CENTRAL NERVOUS SYSTEM CONTRIBUTION TO PHYSIOLOGIC ACTIONS OF ANGIOTENSIN II

By

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ABSTRACT

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A central pressor effect of AII has been postulated to contribute to the development of several models of experimental hypertension. Therefore, the effects of chronic selective stimulation of brain AII receptors were determined in rats and rabbits, and the effect of interruption of central AII mechanisms on several forms of hypertension was assessed.

Chronic ivt AII infusion in rats and rabbits produced a hypertensive response which was enhanced by high sodium intake. Two mechanisms were identified that contribute to this form of hypertension. A small component appeared to be the result of leak of AII from CSF into the periphery. The predominant mechanism supporting elevated arterial pressure was activation of the sympathetic nervous system as demonstrated by enhanced depressor responses to ganglionic and combined alpha- and beta-adrenergic blockade in rats receiving chronic ivt AII infusions. Furthermore, hypertension development was delayed by peripheral sympathectomy.

Chronic ivt infusion of ^{1}sar,^{8}thr-angiotensin II (sarthran), a competitive AII receptor antagonist, was used to assess the role of central

AII pressor mechanisms in several forms of experimental hypertension. The development of DOC-salt hypertension and the established hypertension of spontaneously hypertensive rats were unaffected by chronic ivt sarthran infusion. The hypertensive response to chronic iv AII infusion also was not blocked by ivt sarthran infusion. This latter observation suggested that chronic elevations in plasma AII produce hypertension by an action at brain sites that are relatively inaccessible to ivt sarthran. Since the subfornical organ (SFO) and area postrema (AP) are two circumventricular organs that appear to have a CSF-brain barrier, the participation of these two structures in iv AII-induced hypertension was assessed. SFO ablation and knife cut of SFO efferent pathways did not attenuate chronic iv AII-induced hypertension. Furthermore, lesion of the median preoptic nucleus, an area through which all forebrain AII-sensitive pressor pathways are postulated to pass, did not prevent hypertension development. Preliminary results indicate that AP lesion may protect against this form of hypertension. Therefore, the AP may be the critical central site at which blood-borne AII acts to produce hypertension in the rat.

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The work described in this dissertation could not have been performed without the excellent technical assistance of my friends and coworkers Mark Mann, Corie Pawloski, Doug Shinaver, Dave Walters and Jan Weaver. The work of Ben Kuslikis during his research rotation is gratefully acknowledged. Other investigators also have contributed importantly to this research. Specifically, I would like to thank Dr. Keith Demarest for measurement of tissue catecholamines, Dr. John Thornburg for measurement of plasma catecholamines, and Dr. Ian Reid for determination of plasma AII concentration. Studies involving SFO knife cuts, SFO ablation and median preoptic nucleus ablation were made possible by fruitful collaboration with Dr. Wally Lind and Dr. Michael Mangiapane.

I also would like to thank the faculty of the Department of Pharmacology and Toxicology for setting high standards of performance, and for

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INTRODUCTION

A. Peripheral Renin-Angiotensin System

1. General

It is generally accepted that the peripheral renin-angiotensin system plays an important homeostatic role in the control of fluid and electrolyte balance and cardiovascular function. The first report that a factor of renal origin was capable of increasing arterial blood pressure (Tigerstedt and Bergman, 1898) described increases in blood pressure following intravenous injection of saline extracts of kidney in anesthetized rabbits. However, it was not until the pioneering work of Goldblatt et al. (1934) and Pickering and Prinzmetal (1938) that the link between the kidney and high blood pressure was recognized. The critical finding of these studies was that reduction in renal blood flow can lead to a prolonged increase in arterial pressure. In the 1940's, two groups working independently (Braun-Menendez et al., 1940; Page and Helmer, 1940) demonstrated that renin, the substance released from the kidney in response to a reduction in blood flow, is not pressor in itself. Rather, renin acts on a plasma substrate to produce a heat stable, short-acting vasoconstrictor substance, which is now known as angiotensin II. These initial observations have led to our current understanding of the functions of the renin-angiotensin system.

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Renin is a proteolytic enzyme (Inagami and Murakami, 1977) synthesized and secreted by juxtaglomerular cells, which are specialized cells in the wall of the afferent arteriole of the kidney (Cook, 1971). Davis and Freeman (1976) have classified the mechanisms regulating renin secretion as: a) intrarenal, including the renal vascular receptor in the afferent arteriole sensitive to changes in perfusion pressure and the macula densa receptor in the distal tubule sensitive to changes in tubular sodium and/or chloride load; b) sympathetic, including circulating catecholamines and the renal nerves, and c) humoral, including vasopressin, angiotensin II and prostaglandins. Stimuli such as a decrease in renal perfusion pressure, sodium depletion, or sympathetic activation cause the release of renin from the kidney. Renin catalyzes the conversion of angiotensinogen, a plasma globulin, to angiotensin I (AI), an inactive decapeptide. Converting enzyme, present in high concentration in lung, but also found in plasma and many other tissues (Erdos, 1975) acts to cleave a dipeptide fragment from the carboxyterminus of angiotensin I to form the biologically active octapeptide, angiotensin II (AII). It also has been shown that a metabolite of angiotensin II, des-asp^{1}-angiotensin II (angiotensin III; AIII) has considerable physiologic activity, especially with regards to stimulation of aldosterone secretion from the adrenal gland (Freeman et al., 1976).

2. Physiological effects of blood-borne angiotensin II

Angiotensin II is a potent vasoconstrictor, and the increase in blood pressure produced by acute intravenous infusions of AII is due in large part to its direct vascular constrictor action (DeBono et al.,

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1963; Regioli et al., 1974). However, the magnitude of the sustained increase in blood pressure observed with chronic (days to weeks) elevations in plasma AII concentration is greater than can be accounted for by the acute vasoconstrictor action of AII alone (Bean et al., 1979). These investigators found that chronic (2-week) infusion of acutely subpressor doses of AII produced a sustained increase in blood pressure in dogs. Measurement of plasma AII concentration in these dogs revealed that at any given plasma level of AII, blood pressure was higher in the dogs that had been receiving chronic intravenous AII infusion than in naive dogs in whom plasma AII was acutely raised with an intravenous AII infusion. Similar reports that prolonged infusions of acutely subpressor doses of AII produce sustained increases in blood pressure have been made by others in dogs (Cowley and McCaa, 1976), rabbits (Dickinson and Yu, 1967) and rats (Brown et al., 1981). Thus, it appears that a slowly-developing pressor action of AII, distinct from its acute direct vasoconstrictor effect, plays an important role in maintaining hypertension in response to chronic elevations of plasma AII. Despite efforts by several research groups to identify the mechanism by which chronic elevations in plasma AII produce hypertension, no consensus has been reached. Many of the known physiologic effects of AII could potentially contribute to the hypertension produced by long-term intravenous AII infusions. For example, circulating AII has been demonstrated to enhance activity of the sympathetic nervous system at several levels of the neuraxis. A component of the pressor response to both acute (Lappe and Brody, 1984) and chronic (Sweet et al., 1971; Yu and Dickinson, 1971) infusion of AII into the cerebral circulation has been demonstrated to be the result of a central effect of AII to cause increased sympathetic outflow. Angiotensin II also can act at the level of the

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ganglia to increase sympathetic tone since AII has been shown to be a non-nicotinic ganglionic stimulant (Lewis and Reit, 1965, 1966). At the level of the sympathetic neuroeffector junction, AII has been shown to enhance both the vasoconstrictor responses to nerve stimulation in isolated vascular bed preparations (Zimmerman, 1978; Campbell and Jackson, 1979) and the contractile response of vascular strips to field stimulation (Zimmerman, 1978). In addition, the constrictor effect of exogenous norepinephrine administration *in vivo* is enhanced by AII, but to a much lesser extent than the response to nerve stimulation (Zimmerman, 1973). The AII-induced facilitation of peripheral adrenergic function has been attributed to enhanced transmitter release (Boke and Malik, 1983; Hughes and Roth, 1971), blockade of reuptake (Khairallah, 1972), and increased responsiveness of vascular smooth muscle to norepinephrine (Pals *et al.*, 1968; Zimmerman, 1978). Facilitation of cardiac sympathetic function has been forwarded as an explanation for the positive chronotropic and inotropic effects that have been observed with AII (Lokhandwala *et al.*, 1978). Alternatively, these effects may be due to direct stimulation of myocardial AII receptors (Baker *et al.*, 1984).

One mechanism that has been postulated to contribute to the slowly-developing pressor response to AII is resetting of the baroreceptors (Cowley and DeClue, 1976). Indeed, it has been convincingly demonstrated that peripheral AII, by a central action, inhibits baroreceptor reflex function. Reflex increases in cardiac vagal activity (Lumbers *et al.*, 1979) and decreases in sympathetic activity (Stein *et al.*, 1984; Guo and Abboud, 1984) for a given increment in arterial pressure are blunted by AII.

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Acute intravenous administration of angiotensin II has been reported to increase plasma vasopressin concentration in dogs (Bonjour and Malvin, 1970; Ramsay et al., 1978; Reid et al., 1982). The direct vasoconstrictor effects of AVP could theoretically contribute to hypertension observed with chronic iv AII infusion. However, chronic infusion of the same dose of AII that produces acute increases in plasma AVP (20 ng/kg/min) although producing hypertension does not result in a sustained increase in plasma vasopressin (Cowley et al., 1981). ACTH is another potential contributor to iv AII-induced hypertension, since chronic ACTH infusions will cause hypertension (Scoggins et al., 1984), and plasma ACTH levels have been reported to increase with acute intravenous AII infusions (Ramsay et al., 1978; Reid et al., 1982). However, the doses of AII needed to produce increases in plasma ACTH are relatively large in comparison to the doses needed to produce hypertension.

Circulating angiotensin II functions to conserve sodium, in part by stimulation of aldosterone release from the adrenal glomerulosa (Laragh et al., 1960; Fraser et al., 1965) and in part by a direct renal effect to increase tubular sodium reabsorption (Hall et al., 1980). Chronic aldosterone infusion has been demonstrated to produce hypertension (Garwitz and Jones, 1982), therefore AII-induced aldosterone release may be a mechanism by which chronic iv infusions of AII produce hypertension. In experimental animals, short term (minutes to hours) intravenous infusions of AII cause plasma levels of aldosterone to increase with a concomitant decrease in sodium excretion (Borresen et al., 1982), however this increase in plasma aldosterone is not maintained with longer term (days to weeks) AII infusions (McCaa et al.,

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1975; Cowley et al., 1976). Since chronic intravenous infusion of AII, at doses that produce sustained elevations in arterial pressure, produce only transient increases in plasma aldosterone, it is unlikely that aldosterone is responsible for maintaining hypertension in response to chronic, low-level intravenous AII infusion.

The hypertensive response to chronic intravenous AII infusion, in addition to being "slowly-developing" in nature, is sodium-sensitive. Elevated levels of sodium intake augment the hypertensive response to chronic iv infusions of AII in the dog (Cowley and McCaa, 1976) and rat (Fink et al., 1982b). Not only are the mechanisms by which AII produces a slowly-developing pressor response as yet undefined, but the reason for the sodium-sensitivity of this response also is not yet known. Interactions of sodium and angiotensin have been quite extensively studied. What follows is a summary of the known interactions of sodium and AII, and how these may relate to the sodium-dependent hypertension produced by chronic iv AII infusions.

3. Sodium-Angiotensin Interactions

Plasma renin activity and plasma AII levels are elevated during sodium depletion and depressed during sodium loading (Brown et al., 1964; Laragh et al., 1972). Dietary sodium intake, in addition to being a determinant of endogenous AII levels, is known to modulate the pressor and aldosterone-stimulating effects of exogenous AII infusions. Changes in sodium intake have opposite effects on vascular and adrenal responsiveness to AII. For example, in subjects on low sodium, the pressor response to acute exogenous AII infusion is suppressed while the increase in plasma aldosterone is enhanced relative to normal sodium subjects (Shoback et al., 1983). Efforts have been made to dissociate

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the effects of prior receptor occupancy by endogenous AII and other effects of sodium itself on tissue responsiveness to exogenous AII. In the case of the adrenal responses to AII, the elevated circulating levels of AII seen in sodium deprivation have a trophic effect on the adrenal glomerulosa to increase the number of AII receptors and increase the activity of enzymes in the aldosterone biosynthetic pathway. These trophic effects observed during sodium depletion can be only partially reproduced by AII infusion. Thus, the adrenal responses to changes in sodium intake are due partially to changes in plasma AII and also to an effect of sodium ion or some other factor that is altered with sodium intake (Aguilera and Catt, 1983).

The vascular responses to exogenous AII are suppressed in animals on a low sodium diet and enhanced by high sodium intake. Several in vitro studies have examined the effects of sodium intake on responsiveness of isolated vascular strips to AII. In these studies, receptor occupancy by endogenous AII is eliminated as a factor. It has been shown that the contractile response to AII is potentiated in isolated aortic strips from rabbits maintained on a high sodium intake when compared to aortae obtained from low-sodium animals (Strewler et al., 1972). This change in responsiveness is specific for AII and is not seen with other vasoconstrictors such as norepinephrine. Therefore, even in the absence of the high circulating AII levels seen in sodium depletion, the vasculature from low-sodium animals is less responsive to AII. Aguilera and Catt (1981) have shown that sodium restriction decreases, and sodium loading increases, the number of AII receptors in mesenteric artery. Sodium ion also has been shown to increase binding of ^{125}I-AII to a particulate fraction of rat mesenteric artery (Wright

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et al., 1982). The above studies suggest that receptor alterations may influence the changes in AII pressor responses seen with varying sodium intakes. However, there is convincing evidence that receptor occupancy by endogenous AII is sufficient to explain the pressor responses to exogenous AII seen with varying sodium intakes. Cowley and Lohmeier (1978) have demonstrated in nephrectomized dogs that when plasma sodium concentration is maintained at 140, 146, and 156 meq/liter with body fluid volumes held constant, no change in the AII dose-pressure response is obtained. Similarly, changes in body fluid volumes reflecting those seen in sodium depletion and sodium loading had no effect on the pressor responses to acute iv AII infusion. These authors concluded that changes in sensitivity to exogenous AII infusion with sodium intake are not caused by sodium ion concentration or volume changes that accompany changes in sodium intake, but probably result from changes in the prevailing levels of endogenous AII, which alter the availability of receptor sites. This view is supported by results from other studies in which the diminished pressor responsiveness to exogenous AII infusion observed during sodium depletion can be restored to levels seen in "normal-sodium" subjects by pretreatment with converting enzyme inhibitors (Thurston and Laragh, 1975; Shoback et al., 1983).

As described above, the mechanisms by which alterations in sodium intake effect pressor responsiveness to acute iv AII infusion have been extensively studied. However, it is not known why elevated levels of sodium intake augment the hypertensive response to chronic iv infusions of AII in the dog (Cowley and McCaa, 1976) and rat (Fink et al., 1982b). The degree to which vascular receptors are occupied by endogenous AII is an unlikely factor since chronic iv AII infusions will

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produce sustained hypertension at doses that acutely are subpressor (Bean et al., 1979). In addition, we are unable to lower blood pressure with acute AII antagonist infusions in rats made hypertensive by chronic iv AII infusion (unpublished observation). This result argues against a sodium-sensitive direct vasoconstrictor effect of AII in mediating the hypertensive response to chronic iv AII.

A pivotal role for the kidney in determining the sodium sensitivity of the response to chronic iv AII infusion has been suggested (Cowley and McCaa, 1976; DeClue et al., 1978; Hall et al., 1980). These authors postulate that in an animal on normal sodium intake, the direct renal sodium retaining effect of AII can be offset by other homeostatic mechanisms, such as suppression of renal nerve activity and plasma aldosterone. However, in an animal on high sodium intake, these mechanisms are already maximally suppressed. Therefore, they suggested that elevation of plasma AII in high-sodium animals will cause sodium and water retention, leading to volume expansion. As a means of maintaining sodium and water balance, arterial pressure rises until it reaches a level at which the sodium and water retaining effects of AII are compensated for by a "pressure natriuresis/diuresis" phenomenon. Evidence from our laboratory and others does not support this view. In the rat there is no evidence for sodium retention since urinary electrolyte excretion (unpublished observations) and sodium balance (Brown et al., 1981) remain unchanged during chronic iv AII infusion. Water balance also is unaltered by chronic iv AII infusion in the rat (unpublished observations). Although a transient (1-day) sodium retention is observed in the rabbit and dog in response to chronic iv AII, no change in body fluid volumes is observed. Therefore, although AII undoubtedly has

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direct renal sodium-sparing effects, these effects probably contribute little to the sodium-dependent hypertensive response to chronic iv AII infusion.

It has been demonstrated, however, that an action of AII on the brain, rather than the kidney, is important for the development of hypertension in response to chronic iv AII infusion. Electrolytic ablation of periventricular structures in the anterior hypothalamus (AV3V region) in rats largely prevents the chronic hypertension seen in response to elevated plasma levels of AII (Fink et al., 1982b).

There is abundant evidence that blood-borne AII acts on the brain (as reviewed in the next section). Some of the experiments described in this thesis examine the premise that a central effect of AII, rather than a renal effect, is responsible for chronic elevations in arterial pressure seen with intravenous infusions of this hormone.

4. Evidence that blood-borne angiotensin II acts on the brain

a. Circumventricular organs

A number of the physiological effects of circulating AII can be wholly or partially attributed to an action of AII on the brain. Since AII is a polar peptide, it would not be expected to cross the blood-brain barrier readily. The initial studies that were performed in an effort to examine the degree to which circulating AII had access to brain tissue involved the peripheral administration of radiolabelled AII and subsequent quantitation of the label in CSF or brain tissue (Volicer and Loew, 1971; Johnson and Epstein, 1975). These investigators found that radiolabel did appear in CSF and brain tissue after intravenous AII administration, however, biochemical identification of the label as intact AII was not performed. A subsequent study by Schelling et al.

(1976) employed polyacrylamide gel electrophoresis to separate AII and metabolites. It was demonstrated that after intravenous administration of ^{3}H-AII, the small amount of label that did appear in CSF was not intact AII, but rather a breakdown product. It is now well accepted that blood-borne AII cannot cross the blood-brain barrier, except in situations where large increases in blood pressure disrupt integrity of the barrier (Johansson et al., 1970). However, circulating AII does have access to brain tissue at sites that lack a functional blood-brain barrier. These sites have been collectively termed the circumventricular organs (CVO's) and they are characterized anatomically by the absence of capillary endothelial tight junctions. Instead, the fenestrated capillary endothelium in these areas permits the passage of blood-borne substances that would not normally cross the blood-brain barrier into brain parenchyma (Broadwell and Brightman, 1976). The circumventricular organs include the area postrema, pineal gland, subcomissural organ, subfornical organ, organum vasculosum of the lamina terminalis, and median eminence. Autoradiographic evidence has shown that blood-borne AII binds specifically within the CVO's and is excluded from the rest of the brain (van Houten et al., 1980). It now appears that three of the circumventricular organs, namely the area postrema (AP), subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) are important brain sites at which blood-borne AII acts to produce central effects.

b. Pressor effects

The first demonstration that blood-borne AII could act directly on the brain to produce an increase in arterial pressure was

reported by Bickerton and Buckley (1961). They used a dog cross-circulation preparation in which the head of a recipient dog was vascularly isolated from the trunk to demonstrate that AII, when delivered into the cerebral circulation, could cause an increase in blood pressure that was independent of direct vascular constriction. Experiments in conscious rabbits (Dickinson et al., 1965) showed that infusion of AII into a vertebral artery caused a pressor response of greater magnitude than that seen when the same dose was infused intravenously. Similar findings have been made in anesthetized dogs (Ferrario et al., 1970). The pressor activity of intravertebral AII infusion is associated with increased splanchnic preganglionic activity and is prevented by adrenergic blockade or spinal section at C2 (Ferrario et al., 1972). These experiments indicate that in the dog and rabbit, blood-borne AII acts at a site within the field of vertebral circulation to cause a pressor response mediated by an increase in vasomotor tone. The AP is the only CVO that is perfused by the vertebral arteries, suggesting that blood-borne AII acts at the AP in these species to produce a pressor response. Indeed, it has been shown that electrolytic ablation of the AP in the dog (Joy and Lowe, 1970) and rabbit (Yu and Dickinson, 1971) reduces the pressor effect of intravertebral and intravenous (Ferrario et al., 1979) AII administration. In contrast to these species, the pressor response to intravertebral AII infusion in the rat is not different than the response to intravenous infusion, and AP lesion does not alter the pressor response to acute intravenous AII (Haywood et al., 1980). However, in the rat AII is a more potent pressor agent when given into the carotid circulation than when given intravenously (Fink et al.,

1980a). Thus, a more rostral site appears to be important for the central pressor effect of AII in this species.

The two major candidates for the forebrain site at which blood-borne AII acts to produce a central pressor effect are the SFO and periventricular structures within the anterior ventral third ventricular region (AV3V). Electrolytic ablation of the SFO has been shown to reduce the pressor response to systemic AII administration (Mangiapane and Simpson, 1980). In addition, a knife cut that interrupts ventrally-directed SFO efferent pathways attenuates the pressor response to acute intravenous AII infusion in rats (Lind et al., 1983). Thus, AII-receptive elements within the SFO appear to be sensitive to blood-borne AII and contribute to the pressor response to systemic AII.

In addition to the SFO, structures within the AV3V region also appear to mediate a central pressor effect of blood-borne AII in the rat. The AV3V region encompasses the OVLT, the subcomissural portion of the median preoptic nucleus (MnPO), the periventricular preoptic nucleus and the medial part of the medial preoptic area. It has been demonstrated that the augmented pressor response to AII seen with carotid vs. aortic AII infusion is abolished in rats with electrolytic lesion of the AV3V (Fink et al., 1980a). Furthermore, the pressor response to acute intravenous AII infusion is significantly decreased in rats with AV3V lesions (Buggy et al., 1977), consistent with the idea that AV3V lesion eliminates that component of the iv AII pressor response that is central in origin. Intracerebroventricular administration of AII receptor antagonists also produce similar decrements in the acute iv AII pressor response in the rat (Brody et al., 1978). In addition, the sodium-sensitive hypertensive response to chronic

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intravenous infusions of acutely subpressor doses of AII is largely prevented in rats with AV3V lesions, suggesting that the "slowly developing" pressor response to iv AII is mediated by an action of AII on the brain (Fink et al., 1982). These observations suggest that the CVO within the AV3V region, namely the OVLT, may be responsible for the centrally-mediated pressor effect of blood-borne AII. However, other sites within the AV3V region that are sensitive to AII such as the MnPO (Phillips et al., 1979a) may be the critical site of action for circulating AII. Alternatively, the ability of AV3V lesion to abolish a centrally-mediated AII pressor effect in the rat may be due to disruption of known SFO efferent pathways that project ventrally along the lamina terminalis and terminate, among other areas, in the median preoptic nucleus and OVLT (Miselis, 1981; Saper and Levisohn, 1983).

The importance of the ventral MnPO in mediating central AII pressor responses has recently been examined. Injections of lidocaine into the MnPO attenuate the pressor response to intravenous AII, but these same injection sites are not responsive to local microinjection of AII (O'Neill and Brody, 1984). This observation, coupled with the evidence that an anterior hypothalamic knife cut that interrupts efferents from SFO and OVLT that course through the MnPO also decreases the pressor response to iv AII (Hartle and Brody, 1984), suggest that efferents from forebrain AII sensitive systems converge in the MnPO. Since the ventral MnPO is within the area of AV3V lesion, some effects of AV3V destruction may be due specifically to interruption of pathways in the MnPO.

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An elevation in plasma AII levels is a potent stimulus for drinking behavior. Several experimental manipulations that increase plasma AII concentrations including isoproterenol treatment, caval ligation, and subcutaneous polyethylene glycol (extracellular thirst challenge) result in increased water intake (Leenen et al., 1974; Johnson et al., 1981). In addition, subcutaneous or intravenous administration of AII itself will cause drinking in virtually all mammalian species studied including the rat (Buggy and Johnson, 1977; Hsiao et al., 1977; Eng and Miselis, 1981; Lind and Johnson, 1982) and dog (Fitzsimons et al., 1978; Thrasher et al., 1982a). Chronic intravenous AII infusion (10 days) has been reported to produce a sustained increase in water intake in the dog (Trippodo et al., 1976), whereas comparable infusions in the rat fail to alter water intake (Brown et al., 1981). Although there is evidence that angiotensin is a physiological dipsogen in situations such as water deprivation (Barney et al., 1983), this point is still one of considerable debate (Stricker, 1978; Mann et al., 1980; Johnson et al., 1981).

Blood-borne AII has been proposed to elicit thirst by a direct effect on the brain rather than by altering nervous input from peripheral receptors. One compelling argument that AII acts on the brain to stimulate thirst is that AII is 1,000 times more potent as a dipsogen when delivered directly into the brain as compared to intravenous administration (Epstein et al., 1970). Furthermore, thirst induced by peripheral AII is attenuated by lower doses of AII antagonists when given centrally than peripherally (Johnson and Schwob, 1975). In an effort to localize the specific brain area responsible for

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angiotensin-induced drinking, Johnson and Epstein (1975) mapped cannula sites that were effective in eliciting drinking to central injections of AII. They found that cannula sites bordering the cerebral ventricles or trajectories that passed through the ventricles were the most sensitive sites. Using a method of regional ventricular obstruction with cold cream plugs, it was found that intracerebroventricular (ivt) injections of AII must have access to the AV3V region in order to be dipsogenic (Hoffman and Phillips, 1976a). However, these same plugs were without effect on drinking elicited by systemic AII administration (Johnson and Buggy, 1976). Lesions of the AV3V region, however, abolish drinking to subcutaneous AII injections (Buggy and Johnson, 1977), thereby implicating structures in this area in the dipsogenic response to peripheral AII. Lesions of the MnPO also attenuate the drinking response to subcutaneous AII injections (Mangiapane et al., 1983). In addition to the drinking behavior provoked by blood-borne AII, chronic intravenous AII infusion also has been reported to induce sodium appetite (Findlay and Epstein, 1980).

There is evidence that the SFO also is important in mediating the dipsogenic effects of blood-borne AII. Injections of AII directly into the SFO are more effective in inducing drinking than injections into surrounding brain areas (Simpson and Routtenberg, 1973; Mangiapane and Simpson, 1980a). SFO lesions reduce drinking to intravenous AII in both the rat (Simpson and Routtenberg, 1975) and dog (Thrasher et al., 1982), and transection of SFO efferent projections also attenuates angiotensin-induced drinking (Eng and Miselis, 1981; Lind and Johnson, 1982). Integrity of the SFO (or its efferent pathways)

and structures within the AV3V region appear to be necessary for drinking induced by blood-borne AII.

d. Vasopressin release

Although the cell bodies of magnocellular neurons in the supraoptic and paraventricular nuclei are sensitive to AII (Gregg and Malvin, 1978; Sladek and Joynt, 1979), blood-borne AII does not have direct access to these regions. However, the SFO has known neural connections to the paraventricular nucleus (Miselis, 1981). The release of vasopressin in response to intravenous AII has been found to be attenuated in rats with knife cuts of SFO efferent pathways (Knepel et al., 1980) or SFO lesions (Mangiapane et al., 1984). Structures within the AV3V region also have been implicated in angiotensin-stimulated vasopressin release (Bealer et al., 1979).

B. Brain Renin-Angiotensin System

1. Components present in brain

The actions of angiotensin on the brain are of interest not only because many of the effects of blood-borne AII can be partially or entirely attributed to a central action, but because all the components necessary for the generation of AII are present in the brain (see Phillips, 1978; Ganong, 1984 for reviews).

a. Angiotensinogen

Angiotensinogen (or renin substrate) has been found in brain tissue (Ganten et al., 1971; Printz and Lewicki, 1977; Lewicki et al., 1978) and in CSF (Schelling et al., 1980). The CSF angiotensinogen concentration is lower than that of plasma, but when expressed per mg

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protein, CSF has about 3 times as much angiotensinogen as plasma. The highest tissue concentrations are found in the AP, OVLT, periventricular region of the thalamus and hypothalamus and the median eminence. Printz et al. (1980) also have provided evidence that CSF angiotensinogen is not derived from peripheral sources, but originates in the brain. Manipulations of plasma angiotensinogen are not always reflected in similar changes in brain angiotensinogen (Gregory et al., 1982), suggesting independent regulation of plasma and brain levels.

b. Renin

Renin-like activity has been demonstrated in dog, rat and human brain tissue (Fischer-Ferrario et al., 1971; Daul et al., 1975) and this activity persists after nephrectomy (Ganten et al., 1971), suggesting that brain renin is not of peripheral origin. Renin has been reported to be undectable in CSF (Schelling et al., 1980), or present in extremely low amounts (Brosnihan et al., 1982). Initial studies demonstrated that the pH optimum for brain renin-like activity (4.5-5.5) was lower than that for renal renin (5.0-6.0), and most of the renin-like activity observed in brain was attributed to cathepsin D and similar acid proteases (Day and Reid, 1976). However, chromatographic separation of brain renin and cathepsin D activities has been achieved (Hirose et al., 1978). Independent regulation of plasma and brain renin has been demonstrated in sodium-depleted dogs, where plasma renin increases concomitantly with either no change or decreases in regional brain renin (Brosnihan et al., 1982).

c. Angiotensin converting enzyme

Converting enzyme (when measured by hydrolysis of the substrate hip-his-leu) is found both in CSF (Schelling et al., 1980) and

brain tissue (Yang and Neff, 1972). Immunocytochemical localization of converting enzyme has shown that the enzyme is highly concentrated in the brush border of the choroid plexus (Igli et al., 1977).

d. Angiotensinase

Peptidases that metabolize AII into smaller fragments are collectively termed angiotensinase. Angiotensinase activity is not present in CSF, but AII is degraded during in vivo ventriculo-cisternal perfusion, presumably by contact with tissue angiotensinase. Circumventricular organs (including the subfornical organ, median eminence, subcomissural organ and area postrema) have particularly high angiotensinase activity (Schelling et al., 1980), and it has been suggested that angiotensinase activity is intimately associated with angiotensin receptors (Abhold et al., 1984).

e. Angiotensins

Both angiotensin I and II have been found in CSF (Schelling et al., 1980; Husain et al., 1983), and immunoreactivity for an AII-like material has been detected in brain tissue (Phillips et al., 1980; Quinlan and Phillips, 1981; Phillips et al., 1981; Simonnet et al., 1984). Since available antibodies for AII cross react with the 2-8 (AIII), 3-8 and 4-8 fragments of AII (Hutchinson et al., 1978), some studies have undertaken electrophoretic or chromatographic separation of these peptide fragments to determine the relative amounts of AII and metabolites in biological samples. Using these techniques, some investigators have concluded that AII measured in human CSF may be an artifact (Semple et al., 1980), while others have been able to separate and measure AI, AII, and AIII in rat brain tissue and CSF (Hermann et

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al., 1982; Ganten et al., 1983). Electrophoretic separation of immunoreactive angiotensin from canine CSF has shown that AIII is the major angiotensin from this source (Hutchinson et al., 1978). The concentration of immunoreactive AII in the CSF has been found to increase during the development of 2-kidney Goldblatt hypertension in dogs (Suzuki et al., 1983). The CSF immunoreactive AII found in this study probably was not of peripheral origin since Nicholls (1980) has shown that manipulations that cause changes in the level of plasma immunoreactive AII in the dog (hemorrhage, furosemide, β-adrenergic blockade, and saline infusion) do not result in any changes in CSF immunoreactive AII. In this study it was also demonstrated that AIII, when infused intravenously, does not enter the CSF.

f. Angiotensin receptors

Specific, saturable and reversible binding of ^{125}I-AII to various regions of rat brain has been demonstrated (Sirett et al., 1977; Harding et al., 1981). These in vitro binding studies have shown that high concentrations of AII binding sites are present in area postrema, septum, superior colliculi, midbrain, thalamus and hypothalamus. The technique of in vitro receptor autoradiography has been used to further localize AII receptors to the lateral septal nucleus (Healy and Printz, 1984), superior colliculus, lateral olfactory tract, paraventricular and periventricular nuclei, OVLT, median preoptic nucleus, nucleus of the solitary tract, dorsal motor nucleus of the vagus nerve, and area postrema (Gehlert et al., 1984a,b; Mendelsohn et al., 1984). Angiotensin II has been demonstrated to bind specifically to circumventricular organs (OVLT, SFO, AP, median eminence) after intravenous injection (van Houten et al., 1980). AII binding sites also were found

in the OVLT after intraventricular AII injection by a fluorescent microscopic technique (Landas et al., 1980). It is believed that brain AII binding sites are physiologically relevant for several reasons: 1) microiontophoresis of AII in areas of dense AII binding, such as OVLT (Knowles and Phillips, 1980) and SFO (Phillips and Felix, 1976) cause neuronal excitation; 2) microinjection of AII into these regions results in physiologic responses, such as increases in blood pressure (Mangiapane and Simpson, 1980a), and the binding affinity of AII analogs to brain membrane preparations correlates well with their physiological potency (Mann et al., 1981). Factors that regulate brain AII receptor number and/or affinity, or the activity of the brain renin-angiotensin system as a whole are not well defined.

The regulation of brain AII receptors in response to sodium intake has been studied. One report (Mann et al., 1980a) has shown that low sodium intake results in a decreased number of AII binding sites in the hypothalamus-thalamus-septum-midbrain (HTSM) region in rats, and that sodium restriction also blunts the dipsogenic and pressor responses to acute ivt AII injection. However, others (Speth et al., 1984) have found no alterations in brain AII receptors with sodium intake. Functional studies of brain AII receptor sensitivity have shown that the dipsogenic (Kapsha et al., 1979) and pressor (Brosnihan et al., 1979; Eguchi and Bravo, 1984) actions of acute ivt AII administration appear to be unaltered by dietary sodium intake. However, the hypertensive response to chronic ivt AII injection in the dog is sodium-sensitive (Buckley et al., 1981), suggesting that AII and sodium may interact at the level of the central nervous system to produce chronic increases

in arterial pressure. A central angiotensin-sodium interaction has been demonstrated in the goat, where the pressor, dipsogenic, antidiuretic, natriuretic and plasma renin suppressing activities of acute third ventricular infusions of AII or hypertonic NaCl are potentiated when the AII is given in a hypertonic NaCl solution (Anderson et al., 1971, 1972; Eriksson et al., 1976). Sodium per se appears to be important in this response since hypertonic solutions of non-electrolytes do not have the same effect. In contrast, Buggy et al. (1979) have shown that the dipsogenic effect of ivt infusions of hypertonic NaCl and AII in the rat are not synergistic, but additive, and furthermore that hypertonic sucrose solutions are as effective as hypertonic NaCl in eliciting blood pressure increases. Regardless of whether the effects of AII and sodium are additive or synergistic, it does appear that sodium can augment the central effects of AII.

Although adrenal and vascular AII receptors regulate in response to the elevated plasma AII levels seen in sodium restriction, it is not yet well established whether brain AII receptors are altered in response to changes in endogenous levels of the peptide. During dehydration, a condition in which plasma AII is elevated, AII receptors in the SFO "up-regulate" as reflected by an increase in the number of AII binding sites (Israel et al., 1984). However, chronic ivt AII infusion has no effect on AII binding site number or affinity in the hypothalamus-thalamus-septum-midbrain region (Singh et al., 1984).

At this point it is still uncertain to what extent the central interactions of AII and sodium contribute to the pressor actions of central or peripheral AII administration in animals on varying dietary sodium intakes.

g. Angiotensinergic neurons

Receptor binding studies have localized AII receptors both in sites that would be expected to be exposed to blood-borne AII, and also in sites that are within the blood-brain barrier, and thus would not be expected to "see" circulating AII. The functional importance of AII receptors that are far removed from the ventricular system and inside the blood-brain barrier is not known. However, accumulating evidence suggests that these receptors may indicate the presence of neuronal systems that use angiotensin as a transmitter. It has been demonstrated that AII can be synthesized de novo in brain cells in tissue culture (Fishman et al., 1981; Raizada et al., 1984; Weyhenmeyer et al., 1984), and that AII can be released from these cells upon chemical depolarization with KCl (Meyer et al., 1984). Immunohistochemical studies have localized angiotensin peptides in particular cell groups and fiber systems in the central nervous system (Fuxe et al., 1976; Changaris et al., 1978; Kilcoyne et al., 1981; Phillips et al., 1981; Weyhenmeyer and Phillips, 1982; Lind et al., 1984a). Of particular interest in these studies is that AII immunoreactivity is present in areas that are known to interact with blood-borne AII. The central part of the SFO contains an AII-immunoreactive terminal field which appears to arise from cells in the lateral hypothalamus, zona incerta and nucleus reuniens (Lind et al., 1984). Cell bodies that stain for AII-like peptides are present in the peripheral part of the SFO. Many of the areas that receive SFO efferents are sensitive to AII, and some of these, including the paraventricular nucleus and median preoptic nucleus, receive AII-containing input from the SFO (Lind et al., 1984a).

Among other CVO's, the OVLT was found to contain a plexus of fibers and varicosities that stain for AII. The AP contains AII-stained fibers and adjacent parts of the nucleus of the solitary tract contain immunostained cell bodies.

2. Physiological effects of central AII administration

a. Pressor effects

One method that has been commonly employed to study the central actions of AII is to administer the peptide into the CSF. This route of administration presumably simulates endogenous brain production of AII. Acute or chronic administration of AII into the cerebral ventricles produces a pressor response (Severs et al., 1970; DiNicolantonio et al., 1982; Fink et al., 1982; Fisher and Brown, 1984). Two mechanisms have been proposed to contribute to the pressor response seen with acute ivt injections of AII: the release of pressor quantities of vasopressin (AVP) and activation of the sympathetic nervous system. Acute ivt AII injections have been observed to produce antidiuresis (Severs et al., 1971; Hoffman et al., 1979), an indication of AVP release. In addition, in virtually every study in which it has been measured, plasma AVP increases in response to acute ivt AII (Keil et al., 1975; Haack and Mohring, 1978; Malayan et al., 1979; Scholkens et al., 1982; Fisher and Brown, 1984). Furthermore, the following observations indicate that this increase in plasma AVP exerts an important pressor effect in the rat: 1) hypophysectomy, supraoptic nucleus lesion or median eminence lesion decreases the pressor response to acute ivt AII (Severs et al., 1970; Hoffman et al., 1979), 2) pretreatment with an AVP antibody (Haack and Mohring, 1978; Hoffman et al., 1979) or

with a specific antagonist of the vascular AVP receptor (Unger et al., 1981; Fisher and Brown, 1984) diminishes the magnitude of acute ivt AII-induced pressor responses and 3) Brattleboro rats, genetically deficient in AVP, exhibit markedly reduced pressor responses to central AII when compared to Long-Evans control rats (Hutchinson et al., 1976; Haack and Mohring, 1978). In contrast to the elevated plasma AVP observed after acute ivt AII injection, plasma AVP is not elevated in response to chronic ivt AII infusion in rats or rabbits (Sterling et al., 1980; Fink et al., 1982a). In the dog, AVP appears to contribute less to the ivt AII-induced pressor response than in the rat. Although plasma AVP increases in the dog after acute ivt AII, hypophysectomy does not alter the magnitude of the pressor response (Malayan et al., 1979).

Activation of the sympathetic nervous system also appears to be involved in the pressor response to acute ivt AII, although the evidence for this is somewhat controversial. Some investigators report an increase in plasma catecholamines after ivt AII injection (Mann et al., 1982; Scholkens et al., 1982) while others report no change (Fisher and Brown, 1984). Ganglionic blockade does not decrease the magnitude of the pressor response (Severs et al., 1970; Fisher and Brown, 1984), while α-adrenergic blockade has been reported to either decrease (Severs et al., 1970; Unger et al., 1981) or have no effect on (Mann et al., 1982) the magnitude of the acute ivt AII-induced pressor response. Peripheral sympathectomy with 6-hydroxydopamine does not alter the magnitude of the pressor response to acute ivt AII but prolongs the latency to peak blood pressure (Falcon et al., 1978). Although the above evidence is equivocal, it is known that the reduced pressor response to acute ivt AII seen when the AVP system is blocked by either

hypophysectomy or AVP antagonists can be totally abolished in the presence of ganglionic blockade (Severs et al., 1970), suggesting that activation of the sympathetic nervous system does comprise a component of the total pressor response to acute ivt AII.

The mechanisms involved in maintaining elevated arterial pressure in response to chronic ivt AII infusions have been less well studied. Chronic ivt administration of AII in both rabbits (Fink et al., 1982a) and dogs (Jandhyala et al., 1979) produced persistent hypertension that does not appear to be mediated by elevated sympathetic vasomotor tone. In both studies, pressor responses to exogenous norepinephrine were enhanced in the hypertensive animals, and in the dogs an increased responsiveness to AII also was observed. These results are in contrast to those obtained in rabbits and dogs with chronic intravertebral AII infusion (Dickinson and Yu, 1967; Sweet et al., 1971). Chronic intravertebral AII infusion, at doses that are ineffective when given intravenously, causes hypertension that can be demonstrated to be mediated by the sympathetic nervous system. The apparent difference in the mechanisms supporting hypertension when AII is delivered to the brain by the two routes may reflect differences in the receptor sites reached from blood and from CSF. The area postrema is believed to be the site at which vertebral artery infusions of AII act (Joy and Lowe, 1970; Yu and Dickinson, 1971). In contrast, AII in the CSF probably interacts with structures in the AV3V region to produce both acute (Buggy and Johnson, 1977; Fink and Bryan, 1980) and chronic (Fink et al., 1983) increases in blood pressure. Although chronic ivt AII infusion has been performed in rats (Gronan and York, 1978; Sterling et al.,

1980; DiNicolantonio et al., 1982); the mechanisms involved in the hypertensive response to such infusions have yet to be identified.

b. Thirst and fluid/electrolyte effects

Intracranial administration of angiotensin II is a potent dipsogenic stimulus in many species. Many studies have been undertaken to localize the brain site(s) at which AII acts to induce drinking. Initial studies implicated the preoptic area as an important receptor site for the dipsogenic effect of AII (Epstein et al., 1970). However, subsequent studies demonstrated that the extent to which intracranial AII injections produced drinking was related to the accessibility of the injectate to the ventricular system (Johnson and Epstein, 1975). Both the SFO and structures within the AV3V have been implicated as important receptor areas for the dipsogenic effects of CSF-borne AII. The evidence for the SFO as a receptor site for blood-borne AII is described above. Experiments implicating the SFO as a receptor site for the dipsogenic effects of ivt AII are considerably less convincing. Drinking can be elicited by injection of AII directly into the SFO, and SFO lesion blocks the drinking produced by AII injection into the preoptic area (Simpson and Routtenberg, 1973). However, SFO lesion does not produce consistent thirst deficits to lateral ventricular AII injection (Buggy et al., 1975; Hoffman and Phillips, 1976). Many other studies (as reviewed by Johnson and Buggy, 1977) support the view that access of CSF-borne AII to the SFO is neither necessary nor sufficient for AII-induced drinking. On the other hand, the use of ventricular plugging has demonstrated that access of CSF-borne AII to the AV3V is an absolute requirement for stimulation of drinking (Hoffman and Phillips, 1976;

Buggy et al., 1975). In addition, electrolytic ablation of the AV3V region produces drinking deficits to intraventricular AII and hypertonic saline (Buggy and Johnson, 1977; Fink and Bryan, 1980). With regard to the dipsogenic response to chronic ivt AII infusion, one report has shown that this response is not sustained for more than a few days (DiNicolantonio et al., 1982). However, most investigators report that chronic ivt AII infusions produce sustained increases in water intake (Gronan and York, 1978; Fink and Bryan, 1980; Buckley et al., 1981).

Intracerebroventricular AII administration, in addition to eliciting pressor and drinking responses, can result in a number of other changes related to fluid and electrolyte status. Effects that have been reported to occur after ivt AII administration include natriuresis (Severs et al., 1971; Jandhyala et al., 1979; Halperin et al., 1981; Brooks and Malvin, 1982; Fink et al., 1982), stimulation of ACTH release (Maran and Yates, 1977; Eguchi and Bravo, 1984), suppression of plasma renin activity (Malayan et al., 1979; Eguchi and Bravo, 1984), and stimulation of sodium appetite (Avrith and Fitzsimons, 1980). Plasma aldosterone has been reported to increase (Nicholls et al., 1983) or decrease (Brooks and Malvin, 1980) after acute ivt AII infusion.

C. Central Effects of Angiotensin II in Hypertension

As discussed above, AII can exert a centrally-mediated pressor effect from the blood or from the CSF, in the latter case possibly as the result of AII generation by the endogenous brain renin-angiotensin system. Two lines of experimental evidence have implicated a central

pressor effect of AII as an important pathogenetic factor in many forms of experimental hypertension. First, electrolytic ablation of discrete AII-sensitive brain areas has been demonstrated to prevent the development of hypertension in response to several experimental interventions, or to reverse hypertension once it has become established. Second, central administration of converting enzyme inhibitors or AII receptor antagonists has been reported to decrease blood pressure in hypertensive rats. A discussion of results obtained with each of these methods, and their limitations, is presented below.

