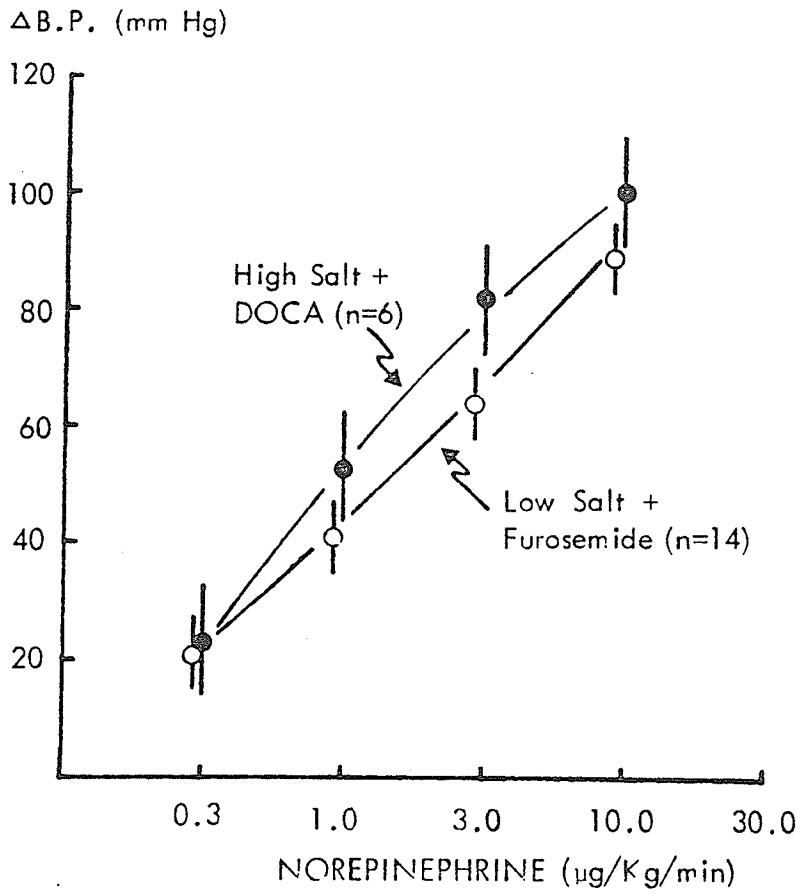

Figure 5: Effect of sodium balance on pressor responsiveness to norepinephrine in the rat. Animals were placed on a high or low salt intake supplemented with DOCA or furosemide.

TABLE 5

Effect of Dietary Salt Intake on Plasma Renin Activity in the Anesthetized Rat

(ng A 1/ml/h)	High Salt	Low Salt	P
Mean	2.18	28.95	<.001
S.D.	2.51	9.12	
S.E.	0.81	3.72	
N	9	6	

in the high salt intake groups than the low salt intake groups. Potassium homeostasis, however, was minimally affected. Only minor differences in potassium excretion were observed.

Plasma renin activity was significantly greater in the rats that had been placed on a low salt intake. In these low salt animals the pressor response to AII was attenuated. The addition of furosemide or DOCA to the dietary regimes resulted in qualitatively similar results. Drug supplementation, however, slightly accentuated the differences between the high and low salt groups. The pressor responsiveness to NE was similar in the high and low salt animals.

The mechanism responsible for the decreased pressor response to AII in the low salt groups is uncertain. In these animals the plasma renin activity is about 14 times greater than in the high salt animals. It is possible that the decreased response may be due to an elevated endogenous level of angiotensin II as reflected by the elevated plasma renin activity. This may artificially lower the pressor response for AII due to the endogenous AII competing with the exogenous AII for the receptor site. Alternatively, the sodium depleted state may induce a decrease in vascular sensitivity to angiotensin II which can result in attenuation of the pressor response for the exogenous vasoconstrictor.

2. Effect of Angiotensin Converting Enzyme Inhibition

The direct relationship between the state of sodium balance and pressor responsiveness to angiotensin II reported in the previous section is consistent with the published literature (Reid and Laragh, 1965; Brunner et al., 1972). The response to norepinephrine remained unaltered.

There is evidence available which indicates that differences

in angiotensin II receptor occupancy may be responsible for these changes (Thurston and Laragh, 1975; Oliver and Cannon, 1978). In the sodium depleted state, plasma renin is elevated (Brown et al., 1964; Coghlan et al., 1972) which results in increased endogenous levels of angiotensin II. This elevated level of endogenous angiotensin could compete with the administrated angiotensin for the receptor site. The result would be a shift in the dose-response curve to the right. Thus, if the endogenous level of angiotensin II is a major determinant of pressor responsiveness, reduction of the endogenous angiotensin level would be expected to increase the pressor response to the exogenously administered angiotensin. This has been demonstrated by increasing sodium intake, as shown earlier, bilateral nephrectomy (Swales et al., 1975) or using a converting enzyme inhibitor, teprotide (Thurston and Laragh, 1975).

The purpose of these experiments was to determine whether the endogenous level of AII affects AII pressor responsiveness in vivo, and if this is the case, whether the observed effect is specific for AII. In these experiments, endogenous AII levels were altered by using animals in high and low states of sodium balance and with a converting enzyme inhibitor, captopril.

a) Methods

Four groups of male Long Evans rats weighing 300 to 400 grams were studied. Two groups of animals were placed on a high salt diet and two groups on a low salt diet, either by changes in the diet alone, or by changes in diet supplemented with furosemide or DOCA, as described earlier.

The experimental protocol required the completion of two dose response curves to AII or NE in each animal. Captopril was administered

between the dose response curves. Following the intravenous captopril (.3 mg/Kg), the blood pressure was recorded for 10 to 15 minutes before the second dose response was done. Preliminary experiments had shown blood pressure to remain stable after this period. To assess the effectiveness of the converting enzyme inhibition, an intravenous bolus injection of angiotensin I (200 ng) was administered prior to the captopril and again at the end of the experiment. This enabled a quantitative estimation of the percent inhibition of the converting enzyme by comparing the pre and post-captopril angiotensin I pressor activity.

b) Results

Administration of captopril generally resulted in a hypotensive response. The response usually occurred in two phases. In all groups, there was a significant and rapid initial decrease in blood pressure. This was followed by a gradual return of the blood pressure towards the pre-captopril level, usually over a period of 10 to 15 minutes. Representative records are shown in Figure 6. The groups' responses are summarized in Table 6. In both high salt groups, the post-captopril steady state blood pressure was similar to the pre-captopril levels. However, in the low sodium intake groups, converting enzyme inhibition resulted in a sustained decrease in blood pressure when compared to the pre-captopril levels. The baseline blood pressures for rats on a low salt intake and a low salt intake with furosemide were suppressed by -10.8 ± 3.5 mm Hg ($P < .01$) and -7.9 ± 3.0 mm Hg ($P < .05$) respectively.

The pressor response for angiotensin was greater in the high salt group as compared to the low salt group. In rats given a high salt intake without DOCA, converting enzyme inhibition had no effect on the pressor response for AII (Figure 7). Animals on a low salt intake alone without

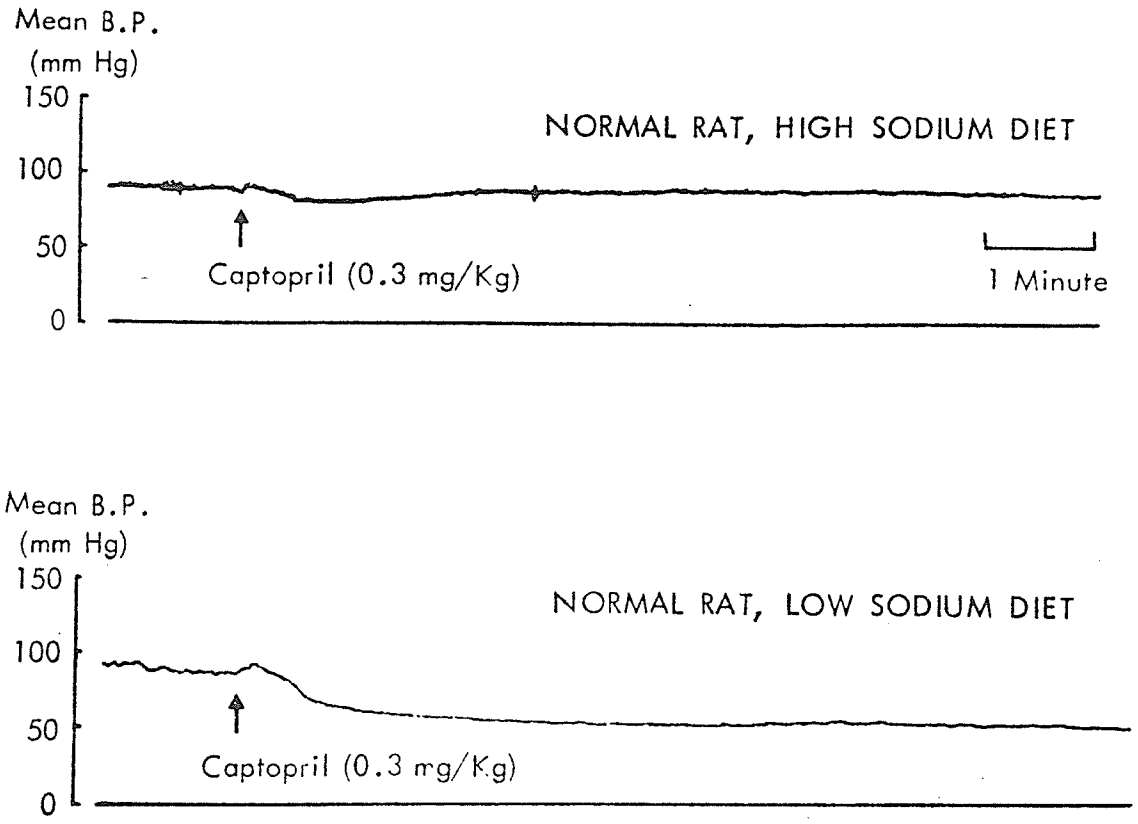

Figure 6: Representative record blood pressure response to captopril in a high salt and low salt rat. One rat was previously placed on a high dietary salt regime, the other on a low salt dietary regime. Note the rapid initial hypotensive response followed by a gradual return of the blood pressure towards pre-captopril levels.

TABLE 6

Effect of Sodium Balance on the Hypotensive Action of Captopril

	Control	Captopril .3 mg/Kg	
	Pre-captopril B.P. (mm Hg)	Maximal Δ B.P. (mm Hg)	Steady State Δ B.P. (mm Hg)
High Salt (n=11)	100.7 ±4.0	-6.1 ±4.0	-8.1 ±3.0
High Salt + DOCA (n=12)	92.2 ±3.7	-16.3 ±3.8	-1.9 ±2.9
Low Salt (n=8)	85.6 ±4.6	-18.1 ±4.6	-10.8** ±3.5
Low Salt + Furosemide (n=11)	117.4 ±4.0	-36.1 ±4.0	-7.9* ±3.0

Values are expressed as means ± S.E.
Comparison of pre-captopril and new steady state blood pressures.
(* = $p < .05$; ** = $p < .01$.)

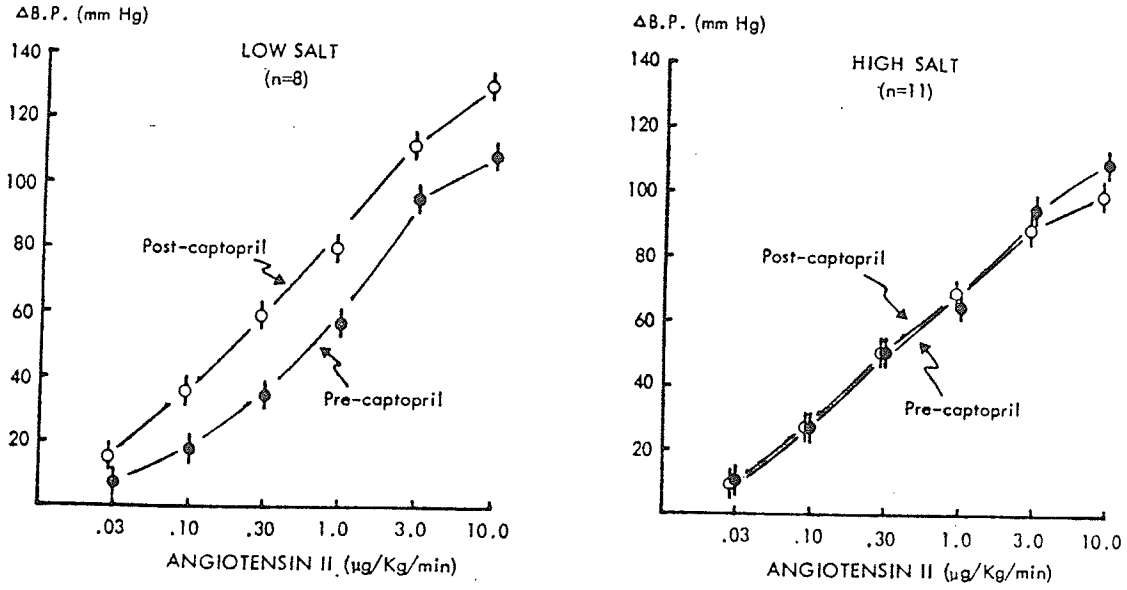

Figure 7: Effect of converting enzyme inhibition with captopril on pressor responsiveness for angiotensin II in high salt and low salt rats. State of sodium balance was induced by dietary changes without drug supplementation.

furosemide demonstrated an enhanced pressor response to AII following the administration of captopril (Figure 7). Following converting enzyme inhibition, AII infusion rates of .10, .30, 1.0, 3.0 and 10.0 μg/Kg/min displayed an enhanced pressor response, ($P < .01$). The percent inhibition of the pressor activity of angiotensin I (200 ng) for these high and low salt intake groups was 91.3 ± 4.1 and 92.5 ± 2.8 respectively. Thus, both groups were effectively blocked by captopril at the dosage used.

The high salt intake plus DOCA group demonstrated an enhanced pressor response for AII following captopril (Figure 8) at .10 and .30 μg/Kg/min ($P < .05$). In animals placed on a low salt intake supplemented with furosemide, the pressor response for angiotensin II following captopril treatment was enhanced, as demonstrated by the shift of the dose response curve to the left (Figure 8). This shift was significant at infusion rates of .10 and .30 μg/Kg/min ($P < .01$). As well, the percent inhibition of the converting enzyme in the high salt and low salt groups were 74.6 ± 5.4 and 81.3 ± 4.5 respectively. Thus, blockade was adequate.

In both the high and low salt groups, the experimental treatment of captopril did not alter the pressor responsiveness to norepinephrine (Figure 9). It appears that the observed shift in response to AII following captopril shown previously, is not a non-specific phenomenon. In addition, the majority of the differences in AII pressor responsiveness in the high and low salt states were abolished by captopril treatment (Figure 10).

c) Comments

In this section, the possible role of the level of endogenous angiotensin II as a determinant of pressor responsiveness to exogenous angiotensin was investigated. The first groups studied were animals

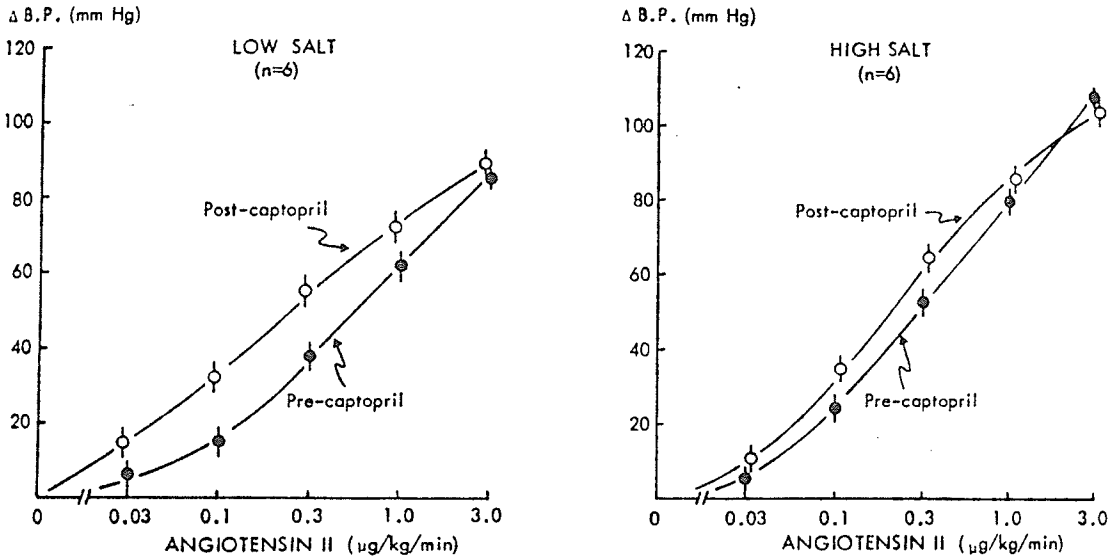

Figure 8: Effect of converting enzyme inhibition with captopril on pressor responsiveness for angiotensin II in rats fed a high salt and low salt diet supplemented with DOCA and furosemide.

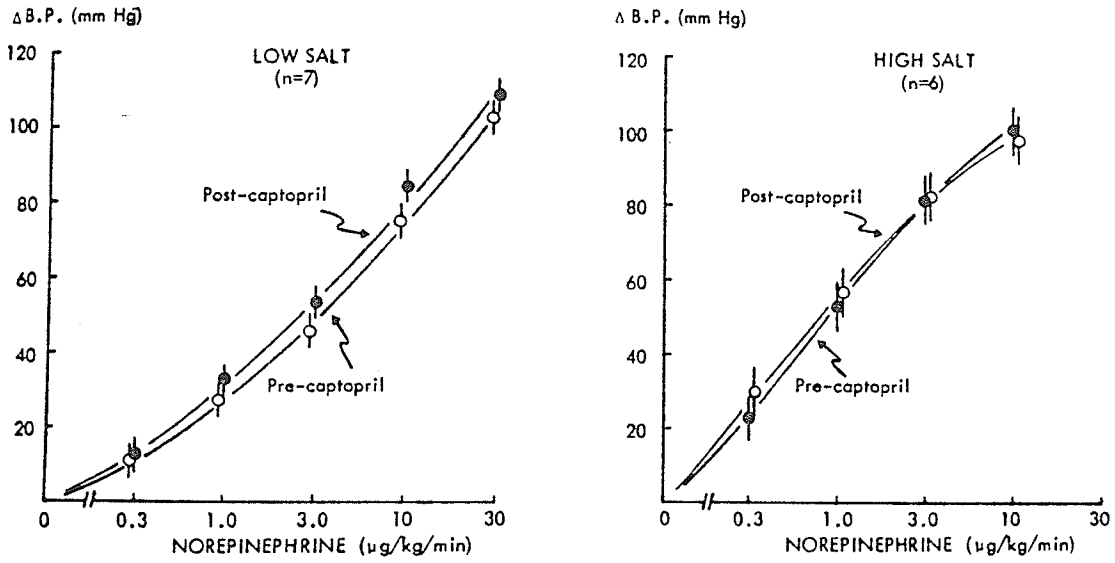

Figure 9: Effect of converting enzyme inhibition with captopril on pressor responsiveness for norepinephrine in rats fed a high salt and low salt diet supplemented with DOCA and furosemide.

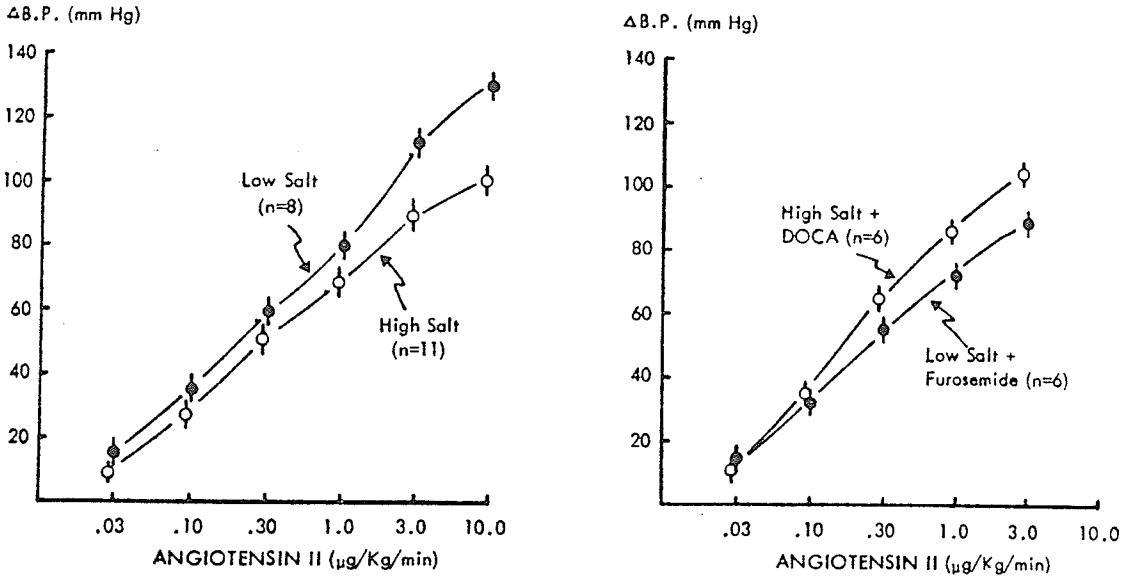

Figure 10: Pressor responsiveness for angiotensin II during converting enzyme inhibition with captopril in high salt and low salt rats.

whose state of sodium balance had been altered by diet alone. The high salt group showed no change in response to AII following captopril in contrast to the low salt group. This suggests that, only in the low salt, but not in the high salt state, endogenous angiotensin may be an important determinant of the pressor activity. This is consistent with previous results which demonstrated an elevated plasma renin activity, and presumably higher circulating AII levels in these low salt animals.

The hypotensive effect of captopril was observed to be greater and more prolonged in low states of sodium balance as has been reported elsewhere (Jaeger et al., 1978). The groups that received a furosemide supplement with the low salt diet showed similar results. Again, captopril increased the responsiveness for AII. However, in the high salt animals supplemented with DOCA, captopril treatment increased significantly the pressor responsiveness for angiotensin II. Since in states of sodium excess the renin-angiotensin system would be suppressed, the change in response observed following captopril may, therefore, be expected to be due to mechanism(s) other than suppressing AII formation.

