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ANGIOTENSIN II AND ARTERIAL PRESSURE REGULATION IN HYPERTENSION

By

Corinn Marie Pawloski

A DISSERTATION

Submitted to Michigan State University
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ABSTRACT

ANGIOTENSIN II AND ARTERIAL PRESSURE REGULATION IN HYPERTENSION

By

Corinn Marie Pawloski

A central action of angiotensin II (ANG II) in the long-term maintenance of the elevated arterial pressure observed in hypertension has been postulated. In this study, three central actions of ANG II were examined to determine their relative importance in the development of hypertension: 1) ANG II-induced drinking, 2) increases in circulating vasopressin and 3) increases in neurogenic vasoconstrictor tone. The results obtained here indicate that neither ANG II-induced drinking nor vasopressin release contributed to chronic ANG II-induced hypertension. However, a slowly developing increase in sympathetically-mediated vasoconstrictor tone occurred during chronic elevations in circulating concentrations of ANG II. Arterial baroreflexes initially prevented the full expression of the neurogenic contributions to hypertension. In rats in which the arterial baroreceptor afferents were removed arterial pressure reached a plateau sooner, and the increased neurogenic component of hypertension was expressed earlier than in reflex intact rats.

Additional experiments were performed to test the hypothesis that ANG II increased arterial pressure by activating a brainstem receptor subtype selectively sensitive to the ANG II metabolite ANG (1-7). Failure of chronic infusion of ANG (1-7) to produce hypertension similar to that seen during chronic ANG II infusion, demonstrated that ANG II hypertension was not the result of selective stimulation of central angiotensin receptor subtypes.

Previous work showed that the integrity of the area postrema (AP) was required for expression of chronic ANG II induced elevations in arterial pressure. The lateral parabrachial nucleus (LPBN) is an integrative brain region involved in neural control of the circulation, and also receives a major neuronal projection from the AP. The current studies demonstrated that the integrity of the LPBN is also required for maintenance of chronic ANG II hypertension. It was concluded that increases in neurogenic vasoconstrictor tone result from an action of circulating ANG II at the AP. The resulting increase in peripheral resistance during ANG II-induced hypertension depends on an interaction between the AP and the LPBN as well. These conclusions may apply to other models of ANG II-dependent hypertension which more closely resemble clinically relevant forms of hypertension: it was demonstrated here that prior lesion of the AP prevented development of ANG II-dependent but not ANG II-independent renovascular hypertension.

In a final set of experiments it was demonstrated that lower concentrations of circulating ANG II were required for expression of the neurogenic actions than for the direct peripheral effects of ANG II. These findings suggest that the contribution of circulating ANG II to models of hypertension previously considered ANG II-independent, as defined by normal or only slightly elevated plasma ANG II concentrations, has been underestimated. Furthermore, they provide a rational basis for the use of angiotensin converting enzyme inhibitors, or the more novel renin inhibitors and angiotensin receptor antagonists, in the treatment of essential hypertension.

To my best friend, Greg

Thank you

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I will dearly miss all these friends and many others in the Pharmacology department who have made East Lansing a pleasant place to live, and Life Sciences, HOME.

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ANG II	Angiotensin II
ANG (1-7)	Angiotensin (1-7)
AP	Area postrema
AVP	Arginine vasopressin
AV3V	Anteroventral third ventricle
BBB	Blood brain barrier
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
CSF	Cerebral spinal fluid
CVO	Circumventricular organ
DMNX	Dorsal motor nucleus of the vagus
EDTA	Ethylenediaminetetraacetic acid
Hex	Hexamethonium
HR	Heart rate
ip	Intraperitoneal
iv	Intravenous
ivt	Intraventricular
LPBN	Lateral parabrachial nucleus
MAP	Mean arterial pressure
ME	Median eminence
MnPO	Median preotic nucleus
NE	Norepinephrine
NTS	Nucleus of the solitary tract

LIST OF ABBREVIATIONS (Continued)