1. Brain lesion studies

In rats, electrolytic ablation of the AV3V region will both prevent and reverse one-kidney, one-wrap Grollman hypertension (Buggy et al., 1977, 1978) and will reduce the severity of aortic coarctation (Hartle et al., 1979), two-kidney one-clip Goldblatt (Haywood et al., 1983), and deoxycorticosterone-salt hypertension (Fink et al., 1977; Berecek et al., 1982). AV3V lesion also has been shown to retard the development of NaCl hypertension in the Dahl salt-sensitive rat strain (Goto et al., 1982). However, lesion of the AV3V does not effect the development of hypertension in young spontaneously hypertensive rats (SHR) or decrease established blood pressure in adult SHR (Buggy et al., 1978; Gordon et al., 1982). SFO lesion also has been shown to attenuate the severity of 2-kidney one-clip renal hypertension (Buggy et al., 1984) and 1-kidney Grollman hypertension (Kneupfer et al., 1984) in the rat.

Perhaps one of the most intriguing observations to come from a lesion study is that AV3V lesions in rats largely will prevent the development of hypertension in response to chronic intravenous infusion

of acutely subpressor doses of AII (Fink et al., 1982b). This suggests that, as opposed to the pressor response to acute iv AII which is predominantly the result of direct vascular constriction, chronic elevations in blood-borne AII produce hypertension by an action on the brain, mediated through the AV3V region.

A species difference between the rat and rabbit with regards to the ability of AV3V lesion to prevent hypertension development has been observed. In contrast to the rat, destruction of the AV3V in the rabbit has no effect on DOCA-salt (Mann et al., 1984) or 2-kidney one-clip renal hypertension (Fink and Bryan, 1982). However, AV3V lesion does prevent the development of one-kidney one-clip renal hypertension in the rabbit (Fink and Mann, 1983). An important distinction bewteen the rat and rabbit lies in the effect of AV3V destruction on pressor responses to acute or chronic intravenous AII infusion. In the rat, the pressor response to acute iv AII infusion is slightly but significantly reduced after AV3V lesion (Buggy et al., 1977), whereas the same responses in the rabbit are unaltered by AV3V lesion (Fink and Bryan, 1980). Furthermore, as mentioned above, AV3V lesion largely prevents the hypertensive response to chronic elevations of plasma AII in the rat. In contrast, the hypertension observed in the rabbit with prolonged intravenous AII infusion is not attenuated in rabbits with prior AV3V lesions (Fink and Mann, 1984). This species difference in the effects of AV3V lesion on various models of hypertension, especially iv AII-induced hypertension, may reflect the relatively greater importance of the area postrema as a central pressor site for circulating AII in the rabbit. Alternatively, the different effects of AV3V lesion in the two

species may relate to the relative size and extent of the lesion. The AV3V lesion is relatively large in the rat, encompassing the OVLT, subcommissural MnPO, and parts of the periventricular and medial preoptic areas, whereas the area of damage in the rabbit is largely confined to the OVLT and immediately surrounding structures. Since the AV3V region in the rat contains both AII-receptive elements (in OVLT and MnPO) and neural pathways from other AII-sensitive sites (notably the SFO), small discrete lesions within the AV3V region will be necessary to discern which specific areas are responsible for "sensing" chronic changes in blood-borne or CSF-borne AII in the rat.

One limitation inherent in lesion studies is the possibility that the lesion produces "non-specific" effects unrelated to the primary intent (in this case, interruption of forebrain AII-sensitive mechanisms). In the case of AV3V lesion, the acute post-lesion period is characterized by adipsia coupled with an inappropriate antidiuretic response to the reduced water intake. As a result, dehydration ensues (Johnson and Buggy, 1978; Brody and Johnson, 1980). Within three weeks, water intake and fluid balance return to normal, but plasma sodium, osmolarity and plasma renin activity remain chronically elevated (Buggy and Johnson, 1977; Shrager and Johnson, 1980). Although fluid balance returns to normal after AV3V lesion, functional deficits persist. Pressor, antidiuretic and dipsogenic responses to ivt or systemic administration of hypertonic solutions or AII are attenuated (Buggy et al., 1977; Bealer et al., 1979; Brody and Johnson, 1980; Fink and Bryan, 1980). In addition, excretion of water and sodium is impaired in response to a volume load (Brody and Johnson, 1980; Bealer et al., 1983).

In short, a rat with an AV3V lesion is not a "normal" rat minus an AII-sensitive brain region, rather it is also a rat with functional deficits in osmoregulation. These "non-specific" lesion effects confound interpretation of experiments in which a centrally-mediated AII pressor effect is estimated by the difference between normal and AV3V lesion animals.

2. Central administration of inhibitors of the renin-angiotensin system

The second method that frequently has been used to assess the effects of AII on the brain is ivt administration of AII receptor antagonists or converting enzyme inhibitors. Acute bolus ivt injections of AII receptor antagonists (most commonly used are ^{1}sar,^{8}ala-AII and ^{1}sar,^{8}ile-AII) will produce slight transient depressor responses in SHR (Ganten et al., 1975; Phillips et al., 1975; Mann et al., 1978; Suzuki et al., 1981), stroke-prone SHR (Phillips et al., 1977), 2-kidney, one-clip renal hypertensive rats (Mann et al., 1978; Suzuki et al., 1981), 2-kidney, 2-clip renal hypertensive rats (Schoelkens et al., 1976) and malignant hypertensive rats (Sweet et al., 1976). In SHR, acute ivt injection of the converting enzyme inhibitor captopril also has been reported to produce decreases in blood pressure (Hutchinson et al., 1980), although this is not a universal finding (Crofton et al., 1981). Since acute ivt injections of AII antagonists do not completely reverse hypertension due to chronic elevation of plasma or CSF AII (Mann et al., 1978; Suzuki et al., 1981; Fink et al., 1982a), such injections may not be sufficient to completely reveal a centrally-mediated AII pressor effect in chronic hypertensive states. This is especially important in view of the suggestion that the central effects of AII are slow to

develop (Brown et al., 1981). Some investigators have reported that the development of hypertension in young SHR is attenuated by chronic ivt infusion of the renin inhibitor N-acetyl-pepstatin (Tonnaer et al., 1981), or captopril (Okuno et al., 1983). Established hypertension in adult SHR can be partially reversed by chronic ivt infusion of ^{1}sar, ^{8}ile-AII (McDonald et al., 1980) and a depressor response to chronic ivt infusion of ^{1}sar,^{8}ala-AII has been observed in stroke-prone SHR (Ganten et al., 1979). However, these depressor responses are greatest within the first 24 hours; blood pressure tending to return toward control levels over the remaining days of ivt antagonist infusion. Although all of the above-mentioned studies support a role for a central pressor effect of AII in hypertension, the acute nature of many of the experiments coupled with the lack of determination to what extent the ivt AII antagonist injections or infusions produce functional blockade of central or peripheral AII responses are limitations of these studies.

STATEMENT OF PURPOSE

Many recent studies have implicated a central pressor effect of AII (of either peripheral or central origin) as an important contributor to some forms of hypertension. Therefore, the first hypothesis tested in this thesis will be whether chronic stimulation of brain AII receptors will produce hypertension. The cardiovascular and fluid/electrolyte responses to chronic stimulation of central AII receptors by ivt AII infusion will be characterized. The sodium dependency of this form of hypertension will be assessed using both rats and rabbits as animal models. Since the sympathetic nervous system and vasopressin contribute to the pressor response to acute ivt AII in the rat, the hypothesis that these mechanisms contribute to the hypertension produced by chronic ivt AII infusion will be investigated. The response to pharmacologic blockade of each of these systems will be used as an index of their contribution to the hypertensive state. The importance of the sympathetic system will be studied further in chemically sympathectomized rats. The role of aldosterone and of the direct vasoconstrictor effects of blood-borne AII also will be examined. Finally, the hypothesis that interactions of the sympathetic nervous system, AVP, and peripheral AII are involved in the maintenance of hypertension in this model will be studied.

Methods will be developed to produce chronic, selective pharmacologic blockade of brain AII receptors or brain angiotensin converting enzyme, in an effort to determine the extent to which central AII effects contribute to several forms of hypertension. These pharmacologic methods should represent an advantage over previous electrolytic lesion studies, since non-specific lesion effects will be dissociated from blockade of central AII receptors. The following models of hypertension will be examined:

1) genetic hypertension: chronic ivt infusion of an AII receptor antagonist or converting enzyme inhibitor will be performed in adult rats with established hypertension

2) DOC-salt hypertension: chronic ivt infusion of AII receptor antagonist prior to and during DOC-salt treatment

3) chronic intravenous AII infusion: chronic ivt infusion of AII receptor antagonist prior to and during chronic iv AII infusion.

The hypothesis that chronic elevations of circulating AII produce hypertension by an action on the brain will be further examined by determining the effects of discrete brain lesions on chronic iv AII-induced hypertension. The brain lesions studied in this regard include: 1) subfornical organ lesion, 2) knife cut of subfornical organ efferents, 3) median preoptic nucleus lesion.

MATERIALS AND METHODS

A. General Methods

1. Animals

Except where otherwise noted, male Sprague-Dawley rats (250-350 g) were used in all experiments. Rats were obtained from either Harlan, SASCO, or Charles River breeding farms. Prior to entry into an experimental protocol they were group housed on corn cob bedding in light-cycled, temperature controlled quarters. Access to standard laboratory chow (Wayne Lab Blox) and distilled water was provided *ad libitum*. Male spontaneously hypertensive rats (SHR) derived from the strain developed by Okamoto and Aoki (1963) were obtained from SASCO and were between 250 and 350 g at the time of experimentation. Male albino New Zealand rabbits supplied by Bailey were housed individually in metal metabolism cages and offered 100 g of Purina high fiber rabbit chow (Lab Rabbit Chow HF) per day with *ad lib* access to distilled water prior to entry into a study.

2. General Surgical Procedures

All major surgical procedures were performed under pentobarbital anesthesia (50 mg/kg, ip for rats; 30 mg/kg, iv for rabbits). Rats also received 0.2 mg atropine sulfate ip to reduce bronchial congestion. Minor procedures were performed under one of the following:

1) gaseous anesthesia with ether or halothane, or 2) methohexital, 10 mg/kg, iv. Postoperatively, rats received a single intramuscular injection of 20,000 U procaine penicillin G and 25 mg dihydrostreptomycin (100,000 U penicillin and 125 mg dihydrostreptomdcin for rabbits). Rats that were subsequently housed in metabolism cages received twice daily injections of ampicillin (10 mg, iv) throughout the experiment. A recovery period of at least 2-3 days after surgery preceded entry into an experimental protocol.

3. Blood pressure measurement

a. Direct catheterization: rats

Chronic indwelling catheters were implanted in the abdominal aorta and vena cava via the left femoral vessels. Catheters were fashioned from either polyvinyl chloride tubing or polyvinyl-silicone rubber. The arterial catheter was filled with a heparinized (1000 U/ml) solution and both catheters were plugged when not in use. Catheters were tunnelled subcutaneously to the suprascapular region and either left beneath the skin or exteriorized and anchored to the skin with a small amount of cyanoacrylate glue. For measurement of arterial pressure, rats were briefly anesthetized with halothane or methohexital and placed in a tethered rat jacket in their home cage. The tether was attached to a swivel mounted above the cage to allow the rat free movement. Arterial and venous catheters were connected to long lengths of polyvinyl chloride tubing that exited the cage via the protective tether. The arterial catheter was connected to a small volume displacement pressure transducer (Gould-Statham P23ID or P50) and pulsatile arterial pressure recorded on a Grass polygraph. When necessary, the

venous catheter was attached to a Harvard syringe infusion pump for intravenous infusion. The duration of anesthesia was typically 1-2 min, and after this time, direct recording of arterial pressure was obtained in the conscious rats. Rats were allowed to sit undisturbed for at least 10-15 minutes before any experimental manipulation was begun. Mean arterial pressure (MAP) was calculated as 1/3 (pulse pressure) plus diastolic pressure. Heart rate (HR) was counted directly from the arterial pressure tracing.

When rats were to be housed in metabolic cages, arterial and venous catheters were tunnelled subcutaneously to the skull, where they were anchored with dental acrylic. Catheters were protected by a flexible metal spring led out the top of the cage and connected to a hydraulic swivel to allow the rat free movement within its cage. A continuous intravenous fluid infusion (either 5% dextrose or 0.9% saline) was maintained throughout the experiment at a rate of 40 ml/24 hr. Mean arterial pressure and heart rate were determined each day between 8:00 and 11:00 a.m., and were taken to be the lowest stable values obtained during a 15-30 min recording session.

b. Indirect blood pressure measurement

Systolic blood pressure was estimated by an indirect tail cuff plethysmographic method (IITC Pulse Amplifier) with photoelectric detection. Rats were restrained in a plexyglass restrainer and warmed under a Tensor lamp for approximately 10 min to obtain dilation of the tail artery. Tail cuff blood pressure was taken as the average of five determinations.

c. Direct measurement: rabbits

Mean arterial pressure was measured directly in conscious rabbits loosely restrained in a head stock. Dilation of a central ear artery was obtained by infiltration of the base of the ear with 1 ml of a 2% lidocaine solution and application of 5% lidocaine ointment to the back of the ear. The artery was punctured percutaneously with a 25-gauge butterfly infusion set. Tubing from the infusion set was connected to a Gould-Statham 23ID pressure transducer and output recorded on a Grass polygraph. Mean arterial pressure and heart rate were calculated in the same manner as for rats.

4. Implantation of intracerebroventricular cannulae

Lateral cerebral ventricular cannulae were implanted in rats under pentobarbital anesthesia. The head was immobilized in a Kopf small animal stereotaxic apparatus. The incisor bar was set at a level 5.0 mm above the interaural line. The skull was exposed by a midline incision and 2-3 small jewelers screws were burrowed a short distance into the skull. A small burr hole was made 1.5 mm lateral to bregma, and a stainless steel 23-guage cannula (12 mm length) was lowered so that its tip was located 4.5 mm ventral to the dura. Dental acrylic was used to anchor the cannula to the skull. In some experiments, bilateral ventricular cannulae were implanted by lowering one cannula 1.5 mm lateral to bregma on either side. Cannulae were occluded with 30-gauge stainless steel obturators when not in use. At the conclusion of each experiment, correct cannula placement was verified by injection of Evans Blue dye into the cannula and visualization of the dye in the ventricular system after the brain had been cut in the frontal plane.

A single lateral ventricular cannula was implanted in rabbits under pentobarbital anesthesia. The skull was leveled in a Kopf stereotaxic apparatus and a burr hole made 1.0 mm anterior and 2.0 mm lateral to bregma. A 23-gauge stainless steel cannula was lowered 8.0 mm ventral to the dura. The cannula was anchored to the skull with jewelers screws and dental acrylic, and was occluded with an obturator when not in use.

5. Metabolic measurements

In some experiments in which rats were housed in metabolic cages, daily measurements of variables related to fluid/electrolyte status were obtained. Fluid intake was quantified as the sum of the volume obtained by intravenous infusion (40 ml) and the volume of water ingested from a calibrated drinking tube. Urine output (UO) was measured by collection in calibrated tubes under the cage. Water balance was calculated as the difference of fluid intake and urine output (assuming a constant insensible loss). Urinary sodium and potassium concentrations were measured by flame photometry and daily urinary sodium and potassium excretions calculated by multiplying urine volume by electrolyte concentration.

6. Statistical analysis

A variety of statistical tests appropriate for the experimental design were used. Many of the experiments take the format of repeated measurements within animals. In these experiments, a randomized block analysis of variance (ANOVA) or a mixed design ANOVA (for comparison or 2 or more groups) was used to detect differences in treatment means. Except where otherwise stated, the "protected" least significant

difference test (lsd) was used for nonconfounded individual within- and between-group comparisons. Values given in the text are mean ± standard error of the mean (within groups) or, when a t-test is used, mean ± standard error of the difference. Specific statistical procedures are mentioned within the context of the particular experiment in the Results section. In all cases a p value of 0.05 was used as the criterion of statistical significance.

B. Experimental Protocols

1. Chronic ivt AII infusion: rat

a. Sodium dependency

In an initial surgical procedure, rats received chronic indwelling arterial and venous catheters and a right lateral cerebral ventricular cannula. The top of the ventricular cannula was fitted with a piece of polyvinyl chloride tubing filled with 0.9% saline and tunnelled subcutaneously to the scapular region, where it was plugged. Rats were housed in metabolic cages and the catheters protected by a metal spring led out the top of the cage and attached to a hydraulic swivel. Rats had free access to standard laboratory chow (0.1 mEq Na, 0.3 mEq K/g). Sodium intake was controlled by the composition of the intravenous infusion; rats maintained on normal Na intake received 40 ml of 5% dextrose/24 hr and high Na intake was achieved by infusion of 40 ml of 0.9% saline/24 hr (equivalent to 6.2 mEq Na). Daily measurements of mean arterial pressure (MAP), heart rate (HR), urine output (UO), water intake (WI), water balance (WB) and urinary sodium ($U_{Na}V$) and potassium (U_KV) excretions were obtained. After two days of control measurements, rats were briefly anesthetized with methohexital and a

prefilled osmotic minipump (Alzet, Model 2001, Alza Corp., Palo Alto, CA) containing a solution of AII (angiotensin II amide, Hypertensin^{R}, CIBA) in isotonic saline (1 or 6 mg/ml) was implanted subcutaneously in the suprascapular region and connected via polyvinyl chloride tubing to the ventricular cannula. Control rats received ivt infusions of isotonic saline. These minipumps deliver solution at a rate of 1 µl/hr, therefore AII was infused into the ventricular system at a dose of 1 or 6 µg/hr. The stability of AII in minipumps over a 7-day period has been previously verified (DiNicolantonio et al., 1982). Intraventricular infusion was maintained for 5 days, after which time the minipumps were removed under methohexital anesthesia, and two recovery days followed. This general protocol was followed in groups of rats receiving the following treatments: 1) AII ivt (6 µg/hr, n=5 or 1 µg/hr, n=5) or saline ivt (n=7), high sodium intake, and access to drinking water only during the final 24 hours of ivt infusion; 2) AII ivt (6 µg/hr, n=6 or 1 µg/hr, n=6) or saline ivt (n=6), high sodium intake and ad lib access to drinking water, and 3) AII ivt (6 µg/hr, n=5 or 1 µg/hr, n=6), normal sodium intake and ad lib water intake.

b. Plasma hormone levels

Rats were maintained on a high sodium intake with ad lib access to water and received ivt infusions of AII (6 µg/hr, n=6) or saline (n=7) for 5 days preceded by two control and followed by two recovery days. MAP and HR were measured daily. On the second control day, the fifth day of ivt infusion, and the second recovery day, after MAP was determined, a 1 ml blood sample was rapidly withdrawn from the arterial catheter into a chilled syringe containing EGTA and glutathione

for measurement of plasma norepinephrine and epinephrine concentration (Cat-a-Kit, Upjohn). Immediately following this first sample, a second arterial sample (3 ml) was withdrawn into a chilled syringe over EDTA for aldosterone determination (radioimmunoassay, Damon). The samples were spun in a refrigerated centrifuge at 7,000 x g for 10 min and the plasma was stored at -70°C until assay. A 60 µl plasma sample was reserved for determination of plasma osmolality (Micro Osmette^{R}) and plasma Na and K concentrations. Hematocrit and body weight also were determined on these days.

In a separate group of rats maintained on a high sodium intake (n=12), arterial blood samples (3 ml) were obtained on the second control day and fifth day of ivt AII infusion for measurement of plasma AII concentration by radioimmunoassay. Red blood cells were resuspended in an equal volume of isotonic saline and returned to the rat. Plasma samples were stored at -70°C until they were sent to the laboratory of Dr. Ian Reid, University of California at San Francisco, for analysis.

c. Adrenalectomy

Bilateral adrenalectomy was performed via a retroperitoneal approach under pentobarbital anesthesia. Rats received a single postoperative injection of dexamethasone (0.2 mg, im), and were maintained on ad lib 0.9% saline drinking fluid and standard rat chow. Rats were housed individually in clear plastic cages. Control rats did not undergo sham operation. Three to four weeks after adrenalectomy, a right lateral ventricular cannula was implanted in adrenalectomized and control rats. Two days after cannula implantation, blood pressure measurements were begun. Blood pressure was measured three times weekly

by tail cuff plethysmography. Body weight and saline intake were measured daily. After one week of control measurements prefilled osmotic minipumps (Alzet Model 2001) containing AII (6 mg/ml) were implanted subcutaneously under ether anesthesia and connected via polyvinyl chloride tubing to the ivt cannula. The ivt AII infusion (6 μg/hr) was maintained for 7 days, during which time blood pressure, body weight, and saline intake were measured. After blood pressure was measured on the seventh day of ivt AII infusion, a plasma sample for aldosterone assay was obtained by cutting the tip of the tail and allowing 1 ml of blood to flow freely into a heparinized tube. Blood samples were centrifuged, the plasma removed and stored at -70°C until assay. Osmotic minipumps were removed under ether anesthesia after the blood sample was taken. One week of recovery measurements followed. On the final day of blood pressure measurement, a second plasma sample was obtained for aldosterone determination.

d. Acute blockade of vascular AII receptors and ganglionic blockade

A series of acute interventions was performed to assess the contributions of the direct vasoconstrictor actions of peripheral AII and of neurogenic tone to maintenance of elevated arterial pressure in response to chronic ivt AII infusion. These interventions were performed in a group of 6 rats maintained on high Na intake in metabolic cages on the second control day, the first, third, and fifth days of ivt AII infusion (6 μg/hr) and the second recovery day. After measurement of basal MAP and HR, rats received an intravenous infusion of ^{1}sar,^{8}ala-angiotensin II (saralasin, 300 ng/min) for 10 min. The change in MAP and HR to saralasin was calculated as the difference of preinfusion

values and the values obtained during the final minute of saralasin infusion. After the iv saralasin infusion was stopped, 10-15 min was allowed for recovery. Hexamethonium (20 mg/kg, iv) was then administered to produce ganglionic blockade, and the change in MAP and HR was determined at 5 min after hexamethonium injection.

e. Acute blockade of vascular AVP receptors

In a group of 5 rats maintained on high Na intake in metabolic cages, an assessment was made of the contribution of the vasoconstrictor effects of AVP to the maintenance of elevated arterial pressure in response to to ivt AII infusion. On the second control day, the first, third and fifth day of ivt AII infusion (6 μg/hr) and the second recovery day, after measurement of basal MAP and HR, a specific antagonist for the vascular AVP receptor (1-(β-mercapto-β,β cyclopentamethylene propionic acid), 2-(o-methyl)tyrosine) arginine-8-vasopressin, 10 μg/kg, iv) was administered. The changes in MAP and HR to the AVP antagonist were measured at 5 min after its administration.

The ability of this dose of AVP antagonist to block the effects of exogenous infusions of AVP was determined in rats on a high sodium intake and housed in metabolic cages (n=5). After measurement of basal MAP and HR, AVP was infused intravenously at successive doses of 0.3, 1.0, and 3.0 mU/min. Each dose was infused for 10 min or until a steady-state blood pressure had been achieved. One to two hours later, the AVP antagonist was given (10 μg/kg, iv), and the dose-response curve to exogenous AVP was repeated starting 5 min after administration of the antagonist. Changes in MAP and HR in response to AVP were calculated as the difference between basal and steady-state values for each infusion rate.

f. Peripheral sympathectomy

Chronic peripheral sympathectomy was produced in rats by the method of Johnson et al. (1976). Pregnant Sprague-Dawley rats were procured at approximately 1 week before parturition. Starting one week after birth, neonatal rats were treated with guanethidine sulfate (50 mg/kg/day, sc) or with an equal volume of isotonic saline vehicle 5 days per week for 3 weeks (total of 15 injections). Solutions were administered in a volume of 5 µl/g body weight. After weaning, rats were separated according to sex and group housed in standard cages with free access to food and water. When rats (of either sex) had reached a body weight of 275-325 g, bilateral adrenal demedullation was performed by a retroperitoneal approach under pentobarbital anesthesia. A small incision was made in the adrenal cortex and the medulla extruded by lightly squeezing the gland with a pair of smooth-tip forceps. After recovery to pre-surgery body weight (approximately 2 wk), a cannula was implanted in the right lateral cerebral ventricle. Rats were housed individually in clear plastic cages. In order to maintain all rats on a relatively fixed, high sodium intake, they were given 50 ml of 0.9% saline to drink per day and had free access to regular rat chow. This regimen provided a daily Na intake of roughly 8.0 mEq. After a two-day recovery period, one week of control blood pressure and body weight measurements were obtained. Blood pressure was measured three times weekly by a tail cuff plethysmographic method. The control period was followed by a 7-day ivt infusion of AII (6 µg/hr). Osmotic minipumps were implanted and removed under ether anesthesia. One week of recovery measurements followed the ivt AII infusion period.

g. Verification of degree of sympathectomy

Three procedures were used to assess the degree of peripheral sympathectomy produced by neonatal guanethidine treatment plus adrenal demedullation at adulthood. In one group of sympathectomized (n=8) and control rats (n=5) a functional assessment of neurogenic tone was made by measuring the depressor response to sequential ganglionic blockade with hexamethonium and alpha-adrenergic receptor blockade with phentolamine. Rats were briefly anesthetized with methohexital and loosely restrained in a tethered rat jacket in their home cage. After 10-15 min of baseline MAP and HR measurements in the conscious rats, a bolus injection of hexamethonium (20 mg/kg, iv) was administered. The change in MAP and HR to hexamethonium was measured at 1 and 5 min after the injection. Phentolamine (2 mg/kg, iv) was then administered and MAP and HR responses were measured at 1 and 5 min.

In another group of sympathectomized (n=9) and control rats (n=5), the MAP response to graded stimulation of sympathetic vasomotor outflow was evaluated in the pithed rat preparation (Gillespie and Muir, 1967). Rats were anesthetized with pentobarbital (50 mg/kg, ip), and the trachea cannulated. Previously implanted arterial and venous catheters were used for blood pressure measurement and iv drug administration. Rats were given atropine (0.2 mg, ip) and were paralyzed with gallamine (10 mg/kg, iv). The animals were then artificially ventilated (Harvard rodent respirator) with room air. Rats were pithed at the level of the seventh cervical vertebra with a steel rod. The change in mean arterial pressure elicited by stimulation of sympathetic vasomotor outflow was determined by stimulating the steel rod with monophasic

square wave pulses (cathodal current, 60 V, 1 msec duration) of varying (1, 2, 3, 5, and 10 Hz) frequency for 20 sec. The stimulations were separated by a period of 5 min. After the stimulus-response curve was obtained, hexamethonium (20 mg/kg, iv) was administered and the 10 Hz stimulation repeated to verify that the pressure rises were mediated by stimulation of sympathetic outflow from the spinal cord.

Norepinephrine and dopamine content also was determined in selected tissues of normal (n=5) and sympathectomized (n=8) rats. Rats were killed by decapitation and the following tissues quickly dissected and frozen on dry ice: renal cortex, pineal gland, hypothalamus, cerebellum, and frontal cortex. Tissues were homogenized in 2 N perchloric acid with 100 mg% EGTA. Norepinephrine and dopamine were analyzed in the supernatant by a radioenzymatic method. Protein was analyzed by the method of Lowry et al. (1951). Results were expressed as ng norepinephrine or dopamine per mg protein.

h. Pharmacologic assessment of sympathetic tone

Rats used in this protocol were instrumented with chronic indwelling arterial and venous catheters and a lateral cerebral ventricular cannula. At the time of surgery, an osmotic minipump (Alzet Model 2001) was implanted subcutaneously and connected via polyvinyl chloride tubing to the ivt cannula. Rats received ivt infusions of either AII (6 μg/hr; n=7) or isotonic saline (n=5). Rats were housed individually and had ad lib access to isotonic saline drinking fluid and standard laboratory chow. Blood pressure was measured in the conscious rats while they were loosely restrained in a rat jacket in their home cage. On day 4 of the ivt infusion, the change in MAP to phentolamine

(2 mg/kg, iv) was determined. On day 5, the change in MAP to sequential administration of propranolol (1 mg/kg, iv) and phentolamine (2 mg/kg, iv) was measured. Phentolamine was given 1 min after the propranolol injection. The depressor response to hexamethonium (20 mg/kg, iv) was determined on day 6 of ivt infusion. Blood pressure responses to each intervention were determined at 1 and 5 min after administration. In 2 of the rats that received ivt AII, the order of the interventions was reversed (i.e., day 4-hexamethonium, day 5-propranolol plus phentolamine, day 6-phentolamine alone).

In a separate group of 4 rats, the depressor response to a 5 minute nitroprusside infusion (12 μg/min, iv) was determined. The MAP response to nitroprusside was measured prior to, and on the fifth day of ivt AII infusion. The rats used in this experiment had ad lib access to isotonic saline drinking fluid and standard rat chow.

i. Interactions of AVP, sympathetic nervous system and peripheral renin-angiotensin system

This experiment followed a 2x2 factorial design. Four groups of rats were used: normal rats received ivt infusions of either AII (n=5) or saline (n=5) and sympathectomized rats (neonatal guanethidine treatment plus adrenal demedullation, see section 1f above) similarly received either AII (n=8) or saline ivt (n=5). The sympathectomized rats used in this study were a mixture of males and females (AII ivt - 3 male, 5 female; saline ivt - 2 male, 3 female). Female rats were ovariectomized at the same time that adrenal demedullation was performed. In a single surgical procedure, chronic indwelling arterial and venous catheters and a lateral cerebral ventricular cannula were implanted. Osmotic minipumps (Alzet Model 2001) containing AII (6

mg/ml) or saline also were implanted at this time and connected to the ivt cannula via polyvinyl chloride tubing. Rats were housed individually and had ad lib access to isotonic saline drinking fluid and standard rat chow. On the fifth day of ivt infusion, rats were loosely restrained in a tethered rat jacket in their home cage for measurement of basal MAP and HR. After a steady-state MAP and HR had been recorded (10-15 min), the following series of interventions was performed. First, AVP antagonist (10 μg/kg, iv) was administered, and MAP and HR were determined at 1 and 5 min after the injection. Five min after the AVP antagonist was given, a 15 min infusion of saralasin (10 μg/min, iv) was begun. MAP and HR were determined at 5, 10, and 15 min after starting saralasin infusion. After 15 min of saralasin infusion, phentolamine (2 mg/kg, iv) was administered. The saralasin infusion was continued and MAP and HR were measured at 1 and 5 min after phentolamine.

On day 6 of ivt AII or saline infusion in normal rats, MAP and HR responses to a 15 min infusion of saralasin (10 μg/min, iv followed by AVP antagonist injection (10 μg/kg, iv) were determined.

j. Spinal cord stimulation

Rats were prepared with chronic indwelling arterial and venous catheters and a lateral cerebral ventricular cannula. Osmotic minipumps containing either AII (6 mg/ml; n=9) or isotonic saline (n=8) were implanted subcutaneously and connected by tubing to the ivt cannula. Rats were housed individually and had ad lib access to isotonic saline drinking fluid and standard rat chow. On the fifth day of ivt AII or saline infusion, rats were placed in a tethered rat jacket in

their home cage and MAP and HR were measured. After basal MAP and HR were measured in the conscious rats, they were anesthetized with pentobarbital (50 mg/kg, ip). Rats were pithed and MAP responses to electrical stimulation of the spinal cord were determined as described in section 1g.

k. Acute ivt sarthran/chronic ivt AII

In an initial surgical procedure, chronic indwelling arterial and venous catheters and bilateral ivt cannulae were implanted in rats. At the same time, an ivt AII infusion (6 μg/hr, n=5) was started by means of osmotic minipump. Rats were housed individually in clear plastic cages with ad lib access to standard rat chow and 0.9% saline drinking fluid. On the fifth day of ivt infusion basal MAP and HR were measured while rats were loosely restrained in a tethered rat jacket in their home cage. Three doses of sarthran (0.3, 1.0, 3.0 μg in 5 μl of isotonic saline) were administered ivt at 15 min intervals. MAP was measured at 5, 10, and 15 min after injection of each dose. Minipumps were then removed, and the same protocol of ivt sarthran injections was repeated two days later. On the fifth day of ivt AII infusion (6 μg/hr) in a separate group of 4 rats, three 5 μl injections of isotonic saline were administered with a 15 min interval between injections. MAP was measured every 5 min.

l. Acute ivt sarthran/acute ivt AII

A group of 4 rats with bilateral ivt cannulae and arterial and venous catheters were used in this experiment. They were housed individually in clear plastic cages with ad lib access to standard rat chow and distilled water. MAP and HR were determined in the

conscious rats while restrained in a tethered rat jacket in their home cage. After a stable MAP had been recorded, an injection of 150 ng AII was administered ivt. One minute after ivt AII injection, sarthran (1 μg in 5 μl volume) was administered ivt in the contralateral cannula. The peak change in MAP to ivt AII was recorded, and MAP also was determined at 2.5, 5, 10, 15, and 20 min after ivt sarthran injection. This protocol was repeated in the same group of rats two days later, with the exception that 5 μl of isotonic saline was administered instead of sarthran.

m. Effect of iv saralasin on response to acute ivt AII

A group of 5 rats was instrumented with chronic indwelling arterial and venous catheters and a lateral cerebral ventricular cannula. They were housed individually and maintained on a normal sodium intake. MAP was measured in the conscious rats while loosely restrained in a tethered rat jacket in their home cage. The peak pressor response to an acute ivt injection of AII (50 ng) was recorded. After a 2-3 hour interval, MAP was recorded again and saralasin (300 ng/min) was infused intravenously for 15 min. The pressor response to ivt AII (50 ng) was retested 1 min after stopping the iv saralasin infusion.

2. Chronic ivt AII infusion in rabbits: Sodium dependency

Male albino rabbits weighing 2.5-3.5 kg were used in these experiments. At least one week prior to the beginning of the study, a cannula was implanted in a lateral cerebral ventricle as described in section A.4. Sodium intake was controlled by the amount of sodium in the diet; all rabbits were offered 100 g/day of either sodium-deficient

(1 mEq Na, 12 mEq K/100 g) or sodium-enriched (163.5 mEq Na, 30.8 mEq K/100 g) rabbit chow (Bioserv, Frenchtown, NJ). Tap water was available ad libitum from calibrated tubes. Rabbits were maintained on either low or high dietary sodium regimens for at least one week prior to study. Daily determinations were made of the following parameters: water intake, urine output, urinary and fecal Na and K excretion, and food intake. Water balance was calculated as the difference of water intake and urine output, assuming constant insensible loss. Sodium and potassium balances were calculated as the difference of dietary intake and the sum of urinary and fecal excretion. The electrolyte content of food and feces was determined by flame photometry after ashing in nitric acid.

Rabbits were brought to the laboratory once weekly for determination of the following variables: mean arterial pressure (MAP), heart rate (HR), body weight (B.Wt.), plasma Na (PNa) and K (PK) concentrations, plasma osmolality (POSM), hematocrit (HCT), plasma volume (PV), extracellular fluid volume (ECFV), blood urea nitrogen (BUN) and creatinine clearance (CCl). MAP and HR were determined in the conscious, lightly restrained rabbits by percutaneous needle puncture of a central ear artery as described in section A.3.c. PNA and PK were determined in triplicate by flame photometry. POSM was determined by the method of freezing point depression (Micro Osmette^{R}). HCT was determined in triplicate by microcentrifugation. PV and ECFV were estimated by determining the 10-min distribution space of Evans Blue dye and the 30-min distribution space of thiocyanate, respectively (Aikawa, 1950). Arterial blood samples (2 ml) were obtained immediately before, and at 10

and 30 min after intravenous injection of 1 ml of a solution containing 5 mg Evans Blue dye and 50 mg sodium thiocyanate. Blood samples were centrifuged, and the concentration of Evans Blue in the 10-min sample and of thiocyanate in the 30-min sample were determined spectrophotometrically against the plasma blank. Fluid volumes were calculated by dividing the concentration of indicator in the plasma by the amount injected intravenously, and subsequently expressed per kg body weight. Blood urea nitrogen was determined using a modified Urease-Berthelow reaction (Sigma Kit #660). Creatinine determinations (Jaffe method; Sigma Kit #555) were made on a plasma aliquot taken during the weekly measurements and on a urine sample taken from the previous 24 hr collection. Creatinine clearance was then calculated as UV/P where U=urine creatinine concentration, V = urine volume/24 hr, and P = plasma creatinine concentration. Baseline measurements of all above-mentioned parameters were made over a two-week period (i.e., 2 weekly determinations of MAP, HR, B.Wt., PNA, PK, POSM, HCT, PV, ECFV, BUN, CCl, and daily determinations of other fluid/electrolyte variables). After the second weekly determination of MAP, an osmotic minipump (Alzet Model 2002) containing either AII (6 mg/ml) or isotonic saline vehicle was implanted subcutaneously at the back of the neck under pentobarbital anesthesia. The minipump was connected to the ivt cannula by a short length of polyvinyl chloride tubing tunnelled subcutaneously. This minipump delivers solution at the rate of 0.5 µl/hr, therefore the nominal infusion rate of AII was 3 µg/hr. Four groups of rabbits were studied (n=5 in each group): AII ivt-high Na intake, saline ivt-high Na intake, AII ivt-low Na intake, and saline ivt-low Na intake. Intraventricular infusion was maintained for two weeks, during which time daily and

weekly measures were obtained. Minipumps were removed and the tubing plugged under local lidocaine anesthesia after the second set of weekly determinations. One week of recovery measures followed the ivt infusion period.

3. Chronic pharmacological blockade of brain AII receptors or brain converting enzyme

a. Chronic ivt saralasin

1) Dose determination. Rats were housed individually with ad lib access to distilled drinking water and standard rat chow. Chronic indwelling arterial and venous catheters were anchored at the neck with a small length of fine copper wire. A lateral cerebral ventricular cannula also was implanted. A control set of pressor responses to iv and ivt administration of AII were determined for each rat in the following manner. Basal MAP and HR were measured while the rat was conscious in its home cage. AII was then infused in successive doses of 10, 30, and 100 ng/min, iv. Each dose was infused for 5-10 min or until arterial pressure had stabilized. After the pressor responses to iv AII were determined, blood pressure was allowed to return to control levels. A 30-gauge cannula was connected to a Hamilton microsyringe by a long, flexible piece of tubing and the syringe and tubing were prefilled with a solution of AII (50 ng/μl). The 30-gauge cannula was introduced into the 23-gauge ivt cannula and a single bolus of AII (150 ng) was delivered into the lateral ventricle and the pressor response recorded. After this control set of pressor responses to iv and ivt AII was determined, rats were anesthetized with ketamine HCl (13 mg/kg, ip) and an osmotic minipump (Alzet Model 2001) filled with a solution of saralasin (6 or 12 mg/ml) in isotonic saline was implanted subcutaneously in

the scapular region. The minipump was connected to the 30-gauge ventricular cannula with a small length of polyvinyl chloride tubing that was tunnelled subcutaneously to the head. Saralasin was infused ivt continuously at doses of 6 μg/hr (n=5) or 12 μg/hr (n=5). Sham rats did not receive an ivt infusion. After 5 days of ivt saralasin infusion (or a 5-day sham period), pressor responses to ivt and iv AII infusion were retested in the same manner as the control measurements were made. Pressor responses to ivt AII were retested by removing the tubing leading to the minipump from the ventricular cannula and attaching the prefilled Hamilton microsyringe and tubing to the ivt cannula.

2) Effects in normal- and high-sodium rats. Rats with chronic indwelling arterial and venous catheters and a lateral cerebral ventricular cannula were housed individually in metabolic cages with ad lib access to standard lab chow. Sodium intake was controlled by the composition of a 40 ml/24 hr fluid infusion; rats maintained on normal sodium intake received dextrose and high sodium intake was achieved by iv infusion of isotonic saline. Four groups of rats were used in the experiment. Two groups were maintained on normal sodium intake and two groups were maintained on high sodium intake. Daily measurements of MAP, HR, urine output, and urinary sodium and potassium excretions were obtained. After two days of control measurements rats were briefly anesthetized with methohexital and osmotic minipumps were implanted subcutaneously and connected to the ivt cannula. Rats received ivt infusions of saralasin, 12 μg/hr (normal Na, n=5; high Na, n=7) or saline vehicle (normal Na, n=5; high Na, n=6) for 5 days, followed by two recovery days. Rats had ad lib access to distilled water from a

calibrated drinking tube only during the final 24 hr of the ivt infusion period.

3) Chronic iv saralasin. Rats were housed in metabolic cages and maintained on high sodium intake as described in the previous section. After 2 days of cardiovascular and fluid/electrolyte measurements, rats received saralasin (18 μg/hr, iv; n=5) for 7 days, followed by 2 recovery days. The saralasin was added directly to the 40 ml/24 hr iv saline infusion. Rats were not allowed to drink during the course of the experiment and received their fluid requirements from the intravenous infusion.

b. Chronic ivt sarthran

1) Dose determination: Sprague-Dawley. In an initial surgical procedure chronic indwelling polyvinyl chloride-silicone rubber arterial and venous catheters and a lateral cerebral ventricular cannula were implanted in male Sprague-Dawley rats (300-325 g). At least two days were allowed for recovery from the surgical procedures. Control pressor responses to intravenous and ivt AII were obtained in the following manner. Rats were briefly anesthetized with methohexital and loosely restrained in a tethered rat jacket. After measurement of basal mean arterial pressure in the conscious rats, AII was infused intravenously at successive doses of 10, 30, and 100 ng/min. Each dose of AII was infused for 5 min or until a steady-state arterial pressure was reached. After the iv pressor response curve was obtained, 10-15 min was allowed for recovery. A prefilled 30-gauge length of stainless steel tubing was then introduced into the ivt cannula so that its tip was at the end of the cannula. The 30-gauge steel tubing was connected to a Hamilton microsyringe by a long length of polyvinyl chloride

tubing. The maximal pressor response to a 150 ng ivt bolus injection of AII (5 μl volume) was then measured. After this set of control responses was obtained, a prefilled osmotic minipump (Alzet Model 2001) was implanted subcutaneously in the suprascapular region and connected to the 30-gauge steel tubing by a length of polyvinyl chloride tubing. This minipump delivers solution at a rate of 1 μl/hr. Minipumps were filled with solutions of sarthran in isotonic saline in varying concentrations to yield doses of 100 ng/hr (n=5), 300 ng/hr (n=6), 1 μg/hr (n=5) or 6 μg/hr (n=4). The ivt sarthran infusion was maintained for 5 days during which time rats were housed individually with ad lib access to food and water. The pressor responses to acute iv and ivt AII were tested after the 5-day ivt infusion period according to the above protocol.

2) Effects in high-sodium rats. A group of 8 rats was instrumented with indwelling arterial and venous catheters and a lateral cerebral ventricular cannula. Rats were housed individually in metabolic cages. A continuous iv infusion of isotonic saline (40 ml/24 hr) provided a sodium intake of 6.2 mEq/day, and was the sole source of fluid intake during the experiment. Low sodium rat chow (10-15 g/day; 0.002 mEq Na, 0.3 mEq K/g) was provided so that sodium intake could be fixed at the level provided by the iv saline infusion. This regimen maintained sodium intake at a level approximately 4-5 times normal, and fluid intake (40 ml/day) at a level comparable to that observed in freely-drinking rats (Halperin et al., 1981). A three-day recovery period followed the surgical procedures. The experimental period consisted of two control days, 5 days of ivt sarthran infusion, and two

post-infusion recovery days. During the experiment, the following cardiovascular and fluid/electrolyte measures were obtained daily: MAP, HR, $U_{Na}V$, U_KV, and UO. After MAP and HR were determined on the second control day, rats were briefly anesthetized with methohexital and pre-filled osmotic minipumps (Alzet Model 2001) were implanted subcutaneously in the suprascapular region and connected to the tubing leading to the ivt cannula. Minipumps were removed in a similar manner after measurements had been obtained on the fifth day of ivt infusion. Minipumps were filled with a solution of sarthran (1 mg/ml in isotonic saline), thus the dose of sarthran infused was 1 μg/hr. Correct placement of the ivt cannula was verified at the end of the experiment by injecting Evans Blue dye into the cannula and visualization of dye in the ventricular system after the brain had been cut in the frontal plane. Rats were included in the study only if dye could be visualized in the right and left lateral and third cerebral ventricles.