The possible explanations include the following:

(1) In both of the low salt groups studied, converting enzyme inhibition significantly lowered the blood pressure. These groups also demonstrated the largest increase in pressor responsiveness to AII following captopril. It is possible that this lowered baseline blood pressure may in part be responsible for the observed shift in responsiveness for angiotensin.

(2) It has been demonstrated in high salt, DOCA treated rats, that pentobarbital anaesthesia increases the level of activity of the renin-angiotensin system (Pettinger et al., 1971). This could account

for the modest shift observed in the high salt rats.

(3) An increase in intrinsic vascular reactivity may have occurred as a result of changes in the state of sodium balance. A high sodium intake may increase the in vivo pressor responsiveness for AII by an increased number or affinity of the angiotensin receptors.

The experiments in the following sections are designed to clarify these considerations.

Thus, in conclusion, acute captopril administration at 0.3 mg/Kg significantly inhibits the converting enzyme activity. Clearly, it also increases the pressor response for AII, particularly in the sodium depleted state. In addition, captopril does not affect NE pressor responsiveness.

These results suggest that the endogenous level of angiotensin could be an important determinant of the pressor responsiveness to exogenous AII. As well, it appears that the elevated levels of circulating AII in states of sodium depletion may only affect specifically the pressor activity of AII.

3. Effect of Nitroprusside-induced Hypotension

To determine whether the depressed baseline blood pressure following captopril could account for observed changes in response to angiotensin II, we studied the pressor response to AII before and during nitroprusside-induced hypotension in low salt rats. If no difference in pressor responsiveness was observed before and during the nitroprusside-induced hypotension, this would exclude an altered baseline blood pressure as a factor in our post-treatment dose response curve.

(a) Methods

Rats that had been fed a low salt diet supplemented with furosemide

were used. Two dose response curves to angiotensin II were done using similar time sequences, separated by the administration of an experimental treatment (nitroprusside). Nitroprusside was infused into the right jugular vein at a rate of 5 µg/Kg/min. The rate of volume delivery was .017 ml/min and blood pressure was allowed to stabilize at a new steady state. Preliminary experiments demonstrated that this rate of nitroprusside infusion produced a sustained drop in blood pressure of 15 to 25 mm Hg that was maintained for at least 30 minutes.

Ten to 15 minutes following the start of the nitroprusside infusion, the second dose response was done in the presence of the nitroprusside-induced hypotension. At the end of the dose response, when blood pressure had returned to baseline, the nitroprusside was discontinued. Changes in blood pressure were recorded.

b) Results

Nitroprusside infusion (5 µg/Kg/min) decreased blood pressure from a baseline of 90.3 ± 7.8 mm Hg to a new steady state of 64.5 ± 9.4 mm Hg. This represented an average drop of 25.8 ± 7.3 mm Hg. At the end of the experiment, termination of the nitroprusside infusion resulted in a rebound increase in blood pressure from 90.3 ± 7.6 mm Hg to 123.7 ± 5.5 mm Hg with a mean rise of 33.3 ± 6.7 mm Hg. This demonstrates the continuing depressor effect of nitroprusside throughout the experiment.

The pressor response for angiotensin during the nitroprusside infusion was not significantly different from the control response (Figure 11).

c) Comments

Nitroprusside effectively maintained a lower baseline blood pressure throughout the period of infusion. The degree of nitroprusside-

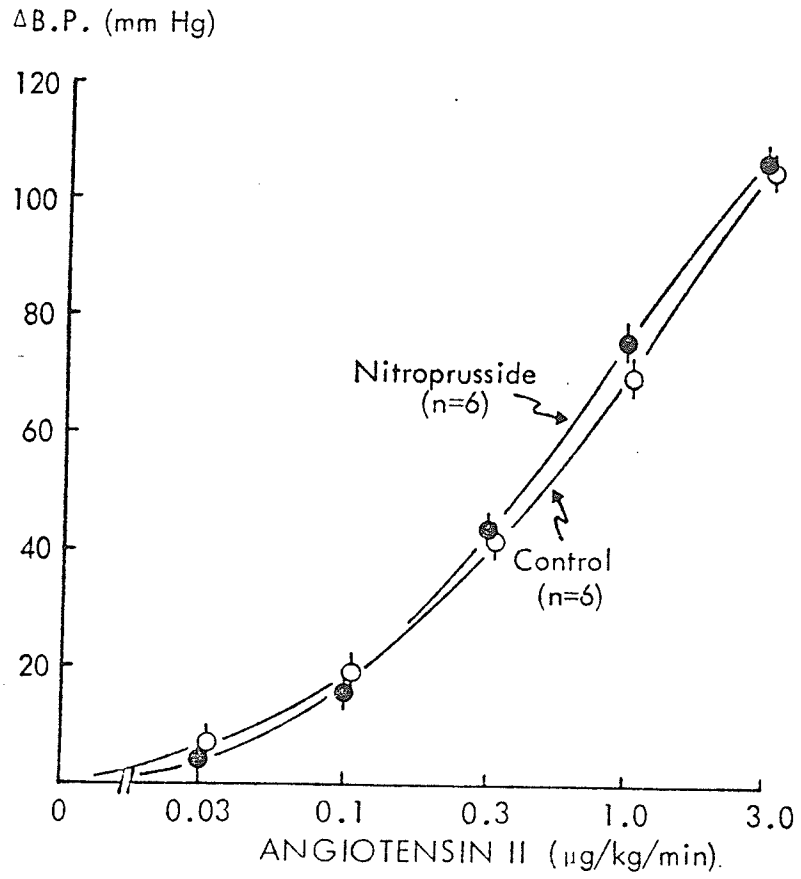

Figure 11: Effect of nitroprusside induced hypotension on the pressor response for angiotensin II in rats fed a low salt diet supplemented with furosemide.

induced hypotension was comparable to or greater than the captopril-induced fall in blood pressures even in the sodium depleted animals. The fact that a lower baseline blood pressure had no significant effect on the pressor responsiveness to AII suggests that the moderate captopril-induced hypotension is not responsible for the enhanced pressor effect in the low salt animals.

In the previous section, we also reported that the pressor responsiveness to norepinephrine following captopril remained unchanged in rats on a low salt intake. This is additional evidence that a moderate degree of hypotension does not produce a non-specific increase pressor responsiveness to pressor agents.

Thus, these experiments provide further evidence that the different depressor responses to captopril treatment were unlikely to be responsible for the shifts in AII pressor responsiveness in the high and low salt rats.

4. Effect of Duration of Anaesthesia

The anaesthetic used in these experiments may have increased the level of activity of the renin-angiotensin system (Pettinger et al., 1971). This may have resulted in an apparent decrease in the pressor responsiveness to AII coincidental with the administration of captopril. As well, the duration of the anaesthetic, hypothermia, or the surgical procedure may alter the response for AII. The purpose of these experiments was to determine whether any secondary effects of the general anaesthesia and the experimental procedures altered the AII pressor responsiveness with respect to time.

a) Methods

The rats (n = 5) used in this study were fed standard rat chow with tap water for drinking. Two dose response curves were done for angiotensin II. Between the dose response curves, a saline bolus was given in the same volume and time sequence as the studies with captopril as the experimental treatment. The second dose response for AII was then completed.

b) Results

The time sequence used to administer our experimental treatments had no effect on the pressor responsiveness to angiotensin II (Figure 12).

c) Comments

The pressor responsiveness to angiotensin II remained unchanged during the course of our experiments when no experimental treatments were administered. Thus, time or duration of anesthesia can be eliminated as a controlling factor in our studies and the dose response curves were reproducible.

5. Effect of Sodium Intake on Renovascular Sensitivity In Vitro

In the previous experiments, it appeared that differences in the in vivo pressor responsiveness to angiotensin in different states of sodium balance could be attributed to the resultant level of endogenous angiotensin II. However, an alternative explanation for the apparent salt sensitivity of the pressor responsiveness does exist. This involves changes in the vascular end-organ sensitivity to specific vasoactive agents or a general increase in the efficiency of the post-receptor excitation contraction process. The majority of the data in support of this theory has been obtained from vascular tissue studied in vitro. Rabbit aortic strips taken from animals previously placed on a low salt

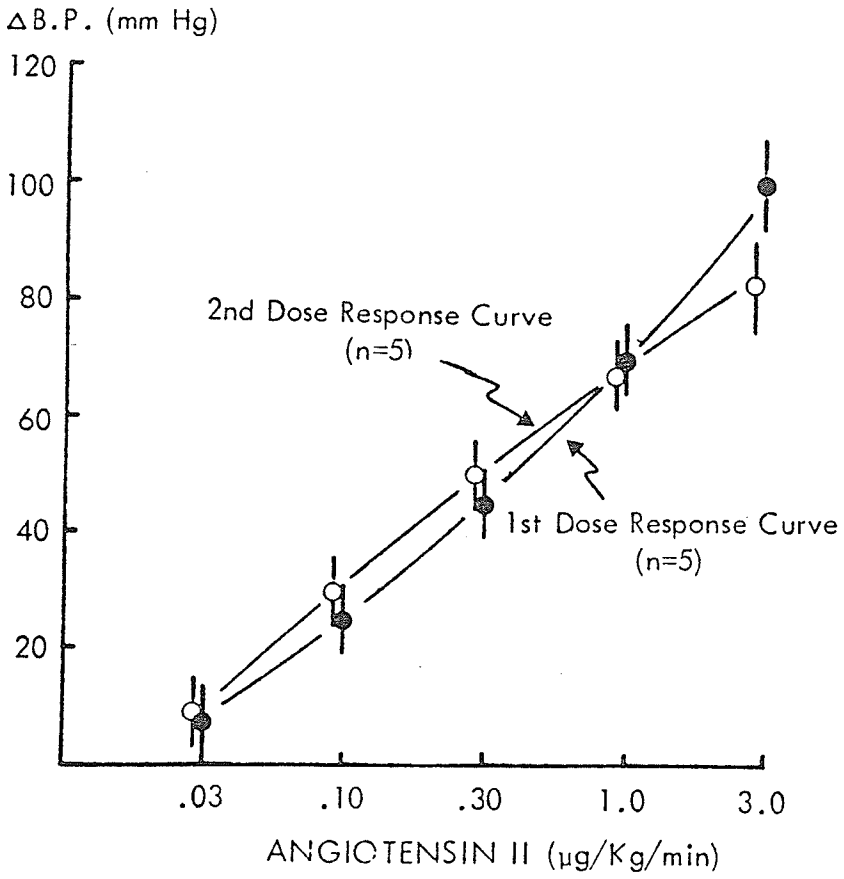

Figure 12: Effect of duration of anesthesia on pressor responsiveness to angiotensin II.

intake were less responsive to AII but the response to NE was enhanced when compared to the high salt counterparts (Strewler et al., 1972). As well, a decrease in angiotensin II receptor numbers and/or affinity have been reported in vascular smooth muscle obtained from sodium depleted rats (Aguilera and Catt, 1980). However, the tissues used in these studies were either obtained from larger arteries or non-vascular smooth muscle and thus may not be representative of the resistance vessels.

The purpose of these experiments was to assess the changes in vascular smooth muscle sensitivity in the renal vascular bed in response to alterations in sodium intake. In order to eliminate systemic influences and the effects of endogenous angiotensin generation, we used the isolated perfused kidney preparation.

a) Methods

Kidneys obtained from rats that had been placed on a high or low salt intake were perfused with a physiological solution, Krebs-Henseleit. Perfusate flow of the Krebs-Henseleit solution was adjusted to produce a perfusion pressure of approximately 100 mm Hg at a constant perfusion rate. Thus, any change in perfusion pressure would reflect changes in renal vascular resistance.

Four groups of animals were studied (see below). Following a 30 minute stabilization period, a timed urine specimen was obtained. A known concentration of creatinine had been included in the perfusate. This enabled the calculation of in vitro creatinine clearance by measuring the creatinine levels in the perfusate and in the collected urine samples.

i) In Vitro Vascular Sensitivity for Angiotensin

Animals used in this study had been placed in a state of high or low sodium balance by dietary changes only.

ii) In Vitro Vascular Sensitivity for Norepinephrine

Animals used in this study had been placed in a state of high or low sodium balance by dietary changes supplemented with DOCA or furosemide, as described previously.

b) Results

i) In Vitro Vascular Sensitivity for Angiotensin

The effect of diet on the in vitro renal function is shown in Table 7. Perfusion pressures in the two groups were similar, although the mean value was greater in the high salt group. The perfusion flows were also similar. Preliminary experiments demonstrated that this degree of variation in perfusion pressure and flow rate did not systematically influence the vascular responsiveness to vasoconstrictors. The renal vascular resistance per gram of kidney weight (RVR-gm) was similar in the two groups. Animals placed in a state of high sodium balance had a greater urinary flow during in vitro perfusion than the low salt groups ($P < .05$). This may be related to the higher perfusion pressures with the resultant slightly higher glomerular filtration rates in the high salt rats. The filtration fraction was similar in the two groups, however, it was slightly elevated in the high salt group.

Kidneys from rats previously placed on a high salt diet showed an enhanced pressor response for angiotensin II (Figure 13), when compared to kidneys from low salt intake rats. Significantly higher perfusion pressures were noted at angiotensin perfusate concentrations

TABLE 7

Effect of High and Low Salt Balance, Induced by Dietary Changes Alone, on In Vitro Renal Function

	High Salt (n= 8)	Low Salt (n= 6)	P
Perfusion Pressure (mm Hg)	100.1 ±5.4	89.3 ±6.3	N.S.
Perfusion Flow (ml/min)	12.8 ±.9	13.6 ±1.1	N.S.
RVR-gm (mm Hg/(ml/min/gm))	13.0 ±1.3	11.4 ±1.8	N.S.
Urinary Volume (ml/min)	.34 ±.03	.23 ±.04	<.05
Creatinine Clearance (ml/min)	.68 ±.07	.65 ±.08	N.S.
Filtration Fraction	.053 ±.007	.046 ±.008	N.S.

Values are expressed as means ± S.E. Comparisons were done with the Student t-test (N.S. = non significant).

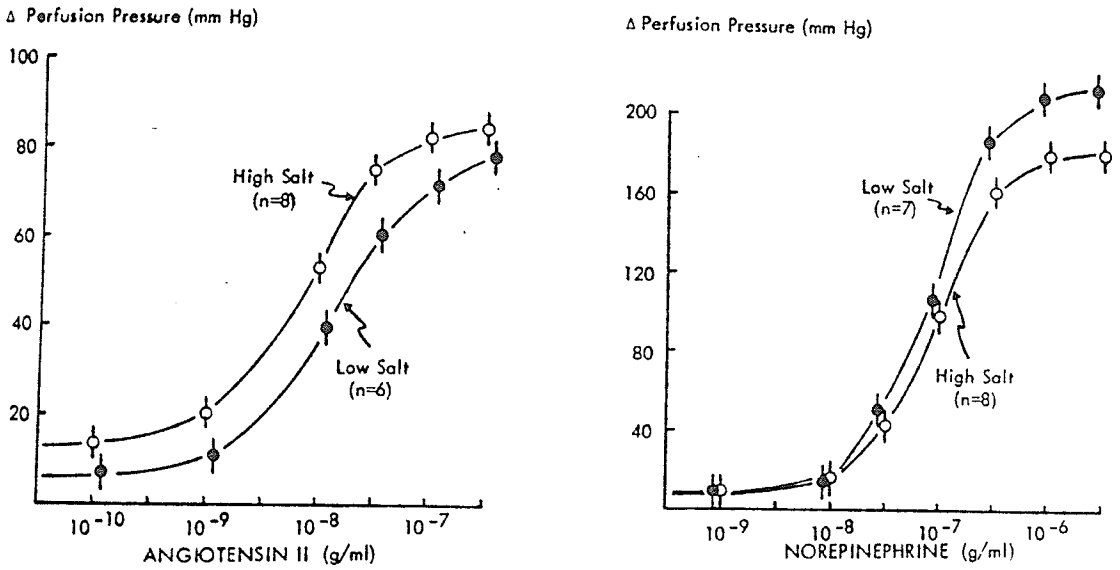

Figure 13: Effect of sodium balance on <u>in vitro</u> renovascular sensitivity to angiotensin II or norepinephrine in the isolated perfused rat kidney. Kidneys were obtained from rats on a high or low salt dietary regime alone (left) or supplemented with DOCA and furosemide (right).

of 10^{-8} g/ml ($P < .01$), 3×10^{-8} g/ml ($P < .01$) and 10^{-7} g/ml ($P < .05$). No differences in the threshold and maximal responses were observed between the two groups. Figure 14 shows the same date transformed into a Linweaver-Burke Plot. The Y-intercepts were comparable. However, the calculated Km values were lower in the high salt group, compared to the low salt group.

ii) In Vitro Vascular Sensitivity for Norepinephrine

The same trends were found as that for the above groups, where diet alone was used to alter sodium blance (Table 8). Again, the perfusion pressures and flows were slightly greater in the high salt group. The RVR-gm in both groups was similar. As well, the urinary flow during in vitro perfusion was significantly greater in the high salt group ($P < .05$). This may be related to the higher perfusion pressures and flows with the resultant slight increase in creatinine clearance and filtration fraction.

Kidneys obtained from animals placed on a low sodium intake supplemented with furosemide showed an enhanced response at norepinephrine perfusate concentrations of 3×10^{-7} g/ml ($P < .05$), 10^{-6} g/ml ($P < .05$) and 3×10^{-6} g/ml ($P < .01$), when compared to the high salt plus DOCA group. The threshold responses were similar in both groups. In contrast to the angiotensin experiments, differences in responsiveness between the two groups were not observed until the perfusion pressures were greater than 100 mm Hg, suggesting non-competitive kinetics (Figure 13).

c) Comments

In the previous sections we have reported studies on the pressor responsiveness to AII or norepinephrine in whole animal preparations. In this section we utilized a specific regional vascular bed (renal) and

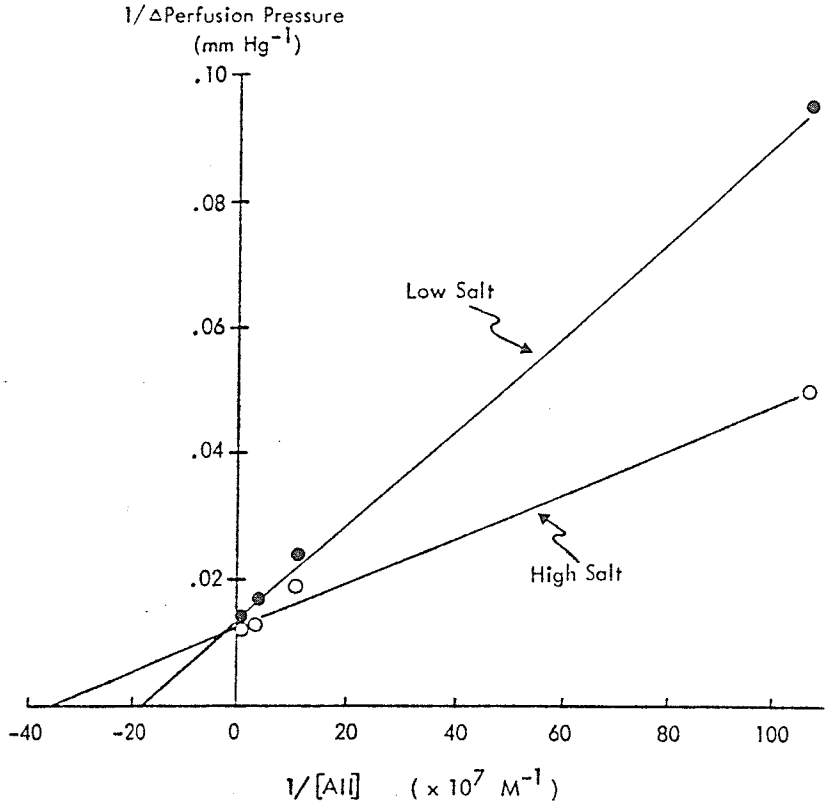

Figure 14: Double reciprocal (Lineweaver-Burke) plot of <u>in vitro</u> renovascular sensitivity to angiotensin II in the isolated perfused rat kidney. Values presented are the means. Low salt n=8, K_m=2.7 x 10^{-9}M; High salt n=11, K_m=5.2 x 10^{-9}M.

TABLE 8

Effect of High and Low Salt Balance, Induced by Dietary Changes Supplemented with DOCA and Furosemide, on In Vitro Renal Function

	High Salt + DOCA (n= 8)	Low Salt + Furosemide (n= 7)	P
Perfusion Pressure (mm Hg)	98.0 ±5.4	82.0 ±5.8	N.S.
Perfusion Flow (ml/min)	12.2 ±.9	9.8 ±1.0	N.S.
RVR-gm (mm Hg/(ml/min/gm))	10.6 ±1.2	11.7 ±1.2	N.S.
Urinary Volume (ml/min)	.36 ±.03	.25 ±.03	<.05
Creatinine Clearance (ml/min)	.64 ±.07	.46 ±.07	N.S.
Filtration Fraction	.054 ±.007	.047 ±.007	N.S.

Values are expressed as means ± S.E. Comparisons were done with the Student t-test (N.S. = non-significant).

an artificial perfusate to study changes in vascular sensitivity *in vitro*. This model would eliminate both neural and circulating humoral factors which may affect the measurement of vascular responsiveness for a drug. Preliminary experiments have shown this to be a reproducible, stable and sufficiently sensitive preparation adequate for the study of vascular responsiveness.

In the absence of these potential influences, there still exists a demonstrable salt sensitive effect on vascular sensitivity. Clearly, high salt intake enhances the response while low salt intake attenuates the vascular reactivity to AII in this vascular bed. The shift in responsiveness also appears to be a parallel one with the threshold and maximal responses remaining unaltered. This is reflected by similar Y-intercepts with different slopes in the double reciprocal plot. In comparison to AII, responses for norepinephrine were different. The maximum response in the kidneys from rats placed on a low sodium intake was actually greater than the response in kidneys from high salt rats.

The cause for the enhanced vascular reactivity to AII cannot be determined from these experiments. In the isolated perfused kidney, any significant effect of circulating angiotensin II can be excluded. The lower Km value for the high salt group suggests an increase in receptor number and/or affinity, which is in accord with the results of other studies that have utilized non-vascular smooth muscle (Devynck *et al.*, 1979; Aquilera and Catt, 1980). Since qualitatively similar results were not observed with norepinephrine, it may be concluded that at least part of these salt-induced changes in reactivity are specific for AII. Thus, in addition to the circulating level of AII, a change in the sensitivity of the vasculature may also be of importance in the

regulation of AII pressor responsiveness in vivo.