1-K,1-C	One-kidney, one-clip
OVLT	Organum vasculosum of the laminae terminalis
pANG	Plasma angiotensin II concentration
pAVP	Plasma vasopressin concentration
P_K	Plasma potassium concentration
P_{Na}	Plasma sodium concentration
P_{osmol}	Plasma osmolality
PRA	Plasma renin activity
RAS	Renin-angiotensin system
SAD	Sino-aortic denervated
SD	Standard deviation
SFO	Subfornical organ
SNP	Sodium nitroprusside
2-K,1-C	Two-kidney, one-clip
UKV	Urinary potassium excretion
UNaV	Urinary sodium excretion
UO	Urine output
WB	Water balance
WI	Water intake

INTRODUCTION

In this country cardiovascular disease or events (i.e. stroke, myocardial infarction) are responsible for more deaths than any other single cause. Often, damage produced in cardiovascular tissues, especially coronary and cerebral vasculature, is associated with many years of hypertension (high blood pressure). It is therefore important to better understand the etiology of this condition. The current use of angiotensin converting enzyme inhibitors, and the anticipated use of both the newly developed renin inhibitors and angiotensin receptor antagonists (all which inhibit the renin-angiotensin system), in the successful treatment of hypertension point to the importance of examining the involvement of the renin-angiotensin system in hypertension.

I. Peripheral Renin-Angiotensin System

The peripheral renin-angiotensin system (RAS) is a well known participant in the regulation of fluid and electrolyte homeostasis and in the control of arterial blood pressure. That kidney extracts contain a substance capable of eliciting a pressor response when intravenously injected into the anesthetized rabbit was first demonstrated almost a hundred years ago (Tigerstedt and Bergman, 1898). It was not until the 1940's that the dissection of the RAS was begun. It was first discovered that in response to a reduction in renal blood flow renin was produced and released from the kidneys (Braun-Menendez *et al.*, 1940; Page and Helmer, 1940). This substance was not directly responsible for the pressor response described by Tigerstedt and Bergman (1898). However, renin initiates the production of the pressor substance, angiotensin II (ANG II). Currently, it is well accepted that ANG II is the effector hormone of the RAS and has a variety of

physiologically relevant actions.

Since the initial discovery of renin, a more detailed understanding of the generation of peripheral ANG II has evolved. The precise site of renin production and release is the juxtaglomerular cells situated in the walls of the afferent arterioles of the renal vasculature (Cook, 1971). The release of renin is controlled by various stimuli classified as intrarenal, sympathetic, or humoral (Davis and Freeman, 1976). Intrarenal regulation of renin release includes an inverse relationship to changes in either perfusion pressure (Skinner *et al.*, 1964), or tubular sodium ion concentrations sensed by the macula densa apparatus in the distal tubule (Shade *et al.*, 1972). Activation of the renal sympathetic nerves or circulating catecholamines stimulate renin release by acting at $beta_1$-adrenergic receptors (Ueda *et al.*, 1970). Additionally, circulating hormones, including ANG II (negative feedback; Vander and Geelhoed, 1965), prostaglandins (stimulate; Whorton *et al.*, 1977) and vasopressin (inhibits; Shade *et al.*, 1973),participate in the control of renin release. Once released into the circulation renin, a proteolytic enzyme (Inagami and Murakami, 1977), catalyzes the conversion of hepatic angiotensinogen to the inactive decapeptide angiotensin I. Subsequently, circulating angiotensin I is cleaved by the actions of angiotensin converting enzyme (ACE), found in greatest concentrations in the endothelial cells in the vasculature of the lung, to the biologically active octapeptide ANG II.

The peripheral RAS described above is one endocrine system believed to be involved in whole body regulation of arterial pressure and fluid and electrolyte balance. Activation of the RAS occurs during states such as sodium deprivation, dehydration, and hemorrhage. The actions of circulating ANG II serve to maintain vascular tone by direct vasoconstriction and to conserve sodium and hence water by stimulating the release of aldosterone. However, more recently,

many have described the existence of a separate tissue RAS that may subserve in a more paracrine capacity in the control of local electrolyte or blood flow homeostasis (Ganten *et al.*, 1983; Dzau *et al.*, 1988; Lindpaintner *et al.*, 1988; Admiraal *et al.*, 1990). That is, all the components of the RAS can be produced locally in various tissues including the adrenals, blood vessels and cardiac tissue (Ganten *et al.*,1983). The significance of the tissue RAS, especially that of the vasculature, will be discussed later.