3) Dose determination: Spontaneously hypertensive rats. Male spontaneously hypertensive rats (SHR) were surgically prepared with chronic indwelling arterial and venous catheters and bilateral ivt cannulae. The day prior to the start of the experiment, correct location of ivt cannulae was verified by measuring the drinking latency to an ivt injection of 150 ng AII. The criterion for acceptable cannula placement was a drinking latency of less than 3 min. The following day, MAP was recorded in the conscious rats while they were loosely restrained in a tethered rat jacket. After a stable blood pressure was recorded, AII was infused iv at successive doses of 10, 30, and 100 ng/min. Each dose was infused for 5-10 min or until a steady-state blood pressure was achieved. Blood pressure was allowed to return

to control levels after termination of iv AII infusion (approximately 10 min). For the purpose of ivt injection of AII, a Hamilton microsyringe was connected to the left lateral ventricular cannula by a long length of polyvinyl chloride tubing. The tubing and syringe were prefilled with a solution of AII and a bolus ivt injection of AII (150 ng) was administered in a 5 μl volume over a 2-3 sec period. Changes in MAP in response to iv infusion and ivt injection of AII were calculated as the difference of the peak steady-state MAP and the resting or pre-injection MAP. After this control set of pressor responses to peripheral and central administration of AII was obtained, a prefilled osmotic minipump (Alzet Model 2001) was implanted subcutaneously under methohexital anesthesia and connected to the right lateral ventricular cannula by polyvinyl chloride tubing. The tubing and right ivt cannula were covered with dental acrylic, leaving the left cannula accessible. Two groups of rats were used in this experiment: one group received sarthran, 1 μg/hr ivt (n=5), and a second group received sarthran, 6 μg/hr ivt (n=6). The vehicle for all infusions was isotonic saline. Intraventricular infusions were maintained for 5 days, and on the fifth day, pressor responses to iv and ivt AII administration were retested in the were then removed, and after a 2-day recovery period, pressor responses to iv and ivt AII were tested a third time.

c. Chronic ivt teprotide

1) Dose determination: Sprague-Dawley. Male Sprague-Dawley rats were instrumented with chronic indwelling arterial and venous catheters and a lateral cerebral ventricular cannula. Changes in MAP in response to successive intravenous infusions of AI (3, 10, 30,

100 ng/min) were recorded in conscious rats. Each dose was infused for 5-10 min or until a steady-state arterial pressure was reached. After the iv pressor response curve was obtained, 10-15 min was allowed for recovery. A prefilled 30-gauge length of stainless steel tubing was then introduced into the ivt cannula so that its tip was at the end of the cannula. The 30-gauge steel tubing was connected to a Hamilton microsyringe by a long length of polyvinyl chloride tubing. The maximal pressor response to a 100 ng ivt bolus injection of AI (5 μl volume) was then measured. After this set of control repsonses was obtained, a prefilled osmotic minipump (Alzet Model 2001) was implanted subcutaneously in the suprascapular region and connected to the 30-gauge steel tubing by a length of polyvinyl chloride tubing. This minipump delivers solution at a rate of 1 μl/hr. Minipumps were filled with solutions of teprotide in isotonic saline in varying concentrations to yield doses of 1 (n=4), 3 (n=3) and 10 μg/hr (n=5). The ivt teprotide infusion was maintained for 5 days during which time rats were housed individually with ad lib access to food and water. The pressor responses to acute iv and ivt AI were retested after 5-day ivt infusion period according to the above protocol.

2) Dose determination: Spontaneously hypertensive rats. The protocol for this experiment was similar to that described for sarthran dose determination in SHR (Section 3.b.3.), except that pressor responses to iv AI (10, 30, 100 ng/min) and ivt AI (100 ng) were measured. These responses to peripheral and central AI administration were measured prior to, on the fifth day of, and 2 days after a 5-day ivt teprotide infusion (10 μg/hr). The pressor response to ivt AII (150 ng) also was determined 30 min after the ivt AI injection was given.

d. Chronic ivt sarthran/chronic iv angiotensin II

Rats used in this experiment were surgically prepared with indwelling arterial and venous catheters and a lateral cerebral ventricular cannula and housed individually in metabolic cages. A minipump (Alzet Model 2002) containing sarthran (2 mg/ml) or isotonic saline was implanted subcutaneously and connected to the ivt cannula at the time of the initial surgery. These minipumps deliver solution at the rate of 0.5 µl/hr for a 14-day period, therefore sarthran was infused at a dose of 1 µg/hr ivt. Rats were maintained on a high sodium intake by continuous iv saline infusion (40 ml/day) and were given 12-15 g of low sodium rat chow per day. After three recovery days, daily measurements of MAP, HR, UO, and urinary sodium and potassium excretions were begun. The experimental period consisted of two control days, 5 days of continuous iv AII infusion, and finally two recovery days after the iv AII infusion was stopped. The ivt infusion of sarthran or saline was maintained throughout the entire experimental protocol (i.e., during the 3-day post-surgery recovery period, and the control, iv AII-infusion, and recovery days). Four groups of rats (n=8 in each group) were used in this experiment: two groups received iv AII infusions at a dose of 10 ng/min for 5 days and two groups received AII at 20 ng/min, iv. At each dose level of iv AII, one group of rats received sarthran 1 µg/hr ivt and the other group received isotonic saline ivt as a control. Correct ivt cannula placement was verified at the end of the experiment by injection of Evans Blue dye in the cannula and visualization of the dye in the ventricular system.

e. Chronic ivt sarthran/DOC-salt hypertension

Right nephrectomy was performed in male Sprague-Dawley rats by a retroperitoneal approach under pentobarbital anesthesia 2 weeks prior to study. Rats were provided with 0.9% saline drinking fluid and standard rat chow ad lib for the duration of the experiment. Two control blood pressure measurements were obtained (tail cuff plethysmography), after which a lateral cerebral ventricular cannula was implanted and an ivt infusion of saline (n=10) or sarthran (1 μg/hr, n=6) was begun. Two days after the start of ivt infusion, DOC (deoxycorticosterone, Percorten pivalate, CIBA) was administered at a dose of 50 mg/kg, sc. This dose of DOC was administered once weekly for the remainder of the study. Blood pressure and body weight were measured twice weekly for 4 weeks, starting on the fourth day after the first DOC injection. Spent minipumps (Alzet Model 2002) were replaced with freshly-filled pumps 14 days after they were implanted. After the final tail cuff blood pressure measurement, femoral arterial and venous catheters were implanted under pentobarbital anesthesia. Two days were allowed for recovery from surgery, at which time MAP and HR were measured in the conscious rats while they were loosely restrained in a rat jacket. Changes in MAP and HR were determined at 1 and 5 min after the following interventions: 1) AVP antagonist, 10 μg/kg, iv; and 2) hexamethonium, 20 mg/kg, iv.

f. Chronic ivt sarthran/spontaneously hypertensive rats

SHR used in this experiment underwent an initial surgical procedure for placement of a cannula in the right lateral cerebral ventricle. Rats were housed individually and had ad lib access to

standard rat chow and distilled water. Blood pressure was measured indirectly by a tail cuff plethysmographic method in conscious, restrained rats. Control blood pressure measurements were made on days 1 and 3 of the experiment. On day 6, an osmotic minipump (Alzet Model 2002) containing sarthran (2 mg/ml; n=7) or isotonic saline vehicle (n=9) was implanted subcutaneously and connected to the ivt cannula. Since this minipump delivers solution at the rate of 0.5 µl/hr, the dose of sarthran infused ivt was 1 µg/hr. This infusion was maintained for 14 days, and blood pressure was measured on the second, fourth, ninth, and eleventh days of ivt sarthran infusion. On the fourteenth day of ivt infusion, the spent minipumps were removed under ether anesthesia and replaced with a minipump (Alzet Model 2001) filled with sarthran (6 mg/ml) or isotonic saline. The ivt sarthran infusion was continued for another five days at a dose of 6 µg/hr. After minipump removal, 2 recovery blood pressure measurements were obtained.

g. Chronic ivt teprotide/spontaneously hypertensive rats

SHR were prepared as described in the previous section for chronic ivt sarthran infusion. Blood pressure was measured by tail cuff plethysmography. Two control blood pressure measurements were obtained on days 1 and 4 of the experiment. On day 6, a prefilled osomotic minipump (Alzet Model 2001) containing teprotide (10 mg/ml, n=10) or isotonic saline vehicle (n=15) was implanted subcutaneously and connected to the ivt cannula. Blood pressure measurements were obtained on days 7, 9, 11, and 13 during ivt teprotide or saline infusion. Minipumps were removed on day 14 under ether anesthesia, and one recovery blood pressure determination was obtained on day 18.

4. Brain lesion studies: chronic intravenous AII infusion

a. Chronic intravenous AII infusion protocol

Male Sprague-Dawley rats (300-400 g) were housed individually in metabolic cages after surgery for implantation of arterial and venous catheters. A sodium deficient rat chow was provided (12-15 g/day; 0.002 mEq Na, 0.3 mEq K per g) so that sodium intake could be strictly controlled at 6.2 mEq/day by iv infusion of 40 ml isotonic saline/24 hr. Distilled water was available ad lib from calibrated drinking tubes. After three recovery days, the experimental protocol was begun. This consisted of 2 control days, five days during which AII was infused continuously iv at a rate of 10 ng/min, then two post-infusion recovery days. Daily measurements of MAP, HR, WI, UO, $U_{Na}V$, and U_KV were obtained.

b. Subfornical organ lesion

Three groups of rats were studied in the preceding chronic iv AII infusion protocol. The first group (n=7) received only saline during the 5-day "hormone infusion" period. The second group of rats (n=8) received AII (10 ng/min) during the same 5-day infusion period. A third group (n=9) was subjected to electrolytic destruction of the SFO 2-4 weeks prior to a 5-day infusion of AII (10 ng/min).

Electrolytic ablation of the SFO was accomplished in the following manner. Rats were anesthetized with a pentobarbital-chloral hydrate mixture and immobilized in a Kopf stereotaxic instrument. A trephine hole was drilled in the skull dorsal to the lesion site and a 30-gauge Teflon-insulated monopolar tungsten electrode was lowered to the lesion site. Because the anterior stalk of the SFO is quite ventral to the posterior stalk, three electrode penetrations were required. A

total of 21 millicoulombs (1 mA for 21 sec) of anodal current was passed, with 7 mC passed per penetration. All penetrations were made in the midline after the superior sagittal sinus had been retracted. The first penetration was to 5.0 mm ventral to the dura, and 0.3 mm posterior to bregma. Each successive penetration was made to a point 0.3 mm posterior and 0.2 mm dorsal to the preceding one. Following surgery, each rat received 100,000 IU of procaine penicillin (im) and was returned to its home cage.

Lesions were produced in 22 rats by Dr. Michael Mangiapane, University of Rochester, following which the animals were sent to our laboratory for AII infusion. At the end of the infusion protocol, the brain of each rat was perfused with buffered formalin, coded, and returned to Dr. Mangiapane for a blind histological analysis of lesion size and location. Frozen sections (35 microns) were cut through the lesion site and stained with cresyl violet. The sections were then examined carefully in a light microscope to determine the location of the lesion, and the examiner had no knowledge of the data for any particular animal. Only after confirmation that the lesion destroyed greater than 80% of the SFO was a given rat included in the "SFO lesion" group. Sixteen rats completed the AII infusion protocol, and nine of these had greater than 80% destruction of the SFO. These nine were included in the analysis. None of these had damage to the median preoptic nucleus. As is typical of SFO lesions (Mangiapane et al., 1984), all sustained a minor degree of damage to the fornical commissure, triangular septal nucleus, and paraventricular nucleus of the thalamus.

b. Knife cut of SFO efferents

A knife cut of SFO efferents was performed in rats by Dr. R.W. Lind according to a previously published method (Lind and Johnson, 1982). Briefly, under ether anesthesia and using stereotaxic guidance, a knife cut of SFO efferents was made with a rotating Knigge wire knife. In sham knife-cut rats, the knife was lowered into the brain, but the blade of the knife was not extruded. Rats were then shipped to our laboratory for performance of the chronic iv AII infusion protocol (10 ng/min, iv for 5 days, see Section 4.a.). On the first control day, MAP and HR changes were determined to acute iv AII infusion (10, 30, and 100 ng/min).

After the chronic iv AII infusion protocol was completed, the brain of each rat was perfused with buffered formalin, coded, and returned to Dr. Lind for blind histological analysis. Brains were embedded in an albumin/gelatin matrix, frozen, and 40 micron sagittal sections were cut. Cresyl violet-stained sections were examined light microscopically to determine position of the knife cut. Five rats completed the iv AII infusion protocol and were found to have knife cuts immediately ventral to the SFO; these rats comprised the "knife cut" group. As described above, sham rats (n=5) underwent surgery but the knife blade was not rotated.

c. Median preoptic nucleus lesion

Chronic iv AII infusion was performed according to the above protocol (Section 4.a.) in two groups of rats: 1) a sham group (n=12), and 2) a gropu which had undergone electrolytic destruction of

the median preoptic nucleus (MnPO) approximately three weeks prior to study (n=5).

Electrolytic destruction of the MnPO was performed by Dr. Michael Mangiapane, University of Rochester, in the following manner. Rats were anesthetized with a pentobarbital-chloral hydrate mixture and immobilized in a Kopf stereotaxic apparatus. The skull was leveled and a hole was drilled in the skull 0.1 mm posterior to bregma. After retraction of the superior sagittal sinus, a monopolar Teflon-coated tungsten electrode (225 micron diameter) was lowered 6.7 mm ventral to the dura on the midline. The MnPO was lesioned by passing 1 mA of anodal current for 15 sec. Following surgery, each rat received 100,000 IU of procaine penicillin (im) and was returned to its home cage. Rats were then shipped to our laboratory for iv AII infusion. After the iv AII infusion protocol was completed, the brain of each rat was perfused with 10% formalin, coded, and returned to Dr. Mangiapane for a blind histological analysis of lesion size and location. Lesion extent was determined by light microscopic examination of cresyl violet-stained sections through the MnPO. The criterion for inclusion of a rat in the "MnPO lesion" group was greater than 90% destruction of the MnPO (ventral to the anterior commissure) with no OVLT damage. Five rats met this criterion and thus were included in the MnPO lesion group. The sham group (n=12) consisted of rats which underwent lesion surgery but no damage to the MnPO or surrounding structures was observed upon histological examination, and rats that had no sham surgery.

RESULTS

A. Chronic ivt AII Infusion: Rat

1. Sodium dependency

In rats maintained on a high sodium intake with free access to drinking water, ivt infusion of AII for 5 days resulted in dose-dependent increases in MAP (Figure 1). Elevations in MAP were not associated with any consistent changes in HR. Rats receiving the lower dose of AII (1 μg/hr) exhibited 3 days of natriuresis, whereas this effect was not seen in rats receiving the higher dose of AII (6 μg/hr). Although WI increased significantly in rats receiving the low dose of AII, and tended to increase in rats receiving the high dose of AII, water balance did not change significantly over the course of the experiment. Similar changes in MAP in response to ivt AII infusion were observed in rats whose fluid intake was restricted to the 40 ml/day provided by the iv infusion (Figure 2). Again, there were no consistent changes in HR or $U_{Na}V$. Urine output remained constant except on the fifth day of ivt infusion, when rats were allowed to drink. The volume of water ingested over 24 hr was significantly greater in rats receiving AII 6 μg/hr ivt than in saline controls ($p<0.05$, rank sum test).

The effect of sodium intake on cardiovascular and fluid and electrolyte responses to chronic ivt AII are depicted in Figure 3 (1 μg/hr) and Figure 4 (6 μg/hr). The magnitude of the ivt AII-induced

Figure 1. Effect of chronic ivt AII infusion on cardiovascular and fluid/electrolyte parameters in the rat. MAP = mean arterial pressure, HR = heart rate, UNA = urinary sodium excretion, WI = water intake, WB = water balance. Points are mean responses in groups of 6 rats maintained on 7.5 mEq Na/day. C1 and C2 are 2 control days, A1-A5 are 5 days of ivt infusion (shaded area), R1 and R2 are recovery days. Vertical bars on C2 value represent standard errors for within groups comparisons. Asterisk (*) represents significant ($p<0.05$) difference from the average of C1 and C2 values (randomized block ANOVA and lsd).

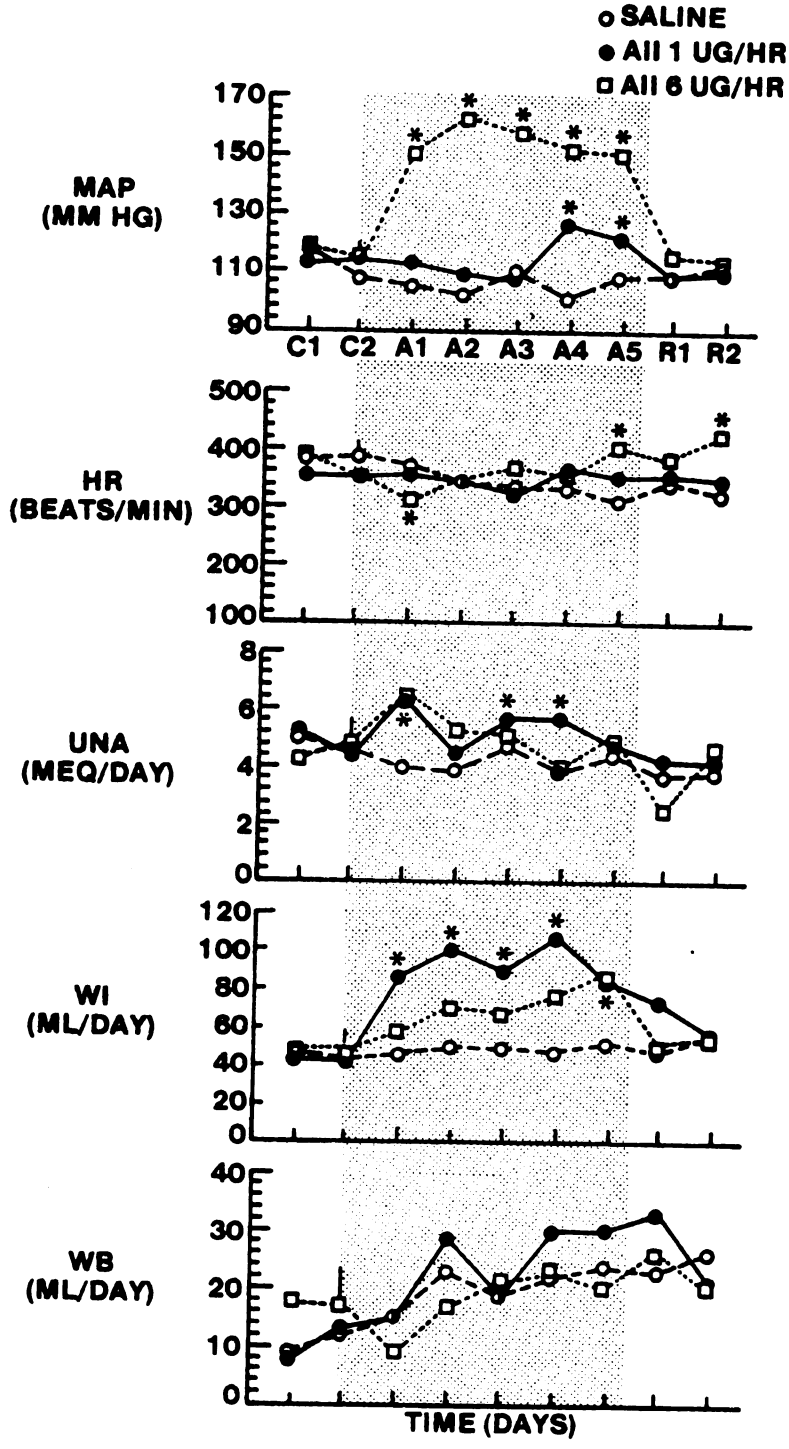
o SALINE
● AII 1 UG/HR
□ AII 6 UG/HR
MAP (MM HG)
170
150
130
110
90
C1 C2 A1 A2 A3 A4 A5 R1 R2
HR (BEATS/MIN)
500
400
300
200
100
UNA (MEQ/DAY)
8
6
4
2
0
WI (ML/DAY)
120
100
80
60
40
20
0
WB (ML/DAY)
40
30
20
10
0
TIME (DAYS)

Figure 1

Figure 2. Chronic ivt AII infusion in rats on restricted fluid intake. Rats were maintained on 7.5 mEq Na/day and allowed access to drinking water only during the final 24 hr of ivt infusion. Vertical bars represent average SEM for within groups comparisons. Units of the abcissa are days. The volume of water ingested during the final 24 hr of ivt infusion was as follows: AII 6 ug/hr ivt: 92 $\pm$ 11 ml, AII 1 ug/hr ivt: 79 $\pm$ 30 ml, saline ivt: 44 $\pm$ 13 ml. Asterisk (*) represents significant (p 0.05) difference from the average of C1 and C2 values (randomized block ANOVA and lsd). Other symbols as in Figure 1.

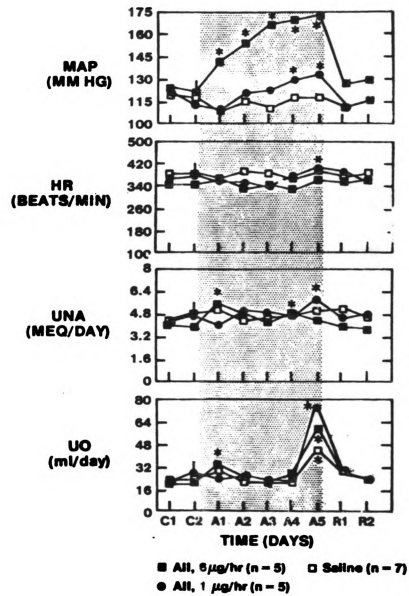
MAP
(MM HG)
175
160
145
130
115
100
HR
(BEATS/MIN)
500
420
340
260
180
100
UNA
(MEQ/DAY)
8
6.4
4.8
3.2
1.6
0
UO
(ml/day)
80
64
48
32
16
0
C1 C2 A1 A2 A3 A4 A5 R1 R2
TIME (DAYS)
■ AII, 6 μg/hr (n = 5)
□ Saline (n = 7)
● AII, 1 μg/hr (n = 5)

Figure 2

Figure 3. Cardiovascular and fluid/electrolyte responses to chronic ivt AII infusion (1 μg/hr) in rats maintained on normal and high sodium intake. Values represent mean responses in groups of 6 rats. Vertical bars on C2 point represent SEM for individual within groups comparisons. Asterisk (*) represents significant ($p<0.05$) difference from average of C1 and C2 values (mixed design ANOVA and lsd). Other symbols as in Figure 1.

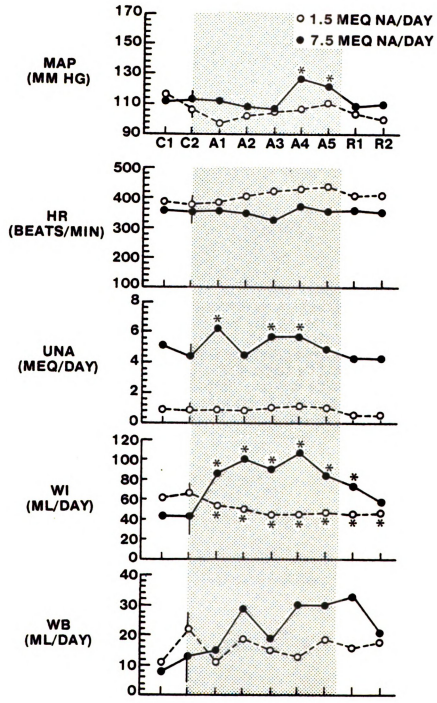
1.5 MEQ NA/DAY
7.5 MEQ NA/DAY
MAP (MM HG)
170
150
130
110
90
C1 C2 A1 A2 A3 A4 A5 R1 R2
HR (BEATS/MIN)
500
400
300
200
100
UNA (MEQ/DAY)
8
6
4
2
0
WI (ML/DAY)
120
100
80
60
40
20
0
WB (ML/DAY)
40
30
20
10
0

Figure 3

Figure 4. Cardiovascular and fluid/electrolyte responses to chronic ivt AII infusion (6 μg/hr) in rats maintained on normal and high sodium intake. Points represent mean responses in groups of 5 (normal sodium) and 6 (high sodium) rats. Units of the abscissa are days. Vertical bars on C2 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values. Cross (+) indicates significant difference between high sodium and normal sodium groups (mixed design ANOVA and lsd). Other symbols as in Figure 1.

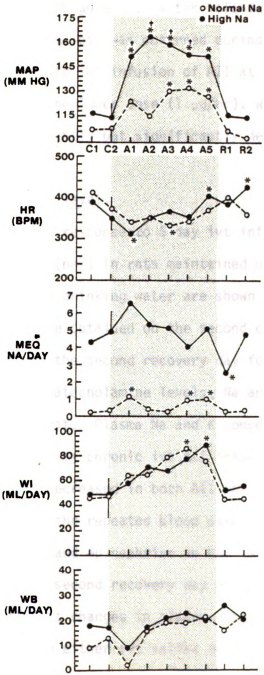
Normal Na
High Na
MAP (MM HG)
HR (BPM)
MEQ NA/DAY
WI (ML/DAY)
WB (ML/DAY)
C1 C2 A1 A2 A3 A4 A5 R1 R2

Figure 4

increase in arterial pressure was significantly greater in high sodium rats than in normal sodium rats, regardless fo the dose of AII infused. This difference was especially pronounced in rats receiving the 6 μg/hr dose of AII (Figure 4). Water balance remained unchanged and a tendency for sodium loss was observed during ivt AII infusion. The dipsogenic response to ivt infusion of AII at 6 μg/hr was identical to ivt AII infusion at the lower dose (1 μg/hr), WI was increased in high sodium rats and slightly but significantly decreased in normal sodium rats (Figure 3).

2. Plasma hormone levels

The MAP and HR response to 5-day ivt infusion of AII (6 μg/hr, n=6) or isotonic saline (n=7) in rats maintained on a high sodium intake with free access to drinking water are shown in Figure 5. Arterial blood samples were obtained on the second control day, the fifth day of ivt infusion and the second recovery day for determination of plasma aldosterone and catecholamine levels, Na and K concentrations, osmolality, and hematocrit. Plasma Na and K concentrations and osmolality were not affected by chronic ivt AII infusion (Figure 6). Hematocrit and body weight decreased in both AII and saline-treated rats, probably due in part to the repeated blood sampling. Plasma concentrations of norepinephrine and epinephrine on the second control day, fifth day of ivt infusion and second recovery day are reported in Table 1. There were no significant changes in plasma norepinephrine or epinephrine in rats receiving either ivt saline or AII when compared to control levels. Plasma aldosterone was significantly elevated on the fifth day of ivt AII infusion when compared to control levels (Table 2).

Figure 5. Cardiovascular response to chronic ivt AII infusion in rats used for plasma hormone determination. Rats received either AII, 6 μg/hr ivt (n=6) or saline ivt (n=7) and were maintained on high sodium intake. Vertical bars on C1 value indicate SEM for individual within groups comparisons. Asterisk (*) represents significant ($p<0.05$) difference from average of C1 and C2 values and cross (+) represents significant difference between AII- and saline-treated rats (mixed design ANOVA and lsd). Other symbols as in Figure 1.

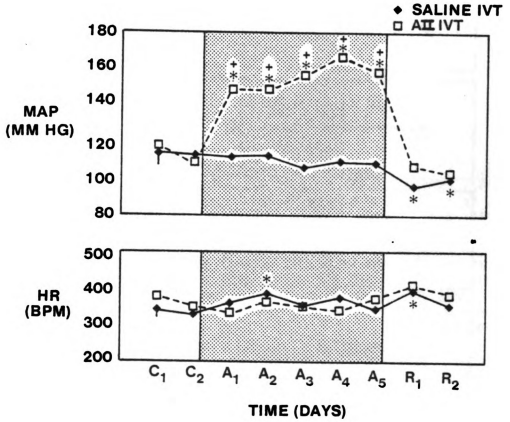
SALINE IVT
AII IVT
MAP
(MM HG)
180
160
140
120
100
80
HR
(BPM)
500
400
300
200
C1 C2 A1 A2 A3 A4 A5 R1 R2
TIME (DAYS)

Figure 5

Figure 6. Effect of chronic ivt AII infusion on plasma electrolytes, osmolality, hematocrit and body weight. P_{Na} = plasma sodium concentration, P_K = potassium concentration, P_{OSM} = plasma osmolality, HCT = hematocrit, B.WT. = body weight. This data was obtained from rats whose MAP and HR responses to ivt AII (n=6) and saline (n=7) are depicted in Figure 5. C2 is the second control day, A5 is the fifth day of ivt infusion and R2 is the second recovery day. Vertical bars on C2 value represent SEM for within groups comparisons. Asterisk (*) denotes significant ($p<0.05$) difference from C2 value and cross (+) indicates significant difference between saline and AII rats (mixed design ANOVA and lsd).

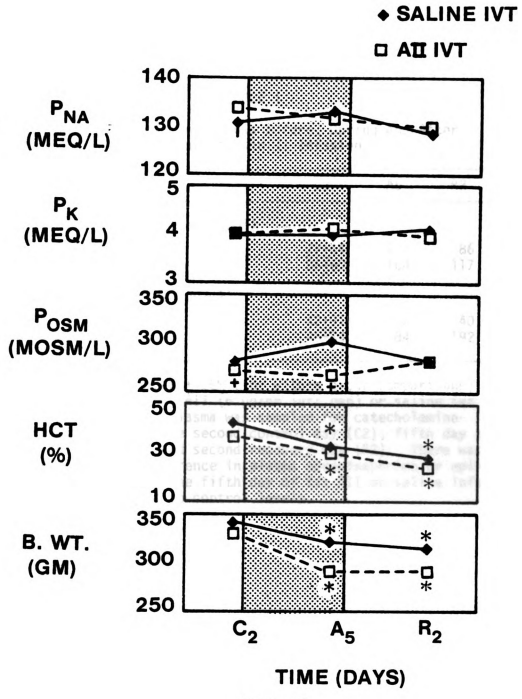
◆ SALINE IVT
□ AII IVT
P_{NA} (MEQ/L)
140
130
120
P_K (MEQ/L)
5
4
3
P_{OSM} (MOSM/L)
350
300
250
HCT (%)
50
30
10
B. WT. (GM)
350
300
250
C_2
A_5
R_2
TIME (DAYS)

Figure 6

TABLE 1

Plasma Catecholamine Levels Before, During and After ivt Angiotensin II Infusion

	C2	A5	R2
Plasma Norepinephrine (pg/ml)			
AII ivt	77±64	101	86
saline ivt	86±60	164	117
Plasma Epinephrine (pg/ml)			
AII ivt	42±91	92	40
saline ivt	45±84	84	192

Values are mean + SEM (for within group comparisons). Rats received either AII (6 μg/hr ivt; n=6) or saline ivt (n=7) for 5 days. Plasma was sampled for catecholamine determinations on the second control day (C2), fifth day of ivt infusion (A5) and second recovery day (R2). There was no significant difference in plasma norepinephrine or epinephrine levels on the fifth day of ivt AII or saline infusion when compared to control levels.

TABLE 2

Plasma Aldosterone Levels Before, During, and After ivt Angiotensin II Infusion

	C2	A5	R2
Plasma Aldosterone (ng/dl)			
AII ivt	17.7±29.0	137.8*	49.1
saline ivt	24.6± 9.1	21.8	34.2

Values are mean ± SEM (within groups). Rats received either AII 6 µg/hr ivt (n=6) or saline ivt (n=7) for 5 days. Plasma was sampled for aldosterone determinations on the second control day (C2), fifth day of ivt infusion (A5) and second recovery day (R2). Asterisk (*) indicates a significant difference from C2 value ($p<0.05$).

Within 2 days after termination of ivt AII infusion, plasma aldosterone returned to pre-infusion levels. Plasma aldosterone did not change in response to ivt infusion of isotonic saline. Plasma AII levels were measured on the second control day and fifth day of ivt AII infusion in a separate group of 12 rats (Figure 7). The rats were divided into two groups depending on their blood pressure response to the ivt AII infusion. Rats in the hypertensive group (average MAP = 151 mmHg; n=7) were found to have ventricular cannulae located in the lateral ventricular space upon post-mortem exam, and thus were receiving the AII infusion directly into cerebrospinal fluid. Normotensive rats (average MAP = 123 mmHg; n=5) had ventricular cannulae tips located in periventricular brain tissue. Therefore, this group of rats did not become hypertensive in response to AII infusion. Regardless of whether AII was infused directly into cerebrospinal fluid or into periventricular brain tissue, plasma AII levels were not significantly elevated on the fifth day of AII infusion when compared to control levels.

3. Adrenalectomy

The effect of adrenalectomy on the response to chronic ivt AII infusion is depicted in Figure 8. The increase in blood pressure seen in response to a 7-day ivt AII infusion was the same in adrenalectomized and intact rats. Blood pressure was still elevated two days after termination of ivt AII infusion in both groups, but returned to control levels by the fourth day after ivt AII was stopped. Saline intake increased in both groups during the period of ivt AII infusion. Body weight was stable in intact rats over the course of the experiment whereas body weight fell in adrenalectomized rats. Plasma aldosterone was undetectable in adrenalectomized rats, and there was no elevation

Figure 7. Plasma AII concentration in rats receiving chronic ivt AII infusion. C2 = second control day; A5 = fifth day of ivt AII (6 μg/hr) infusion. The hypertensive group (n=7) had ivt cannulae located in the ventricular space and the normotensive group (n=5) had cannulae located outside the ventricle. Numbers below data points are means ± SEM. Mixed design ANOVA followed by lsd was performed on the log transform of the data. There were no significant ($p<0.05$) differences between plasma AII concentrations measured on C2 and A5 in either group.

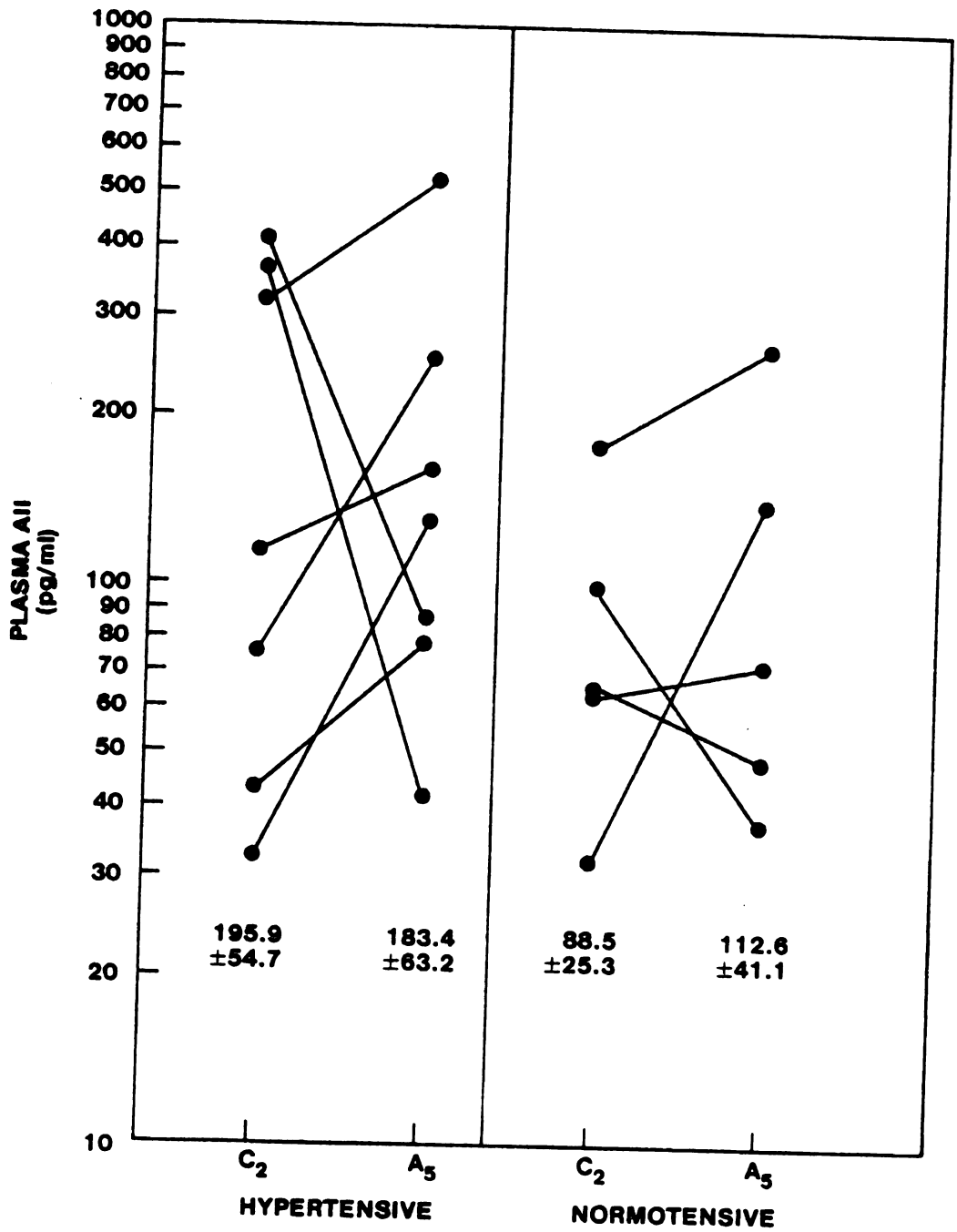
PLASMA AII
(pg/ml)
1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
195.9
±54.7
183.4
±63.2
88.5
±25.3
112.6
±41.1
C2
A5
C2
A5
HYPERTENSIVE
NORMOTENSIVE

Figure 7

Figure 8. Effect of adrenalectomy on the hypertensive response to chronic ivt AII infusion. Adrenalectomized (ADX, n=7) and control (n=4) rats received ivt infusions of AII (6 μg/hr) for the 7-day period denoted by shading. Asterisk (*) represents significant ($p<0.05$) difference from average of all pre-infusion values (mixed design ANOVA and lsd).

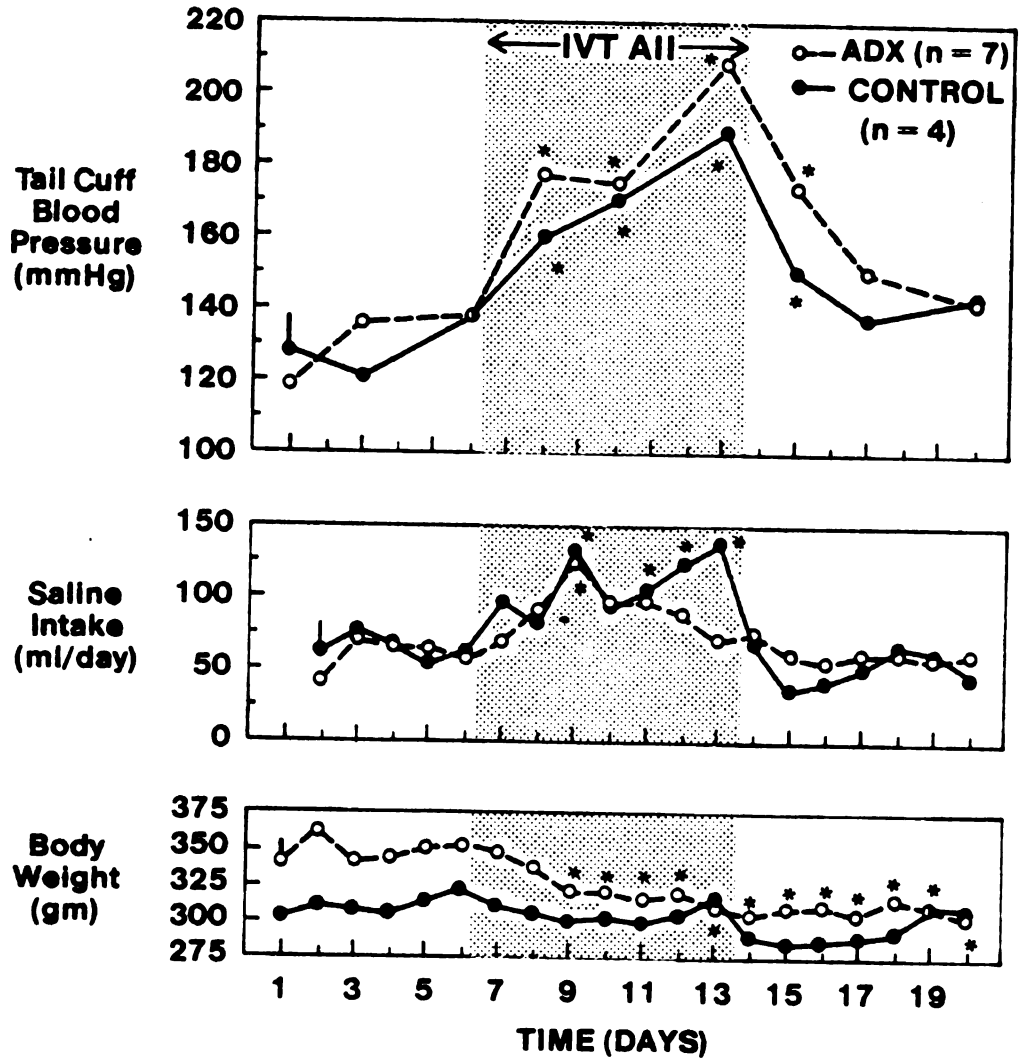
220
200
180
160
140
120
100
IVT AII
ADX (n = 7)
CONTROL
(n = 4)
Tail Cuff
Blood
Pressure
(mmHg)
150
100
50
0
Saline
Intake
(ml/day)
375
350
325
300
275
Body
Weight
(gm)
1
3
5
7
9
11
13
15
17
19
TIME (DAYS)

Figure 8

in plasma aldosterone in intact rats on the final day of ivt AII infusion when compared to levels measured one week after the end of ivt infusion.

4. Acute blockade of vascular AII receptors and ganglionic blockade

Cardiovascular and fluid/electrolyte responses to a 5-day ivt infusion of AII are depicted in Figure 9. A sustained increase in MAP was observed throughout the ivt infusion period. Blood pressure returned to control levels within 24 hr after the infusion was stopped. Heart rate was not altered by ivt AII infusion, although a significant tachycardia occurred on the 2 post-infusion recovery days. Urinary sodium and potassium excretions were not consistently changed by ivt AII infusion. Since sodium intake was held at a fixed level (6.2 mEq/day), sodium balance (assuming constant fecal losses) did not change in response to ivt infusion of AII. Water intake slowly increased during the period of ivt AII infusion, reaching a statistically significant elevation on day 3 of the infusion. Water intake dropped to control levels on the first day after stopping the ivt infusion. Water balance remained unchanged throughout the experiment.

The response of the group of rats shown in Figure 9 to blockade of the direct vasoconstrictor effects of AII is depicted in Figure 10. During the control period, acute iv infusion of saralasin (300 ng/min for 10 min) produced a small increase in MAP and a decrease in HR. When saralasin was administered on the first, third, and fifth days of ivt AII-induced hypertension, a moderate decrease in MAP occurred, associated with tachycardia. The decreases in MAP and increases in HR produced by iv saralasin during the ivt AII infusion period were

Figure 9. Cardiovascular and fluid/electrolyte response to chronic ivt AII in rats that were subject to acute saralasin and hexamethonium treatment. Rats were maintained on a high sodium intake and received AII, 6 μg/hr ivt for 5 days (n=6). MAP = mean arterial pressure, HR = heart rate. Units of the abscissa are days. C1 and C2 are control days, A1-A5 are 5 days of ivt infusion, R1 and R2 are recovery days. Vertical bars on C2 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (randomized block ANOVA and lsd).