6. Effect of Isotonic Saline Volume Expansion

Generally, sodium excess in a normal animals is associated with an expansion of the plasma and blood volumes, whereas sodium depletion is associated with a definite contraction of the blood and plasma volume (Brown et al., 1971). It is conceivable that the contracted volume may partially account for the depressed angiotensin II responsiveness in low sodium states observed in the previous experiments. The purpose of these experiments was to determine whether the pressor response to AII or NE would be altered following restoration of plasma volume by acute isotonic saline administration in a group of low salt rats. The failure of acute volume expansion to enhance pressor responsiveness in this group would suggest that this factor cannot explain the differences between the high and low salt rats.

a) Methods

A total of 16 rats that had been fed a low sodium diet supplemented with furosemide were used. Two dose response curves for angiotensin II or norepinephrine were done. Again, an experimental treatment (volume expansion) was administered between the two dose response curves. Following the first dose response (control), the blood pressure was allowed to stabilize for 10 to 15 minutes. At this time, 0.9% saline was infused at a rate of 6% of the animals' body weight per hour for 30 minutes, (i.e. 3% body weight). Then the rate of infusion was decreased to 3% body weight per hour. During this final infusion rate the second dose response was done. Preliminary experiments have shown that mean blood pressures were unaffected by this rate of saline infusion.

b) Results

The pressor responses for AII and norepinephrine obtained before and during saline volume expansion are shown in Figure 15 . The response for AII was unchanged following volume expansion. As well, the dose response curve for norepinephrine remained unchanged following the experimental treatment. During the saline infusions, the pulse pressure did widen but mean blood pressure remained the same.

c) Comments

The administration of 3% body weight of isotonic saline over a 30 minute period represents a considerable volume load and a significantly high rate of volume expansion (Fraley and Zett, 1981). These findings indicate that an acute change in vascular volume is not an important factor in the determination of vascular responsiveness to pressor agents in the low salt state. Our observations agreewith those of Cowley and Lohmeier (1978) who demonstrated in the anaesthetized anephric dog, that changes in the plasma volume of a magnitude of 5-6% body weight did not alter the pressor response for angiotensin II. In these experiments, hemodialysis was used to control plasma volume. Since, in our experiments, restoration of plasma volume did not increase pressor responsiveness in the low salt rat, one may argue that moderate volume contraction per se is an unlikely explanation for the lower AII pressor responsiveness when compared to the high salt animals.

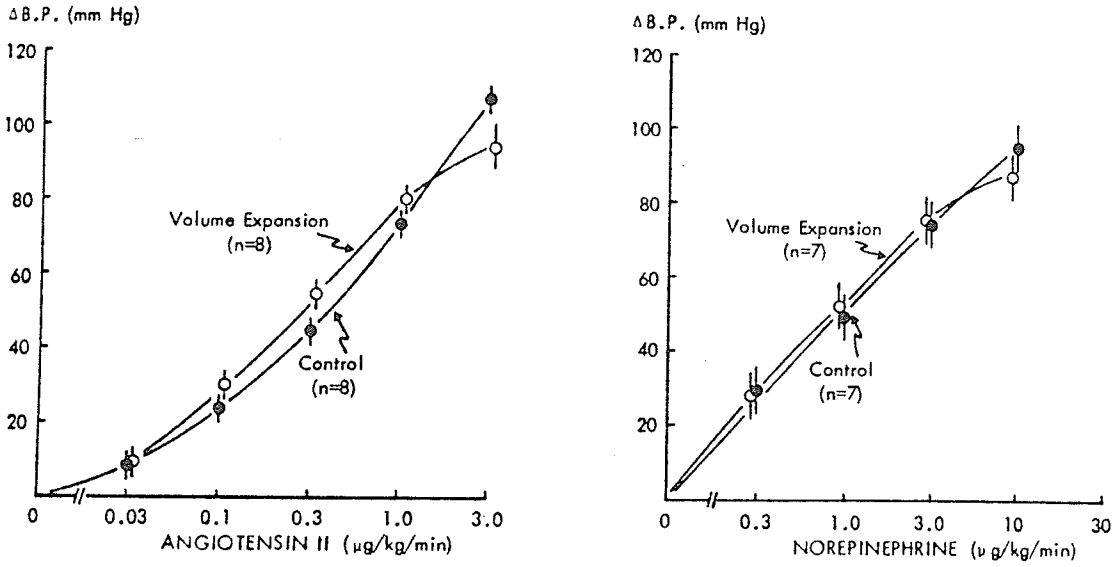

Figure 15: Effect of acute saline volume expansion on the pressor responsiveness to angiotensin II or norepinephrine in low salt rats supplemented with furosemide.

7. Discussion

The decreased pressor response to exogenous AII in animals in the sodium depleted state as compared to a state of sodium excess has been well documented (Reid and Laragh, 1965; Brunner et al., 1972; Hollenberg et al., 1972; Thurston and Laragh, 1975; Slack and Ledingham, 1976; Oliver and Cannon, 1978). A number of mechanisms have been proposed to explain this observation. Thurston and Laragh (1975) and Brunner et al., (1972), suggested that increased circulating levels of angiotensin II, secondary to sodium depletion, resulted in the attenuated response to exogenous AII by competition for the receptor site. However, it has been shown, using isolated aortic strips, that sodium depletion decreases and high salt intake increases the vascular sensitivity to AII (Strewler et al., 1972). The effect of sodium intake on norepinephrine vascular responsiveness was opposite to that of angiotensin II, suggesting that this was not a total non-specific change in vascular sensitivity. Similarly, others using a radioreceptor assay have noted an increase in the number of angiotensin II receptors in smooth muscle from animals previously on a high sodium diet (Aquilera and Catt, 1980; Devynck et al., 1979). Therefore, a specific change in end-organ sensitivity can also explain the difference in AII pressor responsiveness with different sodium intakes. Thus, it appears difficult to dissociate the role of end-organ sensitivity from the level of endogenous angiotensin with regard to pressor responsiveness for angiotensin II in vivo.

Since AII pressor responsiveness is clearly a significant element in the regulation of blood pressure, it would be of value to determine the variables which regulate the pressor responsiveness to this hormone in different states of sodium balance. The purpose of this section was to

dissociate the influences of sodium intake and the activity of the renin-angiotensin system by the use of the isolated perfused kidney preparation and by the use of captopril *in vivo*.

In the anaesthetized rat, we found a decrease in pressor responsiveness to AII in animals fed a salt deficient diet as compared to animals fed a high salt diet. This is consistant with published data. The addition of DOCA to the high salt group and furosemide to the low salt group failed to accentuate this difference. In both low salt groups, converting enzyme inhibition by captopril (0.3 mg/Kg) resulted in a parallel shift of the dose response curve for AII to the left, suggesting an increase in response. However, the high salt diet supplemented with DOCA also demonstrated a slight shift in the AII dose response curve following converting enzyme inhibition. The response for norepinephrine was unaltered in high and low states of salt balance and following the addition of captopril (0.3 mg/Kg). In all of these experiments, captopril effectively inhibited the converting enzyme activity. Thus, these observations suggest that in situations where the pressor response is increased following captopril, the endogenous level of angiotensin substantially influenced the AII pressor responsiveness.

The potentiation of the pressor response to angiotensin in the high salt plus DOCA group following captopril appears to conflict with the proposed hypothesis. In high salt animals, captopril would be expected to have little effect due to the endogenous angiotensin II levels being suppressed. However, it has been demonstrated that barbiturate anaesthesia will increase the level of activity of the renin-angiotensin system in rats fed a high salt diet supplemented with DOCA

(Pettinger et al., 1971). In our experiments, pentobarbital may have elevated the endogenous levels of angiotensin II. As well, captopril has been shown to increase the endogenous levels of bradykinin and prostaglandins (Swartz et al., 1979). Thus, it may be postulated that the leftward shift of the dose response curve following captopril in the low salt animals may be related to the increased levels of bradykinin or prostaglandins. Oliver and Cannon (1978), found that bradykinin infusion or prostaglandin synthetase inhibitors had little effect on the renal vascular response to AII. The relationship between the salt sensitive pressor response to AII and the endogenous level of prostaglandins is discussed later in this thesis.

In the high salt groups studied, the drop in blood pressure following converting enzyme inhibition was only transient. In the low salt groups, the blood pressure remained significantly suppressed. It may be argued that this reduced blood pressure may have resulted in a non-specific increase in responsiveness to vasopressor agents. The failure of the pressor activity of norepinephrine to be enhanced is evidence against this. To more specifically answer this question, blood pressure was reduced 15-20 mm Hg by the infusion of nitroprusside. Even at this lowered baseline blood pressure, the response to AII was unaltered. A non-specific action of the moderate captopril-induced hypotension can be ruled out.

It is generally appreciated that a chronic diet low in sodium is associated with a contraction of the plasma volume (Romero et al., 1968; Brown et al., 1971). This decrease in extracellular fluid volume and plasma volume may be a factor in the attenuation of the pressor response for angiotensin in the sodium depleted rats. However, in the low salt

rat, acute volume expansion with isotonic saline did not alter the pressor response for angiotensin. Others have demonstrated a slight, but significant, reduction in plasma renin following volume expansion of this magnitude (Oliver and Cannon, 1978). If this was true, this should have further biased the results towards increasing pressor responsiveness to AII. However, no such changes were observed. These results are similar with the experiments reported by Cowley et al., (1978), who showed that changes in extracellular fluid volume did not affect pressor responsiveness to angiotensin II in the anephric dog, where circulating AII levels would be essentially zero.

The effect of sodium balance on vascular responsiveness to norepinephrine as reported in the literature has been less consistent. Firstly, there has been substantial variation in the qualitative response to norepinephrine (Hollenberg et al., 1972; Oliver and Cannon, 1978). Similarly, the observed pressor response to NE would be the sum total of the response found in a number of peripheral vascular beds, which individually may vary enormously. Norepinephrine may also potentially induce significant beta-adrenergic activation. Thus, it is not surprising that the pressor response to an intravenous infusion of norepinephrine may be a relatively insensitive method of assessing vascular responsiveness. Furthermore, the ability of norepinephrine to increase cardiac output at certain dose levels may vary depending on the experimental condition (i.e., high or low salt balance). This would add another variable to an already complex situation.

The rise in blood pressure following the infusion of angiotensin II may be due to changes in cardiac output and/or total peripheral resistance. In our experiments, however, it is likely that cardiac output was

not changed significantly at the infusion rates used. We studied the effect of angiotensin II infusion (.10 and .30 μg/Kg/min) on cardiac output in a group of normal rats (Appendix D). Both blood pressure and total peripheral resistance were increased at these rates of infusions, but the cardiac output was unaltered (Figure D1, page 190). Similar results were found for norepinephrine (Figure D2, page 191). Unfortunately, there is an increase in cardiac output in our control experiments (Figure D3, page 192) associated with a drop in total peripheral resistance, but not blood pressure. Although highly unlikely, if the cardiac output did in fact increase spontaneously during the course of the experiment, this would suggest that AII or NE actually decreased the cardiac output. The fact that the cardiac output may be unchanged or decreased, but not increased, is added support that a change in blood pressure following AII or NE infusion is an approximate measure of the perhaps under estimated change in peripheral resistance. A recent study did not report any change in cardiac output following the administration of AII at infusion rates comparable to our study (Hsu *et al.*, 1980).

In order to circumvent the large number of uncontrolled variables that regulate blood pressure *in vivo*, we utilized the constant perfusion isolated kidney preparation to study the effect of dietary sodium intake on vascular sensitivity for AII and for NE. In this isolated system, it may be safely assumed that the circulating level of AII is insignificant. In these studies, sodium depletion still resulted in a parallel shift of the dose response curve to the right of the high salt rats. A double reciprocal plot (Lineweaver-Burke) showed the Y-intercept to be the same in the high and low salt groups, suggesting similar maximal responses.

The K_m, however, was lower in the high salt animals than the low salt animals. This indicates a difference in the receptor characteristics. Whether this is due to an increase in receptor number and/or affinity in kidneys from high salt rats cannot be determined from these results. Radioreceptor assay done on isolated aortic strips, however, would suggest that this is due to an increase in receptor number following the high salt intake (Aguilera and Catt, 1980; Devynck et al., 1979).

In contrast to the in vivo studies, the renovascular response for norepinephrine was greater in the low salt group. In the in vitro studies, only doses of norepinephrine in excess of 3×10^{-7} g/ml demonstrated significantly different responses. As well, the changes in perfusion pressure at this dose were in excess of 120 mm Hg. Therefore, due to the large dose of norepinephrine used and the large pressure changes observed, it is questionable whether these results are of any physiological value. Similar results, but within physiological ranges, have been observed in the isolated hind limb preparation and in aortic strips from rabbits placed in high and low states of sodium balance (Strewler et al., 1972). This suggests that the increased response observed for angiotensin II in rats on a high salt intake is not due to a non-specific increase in vascular responsiveness. This is further supported by our findings that sodium deprivation suppresses the in vitro vasoconstrictor activity for angiotensin and enhances the activity for norepinephrine.

A difference in the rate of angiotensin degradation may also account for differences in pressor responsiveness to exogenous angiotensin II. Angiotensinase activity has been found to be lower in some patients with hypertension (Hickler et al., 1963; Birbar and Hickler,

1965). These authors proposed that the rate of AII degradation may be important in the regulation of blood pressure. Thus, it is possible that differences in pressor responses for AII in different states of sodium balance may be attributed to alterations in the rates of angiotensin II degradation.

Leary and Ledingham (1970) studied the rate of angiotensin II disappearance in the isolated perfused kidney in vitro obtained from rats previously on a seven day high or low salt intake. The rate of disappearance of AII was less in the high salt group as compared to their normal sodium intake controls. Conversely, the rate of AII disappearance in the low salt group was enhanced when compared to their respective controls. The authors concluded that low sodium intake enhanced angiotensinase activity. However, "angiotensinase" activity in the control group for the high salt animals was significantly greater than the controls for the low salt group. Consequently, AII degradation rates were similar in both the high and low salt groups. In addition, in vitro data of this sort cannot be easily translated into in vivo terms.

In contrast to Leary and Ledingham's observations, Strewler et al. (1972) incubated angiotensin in whole blood obtained from rabbits previously on a high or low salt intake. Angiotensinase activity was similar in both groups. Although, it is relevant to point out that plasma angiotensinase activity may not be significant in the inactivation of AII as compared to the role of organ vascular beds (Bakhle et al., 1969; Vane, 1970; Leary and Ledingham, 1969).

Our experiments do not prove or disprove AII degradation as a regulator of the vascular reactivity for AII in vitro. However, this

mechanism is not a likely explanation for our in vivo observations since captopril treatment resulted in similar AII pressor responsiveness in both high and low salt rats.

Although the relative contribution of endogenous angiotensin II or the vascular sensitivity to the in vivo AII pressor response is difficult to determine, the importance of endogenous AII levels are clearly supported by our experiments. In addition, the fact that AII pressor responsiveness became similar following captopril treatment regardless of previous salt intake further emphasizes the significance of the role of endogenous AII. If changes in vascular sensitivity were important in affecting pressor responsiveness in vivo, one would expect a significant greater responsiveness to AII in the high salt animal in spite of captopril treatment. Thus, the differences in pressor responsiveness for AII between rats on a high salt or low salt intake can be almost completely attributed to the differences in the endogenous levels of angiotensin II. This is consistent with the findings of Chinn and Dusterdieck (1972), who noted that pressor responsiveness to AII in normotensive and hypertensive man was inversely proportioned to the baseline plasma AII levels.

Numerous investigators in the past have used the blood pressure response to angiotensin II as an index of vascular responsiveness. Based on our observations, it appears that this approach is only valid if the endogenous levels of angiotensin II have been suppressed. Otherwise, a high baseline endogenous AII level may artificially shift the dose response curve to the right. This has been demonstrated in our experiments where low salt and high salt rats were treated with captopril. The apparent differences in the vascular responsiveness

between the two groups were no longer apparent following converting enzyme inhibition. This argument can be extended to a number of pathological states, such as Bartter's syndrome (Bartter, 1981), hepatic ascites (Ames et al., 1965) and hypertension (Kaplan and Silah, 1964), where a suppressed pressor response for angiotensin II has been reported. In order to determine the pressor responsiveness for AII, the endogenous levels of AII should be suppressed prior to the test. If this is not done, one may erroneously conclude that the pressor responsiveness is decreased when, in fact, it is not.

The arbitrary shift of the dose response curve due to alterations in the endogenous level of the agonist does not appear to exist only for angiotensin II. It is well documented that the plasma level of norepinephrine varies inversely with the subject's state of sodium balance (Luft et al., 1979; Nicholls et al., 1980; Rankin et al., 1981). Recently, Rankin et al. (1981) have demonstrated a suppression of the pressor response for norepinephrine in states of sodium depletion where plasma norepinephrine levels are elevated. Conversely, a high salt intake was associated with an increased responsiveness for NE. Although the response for NE was not repeated following the suppression of endogenous NE release, Touw et al. (1980) found an enhanced response for NE following the administration of hexamethonium in the rat. It would, however, be of interest to study the pressor response for NE in high salt and low salt rats, before and after the administration of hexamethonium.

In summary, these results demonstrate that changes in the level of salt intake alter the pressor responsiveness to exogenous angiotensin II. The whole animal studies suggested that the degree of pressor

responsiveness for exogenous angiotensin is largely determined by the endogenous level of circulating angiotensin. Our *in vitro* studies further suggested that an actual change in the vascular sensitivity for angiotensin may play some role in the determination of the pressor responsiveness *in vivo*.

B. SODIUM BALANCE AND THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE REGULATION OF PRESSOR RESPONSIVENESS IN VIVO

1. Effect of Alpha Adrenergic Blockade
2. Effect of Ganglionic Blockade
3. Effect of Beta Adrenergic Blockade
4. Discussion

The pressor effect of angiotensin is thought to be mediated at least partially, by interacting with the sympathetic nervous system. Bell (1972) has demonstrated that subthreshold doses of AII potentiate the contractile response of the rat uterine artery to low frequency stimulation but not to low doses of norepinephrine. Khairallah et al, (1971) showed that low rates of angiotensin II infusion decreased the neuronal uptake of tritiated norepinephrine in a number of organs in the rat. This decreased uptake was associated with an increased norepinephrine pressor response. Whether the action of angiotensin II is to increase norepinephrine release or inhibit the neuronal uptake of norepinephrine, these observations indicate that subpressor and pressor levels of angiotensin II potentiate the pressor effects of nerve stimulation and norepinephrine infusion (Ekboir and Enero, 1980; Jackson and Campbell, 1981). Conversely, this also suggests that endogenous angiotensin may alter the physiological response to norepinephrine and sympathetic stimulation.

Since an increase in sodium intake increases the pressor response for angiotensin II, it is conceivable that this may be due to a change in the response of the sympathetic nervous system to angiotensin II.

The purpose of the experiments in this section is to study the role of the adrenergic system in the modulation of the AII pressor responsiveness in different states of sodium balance.

1. Effect of Alpha Adrenergic Blockade

The role of the α-adrenergic receptor in the pressor response for AII is unclear. The classic experiments of Bickerton and Buckley (1961) demonstrated a greatly reduced pressor response to angiotensin II

in the presence of an α-adrenergic blocking agent, piperoxane, in the dog. Others have found only a small decrease in the pressor activity of AII in the rat following phentolamine (Chryssanthou et al., 1971). Similarly in man, vasoconstriction in the hand during intravenous angiotensin infusions was abolished by intravenous phentolamine. In these same experiments, the blood pressure response to AII was unaffected by the α-adrenergic blocking agent (Scroop and Whelan, 1968). Thus, a sympathetic mediated vasoconstriction does appear to occur during angiotensin infusion. Whether this vasoconstriction contributes uniformly to the blood pressure response for AII is uncertain. In studies using isolated blood vessels and isolated perfused organs, no changes were found in the response to AII in the presence of an α-blocking agent (Bohr, 1974 and Peach, 1977).

Since the AII pressor response is dependent on the state of sodium balance, the purpose of these experiments was to assess the effect of α-adrenergic blockade, with phenoxybenzamine, on the blood pressure response for intravenous AII. More specifically, we wished to evaluate the role of the α-adrenergic receptor in the pressor response for AII in high salt and low salt rats.

a) Method

Male Long Evans rats were fed high salt (n=6) and low salt (n=5) intakes supplemented with DOCA and furosemide, respectively. Two dose response curves were done for angiotensin in high salt and low salt rats. Between the dose response curves, an experimental treatment was applied. The treatment administered in the acute blockade experiments was phenoxybenzamine (POB) at a dose of 5 mg/Kg, intravenously. A bolus dose of norepinephrine (100 ng) was given before and after the POB to

evaluate the effectiveness of the α-blockade. The kinetics of α-blockade with POB is known to change from a competitive type of inhibition to a non-equilibrium type of blockade in a few hours (Nickerson, 1957). This change may have occurred during our acute blockade experiments. Therefore, in another two groups of high (n=6) and low (n=6) salt rats, we studied the effect of chronic blockade by administering POB on the day prior to the experiment as well as two hours before the experiment (5 mg/Kg; IP). Following the dose response curves for angiotensin, norepinephrine was infused in incremental doses to assess the effectiveness of the chronic α-blockade.

b) Results

In the high and low salt groups the percent inhibition of the pressor response to a bolus injection of NE was 70.6 ± 9.6 and 80.1 ± 8.7, respectively (P=NS). Thus, both groups showed similar levels of blockade. The drop in blood pressure following the POB was comparable in the high salt (-13.3 ± 7.2 mm Hg) and low salt (-15.0 ± 7.9 mm Hg) groups. The effect of acute POB on the pressor response to AII as well was similar in the high and low salt animals (Figure 16).

The control AII pressor responsiveness was higher in the high salt group when compared to the low salt group. This is similar to the observations reported in Part A of the Specific Experiments. In both groups, following the POB administration, there was a significant decrease in response to AII at infusion rates of .10, .30, 1.0, 3.0 and 10.0 μg/Kg/min ($P < .01$). However, the post-treatment pressor response to AII in high salt rats was still significantly greater than the response obtained in low salt rats post-POB (Figure 17).