II. Peripheral Actions of Circulating Angiotensin II

As previously stated ANG II is an active participant in the maintenance of arterial pressure and body fluid volume. Since body fluid volume influences cardiac output, which is directly related to mean arterial pressure, it is not surprising that chronic elevations in circulating ANG II result in hypertension. What is surprising is that only a few other hormones [aldosterone (Kanagy *et al.*, 1990) and endothelin (Mortensen *et al.*, 1990)] are capable of eliciting similar results. There are many compensatory mechanisms to counter adverse (pressor, antinatriuretic, etc.) effects of excess or inappropriate elevations in various hormonal substances. It is of great interest to elucidate which actions of ANG II participate in the hypertension produced by exogenous infusion of the hormone, and which actions may or may not be countered by compensatory mechanisms. Following is a discussion of the acute interactions of circulating ANG II with peripheral tissues. Particular emphasis is placed on how each effect may be important in chronic ANG II hypertension.

A. Pressor effect

The most obvious effect of increases in circulating ANG II is an increase in arterial pressure. Although the existence of ANG II was not known at the time, the acute pressor effect of the hormone was demonstrated by the injection of renal

extracts into rabbits (Tigerstedt and Bergman, 1898). The first description of the chronic pressor actions of ANG II (again, prior to its discovery) were made by Goldblatt *et al.* (1934) and Pickering and Prinzmetal (1938). In their experiments it was reported that a chronic increase in arterial pressure accompanied a long-term reduction in renal blood flow. These studies first demonstrated the importance of the kidneys and subsequently, of ANG II in the development and maintenance of systemic hypertension.

Indeed, ANG II is a potent pressor agent when intravenously injected. A large component of the acute or fast (Brown *et al.*, 1981) pressor effect (minutes to hours) of systemically administered ANG II is direct constriction of the vascular smooth muscle (DeBono *et al.*, 1963; Regioli *et al.*, 1974). This direct effect is inhibited by prior administration of either saralasin, a partial agonist of the actions of ANG II (Zimmerman, 1973), or nonpeptide ANG II receptor antagonists (Wong *et al.*, 1989a; 1989b).

The ability of intravenous infusion of ANG II to elevate arterial pressure persists up to weeks, until the infusion is discontinued. However, the chronic or slow (Brown *et al.*, 1981) pressor effect (days to weeks) of ANG II differs from the acute pressor actions of the hormone. The most striking difference between the acute and chronic pressor actions of ANG II is the magnitude of the blood pressure increase. An intravenous infusion of ANG II continued for days results in a much greater rise in arterial pressure from control than an acute (minutes) infusion of the same dose (Cowley and McCaa, 1976; Hall *et al.*, 1978; Fink *et al.*, 1987). In fact, doses that are subpressor when administered acutely are capable of producing long-term arterial hypertension when chronically infused (McCubbin *et al.*, 1965; DeClue *et al.*, 1978). The half-life of circulating ANG II is approximately 15 seconds (Al-Merani *et al.*, 1978). Therefore, it is highly unlikely that the

increased magnitude of the pressor response is simply due to a progressively greater level of circulating ANG II due to "build up" during chronic infusion.

The chronic hypertensive actions of ANG II are referred to as "slow" (Brown *et al.*, 1981) simply because a pressure plateau is not attained for days to weeks. Further, hypertension produced by chronic ANG II infusion is slower to reverse than the acute pressor effect. That is, when the infusion is stopped after days of ANG II administration, it is hours before control pressures are again reached, rather than minutes required to return to control pressures after cessation of an acute, ten minute infusion (Brown *et al.*, 1981). Additional support for the claim that the slow pressor effect of ANG II is less readily reversed than the acute effect is evidenced by the inability of saralasin to lower blood pressure in the rat made hypertensive by chronic iv ANG II infusion (unpublished results). Taken together, it is highly unlikely that the long-term hypertensive actions of ANG II are completely attributable to a chronic direct constriction of the vasculature by the hormone. Additional mechanisms must contribute as well.