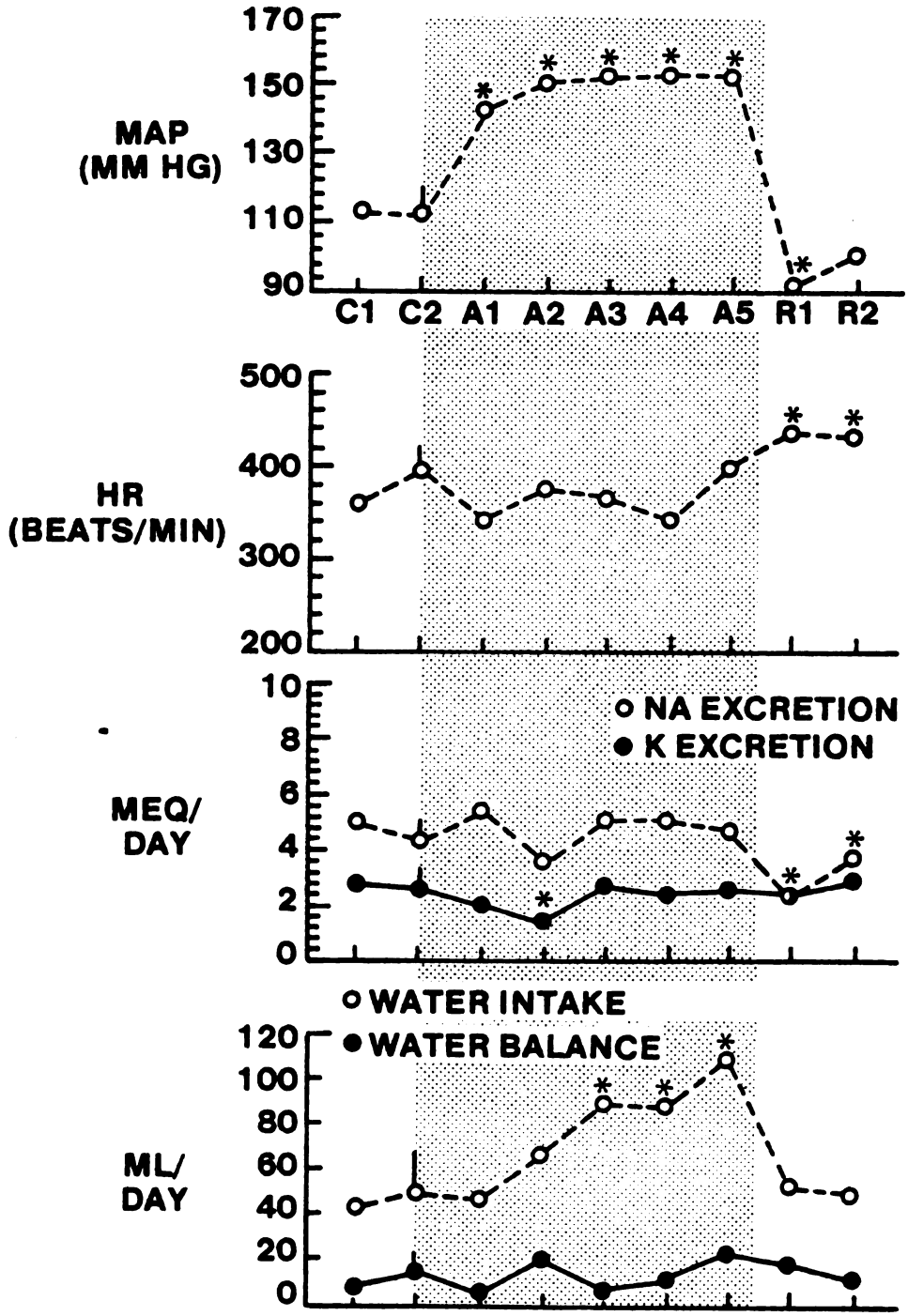
MAP
(MM HG)
170
150
130
110
90
C1 C2 A1 A2 A3 A4 A5 R1 R2
HR
(BEATS/MIN)
500
400
300
200
MEQ/
DAY
10
8
6
4
2
0
NA EXCRETION
K EXCRETION
WATER INTAKE
WATER BALANCE
ML/
DAY
120
100
80
60
40
20
0

Figure 9

Figure 10. Change in mean arterial pressure and heart rate in response to acute iv saralasin infusion before, during, and after chronic ivt AII infusion. C2 is the second control day; A1, A3, and A5 are the first, third and fifth days of ivt AII infusion; R2 is the second recovery day. Saralasin was infused at the rate of 300 ng/min for 10 min. Data was obtained from rats (n=6) whose cardiovascular and fluid/electrolyte response to chronic ivt AII is depicted in Figure 9. Vertical lines on C2 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant difference from C2 value (randomized block ANOVA and lsd).

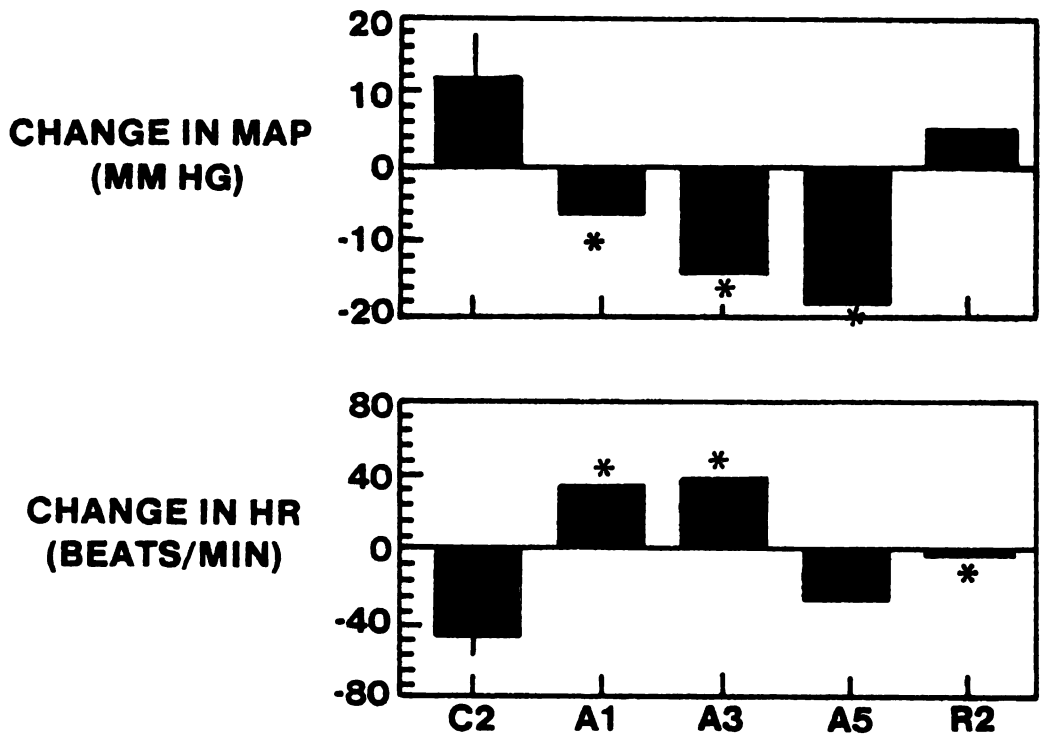
CHANGE IN MAP
(MM HG)
20
10
0
-10
-20
CHANGE IN HR
(BEATS/MIN)
80
40
0
-40
-80
C2
A1
A3
A5
R2

Figure 10

significantly different from the response to saralasin observed in the control period. By the second recovery day, the MAP response to iv saralasin was not significantly different from the control response. Acute ganglionic blockade with hexamethonium produced a decrease in MAP and HR during the control period (Figure 11). Hexamethonium administration on the first, third, and fifth days of ivt AII infusion resulted in a greater depressor response than that observed during the control period, but this difference was not statistically significant.

5. Acute blockade of vascular AVP receptors

The change in MAP and HR in response to acute iv administration of the vascular AVP receptor antagonist (1-(β-mercapto-β,β cyclopentamethylene propionic acid), 2-(o-methyl)tyrosine) arginine-8-vasopressin, 10 μg/kg) is depicted in Figure 12. Blockade of the vasoconstrictor actions of blood-borne AVP on the second control day did not effect MAP or HR. Similarly, administration of AVP antagonist on the first, third or fifth day of ivt AII infusion or the second post-infusion recovery day resulted in no change in MAP or HR. Shown in Figure 13 is the effect of AVP antagonist (10 μg/kg, iv) on the MAP and HR responses to exogenous AVP infusion. At 2 hours after administration of the AVP antagonist, exogenous AVP infusion produced negligible changes in MAP and HR when compared to control responses (blocked factorial ANOVA followed by lsd).

6. Peripheral sympathectomy

The role of the peripheral sympathetic nervous system in the hypertension produced by a 7-day ivt AII infusion was assessed in rats which had undergone neonatal guanethidine treatment plus adrenal

Figure 11. Change in mean arterial pressure and heart rate in response to hexamethonium before, during, and after chronic ivt AII infusion. Hexamethonium was administered as a bolus (20 mg/kg, iv). Data was obtained from rats (n=6) whose cardiovascular and fluid/ electrolyte response to chronic ivt AII is depicted in Figure 9. Vertical lines on C2 value represent SEM for individual within groups comparisons (mixed design ANOVA and lsd). Other symbols as in Figure 10.

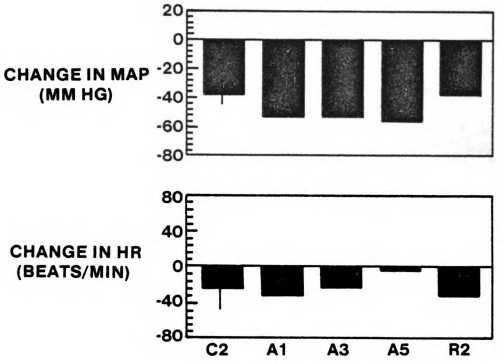
CHANGE IN MAP
(MM HG)
20
0
-20
-40
-60
-80
CHANGE IN HR
(BEATS/MIN)
80
40
0
-40
-80
C2
A1
A3
A5
R2

Figure 11

Figure 12. Change in mean arterial pressure and heart rate in response to AVP antagonist before, during, and after chronic ivt AII infusion. The top panel depicts mean arterial pressure (MAP) in response to a 5-day ivt AII infusion (6 μg/hr) in high sodium rats (n=5). The middle and bottom panels depict the change in MAP and heart rate (HR) to acute administration of the AVP antagonist (10 μg/kg, iv). Vertical lines on C2 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (MAP) or from C2 value (ΔMAP and ΔHR). Data was analyzed by randomized block ANOVA followed by lsd.

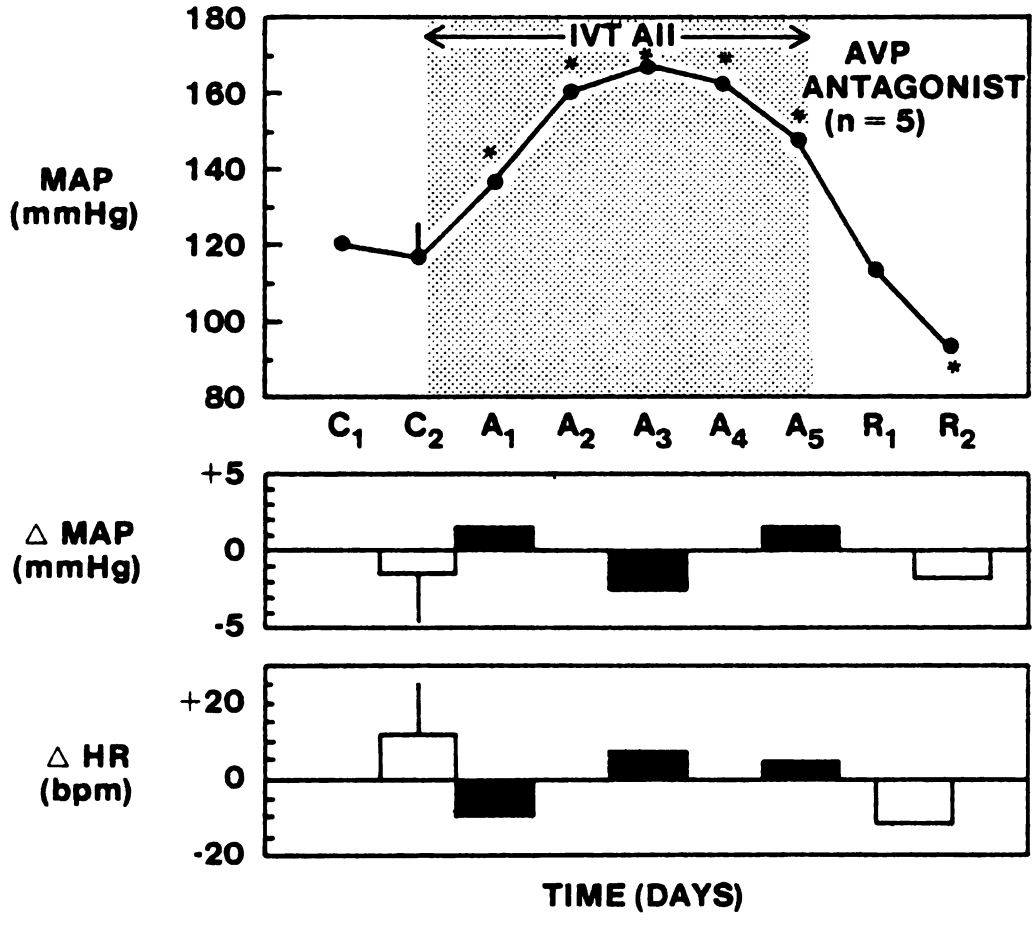
180
160
140
120
100
80
MAP
(mmHg)
IVT AII
AVP
ANTAGONIST
(n = 5)
C1 C2 A1 A2 A3 A4 A5 R1 R2
+5
0
-5
Δ MAP
(mmHg)
+20
0
-20
Δ HR
(bpm)
TIME (DAYS)

Figure 12

Figure 13. Efficacy of AVP antagonist in blocking responses to exogenous AVP infusion. Change in mean arterial pressure (ΔMAP) and heart rate (ΔHR) in response to 10-min iv AVP infusions (0.3, 1.0, 3.0 mU/min) was determined before, and 5 min after AVP antagonist administration (n=5). Crosses (+) represent significant ($p<0.05$) difference between pre- and post-antagonist values (mixed design ANOVA and lsd).

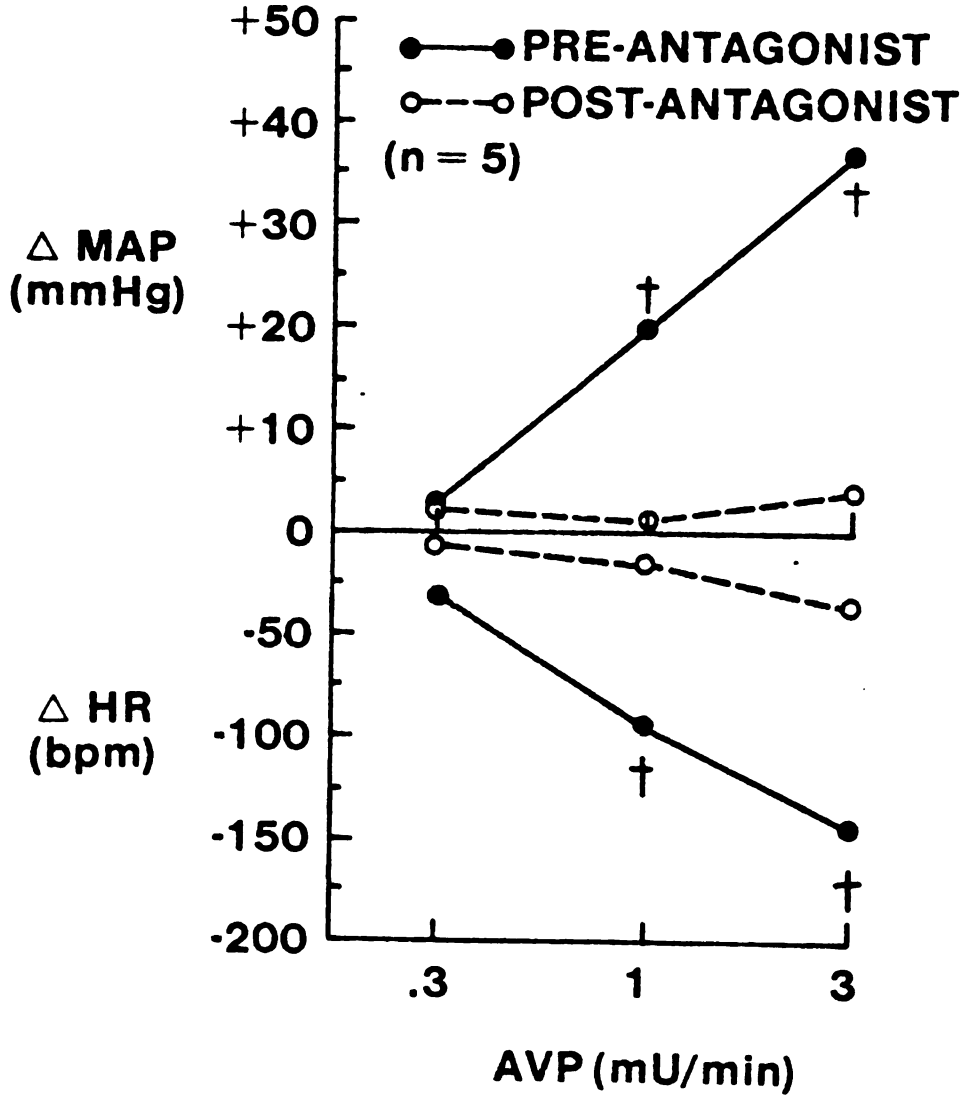
+50
+40
+30
+20
+10
0
-50
-100
-150
-200
PRE-ANTAGONIST
POST-ANTAGONIST
(n = 5)
Δ MAP
(mmHg)
Δ HR
(bpm)
†
.3
1
3
AVP (mU/min)

Figure 13

demedullation at adulthood. Chronic ivt AII infusion produced similar elevations in blood pressure in sympathectomized and normal rats (Figure 14). The onset of hypertension was significantly delayed in the sympathectomized group. On the second day of ivt AII infusion, the sympathectomized group was normotensive while normal rats exhibited a significant elevation in blood pressure at that time. By the fourth day of ivt infusion, both groups were hypertensive. The offset of the hypertensive response to chronic ivt AII also was significantly delayed in sympathectomized rats.

7. Verification of degree of sympathectomy

The effect of sequential ganglionic blockade and alpha-adrenergic blockade on MAP and HR of sympathectomized and normal rats is shown in Figure 15. The drop in MAP at 1 and 5 min after hexamethonium administration was significantly less in sympathectomized rats than in normal rats. Subsequent administration of phentolamine resulted in no further depressor response in either group of rats. The overall depressor response to combined ganglionic and alpha-adrenoceptor blockade was less in sympathectomized rats than control rats. Changes in heart rate in response to these interventions did not differ in the two groups.

Increases in MAP elicited by electrical stimulation of sympathetic vasomotor outflow in the pithed rat preparation are shown in Figure 16. Normal rats exhibited graded increases in MAP in response to spinal cord stimulation over the range of stimulation frequencies (1-10 Hz). Sympathectomized rats exhibited pressor responses that were significantly less than those seen in normal rats at all stimulation

Figure 14. Effect of peripheral sympathectomy on hypertensive response to chronic ivt AII infusion. Sympathectomized (SYMX, n=5) and control (n=6) rats were given ivt infusions of AII (6 μg/hr) for 7 days (shaded area). Vertical bars on first data point represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of the three pre-infusion values. Cross (+) indicates significant difference between sympathectomized and control rats (mixed design ANOVA and lsd).

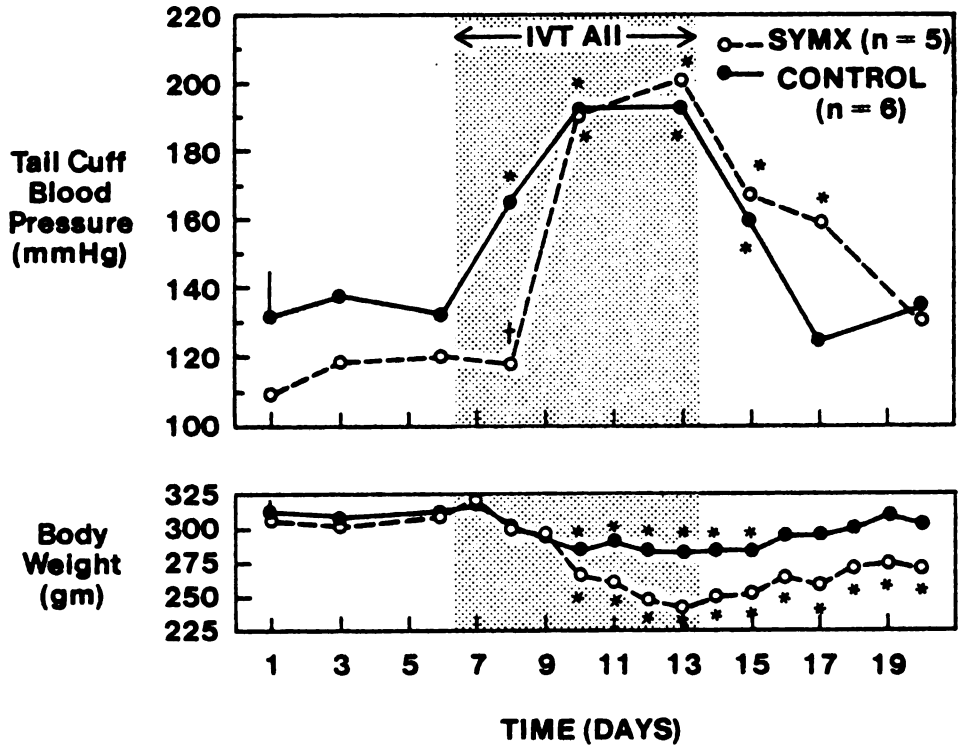
220
200
180
160
140
120
100
Tail Cuff Blood Pressure (mmHg)
IVT AII
SYMX (n = 5)
CONTROL (n = 6)
325
300
275
250
225
Body Weight (gm)
1
3
5
7
9
11
13
15
17
19
TIME (DAYS)

Figure 14

Figure 15. Blood pressure and heart rate response to hexamethonium and phentolamine in normal and sympathectomized rats. Changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) were measured at 1 and 5 min after sequential administration of hexamethonium (20 mg/kg, iv) and phentolamine (2 mg/kg, iv) in normal (n=5) and sympathectomized (SYMX, n=8) rats. Points represent means ± SEM. Differences between normal and sympathectomized rats were tested at each time point using Student's t-test. Cross (+) indicates significant ($p<0.05$) difference between normal and sympathectomized rats.

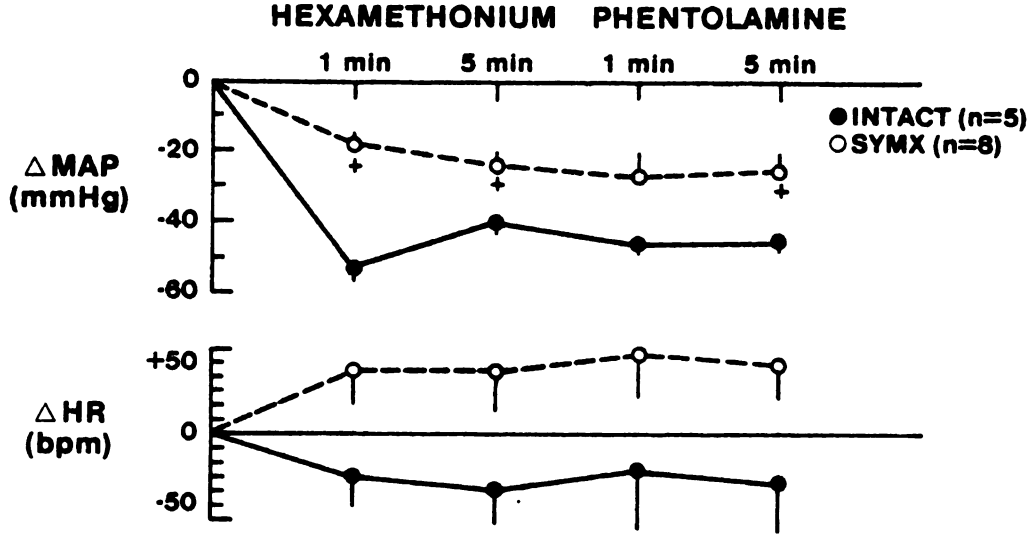
HEXAMETHONIUM
PHENTOLAMINE
1 min
5 min
1 min
5 min
0
-20
-40
-60
△MAP
(mmHg)
●INTACT (n=5)
○SYMX (n=8)
+50
0
-50
△HR
(bpm)

Figure 15

Figure 16. Change in mean arterial pressure in response to electrical stimulation of sympathetic vasomotor outflow in normal and sympathectomized pithed rats. Points represent average changes in mean arterial pressure (ΔMAP) in intact (n=5) and sympathectomized (SYMX, n=8) rats. Vertical bars represent SEM for individual between groups pairwise comparisons. Cross (+) indicates significant ($p<0.05$) difference between normal and sympathectomized rats (mixed design ANOVA and lsd).

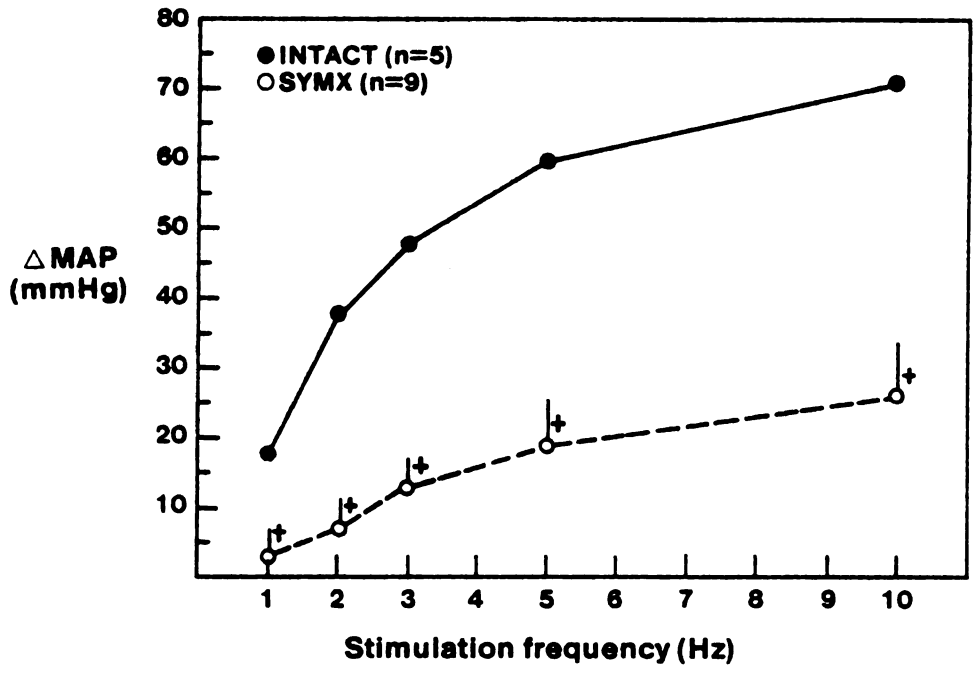
80
70
60
50
40
30
20
10
● INTACT (n=5)
○ SYMX (n=9)
△MAP
(mmHg)
1
2
3
4
5
6
7
8
9
10
Stimulation frequency (Hz)

Figure 16

frequencies. The pressor response to the 10 Hz stimulation was completely abolished by hexamethonium (20 mg/kg, iv) in all rats, indicating that pressor responses were neurogenically mediated.

Tissue norepinephrine and dopamine content from normal and sympathectomized rats is reported in Table 3. Norepinephrine content was significantly lower in the pineal and higher in the cerebellum of sympathectomized rats than control rats. Dopamine content of hypothalamus was lower in sympathectomized rats than in normal rats.

8. Pharmacologic assessment of sympathetic tone

Resting MAP of rats used in these experiments is presented in Table 4. The depressor responses to phentolamine, propranolol plus phentolamine, hexamethonium, and nitroprusside in rats receiving chronic ivt AII infusions are depicted in Figure 17. In control rats (saline ivt, n=5), phentolamine (2 mg/kg, iv) produced an average fall in MAP of 47±8 mmHg. The combination of propranolol (1 mg/kg, iv) followed by phentolamine produced a depressor response of 19 mmHg, which was significantly different than the response to phentolamine alone. Hexamethonium (20 mg/kg, iv) decreased MAP by 30 mmHg, a value not different from the response to phentolamine alone. The depressor responses to all of these interventions were significantly greater in rats receiving AII ivt (n=7), averaging 64±7 mmHg for phentolamine alone, 43 mmHg for propranolol plus phentolamine, and 66 mmHg for hexamethonium. In the rats that received AII ivt, the depressor response to propranolol plus phentolamine was significantly less than the response to phentolamine alone or hexamethonium.

TABLE 3

Tissue Norepinephrine and Dopamine Content in Normal and Sympathectomized Rats

	Renal Cortex	Pineal	Hypothalamus	Cerebellum	Frontal Cortex
Norepinephrine (ng/mg protein)					
Normal	4.05±1.79	3.04±0.67	21.43±8.93	5.94±0.77	5.40±1.55
Sympathectomized	3.60±1.74	1.18±0.44*	8.62±5.51	7.72±1.58*	5.30±1.49
Dopamine (ng/mg protein)					
Normal	0.41±0.12	0.79±0.11	3.74±0.33	0.32±0.04	6.38±3.77
Sympathectomized	0.25±0.04	0.53±0.09	2.71±0.86*	0.94±0.38	1.61±0.84

Values represent mean ± SEM (normal rats: n=5, sympathectomized rats: n=8). Asterisk (*) indicates significant difference between normal and sympathectomized rats (Student's t-test).

TABLE 4

Resting Mean Arterial Pressure Before Various Vasodepressor Interventions

Intervention	Resting MAP (mmHg)	
	Saline ivt (n=5)	AII ivt (n=7)
Phentolamine	111±6	115±5*
Propranolol plus Phentolamine	111	148*
Hexamethonium	116	144*

Values represent mean ± SEM for within groups comparisons. Asterisk (*) indicates significant difference from saline ivt value (mixed design ANOVA and lsd).

Figure 17. Pharmacologic assessment of sympathetic tone in rats receiving chronic ivt infusions of AII. Change in mean arterial pressure (ΔMAP) was measured at 5 min after each intervention (see text for details of experimental protocol). PHENT = phentolamine, PROP = propranolol, HEX = hexamethonium, NITRO = nitroprusside. Vertical lines on each pair of bars represent SEM for individual between groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference between AII- and saline-infused rats (mixed design ANOVA and Tukey's test). Nitroprusside data was analyzed using a paired t-test.

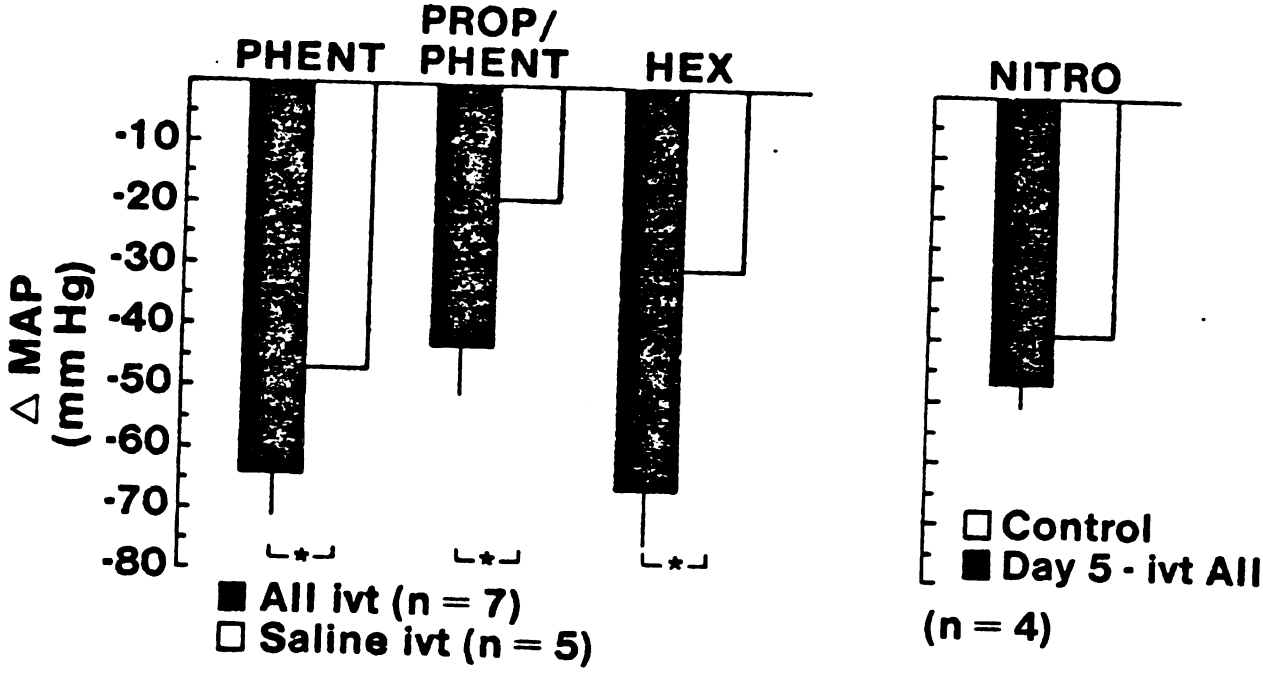
PHENT
PROP/
PHENT
HEX
NITRO
-10
-20
-30
-40
-50
-60
-70
-80
Δ MAP
(mm Hg)
*
*
*
All ivt (n = 7)
Saline ivt (n = 5)
Control
Day 5 - ivt All
(n = 4)

Figure 17

In a separate group of rats (n=4), the decrease in MAP produced by a 5 min iv nitroprusside infusion (12 μg/min) was not significantly different before, and on the fifth day of ivt AII infusion (39±4 vs. 47 mmHg, paired t-test). Resting MAP in these rats before nitroprusside infusion was 118±6 mmHg on the control day and 155 mmHg on the fifth day of ivt AII infusion ($p<0.05$, paired t-test).

9. Interactions of AVP, sympathetic nervous system and peripheral renin-angiotensin system

The effect of sequential administration of AVP antagonist (10 μg/kg, iv), saralasin (10 μg/min, iv for 15 min) and phentolamine (2 mg/kg, iv) on MAP and HR in normal rats receiving chronic ivt saline (n=5) or AII infusions (n=5) is shown in Figure 18. On the fifth day of ivt infusion, MAP was significantly greater in rats receiving AII (6 μg/hr) than in saline controls (165 vs. 114 mmHg, respectively). Blood pressure was not significantly different at 1 and 5 min after administration of the AVP antagonist in either group of rats. Heart rate was significantly increased by this intervention in the AII group but not in the saline group. Subsequent intravenous infusion of saralasin (10 μg/min) for 15 min caused no change in MAP in the ivt-saline control rats, but caused a significant fall in MAP in rats receiving AII ivt. After the combined treatment with AVP antagonist and saralasin, MAP was still significantly higher in AII rats when compared to saline rats. Phentolamine administration (2 mg/kg, iv) produced a significant fall in blood pressure in both groups of rats. After the administration of phentolamine, MAP was not significantly different between the ivt saline and ivt AII groups of rats.

Figure 18. Effect of sequential administration of AVP antagonist, saralasin, and phentolamine on blood pressure and heart rate in normal rats receiving chronic ivt infusions of AII. Rats received either AII (6 μg/hr, n=5) or saline (n=5) ivt and were tested on the fifth day of infusion (see text for complete description of protocol). CON = control; numbers along abscissa indicate time in minutes after each intervention (denoted by arrows). Vertical bars represent SEM for individual within groups comparisons. Asterisk (*) denotes significant ($p<0.05$) difference from control value. Cross (+) denotes significant difference between AII and saline rats (mixed design ANOVA and lsd).

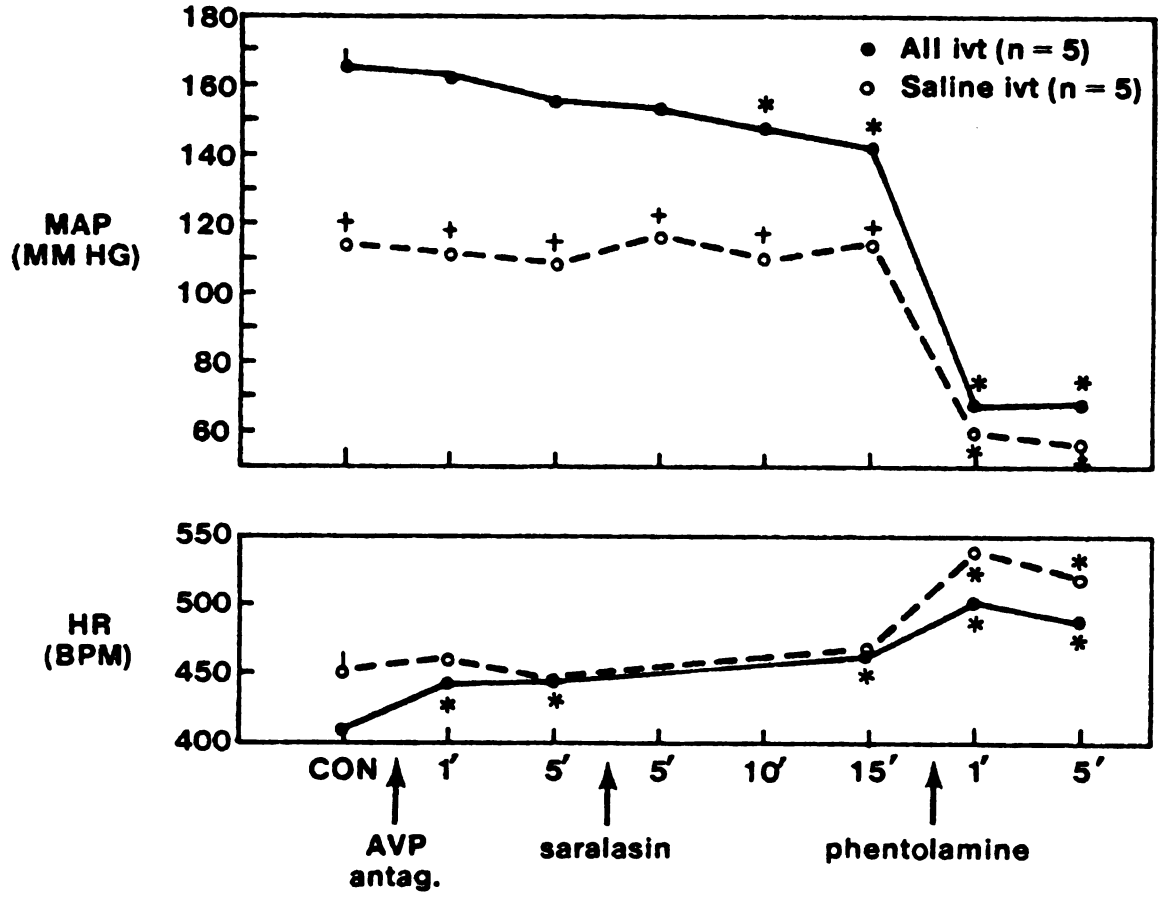
180
160
140
120
100
80
60
MAP
(MM HG)
All ivt (n = 5)
Saline ivt (n = 5)
550
500
450
400
HR
(BPM)
CON
1'
5'
5'
10'
15'
1'
5'
AVP
antag.
saralasin
phentolamine

Figure 18

Figure 19 shows the MAP and HR responses to 15 min iv saralasin infusion followed by AVP antagonist injection on the sixth day of ivt saline or AII infusion. Saralasin infusion produced a significant depressor response in rats receiving ivt AII, but not in saline control rats. Subsequent injection of AVP antagonist produced no further change in pressure in either group of rats.

Mean blood pressure and heart rate responses to sequential administration of AVP antagonist and saralasin in sympathectomized rats on the fifth day of ivt saline or AII infusion is depicted in Figure 20. Resting MAP was significantly higher in sympathectomized rats given ivt AII infusions than in saline-treated sympathectomized rats (154 vs. 121 mmHg, respectively). Injection of the AVP antagonist did not alter blood pressure in either group of rats. Intravenous saralasin infusion, after AVP antagonist administration, produced a significant fall in pressure in the sympathectomized-ivt AII group, but not in the sympathectomized-ivt saline group. MAP was not significantly different in AII- and saline-treated sympathectomized rats after combined treatment with AVP antagonist and saralasin. The MAP responses of individual sympathectomized rats given chronic ivt AII infusion (n=8) to sequential administration of AVP antagonist and saralasin are given in Table 5. As can be seen from this table, there was considerable heterogeneity in the response to AVP antagonist plus saralasin in this group of rats. Notably, 3 rats (#2, 4, and 6) experienced large depressor responses to the combination of AVP antagonist and saralasin, while MAP in one rat (#3) remained unchanged in response to these interventions. The other rats exhibited intermediate responses.

Figure 19. Effect of sequential administration of saralasin and AVP antagonist on blood pressure and heart rate in normal rats receiving chronic ivt infusions of AII. Rats received either AII (6 μg/hr, n=4) or saline (n=5) ivt and were tested on the sixth day of ivt infusion (see text for complete description of protocol). CON = control; numbers along abscissa denote time in minutes after each intervention (indicated by arrows). Vertical bars represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from control value. Cross (+) indicates significant difference between AII and saline rats (mixed design ANOVA and lsd).

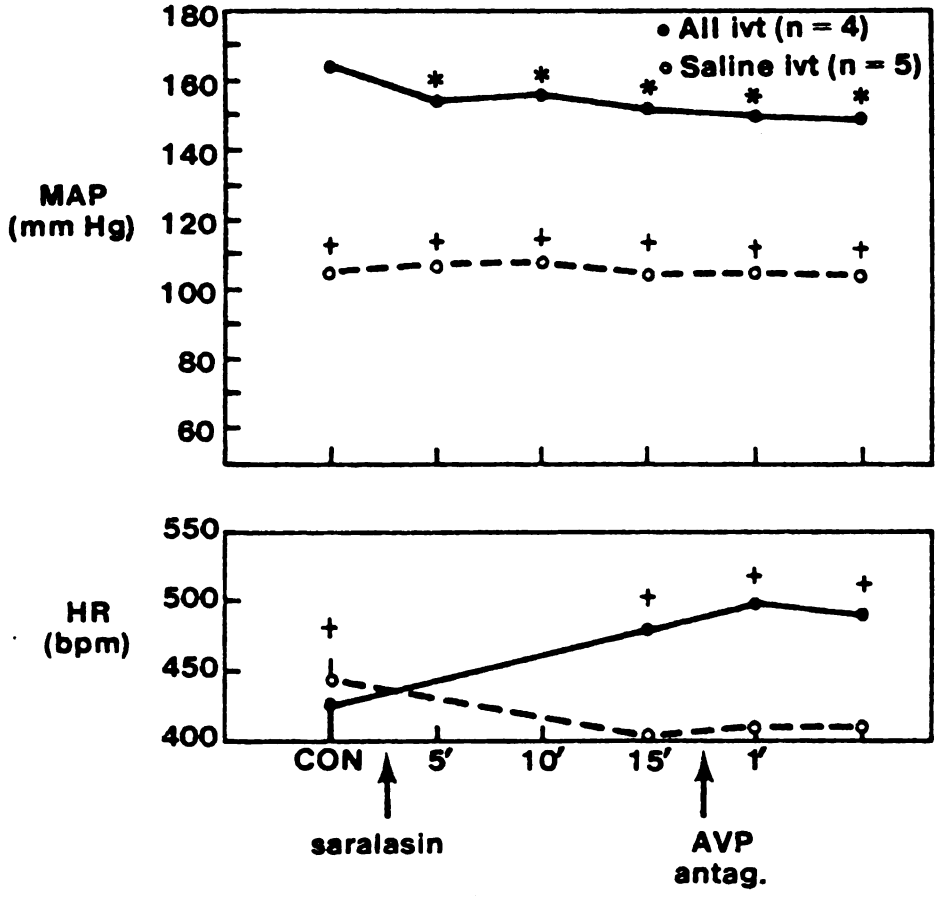
• All ivt (n = 4)
o Saline ivt (n = 5)
MAP
(mm Hg)
180
160
140
120
100
80
60
HR
(bpm)
550
500
450
400
CON
5′
10′
15′
1′
saralasin
AVP
antag.

Figure 19

Figure 20. Effect of sequential administration of AVP antagonist and saralasin on blood pressure and heart rate in sympathectomized rats receiving chronic ivt infusions of AII. Sympathectomized rats received either AII (6 μg/hr, n=8) or saline (n=5) ivt and were tested on the fifth day of ivt infusion (see text for complete description of protocol). CON = control; numbers along abscissa denote time in minutes after each intervention (indicated by arrows). Vertical bars represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from control value. Cross (+) indicates significant difference between AII and saline rats (mixed design ANOVA and lsd).