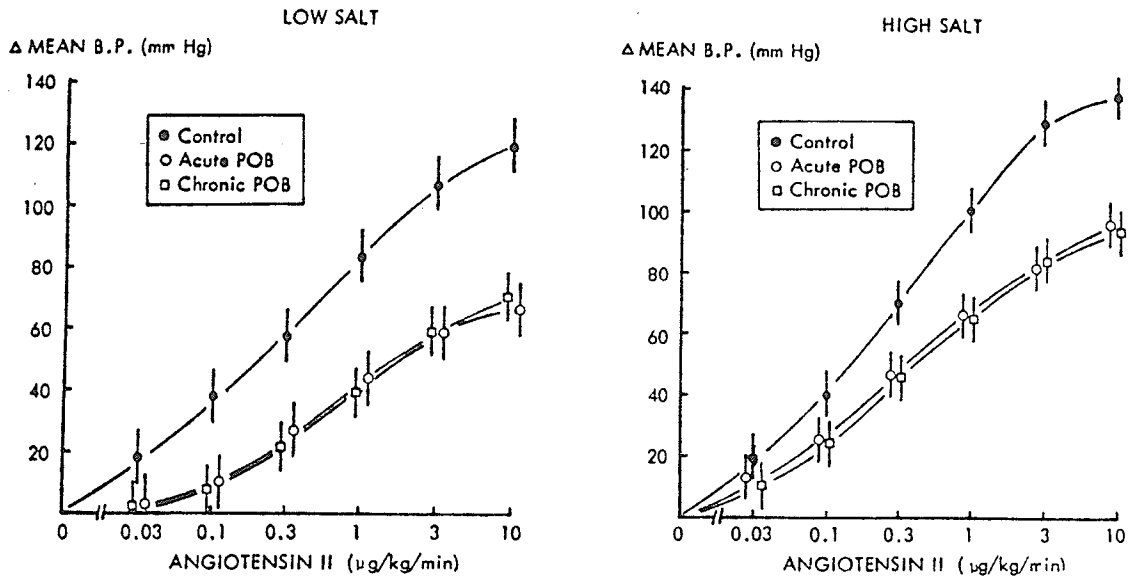

Figure 16: Effect of acute and chronic α-adrenergic blockade with phenoxybenzamine on the pressor responsiveness to angiotensin II in high and low salt rats supplemented with DOCA and furosemide. In a high salt (n=6) and low salt (n=5) group of rats, a dose response curve was done to AII before and after acute phenoxybenzamine (5 mg/Kg). In another group of high salt (n=6) and low salt (n=5) rats, only one dose response curve for AII was done following chronic phenoxybenzamine.

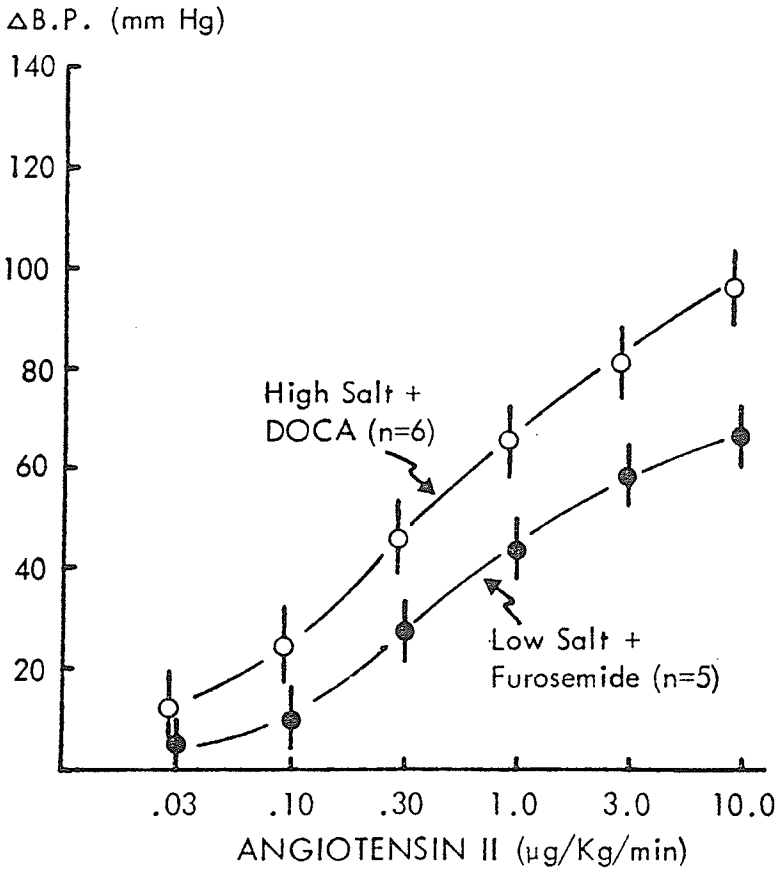

Figure 17: Pressor responsiveness to angiotensin II in high salt and low salt rats following acute α-adrenergic blockade with POB.

In the chronic POB experiments, the effectiveness of blockade was assessed by the infusion of graded doses of norepinephrine. The results were similar in the high and low salt rats (Figure 18). The lowest infusion rates demonstrated a slight pressor response to the NE. However, at infusion rates greater than 3.0 μg/Kg/min, the response was vasodepressor, suggesting α-blockade had occurred. The only significant difference between the two groups was at the highest infusion rate of 100 μg/Kg/min.

The pressor responses for AII following chronic α-blockade are presented with the responses obtained in the acute experiments (Figure 16) for comparison. In both the high and low salt groups studied, the response obtained following chronic blockade was not significantly different from the response to AII following acute blockade.

To assess the contribution of the α-adrenergic receptor to the pressor response for AII, the decrease in the blood pressure response for AII following POB at each infusion rate was tabulated (Table 9). This decrease in responsiveness to AII at each dose level would serve as an estimate of the portion of the AII pressor response that was due to activation of the α-adrenergic receptor. At all infusion rates studied, POB had a similar effect in both the high salt and low salt rats. This suggests that the contribution of the sympathetic nervous system to the pressor response for AII was not altered by the level of dietary salt intake.

c) Comments

The presence of an α-adrenergic blocking agent (POB) had a significant effect on the pressor response for angiotensin in high salt and low salt rats. It was previously mentioned that angiotensin II

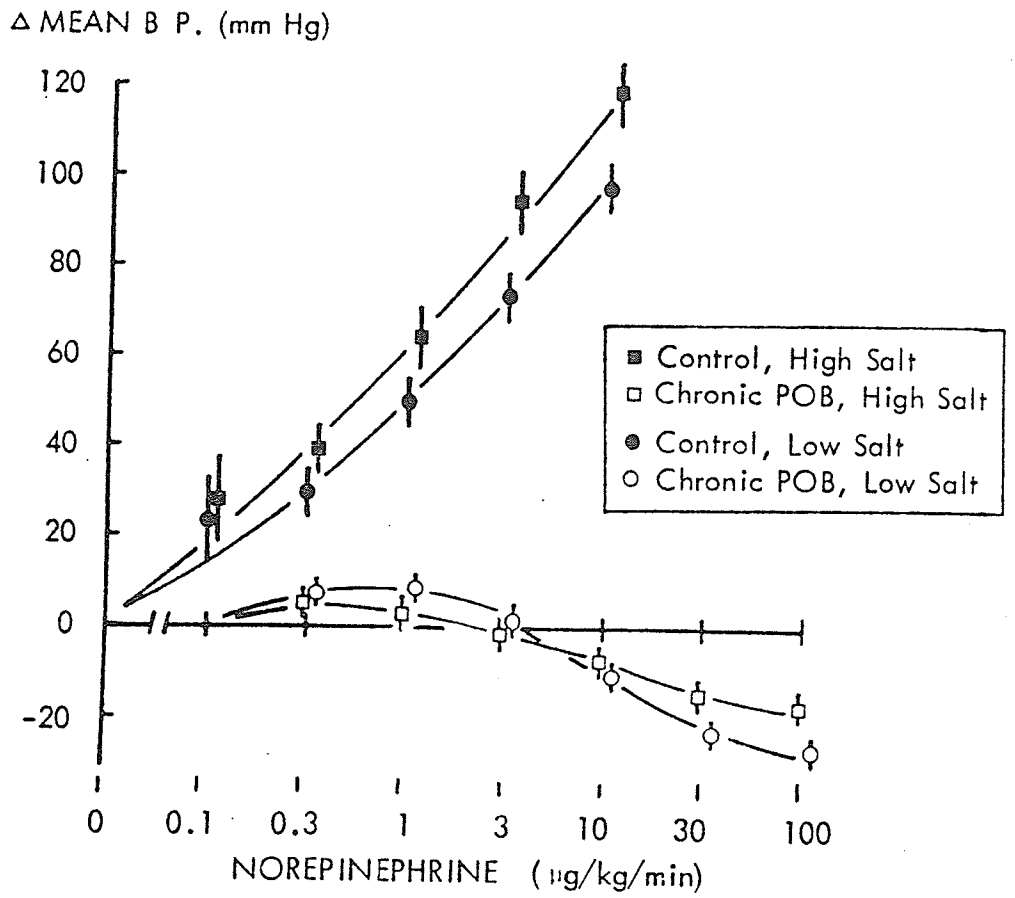

Figure 18: Effect of chronic α-adrenergic blockade with phenoxybenzamine on the pressor response for norepinephrine in high salt and low salt rats. The control response curves for the high salt (n=6) and low salt (n=6) rats were obtained from another section for comparison (Figure 21). Chronic POB high salt (n=6), low salt (n=5).

TABLE 9

Decrease in Pressor Responsiveness to Angiotensin II Following Phenoxybenzamine Treatment in High and Low Salt Rats

Angiotensin II (µg/Kg/min)	0.03	0.1	0.3	1.0	3.0	10.0
High Salt (n=6)	7.0 ±5.7	15.8 ±5.7	24.3 ±5.7	35.7 ±5.7	48.3 ±5.7	42.8 ±5.7
Low Salt (n=5)	14.4 ±6.2	27.6 ±6.2	30.4 ±6.2	40.0 ±6.2	49.0 ±6.2	54.0 ±6.2
P	NS	NS	NS	NS	NS	NS

Values represent decrease in pressor response (mm Hg).

Values are expressed as means ± pooled S.E.

could increase the levels of norepinephrine by an action on the sympathetic nervous system. If this played an important role in the peripheral pressor action of angiotensin II, it would do so by an involvement with peripheral α-adrenergic receptors. The suppression of the pressor action of angiotensin by phenoxybenzemine suggests that an endogenous α agonist (norepinephrine) is required for angiotensin to exert its full pressor effect. In addition, this α-adrenergically mediated component of the AII pressor activity is not related to the state of sodium balance.

Some earlier experiments failed to show significant attenuation of the AII pressor effects following the administration of α-adrenergic blocking agents. In general, these studies utilized phentolamine, which is known to have a much shorter duration of action and a less stable blockade than phenoxybenzamine. As well, an adequate degree of α-adrenergic blockade was usually not established in these studies.

The statistically negative results may also be due to the relatively low dose of AII administered. This is especially true in human experiments where, for ethical reasons, high doses of AII are unacceptable.

It may be argued that POB treatment resulted in a decrease in cardiac output and thus a decrease in pressor response for AII. If this was true, a 50% reduction in cardiac output would be necessary to explain the observed decrease in AII pressor responsiveness. Cardiac output was not measured in these experiments. However, data from the literature indicate that phenoxybenzamine usually does not change or may actually increase cardiac output slightly (Nickerson and Hollenberg, 1967). Thus, it appears unlikely that the attenuated AII pressor

responsiveness following POB treatment is due to the cardiac effects of the latter.

In summary, POB treatment results in attenuation of the pressor response for AII in the rat. This effect does not depend on the state of sodium balance.

2. Effect of Ganglionic Blockade

The reported effects of ganglionic blockade on the pressor response to angiotensin II have been inconsistent. Haas and Goldblatt (1960) reported that the pressor response to AII was enhanced following pentolinium in the dog. In fact, pentolinium pre-treatment is a standard laboratory procedure in the bioassay of renin activity. However, others have found either a decreased response (Samwer et al., 1974), or no change (Gableman and Rondell, 1966) following pentolinium administration. All three studies used similar doses of pentolinium.

The variation in results may have been due to the magnitude of the blood pressure lowering following blockade. Usually, the greater the depressor response to pentolinium the greater the pressor response to AII. Elijovich and Krakoff (1980) were unable to demonstrate a change in the pressor response to AII in captopril-treated low salt rats following pentolinium treatment in spite of a substantial fall in in blood pressure. In view of the effect of AII on adrenergic mechanisms, it is conceivable that the observed responses may be due to differences in the level of circulating AII as a result of either ganglionic blockade or the state of sodium balance.

The purpose of this study was to further identify the probable site of action of AII in its interaction with the sympathetic nervous

system and the effects of changes in the state of sodium balance. Specifically, we evaluated the effect of ganglionic blockade with pentolinium on the pressor activity of angiotensin II in low salt and high salt rats.

a) Method

Two groups of animals used in this study had been placed in a high state (n=5) or low state (n=6) of sodium balance with diet, as well as supplementary DOCA and furosemide injections respectively, as described previously. Two dose response curves to AII were done. An experimental treatment, intravenous pentolinium tartrate (1 mg/Kg), was administered between dose response curves. The second dose response was done approximately 20 minutes after the administration of the pentolinium.

b) Results

Pentolinium treatment had no effect on the pressor response for AII in a low salt group of rats (Figure 19). In these animals, blood pressure dropped 16.7 ± 3.0 mm Hg following ganglionic blockade. Similarly, in the high salt group of rats (Figure 19), the pressor response for AII was unaltered by pentolinium treatment. In these rats, blood pressure fell 27.6 ± 3.3 mm Hg following ganglionic blockade.

c) Comments

The degree of ganglionic blockade achieved in these experiments had no effect on the pressor response for angiotensin II in animals placed on either a low salt or high salt intake. Pentolinium produced a significant degree of ganglionic blockade. This is demonstrated by the fall in blood pressure following the pentolinium administration.

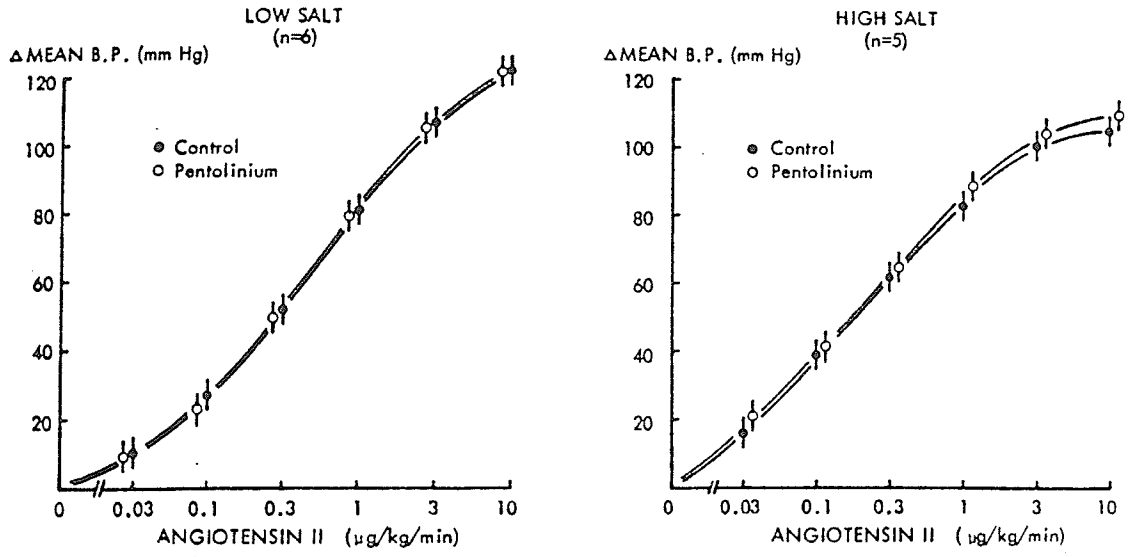

Figure 19: Effect of ganglionic blockade with pentolinium on the pressor responsiveness for angiotensin II in high salt and low salt rats supplemented with DOCA and furosemide.

3. The Effect of Beta-Adrenergic Blockade

Angiotensin in subpressor and pressor doses potentiates the vasoconstrictor response of nerve stimulation and norepinephrine infusion (Benelli et al., 1964; Zimmerman and Gomez, 1965; Zimmerman et al., 1972). Several lines of evidence suggest that this potentiation by AII is through the enhancement of the activity of the peripheral sympathetic nevous system by increasing levels of norepinephrine at the receptor site (Khairallah et al., 1971; Bell, 1972; Roth, 1972). However, norepinephrine released from the sympathetic nerve ending also acts on pre-synaptic β-receptors promoting further release of the neurotransmitter (Adler-Graschinsky and Langer, 1975). Thus, it is possible that β-adrenergic blockade could attenuate the potentiation of nerve stimulation by AII. This latter feature has been proposed as one of the possible mechanisms of anti-hypertensive actions of β-blockers.

In the isolated mesenteric vascular preparation with a protein free perfusate, Jackson and Campbell (1980, 1981) have shown recently that β-adrenergic blockers inhibit the enhancement of nerve stimulation by AII. As well, β-blockers have been shown to produce a general decrease in vascular contractility in response to norepinephrine and barium in vitro (Moulds et al., 1978). Both of these actions, if significant in vivo, would result in a decreased pressor response to AII in the presence of β-blockade. On the other hand, beta agonism is known to play an important role in the regulation of renin release (Davis and Freeman, 1976). In fact, propranolol is well recognized in producing a significant drop in plasma renin activity (Buhler et al., 1972; Antonaccio et al., 1979a). Our previous experiments would suggest any sufficient reduction in endogenous AII, should increase AII sensitivity in vivo. Thus, it is

difficult to predict the effect of β-blockade on AII pressor responsiveness.

The purpose of these experiments was to assess the role of β-adrenergic blockade in the pressor response to angiotensin II. More specifically, we studied the effect of short term and long term propranolol treatment on the pressor response for AII in high salt and low salt rats.

a) Methods

Two groups of rats, placed in a state of high or low sodium balance by dietary changes only, were studied. In both groups, animals received (i) propranolol treatment for one day, (ii) propranolol treatment for seven days, or (iii) no treatment. Propranolol was added to the drinking water (tap water or 0.9% saline) at a final concentration of 0.1 mg/ml for one day or 0.2 mg/ml for seven days. The volume of drinking water was recorded during this period to obtain an approximation of the quantity of propranolol or water consumed. Only one dose response curve for AII was done in each animal.

b) Results

The approximate daily propranolol intake and baseline blood pressures for the four groups studied are shown in table 10. Only the high salt group on seven days of propranolol had a significantly greater intake of propranolol than the other three groups ($P < .01$). Blood pressures were not significantly different.

The pressor response for AII in these rats is shown in figure 20. In the no treatment controls, the pressor response for AII was greater in the high salt group. In rats on a high salt intake, one day or seven day propranolol treatment did not alter the pressor response

TABLE 10

Daily Oral Propranolol Intake and Blood Pressures in High and Low Salt Rats

	1 Day Propranolol			7 Day Propranolol		
	Dose mg/Kg/day	B.P. mm Hg	n	Dose mg/Kg/day	B.P. mm Hg	n
High Salt	4.1 ±.85	98.3 ±7.0	6	14.7 ±.85	80.3 ±7.0	6
Low Salt	5.3 ±.85	90.7 ±7.0	6	5.6 ±.85	85.3 ±7.0	6

Values are expressed as means ± S.E.

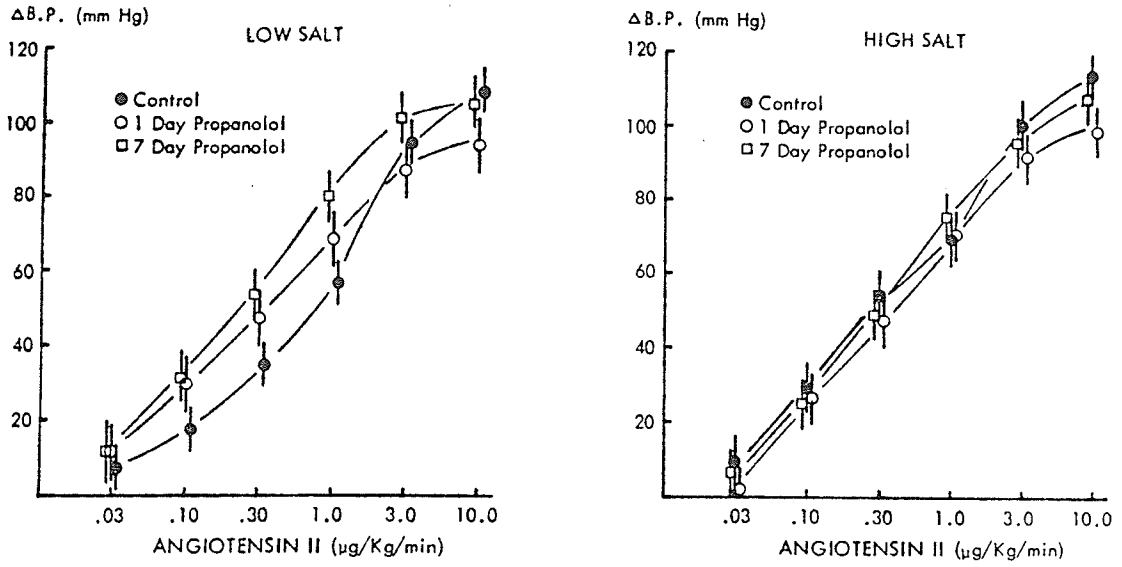

Figure 20: Effect of one day and seven day oral propranolol treatment on pressor responsiveness for angiotensin II in high salt and low salt rats. Three groups of low salt rats are presented, control (n=8), one day propranolol (n=5) and seven day propranolol (n=6). Three groups of high salt rats are presented, control (n=11), one day propranolol (n=6) and seven day propranolol (n=6).

for AII. In the low salt rats, one day propranolol treatment produced a slight, but non-significant increase in the pressor responsiveness to AII. However, the group receiving seven days of oral propranolol demonstrated an enhanced pressor responsiveness for AII at infusion rates of .30 and 1.0 μg/Kg/min ($P < .05$). As well, the pressor responses for the high and low salt groups were similar following either one day or seven days of oral propranolol treatment.

c) Comments

The effect of propranolol treatment on the pressor responsiveness for AII appears to be dietary salt sensitive. The high salt intake group were unaffected by the oral propranolol. The degree of β-blockade achieved was not assessed in these experiments by the infusion of a β-agonist. However, the quantities of propranolol ingested were comparable to those used by others where plasma renin activity was suppressed (Antonaccio, 1979).

In the sodium restricted rat, one day propranolol treatment probably had a slight effect on the AII pressor responsiveness. Following seven days of oral propranolol treatment, the pressor responsiveness for AII was significantly enhanced. These findings are consistent with the hypothesis that beta blockade alters the pressor effects of AII by inhibiting renin release and endogenous AII production. This is further supported by the observation that the pressor response curves for AII were similar in the high and low salt rats, following propranolol treatment. In addition, if AII pressor responsiveness is an important mechanism in the pathogenesis of some types of hypertension, our data suggest that propranolol does not lower blood pressure by reducing sensitivity to AII.