B. Antinatriuretic effects

Circulating ANG II causes sodium retention both by directly stimulating sodium reabsorption in the distal tubules (Hall *et al.*, 1978) and by stimulating the release of aldosterone from the adrenal glomerulosa (Laragh *et al.*, 1960; Fraser *et al.*, 1965). One hypothesis to explain the chronic hypertensive actions of ANG II was formulated based on these antinatriuretic actions of the hormone. Sodium and water retention by ANG II could lead to volume expansion which in turn would raise arterial pressure by a resulting increase in cardiac output. However, long-term infusion of ANG II is not always accompanied by a significant increase in sodium retention in either the rat (Brown *et al.*, 1981; Fink *et al.*, 1985) or dog (Trippodo *et al.*, 1976; Carroll *et al.*, 1984). Further, chronic (3 week) ANG II

infusion in the dog recently has been reported to be accompanied by a decrease in cardiac output (Granger *et al.*, 1990). Therefore, the chronic pressor effect of ANG II is not attributable solely to antinatriuretic and/or fluid retentive actions of the hormone.

C. Aldosterone release

A second hypothesis to explain ANG II hypertension is based on the ability of circulating ANG II to stimulate the release of aldosterone. When administered chronically to either uninephrectomized (Garwitz and Jones, 1982) or intact (Kanagy *et al.* , 1990) rats, aldosterone produces hypertension that is slow to develop (one to two weeks). It follows that ANG II stimulated increases in circulating aldosterone could lead to mineralocorticoid hypertension similar to that seen in patients with primary hyperaldosteronism (Biglier *et al.*, 1972). Acute increases in circulating ANG II do produce significant increases in circulating aldosterone in humans (Laragh *et al.*, 1960), rats (Kanagy *et al.*, 1990) and dogs (McCaa *et al.*, 1975; Bean *et al.*, 1979; Cowley and McCaa *et al.*, 1976). However, the chronic actions of ANG II are not readily predicted by the acute actions of the hormone. Thus it has been demonstrated in the dog (Cowley and McCaa, 1976; Bean *et al.*, 1979; Young and McCaa, 1980) and rat (Kanagy *et al.*, 1990) that chronic elevation in circulating ANG II is not accompanied by chronic increases in circulating aldosterone. However, these findings are not always consistent as Carroll *et al.* (1984) reported increases in circulating aldosterone in the ANG II-hypertensive dog, and Luft *et al.* (1989) reported a slight but significant increase in urinary aldosterone excretion in the ANG II-hypertensive rat. Regardless, the levels of plasma aldosterone concentrations required to produce hypertension in the rat (Garwitz and Jones, 1982; Kanagy *et al.,* 1990) are not approached during chronic ANG II hypertension (Kanagy *et al.*, 1990). Further, it has been reported

that six day treatment with an aldosterone receptor antagonist, spironolactone, does not lower arterial pressure in ANG II-induced hypertensive dogs (DeClue *et al.*, 1978). Thus, it is unlikely that the chronic hypertensive actions of ANG II are related to increases in circulating aldosterone.

D. Interactions with peripheral sympathetic neurotransmission

There is convincing evidence in the literature that ANG II interacts with the peripheral sympathetic nervous system to enhance both the response to evoked release of norepinephrine (NE) from noradrenergic nerve terminals and to exogenously applied NE. Specifically, it has been shown that ANG II facilitates the electrically stimulated release of NE (Hughes and Roth, 1971) by a receptor mediated reaction that is attenuated by pretreatment with saralasin (Zimmerman, 1973; Lokhandwala *et al.*, 1978; Boke and Malik, 1983). Further, this effect is augmented by pretreatment with an alpha$_2$-adrenergic receptor antagonist (Costa and Majewski, 1988). The concentration of NE in the neuroeffector junction may be increased further by ANG II inhibition of NE reuptake (Khairallah, 1972; Campbell and Jackson, 1979). Finally, ANG II enhances the vascular response to NE by a post-synaptic interaction also blocked by saralasin pretreatment (Zimmerman, 1978; Purdy and Weber, 1988). Taken together, these results indicate that in the presence of ANG II there is an enhanced vascular resistance in response to both sympathetic nerve stimulation and exogenously administered NE (Zimmerman, 1978). The importance of these facilitatory actions of ANG II on the peripheral sympathetic nervous system in ANG II hypertension is of interest. It is not known whether the low doses of ANG II required to produce chronic hypertension result in ANG II concentrations in the neuroeffector junction sufficient to enhance noradrenergic neuronal transmission. However, in the absence of converting enzyme inhibition, ANG I can enhance neuronal

transmission similar to ANG II in the isolated kidney (Boke and Malik, 1983). These observations raise the question of the importance of local tissue generation of ANG II in local regulation of peripheral sympathetic nerve discharge.