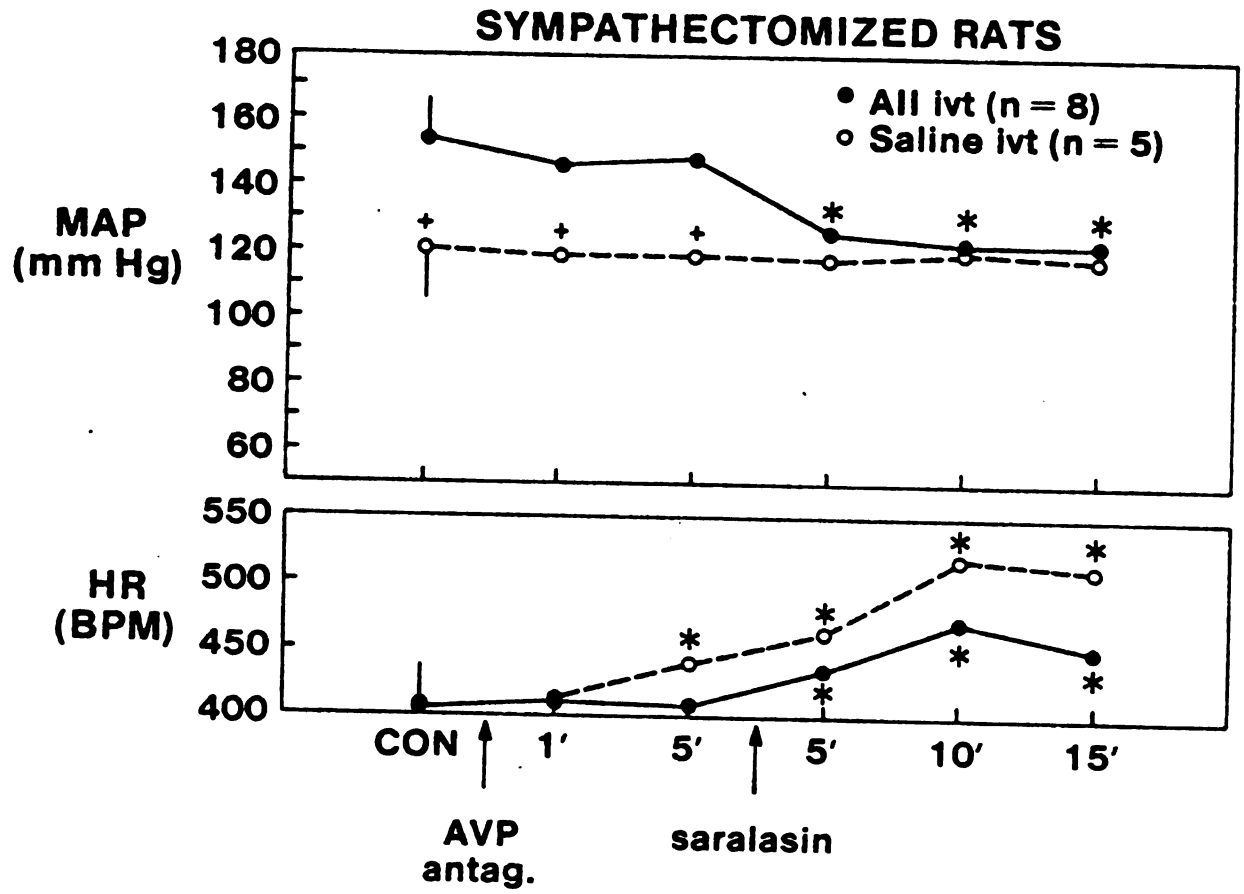
SYMPATHECTOMIZED RATS
All ivt (n = 8)
Saline ivt (n = 5)
MAP
(mm Hg)
180
160
140
120
100
80
60
HR
(BPM)
550
500
450
400
CON
1'
5'
5'
10'
15'
AVP
antag.
saralasin

Figure 20

TABLE 5

Effect of Sequential Administration of AVP Antagonist and Saralasin on Blood Pressure in Sympathectomized Rats Receiving Chronic ivt Infusions of AII

Rat	Resting	Mean Arterial Pressure (mmHg)				
		AVP Antagonist		Saralasin		
		1 min	5 min	5 min	10 min	15 min
1	122	132	131	128	128	126
2	219	177	207	124	121	124
3	178	178	170	179	171	171
4	111	125	119	76	76	80
5	167	146	145	150	148	146
6	156	143	144	109	105	104
7	132	125	131	115	111	110
8	146	143	138	125	120	125

Values represent mean arterial pressure (MAP). "Resting" denotes basal MAP before any intervention. MAP was measured at 1 and 5 min after administration of AVP antagonist (10 μg/kg, iv) and at 5, 10, and 15 min after subsequent iv saralasin infusion (10 μg/min).

10. Spinal cord stimulation

Figure 21 depicts the pressor responses to electrical stimulation of the spinal cord in pithed rats on the fifth day of ivt saline (n=8) or AII (6 μg/hr, n=9) infusion. The resting MAP in the conscious rats was measured immediately before the pithing procedure and averaged 152±6 mmHg in AII-infused rats and 120±4 mmHg in saline controls ($p<0.05$, Student's t-test). Blood pressure after anesthesia and pithing was not significantly different in the two groups (62±5 mmHg for saline controls, 60±6 mmHg for AII-treated rats, Student's t-test). Electrical stimulation of the spinal cord produced frequency-dependent increases in MAP in both groups of rats. There were no significant differences between AII- and saline-treated rats in the pressor response to spinal stimulation.

11. Acute ivt sarthran/chronic ivt AII

The ability of acute ivt sarthran injection to reverse hypertension induced by chronic ivt AII infusion is depicted in Figure 22. On the fifth day of hypertension induced by chronic ivt AII infusion, acute ivt sarthran injection (0.3 μg) had no effect on MAP (n=5). However, successive sarthran injections (1 and 3 μg, ivt), each separated by 15 min, produced a significant decrease in MAP (ivt AII/sarthran group). This series of ivt sarthran injections had no effect on blood pressure in the same group of rats on the second day after stopping the ivt AII infusion (recovery/sarthran group). Three ivt injections of 5 μl isotonic saline in a separate group of rats (n=4) on the fifth day of ivt AII infusion also had no effect on MAP (denoted ivt AII/saline).

Figure 21. Effect of chronic ivt AII infusion on pressor responses to electrical stimulation of sympathetic vasomotor outflow in pithed rats. Points represent average changes in mean arterial pressure (ΔMAP) of rats treated with saline (n=8) or AII (6 μg/hr, n=9) ivt for 5 days. Vertical bars represent SEM for individual within groups comparisons. There were no significant differences between the AII and saline group (mixed design ANOVA and lsd).

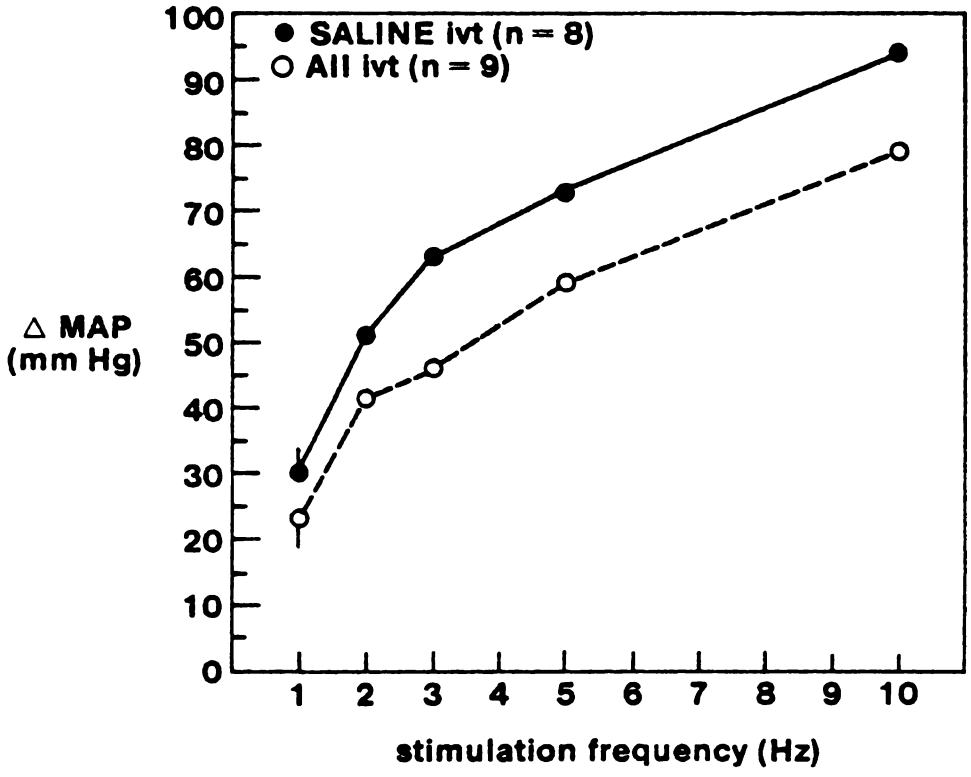
100
90
80
70
60
50
40
30
20
10
0
● SALINE ivt (n = 8)
○ All ivt (n = 9)
△ MAP
(mm Hg)
1
2
3
4
5
6
7
8
9
10
stimulation frequency (Hz)

Figure 21

Figure 22. Ability of acute ivt sarthran injection to reverse hypertension induced by chronic ivt AII infusion. MAP = mean arterial pressure; BASAL = resting MAP. For explanation of experimental groups, see description in text. Vertical bars represent SEM for within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from basal value (mixed design ANOVA and lsd).

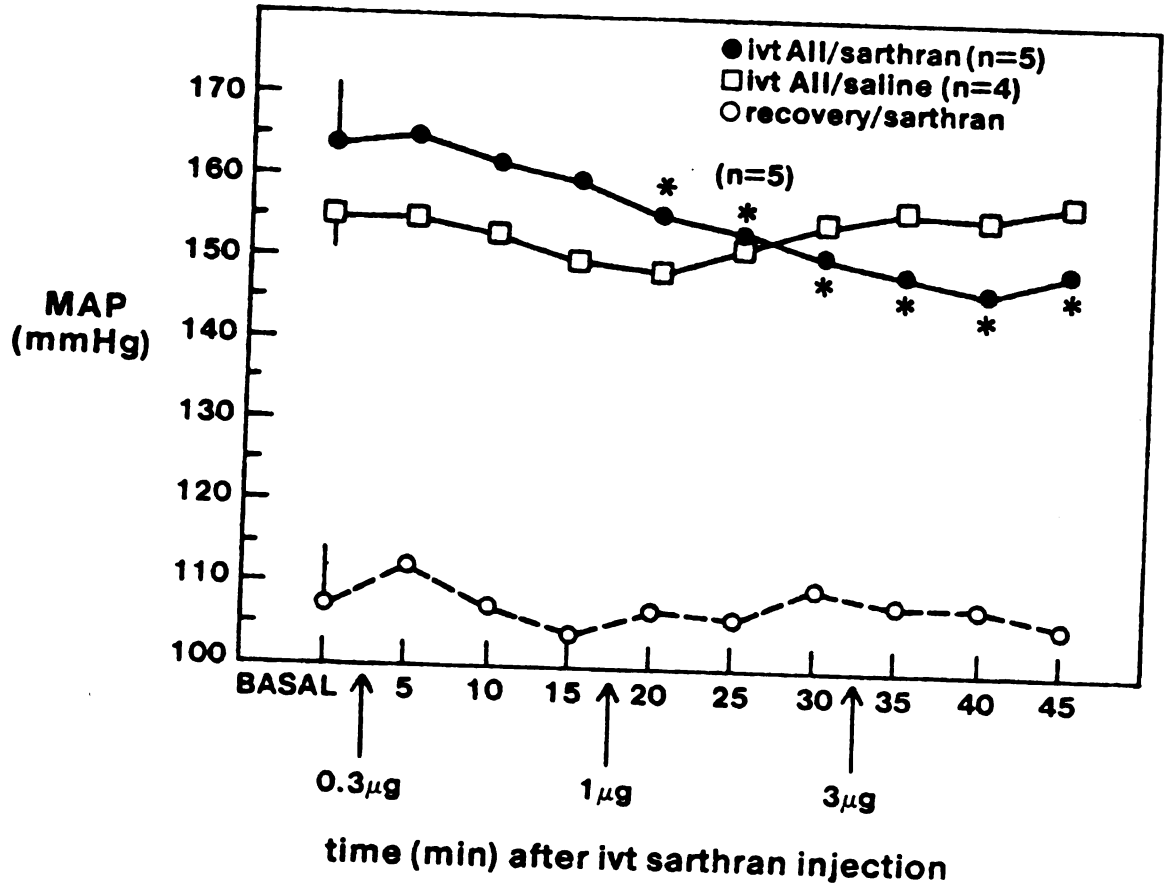
ivt AII/sarthran (n=5)
ivt AII/saline (n=4)
recovery/sarthran
(n=5)
MAP (mmHg)
170
160
150
140
130
120
110
100
BASAL
5
10
15
20
25
30
35
40
45
0.3μg
1μg
3μg
time (min) after ivt sarthran injection

Figure 22

12. Acute ivt sarthran/acute ivt AII

Figure 23 illustrates the ability of acute ivt sarthran injection to reverse the pressor effect of acute ivt AII injection. There was no significant difference in the time course of the decay of the pressor response to an acute ivt AII injection (150 ng) whether the injection was followed one minute later by an ivt saline injection or an ivt injection of 1 μg sarthran.

13. Effect of iv saralasin on response to acute ivt AII

The pressor response to acute ivt AII (50 ng) was evaluated in a group of 5 rats before and after a 15 min iv saralasin infusion (300 ng/min). Resting MAP was unaltered over the course of the experiment and averaged 120±7 mmHg before the first ivt AII injection, 117 mmHg before starting the iv saralasin infusion (after a 2-hour interval) and 124 mmHg at the end of the 15-min saralasin infusion (immediately preceding the second ivt injection of AII). The pressor response to the initial AII injection was 13±2 mmHg, and averaged 9 mmHg after the iv saralasin infusion. This difference was not statistically significant ($p>0.05$, paired t-test).

B. Chronic ivt AII Infusion in Rabbits: Sodium Dependency

The effect of chronic ivt AII infusion (3 μg/hr) on MAP, HR, and B.Wt. in rabbits on low and high sodium intake is shown in Figure 24. In rabbits on low sodium intake, MAP, HR, and B.Wt. were unaltered during ivt AII or saline infusion. In contrast, ivt AII infusion produced a significant increase in MAP in high sodium rabbits which was evident on both the first and second weeks of measurement. Blood

Figure 23. Ability of acute ivt sarthran injection to reverse the pressor effect of acute ivt AII injection. The peak change in mean arterial pressure (ΔMAP) to acute ivt AII (150 ng) and the subsequent decay in this response was measured in rats (n=5) receiving saline or sarthran (1 μg) ivt at 1 min after ivt AII injection. Vertical bar represents SEM for individual between groups comparisons (blocked factorial ANOVA and lsd).

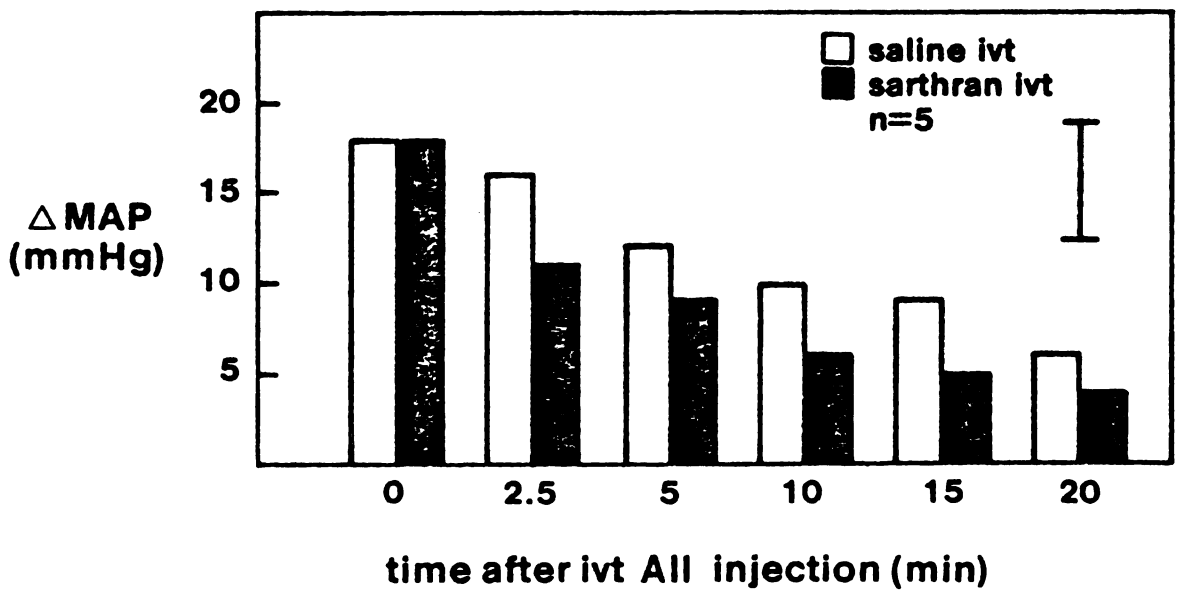
saline ivt
sarthran ivt
n=5
20
15
10
5
△MAP
(mmHg)
0
2.5
5
10
15
20
time after ivt AII injection (min)

Figure 23

Figure 24. Mean arterial pressure, heart rate, and body weight responses to chronic ivt AII infusion in rabbits maintained on low and high sodium intake. MAP = mean arterial pressure, HR = heart rate, B.WT. = body weight. C1 and C2 are two control weeks, P1 and P2 are 2 weeks of ivt AII infusion (3 μg/hr, n=5) or saline infusion (n=5), P3 is a recovery week. Vertical bars on C1 value represent SEM for individual within group comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values. Cross (+) indicates significant difference between AII- and saline-infused rabbits (mixed design ANOVA and lsd).

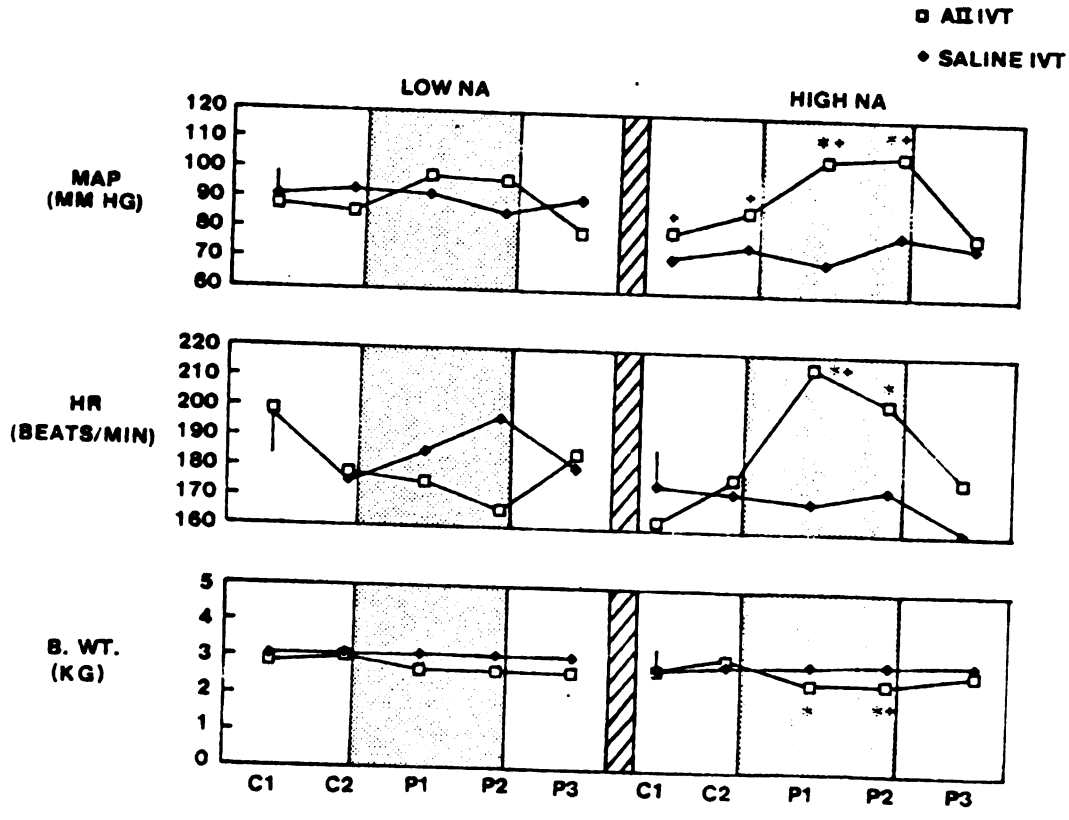
AII IVT
SALINE IVT
LOW NA
HIGH NA
MAP
(MM HG)
120
110
100
90
80
70
60
HR
(BEATS/MIN)
220
210
200
190
180
170
160
B. WT.
(KG)
5
4
3
2
1
0
C1
C2
P1
P2
P3
C1
C2
P1
P2
P3

Figure 24

pressure returned to control levels within 1 week after stopping ivt AII infusion. MAP remained unchanged in high sodium rabbits receiving saline ivt. The hypertensive response to chronic ivt AII infusion in the high sodium rabbits was associated with a significant tachycardia and decrease in body weight, neither of which were observed in ivt saline-infused control rabbits. Plasma Na and K concentrations fell significantly during the period of ivt AII infusion in both low and high sodium rabbits (Figure 25). Plasma Na concentration was still significantly lower than control levels one week after stopping ivt AII infusion, however, plasma K concentration had recovered to a value not significantly different from control by that time. Plasma osmolality was decreased during ivt AII infusion only in the high sodium group.

As shown in Figure 26, daily water intake did not change significantly in high-sodium rabbits over the first week of ivt AII infusion. Water intake slowly increased over the second week of ivt infusion, reaching a statistically significant elevation only on the final day of the ivt infusion period. Urine output paralleled water intake, therefore water balance did not change over the course of the experiment in this group of rabbits. Low-sodium rabbits exhibited significant increases in water intake and urine output during ivt AII infusion, however, water balance remained unchanged (Figure 27). Since food intake tends to decrease in response to ivt AII infusion and drinking is associated with ingestion of food in rabbits, the ratio of water intake to food intake was calculated and is presented in Figure 28. The ratio of water/food in low-sodium rabbits was dramatically increased over the first week of AII infusion, and decreased to a level not significantly

Figure 25. Effect of chronic ivt AII infusion on plasma sodium and potassium concentrations and plasma osmolality in rabbits maintained on low and high sodium intake. P_{Na} = plasma sodium concentration, P_K = plasma potassium concentration, P_{OSM} = plasma osmolality. Data was analyzed using a mixed design ANOVA followed by lsd. Other symbols as in Figure 24.

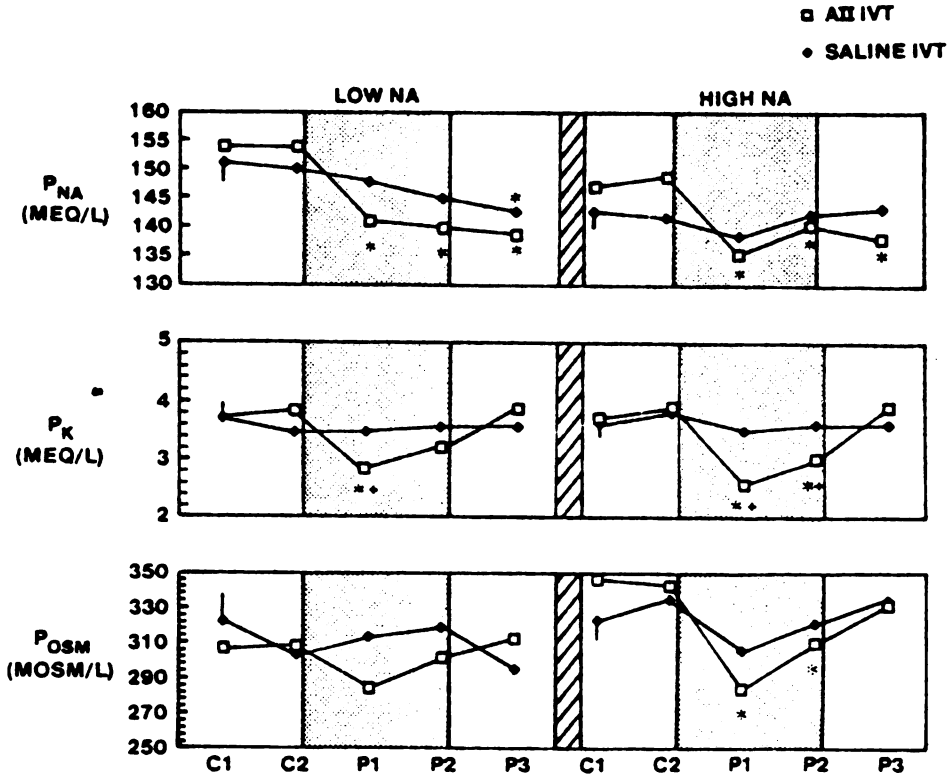
AII IVT
SALINE IVT
LOW NA
HIGH NA
P_{NA} (MEQ/L)
160
155
150
145
140
135
130
P_K (MEQ/L)
5
4
3
2
P_{OSM} (MOSM/L)
350
330
310
290
270
250
C1
C2
P1
P2
P3
C1
C2
P1
P2
P3

Figure 25

Figure 26. Water intake, urine output, and water balance in response to chronic ivt AII infusion in high-sodium rabbits. Top panel depicts responses in rabbits (n=5) that received AII ivt (3 μg/hr), and lower panel depicts responses in saline control rabbits (n=5). Vertical lines on day 1 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of values obtained during the control week (mixed design ANOVA and lsd).

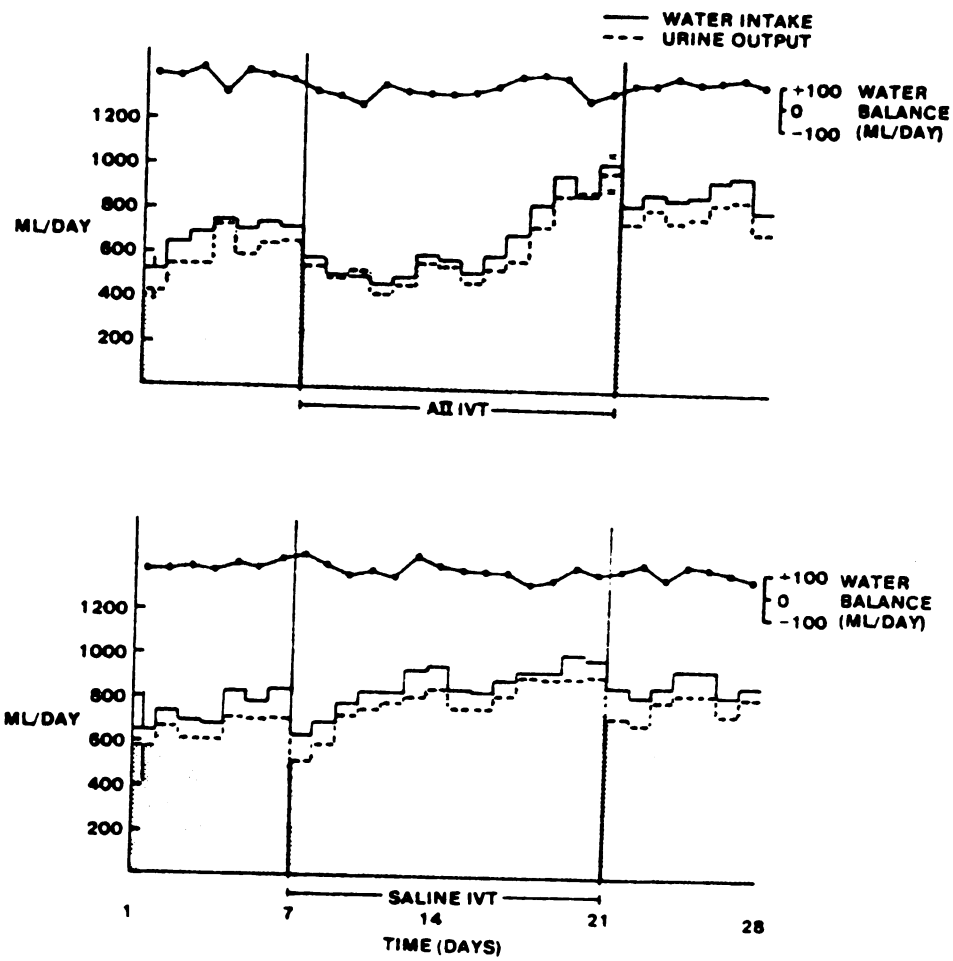
WATER INTAKE
URINE OUTPUT
+100 WATER
0 BALANCE
-100 (ML/DAY)
1200
1000
800
600
400
200
ML/DAY
SALINE IVT
1
7
14
21
28
TIME (DAYS)

Figure 26

Figure 27. Water intake, urine output, and water balance in response to chronic ivt AII infusion in low-sodium rabbits. Top panel depicts responses in rabbits (n=5) that received AII ivt (3 μg/hr) and lower panel depicts responses in saline control rabbits (n=5). Vertical lines on day 1 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of values obtained during the control week. Cross (+) indicates significant difference between AII- and saline-infused rabbits (mixed design ANOVA and lsd).

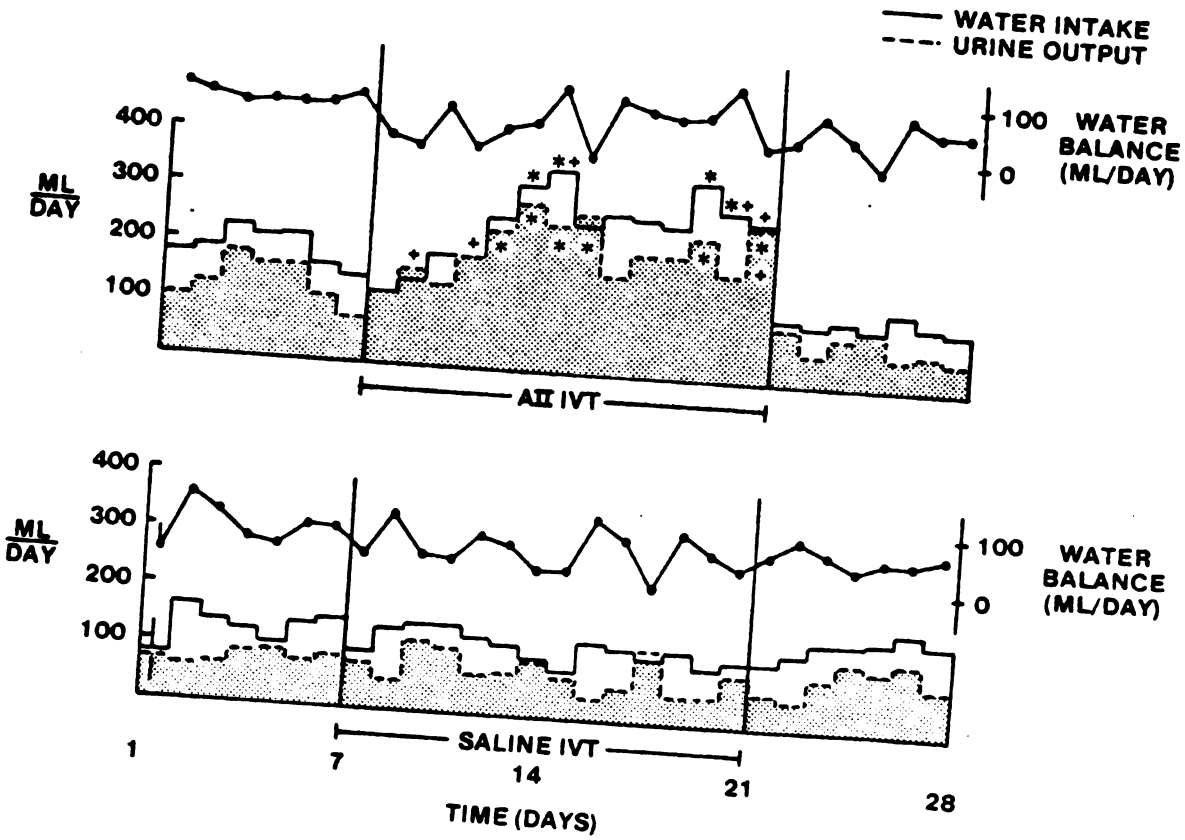
WATER INTAKE
URINE OUTPUT
ML/DAY
400
300
200
100
100
0
WATER BALANCE (ML/DAY)
AII IVT
ML/DAY
400
300
200
100
100
0
WATER BALANCE (ML/DAY)
SALINE IVT
1
7
14
21
28
TIME (DAYS)

Figure 27

Figure 28. Ratio of water intake to food intake in rabbits maintained on low and high sodium intake in response to chronic ivt AII infusion. Open circles represent ivt AII-infused rabbits and closed circles represent saline controls (n=5 in all groups). Vertical bars on day 1 values represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of control week values. Cross (+) indicates significant difference between AII- and saline-infused rabbits (mixed design ANOVA and lsd).

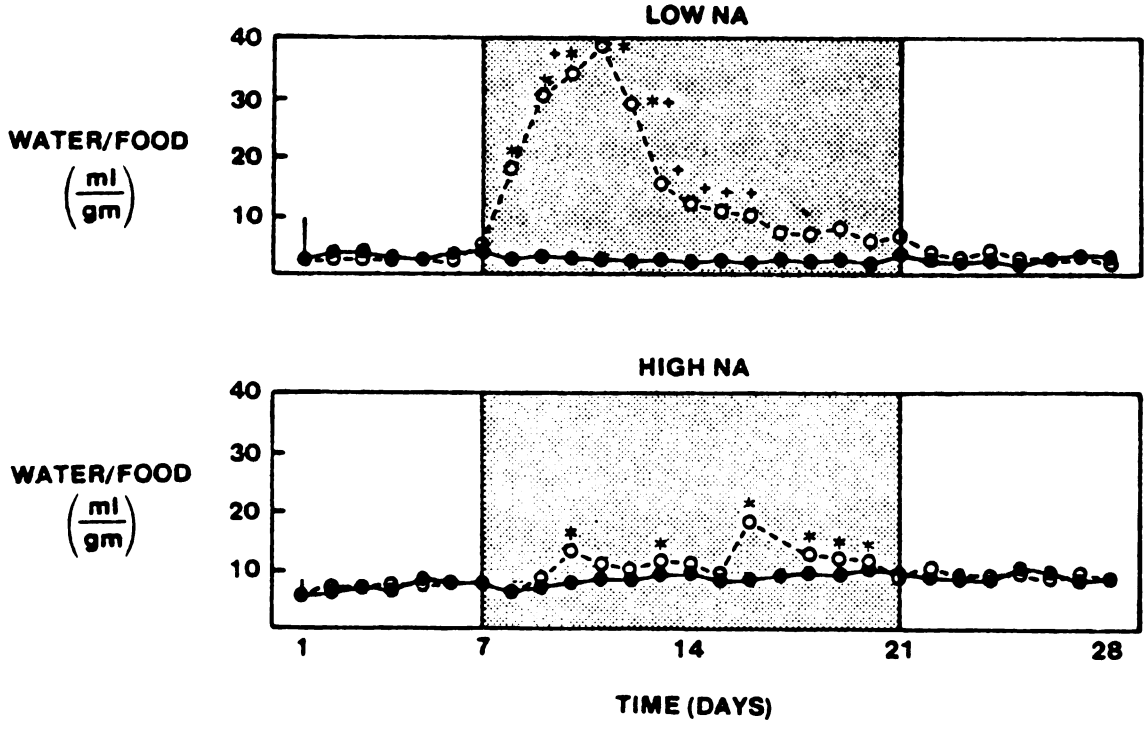
LOW NA
WATER/FOOD
(ml/gm)
40
30
20
10
HIGH NA
WATER/FOOD
(ml/gm)
40
30
20
10
1
7
14
21
28
TIME (DAYS)

Figure 28

different from saline-infused rabbits during the second week of ivt AII infusion. Water/food intake was significantly elevated on 6 out of the 14 days of ivt AII infusion in high-sodium rabbits, but the increase was not as marked as that seen in low-sodium rabbits.

Figure 29 illustrates the effect of chronic ivt AII infusion on body fluid compartment volumes and hematocrit. In high-sodium rabbits, plasma volume (PV), extracellular fluid volume (ECFV) and hematocrit (HCT) were unchanged in response to ivt AII infusion. However, in low-sodium rabbits, ECFV increased significantly during the ivt AII infusion and remained elevated one week after the infusion was stopped. PV also was significantly elevated on the recovery week. Hematocrit did not change in the low-sodium rabbits over the course of the experiment.

Daily sodium and potassium balance are shown in Figure 30. Sodium balance did not change significantly during ivt AII infusion in high-sodium rabbits, but low-sodium rabbits went into negative sodium balance over the first 8 days of ivt AII infusion. The sodium lost during this time period was not regained once the ivt AII infusion was stopped. Potassium balance decreased significantly in both low- and high-sodium rabbits in response to ivt AII infusion. In high-sodium rabbits, K balance was significantly more negative in the group receiving AII ivt than in saline control rabbits over the first week of ivt AII infusion. Low-sodium rabbits exhibited only one day of decreased potassium balance. Intraventricular AII infusion had no effect on creatinine clearance or blood urea nitrogen in either low- or high-sodium rabbits (Figure 31).

Figure 29. Effect of chronic ivt AII infusion on body fluid compartment volumes and hematocrit in rabbits maintained on low and high sodium intake. PV = plasma volume, ECFV = extracellular fluid volume, HCT = hematocrit. Vertical bars on C1 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (mixed design ANOVA and lsd). Other symbols as in Figure 24.

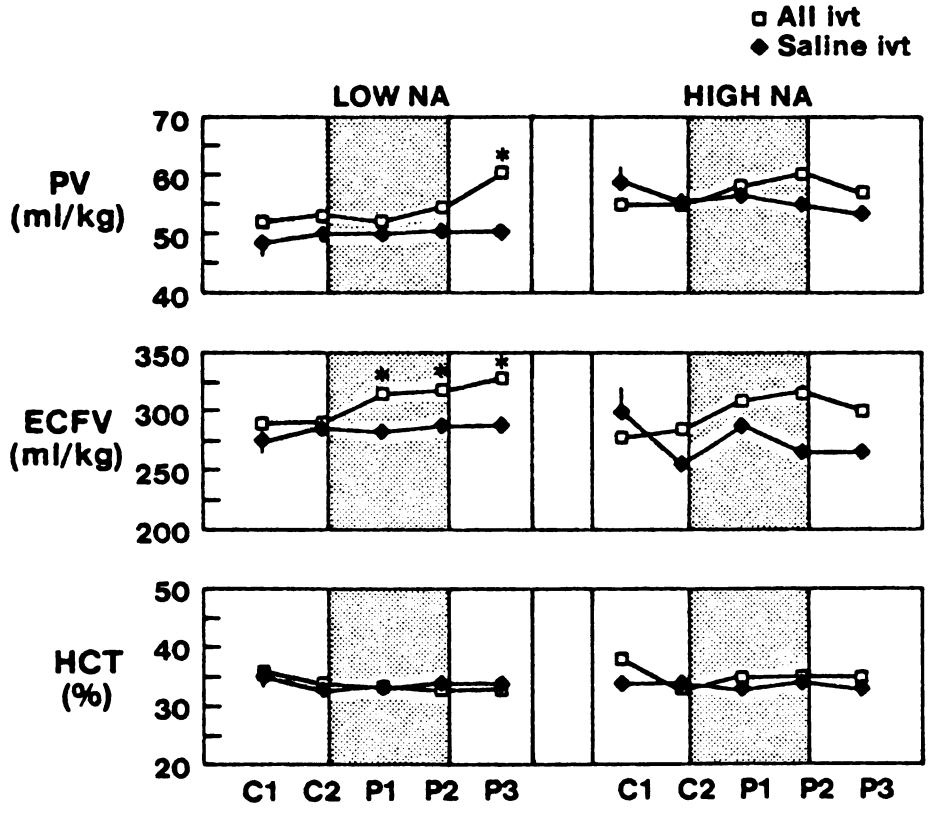
All ivt
Saline ivt
LOW NA
HIGH NA
PV (ml/kg)
70
60
50
40
ECFV (ml/kg)
350
300
250
200
HCT (%)
50
40
30
20
C1 C2 P1 P2 P3
C1 C2 P1 P2 P3

Figure 29

Figure 30. Effect of chronic ivt AII infusion on sodium and potassium balance in rabbits maintained on low and high sodium intake. Upper two panels depict daily sodium balance in high and low sodium rabbits receiving either AII (3 μg/hr) or saline ivt (n=5 in each group). Lower two panels depict daily potassium balance. Vertical bars on day 1 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of control week values. Cross (+) indicates significant difference between AII- and saline-infused rabbits (mixed design ANOVA and lsd).

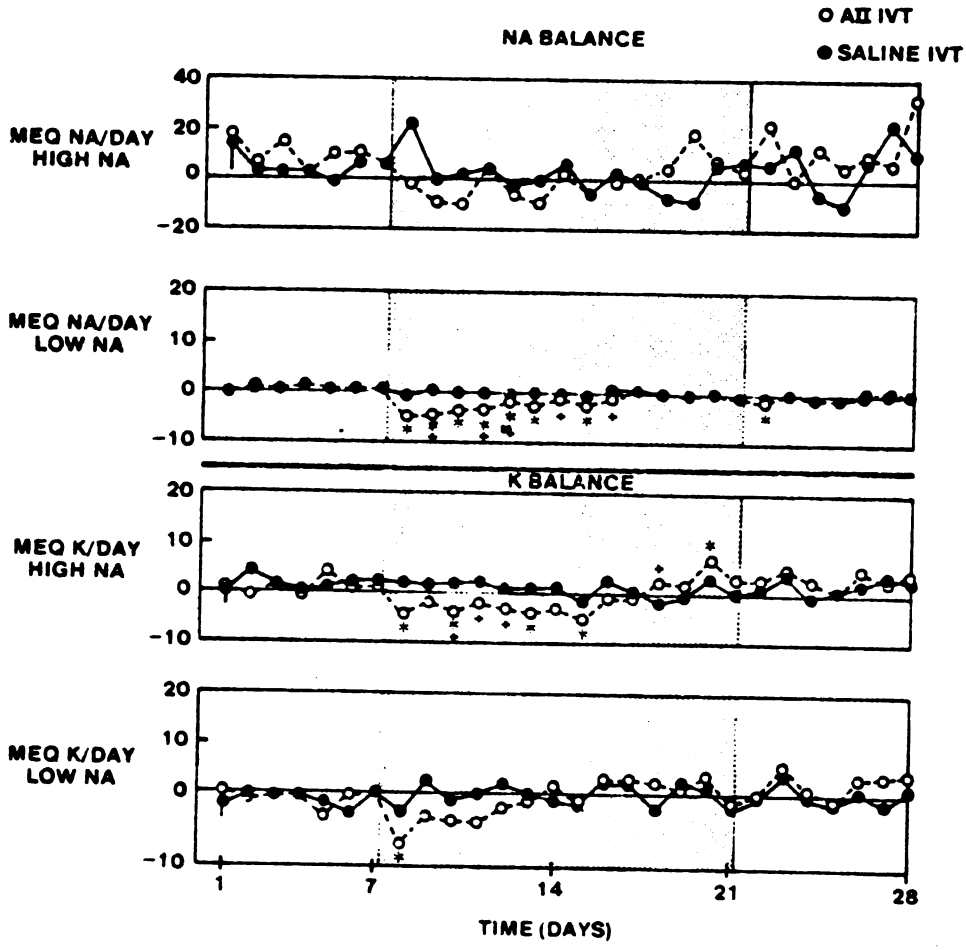
NA BALANCE
O All IVT
● SALINE IVT
MEQ NA/DAY HIGH NA
MEQ NA/DAY LOW NA
K BALANCE
MEQ K/DAY HIGH NA
MEQ K/DAY LOW NA
40
20
0
-20
20
10
0
-10
1
7
14
21
28
TIME (DAYS)

Figure 30

Figure 31. Effect of chronic ivt AII infusion on creatinine clearance and blood urea nitrogen in rabbits maintained on low and high sodium intake. Cl_{CR} = creatinine clearance; BUN = blood urea nitrogen. Vertical bars on day 1 value indicate SEM for individual within groups comparisons. Other symbols as in Figure 24.