4. Discussion

Angiotensin II is a potent vasoconstrictor. It is known to act directly by interacting with specific angiotensin receptors. Indirectly, angiotensin also interacts with the peripheral sympathetic nervous system. Previous studies have shown that subpressor doses of AII potentiate the vasoconstrictor effects of nerve stimulation and/or norepinephrine infusion in certain vascular beds and isolated tissues (Benelli et al., 1964; Zimmer and Gomez, 1965; Ekboir and Enero, 1980; Jackson and Campbell, 1981). This potentiation has been proposed to be due to either an increased release (Bell, 1972), decreased uptake (Khairallah et al., 1971), and/or decreased metabolism (Roth, 1972) of norepinephrine in the presence of AII. Thus, part of the pressor response for AII may be due to a sympathomimetic effect. However, pharmacological interruption of the adrenergic system has resulted in inconclusive findings. Alpha-adrenergic blockade in isolated vascular beds results in either a decrease (Scroop and Whelan, 1968) or no change (Bohr, 1974; Peach, 1977) in the response for AII. Similarly, the blood pressure response to AII is either decreased (Bickerton and Buckley, 1961; Chryssanthou et al., 1971) or unaffected (Scroop and Whelan, 1968) by α-adrenergic blockade. Discrepancies reported in these studies may be related to an inadequate dose or an inappropriate choice of α-blocking agent resulting in an inadequate degree of α-blockade. In the reported clinical studies, for obvious ethical reasons, the administered dose of AII may have been too low to detect a difference. As well, although the α-adrenergic blockade may partially attenuate the AII effects in some vascular beds, this may not be sufficient to alter the systemic pressor response for angiotensin.

In the present study, we assessed the effect of high and low salt intake on AII pressor responsiveness before and after α-adrenergic blockade with phenoxybenzamine. In both acute and chronic experiments, the degree of α-blockade was tested and found to be adequate. The kinetics of POB blockade changes from an initial competitive type to a non-equilibrium type blockade once a stable covalent bond is formed (Nickerson, 1957). In both acute and chronic experiments, phenoxybenzamine markedly attenuated the AII pressor responses. These results agree with earlier experiments of Bickerton and Buckley (1961), who reported a greatly suppressed response to AII following piperoxane treatment in the dog. Thus, it appears that α-adrenergic receptor activation is required to observe the full pressor effect of angiotensin.

Sybertz and Peach (1980) have found that the degree of AII potentiation of the response to nerve stimulation of isolated vascular strips is related directly to the donor animals' previous state of sodium balance. However, we were unable to demonstrate any difference between the high and low salt groups following POB treatment. Thus, in certain vascular beds, the level of interaction of the sympathetic nervous system and angiotensin II may be dietary salt sensitive. However, this effect does not appear to be great enough to alter the systemic pressor response for AII.

To establish the site of interaction, we studied the pressor response for AII before and following ganglionic blockade with pentolinium. Pentolinium produced a modest degree of hypotension, indicative of ganglionic blockade. The dosage was chosen to avoid excessive hypotension since this may potentiate AII pressor responsiveness. In our study, ganglionic blockade had no effect on the pressor response for AII

in either the high or low salt group. This is consistent with the findings of Elijovich and Krakoff (1980), who noted no change in the pressor responsiveness to AII following ganglionic blockade in the captopril treated rat, despite a significant drop in blood pressure following pentolinium and captopril. Thus, our findings indicate that the interaction between angiotensin and the sympathetic nervous system is likely to be post-ganglionic.

The effect of β-adrenergic blockade on the pressor response for AII was also assessed. The pressor response for AII was unaltered in the high salt groups following one day or seven days of propranolol treatment. However, in the low salt groups, one day propranolol treatment produced a slight, although insignificant, enhancement and seven days of propranolol resulted in a significant enhancement of the pressor response for AII. As well, the pressor response for AII was not significantly different between the high and low salt groups following one day or seven days of oral propranolol. These results suggest that the effect of β-adrenergic blockade on the AII pressor responsiveness is salt sensitive, only increasing the responsiveness in sodium depleted animals.

The enhancement of the response in the low salt animals may be by a mechanism similar to that shown for captopril in a previous section. That is, the suppression of endogenous AII levels in the low salt groups. The β-adrenergic receptor is known to be important in the control of renin release (Davis and Freeman, 1976; Oates et al., 1979). Antonaccio et al. (1979) have shown that oral propranolol treatment in spontaneously hypertensive and normotensive rats significantly lowers plasma renin activity. Thus, in our low salt groups, β-blockade may have resulted

in a dose related drop in endogenous plasma renin activity and, therefore, a decrease in the endogenous level of circulating AII. As well, this would explain why the high salt groups were unaffected.

Beta blockers are effective antihypertensive agents. A number of mechanisms have been proposed to explain their effects. One of these include a diminished vascular responsiveness. Our finding of an enhanced AII responsiveness following propranolol treatment is thus surprising when we consider the work of Jackson and Campbell (1980). These authors found that β-blockers inhibited the potentiation of the pressor response to nerve stimulation by angiotensin. This inhibition by β-blockers has been attributed to an increased release of prostaglandins, which probably act to prevent the release of norepinephrine (Jackson and Campbell, 1981). In a previous section, we demonstrated the importance of an intact peripheral α-adrenergic system to observe the full pressor effect of angiotensin. If this mechanism played an important role in the hypotensive response to β-blockers, then a suppression of the pressor response to AII would be expected. Similarly, Moulds _et al_. (1978) found a non-specific decrease in vascular smooth muscle contraction in the presence of a β-blocker. Again, if this played a significant role in the hypotensive action of β-blockers, a decreased response to AII would be expected. However, we observed a significant enhancement of the pressor response for AII. These discrepancies may be related to the model studied and dose administered. In the studies cited, _in vitro_ preparations or relatively higher doses were used. More importantly, these are probably not real discrepancies. A decrease in vascular responsiveness probably occurred _in vitro_ following propranolol, but this effect is overridden by the effect of inhibition of renin release

in vivo. The net result is an enhanced pressor responsiveness to AII in the low salt rat. These studies further illustrate the significance of endogenous AII activity in the regulation of AII pressor response.

In summary, our results suggest that the sympathetic nervous system plays an important role in the determination of the pressor response for AII. As well, it appears that activation of the α-adrenergic receptor is required to observe the full pressor effect of angiotensin. However, the contribution of the α-receptor to the pressor response for AII does not appear to be salt sensitive. The role of β-adrenergic blockade was also evaluated. It possibly plays a role in the regulation of AII responsiveness through the regulation of renin release. Consequently, the effect of β-blockade on the pressor response for AII was found to be dietary salt sensitive.

C. SODIUM BALANCE AND THE ROLE OF ENDOGENOUS PROSTAGLANDIN IN THE REGULATION OF PRESSOR RESPONSIVENESS IN VIVO

1. Effect of Dietary Salt Intake
2. Effect of the State of Sodium Balance on Pressor Responsiveness to Angiotensin II Before and After Indomethacin and Captopril Treatment
3. Effect of the State of Sodium Balance on Pressor Responsiveness to Norepinephrine Before and After Indomethacin and Captopril Treatment
4. Effect of the State of Sodium Balance on Pressor Responsiveness to Angiotensin II Before and During Ganglionic Blockade with Pentolinium
5. Effect of the State of Sodium Balance on Pressor Responsiveness to Angiotensin II Before and During Alpha Adrenergic Blockade with Phenoxybenzamine
6. Effect of Indomethacin on the Hypotensive Action of Captopril
7. Discussion

The *in vivo* pressor responsiveness to exogenous angiotensin II is dependent on sodium intake. Previous sections have demonstrated that a high salt intake enhances and a low salt intake attenuates the pressor response to AII, which is consistent with the published literature (Thurston and Laragh, 1975; Slack and Ledingham, 1976; Oliver and Cannon, 1978; Hollenberg, *et al.*, 1972). The previous sections also suggested that this change may be due to secondary changes in the endogenous level of AII and/or due to changes in the vascular sensitivity as a result of the altered sodium intakes. It was also determined that the α-adrenergic receptor played a role in the AII pressor activity, although this role did not appear to be altered by the level of sodium intake.

Recently, it has been demonstrated that the endogenous production of some prostaglandins may be salt sensitive (Zusman *et al.*, 1973; Davila *et al.*, 1978; Stahl *et al.*, 1979; Oliver *et al.*, 1980; Yoshimura *et al.*, 1980). It has also been shown that endogenous prostaglandins may be an important determinant of the pressor response to AII (McGiff *et al.*, 1976; Obrien and Pipkin, 1979; Aiken and Vane, 1973). It is, therefore, conceivable that the observed changes in prostaglandin levels associated with altered states of sodium balance may potentially be responsible for the associated changes in pressor responsiveness to AII. The purpose of this section was to establish if endogenous levels of prostaglandins are important determinants of the pressor activity of angiotensin and norepinephrine. More specifically, we wished to determine the pressor responsiveness for AII and NE before and during prostaglandin synthetase inhibition in the high and low salt rat. In addition, the effect of converting enzyme inhibition with captopril was

also studied under these conditions.

1. Effect of Dietary Salt Intake

In this section, the effect of salt intake on the pressor responsiveness for AII and NE are reported.

a) Method

Animals in these studies had been placed in a state of high salt (n=12) or low salt (n=18) balance by using the previously described dietary regimes that had been supplemented with DOCA or furosemide. Again, as previously described, the control dose response curves for AII or NE were compared between the high and low salt intake rats.

b) Results

Rats placed on a high salt intake, when compared to those on low salt intake, showed an enhanced pressor response to AII at infusion rates of .30, 1.0 and 3.0 μg/Kg/min ($P < .01$). The pressor response for norepinephrine was similar between the two groups studied (Figure 21).

c) Comments

Results reported in this section are similar to that reported previously (Specific Experiments, Part A). Thus, it is valid to use these animals as controls in the corresponding experiments described in the following sections.

2. Effect of the State of Sodium Balance on Pressor Responsiveness to Angiotensin II Before and After Indomethacin and Captropril Treatment

Previous investigators have demonstrated a possible role of prostaglandins as determinants of vascular reactivity to a number of

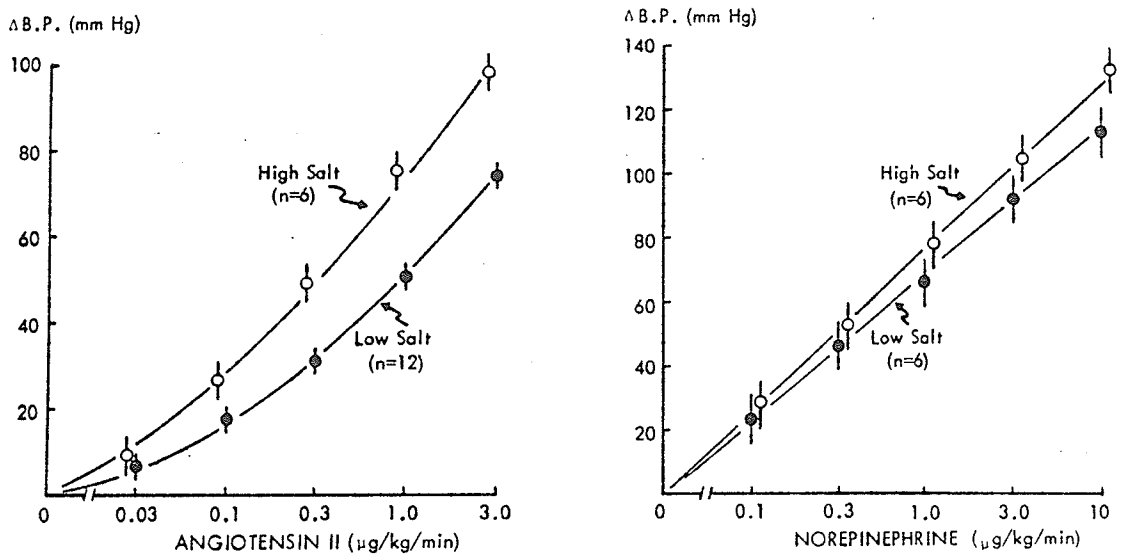

Figure 21: Effect of dietary salt intake on the pressor responsiveness for angiotensin II or norepinephrine in high salt and low salt rats supplemented with DOCA and furosemide.

vasoactive agents (McGiff et al., 1976; Aiken and Vane, 1973). Aiken and Vane (1973), showed that following indomethacin, a prostaglandin synthetase inhibitor, the renovascular response to angiotensin II is enhanced. Murthy et al. (1978), however, observed no change in the pressor response to angiotensin II or norepinephrine following indomethacin treatment in the conscious rabbit. The inconsistant results may be due to the vascular bed studied or the use of general anesthesia. As well, the amount and type of endogenous prostaglandin present in different tissues in different animal species may also be a factor.

Recently, several investigators (Zusman et al., 1973; Davila et al., 1978; Stahl et al., 1979; Oliver et al., 1980; Yoshimura et al., 1980) have demonstrated that the synthesis of certain prostaglandins may also be salt sensitive. The purpose of these experiments was to assess the role of prostaglandins in the pressor response to AII in rats in high and low states of salt balance. As well, it has been reported that the endogenous level of prostaglandins are increased following captopril administration (Swartz et al., 1979). Therefore, we also attempted to determine whether the increased pressor response to AII in low salt rats following captopril treatment is altered by the administration of a prostaglandin synthetase inhibitor.

a) Method

Four groups of animals were studied. In three groups studied, two or three dose response curves were obtained for AII using incremental infusion rates. Between pressor infusions, experimental treatments were administered. In two experimental groups, one high salt (n=6) and one low salt (n=6), indomethacin (dissolved in phosphate

buffer pH = 7.4) (5 mg/Kg) was administered intraperitoneally as the first treatment. Following a period of one hour, the second response curve for AII was done. The second experimental treatment was captopril (1 mg/Kg) IV. The preparation was again allowed to reach a steady state. Then, the final response curve for AII was completed. In one group of low sodium rats studied (n=6) the order of administration of the treatment was reversed. In a final group of low salt rats (n=5), only two dose response curves for AII were done. The experimental treatment was meclofenamate (5 mg/Kg) administered intravenously. The second dose response curve was done one hour following the meclofenamate.

b) Results

Following indomethacin treatment (5 mg/Kg; IP), there was no change in the response to AII in animals previously placed on a high salt intake (Figure 22). In the low salt rats, however, treatment with indomethacin resulted in a significant increase in response to angiotensin II (Figure 22). Pressor responses to infusion rates of .10, .30, 1.0 and 3.0 µg/Kg/min were significantly greater than the control responses ($P < .01$). In these same low salt rats, the further addition of captopril (1 mg/Kg), resulted in another significant shift of the response curve for AII to the left. Infusion rates of .10, .30, 1.0 and 3.0 µg/Kg/min of AII showed further increased response ($P < .01$) when compared to the previous curve obtained when indomethacin alone was present.

Figure 23 shows the results when the order of these two treatments were reversed in a low salt group. As demonstrated in a previous section, the administration of captopril resulted in a significant

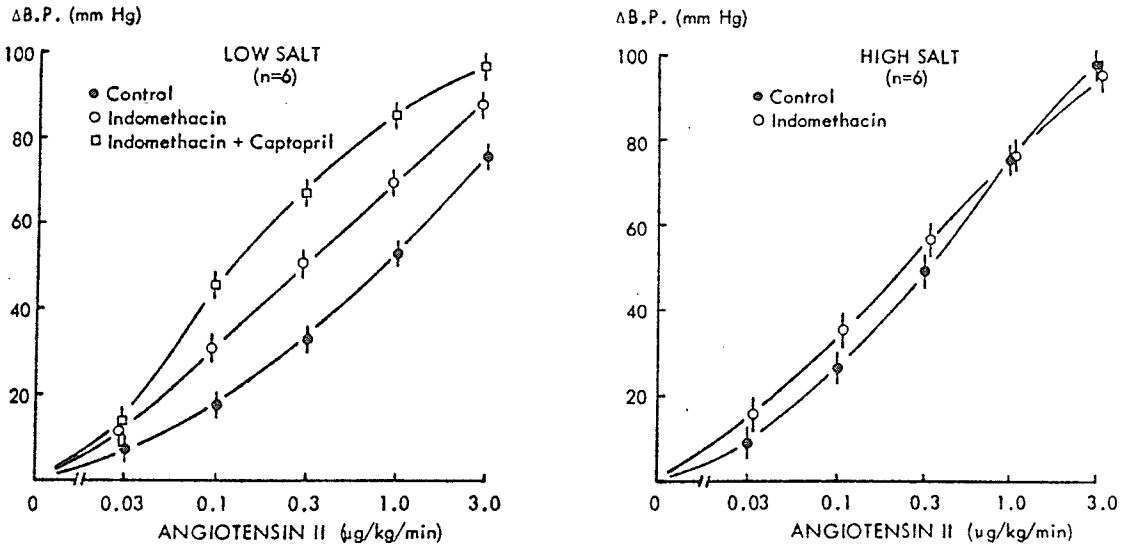

Figure 22: Pressor responsiveness to angiotensin II following indomethacin and then captopril treatments in high salt and low salt rats supplemented with DOCA and furosemide. Low salt group n=6, high salt group n=6.

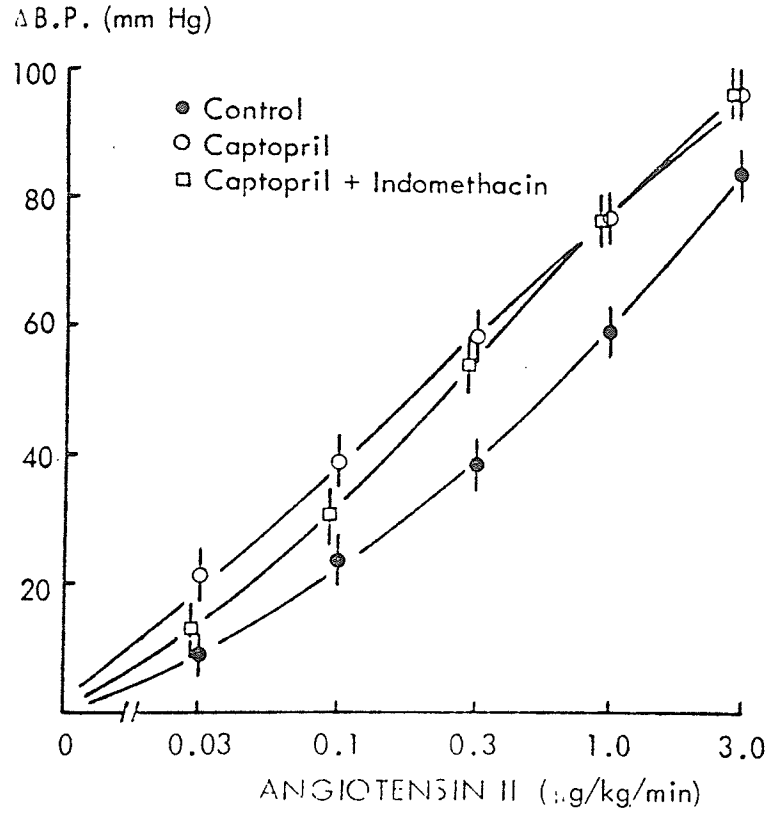

Figure 23: Pressor responsiveness to angiotensin II following captopril and then indomethacin treatments in low salt rats supplemented with furosemide (n=7).

increase in response at infusion rates of .30, 1.0, 3.0 μg/Kg/min, when compared to the control. When indomethacin was administered in the presence of captopril, however, there was no further change in pressor response as compared to the captopril treatment alone curve. The dose response curve for AII in the presence of captopril and indomethacin was still significantly shifted to the left of the control curve.

With another prostaglandin synthetase inhibitor, meclofenamate, the results were similar (Figure 24). In a group of low salt rats, meclofenamate treatment resulted in an increased pressor response when compared to the control response at AII infusion rates of .10 ($P < .01$), .30 ($P < .01$) and 1.0 ($P < .05$) μg/Kg/min.

c) Comments

Indomethacin treatment resulted in an increased pressor response to AII only in animals that had been previously on a low salt intake. High salt rats were unaffected. This suggests that prostaglandins play a role in determining pressor responsiveness for AII only in low states of sodium balance. The proposal that indomethacin is acting by inhibiting prostaglandin synthesis is supported by the fact that meclofenamate treatment resulted in similar observations. It is interesting to note that captopril pre-treatment abolished the AII potentiating effect of indomethacin. It is now important to determine whether this action of indomethacin is specific for AII or for vasopressor agents in general.

3. Effect of the State of Sodium Balance on the Pressor Responsiveness to Norepinephrine Before and After Indomethacin and Captopril

It has been proposed that prostaglandins, acting as vasodilators,

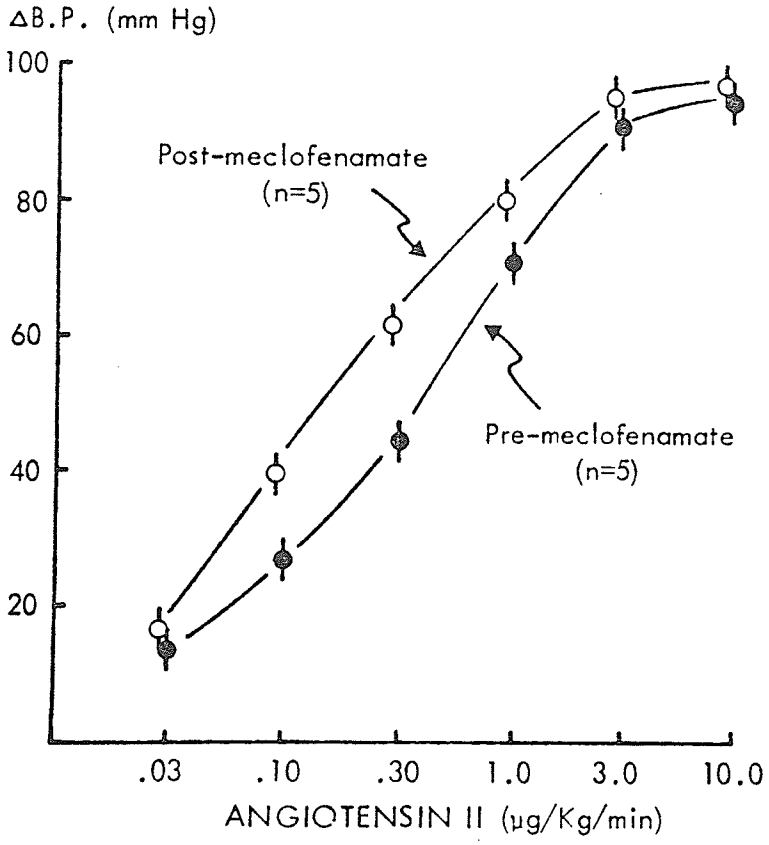

Figure 24: Effect of meclofenamate on the pressor responsiveness to angiotensin II in low salt rats supplemented with furosemide (n=5).

are released in response to any general vasoconstrictor stimuli (McGiff et al., 1976). This would suggest that the potentiation for AII following prostaglandin synthesis inhibition, would be due to the removal of a prostaglandin functioning as a physiological antagonist. To test this hypothesis, the following experiments were done to determine if the effect of indomethacin on AII is non-specific, with NE as the vasoconstrictor.

a) Method

High salt (n=7) and low salt (n=7) rats were used. Three dose response curves for NE were done. Indomethacin (5 mg/Kg) IP was the first treatment. The second dose response was done one hour after indomethacin administration. Captopril (1 mg/Kg) IV was given as the second treatment. The third dose response curve was done 15 to 20 minutes following the captopril administration.

b) Results

The effect of indomethacin (5 mg/Kg) IP on pressor responsiveness to norepinephrine was studied in low salt and high salt rats (Figure 25). In rats previously on a high sodium intake, the treatment of indomethacin had no effect on the pressor response to norepinephrine. The further addition of captopril had no effect. These results were similar to those observed for AII in high salt rats.