III. Angiotensin II and the Central Nervous System

Accumulating evidence demonstrates that circulating ANG II can influence central nervous system regulation of body fluid volume and arterial pressure. Since it was revealed that infusion of ANG II into the vertebral arteries produced chronic hypertension at doses ineffective when infused intravenously, there has been little doubt that an interaction between the central nervous system and ANG II is operative in ANG II-induced hypertension.

A. Brain renin-angiotensin system

Prior to addressing specific effects and sites of action of ANG II it may be helpful to discuss the well established brain RAS. Similar to peripheral tissues, the brain also appears to contain its own RAS. Renin (Ganten *et al.*, 1971), angiotensinogen (Lewicki *et al.*, 1978; Gregory *et al.*, 1982), ANG I (Husain *et al.*, 1983), ACE (Yang and Neff, 1972; Daul *et al.*, 1975) and ANG II (Nicholls, 1979) have been isolated from brain tissue. Both renin (dog- Ganten *et al.*, 1971) and angiotensinogen (rat- Gregory *et al.*, 1982) appear in the brain tissue of bilaterally nephrectomized animals. Further, manipulations that cause large increases in circulating ANG II do not alter ANG II concentrations in cerebral spinal fluid (CSF; Nicholls, 1979). These findings suggest independent regulation of the peripheral and brain RAS. The existence of the brain RAS suggests that ANG II may have a greater influence on blood pressure and body fluid control than originally suspected, and potentially acts through the central nervous system as well as the kidneys and vasculature. Additionally, there is speculation that ANG

II may be a neurotransmitter or cotransmitter serving as a local modulator of adrenergic and noradrenergic neurotransmission as postulated for peripheral tissues. Further, ANG II immunohistological studies identify ANG II immunoreactivity associated with vasopressinergic cells (Kilcoyne, 1980). The role of ANG II as a central neurotransmitter requires further investigation. However, the brain possesses the capability to produce ANG II, shows ANG II immunoreactivity, and as will be discussed later, has ANG II receptors. Therefore, it is likely that ANG II can interact with the brain in some physiologically relevant capacity that is ANG II-specific.

B. Central vs. systemic angiotensin II administration

Intracerebral ventricular (icv) administration of ANG II or precursors (ie. renin or angiotensin I) elicits a variety of responses including an increase in arterial pressure, antidiuresis, natriuresis, and drinking (Hoffman *et al.*, 1979; Phillips *et al.*, 1979; Breuhaus and Chimoskey, 1990). These central actions (except natriuresis) parallel the actions of acute systemic administration of ANG II. Three possibilities exist to explain the similar actions of both central and peripheral administration of ANG II. 1.) ANG II administered icv acts at sites separate from those when the hormone is given systemically. 2.) ANG II acts at similar sites regardless of the route of administration but crosses the blood brain barrier (BBB) from the CSF to the circulation or vice versa. 3.) Central and circulating ANG II act at similar sites that are accessible from both the CSF and the peripheral circulation.

First, there is convincing evidence that ANG II infused intravenously does not cross the BBB into the CSF unless there is concurrent or previous disruption of the BBB (Schelling *et al.*, 1980). Second, ANG II injected icv does not result in an increase in circulating ANG II concentrations (Breuhaus and Chimoskey, 1990).

These observations suggest that ANG II neither leaks into the CSF from the circulation nor into the circulation from the CSF.