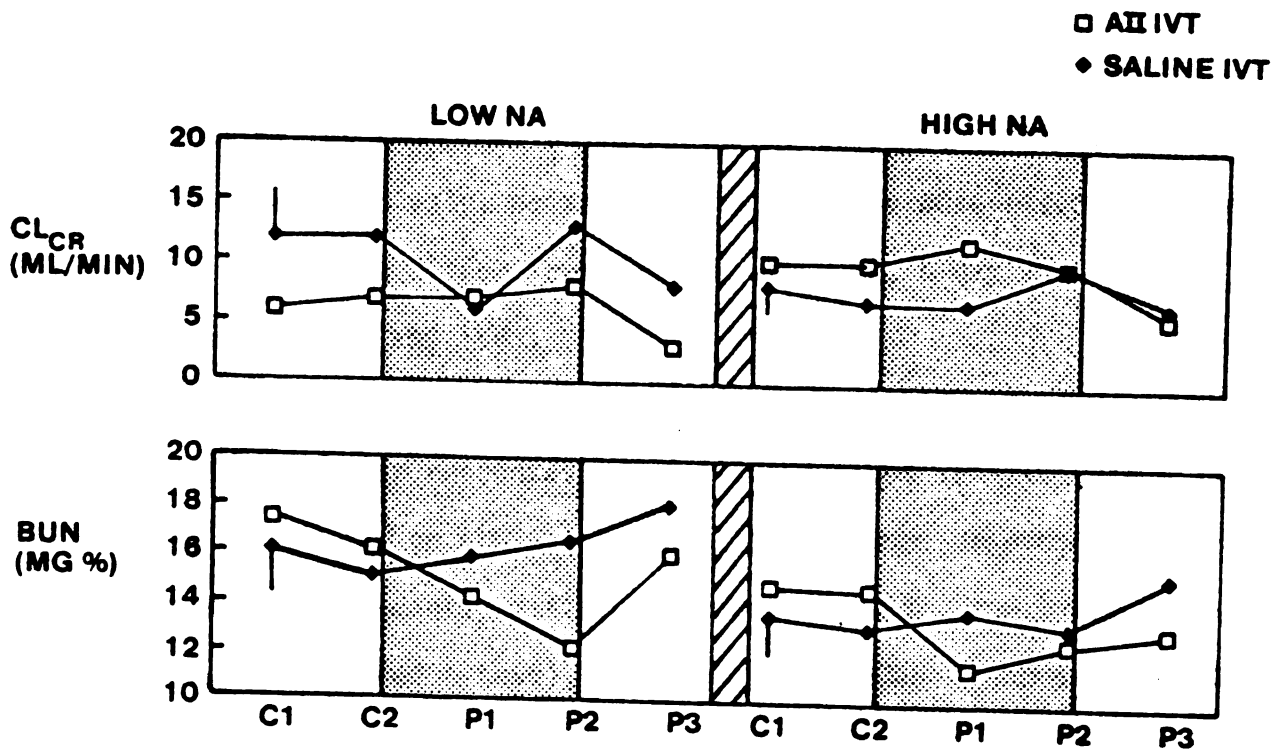
AII IVT
SALINE IVT
LOW NA
HIGH NA
CL_{CR} (ML/MIN)
20
15
10
5
0
BUN (MG %)
20
18
16
14
12
10
C1
C2
P1
P2
P3
C1
C2
P1
P2
P3

Figure 31

C. Chronic Pharmacological Blockade of Brain AII Receptors or Brain Converting Enzyme

1. Chronic ivt saralasin

a. Dose determination

As shown in Table 6, 5-day ivt infusion of saralasin at a dose of either 6 or 12 μg/hr does not alter resting mean arterial blood pressure in rats maintained on normal sodium intake. To assure that pressor responses to ivt and iv AII are reproducible after 5 days, control pressor responses were measured in a group of 5 rats and were measured again after a 5-day sham period, during which no ivt infusion was performed (Figure 32). The control pressor response to 150 ng AII ivt did not differ from that measured in the same rats after 5 days (19 vs. 21 mmHg, respectively, paired t-test). The iv dose-response curve for AII also did not differ from control after the 5-day sham period (mixed design ANOVA and lsd).

Intraventricular saralasin infusion (6 μg/hr) for 5 days did not alter pressor responses to iv AII infusions as illustrated in Figure 33 (mixed design ANOVA and lsd). The pressor response to ivt AII also was not significantly different before and after the 5-day saralasin infusion (paired t-test), although the pressor response after the ivt saralasin infusion tended to be less than the control response (15 vs. 24 mmHg, respectively). As shown in Figure 34, ivt infusion of saralasin (12 μg/hr) for 5 days completely abolished pressor responsiveness to centrally administered AII. Pressor responses to iv AII were modestly reduced after ivt saralasin infusion, significantly so at the 30 ng/min infusion rate of AII.

TABLE 6

Resting Mean Arterial Pressure Before and After ivt Saralasin Infusion

	Mean Arterial Pressure (mmHg)	
	Control	Treatment
5-Day Sham Period	120±4	121±6
5-Day Saralasin 6 μg/hr ivt	118±2	127±3
5-Day Saralasin 12 μg/hr ivt	118±3	122±5

Values are mean ± SEM; n=5 in each group. There were no significant differences in resting mean arterial pressure before and after any treatment ($p<0.05$).

Figure 32. Pressor responses to acute intravenous and intraventricular AII administration in rats before and after a 5-day sham period. Open symbols represent control pressor responses and filled symbols represent responses in the same group of rats (n=5) after a 5-day sham period. ΔMAP = change in mean arterial pressure. Values represent mean ± SEM.

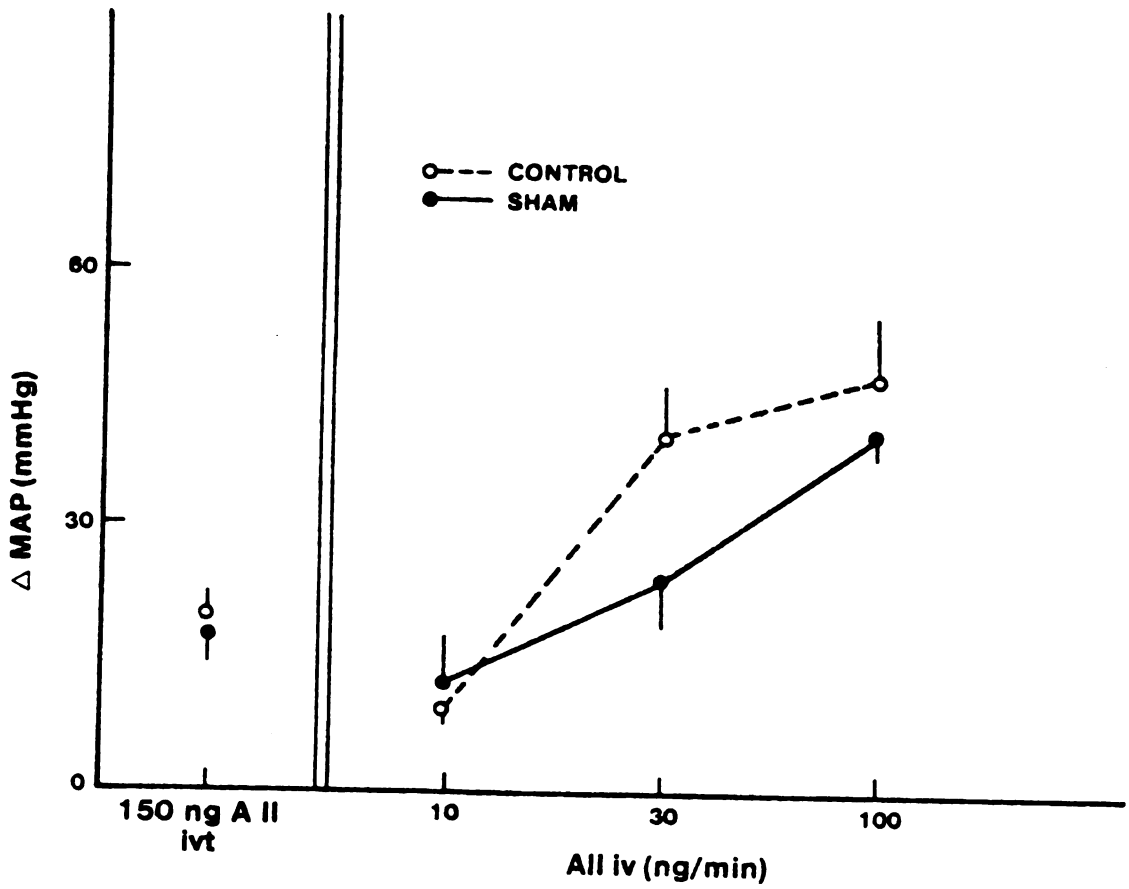
CONTROL
SHAM
60
30
0
Δ MAP (mmHg)
150 ng A II
ivt
10
30
100
AII iv (ng/min)

Figure 32

Figure 33. Effect of continuous ivt infusion of saralasin (6 μg/hr) on pressor responses to acute intravenous and intraventricular AII administration. Open symbols represent control pressor responses and filled symbols represent responses in the same group of rats (n=5) after a 5-day sham period. ΔMAP = change in mean arterial pressure. Values represent mean ± SEM.

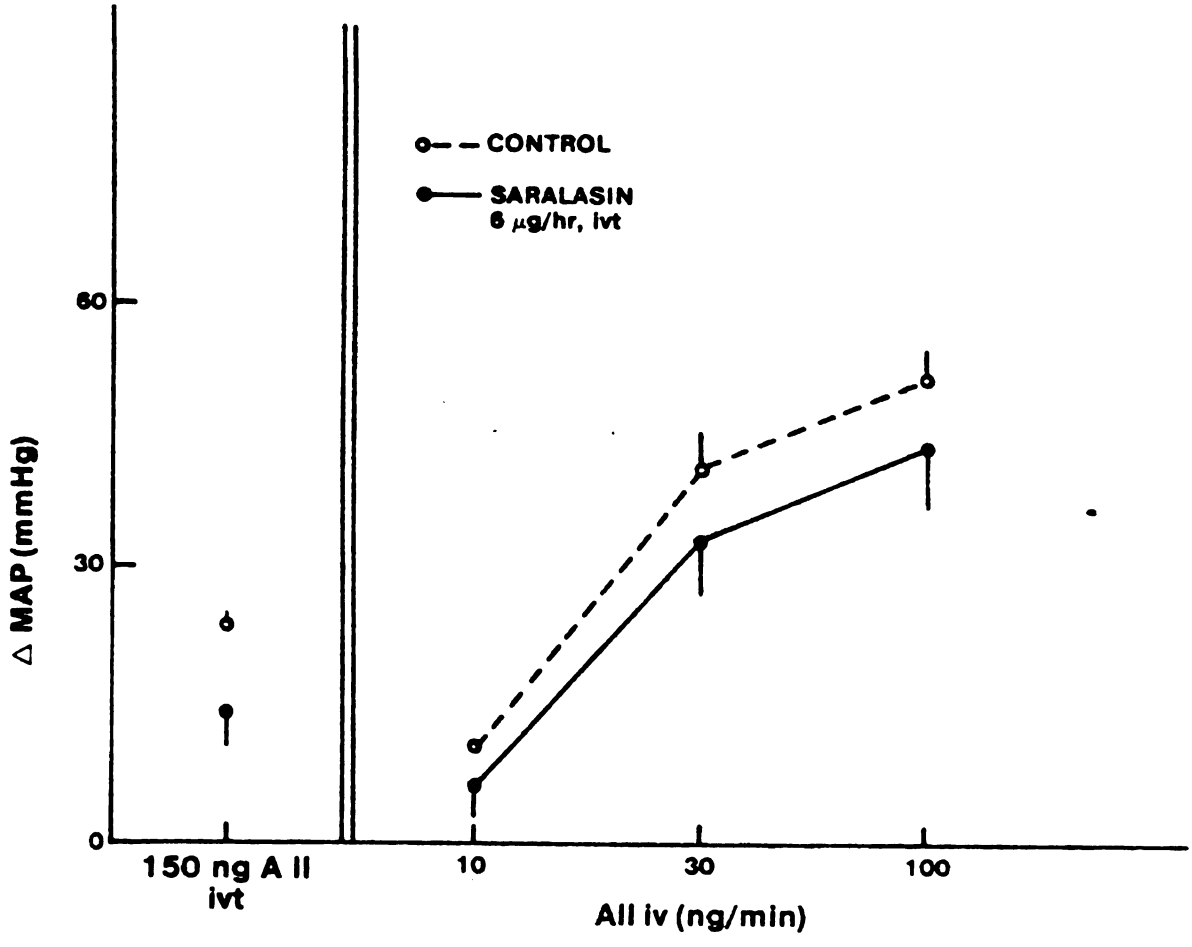
CONTROL
SARALASIN
6 μg/hr, ivt
Δ MAP (mmHg)
60
30
0
150 ng A II
ivt
10
30
100
AII iv (ng/min)

Figure 33

Figure 34. Effect of continuous ivt infusion of saralasin (12 μg/hr) on pressor responses to acute intravenous and intraventricular AII administration. Open circles represent control pressor responses and filled circles represent pressor responses in the same group of rats (n=5) after a 5-day ivt infusion of saralasin (12 μg/hr). Values represent mean ± SEM. Asterisk (*) indicates significant difference from corresponding control value (paired t-test for ivt response, mixed design ANOVA and lsd for iv dose-response).

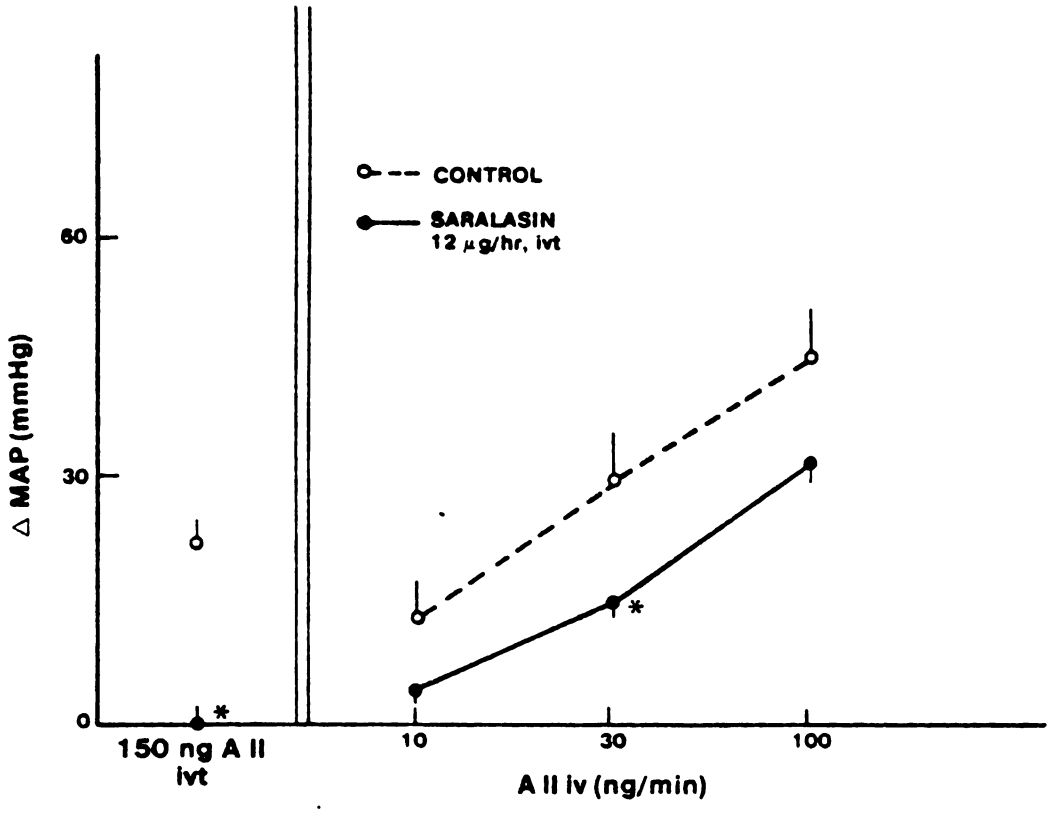
CONTROL
SARALASIN
12 μg/hr, ivt
Δ MAP (mmHg)
60
30
0
*
*
150 ng A II
ivt
10
30
100
A II iv (ng/min)

Figure 34

b. Effects in normal- and high-sodium rats

In rats maintained on a normal sodium intake, 5-day ivt saralasin infusion (12 μg/hr) had no effect on MAP, HR, $U_{Na}V$ and UO (Figure 35). The amount of water ingested over the final 24 hr of ivt saralasin infusion was not different from saline controls (rank sum test). However, in rats maintained on a high sodium intake, ivt saralasin infusion produced a significant increase in MAP by day 3 of the infusion, which was reversed within 24 hr after the infusion was stopped (Figure 36). This increase in MAP was associated with a bradycardia and transient sodium retention. Rats receiving saralasin tended to drink more than saline controls during the final 24 hr of ivt infusion, although this difference was not statistically significant.

Intravenous saralasin infusion (18 μg/hr) produced a significant increase in MAP in rats maintained on high sodium intake, although the pressor response was of a lesser magnitude than that seen with ivt saralasin (Figure 37). In addition, the pressor response to iv saralasin was evident within 24 hr after the infusion was started, whereas a 3-day latent period was observed with ivt saralasin infusion. Intravenous infusion of saralasin caused no significant changes in HR, $U_{Na}V$ or UO.

A comparison of results obtained in rats receiving chronic ivt saralasin to rats receiving chronic ivt AII infusions is depicted in Figure 38. In rats maintained on a high sodium intake, both ivt saralasin (12 μg/hr) and AII (1 μg/hr) infusion increased MAP with approximately a 3-day latency. No remarkable differences in the response of HR, $U_{Na}V$, or UO to infusion of saralasin or AII were seen between the two groups of rats.

Figure 35. Cardiovascular and fluid/electrolyte responses to chronic ivt saralasin infusion in normal sodium rats. Rats were maintained on a sodium intake of 1.5 mEq/day. Units on the abscissa are days. C1 and C2 are two control days, A1-A5 are five days of ivt infusion, R1 and R2 are recovery days. Rats received either saralasin (12 μg/hr, n=5) or saline ivt (n=5) during the ivt infusion period. Vertical bars on C2 value represent SEM for individual within groups comparisons. Numbers in parentheses indicate the volume of water ingested during the final 24 hr of ivt infusion (mean ± SEM). Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (randomized block ANOVA and lsd).

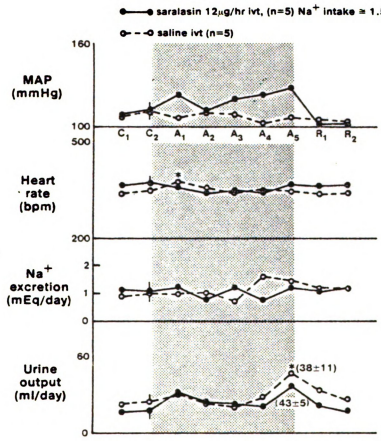
saralasin 12μg/hr ivt, (n=5) Na+ intake ≈ 1.5 mEq/day
saline ivt (n=5)
MAP (mmHg)
160
100
C1 C2 A1 A2 A3 A4 A5 R1 R2
Heart rate (bpm)
500
200
Na+ excretion (mEq/day)
2
1
0
Urine output (ml/day)
60
0
*(38±11)
(43±5)

Figure 35

Figure 36. Cardiovascular and fluid/electrolyte responses to chronic ivt saralasin infusion in high sodium rats. Rats were maintained on a sodium intake of 7.5 mEq/day. Rats received either saralasin (12 μg/hr, n=6) or saline (n=7) during the ivt infusion period. Vertical bars on C2 value indicate SEM for individual within groups comparisons. Values in parentheses represent the volume of water ingested during the final 24 hr of ivt infusion (mean ± SEM). Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (randomized block ANOVA and lsd). Other symbols as in Figure 34.

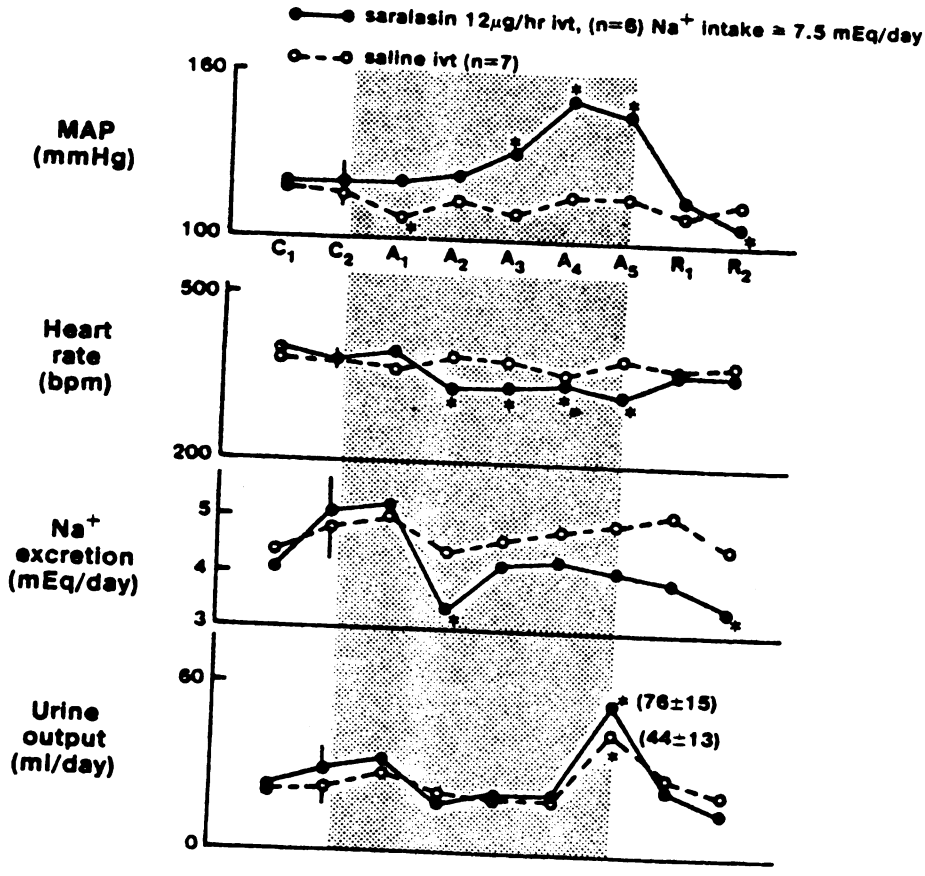
saralasin 12μg/hr ivt, (n=6) Na+ intake ≈ 7.5 mEq/day
saline ivt (n=7)
MAP (mmHg)
160
100
C1 C2 A1 A2 A3 A4 A5 R1 R2
Heart rate (bpm)
500
200
Na+ excretion (mEq/day)
5
4
3
Urine output (ml/day)
60
0
(76±15)
(44±13)

Figure 36

Figure 37. Effect of chronic intravenous saralasin infusion (18 μg/hr) on cardiovascular and fluid/electrolyte parameters. Rats received intravenous infusions of saralasin (18 μg/hr) or saline during the 7day infusion period (denoted S1-S7). Vertical bars on C2 value indicate SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (randomized block ANOVA and lsd). Other symbols as in Figure 34.

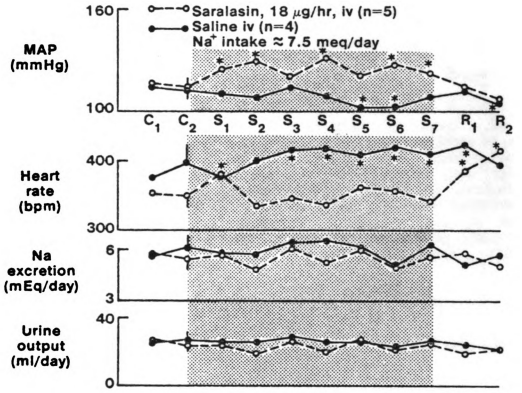
Saralasin, 18 μg/hr, iv (n=5)
Saline iv (n=4)
Na+ intake ≈ 7.5 meq/day
MAP (mmHg)
160
100
C1 C2 S1 S2 S3 S4 S5 S6 S7 R1 R2
Heart rate (bpm)
400
300
Na excretion (mEq/day)
6
3
Urine output (ml/day)
40
0

Figure 37

Figure 38. Comparison of cardiovascular and fluid/electrolyte responses to chronic ivt saralasin and AII. Rats were maintained on high sodium intake and received either AII (1 μg/hr, n=5) or saralasin (12 μg/hr, n=6) during the ivt infusion period. Vertical bars on C2 value indicate SEM for individual within groups comparisons. Values in parentheses represent the volume of water ingested during the final 24 hr of ivt infusion (mean ± SEM). Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (randomized block ANOVA and lsd). Other symbols as in Figure 34.

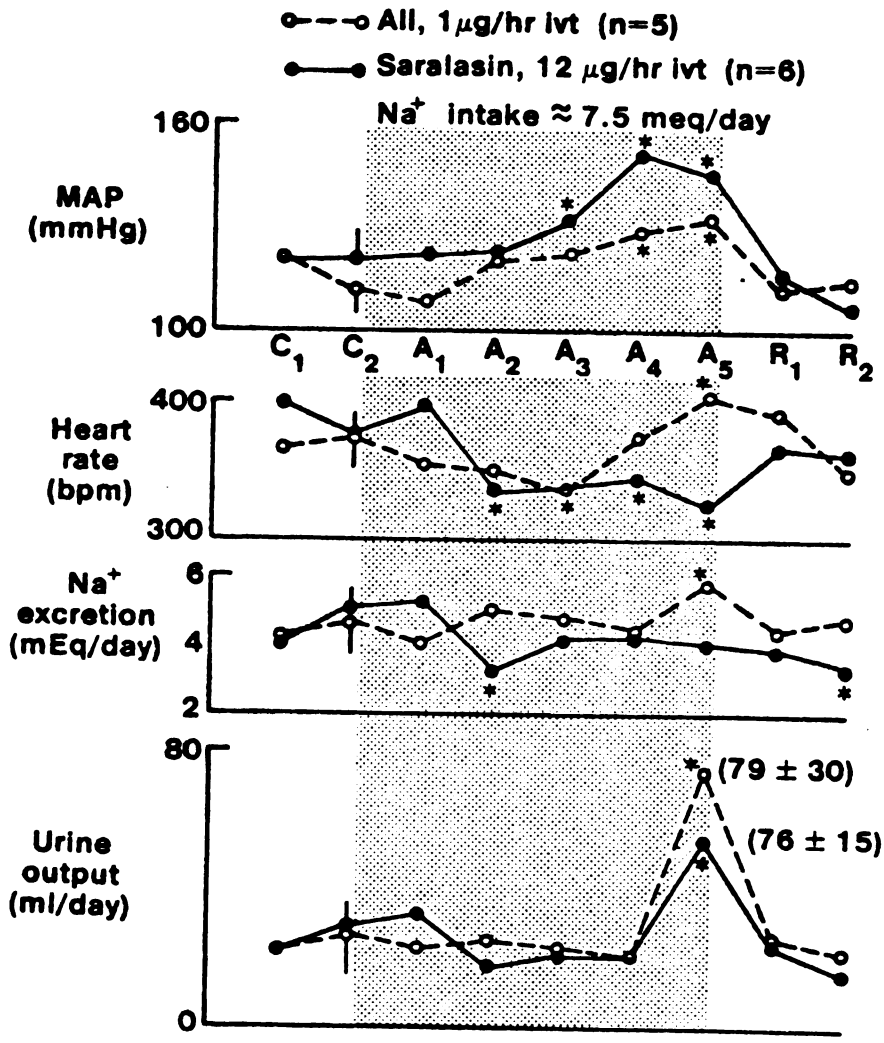
AII, 1μg/hr ivt (n=5)
Saralasin, 12 μg/hr ivt (n=6)
Na+ intake ≈ 7.5 meq/day
MAP (mmHg)
160
100
C1 C2 A1 A2 A3 A4 A5 R1 R2
Heart rate (bpm)
400
300
Na+ excretion (mEq/day)
6
4
2
Urine output (ml/day)
80
0
(79 ± 30)
(76 ± 15)

Figure 38

2. Chronic ivt sarthran

a. Dose determination: Sprague-Dawley

The effect of chronic ivt sarthran infusion at various doses on the pressor responses to acute iv and ivt AII is depicted in Figure 39. Infusion of sarthran at a dose of 100 ng/hr ivt for 5 days did not effect the pressor response to acute iv AII infusion. However, this dose of sarthran did produce a significant attenuation of the pressor response to an acute ivt bolus of AII. Chronic ivt sarthran infusion at 300 ng/hr for 5 days did not alter the pressor response to iv or ivt AII. Marked suppression of the pressor response to acute ivt AII was obtained after 5 days of ivt sarthran infusion at doses of 1 μg/hr and 6 μg/hr. However, the 6 μg/hr dose produced significant inhibition of iv AII pressor responses at all 3 doses of AII infused. In contrast, the 1 μg/hr dose produced significant inhibition of the iv AII dose-response curve only at the highest infusion rate of AII. In view of the above findings, the ivt sarthran dose of 1 μg/hr for 5 days was chosen as the optimum dose that would produce relatively complete functional blockade of brain AII receptors with minimal antagonism of peripheral vascular receptors. This dose of sarthran was used in all subsequent experiments.

b. Effects in high-sodium rats

Figure 40 shows the cardiovascular and fluid/electrolyte responses to a 5-day ivt sarthran infusion (1 μg/hr) in rats maintained on a high sodium intake (n=8). MAP and HR were not significantly changed by the ivt sarthran infusion. Urinary sodium excretion also was basically unchanged although on the third day of ivt sarthran infusion,

Figure 39. Effect of chronic ivt sarthran infusion on pressor responses to acute ivt and iv AII administration. Change in mean arterial pressure (ΔMAP) was measured in response to 150 ng AII ivt (left panel) and 10, 30, and 100 ng/min AII iv (right panel). Filled symbols are control responses and open symbols are responses in the same group of rats after the 5-day ivt infusion period. Doses of sarthran infused were: 6 μg/hr (n=4, top panel), 1 μg/hr (n=5, second panel), 300 ng/hr (n=6, third panel), and 100 ng/hr (n=5, bottom panel). Vertical bars represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from corresponding control value (paired t-test for ivt response; mixed design ANOVA and lsd for iv dose-response curves).

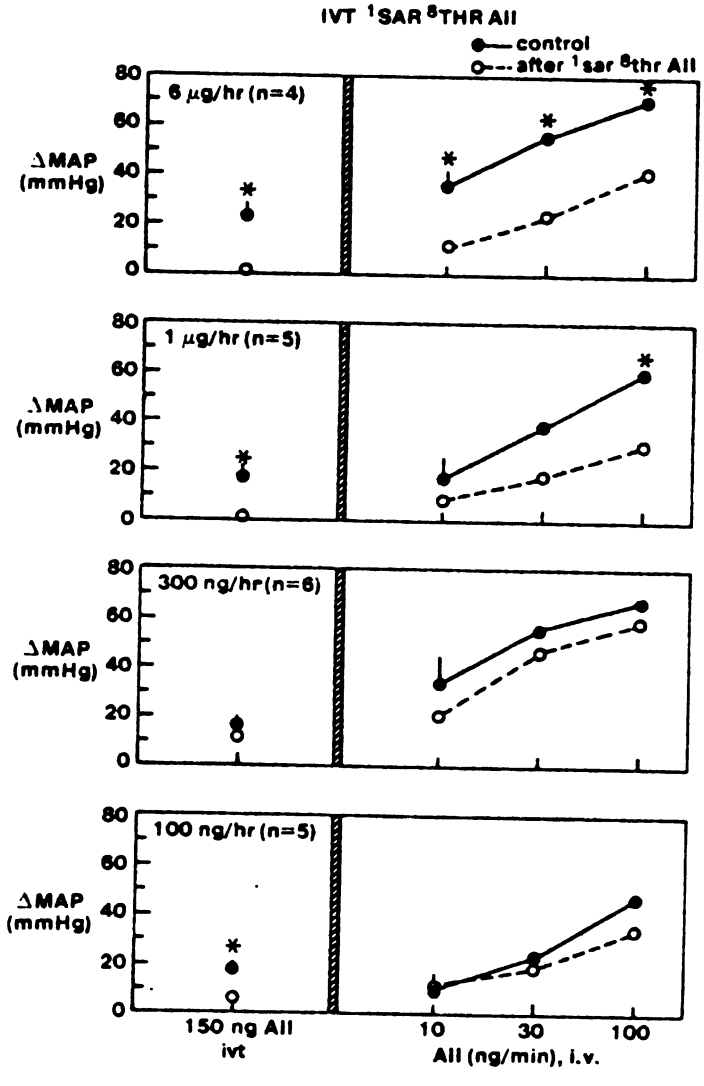
IVT [1]SAR [8]THR AII
control
after [1]sar [8]thr AII
6 μg/hr (n=4)
1 μg/hr (n=5)
300 ng/hr (n=6)
100 ng/hr (n=5)
ΔMAP (mmHg)
0
20
40
60
80
150 ng AII ivt
10
30
100
AII (ng/min), i.v.

Figure 39

Figure 40. Cardiovascular and fluid/electrolyte responses to chronic ivt sarthran infusion (1 μg/hr) in rats on high sodium intake. C1 and C2 are control days, S1-S5 are 5 days of ivt sarthran infusion (shaded area, R1 and R2 are recovery days. MAP = mean arterial pressure, HR = heart rate, UO = urine output. Vertical bars on C2 value indicate SEM for within groups comparisons (n=8). Asterisk (*) represents significant ($p<0.05$) difference from average of C1 and C2 values (randomized block ANOVA and lsd).

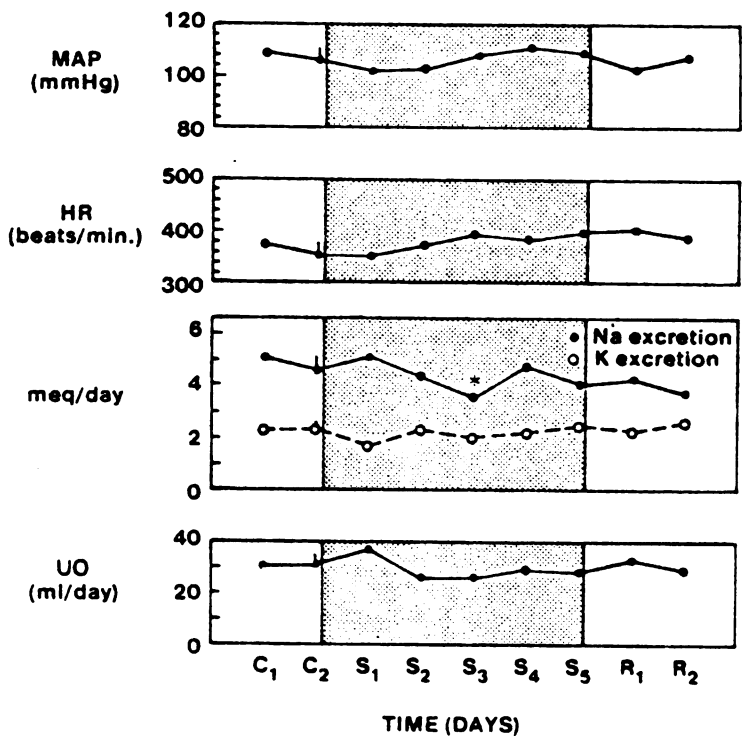
MAP
(mmHg)
120
100
80
HR
(beats/min.)
500
400
300
meq/day
6
4
2
0
Na excretion
K excretion
*
UO
(ml/day)
40
20
0
C1 C2 S1 S2 S3 S4 S5 R1 R2
TIME (DAYS)

Figure 40

sodium excretion fell significantly when compared to control values. Urinary potassium excretion and urine output also remained stable throughout the ivt sarthran infusion period.

c. Dose determination: spontaneously hypertensive rats

The pressor responses to acute ivt and iv AII administration before, during, and after 5-day ivt sarthran infusion (1 and 6 μg/hr) are depicted in Figure 41. The pressor response to 150 ng AII ivt was significantly reduced from a control value of 29 mmHg to 15 mmHg on the fifth day of ivt sarthran infusion (1 μg/hr, n=5; paired t-test). Recovery measurements were obtained 2 days after termination of ivt sarthran in 2 rats and averaged 30 mmHg in response to acute ivt AII. The pressor responses to graded iv infusions of AII were not significantly different on the fifth day of ivt sarthran infusion when compared to control and recovery days (mixed design ANOVA and lsd). Chronic ivt infusion of sarthran at a dose of 6 μg/hr (n=6) caused a reduction in the acute ivt AII pressor response from 35 mmHg to 9 mm Hg. The response to ivt AII on the second day after pump removal (29 mmHg) was restored to a value not significantly different from the control value. The dose-response curves for acute iv AII were not significantly different at any time. As shown in Table 7, resting MAP was elevated by 20-25 mmHg on the fifth day of ivt sarthran infusion at either dose when compared to control values.

3. Chronic ivt teprotide

a. Dose determination: Sprague-Dawley

Figure 42 depicts the pressor responses to acute ivt and iv AI administration before and on the fifth day of ivt teprotide infusion

Figure 41. Effect of chronic ivt sarthran infusion on pressor responses to acute ivt and iv AII in SHR. Sarthran was infused continuously ivt at 1 μg/hr (n=5, upper panel) or 6 μg/hr (n=6, lower panel). C = control response, S = response on fifth day of ivt sarthran infusion, R = recovery response. Change in mean arterial pressure (ΔMAP) was measured in response to 150 ng AII ivt (histograms) and to 10, 30, and 100 ng/min AII iv (line plots). Vertical bars represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from control response (randomized block ANOVA for ivt response, mixed design ANOVA and lsd for iv dose-response).

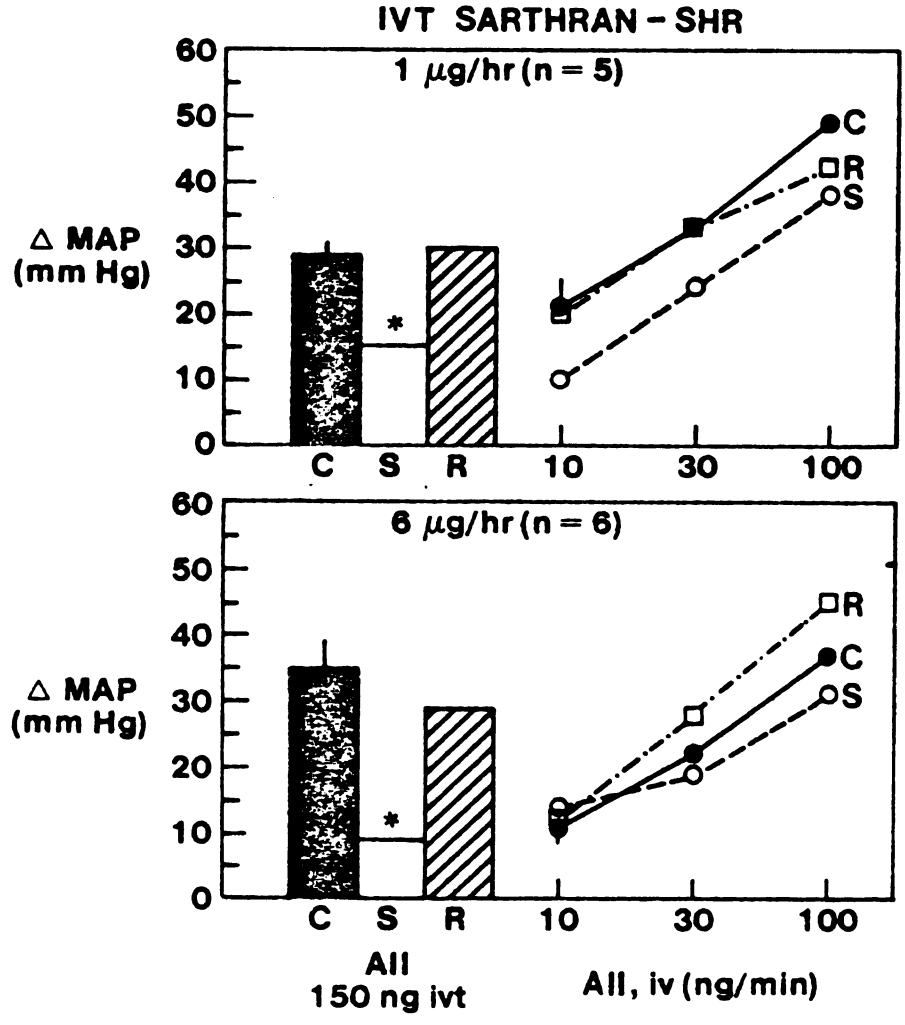
IVT SARTHRAN - SHR
1 μg/hr (n = 5)
6 μg/hr (n = 6)
Δ MAP (mm Hg)
Δ MAP (mm Hg)
60
50
40
30
20
10
0
C
S
R
10
30
100
*
AII 150 ng ivt
AII, iv (ng/min)

Figure 41

TABLE 7

Resting Mean Arterial Pressure Before, During, and After ivt Sarthran or Teprotide Infusion in SHR

Treatment	Control	Day 5-ivt	Recovery
Sarthran 1 μg/hr ivt (n=5)	162±8	183*	---
Sarthran 6 μg/hr ivt (n=6)	161±5	186*	155
Teprotide 10 μg/hr ivt (n=6)	152±5	174*	156

Values represent mean ± SEM for individual within groups comparisons. Asterisk (*) represents significant ($p<0.05$) difference from control value (randomized block ANOVA and lsd).

Figure 42. Effect of chronic ivt teprotide infusion on pressor responses to acute ivt and iv AI administration. Teprotide was infused continuously ivt at 1 μg/hr (n=4, upper panel), 3 μ/hr (n=3, middle panel), and 10 μg/hr (n=5, lower panel). Change in mean arterial pressure (ΔMAP) was measured in response to 100 ng AI ivt (left side) and to 3, 10, 30, and 100 ng/min AI iv (right side). Filled symbols represent control responses and open symbols represent responses in the same group of rats on the fifth day of ivt teprotide infusion. Vertical bars represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from control value (paired t-test for ivt response, mixed design ANOVA and lsd for iv dose-response).

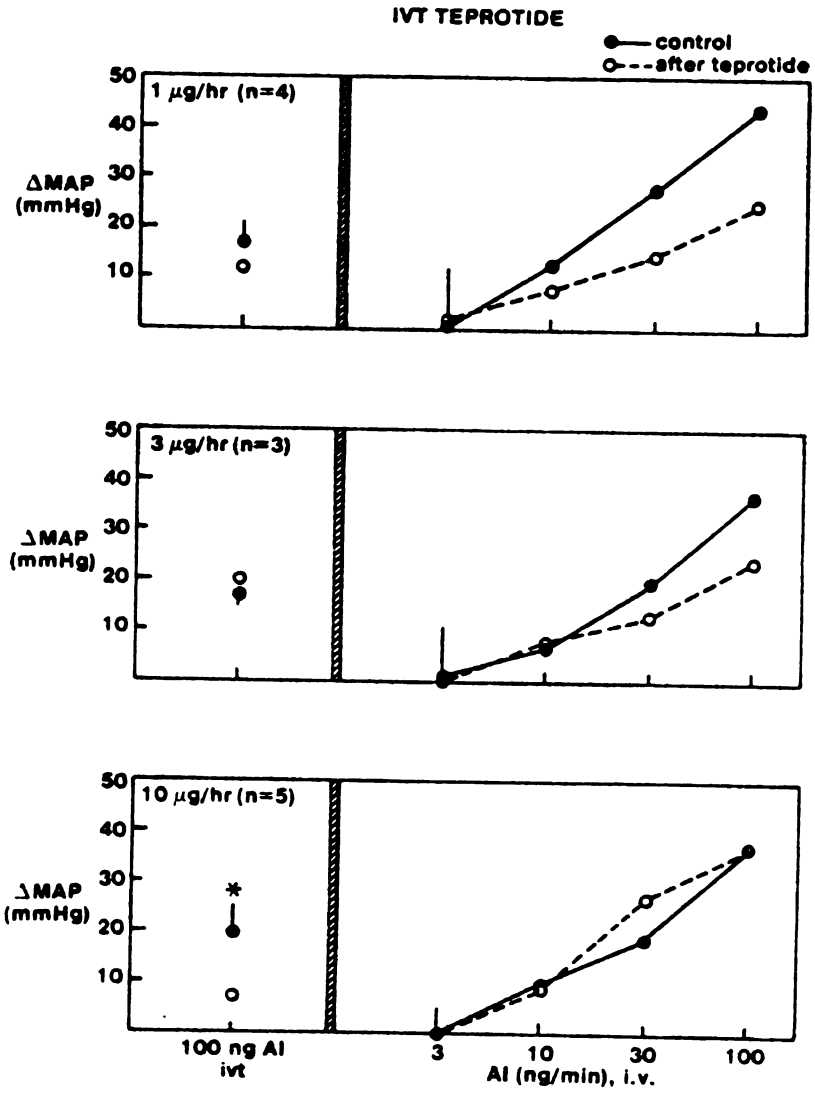
IVT TEPROTIDE
control
after teprotide
1 μg/hr (n=4)
3 μg/hr (n=3)
10 μg/hr (n=5)
ΔMAP (mmHg)
50
40
30
20
10
100 ng AI ivt
3
10
30
100
AI (ng/min), i.v.

Figure 42

at 1, 3, or 10 μg/hr. Infusion of teprotide at any dose did not produce a significant reduction in the pressor response to intravenous AI infusion. The pressor response to ivt AI injection was not significantly different before or on the fifth day of ivt teprotide infusion at the 1 and 3 μg/hr doses. However, the pressor response to ivt AI was significantly reduced on the fifth day of ivt teprotide infusion at 10 μg/hr when compared to the control response.

b. Dose determination: spontaneously hypertensive rats

The effect of 5-day ivt infusion of teprotide (10 μg/hr) on the pressor responses to ivt and iv AI administration is shown in Figure 43. On the fifth day of ivt teprotide infusion, the pressor response to ivt AI was slightly, but significantly decreased when compared to the control response (22 vs. 30 mmHg, respectively). Two days after stopping the ivt teprotide infusion, the pressor response to ivt AI was significantly elevated when compared to control (38 vs. 30 mmHg, respectively). The dose-response relationship for intravenous infusions of AI was unaltered by ivt teprotide infusion. The pressor response to ivt injection of AII (150 ng) averaged 26±3 mmHg on the fifth day of ivt teprotide infusion and 27 mmHg on the second recovery day (no significant difference, paired t-test). The fact that ivt teprotide infusion caused significant changes in the pressor response to ivt AI but not to ivt AII verifies that the effect of teprotide was specific for the conversion of AI to AII, and did not represent a general decrease in responsiveness to AII.