In the low salt group, unlike the results obtained for AII, there was no enhancement of the pressor response obtained for norepinephrine following indomethacin. As well, the addition of captopril also had no effect on the pressor response to norepinephrine in these animals.

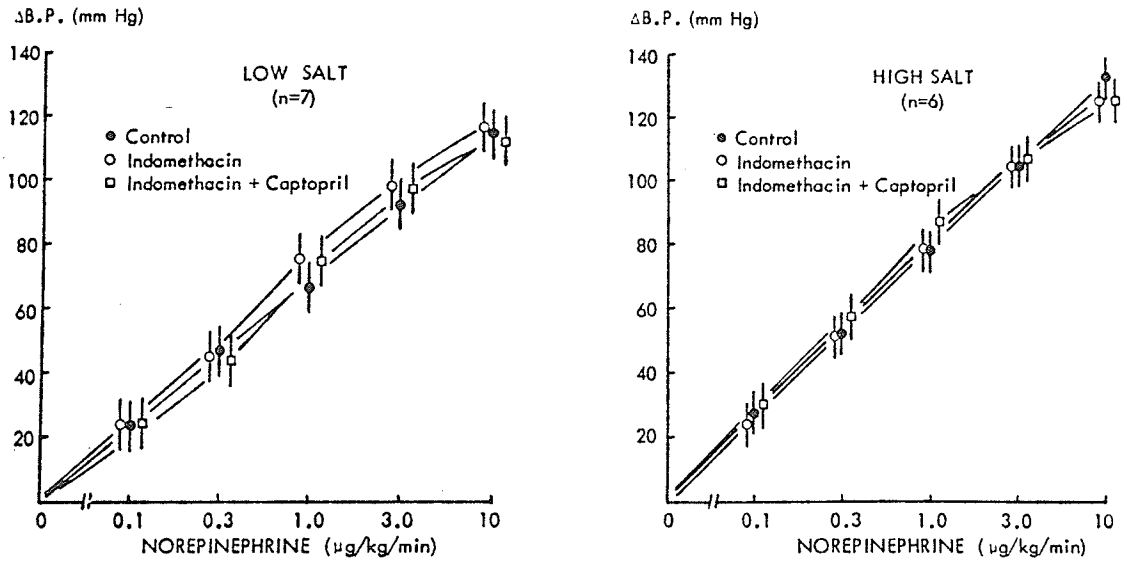

Figure 25: Pressor responsiveness to norepinephrine following indomethacin and then captopril treatments in high salt (n=6) and low salt (n=7) rats supplemented with DOCA and furosemide.

c) Comments

The potentiation by indomethacin of the pressor response for angiotensin II in low salt rats was not also observed for norepinephrine. This suggests that the ability of indomethacin to enhance pressor responsiveness may be an angiotensin-specific phenomenon. Particularly, it appears that endogenous prostaglandins are not acting as non-specific physiological antagonists.

4. Effect of the State of Sodium Balance on Pressor Responsiveness to Angiotensin II Before and During Ganglionic Blockade with Pentolinium

Indomethacin has been shown to potentiate the response to AII in low salt rats, most probably by interfering with prostaglandin synthesis. Subpressor doses of angiotensin II will potentiate the pressor response to low frequency nerve stimulation and norepinephrine infusion (Ekboir and Enero, 1980; Jackson and Campbell, 1980). It has also been proposed that this potentiation by AII may be antagonized by prostaglandins, such as prostaglandin E_2 but not by prostaglandin I_2 (Jackson and Campbell, 1980). It is well known that certain prostaglandins inhibit the release of NE from nerve endings (Frame and Hedqvist, 1975; Hedqvist, 1976; Hedqvist, 1978). Previously, we demonstrated the importance of an intact adrenergic nervous system for the full activity of infused AII to be observed. Thus, it appears that the potentiation observed following indomethacin may be due to the removal of the antagonist (prostaglandin) which affects the AII potentiation of the sympathetic nervous system. The purpose of these experiments was to assess the effect of ganglionic blockade on the potentiation for AII

following prostaglandin synthetase inhibition in low salt rats.

a) Method

Rats given a sodium deficient diet plus furosemide injection were used (n=6). Three dose response curves were done for AII. The first treatment was pentolinium (1 mg/Kg) administered intravenously. Blood pressure was allowed to restabilize for 20 minutes before the second dose response curve was started. The second treatment was indomethacin (5 mg/Kg). Following this, one hour elapsed before the final dose response was done.

b) Results

The control and post-pentolinium dose response for AII has been reported in a previous section. The blood pressure decreased from 87.3 ± 2.7 mm Hg to 70.7 ± 2.7 mm Hg ($P < .01$) following pentolinium. Pentolinium did not alter the pressor response for AII (Figure 26). Similarly, in the presence of pentolinium, indomethacin failed to potentiate the pressor response for AII in these low salt rats.

c) Comments

In the presence of ganglionic blockade in the low salt rat, indomethacin treatment failed to enhance AII pressor activity. These experiments suggest that an intact autonomic nervous system is required for the potentiating effect of indomethacin on the AII pressor response. As reported previously, ganglionic blockade did not alter the response for AII.

5. Effect of the State of Sodium Balance on Pressor Responsiveness to Angiotensin II Before and During Alpha Adrenergic Blockade with Phenoxybenzamine

Prostaglandins have been proposed as antagonists of the

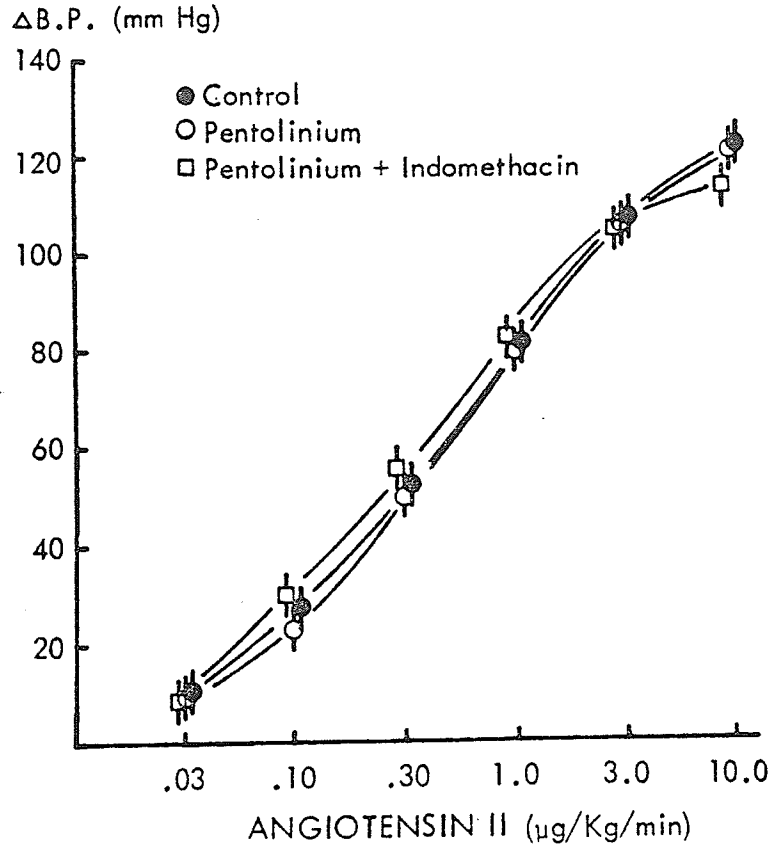

Figure 26: Pressor responsiveness to angiotensin II before and during ganglionic blockade with pentolinium and indomethacin treatment in low salt rats supplemented with furosemide (n=6).

potentiating action of angiotensin on the pressor response to nerve stimulation (Jackson and Campbell, 1981). This antagonism of the pressor response to nerve stimulation suggests an indirect interaction with an α-agonist or the α-receptor site. Thus, in our experiments, the potentiating action of indomethacin on AII pressor response may be due to the removal of a prostaglandin that is somehow acting peripherally on the α-adrenergic nervous system. If this is so, then an α-adrenergic blocker should abolish or attenuate the potentiating effect of indomethacin on the pressor response for AII. The purpose of these experiments was to assess the effect of an α-adrenergic blocking agent, phenoxybenzamine, on the ability of indomethacin to potentiate the pressor response for AII in a group of sodium-deficient rats.

a) Method

Rats given a sodium deficient diet plus furosemide injections were used (n=5). Three dose response curves for AII were done. The first experimental treatment was phenoxybenzamine (5 mg/Kg) given intravenously. Approximatley 20 minutes following this, the second dose response to AII was done. The second experimental treatment was indomethacin (5 mg/Kg). Approximately one hour following this, the third dose response to AII was done.

b) Results

The effect of acute α-blockade on the pressor response for AII has been reported in a previous section. In these experiments, phenoxybenzamine resulted in a significant decrease of the pressor response for AII at infusion rates of .10, .30, 1.0, 3.0 and 10.0 μg/Kg/min ($P < .01$) (Figure 27). In the presence of POB, indomethacin failed to potentiate the pressor response for AII. In fact, the AII

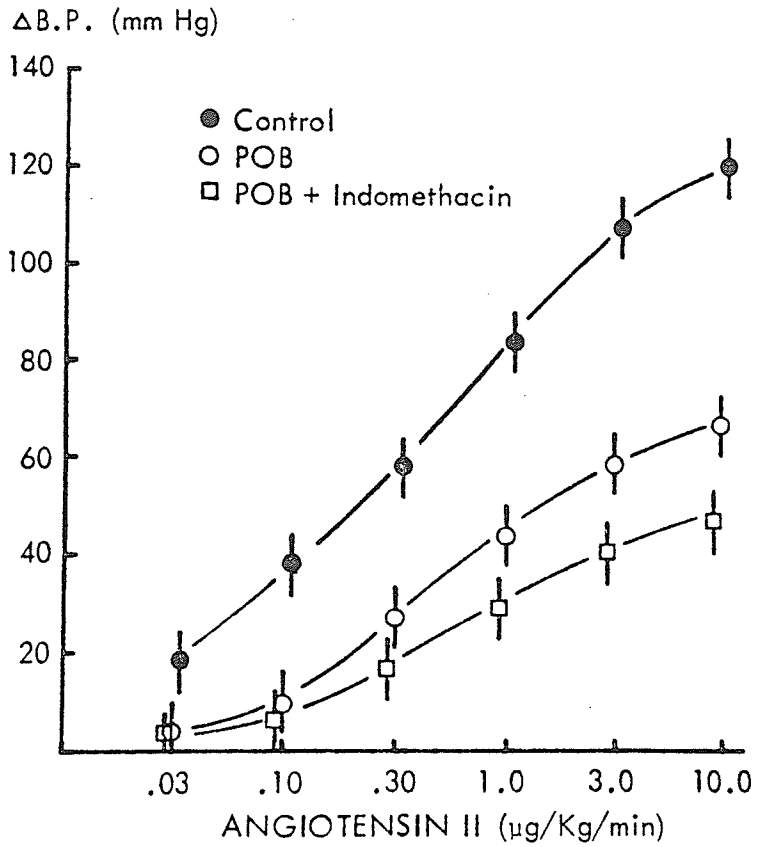

Figure 27: Pressor responsiveness to angiotensin II before and during α-adrenergic blockade and indomethacin treatments in low salt rats supplemented with furosemide (n=5).

infusion rate of 10.0 μg/Kg/min demonstrated a significant decrease in the pressor response for AII ($P < .05$).

c) Comments

Acute α-adrenergic blockade significantly decreased the response for AII, suggesting AII increases blood pressure partly by indirectly activating the α receptor. This probably occurs through the release of norepinephrine. When this component of the AII pressor response was blocked by POB, indomethacin failed to potentiate the response for AII.

6. Effect of Indomethacin on the Hypotensive Action of Captopril

The mechanism responsible for the hypotensive action of captopril is uncertain. Recent evidence has suggested that the blood pressure lowering effect of captopril may correlate best to changes in bradykinin (Mimran et al., 1980) and/or prostaglandin levels (Swartz et al., 1980; Moore et al., 1981) and not necessarily to changes in endogenous AII levels (Swartz et al., 1979). We, therefore, assessed the effect of indomethacin pre-treatment on the hypotensive action of captopril.

a) Method

The methods are as previously described. In the previously mentioned experiments, captopril treatment preceded or followed indomethacin treatment, depending on the experimental protocol. The effect of indomethacin on the hypotensive effect of captopril could, therefore, be assessed. Data reported in this study are taken from animals mentioned in the previous sections (Specific Experiments, Part C, no. 1-5).

b) Results

Blood pressures before captopril administration were not significantly different in the control and indomethacin-treated groups (Table 11). Following captopril administration, a biphasic effect (described previously) was observed in both groups. There was a transient fall in blood pressure over a 4 to 6 minute period. Following this, the blood pressure slowly rose towards control levels and restabilized. In the presence of indomethacin, captopril produced a significantly smaller hypotensive effect than when no indomethacin was given. The values were -13.2 ± 5.7 mm Hg and -38.5 ± 5.7 mm Hg, respectively ($P < .01$). The new steady state blood pressure also appeared to be less suppressed in the presence of indomethacin, although it was not significantly different from the captopril alone animals ($P = .052$). In the presence and absence of indomethacin treatment, captopril produced a significant maintained fall in blood pressure when the pre-captopril blood pressure and the new steady state levels were compared ($P < .05$).

c) Comments

These experiments show that indomethacin pre-treatment will decrease, but not totally abolish the hypotensive action of captopril. The data suggest that part of the acute hypotensive action of captopril may be due to prostaglandin production.

TABLE 11

Effect of Indomethacin on the Hypotensive Action of Captopril in Low Salt Plus Furosemide Rats

	Control	Captopril 1mg/Kg	
	Pre-Captopril B.P. (mm Hg)	Maximal ΔB.P. (mm Hg)	Steady State ΔB.P. (mm Hg)
No Pre-Treatment (n=7)	110.0 ±5.1	-38.3 ±5.0	-11.3 ±2.5
Indomethacin Pre-Treatment (n=11)	103.6 ±4.1	-15.6 ±4.0	-6.9 ±2.0
P	N.S.	P < .001	P = .052

7. Discussion

It has been well established that a direct relationship exists between the state of sodium balance and the pressor responsiveness for angiotensin II. A number of vasoactive systems are altered by sodium intake, which include the renin-angiotensin system (Brown et al., 1964; Churchill et al., 1973), the kallikrein-kinin system (Levinsky, 1979; Margolius et al., 1974), the prostaglandin system (Zusman et al., 1973; Davila et al., 1978; Stahl et al., 1979; Oliver et al., 1980; Yoshimura et al., 1980), and the adrenergic system (Luft et al., 1979; Nicholls et al., 1980). The role of endogenous angiotensin II on the pressor response to exogenous AII has been demonstrated both in a previous section and in the published literature (Reid and Laragh, 1965; Hollenberg, et al., 1972; Thurston and Laragh, 1975; Slack and Ledingham, 1976; Oliver and Cannon, 1978). The role of kinins appears to be insignificant (Oliver and Cannon, 1978). However, endogenous prostaglandins have been proposed as important determinants of the pressor response to AII (Aiken and Vane, 1973; McGiff et al., 1976; Obrien and Pipkin, 1979). In this section, we attempted to investigate whether the salt sensitive changes in pressor response to AII were partially mediated by the endogenous level of prostaglandins.

In our experiments, the pressor response for AII was greater in animals previously on a high salt intake. In these high salt rats, indomethacin treatment had no effect on the pressor response for AII. In the low salt rats, prostaglandin synthetase inhibition with indomethacin resulted in a significant enhancement of the pressor activity of angiotensin. Although plasma prostaglandin levels were not measured before and after indomethacin treatment, it appears that this dosage was

sufficient to inhibit prostaglandin synthesis significantly as reported by others under comparable experimental conditions (Campbell et al., 1979). Similar results were obtained with meclofenamate, a different class of prostaglandin synthetase inhibitor. This is added support that the observed response was due to the inhibition of prostaglandin synthesis.

The mechanism by which endogenous prostaglandins antagonize the pressor response for AII is unclear, but a number of possible explanations exist. These are: a) a direct physiological antagonism by vasodilation; b) stimulation of renal renin release resulting in an elevation of endogenous AII levels; and/or, c) inhibition of AII mediated norepinephrine release from adrenergic nerve endings.

The infusion of angiotensin II or norepinephrine into the dog renal artery results in the increased release of a prostaglandin E_2 - like substance (McGiff et al., 1970a; McGiff et al., 1970b; Aiken and Vane, 1973). These experiments suggested that the release of this vasodilatory prostaglandin acted as a physiological antagonist to any general vasoconstrictor response in the kidney (Vander, 1968). Aiken and Vane (1973), however, could only demonstrate an enhancement of the pressor effect of angiotensin and norepinephrine following indomethacin in the renal vascular bed and not the hind limb of the dog. Thus, this does not occur in all vascular beds and the pressor response to AII following indomethacin cannot be predicted on the basis of these findings. As well, if a non-selective vasodilatory action of prostaglandins were an important factor, one would expect an effect of prostaglandin synthetase inhibition on the pressor response to another vasoconstrictor agent, such as norepinephrine. Indomethacin had no affect on the

norepinephrine response which would imply that a significant direct physiological antagonism was unlikely. Although a number of localized vascular beds may have been affected by the indomethacin treatment, the sum total of these events in the whole animal preparation demonstrates no change in the pressor response for norepinephrine.

A second mechanism whereby prostaglandins may antagonize the pressor response for AII may be related to the affects of prostaglandins on renin release. Arachidonic acid and PGE_2, but not $PGF_{2\alpha}$, when infused intravenously or directly into the renal artery, result in an increase in plasma renin activity (Larsson et al., 1974; Yun et al., 1978). Similarly, indomethacin treatment, at doses comparable to that used in our study, has been shown to significantly decrease plasma renin activity (Yun et al., 1977; Data et al., 1978; Campbell et al., 1979). In a previous section, we established the importance of endogenous AII on the pressor response for AII. In the sodium depleted state, where the level of plasma angiotensin is high, indomethacin would presumably cause a decrease in renal renin release through its inhibitory effects on prostaglandin synthesis. The resulting decrease in endogenous AII levels would have an effect similar to that produced by captopril, which has been shown to increase the pressor response to AII in low salt rats. This hypothesis is attractive since it explains why indomethacin has no effect in the high salt animals where the endogenous AII levels are suppressed. It also accounts for why norepinephrine pressor response is unchanged. The proposed drop in plasma renin activity, however, was probably not reduced to levels seen in the high salt animals. Deforrest et al., (1980), have shown that prostaglandin synthetase inhibitors do lower plasma renin activity in sodium depleted

dogs, but the new level is still approximately seventeen times the normal level. If qualitatively similar renin levels are present in our experiments, this would explain why captopril, when given in the presence of indomethacin pre-treatment, further potentiated the response for angiotensin.

The findings in our experiments when captopril was administered prior to indomethacin treatment further substantiates this hypothesis. In this situation, endogenous levels of angiotensin II would be maximally decreased in the low salt rats following captopril. A relatively high dose of captopril (1 mg/Kg) was used to ensure adequate blockage of the converting enzyme. In these animals, captopril treatment resulted in an enhancement of the pressor response to AII. If indomethacin acts by inhibiting renin release, one would expect no further enhancement of the AII pressor responsiveness. This was, in fact, the case following indomethacin treatment. Thus, in our experiments, when endogenous angiotensin II levels were suppressed by either converting enzyme inhibition or a high salt intake, indomethacin treatment failed to potentiate the pressor response for exogenous angiotensin II.

Although the previous hypothesis is attractive, another possibility exists. Prostaglandins may be interfering with the adrenergic component of the angiotensin pressor response. It is well established that AII potentiates the pressor response to norepinephrine and nerve stimulation, most conceivably by increasing the effective concentration of norepinephrine at the neurojunction (Khairallah et al., 1971; Bell, 1972; Roth, 1972). Jackson and Campbell (1981), have shown that the angiotensin II potentiation of nerve stimulation, but not norepinephrine

infusion, is antagonized by prostaglandin E_2 but not prostaglandin I_2. As well, these authors have shown that the inhibition of the AII potentiation of nerve stimulation associated with increased prostaglandin production was reversed by indomethacin treatment. This evidence suggests that prostaglandins may antagonize the adrenergic component of the angiotensin II pressor response. This is most probably due to inhibition of the release of norepinephrine from the nerve ending (Frame and Hedqvist, 1975; Hedqvist, 1976; Hedqvist, 1978). We have demonstrated in the previous section the importance of α-adrenergic receptor activation for the full pressor response of AII to be observed. If prostaglandins act by inhibiting norepinephrine release and, therefore, α-receptor activation, any interference with the adrenergic nervous system should abolish the potentiation observed following indomethacin. Indeed, following ganglionic blockade or α-adrenergic blockade, indomethacin treatment fails to potentiate the pressor response for angiotensin in low salt rats. As well, the interaction between prostaglandins, AII and the adrenergic nervous system does not appear to be only a post-ganglionic phenomenon. This is demonstrated by the fact that indomethacin fails to potentiate the pressor response for AII following ganglionic blockade.