4. Chronic ivt sarthran/chronic iv angiotensin II

Cardiovascular and fluid/electrolyte responses to chronic intravenous AII infusion (10 ng/min) in the presence or absence of chronic

Figure 43. Effect of chronic ivt teprotide infusion on pressor responses to acute ivt and iv AI administration in SHR. Teprotide was infused continuously ivt at 10 μg/hr (n=6). C = control response, T = response on fifth day of ivt teprotide infusion, R = recovery response. Change in mean arterial pressure (ΔMAP) was determined in response to 100 ng AI ivt (histograms) and to 10, 30, and 100 ng/min AI iv (line plots). Vertical lines represent SEM for individual within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from control response (randomized block ANOVA for ivt response, mixed design ANOVA and lsd for iv dose-response).

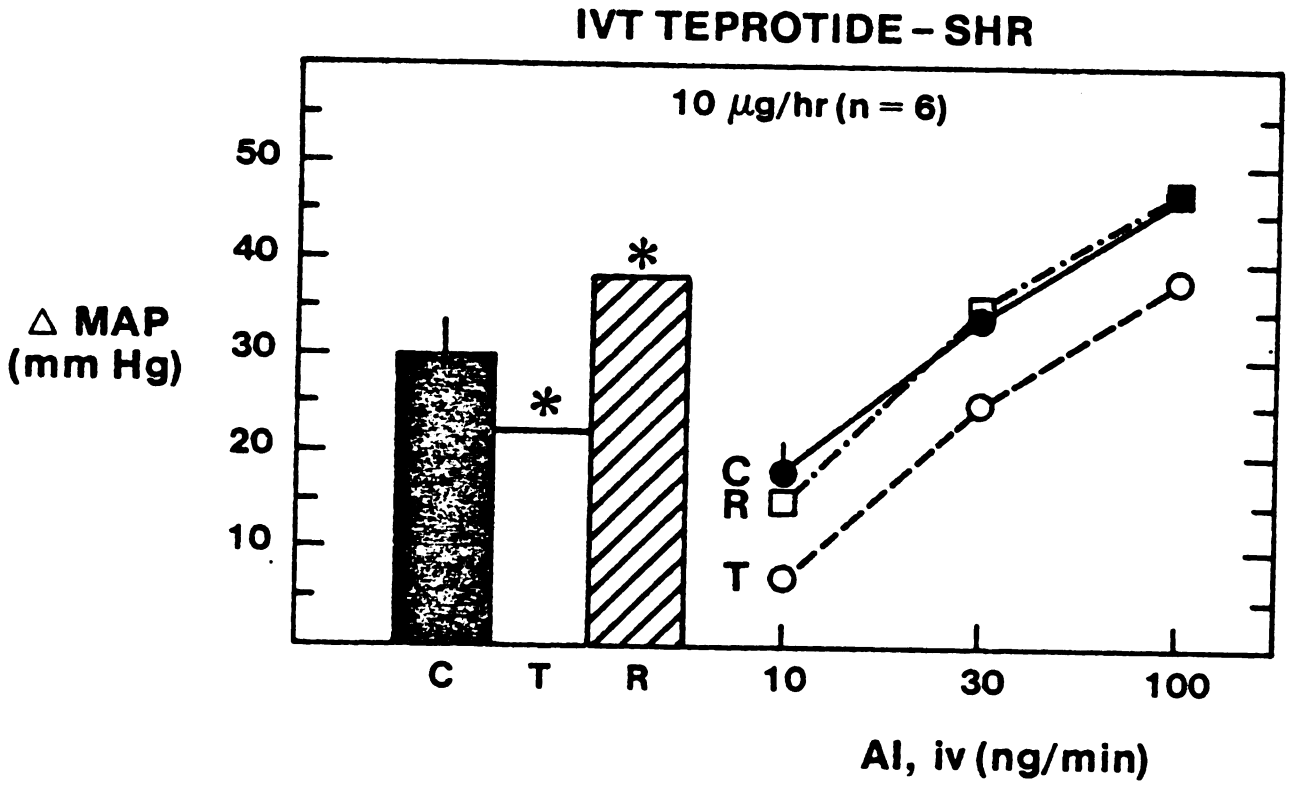
IVT TEPROTIDE - SHR
10 μg/hr (n = 6)
Δ MAP
(mm Hg)
50
40
30
20
10
*
*
C
R
T
C
T
R
10
30
100
AI, iv (ng/min)

Figure 43

ivt sarthran infusion are shown in Figure 44. Chronic iv AII infusion at this dose produced a sustained increase in MAP in rats receiving saline ivt. The hypertensive response to iv AII infusion in rats receiving sarthran ivt was similar, however, this group of rats did not exhibit a statistically significant increase in MAP until the fourth day of iv AII infusion. In addition, there was a significant difference in MAP between saline- and sarthran-treated rats on the first day of iv AII infusion. Rats receiving sarthran exhibited a significant decrease in $U_{Na}V$ on the first day of AII infusion, but $U_{Na}V$ was unchanged thereafter. There were no significant changes in any of the other variables measured (HR, U_KV or UO) in either saline- or sarthran-treated rats in response to chronic iv AII infusion. Figure 45 depicts responses of saline- and sarthran-treated rats to chronic iv AII infusion at a dose of 20 ng/min. In this experiment, there were virtually no differences between the two groups of rats in their response to AII. MAP was significantly elevated to the same extent in both groups of rats, while HR, $U_{Na}V$, U_KV, and UO were unchanged in both groups over the course of the experiment.

5. Chronic ivt sarthran/DOC-salt hypertension

The hypertensive response to DOC-salt treatment in rats receiving chronic ivt sarthran infusion (1 μg/hr) is reported in Table 8. A significant increase in tail cuff blood pressure was observed in both saline- and sarthran-treated rats in response to DOC administration. The increase in blood pressure was evident 4 days after DOC injection (the first point at which it was measured) and persisted in both groups of rats for the duration of the study (3.5 wk). At no time was there a significant difference in blood pressure between rats receiving

Figure 44. Effect of chronic ivt sarthran infusion (1 μg/hr) on cardiovascular and fluid/electrolyte responses to chronic iv AII infusion (10 ng/min). Rats received either sarthran (1 μg/hr, n=8) or saline ivt (n=8) during the entire protocol. MAP = mean arterial pressure, HR = heart rate, UNAV = urinary sodium excretion, UKV = urinary potassium excretion, UO = urine output. Units of the abscissa are days. A1-A5 (shaded area) are the 5 days of iv AII infusion. Vertical lines on C2 value represent SEM for within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values. Cross (+) indicates significant difference between sarthran- and saline-infused rats (mixed design ANOVA and lsd).

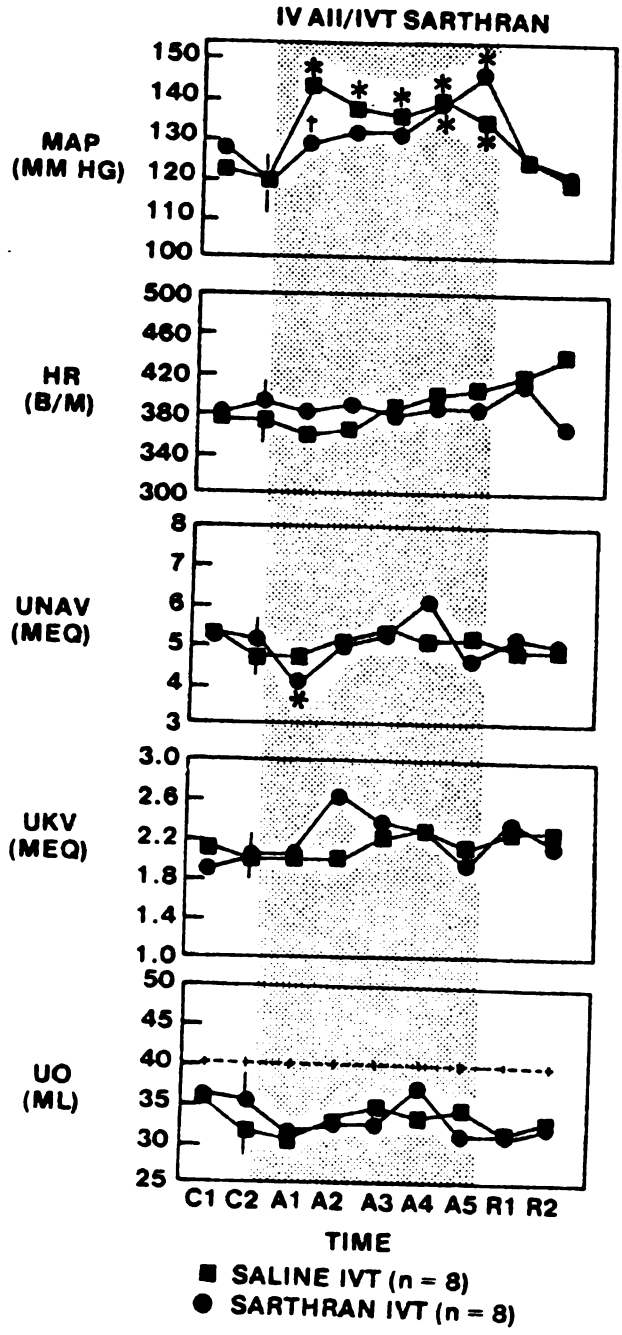
IV AII/IVT SARTHRAN
MAP (MM HG)
150
140
130
120
110
100
HR (B/M)
500
460
420
380
340
300
UNAV (MEQ)
8
7
6
5
4
3
UKV (MEQ)
3.0
2.6
2.2
1.8
1.4
1.0
UO (ML)
50
45
40
35
30
25
C1 C2 A1 A2 A3 A4 A5 R1 R2
TIME
SALINE IVT (n = 8)
SARTHRAN IVT (n = 8)

Figure 44

Figure 45. Effect of chronic ivt sarthran infusion (1 μg/hr) on cardiovascular and fluid/electrolyte responses to chronic iv AII infusion (20 ng/min). Rats received either sarthran (1 μg/hr, n=8) or saline ivt (n=8) during the entire protocol. MAP = mean arterial pressure, HR = heart rate, UNAV = urinary sodium excretion, UKV = urinary potassium excretion, UO = urine output. Units of the absicssa are days. A1-A5 (shaded area) are the 5 days of iv AII infusion. Vertical lines on C2 value represent SEM for within groups comparisons. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (mixed design ANOVA and lsd).

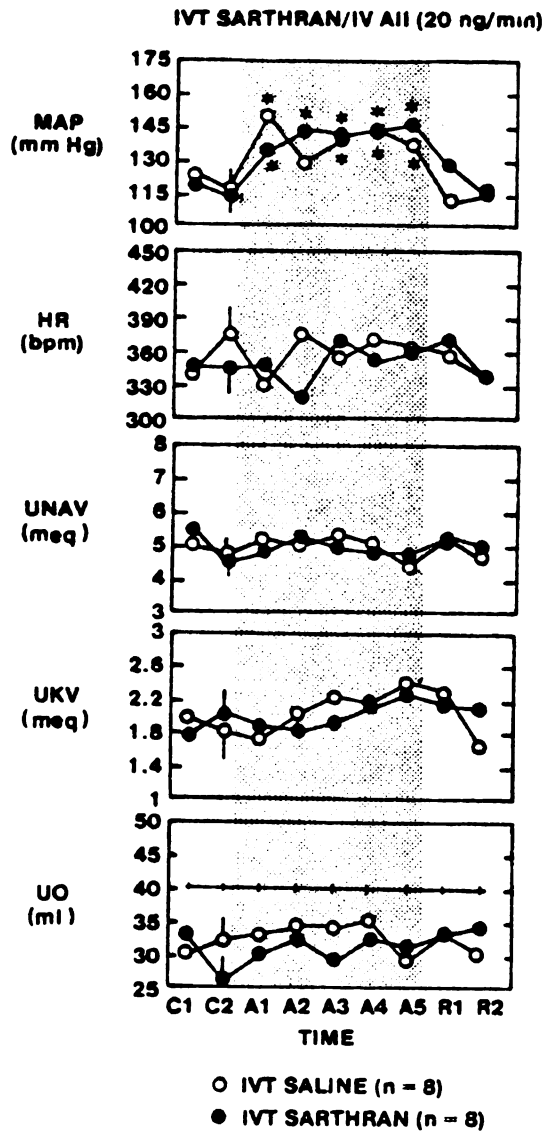
IVT SARTHRAN/IV AII (20 ng/min)
MAP (mm Hg)
175
160
145
130
115
100
HR (bpm)
450
420
390
360
330
300
UNAV (meq)
8
7
6
5
4
3
UKV (meq)
3
2.6
2.2
1.8
1.4
1
UO (ml)
50
45
40
35
30
25
C1 C2 A1 A2 A3 A4 A5 R1 R2
TIME
IVT SALINE (n = 8)
IVT SARTHRAN (n = 8)

Figure 45

TABLE 8

Effect of Chronic ivt Sarthran Infusion on DOC-salt Hypertension

	Tail cuff blood pressure (mmHg)								
	C1	C2	P1	P2	P3	P4	P5	P6	P7
Saline ivt (n=10)	140±5	141	154*	153*	175*	167*	178*	187*	196*
Sarthran ivt (n=6)	139±7	143	161*	157*	177*	181*	187*	193*	185*

Values represent mean ± SEM (within groups). Average SEM for between groups comparisons = 7.2. C1 and C2 represent control measurements, P1-P7 represent measurements made after the start of DOC-salt treatment. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (mixed design ANOVA and lsd).

chronic ivt sarthran infusions and rats receiving ivt saline infusions. Body weight also did not differ between the two groups (not shown). The arterial pressure responses to sequential administration of AVP antagonist and hexamethonium in DOC-salt rats are shown in Table 9. Resting MAP (direct catheterization) tended to be higher in DOC-salt rats receiving sarthran ivt than in saline controls, although this difference was not significant. AVP antagonist did not cause a change in MAP in either group of rats. Subsequent administration of hexamethonium produced depressor responses in both groups of rats. MAP was significantly higher in sarthran-treated rats than in saline-treated rats after combined AVP antagonist-hexamethonium treatment.

6. Chronic ivt sarthran/spontaneously hypertensive rats

The effect of chronic ivt infusion of sarthran or isotonic saline on tail cuff blood pressure in SHR is shown in Figure 46. Infusion of sarthran at 1 μg/hr, ivt for 2 weeks followed by 1 week of ivt sarthran infusion at 6 μg/hr had no significant effect on blood pressure in adult SHR. Similarly, blood pressure was not affected by ivt infusion of vehicle (isotonic saline). Body weight was stable in both groups over the experimental period.

7. Chronic ivt teprotide/spontaneously hypertensive rats

Teprotide (10 μg/hr, ivt) produced no significant changes in tail cuff blood pressure over a 1 week continuous infusion period in adult SHR (Figure 47). Infusion of the saline vehicle also was without effect on blood pressure. Body weight was stable in both groups of rats.

TABLE 9

Effect of AVP Antagonist and Hexamethonium on Blood Pressure in DOC-salt Hypertensive Rats

	Resting	Mean Arterial Pressure (mmHg)			
		AVP Antagonist		Hexamethonium	
		1 min	5 min	1 min	5 min
Saline ivt (n=10)	167±5	166	167	73*	80*
Sarthran ivt (n=6)	181±7	182	179	87*+	106*+

Values represent mean ± SEM (within groups). Average SEM for individual between groups comparisons = 7.2. See text for description of experimental protocol. Asterisk (*) indicates significant ($p<0.05$) difference from resting value. Cross (+) indicates significant difference between saline ivt and sarthran ivt rats (mixed design ANOVA and lsd).

Figure 46. Tail cuff blood pressure and body weight responses of SHR to ivt sarthran infusion (1 μg/hr for 2 weeks followed by 6 μg/hr for 1 week). Rats received either saline (n=9) or sarthran ivt (n=7) for the infusion period denoted by the shaded area. Vertical bars represent SEM for individual within groups comparisons (mixed design ANOVA).

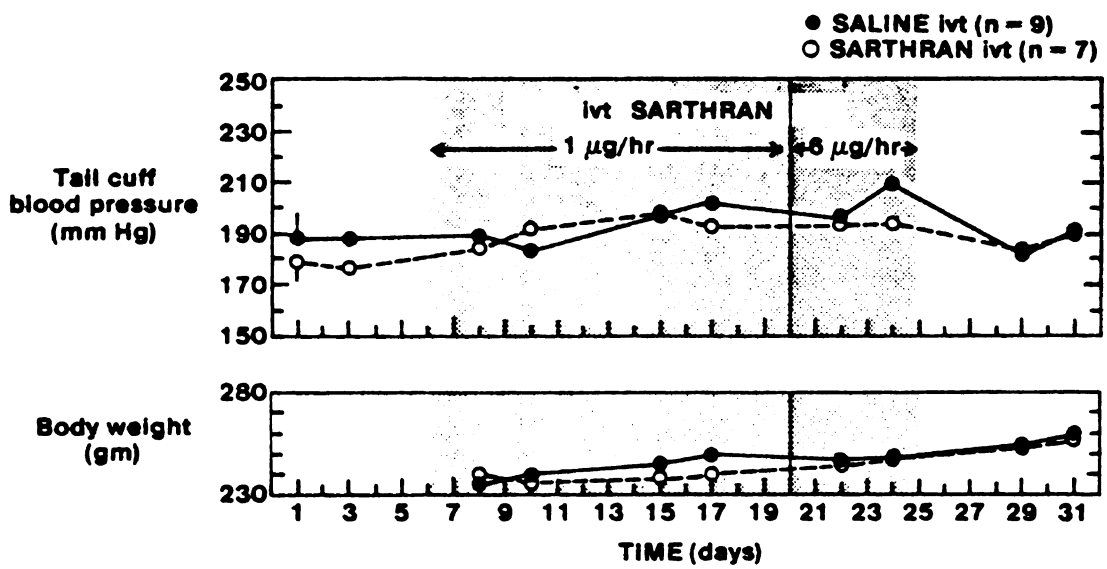
● SALINE ivt (n = 9)
○ SARTHRAN ivt (n = 7)
ivt SARTHRAN
1 µg/hr
6 µg/hr
Tail cuff blood pressure (mm Hg)
250
230
210
190
170
150
Body weight (gm)
280
230
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
TIME (days)

Figure 46

Figure 47. Tail cuff blood pressure and body weight responses of SHR to ivt teprotide infusion (10 μg/hr for 1 week). Rats received either saline (n=15) or teprotide ivt (n=10) for a 7-day interval denoted by the shaded area. Vertical bars on day 1 value represent SEM for individual within groups comparisons. Asterisk (*) indicates significant difference from average of control week values (mixed design ANOVA and lsd).

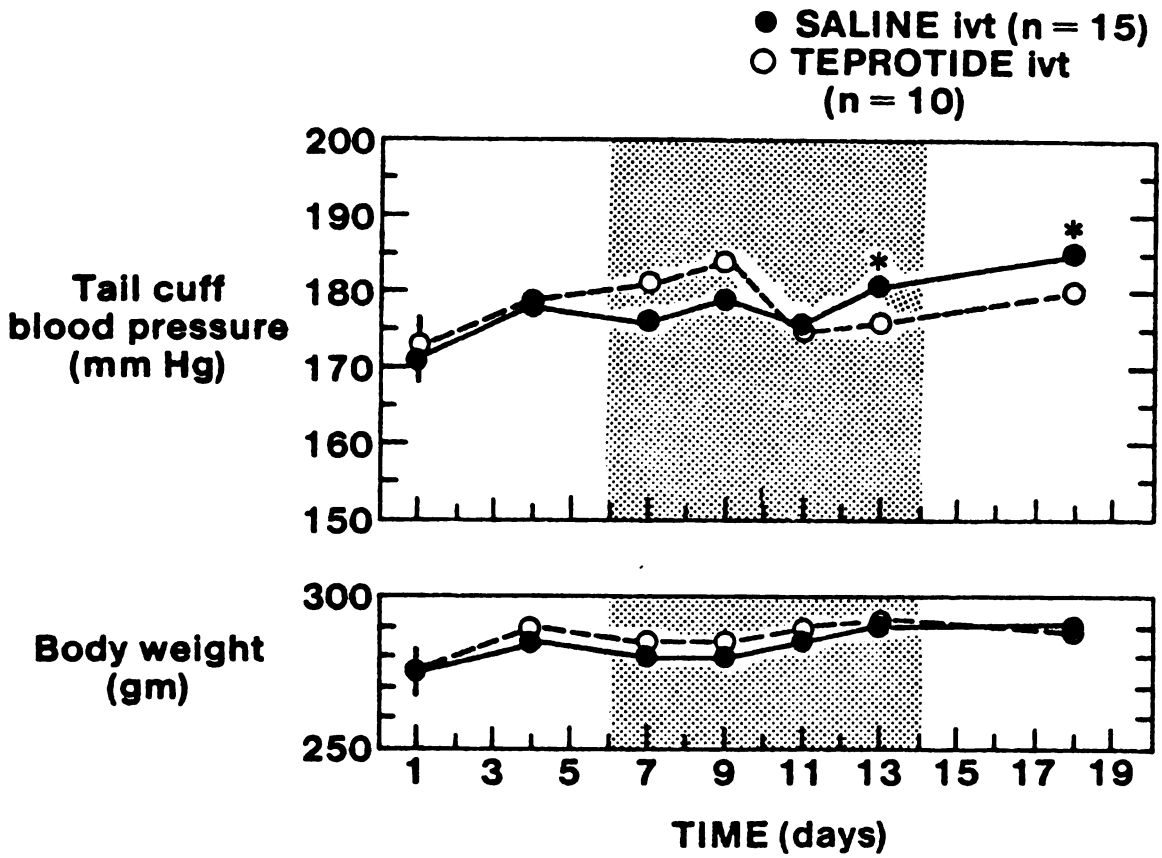
● SALINE ivt (n = 15)
○ TEPROTIDE ivt (n = 10)
Tail cuff blood pressure (mm Hg)
200
190
180
170
160
150
*
*
Body weight (gm)
300
250
1
3
5
7
9
11
13
15
17
19
TIME (days)

Figure 47

D. Brain Lesion Studies

1. Subfornical organ lesions

The results of 5 days of continuous AII infusion (10 ng/min, iv) in normal rats and SFO-ablated rats are illustrated in Figure 48. In control rats receiving only saline iv, MAP, HR, WI, $U_{Na}V$, and $U_{K}V$ were unchanged during the 5-day infusion period when compared to values obtained on the control days. In both normal and SFO-lesion rats, MAP rose significantly during the 5-day iv AII infusion period and returned to control levels within 24 hr after the infusion was stopped. In SFO-lesion rats, MAP was higher than in normal rats during the entire period of iv AII infusion, this difference reaching statistical significance only on days 1, 4, and 5. Normal rats exhibited a gradual rise in HR over the period of iv AII infusion, reaching statistical significance by the fifth day of iv AII administration, and HR remained elevated on the 2 recovery days. No significant change in HR was observed in SFO-lesion rats at any time during the protocol. The administration of AII did not produce changes in WI in normal rats, whereas WI was significantly increased by the third day in SFO-lesion rats and remained significantly elevated for the remainder of the AII infusion period. Water intake returned to normal within 24 hr after stopping the AII infusion in SFO-lesion rats. In all groups of rats, urine output paralleled water intake; therefore, water balance remained unchanged throughout the experiment (not shown). Neither $U_{Na}V$ nor $U_{K}V$ were significantly changed by AII infusion in either normal or SFO-lesion rats.

2. Knife cut of SFO efferents

Cardiovascular and fluid/electrolyte responses to chronic intravenous AII infusion (10 ng/min) in rats with knife cuts of SFO

Figure 48. Effect of chronic iv AII infusion on cardiovascular and fluid/electrolyte responses in rats with electrolytic ablation of the subfornical organ. MAP = mean arterial pressure, HR = heart rate, WI = water intake, UNAV = urinary sodium excretion, UKV = urinary potassium excretion. Units of the time axis are days, where C1 and C2 are control days, A1-A5 represent the iv infusion period (shaded area); R1 and R2 are recovery days. CONTROL rats (n=8) received only saline during the iv infusion period. SHAM/AII rats (n=7) received AII (10 ng/min) during the iv infusion period, and LESION/AII rats (n=9) underwent prior electrolytic ablation of the SFO and received AII (10 ng/min) during the iv infusion period. Vertical bars on C2 value indicate SEM for individual within groups comparisons, and bars at upper right of each panel indicate SEM for any between group comparison on a given day. Asterisk (*) denotes significant ($p<0.05$) difference from average of C1 and C2 values. Cross (+) indicates significant difference between SHAM/AII and LESION/AII groups (mixed design ANOVA and lsd).

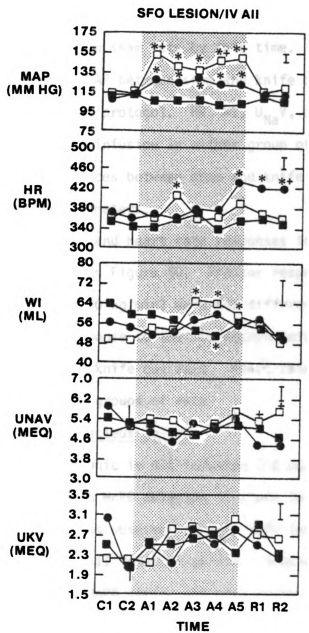
SFO LESION/IV AII
MAP (MM HG)
175
155
135
115
95
75
HR (BPM)
500
460
420
380
340
300
WI (ML)
80
72
64
56
48
40
UNAV (MEQ)
7.0
6.2
5.4
4.6
3.8
3.0
UKV (MEQ)
3.5
3.1
2.7
2.3
1.9
1.5
C1 C2 A1 A2 A3 A4 A5 R1 R2
TIME
■ CONTROL (n = 8)
□ LESION/AII (n = 9)
● SHAM/AII (n = 7)

Figure 48

efferent pathways are illustrated in Figure 49. Both sham rats and knife cut rats exhibited significant increases in MAP over the 5-day iv AII infusion period. MAP was significantly elevated 24 hr after stopping the iv AII infusion in knife cut rats, but had returned to a value not different from control in sham rats by this time. There were no significant differences in MAP between sham and knife cut rats at any time during the experimental protocol. HR, WI, $U_{Na}V$, $U_{K}V$, and WB were unaltered by chronic iv AII infusion in either group of rats. Furthermore, there were no differences between sham and knife cut rats with respect to any of these parameters.

Arterial pressure and heart rate responses to acute graded iv infusions of AII are shown in Figure 50. Pressor responses to the two lower doses of AII (10 and 30 ng/min) were not different in sham and knife cut rats, but the response to the 100 ng/min infusion rate of AII was significantly higher in knife cut rats. Heart rate responses were not different between the 2 groups of rats.

3. Median preoptic nucleus lesion

The effect of chronic iv AII infusion (10 ng/min) on mean arterial pressure in rats with MnPO ablation is shown in Table 10. A sustained increase in MAP was observed in both MnPO lesion and sham lesion rats over the 5-day AII infusion period. However, there were no differences in MAP between MnPO lesion and sham lesion rats at any time during the protocol. Heart rate and water intake (not shown) did not change in either group in response to iv AII infusion, and there were no differences between groups with respect to these measures at any time.

Figure 49. Responses to chronic ivt AII infusion in rats with knife cuts of SFO efferent pathways. MAP = mean arterial pressure, HR = heart rate, FI = fluid intake, WB = water balance, UNAV = urinary sodium excretion, UKV = urinary potassium excretion. C1 and C2 are control days, A1-A5 (shaded area) represents the iv AII infusion period (10 ng/min), R1 and R2 are recovery days. SHAM = sham-operated rats, KC = rats with knife cut of SFO efferents. Vertical bars on C1 value indicate SEM for individual within groups comparisons. Asterisk (*) denotes significant difference from average of C1 and C2 values (mixed design ANOVA and lsd).

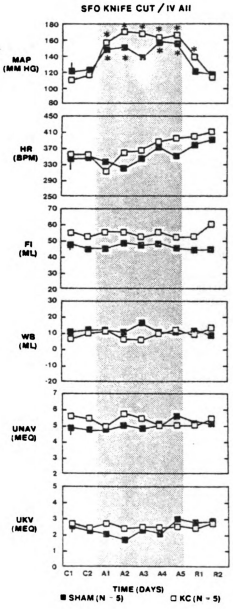
SFO KNIFE CUT / IV AII
MAP (MM HG)
HR (BPM)
FI (ML)
WB (ML)
UNAV (MEQ)
UKV (MEQ)
C1 C2 A1 A2 A3 A4 A5 R1 R2
TIME (DAYS)
SHAM (N = 5)
KC (N = 5)

Figure 49

Figure 50. Arterial pressure and heart rate responses to acute intravenous AII infusion in rats with knife cuts of SFO efferent pathways. ΔMAP = change in mean arterial pressure; ΔHR = change in heart rate; KC = knife cut rats; SHAM = sham-operated rats. Vertical bar on 10 ng/min value represents SEM for individual within groups comparisons. Average SEM for between groups comparisons at any given dose of AII was 5.7 for ΔMAP and 37.8 for ΔHR. Cross (+) represents significant ($p<0.05$) difference between SHAM and KC rats (mixed design ANOVA and lsd).

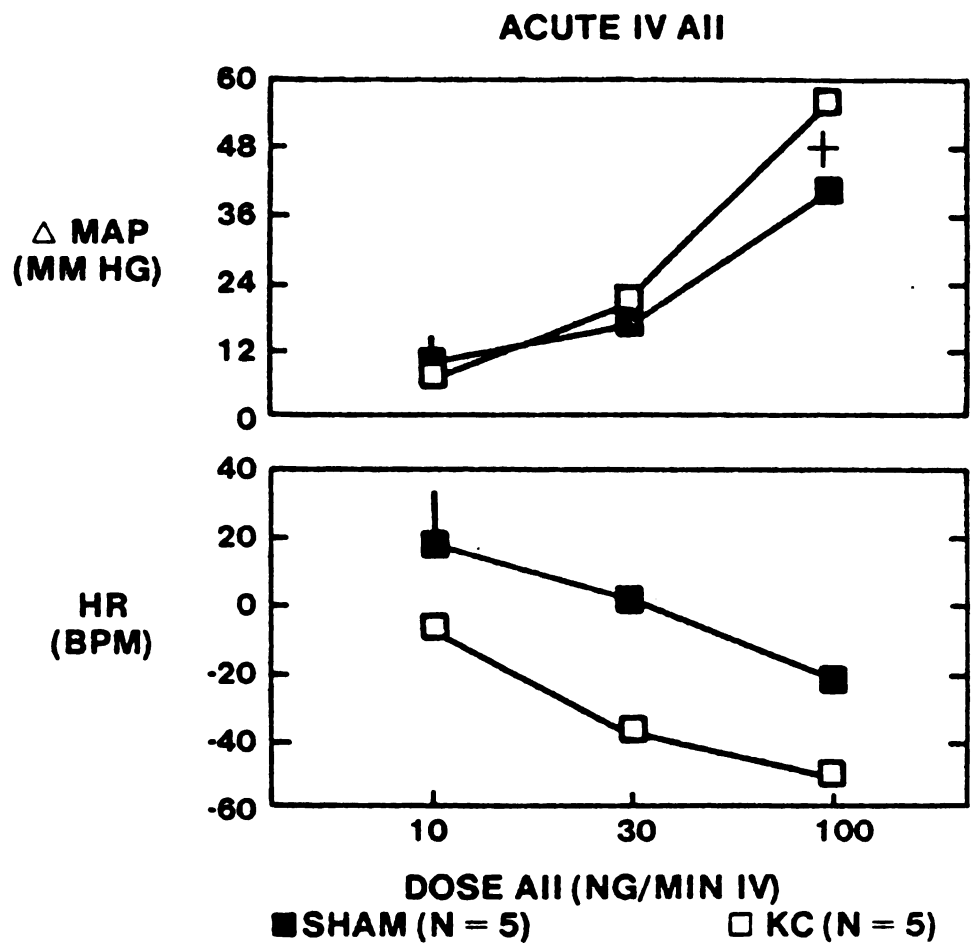
ACUTE IV AII
Δ MAP
(MM HG)
60
48
36
24
12
0
HR
(BPM)
40
20
0
-20
-40
-60
10
30
100
DOSE AII (NG/MIN IV)
SHAM (N = 5)
KC (N = 5)

Figure 50

TABLE 10

Effect of Chronic iv AII Infusion on Mean Arterial Pressure in Rats with Electrolytic Ablation of the Median Preoptic Nucleus

	MAP (mmHg)								
	C1	C2	A1	A2	A3	A4	A5	R1	R2
MnPOX (n=5)	110±10	108	149	142	150	150	140	108	110
Sham (n=12)	117± 6	112	150	148	152	152	154	115	108

Values represent mean ± SEM (within groups). Average standard error for between groups comparisons on any given day = 7.3. Asterisk (*) indicates significant ($p<0.05$) difference from average of C1 and C2 values (mixed design ANOVA and lsd).

DISCUSSION

A. Cardiovascular and Fluid/Electrolyte Response to Chronic ivt AII Infusion

Most literature concerning the central effects of AII in hypertension has focused on two areas: 1) the physiological effects produced by acute stimulation of brain AII receptors, and 2) the consequences of either acute or chronic antagonism of the central effects of AII in hypertensive animals. Such studies suggest that a central pressor effect of AII contributes to hypertension based on observations that acute ivt AII injections cause a rise in blood pressure (Severs et al., 1970; Unger et al., 1971) and that central administration of AII antagonists causes depressor responses in many models of hypertension (Sweet et al., 1976; Mann et al., 1978; McDonald et al., 1980). The argument that central actions of AII contribute to chronic hypertension would be strengthened by a demonstration that chronic central stimulation with AII alone will cause a sustained increase in arterial pressure. The results of the present experiments in rats and rabbits support previous work by others (Buckley et al., 1981; DiNicolantonio et al., 1981; Fink et al., 1982a) showing that chronic central administration of AII will produce a sustained rise in arterial pressure. In addition, these results demonstrate that the magnitude of the rise in arterial pressure in response to chronic ivt AII infusion in the rat is dose-dependent. Furthermore, the current experiments show that sodium

intake is a critical determinant of the magnitude of the hypertensive response to chronic ivt AII infusion. In the rat, high sodium intake enhances the hypertensive response to chronic ivt AII infusion over that observed in rats maintained on a "normal" sodium intake. The present work and a previous study (Fink et al., 1982a) demonstrated that chronic hypertension can be produced by long-term ivt AII infusion in rabbits on either normal- or high-sodium intake. This hypertensive response can be abolished by dietary sodium restriction. It also has been reported that the hypertensive response to repeated ivt injections of AII in the dog requires a high sodium intake (Buckley et al., 1981). However, the present report of sodium sensitivity of ivt AII-induced hypertension differs somewhat from the results of DiNicolantonio and coworkers (1982). They found that a 6-day ivt infusion of AII at 6 μg/hr resulted in a 70 mmHg rise in arterial pressure in rats maintained on a normal sodium intake. In contrast, under the conditions of the present experiments, ivt AII infusion at 6 μg/hr for 5 days produced a 25 mmHg rise in arterial pressure in normal-sodium rats and a 45 mmHg rise in high-sodium rats. The comparatively large increase in blood pressure seen by DiNicolantonio et al. in normal-sodium rats may be attributable to a strain difference, since these investigators used Wistar-Kyoto rats whereas Sprague-Dawley rats were used in the present study.

The mechanism by which high sodium intake causes sensitization to the chronic central pressor effect of AII is not known. An argument could be made that a significant amount of AII leaks from CSF into the peripheral circulation, and since the hypertensive response to chronic intravenous AII infusion is known to be sodium sensitive (Cowley and McCaa, 1976; Fink et al., 1982b) perhaps the sodium-sensitivity of ivt

AII-induced hypertension is a peripheral and not a central phenomenon. This is probably not the case since the present experiments showed that plasma AII levels were not consistently increased during chronic ivt AII infusion. Therefore, leak of AII from CSF into the bloodstream in amounts sufficient to cause a significant rise in plasma AII levels cannot completely account for either the hypertensive response to chronic ivt AII infusions or the sodium-sensitivity of this response. An alternate explanation for the sodium dependency of this form of hypertension is that changes in sodium intake may alter brain AII receptor binding characteristics. High sodium intake may increase the number and/or affinity of brain AII receptors. Conflicting evidence exists for this hypothesis. Mann and coworkers (1980a) found that low sodium intake blunted the pressor and dipsogenic effects of acute ivt AII administration, and decreased ^{125}I-AII binding *in vitro* to brain membrane preparations. However, Speth *et al.* (1984) reported no change in brain AII receptors in response to low sodium intake. Further work needs to be done to clarify the issue of whether changes in dietary sodium intake result in alterations in brain AII receptor binding characteristics, specifically in discrete regions known to be sensitive to AII.

Unlike the pressor response to chronic ivt AII infusion, the magnitude of the dipsogenic response does not appear to be sodium-sensitive. The dipsogenic response to AII (6 μg/hr, ivt) for 5 days was identical in normal-sodium and high-sodium rats. In rabbits, the volume of water drank in response to chronic ivt AII infusion, when expressed as a function of food intake, was actually higher in animals maintained on low sodium intake than in animals on high dietary sodium.

These findings are at variance with those of Buggy et al. (1979) and Andersson et al. (1971, 1972). They have shown that in the goat and rat, the dipsogenic response to acute ivt AII is enhanced by concomitant central osmotic stimulation with sodium ion. There are several possible explanations for the failure to demonstrate a sodium-sensitive dipsogenic response to chronic ivt AII infusion: 1) CSF-borne AII may act at two distinct AII receptor subtypes -- one subtype is "sodium sensitive" and mediates pressor responses and the other is "sodium-insensitive" and mediates drinking responses, 2) the greater magnitude of ivt AII-induced hypertension observed in high-sodium animals may suppress the drinking response, and 3) the doses of AII used in these experiments are supramaximal for the drinking response (Gronan and York, 1978), and a sodium-dependent dipsogenic response may be seen with much lower doses of AII.

The present experiments provide no evidence for dissociation of the pressor and dipsogenic effects of chronic ivt AII infusion in rats as seen by DiNicolantonio et al. (1982). These investigators observed an increase in water intake that persisted only for the first 3-4 days of ivt AII infusion, while the increase in blood pressure was maintained over the 6-day infusion period. In contrast, the present experiments in rats demonstrated that increases in both arterial pressure and water intake occurred within one day and were maintained during the 5 days of ivt infusion.

Although chronic ivt AII infusion causes increases in both blood pressure and fluid intake, studies reported here in rats on fixed fluid intake demonstrated that increased water drinking is not required

for a hypertensive response to chronic ivt AII. In addition, water balance and urinary sodium excretion were not consistently effected by ivt AII infusion. Strict electrolyte balance measurements were not performed in these experiments (dietary intake and fecal excretion were not quantified), however, additional experiments in which sodium intake was controlled by iv infusion have confirmed that ivt AII does not alter urinary sodium excretion over a 5-day period (unpublished results).

In rabbits, although water intake increased in response to ivt AII infusion, water balance, plasma volume and extracellular fluid volume were unchanged in animals on a high sodium intake. ECFV was increased in low-sodium rabbits by ivt AII infusion, but since these rabbits did not become hypertensive, the increased fluid volume alone apparently is not sufficient to produce an increase in arterial pressure. Rabbits maintained on high sodium exhibited no significant change in sodium balance during ivt AII infusion, whereas low-sodium rabbits experienced a significant sodium loss during the first week of infusion. The results obtained in both rats and rabbits in response to chronic ivt AII infusion suggest that sodium and water retention with expansion of body fluid volumes is not likely to be a pathogenetic factor in this form of hypertension, as has been proposed for hypertension resulting from iv infusion of AII (Cowley and McCaa, 1976; Hall et al., 1984).

A species difference appears to exist with respect to electrolyte handling in response to chronic ivt AII infusion. We have found that plasma sodium and potassium concentrations were unaltered by ivt AII infusion in the rat. These results are in agreement with those of

DiNicolantonio et al. (1982) but at variance with those of Sterling et al. (1980), who found hyponatremia as a consequence of ivt AII infusion. In rabbits, however, chronic ivt AII infusion results in hyponatremia and hypokalemia. The mechanisms responsible for these effects are not known, but it seems that a redistribution of the ions within body fluid compartments is likely. Increased excretion of the ions is not sufficient to explain the hypokalemia and hyponatremia in high sodium rabbits, since these effects are observed with no change in sodium balance and only one day of significant potassium loss. In addition, a "dilution" effect of volume retention cannot be invoked since the rabbits do not exhibit increases in plasma or extracellular fluid volumes.

In summary, chronic ivt infusion of AII in rats and rabbits produced a sodium-dependent form of hypertension associated with increased water intake. These studies are the first to provide a quantitative estimate of the sodium-sensitivity of the hypertensive response to chronic ivt AII, and to demonstrate that the dipsogenic response to chronic central AII administration is not required for expression of the hypertension. AII has been postulated to serve an important function in the conservation of sodium and water. However, in the present studies, hypertension observed with chronic central AII infusion was not associated with sodium or water retention. In fact, ivt AII infusion in rabbits actually caused significant sodium and potassium loss.

B. Physiologic Mechanisms Involved in the Hypertensive Response to Chronic ivt AII Infusion

Despite the growing body of evidence that a central action of AII may be of physiological significance in hypertension, relatively few

(Jandhyala et al., 1979; Buckley et al., 1981; Fink et al., 1982a) studies to date have examined the mechanisms by which chronic stimulation of brain AII receptors cause sustained increases in arterial pressure. Some of the experiments in this thesis were designed to examine the role of several pressor mechanisms in the hypertension produced by chronic ivt AII infusion in the rat.

One obvious factor that could contribute to this form of hypertension is leak of AII from CSF into the peripheral circulation. Measurement of plasma AII concentration revealed that, on the average, plasma AII levels were not significantly increased by 5-day ivt AII infusion. However, it is possible that a small amount of AII, not enough to measurably elevate plasma levels, could pass into the bloodstream as a result of ivt infusion. A peripheral effect of blood-borne AII could then contribute to the elevation in arterial pressure observed with chronic ivt AII infusion. To test this possibility, acute iv infusions of the AII receptor antagonist saralasin were administered before and during ivt AII infusion. Saralasin produced a slight (10-15 mmHg) but significant depressor response when administered during the period of ivt AII-induced hypertension. Thus, it appears that a small component of the hypertension is due to a peripheral effect of AII. An alternative explanation for this result is that iv saralasin may have access to brain sites at which ivt AII acts to produce increases in arterial pressure. Hoffman and Phillips (1976) have shown that large doses of ivt saralasin (72 μg/min) are required to significantly attenuate the pressor effect of an acute ivt AII injection in the rat. The two lower doses of saralasin that these investigators used (500 and 1800 ng/min, iv) were without effect on the acute ivt AII pressor response. In

agreement with their study, we also found that iv saralasin infusion (300 ng/min for 15 min) did not effect the magnitude of the pressor response to an acute ivt injection of AII. This result suggests that iv saralasin, at the doses employed here, cannot reach brain sites at which ivt AII acts to produce acute increases in MAP. The possibility still remains that during chronic hypertension produced by ivt AII infusion, the accessibility of iv saralasin to critical central sites is increased.

Another approach also was used to assess the degree to which AII, when delivered chronically into the cerebral ventricles, might "leak" from CSF into the bloodstream. This approach was based on the observations that AII, even at concentrations lower than the direct vasoconstrictor threshold, enhances the vasoconstrictor response to nerve stimulation (Zimmerman, 1978; Campbell and Jackson, 1979). Therefore, if AII was leaking into the periphery as a result of ivt infusion, the pressor responses to stimulation of sympathetic vasomotor outflow should be greater in rats receiving AII ivt than in rats receiving saline ivt. However, we found that pressor responses to electrical stimulation of the spinal cord in the pithed rat preparation were not significantly different on the fifth day of ivt AII or saline infusion. In fact, the pressor responses in rats receiving AII ivt tended to be less than saline controls at all frequencies tested. In addition, resting blood pressure of anesthetized, pithed rats was not different in rats receiving AII ivt and saline ivt. If AII was leaking into the periphery, blood pressure should have been higher in rats

receiving AII ivt. This evidence suggests that AII does not cross from CSF into the peripheral circulation in appreciable quantities. Whatever the mechanism by which iv saralasin lowers blood pressure in this model, the depressor response is not of sufficient magnitude to suggest that leak of AII from the brain accounts for the full hypertensive response to chronic ivt AII infusions.

Plasma aldosterone concentrations were measured during ivt AII infusion since there is a report that acute ivt administration of AII causes aldosterone release (Nicholls et al., 1983), and the sodium-dependency of ivt AII-induced hypertension suggests that it may share similarities with steroid-salt hypertension. Plasma aldosterone concentrations were significantly elevated on the fifth day of ivt AII infusion when compared to pre-infusion levels in rats maintained on 7.5 mEq Na/day. However, adrenalectomy failed to alter the course of hypertension development. In addition, plasma aldosterone concentration was unaltered in rats that had unlimited access to 0.9% saline drinking fluid during ivt AII infusion, although the rats did become hypertensive. The increase in plasma aldosterone seen in response to ivt AII in rats maintained on a sodium intake of 7.5 mEq/day appears to be suppressed by higher sodium intakes. This fact, coupled with the observation that adrenalectomy was without effect on this form of hypertension obviates a critical role for aldosterone in the hypertension produced by chronic ivt AII infusion.

As detailed in the INTRODUCTION, numerous studies have investigated the role of both AVP and the sympathetic nervous system in the pressor response to acute ivt AII injections. Additional experiments for this

thesis were designed to determine the extent to which these two systems participate in the maintenance of chronic hypertension resulting from continuous ivt infusion of AII. Administration of an AVP antagonist to rats before the start of chronic ivt AII infusion caused no measurable changes in blood pressure or heart rate. Similarly, on the first, third, and fifth days of ivt AII infusion, the AVP antagonist caused no change in MAP. It was verified that the dose of AVP antagonist used was sufficient to block increases in MAP elicited by iv infusion of graded doses of AVP. The finding that the AVP antagonist did not cause a depressor response in this form of hypertension was surprising in light of the strong evidence supporting a role for AVP in the pressor response to acute central AII injections. However, it is possible that the effector mechanisms involved in the pressor response to acute vs. chronic ivt AII may be different. For example, in contrast to the increase in plasma AVP observed after acute ivt AII injection (Haack and Mohring, 1978; Fisher and Brown, 1984), plasma AVP is not elevated in response to chronic ivt AII infusion (Sterling et al., 1980; Fink et al., 1982). Therefore, one may not expect to see a depressor response to AVP antagonists during chronic ivt AII infusion as one sees with acute ivt AII injection. The present experiments indicate that the pressor actions of AVP do not contribute to the hypertension seen in response to chronic ivt AII infusion.

Three approaches were used in the present experiments to address the role of the sympathetic nervous system in the maintenance of chronic ivt AII-induced hypertension. While the limitations of using plasma catecholamines as an index of peripheral sympathetic nervous

system activity have been recognized (Folkow et al., 1983; Mancia et al., 1983), the observation that plasma norepinephrine and epinephrine were not elevated on the fifth day of ivt infusion is at least suggestive that the sympathetic system is not activated in response to chronic ivt AII infusion. The depressor responses to various acute pharmacologic interventions, including ganglionic blockade, alpha-adrenergic blockade, and combined alpha and beta-adrenergic blockade also were used in these studies as indices of neurogenic tone. The depressor response to acute ganglionic blockade with hexamethonium was determined in two separate experiments. In the first, rats were housed in metabolic cages on a fixed, high sodium intake of 7.5 mEq/day, and hexamethonium was administered on the second control day, the first, third and fifth days of ivt AII infusion, and the second recovery day. The decrease in MAP in response to hexamethonium averaged 37 mmHg on the control and recovery days and 53 mmHg on the 3 days of ivt AII-induced hypertension. Although the depressor response to hexamethonium was greater than the control response on all 3 days of ivt infusion, the difference was not statistically significant. In the second experiment, rats were given ad libitum access to 0.9% saline drinking fluid and received ivt infusions of either saline or AII. In this case, the average depressor response to hexamethonium in AII-infused rats (66 mmHg) was significantly greater than that observed in saline-infused control rats (30 mmHg). This result suggests that neurogenic mechanisms are engaged to a greater degree in the support of blood pressure in rats made hypertensive with ivt AII infusions than in normotensive rats. The degree of sympathetic vasoconstrictor tone was assessed more specifically by measuring the depressor response to

phentolamine (alpha-adrenergic blockade) and the combination of propranolol and phentolamine (alpha- and beta-adrenergic blockade). Phentolamine produced a significantly greater fall in MAP in AII-infused rats (64 mmHg) than in saline-infused rats (47 mmHg). At least two mechanisms have been suggested to contribute to the hypotensive effect of phentolamine: 1) blockade of post-synaptic alpha-1 receptors and 2) beta-adrenergic vasodilation due to presynaptic alpha-2 receptor blockade with resultant increased catecholamine release (Saeed et al., 1982). Therefore, the depressor response to phentolamine also was measured after beta-adrenergic blockade with propranolol in order to determine more selectively the degree of alpha-adrenergic vasoconstrictor tone. Combined alpha- and beta-adrenergic blockade also produced a significantly greater depressor response in AII-infused rats (43 mmHg) when compared to normotensive saline-infused rats (19 mmHg). However, these responses were of considerably lesser magnitude than those seen with phentolamine alone. Thus, a component of the hypotensive response to phentolamine is beta-receptor mediated, but whether it is due to direct beta-receptor stimulation by phentolamine or beta-stimulation due to increased catecholamine release (an alpha-2 antagonist effect) cannot be determined from the present experiments. Regardless, pharmacologic assessment of neurogenic tone using hexamethonium, phentolamine, and propranolol plus phentolamine indicates that sympathetic tone is greater in rats receiving AII ivt than in rats receiving saline ivt.

It has been suggested that in established hypertension, structural adaptation of blood vessel walls (i.e., hyperplasia and hypertrophy of vascular smooth muscle leading to vessel wall thickening and luminal

narrowing) may render the vasculature supersensitive to a number of vasoconstrictor and vasodepressor stimuli (Folkow, 1983). If this was the case in the present studies, then the augmented depressor response to hexamethonium, phentolamine, and propranolol plus phentolamine observed in hypertensive rats could represent a non-specific change in vascular reactivity due to structural factors rather than an actual change in neurogenic vasoconstrictor function. Therefore, the depressor response to nitroprusside, a direct vasodilator, was compared in AII- and saline-infused rats. Nitroprusside produced decreases in blood pressure of a similar magnitude in both groups of rats. Therefore, the enhanced depressor responses to ganglionic and alpha- and beta-adrenergic blockade in rats made hypertensive by chronic ivt AII infusion in all likelihood are indicative of actual increases in the degree of sympathetic neurogenic vasoconstriction, rather than a generalized increase in sensitivity to vasodilator stimuli.

The increase in sympathetic tone (as measured by the depressor response to phentolamine alone or phentolamine plus propranolol) accounts for between 40 and 65% of the total hypertensive response to chronic ivt AII infusion. However, acute pharmacological interruption of neurogenic mechanisms may underestimate the true contribution of the sympathetic nervous system to the development and maintenance of chronic ivt AII-induced hypertension. The sympathetic nervous system has been demonstrated to exert "trophic" effects on smooth muscle that develop over a period of days (Fleming, 1978; Aprigliano and Hermsmeyer, 1977). Therefore, acute interventions may not be sufficient to uncover long-term effects of sympathetic activation. To address the

possibility that the sympathetic nervous system may have a trophic role in this form of hypertension, chronic ivt AII infusion was performed in rats which had undergone prior peripheral sympathectomy by neonatal treatment with guanethidine and adrenal demedullation at adulthood. Rats with markedly impaired peripheral sympathetic nervous system function and normal control rats became equally hypertensive in response to a 7-day ivt AII infusion. However, the onset of hypertension was significantly delayed in the sympathectomized rats. Two days after the start of ivt AII infusion (the first time point at which blood pressure was measured), sympathectomized rats were still normotensive while blood pressure was significantly elevated in normal rats. By four days after the start of ivt infusion, both groups were hypertensive. These data suggest that the sympathetic nervous system is required in the early phase, but its presence is not ultimately necessary for full expression of the hypertension in this model.

There are several possible explanations for the failure of peripheral sympathectomy to effect the ultimate blood pressure level achieved in response to chronic ivt AII infusion: 1) the sympathetic system is not activated by chronic ivt AII infusions, 2) the sympathetic system may be activated by chronic ivt AII infusion in a normal animal, but in an animal with extensive peripheral adrenergic deficits, other pressor mechanisms are activated and play a greater role in the hypertensive response, and 3) the degree of sympathectomy was not "complete" enough, and the remaining sympathetic function was sufficient to allow hypertension to develop. In the present experiments, an attempt was made to assess the degree of sympathectomy produced by neonatal guanethidine treatment plus adrenal demedullation at adulthood. In our

hands, sympathectomized rats exhibited a significantly attenuated depressor response to hexamethonium and phentolamine when compared to normal rats, indicating that the degree of neurogenic tone indeed was less in the sympathectomized rats. However, the fact that in the sympathectomized rats MAP fell at all in response to ganglionic blockade is suggestive that a degree of neurogenic tone remains. Tissue norepinephrine levels in renal cortex and pineal gland were reduced by 11 and 62%, respectively, again indicating that "complete" sympathectomy was not achieved. In addition, significant but not complete attenuation of the pressor response to spinal cord stimulation was observed at all frequencies tested in sympathectomized rats when compared to normal rats. By the functional measures reported here, it appears that the "sympathectomized" rats used in these experiments had approximately 20-30% of the sympathetic vasoconstrictor function of normal rats. Others also have demonstrated that, although significant functional deficits are present, this method of producing sympathectomy is not complete (Bennett et al., 1982). Despite its limitations, guanethidine-induced sympathectomy was judged to be superior to 6-hydroxydopamine treatment for the purpose of these experiments, since the sympathetic innervation of the vasculature is remarkably resistant to the effects of 6-hydroxydopamine (Berkowitz et al., 1972; Finch et al., 1973).

The most obvious interpretation of the results of the chronic sympathectomy experiments is that the presence of the sympathetic nervous system ultimately is not required for the development of hypertension in response to chronic ivt AII infusion. However, pharmacologic assessment of vasoconstrictor tone revealed that an elevation in sympathetic

nervous system activity does play a role in this form of hypertension. The residual sympathetic vasoconstrictor function present in the "sympathectomized" rats may be sufficient to allow hypertension to develop. Since we were unable to produce chronic total (100%) destruction of adrenergic innervation to the vasculature, resolution of the issue using these methods was not possible.

On the other hand, the failure of peripheral sympathectomy ultimately to effect hypertension in this model could be explained by postulating an interaction between 2 or more pressor systems in this form of hypertension. In the absence of one system, another system may compensate to maintain arterial pressure at its prevailing level. This could explain the lack of effect of interruption of only one system (by chronic sympathectomy or administration of an AVP antagonist) in hypertension induced by chronic ivt AII infusion. Interaction of arterial pressure control mechanisms has been demonstrated in other physiological situations. For example, the renin-angiotensin system and AVP appear to interact to maintain arterial pressure during alpha-blockade with prazosin. Plasma renin activity and plasma AVP are markedly increased by prazosin administration, and both appear to limit its hypotensive effect (Waeber et al., 1983). In addition, Paller and Linas (1984) found that in normal, conscious rats, MAP is markedly reduced when the three major blood pressure regulatory systems (AVP, renin-angiotensin system, and the sympathetic nervous system) are interrupted, but arterial pressure can be well maintained as long as a single pressor system remains intact. This type of compensatory interaction of blood pressure regulatory mechanisms may occur in the present experiments when one system is interrupted during ivt AII-induced

hypertension. Specifically, in sympathectomized rats, chronic ivt AII infusion may produce a hypertensive state that is more AVP-dependent than that seen in normal rats. Indeed, it has been suggested that, in contrast to a normal rat, resting blood pressure in a sympathectomized rat is dependent on the pressor actions of AVP. Therefore, it may be reasonable to postulate that ivt AII-induced hypertension is relatively more AVP-dependent than that seen in a normal rat. Similarly, administration of an AVP antagonist may stimulate a compensatory increase in sympathetic activity so that arterial pressure is maintained at its prevailing level.

To test the hypothesis that a compensatory interaction of AVP, the sympathetic nervous system, and renin-angiotensin system may occur in hypertension produced by chronic ivt AII infusion, all 3 of these systems were blocked pharmacologically in rats receiving chronic ivt infusions of saline or AII. In normal rats given AII or saline ivt, the AVP antagonist did not alter blood pressure. Subsequent administration of saralasin produced a slight, but significant depressor response only in rats receiving ivt AII, and not in ivt-saline control rats. The depressor response to saralasin observed in rats receiving ivt AII infusions was not secondary to previous blockade of AVP receptors, since this depressor response also was observed when the order of these two interventions was reversed. Thus, a "saralasin-blockable" component can be identified in this form of hypertension, but whether this is a central or peripheral effect of saralasin is yet to be resolved. The observation that acute iv saralasin infusion does not diminish the pressor response to acute ivt AII injection suggests that

iv saralasin may not act centrally at the doses used to antagonize AII effects. Therefore, one must conclude that iv saralasin acts peripherally to block a "leak" component of ivt AII infusions. One observation that argues against this is that acute iv saralasin does not produce a depressor response in rats made hypertensive by chronic intravenous AII infusion (unpublished observation). Therefore, if a slow, continual leak of AII from CSF to blood were occurring as a result of ivt infusion, it does not appear that the pressor effect of this "leak" would be antagonized by acute iv saralasin infusion. Whatever the mechanism by which iv saralasin lowered pressure, blood pressure after AVP antagonist and saralasin was still significantly higher in rats receiving AII ivt than in saline control rats. Addition of phentolamine now produced a fall in blood pressure in both groups of rats, and the level of arterial pressure was not significantly different in AII- and saline-infused rats after blockade of all three systems. Therefore, in a normal rat on high sodium intake, the hypertensive response to chronic ivt AII infusion appears to be due, in small part, to leak of AII from CSF into the peripheral circulation, and, to a greater extent, to activation of the sympathetic nervous system. However, the hypertension observed in response to ivt AII infusion in chronically sympathectomized rats on the average appears to be due exclusively to leak of AII from CSF into the periphery, since AVP antagonist plus saralasin brought MAP to the level seen in saline-infused sympathectomized rats. A puzzling aspect of this experiment was that AVP antagonist plus saralasin did not lower blood pressure in sympathectomized rats receiving ivt saline infusions. One might expect that in a sympathectomized rat, blockade of the other two major blood pressure regulatory

mechanisms would produce a profound fall in MAP to the levels seen in a normal rat given AVP antagonist, saralasin, and phentolamine (approximately 60-70 mmHg). The fact that MAP is well maintained in chronically sympathectomized rats after AVP and AII blockade suggest that either 1) residual sympathetic function is able to maintain blood pressure or 2) chronic sympathectomy may produce compensatory cardiovascular changes, such as volume expansion, that may help to maintain arterial pressure. The present experiments did not address which of these may be the case. However, one can conclude from these experiments that AVP and the sympathetic nervous system do not appear to interact in a compensatory manner to maintain elevated arterial pressure in response to chronic ivt AII since the AVP antagonist did not produce a depressor response in sympathectomized rats given chronic ivt AII infusions.

C. Chronic Pharmacologic Interruption of Central AII Pressor Mechanisms in Several Forms of Experimental Hypertension

The purpose of these experiments was to develop a method to produce chronic, selective blockade of brain AII receptors. To date, electrolytic lesion of discrete brain areas or acute ivt injection of AII antagonists are the two methods that have been used to assess the effects of AII on the brain. As described in the INTRODUCTION, both of the above methods have limitations. Some areas of the brain that are sensitive to AII (i.e., AV3V or SFO) also are sensitive to osmotic stimuli (Brody and Johnson, 1980). Electrolytic lesion of these areas results in drinking deficits not only to ivt AII but also to ivt injections of hypertonic saline (Johnson et al., 1978). In addition, AV3V lesion produces chronic alterations in fluid and electrolyte regulation.

Therefore, it is difficult to attribute the effects of AV3V lesion solely to destruction of central AII receptors.

The other method that frequently has been used to assess the effects of AII on the brain is acute ivt injection of AII antagonists. Although such injections will result in a decrease in blood pressure in some types of hypertensive rats (Ganten et al., 1975; Phillips et al., 1975, 1977; Sweet et al., 1976; Mann et al., 1978; Suzuki et al., 1981), the doses used are largely empirical and have not been shown to produce functional central AII receptor blockade. Furthermore, acute ivt injections of AII antagonists are incapable of completely reversing hypertension in experimental models characterized by chronic elevations of plasma or CSF AII (Suzuki et al., 1981; Fink et al., 1982a). We, therefore, have postulated that it may be necessary to produce chronic blockade of central AII receptors in order to assess the cardiovascular effects of chronic elevation of plasma AII levels.

In order to develop a method suitable to assess the contribution of central AII effects to various forms of experimental hypertension, saralasin, a competitive AII receptor antagonist, was infused into the cerebral ventricles of rats for 5 days. We chose saralasin since it is the most readily available and widely studied AII receptor antagonist. Intraventricular infusion of saralasin (12 μg/hr) for 5 days blocked the pressor response to a supramaximal ivt dose of AII (150 ng) and slightly diminished, but did not abolish, pressor responses to iv AII. The slight inhibition of the pressor response to iv AII after central saralasin infusion may have been due to blockade of some vascular AII receptors, and more selective blockade may have been achieved with a dose of saralasin between 6 and 12 μg/hr. However, there is

evidence that a component of the pressor response to acute iv AII infusion is centrally mediated and that this component can be blocked by ivt saralasin. It has been demonstrated that AV3V lesions significantly reduce the pressor response to acute iv infusions of AII (Buggy et al., 1977) and that ivt saralasin injections abolish the pressor difference between acute intracarotid and intra-aortic AII infusions (Fink et al., 1980a). Blockade of the central pressor action of blood-borne AII by ivt saralasin may prevent a complete separation of central and peripheral effects of AII antagonists delivered ivt using the methods described here. Our initial goal was to use chronic ivt saralasin infusion to determine if hypertension produced by chronic iv infusions of AII could be attenuated by concomitant blockade of brain AII receptors. It has been found previously, however, that chronic iv AII infusions produce sustained hypertension only in rats maintained on a high sodium intake (Fink et al., 1982b). Saralasin was infused ivt for 5 days in rats maintained on a high sodium intake to ensure that saralasin alone did not alter the physiological variables we wished to measure during AII infusion. In rats maintained on a high sodium intake, saralasin produced a pressor response that was not seen in rats maintained on a normal sodium intake. The pressor response could not be explained by leakage of saralasin into the periphery for two reasons: 1) we demonstrated that chronic ivt saralasin infusion did not produce functional blockade of peripheral AII receptors in rats on normal sodium intake; and 2) iv saralasin infusion (18 μg/hr) for 7 days in rats maintained on high sodium intake produced a pressor response (approximately 12 mmHg) that appeared within the first 24 hr of infusion and lasted for the duration of saralasin administration. This is in contrast to the

3-day latency and 30 mmHg rise in MAP seen with ivt saralasin infusion at 12 μg/hr. Even if all of the ivt saralasin passed from the CSF into the bloodstream and exerted peripheral effects, it could not explain the magnitude of the pressor response observed during ivt infusion.

Because the pressor effect of ivt saralasin infusion suggested an agonistic action at central AII receptors, we compared the effects seen with ivt saralasin to those seen with chronic ivt infusion of AII itself. Intraventricular infusion of AII (1 μg/hr) or saralasin (12 μg/hr) for 5 days in rats on high sodium intake produced very similar cardiovascular changes. Both compounds produced increases in blood pressure over the duration of the infusion that were reversed within 24 hr of cessation of ivt infusion. Patterns of fluid and electrolyte excretion were similar in rats receiving either ivt saralasin or AII. From these experiments, it appears that in rats maintained on a high sodium intake, saralasin produces a pressor response due to an agonistic effect at central AII receptors.

Although the agonistic effects of iv saralasin administration are well documented, we could not predict a priori that the same effects would be seen with ivt administration under the conditions of our experiment. In human subjects with high circulating AII levels as a result of either high-renin hypertension or low dietary sodium intake (Case et al., 1980; Pals and Masucci, 1973; Brunner et al., 1974; Streeten et al., 1975), acute iv saralasin infusion will cause a fall in blood pressure. Saralasin infusion will increase blood pressure in humans with suppressed plasma AII concentration secondary to a high level of sodium intake or in low-renin forms of hypertension (Brunner et al., 1976; Case et al., 1980). It has been proposed that saralasin

is a very weak partial agonist and as such its agonistic activity is dependent upon prior occupancy of AII receptors (Case et al., 1980). In physiological states where plasma renin activity (and therefore plasma AII levels) are elevated (i.e., sodium depletion), saralasin acts as a competitive antagonist, displacing a potent agonist with a very weak agonist. In contrast, when plasma renin activity and plasma AII are low (sodium loading), the weak agonistic activity of saralasin is expressed. The same reasoning could be used to explain the sodium-dependency of the central agonistic action of saralasin. An alternate explanation of our findings is provided by Mann et al. (1980). They showed a reduction in both the number and affinity of brain AII receptors in sodium-depleted rats relative to sodium-replete rats. If brain AII receptors in rats on a high sodium intake are increased either in number or affinity, this could explain why saralasin acts as an agonist only in rats maintained on a high sodium intake. Whatever the mechanism, as central saralasin infusion did prove to have agonistic effects in rats on a high sodium intake, an attempt was made to use an antagonist with reportedly less agonistic potency than saralasin. Sarthran (1-sar-8-thr-angiotensin II) was chosen since it is reported to possess less agonistic activity than saralasin. We found that chronic (5-day) ivt infusion of sarthran produced relatively complete functional blockade of brain AII receptors. A dose of 1 μg/hr was judged to be optimal in this regard since this dose produced blockade of the pressor response to acute ivt AII but little suppression of the iv dose-response curve for AII. Infusion of this dose of sarthran (1 μg/hr, ivt) in rats on high sodium intake was performed to verify that sarthran had no agonistic properties that would make it unsuitable for use in further

studies. Sarthran, in contrast to saralasin, had no agonistic effects (i.e., did not increase blood pressure) in rats on high sodium intake. The central agonistic properties of saralasin, and relative lack of these effects with sarthran at the doses used, agrees with the known relative agonistic potencies of these two compounds at peripheral AII receptors (Munoz-Ramirez et al., 1976).

The evidence that a central pressor effect of AII may be involved in the hypertension of SHR is discussed in the INTRODUCTION. In light of the equivocal results of previous studies, the present experiments were designed in an attempt to further delineate the role of central AII mechanisms in the established hypertension of SHR using pharmacological methods. Chronic interruption of central AII mechanisms by two different means -- blockade of central AII receptors or inhibition of brain converting enzyme -- had no effect on established hypertension in adult SHR. Continuous ivt infusion of sarthran at doses (1 and 6 μg/hr for 5 days) that inhibited the pressor response to a supramaximal ivt dose of AII by 48 and 75%, respectively, did not lower blood pressure. In addition, by functional estimates, ivt teprotide infusion (10 μg/hr) caused a 25% inhibition of central converting enzyme, with no effects on the peripheral conversion of AI to AII. It has been found that central infusion of captopril (another converting enzyme inhibitor), at a dose that decreases the ivt AI pressor response by 50%, attenuates hypertension development in young SHR (Okuno et al., 1983). Therefore, if a central pressor effect of AII was at least partially responsible for hypertension in the adult SHR, a depressor response to either sarthran or teprotide should have been observed in the current experiments.

The results reported herein are in contrast to those of several other studies in which acute ivt injection of saralasin (Phillips et al., 1975; Mann et al., 1978) or captopril (Hutchinson et al., 1980) or chronic ivt infusion of 1-sar-8-ile-AII (McDonald et al., 1980) produced depressor responses in adult SHR. Mann and coworkers found that acute ivt saralasin injections produced dose-dependent decreases in blood pressure, however, the possibility that these injections had peripheral effects was not ruled out. In another study that showed a depressor response to acute ivt saralasin, the weight range of rats used included both young and mature stroke-prone SHR (Phillips et al., 1979). Since several studies have revealed a centrally-mediated AII pressor effect in developing SHR (Okuno et al., 1983; Tonnaer et al., 1981; Tonnaer et al., 1984), the age of rats used in the study may influence the outcome. Although chronic ivt infusion of 1-sar-8-ile-AII caused a significant depressor response in adult SHR, the response was most pronounced on the first day of infusion and waned with time, averaging about 15 mmHg (McDonald et al., 1980). Disparate results have been obtained with acute ivt captopril, another study using the same dose (Crofton et al., 1981) concluded that the depressor effect was nonspecific, as it was seen with vehicle injection. Although some of the above-mentioned studies suggest that a central pressor effect of AII may be important in maintenance of blood pressure in adult SHR, other studies using AII antagonists (Elghozi et al., 1976; Schoelkens et al., 1976) or AV3V lesion (Buggy et al., 1978) support the results of the present work -- that is, that blockade of central AII pressor effects does not influence established hypertension in SHR. In fact, in contrast to the depressor effects that others have reported, direct

blood pressure measurements in catheterized rats showed that 5-day ivt infusions of sarthran or teprotide actually caused blood pressure to increase by approximately 20 mmHg. This increase in arterial pressure was not observed in rats whose pressure was measured by the tail cuff method. These pressor effects are peculiar to SHR and are not observed in normotensive Sprague-Dawley rats at the same doses of sarthran and teprotide. Since angiotensin converting enzyme and kininase II are the same protein, and bradykinin is pressor when administered centrally (Lewis and Phillips, 1984), the pressor effect of teprotide could be related to inhibition of bradykinin metabolism in the brain.

We also found that chronic ivt sarthran infusion in DOC-salt rats did not prevent development of hypertension, in fact rats that received sarthran ivt actually had higher blood pressures than rats that received saline ivt. This was surprising since AV3V lesion is particularly effective in preventing hypertension development in this model (Fink et al., 1977; Berecek et al., 1982). The effect of AV3V lesion in DOC-salt rats, rather than specifically being related to destruction of AII receptors, may be due to disruption of osmoreceptor mechanisms. In agreement with the current results, Mann et al. (1978) found that acute ivt injection of AII receptor antagonists caused a paradoxical increase in blood pressure in DOC-salt rats. The mechanisms supporting blood pressure in sarthran-treated and saline-treated DOC-salt rats were not different. AVP antagonist did not lower blood pressure in either group, and ganglionic blockade produced equivalent decreases in blood pressure in both groups. Thus, this study provides no evidence that a central pressor effect of AII contributes to the hypertension produced by DOC-salt treatment.

The pressor response to ivt sarthran infusion in SHR and DOC-salt hypertensive rats may be the result of a partial agonistic effect. Lower doses of sarthran could not be used to circumvent the apparent agonistic effect since the doses used in these studies were necessary to achieve significant functional blockade of brain AII receptors. Since the partial agonistic effects of angiotensin antagonists are the greatest when endogenous AII levels are low (Wallace et al., 1979), occupancy of central AII receptors by endogenous AII is probably minimal in SHR and DOC-salt rats. This being the case, it is unlikely that a central pressor effect of AII contributes to the maintenance of hypertension in either experimental model. In summary, studies in adult SHR have shown that chronic ivt infusion of an AII receptor antagonist or converting enzyme inhibitor at doses that were demonstrated specifically to effect central, but not peripheral, angiotensin responses, did not lower blood pressure. Although other studies provide evdience that a central AII pressor effect contributes to hypertension development in young SHR, a role for this system in the established hypertension of adult SHR could not be revealed in the current experiments by chronic pharmacological blockade of central AII receptors or endogenous brain AII production.

In our hands, chronic blockade of brain AII receptors with ivt sarthran infusion also had no effect on the ultimate development of hypertension induced by chronic intravenous AII infusion. However, at the lower dose of iv AII, ivt sarthran appeared to delay development of hypertension by several days. These studies indicate that a central pressor effect of iv AII, antagonized by ivt sarthran, may participate in the early increase in blood pressure (1-2 days) in response to

elevated plasma AII. This observation agrees with previous work showing that the pressor response to a 10-30 minute iv AII infusion is attenuated by ivt sarthran administration (Brody et al., 1978). However, the hypertension produced by more long-term increases in plasma AII levels does not appear to be mediated by central AII mechanisms blockable by ivt sarthran. This result is in contrast to the complete blockade of this form of hypertension observed when central AII mechanisms are interrupted, not by pharmacological means, but by electrolytic destruction of the AV3V region. There are several possible explanations for the apparent contradiction that ivt sarthran failed to block chronic iv AII-induced hypertension whereas AV3V lesion was effective in preventing hypertension development. First, AV3V lesion, rather than eliminating a critical central receptor site for AII -- an effect presumably shared by ivt sarthran -- may block iv AII-induced hypertension by a mechanism unrelated to disruption of central AII effects. Previous studies have demonstrated that the development of sustained hypertension in response to chronic iv AII infusion in the rat is dependent on sodium intake. If dietary sodium intake is not maintained at a sufficiently elevated level, chronic iv AII infusion will not produce hypertension, regardless of the dose of AII infused (Fink et al., 1982b). Thus, it appears that the stimulus of elevated sodium intake is necessary for the chronic hypertensive effects of blood-borne AII to be manifest. The effect of AV3V lesion to prevent this form of hypertension may be related to disruption of osmoreceptor mechanisms (Brody and Johnson, 1980) with the result that the rat no longer can "sense" the prevailing level of sodium intake. The rat with an AV3V lesion, when maintained on a high sodium intake, may respond to chronic iv AII infusion in a manner similar

to an intact rat on a normal sodium intake -- that is, with an insignificant increase in arterial pressure.

A second possibility that could explain the lack of effect of ivt sarthran infusion on the hypertensive response to iv AII is that the central AII receptors that mediate the pressor response to chronic AII administration may not be blocked by sarthran. In the present experiments, we have demonstrated that chronic ivt sarthran infusion blocks receptors that mediate the pressor response to an acute ivt injection of AII. However, if a different set of brain AII receptors was activated in response to chronic, as opposed to acute, AII administration, these receptors may not be blocked by sarthran, which was developed as an antagonist of the vascular AII receptor. Intracellular receptors for AII have been demonstrated on nuclei (Re et al., 1981) and mitochondria (Goodfriend et al., 1972), and activation of nuclear AII receptors has been shown to stimulate RNA synthesis in isolated nuclei (Re and Parab, 1984). Furthermore, it has been suggested that AII-receptor complexes can be internalized in brain tissue (Erickson et al., 1984). One could hypothesize that the central receptors activated by chronic AII administration are intracellular, or that AII bound to membrane receptors may be internalized as an AII-receptor complex, translocated to the nucleus, where AII could influence DNA transcription, and ultimately the products of cellular metabolism. If this were the case, sarthran may not be a potent antagonist at the receptors activated by chronic AII administration. Although this hypothesis is highly speculative, it is worthwhile considering especially in light of the observations that acute ivt injections of AII antagonists cannot reverse hypertension caused by chronic elevations in plasma or CSF AII (Suzuki et al.,

1981; Fink et al., 1982a). The present studies also have demonstrated that hypertension produced by chronic ivt AII infusion can be reversed only to a small extent by acute ivt sarthran administration.

A third possible explanation for the failure of ivt sarthran infusion to block chronic iv AII-induced hypertension is that sarthran, when administered into the cerebrospinal fluid, may not have access to the critical receptor site(s) at which iv AII acts to produce hypertension. Blood-borne AII appears to have ready access to the OVLT, SFO, and AP since high amounts of binding are found in these regions after iv administration of ^{125}I-AII (van Houten et al., 1983). The binding is specific for AII since it is completely eliminated by iv saralasin infusion. However, the binding of peripherally-administered ^{125}I-AII to CVO's is differentially affected by ivt saralasin administration. Some CVO's, such as the OVLT, appear to be equally accessible from blood or CSF since binding of intravenously-administered AII is prevented to the same extent by either iv or ivt saralasin pretreatment. In contrast, binding of blood-borne ^{125}I-AII to the SFO and AP is not completely blocked by ivt saralasin infusion. It appears that AII-like peptides have preferential access to these brain regions from the blood-side over the CSF-side. In addition to evidence from binding studies, anatomical evidence also exists that substances may have limited access to CVO's from the CSF. It has been demonstrated that, in contrast to ependyma lining most of the ventricular surface at the CSF interface, ependymal cells lining the OVLT, SFO, and AP are connected by tight junctions that preclude the passage of CSF-borne tracers into the body of the CVO (Brightman et al., 1975; Krisch and Leonhardt, 1978). On the other hand, these same tracers have access to OVLT, SFO, and AP when given

into the blood. The results of the present experiments suggest that sarthran, when administered into the CSF, may not gain access to the same brain sites as blood-borne AII. The AP and SFO are two CVO's that are sensitive to AII, and the accessibility of AII receptors in these areas appears to be greater from blood than from CSF. If the critical receptor site for the hypertensive action of blood-borne AII is located in one of these regions, or some other region where peptides have good access from blood and relatively poor access from CSF, ivt sarthran infusion may not reach the critical receptor population in sufficient concentration to block the effect of iv AII. In contrast, AV3V lesion may prevent iv AII-induced hypertension either by destroying AII receptors to which ivt sarthran has restricted access or interrupting pathways that are known to course through the region from other AII-sensitive sites, notably the SFO (Carithers et al., 1980; Miselis, 1981; Saper and Levisohn, 1983).

In summary, blockade of CSF-accessible AII receptors with chronic ivt sarthran infusion does not attenuate hypertension produced by chronic elevation in plasma AII levels coupled with high sodium intake. The inability of ivt sarthran to prevent hypertension development in this model most probably reflects poor access of CSF-borne sarthran to central receptor sites at which iv AII exerts hypertensive effects.

D. Central Site of Action of Blood-borne AII

This set of experiments was initiated as the result of the finding that chronic intraventricular sarthran infusion does not block hypertension produced by chronic iv AII infusion, although it does block

acute increases in blood pressure produced by ivt AII injections. These observations suggest that the site at which iv AII acts to produce increases in arterial pressure is different than the site at which CSF-borne AII acts. Furthermore, the chronic ivt sarthran experiments indicated that receptors within the OVLT probably are not a critical site of action of blood-borne AII, since according to van Houten et al. (1983), AII has equal access to this area from blood and from CSF. The ability of AV3V lesion to block chronic iv AII-induced hypertension is probably not due to destruction of AII receptors in the OVLT. Rather, the effect of AV3V lesion is most likely the result of interruption of pathways from other AII-sensitive brain areas, or to disruption of osmoregulation. Therefore, an attempt was made to localize the critical receptor field at which blood-borne AII acts to produce hypertension. The work of van Houten et al. (1983) showed that, when given into CSF, AII-like peptides have limited access to the AP and SFO. This observation made these two areas likely candidates for the critical receptor area for blood-borne AII. The literature is replete with studies that indicate that integrity of the SFO or its efferent pathways is necessary for many of the central effects of blood-borne AII, including increases in blood pressure, vasopressin secretion, and thirst (Knepel et al., 1980; Simpson and Routtenberg, 1975; Lind and Johnson, 1982; Lind et al., 1983; Mangiapane and Simpson, 1980). However, a common denominator in all of these studies is that they employed acute stimulation with AII. It is not known to what extent the SFO is involved in central responses to chronic elevations in plasma AII concentration. To determine the importance of the SFO in mediating cardiovascular and fluid/electrolyte responses to AII, chronic intravenous AII

infusions were performed in rats with electrolytic ablation of the SFO. In contrast to the studies cited above, no decrement in the hypertensive response to chronic intravenous AII infusions was observed in rats with SFO lesions. In fact, SFO-lesion rats were significantly more hypertensive than normal rats on 3 out of the five days of iv AII infusion. In addition, normal rats did not increase their water intake over the iv AII infusion period, whereas SFO-lesion rats exhibited a significant increase in water intake. There were no differences in water balance or urinary electrolyte excretion between the two groups of rats. The finding that integrity of the SFO is not important for the chronic hypertension produced by iv AII infusions was unexpected because of the studies that have shown that the SFO is an important neural sensor of acute increases in blood-borne AII. Another study was performed to confirm that under the conditions of our chronic iv AII infusion protocol, the SFO is not important for hypertension development. In the second experiment, rather than ablating the body of the SFO, the majority of the efferent pathways from the SFO were cut, leaving the organ itself intact. Rats with knife cuts of SFO efferents and normal rats became equally hypertensive in response to chronic iv AII infusion. No other parameter measured (heart rate, water intake, water balance, urinary electrolyte excretion) was different between the 2 groups of rats. Therefore, two different experimental approaches, one involving ablating the body of the SFO (and the AII receptors within it), and another involving cutting neural efferents from the SFO but leaving the body of the organ intact, indicate that the SFO is not an AII-receptive area important for the hypertensive response to chronic elevations in circulating levels of AII. In fact, these studies indicate that the presence

of the SFO actually inhibits the increase in arterial pressure in response to chronic iv AII infusion, since rats with SFO lesions and knife cuts of SFO efferent pathways actually became more hypertensive than normal rats. Although MAP was not statistically different in the knife-cut and normal rats over the iv AII infusion period, a calculation of the "average" pressor response to iv AII is illustrative. In sham knife-cut rats, MAP rose from an average of 120 mmHg on the two control days to 149 mmHg during the 5 days of iv AII infusion (Δ29 mmHg). MAP went from 112 mmHg to an average of 164 mmHg in knife-cut rats (Δ52 mmHg), an "average" pressor response that was almost twice that seen in normal rats. Similarly, in response to chronic iv AII, SFO-lesion rats exhibited an average rise in MAP of 32 mmHg compared to 16 mmHg for the sham rats. Thus, SFO lesion or knife cut of SFO efferents actually appears to render the rat more sensitive to the hypertensive effects of blood-borne AII. An explanation for this finding can be postulated based on the work of Lind and Johnson (1982a) demonstrating the existence of an efferent neural pathway from the SFO to the median preoptic region which exhibits AII-like immunoreactivity. The destruction of this pathway by SFO lesions or knife cuts of SFO efferents might produce a type of "denervation supersensitivity" of AII receptors in the MnPO. If circulating AII exerts some of its neural actions on the MnPO, as has been proposed (Hartle and Brody, 1984), then receptor supersensitivity in this region could explain the augmented pressor responses of SFO-ablated or knife-cut rats to chronic iv infusion of AII.

There are several possible explanations for the discrepancy between the present chronic infusion experiments and those in which acute stimulation with AII was used to study the importance of the SFO. First,

although there is little evidence for this postulate in the literature, the central AII receptors that mediate AII-induced pressor responses may be different during acute and chronic stimulation with AII. Perhaps neural elements in the SFO are activated in response to acute elevations in plasma AII, whereas other areas are activated in response to more chronic elevations in blood levels of the peptide. Alternatively, sodium intake may influence the neural response to circulating AII. In the present experiments, all rats were receiving a high sodium intake, since this has been shown to facilitate hypertension development in this model (Fink et al., 1982b). Previous acute studies have used rats on a normal sodium intake. Since some CVO's (e.g., OVLT) stimulated by AII also are osmosensitive (Brody and Johnson, 1980; Thrasher et al., 1982), it is possible that a high sodium intake alters the relative sensitivity of the circumventricular organs to circulating AII. It has been demonstrated that the enhanced pressor response to acute vertebral artery vs. intravenous AII infusions in the dog is abolished by sodium depletion, suggesting that sodium status alters the AII-sensitivity of the area postrema (Szilagyi and Ferrario, 1980). This potentially could occur in other CVO's as well. Thus, blood-borne AII may act to a greater extent at the SFO in rats on normal sodium intake, but preferentially at other CVO's during the osmotic stimulation accompanying higher sodium intakes. This also may explain why in the current experiments (high sodium intake), in contrast to previous studies (normal sodium intake, Lind et al., 1983), SFO-efferent knife cuts did not alter the pressor response to acute iv AII. Whatever the differences between current and previous studies, the present work clearly demonstrates that the SFO alone is not the critical central receptor site involved in mediating cardiovascular responses to blood-borne AII.

Since the SFO does not appear to be the critical forebrain site at which chronic elevations in blood-borne AII act to cause hypertension, another study was undertaken to examine the role of a second forebrain area, the MnPO, in pressor responses to chronic iv AII infusion. The MnPO is of interest for several reasons. First, cells in the ventral MnPO are sensitive to microiontophoretic application of AII (Phillips et al., 1979a). Second, according to the model of the AII pressor system of the rat forebrain proposed by Hartle and Brody (1984), all efferents from the SFO and AV3V involved in pressor responses to blood-borne or CSF-borne AII converge in the MnPO before descending through the anterior hypothalamus. Therefore, if chronic iv AII infusion produces hypertension by acting on this forebrain system, MnPO lesion should block hypertension development. However, the present experiments showed that selective MnPO abalation had no effect on the hypertensive response to chronic iv AII infusion. This result suggests that the hypertension produced by chronic intravenous AII infusion is not dependent on forebrain AII-sensitive neural mechanisms, or at least on pathways that originate in or pass through the MnPO.

The area postrema is the other AII-sensitive CVO that van Houten and coworkers (1983) have suggested may be relatively inaccessible to CSF-borne peptides. If blood-borne AII were acting at the AP to produce chronic hypertension, ivt sarthran infusion would not be expected to block the response. Although the AP does not appear to be involved in pressor responses to acute iv AII infusion in the rat (Haywood et al., 1980), it may be activated in response to the conditions of high sodium intake and chronic iv AII infusion in our protocol.

Preliminary experiments in our laboratory suggest that AP lesion largely prevents chronic hypertension in response to long-term iv AII infusion in the rat. Further work is being done to confirm this initial finding. It is interesting to speculate that the AP may contain the critical receptor field for the hypertensive response to chronic intravenous AII infusions. If this were the case, the ability of AV3V lesions to attenuate this form of hypertension would probably relate to interruption of normal osmoreception, rather than destruction of a critical AII receptor area.

CONCLUSIONS

The experiments presented in this thesis were designed to address three hypotheses relating to the central nervous system actions of angiotensin II. First, the ability of chronic intracerebroventricular AII infusion to produce chronic hypertension was assessed. A sodium-dependent hypertension associated with increased water turnover was produced by chronic ivt AII infusion in both rats and rabbits. This form of hypertension could not be attributed to sodium and water retention. Although plasma aldosterone concentration increased in some situations during ivt AII infusion, adrenalectomy was without effect on hypertension development. The use of acute pharmacological interventions uncovered two mechanisms that contribute to elevated arterial pressure resulting from chronic ivt AII infusions. A small component of the hypertension appeared to be attributable to leak of AII from CSF into the peripheral circulation, and the remainder due to activation of the sympathetic nervous system. A role for the pressor actions of vasopressin could not be demonstrated in this form of hypertension. The hypothesis that the sympathetic nervous system, AVP, and peripheral AII interact to maintain hypertension could not be substantiated in these experiments.

Another set of experiments was designed to develop a method to produce chronic pharmacologic blockade of brain AII receptors, and to

use this method to assess the contribution of central AII pressor effects to several forms of experimental hypertension. Chronic ivt infusions of saralasin at doses demonstrated to block the pressor response to acute ivt AII injection produced apparent agonistic effects. Sarthran, another AII receptor antagonist, produced functional blockade of central AII receptors, and was devoid of agonistic activity. Chronic ivt infusion of sarthran failed to prevent the development of DOC-salt hypertension or to lower blood pressure in adult SHR. In addition, chronic ivt infusions of the converting enzyme inhibitor teprotide failed to produce a depressor response in SHR. Therefore, a central AII pressor effect does not appear to contribute to either of these forms of hypertension.

Previous studies in rats with electrolytic destruction of the AV3V region (Fink et al., 1982b) indicate that the hypertension produced by chronic iv infusion of AII is dependent on the integrity of this forebrain region. The finding that chronic ivt sarthran infusion did not prevent this form of hypertension suggested that the effects of AV3V lesion probably were not specifically due to destruction of AII receptors in that region. Furthermore, the critical site at which blood-borne AII exerted hypertensive effects was most likely a brain area to which CSF-borne sarthran had poor access. This postulate was the impetus for the third set of experiments, in which selective lesions were made of circumventricular organs (SFO, AP) to which CSF-borne peptides are reported to have somewhat limited access (van Houten et al., 1983). Electrolytic ablation of the SFO and knife cuts of SFO efferents both were without effect on the hypertension produced by chronic intravenous infusions of AII. In addition, lesions of the median preoptic nucleus, an area thought to receive all AII-sensitive input from SFO and AV3V

(Hartle and Brody, 1984), was without effect on this form of hypertension. Thus, integrity of AII-sensitive forebrain areas does not appear to be crucial for the hypertensive response to chronic elevations in plasma AII levels in the rat. However, preliminary studies indicate that chronic elevations in plasma AII concentration may produce hypertension by an action at the area postrema. Thus, the AP, which was thought to contribute little to the pressor actions of blood-borne AII based on acute experiments (Haywood et al., 1980) may be a critical sensor for chronic elevations in plasma AII levels.

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