The proposed interference of norepinephrine release by endogenous prostaglandins does not directly explain why captopril pre-treatment abolished the potentiating action of indomethacin. A possible explanation for this may be that in the low salt state, endogenous prostaglandin synthesis is increased in response to the elevated levels of angiotensin II. The elevation of prostaglandin synthesis in response to exogenous AII is well documented (McGiff et al., 1970a,b; Aiken and

Vane, 1973). Thus, when captopril inhibits the endogenous AII levels, the endogenous prostaglandin levels may be reduced as well. Indomethacin, therefore, would be inaffective due to the prostaglandin levels already being suppressed. However, captopril also increases plasma prostaglandin levels (Swartz et al., 1979, 1980). Whether these prostaglandins antagonize norepinephrine release is not known.

Although it has often been assumed that the hypotensive action of captopril was due to inhibition of AII formation, several lines of evidence suggest that non-angiotensin mechanisms may also be important (Swartz et al., 1979, 1980; Moore et al., 1981). Murthy et al. (1978), first proposed that the hypotensive action of captopril may be due to associated increases in bradykinin and prostaglandin levels. To determine the role of prostaglandins in the hypotensive action of captopril, the drop in blood pressure following captopril was measured in the presence and absence of indomethacin pre-treatment. In all cases, a biphasic response was observed following converting enzyme inhibition which has been reported elsewhere (Jaeger et al., 1978). The animals on a low salt intake demonstrated a greater depressor response than the high salt animals. In the low salt rats studied, indomethacin significantly decreased the transient depressor response to captopril. This suggests that prostaglandin synthesis is necessary to observe the full blood pressure lowering effect. This supports the findings and conclusions of others (Moore et al., 1981). However, another possible explanation must be considered. Captopril may still be acting mainly by decreasing endogenous AII levels. In low salt rats, indomethacin treatment may potentially lower the endogenous levels of AII as discussed. Thus, converting enzyme inhibition in the presence of this lower level

of endogenous AII would be expected to produce a smaller drop in blood pressure.

In summary, indomethacin or meclofenamate treatment will result in an increase in pressor responsiveness to AII in animals previously on a low salt, but not on a high salt, intake. This action is most likely due to the inhibition of prostaglandin synthesis. Whether these prostaglandins antagonize the response for AII by increasing plasma renin activity and/or by inhibiting the adrenergic component of the AII response, is unclear. In addition, the hypotensive action of captopril appears to be dependent, at least in part, on the generation of prostaglandins. However, other possible mechanisms may exist.

SECTION V

GENERAL DISCUSSION

In this thesis, the effect of sodium balance on vascular responsiveness in the rat has been studied. States of high or low sodium balance, achieved by dietary manipulation, and various pharmacological interventions were used to investigate the mechanisms responsible for changes in vascular responsiveness.

In rats placed on a low sodium intake, the pressor response for angiotensin II was attenuated as compared to the response obtained from rats on a high sodium intake. The response for norepinephrine was unaltered by sodium intake. Suppression of endogenous angiotensin II levels with captopril enhanced the response for angiotensin II but not norepinephrine in low salt rats, indicating that the salt-induced changes were relatively specific for angiotensin. It is also quite clear from our results that other variables, such as the hypotensive action of captopril, duration of anaesthesia or a reduced plasma volume in the sodium depleted state were unlikely to be important determinants of pressor responsiveness as far as angiotensin II was concerned. Collectively, the data suggest that the endogenous AII level is an important factor in determining vascular reactivity to AII.

In order to investigate the effect of sodium balance on the vasculature in the absence of neural and humoral influences, we utilized the isolated perfused kidney. Kidneys obtained from rats on a low salt intake still demonstrated a suppressed response for AII as compared to kidneys obtained from rats on a high salt intake. The shifts in the log-dose response curves were parallel. The reverse was found for norepinephrine. These findings are comparable to those reported by Strewler _et al_. (1972) in rabbit aortic strips and in the isolated hindlimb preparation. Thus, sodium balance altered the vascular sensitivity

to vasoactive substances. However, this was unlikely to be a non-specific increase in the contractile response since AII and NE produced qualitatively different results. Whether changes in vascular sensitivity of this magnitude in vitro can explain the in vivo observations are unclear. In a quantitative sense, endogenous AII levels are likely to be a more significant factor in determining the systemic pressor response to AII since acute captopril treatment results in comparable responses in both the low and high salt groups.

We also studied the interaction between the sympathetic nervous system and the pressor effects of angiotensin II. In the presence of an α-adrenergic blocker, the response to AII was attenuated. However, sodium intake had no significant effect on the degree of attenuation. Ganglionic blockade did not affect AII pressor responsiveness. In other words, α-receptor activation was required for the full pressor effect of AII, but this was not affected by sodium intake. Following β-adrenergic blockade with propranolol, the pressor response for AII was only enhanced in the low salt intake groups. Possibly this was due to a suppression of endogenous AII levels, an effect similar to that of captopril.

The role of endogenous prostaglandins were also found to be dietary salt sensitive. Prostaglandin synthetase inhibition accentuated the response for AII only in rats on a low salt intake. Prostaglandin synthetase inhibition had no effect on the pressor response for NE in rats on either a high or low salt intake. In the low salt group, suppression of the endogenous AII levels with captopril prevented the previously observed potentiating effect of indomethacin. Similarly, α-adrenergic or ganglionic blockade also prevented the potentiation of

the AII response in low salt rats following indomethacin treatment. Thus, it appears that the state of sodium balance affects the interaction between the prostaglandin system and the angiotensin system.

In the sodium depleted state, two potent vasoconstrictor systems, the renin-angiotensin system (Brown et al., 1964; Churchill et al., 1973) and the sympathetic nervous system (Luft et al., 1979; Nicholls et al., 1980) are activated. In spite of this, blood pressure is generally normal or slightly lowered. In this regard, it may be related to a slightly reduced cardiac output as well as to a suppressed pressor response in this state for endogenous substances such as angiotensin II (Swales et al., 1975; Thurston and Laragh, 1975) and possibly norepinephrine (Rankin et al., 1981). An understanding of the physiological mechanisms which regulate vascular responsiveness may, therefore, be important in an understanding of the regulation of blood pressure.

In states of low sodium balance, the renin-angiotensin system plays an important role in the maintenance of peripheral resistance. This is demonstrated by a substantial drop in blood pressure following the administration of either a competitive antagonist of angiotensin II (Mimran et al., 1974; Johnson and Davis, 1973) or a converting enzyme inhibitor (Mimran et al., 1974; Coleman et al., 1975; Thurston and Laragh, 1975; Jaeger et al., 1978). However, the drop in blood pressure is not accompanied by a significant change in cardiac output (Mimran et al., 1974; Coleman et al., 1975), suggesting the contribution of the angiotensin system in maintaining blood pressure is by acting on the peripheral vasculature.

Teleologically, this attenuated response may appear paradoxical

for a situation where blood pressure needs to be elevated. However, the contribution of a vasoconstrictor to the maintenance of blood pressure is dependent on both the effective concentration of the substance and the vascular sensitivity to that substance. In states of low sodium balance, it appears that a high circulating level of angiotensin II is necessary partly for the stimulation of the adrenal glands to produce aldosterone (Hollenberg et al., 1974). Had there been no reduction in AII vascular responsiveness, blood pressure would have been inordinately high. The presence of a negative feedback mechanism involving vascular reactivity thus plays an important regulatory role.

Available data suggest that two mechanisms may operate in regulating AII vascular sensitivity. In the sodium depleted state, it appears that this decreased effectiveness could be due to the occupation of the receptor sites by the higher levels of endogenous AII, leaving fewer sites available for subsequent binding (Thurston and Laragh, 1975; Cowley and Lohmeier, 1978; Oliver and Cannon, 1978). The shift of the dose response curve to the left following captopril treatment only in the low salt rat in our experiments would support this explanation. However, our results and the data from others (Strewler et al., 1972), showed a decreased vascular reactivity in the sodium depleted state. Recently, Aquilera and Catt (1980) found a decrease in number of AII receptors in the mesenteric artery obtained from sodium depleted rats. In these same studies, the infusion of AII to elevate blood AII levels to that observed in sodium restricted rats produced a similar decrease in vascular AII receptor number. Thus, the suppressed AII response may be related to the elevated endogenous AII levels as well as the

resulting change in vascular sensitivity, both of which can contribute to pressor responsiveness. Although it is not possible to define the quantitative contributions of these two mechanisms from our experiments, it is quite clear that, with captopril, we were able to dissociate the effects of dietary sodium per se from that of endogenous AII *in vivo*.

The pressor response for norepinephrine was unaltered by sodium intake in our experiments. Since plasma norepinephrine levels are elevated in states of sodium depletion, it is conceivable that this should attenuate the response to exogenous norepinephrine. However, an increased vascular sensitivity would offset this effect. In our low salt group an enhanced renovascular sensitivity has been observed, although the enhancement occurred only at large norepinephrine perfusate concentrations.

Rankin *et al.* (1981) reported an inverse relationship between the endogenous norepinephrine levels and the pressor response for exogenous norepinephrine. Although the effect of suppressing the endogenous NE levels on the pressor response for NE was not reported, Touw *et al.* (1980) has observed an enhanced response to norepinephrine following ganglionic blockade with hexamethonium. Thus, although in our experiments the response for NE was not significantly suppressed, this responsiveness may be increased following suppression of endogenous adrenergic activity.

There may be an explanation as to why sodium intake does not appear to effect vascular responsiveness for AII and NE to similar degrees. In states of low sodium balance, plasma angiotensin II levels may be elevated as much as 20 times that observed in high salt states. These markedly elevated levels may be required to ensure an adequate release

of aldosterone to maintain plasma volume (Hollenberg et al., 1974). The endogenous angiotensin II levels may be sufficiently elevated to result in a down-regulation of receptor synthesis. In contrast, the effect of sodium intake on the endogenous levels of norepinephrine is not as drastic. An excessively high salt intake only decreases the plasma norepinephrine levels by one half when compared to a low salt intake (Luft et al., 1979; Rankin et al., 1981). This level is most probably substantially higher than is required to result in a situation comparable to "denervation supersensitivity". In states of sodium excess, a certain degree of sympathetic drive will still be required to maintain a normal cardiac output.

The difference in physiologic functions of the angiotensin and the adrenergic system thus can explain the desirability of preferential changes in AII pressor responsiveness following alterations in dietary salt intake.

Our data indicate that part of the vascular effects of angiotensin is mediated through interaction with the adrenergic nervous system and the prostaglandin system. Although the former is insensitive to dietary salt intake, the state of sodium balance appears to modulate the contribution of prostaglandins to the pressor effects of AII. In our experiments, indomethacin potentiated the pressor response to AII only in the low salt state where prostaglandin synthesis is enhanced. The complex relationship between sodium balance and AII pressor responsiveness is summarized schematically in Figure 28.

Evidence from the literature has been presented supporting the pathogenetic role of salt intake in various forms of hypertension. There is also considerable data suggesting that in some forms of hyper-

tension, an inappropriate pressor responsiveness for angiotensin is involved. It is clear from this discussion that if pressor responsiveness is a key factor in blood pressure control, the effects of changes in sodium balance is likely to be largely modulated by corresponding changes in AII pressor responsiveness. The effects of sodium balance on other vasoactive systems may be substantially less important in terms of blood pressure regulation.

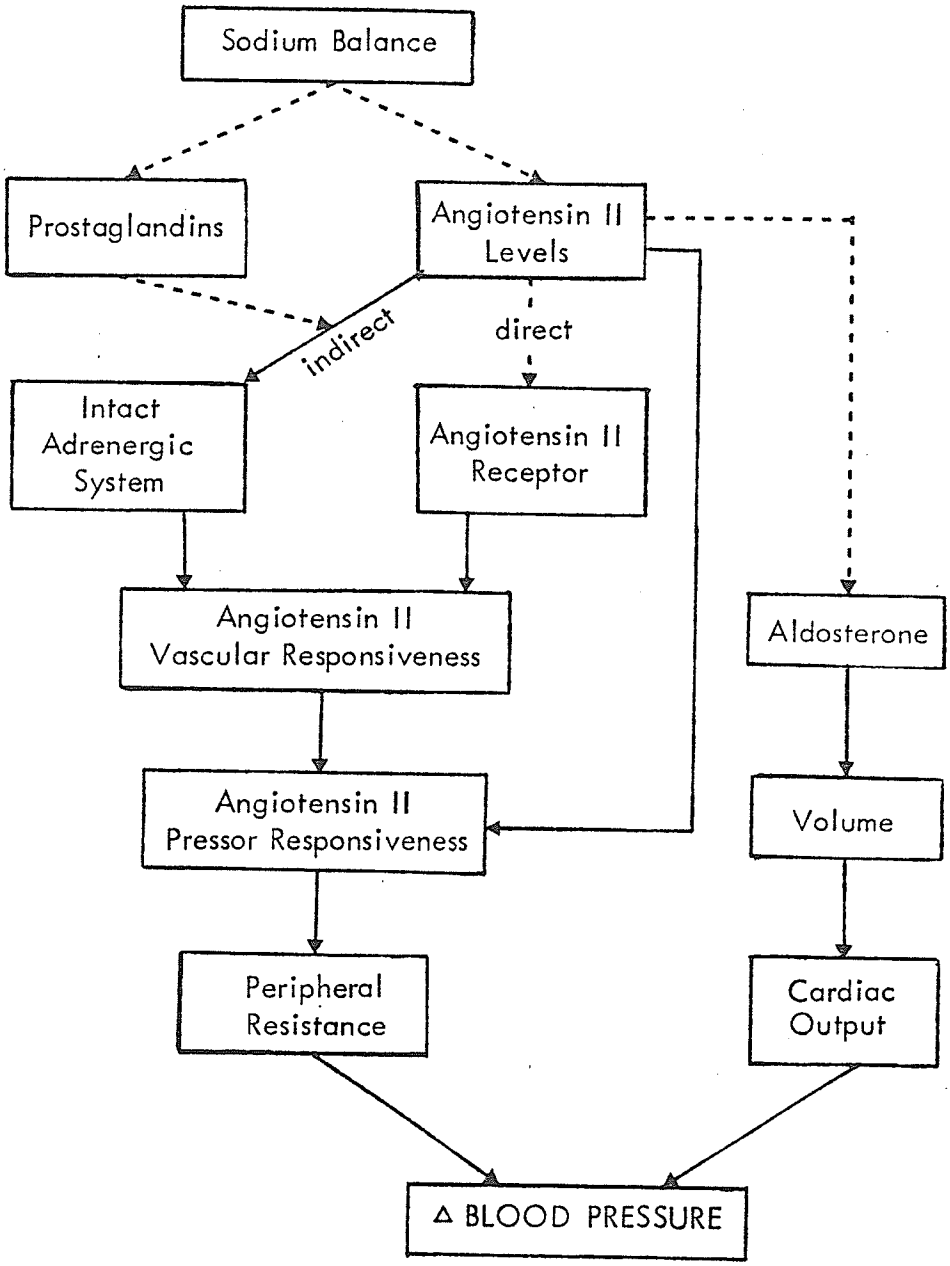

Figure 28: Schematic diagram of the effect of sodium balance on pressor responsiveness for angiotensin II and blood pressure regulation. Broken lines represent the parameters which are altered by changes in dietary sodium.

SECTION VI

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SECTION VII

APPENDIX

Appendix A: Equations used for the calculation of renal functional parameters.

The calculations presented were done as follows:

Creatinine Clearance (C_{Cr}) ml/min:

$$C_{Cr} = \frac{U_{Cr} \times \dot{V}}{P_{Cr}}$$

Where: U_{Cr} = urinary creatinine (mg/100 ml)
$\dot{V}$ = urinary volume (ml/min)
P_{Cr} = plasma creatinine (mg/100 ml)

Urinary Sodium Excretion (UV_{Na}) mEq/day:

$$UV_{Na} = U_{Na} \times \dot{V}$$

Where: $\dot{V}$ = urinary volume (ml/day)
U_{Na} = urinary sodium (mEq/ml)

Urinary Potassium Excretion (UV_K) mEq/day:

$$UV_K = U_K \times \dot{V}$$

Where: $\dot{V}$ = urinary volume (ml/day)
U_K = urinary potassium (mEq/ml)

Percent Fractional Excretion of Sodium ($\%FE_{Na}$):

$$\%FE_{Na} = \frac{U\dot{V}_{Na}}{P_{Na} \times C_{Cr}} \times 100$$

Where: P_{Na} = plasma sodium (μEq/ml)
C_{Cr} = creatinine clearance (ml/min)
$U\dot{V}_{Na}$ = urinary excretion of sodium (μEq/min)

Appendix B: Preparation of study drugs.

ANGIOTENSIN I:

Supplier Manufacturer:	Peninsula Inc., California
Diluent:	.01 N HCl
Stock Solution:	1.333 µg/ml
Storage:	Frozen 4 ml aliquots
Comments:	Bolus injection of .15 ml delivered 200 ng.

ANGIOTENSIN II:

Supplier Manufacturer:	Hypertensin^{R} - CIBA
Diluent:	Water
Stock Solution:	1 mg/ml
Storage:	Refrigerated
Comments:	Used for infusion study

CAPTOPRIL:

Supplier Manufacturer:	Capoten^{R} - Squibb Ind., N.J.
Diluent:	.001 N HCl or 5% Dextrose
Stock Solution:	6 mg/ml or 1 mg/ml
Storage:	Frozen 4 ml aliquots
Comments:	Bolus injection of approx. .4 - .5 ml (.3 or 1 mg/Kg)

DESOXYCORTICOSTERONE ACETATE:

Supplier Manufacturer:	Percorten^{R} - CIBA-GEIGY
Diluent:	Sesame oil and 5% Chlorobutanol
Stock Solution:	5 mg/ml
Storage:	Refrigerated
Comments:	Intraperitoreal injection, .5 mg/Kg

FUROSEMIDE:

Supplier Manufacturer:	LASIX^{R} - Hoechst
Diluent:	Saline
Stock Solution:	5 mg/ml
Storage:	Refrigerated
Comments:	Intraperitoneal injection, 5 mg/Kg

INDOMETHACIN:

Supplier Manufacturer:	Indocid^{R} - Merck, Sharpe and Dohme
Diluent:	Phosphate buffer, pH = 7.4
Stock Solution:	5 mg/ml
Storage:	Frozen 4 ml aliquots
Comments:	Intraperitoneal injection approx. .5 ml (5 mg/Kg)

MECLOFENAMATE:

Supplier Manufacturer:	Warner Lambert
Diluent:	Saline
Stock Solution:	5 mg/ml
Storage:	Refrigerated
Comments:	Intraperitoneal injection, 5 mg/Kg

NOREPINEPHRINE:

Supplier Manufacturer:	Arternol^{R} - CALBIOCHEM
Diluent:	.01 N HCl
Stock Solution:	.667 µg/ml or 1 mg/ml
Storage:	.667 µg/ml frozen 4 ml aliquots; 1 mg/ml Refrigerated
Comments:	Bolus injection (.667 µg/m) of .15 ml delivered 100 ng. 1 mg/ml used for infusion study

PENTOBARBITAL SODIUM:

Supplier Manufacturer:	Nembutal^{R} - British Drug House
Diluent:	Saline, 20% alchohol, 10% propylene glycol
Stock Solution:	60 mg/ml
Storage:	Room temperature
Comments:	Dosage approx. 50 mg/Kg

PENTOLINIUM TARTRATE:

Supplier Manufacturer:	Ansolysen^{R} - Wyeth
Diluent:	Saline
Stock Solution:	1 mg/ml
Storage:	Frozen 4 ml aliquots
Comments:	Bolus injection 1 mg/Kg

PHENOXYBENZAMINE:

Supplier Manufacturer:	Dibenzyline^{R} - Smith, Kline and French
Diluent:	Saline
Stock Solution:	5 mg/ml
Storage:	Refrigerated
Comments:	Intraperitoneal injection approx. .5 ml (5 mg/Kg)

SODIUM NITROPRUSSIDE:

Supplier Manufacturer:	Nipride^{R} - Hoffman-LaRoche
Diluent:	5% Dextrose
Stock Solution:	1 mg/ml
Storage:	Prepared as required
Comments:	Infusion studies, prepared solution light sensitive, therefoe syringe and tubing wrapped in tin foil.

Appendix C: Program for the computation of cardiac output.

```
10 REM---            DON SMYTH                MARCH 1981
20 REM---
30 REM
40 REM--- ########## CALC. C.O. USING LABELLED MICROSPHERES ######
50 REM
60 PRINT TAB(15)'CALC. OF CARDIAC OUTPUT'
70 PRINT TAB(15)'########################'
80 PRINT
90 DIM J$(20),K$(20),L$(20),X(4,30),N(20),W(4,30)
100 PRINT 'EXP. TITLE' \ INPUT A1$
110 PRINT 'DATE:' \ INPUT A2$
120 PRINT 'EXP. NO.' \ INPUT A3$
130 PRINT 'WT OF ANIMAL (GM)' \ INPUT Y3$
140 Y5=VAL(Y3$)/1000
150 PRINT
160 PRINT 'NO. OF BLANK TUBES' \ INPUT A1
170 PRINT
180 PRINT 'TOTAL TIME COUNTED(min)' \ INPUT S7
190 PRINT 'ENTER ISOTOPES IN ORDER COUNTED ie HIGHEST TO LOWEST'
200 FOR I=1 TO 3
210 PRINT 'ISOTOPE NAME' \ INPUT B$(I)
220 PRINT 'DRUG STUDIED ie. CONTROL:A2.10:NE.30' \ INPUT Y$(I)
230 PRINT 'TIME OF INJECTION' \ INPUT Z$(I)
240 PRINT 'B.P. (mmHg)' \ INPUT C$(I)
250 PRINT 'VOL. INJ. (ml)' \ INPUT D$(I)
260 PRINT 'SPHERES/ML' \ INPUT E$(I)
270 PRINT 'VOL. OF STANDARDS' \ INPUT F$(I)
280 PRINT '# OF STANDARD TUBES' \ INPUT G$(I)
290 PRINT 'BLOOD WITHDRAWL RATE (ML/MIN)' \ INPUT H$(I)
300 Q(I)=VAL(D$(I))/VAL(F$(I))
310 PRINT  \ NEXT I
320 PRINT  \ PRINT
330 PRINT 'NO. OF ORGANS' \ INPUT B5
340 FOR J=7 TO B5+6
350 PRINT 'ORGAN NAME' \ INPUT J$(J)
360 PRINT 'WT. OF ORGAN' \ INPUT K$(J)
370 PRINT 'NO. OF TUBES' \ INPUT L$(J)
380 NEXT J
390 PRINT '# BLOOD SAMPLE TUBES' \ INPUT B6
400 PRINT 'ENTER BLANKS'
410 FOR I=1 TO 3
420 PRINT 'BLANK C.P.M. ';B$(I);' WINDOW'
430 FOR J=1 TO A1
440 E=0 \ D=0
450 INPUT D
460 E=E+D \ NEXT J \ M(I)=E/A1 \ E=0
470 NEXT I
480 PRINT  \ PRINT
490 PRINT 'ENTER CPM BY COLUMN PRINTED OUT, START AFTER BLANKS'
500 FOR I=1 TO 3
510 PRINT
520 PRINT 'ENTER CPM BY COLUMN PRINTED OUT'
530 PRINT 'AFTER EACH GROUP ENTER #0:ieENTER FOUR STDS, ENTER 0'
540 PRINT 'IF STANDARDS ENTERED, BEGIN AT BLOODS'
```

```
550 PRINT 'INPUT CPM FOR ';B$(I);' WINDOW'
560 P3=1
570 IF P5=3 THEN P3=4
580 FOR J=P3 TO B5+6
590 X(I,J)=0
600 INPUT X
610 IF X=0 THEN X(I,J)=(X(I,J)/N)-M(I) \ N=0 \ GO TO 640
620 X(I,J)=X(I,J)+X \ N=N+1
630 GO TO 600
640 NEXT J
650 NEXT I
660 REM-- #######CALC. CONSTANTS######
670 REM---         Z7= RATE OF WITHDRAWL / BLOOD CPM
680 REM---
690 REM---          Z8= CORRECTION OVERFLOW CERIUM
700 REM---
710 REM---            Z9=CORRECTION OVERFLOW STRONTIUN
720 REM---
730 FOR I=1 TO 3
740 FOR J=7 TO B5+6
750 IF I=1 THEN Z7=VAL(H$(1))/X(1,4) \ Z8=0 \ Z9=0
760 IF I=2 THEN Z7=VAL(H$(2))/X(2,5) \ Z8=(X(2,1)/X(1,1))*X(I-1,J) \ Z9=
0
770 IF I=3 THEN Z7=VAL(H$(3))/X(3,6) \ Z8=(X(3,1)/X(1,1))*X(I-2,J) \ Z9=
(X(3,2)/X(2,2))*(X(I-1,J)-((X(2,1)/X(1,1))*X(I-2,J)))
780 W(I,1)=Q(I)*X(I,I)*Z7
790 W(I,J-5)=(X(I,J)-(Z8+Z9))*Z7
800 NEXT J
810 NEXT I
820 PRINT  \ PRINT
830 IF P5=3 GO TO 860
840 OPEN "DON.LST" FOR OUTPUT AS FILE #1
850 PRINT #1,DAT$,CLK$
860 A$="'CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
CC"
870 PRINT #1,USING A$,A1$
880 B$="'LLLLLLLLLLL'LLLLLLLLL                    'RRRRRRRRR 'LLLLLLLLL"
890 PRINT #1,"-----------------------------------------------------------
-------"
900 PRINT #1,USING B$,"ISOTOPE #1:",B$(1),"EXP DATE:",A2$
910 PRINT #1,USING B$,"ISOTOPE #2:",B$(2),"EXP. NO.:",A3$
920 PRINT #1,USING B$,"ISOTOPE #3:",B$(3)
930 PRINT #1,"-----------------------------------------------------------
-------"
940 PRINT #1 \ PRINT #1
950 PRINT #1
960 C$="'CCCC   'CCCC   'CCCC   'CCCCCCC   'CCCCCCC   'CCCCCCCCCC   'CCC
CCC   'CCCCC"
970 PRINT #1,USING C$,"DOSE","TIME","B.P.","ISOTOPE","VOL INJ","SPHERES/
ML","VOL OF","NO OF"
980 PRINT #1,USING C$,"  ","  ","mmHG","  ","(ml)","  ","STAND","TUBES"
990 FOR I=1 TO 3
1000 PRINT #1,USING C$,Y$(I),Z$(I),C$(I),B$(I),D$(I),E$(I),F$(I),G$(I)
```

```
1010 NEXT I
1020 PRINT #1 \ PRINT #1
1030 D$="'RRRRRRRRRRRRRRRR'LLLLLL      'CCCCCCC     'CCCCCCC     'CCCCC
CCC"
1040 PRINT #1,USING D$,"WT OF ANIMAL GM:",Y3$,"ORGAN","WEIGHT","NO TUBES"
1050 PRINT #1,USING D$,"  ","  ","#####","######","########"
1060 FOR J=7 TO B5+7
1070 PRINT #1,USING D$,"  ","  ",J$(J),K$(J),L$(J)
1080 NEXT J
1090 PRINT #1 \ PRINT #1
1100 PRINT #1,'A. HEMODYNAMICS:'
1110 PRINT #1,'################'
1120 PRINT #1
1130 G$="'CCCCCCCCCCCCCCCCCCCCCCCCCCCC    'CCCCCCCCCC  'CCCCCCCCCC  'CCC
CCCCCCC"
1140 PRINT #1,USING G$,"   ","ISOTOPE #1","ISOTOPE #2","ISOTOPE #3"
1150 PRINT #1,USING G$,"  ",B$(1),B$(2),B$(3)
1160 PRINT #1,USING G$,"DRUG STUDIED",Y$(1),Y$(2),Y$(3)
1170 H$="'CCCCCCCCCCCCCCCCCCCCCCCCCCCC    #####.##     #####.##      ###
##.##  "
1180 I$="'CCCCCCCCCCCCCCCCCCCCCCCCCCCC    #.##^^^^     #.##^^^^      #>#
#^^^^  "
1190 PRINT #1
1200 PRINT #1,USING H$,"B.P. mmHg.",VAL(C$(1)),VAL(C$(2)),VAL(C$(3))
1210 PRINT #1,USING H$,"C.O. ml/min.",W(1,1),W(2,1),W(3,1)
1220 PRINT #1,USING H$,"C.O. ml/min/Kg",W(1,1)/Y5,W(2,1)/Y5,W(3,1)/Y5
1230 PRINT #1,USING H$,"TPR mmHg/(ml/min)",VAL(C$(1))/W(1,1),VAL(C$(2))/
W(2,1),VAL(C$(3))/W(3,1)
1240 PRINT #1
1250 PRINT #1,USING H$,"LEFT RBF ml/min",W(1,3),W(2,3),W(3,3)
1260 PRINT #1,USING H$,"RIGHT RBF ml/min",W(1,4),W(2,4),W(3,4)
1270 PRINT #1,USING H$,"TOTAL RBF ml/min",W(1,3)+W(1,4),W(2,3)+W(2,4),W(
3,3)+W(3,4)
1280 PRINT #1
1290 PRINT #1,USING H$,"LEFT RVR",VAL(C$(1))/W(1,3),VAL(C$(2))/W(2,3),VA
L(C$(3))/W(3,3)
1300 PRINT #1,USING H$,"RIGHT RVR",VAL(C$(1))/W(1,4),VAL(C$(2))/W(2,4),V
AL(C$(3))/W(3,4)
1310 PRINT #1,USING H$,"TOTAL RVR",VAL(C$(1))/(W(1,3)+W(1,4)),VAL(C$(2))
/(W(2,3)+W(2,4)),VAL(C$(3))/(W(3,3)+W(3,4))
1320 PRINT #1
1330 PRINT #1,USING H$,"LEFT RBF ml/min/g",W(1,3)/VAL(K$(8)),W(2,3)/VAL(
K$(8)),W(3,3)/VAL(K$(8))
1340 PRINT #1,USING H$,"RIGHT RBF ml/min/g",W(1,4)/VAL(K$(9)),W(2,4)/VAL
(K$(9)),W(3,4)/VAL(K$(9))
1350 PRINT #1
1360 PRINT #1,USING H$,"ILEAL BLOOD FLOW ml/min/g",W(1,5)/VAL(K$(10)),W(
2,5)/VAL(K$(10)),W(3,5)/VAL(K$(10))
1370 PRINT #1
1380 PRINT #1,USING I$,"CPM/ml OF STANDARD",(1/VAL(F$(1)))*X(1,1),(1/VAL
(F$(2)))*X(2,2),(1/VAL(F$(3)))*X(3,3)
1390 PRINT #1,USING H$,"ACTIVITY OF LUNG (%CO)",(W(1,2)/W(1,1))*100,(W(2
,2)/W(2,1))*100,(W(3,2)/W(3,1))*100
1400 PRINT #1 \ PRINT #1
```

```
1410 PRINT #1,CHR$(12)
1420 PRINT #1,'B. NUMBER OF MICROSPHERES:'
1430 PRINT #1,'##########################'
1440 PRINT #1,USING G$,"  ","ISOTOPE#1","ISOTOPE#2","ISOTOPE#3"
1450 PRINT #1,USING G$,"  ",B$(1),B$(2),B$(3)
1460 PRINT #1,USING I$,"TOTAL INJECTED",VAL(D$(1))*VAL(E$(1)),VAL(D$(2))
*VAL(E$(2)),VAL(D$(3))*VAL(E$(3))
1470 FOR I=1 TO 3
1480 U(I)=VAL(F$(I))*VAL(E$(I))
1490 NEXT I
1500 PRINT #1,USING H$,"REFERENCE BLOOD SAMPLE",U(1)*X(1,4)/X(1,1),U(2)*
X(2,5)/X(2,2),U(3)*X(3,6)/X(3,3)
1510 PRINT #1
1520 A=(U(1)/X(1,1))*X(1,8)
1530 B=(U(2)/X(2,2))*W(2,3)/(VAL(H$(2))/X(2,5))
1540 C=(U(3)/X(3,3))*W(3,3)/(VAL(H$(3))/X(3,6))
1550 D=(U(1)/X(1,1))*X(1,9)
1560 E=(U(2)/X(2,2))*W(2,4)/(VAL(H$(2))/X(2,5))
1570 F=(U(3)/X(3,3))*W(3,4)/(VAL(H$(3))/X(3,6))
1580 PRINT #1,USING H$,"LEFT KIDNEY",A,B,C
1590 PRINT #1,USING H$,"RIGHT KIDNEY",D,E,F
1600 PRINT #1,USING H$,"BOTH KIDNEYS",A+D,B+E,C+F
1610 PRINT #1
1620 G=(U(1)/X(1,1))*X(1,10)
1630 H=(U(2)/X(2,2))*W(2,5)/(VAL(H$(2))/X(2,5))
1640 I=(U(3)/X(3,3))*W(3,5)/(VAL(H$(3))/X(3,6))
1650 PRINT #1,USING H$,"ILEUM/GM",G/VAL(K$(10)),H/VAL(K$(10)),I/VAL(K$(1
0))
1660 PRINT #1 \ PRINT #1
1670 PRINT #1,USING A$,"##########################"
1680 PRINT #1,CHR$(12)
1690 P5=3
1700 PRINT 'FINISHED Y OR N ' \ INPUT Y6$
1710 IF Y6$="Y" GO TO 1740
1720 IF Y6$="N" GO TO 60
1730 GO TO 1700
1740 CLOSE #1
1750 PRINT
1760 PRINT "TO OBTAIN PRINTOUT FIRST TYPE   BYE"
1770 PRINT "THEN TYPE     PRINT TT3:=DON.LST  "
1780 END
```

Appendix D: Effect of angiotensin II, norepinephrine or 0.9% saline on blood pressure (B.P.), total peripheral resistance (T.P.R.) and cardiac output (C.O.).

Iodine^{125}, cerium^{141} and strontium^{85} labelled microspheres were used. Activity overflow by higher energy to lower energy isotopes were corrected by assuming that lower energy isotopes did not differ from background activity at the upper level windows.

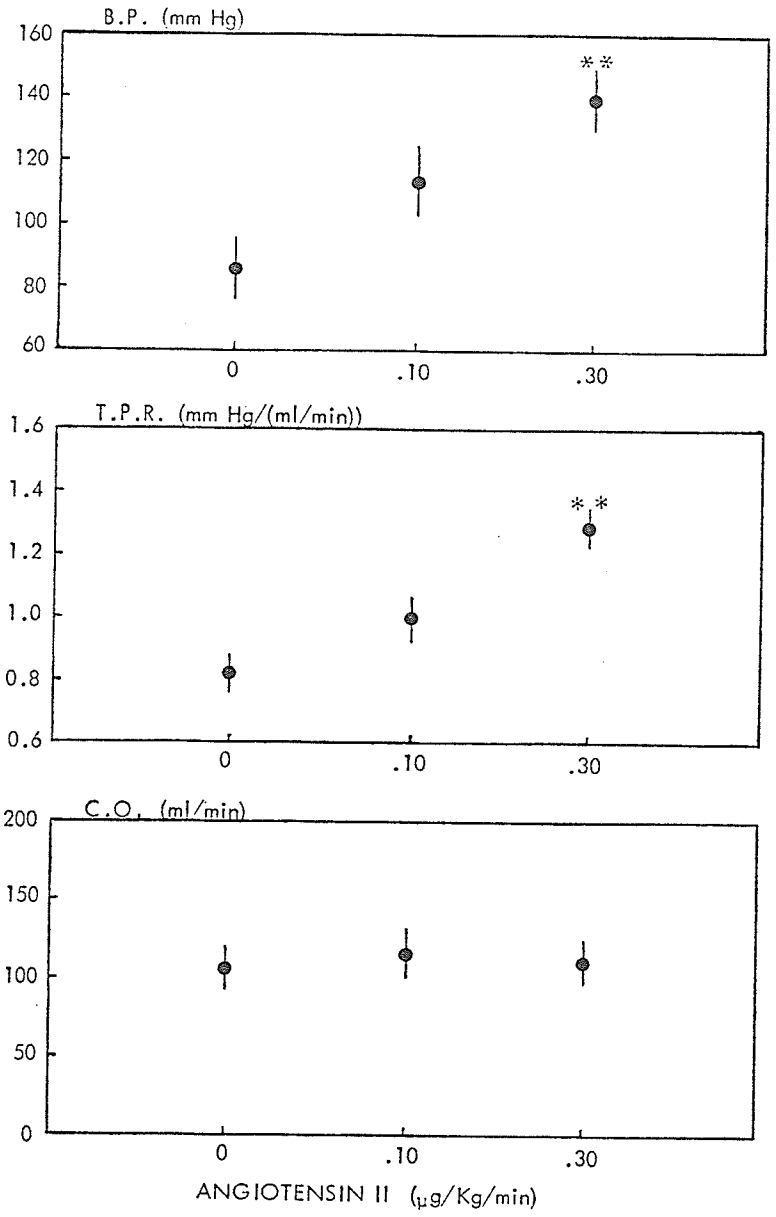

Figure D1: Effect of angiotensin II on blood pressure (B.P.), total peripheral resistance (T.P.R.) and cardiac output (C.O.) in the anaesthetized rat. Values represent mean ± pooled standard error (n=5). Comparisons are made with the first cardiac output determination (AII 0 µg/Kg/min); (*=p<.01).

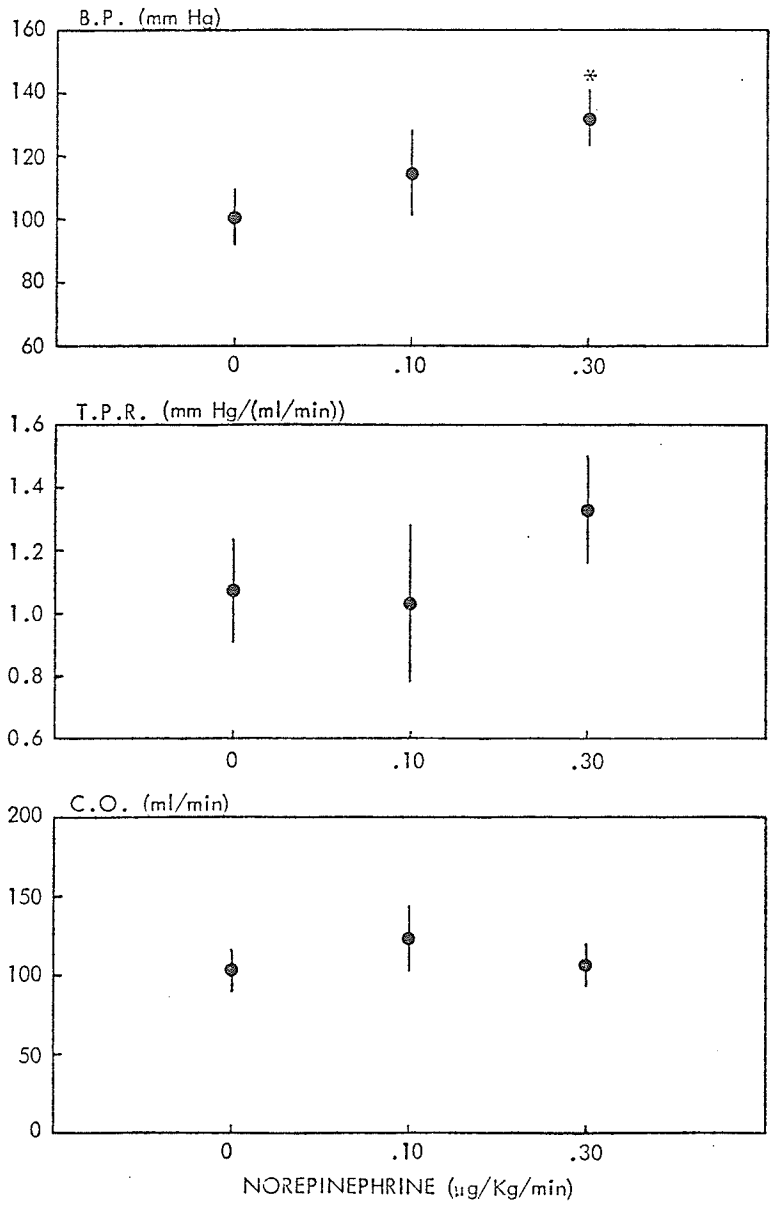

Figure D2: Effect of norepinephrine on blood pressure (B.P.), total peripheral resistance (T.P.R.) and cardiac output (C.O.) in the anaesthetized rat. Values represent mean ± pooled standard error. Comparisons are made with the first cardiac output determination (NE 0 µg/Kg/min); (*=p<.05). (n=7 replicates were done except at NE .10 µg/Kg/min where n=3).

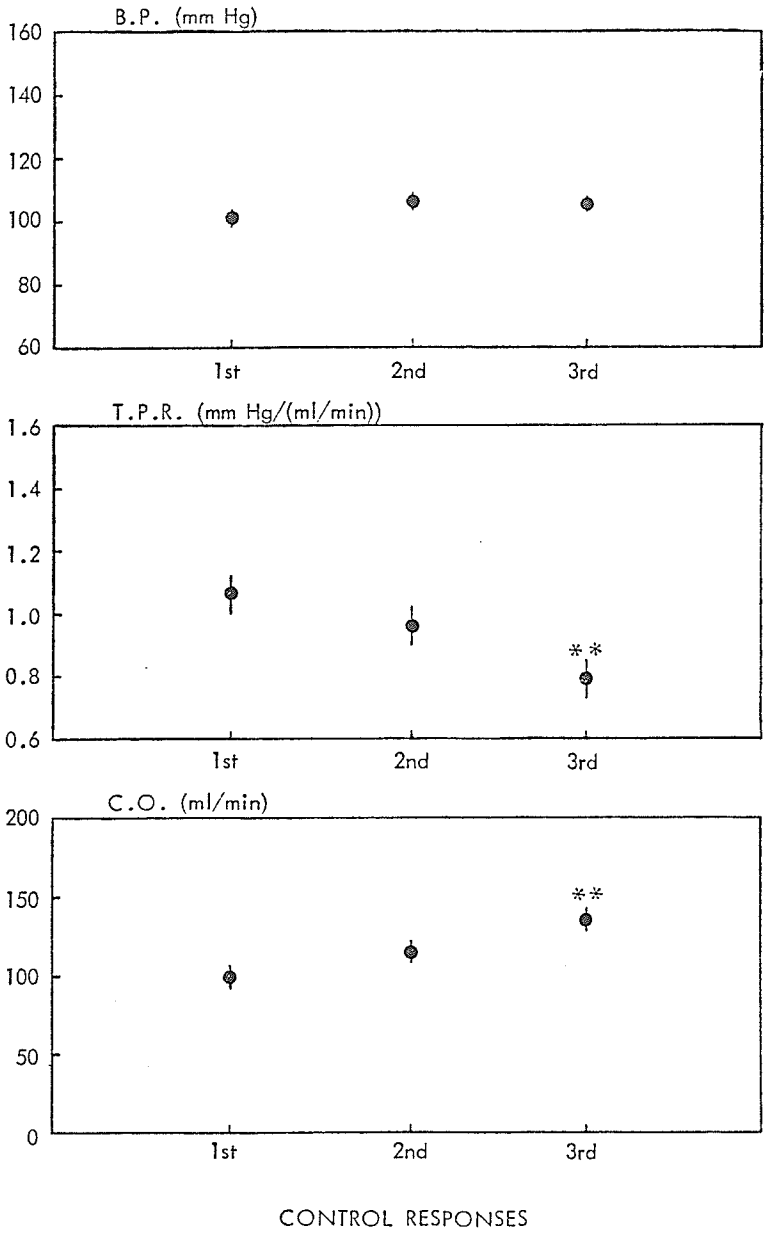

Figure D3: Effect of radiolabelled microspheres on blood pressure (B.P.), total peripheral resistance (T.P.R.) and cardiac output (C.O.) in the anaesthetized rat. Values represent mean ± pooled standard error (n=6). Comparisons were made with the first cardiac output determination (**=p<.01). In this series of experiments a vasoconstrictor was not administered.

Appendix E

Table E1

A. Low Salt Chow

I.C.N. Nutritional Biochemical

Ohio, U.S.A.

Sodium Deficient Diet, Rat, Modified Pellet.

Composition (%):

Alphacel	2.0
Corn Oil	7.0
Special Sodium Free Salt Mixture	4.0
Sucrose	67.0
Casein Purified High Nitrogen	20.0

Plus I.C,N, Vitamine Diet Fortification Mixture

B. Normal Rat Chow

Wayne Lab

Blox F6

Composition (%):

Protein	24.48
Fat	4.27
Carbohydrate	≈60.00
Fiber	3.86

Plus added multivitamines.

Gross Energy, kcal/gm	3.92
Digestible Energy, kcal/gm	3.23
Metabolizable Energy, kcal/gm	2.97