The observation that chronic icv administration of an ANG II receptor antagonist, sarthran, does not attenuate chronic hypertension produced by intravenous infusion of ANG II (Bruner and Fink, 1985) supports the hypothesis that ANG II hypertension does not necessitate the hormone entering the CSF. This study further suggests that the central site of action responsible for the neural component of the pressor response to ANG II may depend on the route of administration. However, central administration of saralasin, an alternate ANG II antagonist, markedly attenuates the acute pressor response to intracarotid infusion of ANG II (Fink, *et al.*, 1980) and the drinking response to subcutaneous injection of ANG II (Johnson and Schwob, 1975). Further, electrolytic ablation of the tissue surrounding the anteroventral third ventricle (AV3V) abolished both the acute icv ANG II pressor response (Brody and Johnson, 1980) and the central component of circulating ANG II pressor effect in the rat (Fink *et al.*, 1980). These results support the claim that ANG II may act at similar sites regardless of the route of administration.

Two approaches have been employed to elucidate where in the brain ANG II acts. The first approach entails the localization of ANG II binding in the brain. A second approach involves removing tissue exhibiting ANG II receptor binding and then evaluating the necessity of that tissue for a given response. A third approach, stimulation of specific receptor subtypes, may be useful to elucidate the exact interactions of ANG II with the brain that are vital to hypertension development.

1. Angiotensin receptor localization

Autoradiographic studies demonstrate a map of receptors unique for ANG II binding. These studies demonstrate saturable, high affinity binding sites.

Further, radiolabeled ANG II is readily displaced by unlabeled (cold) ANG II and relative affinity of ANG II analogues correlates well with the relative potency of each to cause direct constriction of the vasculature (i.e. Ang II > Ang III) (Saavedra *et al.*, 1986). Experiments in rat (Gehlert, *et al.*, 1984; Mendelsohn *et al.*, 1984) and dog (Speth *et al.*, 1985) demonstrate ANG II receptor binding in nuclei associated with a variety of functions including memory and olfaction. However, the sites which are most noteworthy for the current discussion are those that may be accessible to circulating peptides. Specifically, the circumventricular organs (CVOs), which have fenestrated capillary networks that lack the tight junctions of a true blood brain barrier, are of interest. All studies demonstrate heavy ANG II binding in the CVOs, such as the subfornical organ (SFO), median eminence (ME), paraventricular nucleus, and organum vasculosum laminae terminalus (OVLT) (Saavedra, 1986; Mendelsohn *et al.*, 1984; Speth *et al.*, 1985; Gehlert *et al.*, 1984) All but Gehlert *et al.* (1984) claim the area postrema (AP) contains many ANG II receptors. Additionally the nucleus of the solitary tract (NTS) contains a dense ANG II receptor population. The NTS is not easily accessible to circulating hormones. However, tanicytes in the AP display both ANG II receptor binding and immunohistochemically labeled intracellular ANG II. Immunohistochemical studies also demonstrate ANG II labeling in neuronal cells with terminals in the NTS especially along the AP margin. (Lind, 1985). These observations led to the postulation that ANG II can be internalized from the periphery to be released into the NTS as a modulator of NTS neuronal transmission (Gehlert, *et al.*, 1984). In addition, there are ANG II receptors associated with vagal afferents in both the NTS and dorsal motor nucleus (Healy *et al.*, 1989). Therefore, numerous regions of the central nervous system to which circulating ANG II may obtain access all show receptor labeling. Circulating ANG

II does gain access to these regions as demonstrated by radiolabeled ANG II in the SFO, ME, OVLT, and AP, 3 minutes after a systemic (van Houten *et al.*, 1980) or icv (van Houten *et al.*, 1983) injection of radiolabeled ANG II. Thus, the ANG II receptor studies only slightly narrow the possibilities in the determination of the brain site(s) crucial to development of ANG II hypertension.

2. Lesion studies

Discrete electrolytic ablation of brain tissue destroys cell bodies within and axons passing through the selected region. Studies using such techniques have been particularly useful in discerning the regions of the CNS most critical to various actions of ANG II. Of particular interest are the studies concerned with localizing the central sites essential for ANG II hypertension development.

For some time it was believed that the dog and rat were different with regard to the interactions between circulating ANG II and the brain. The acute pressor response to ANG II was diminished in the dog by prior ablation of the AP (Ferrario, 1983). However, in the AP-lesioned rat the acute pressor response to ANG II was unaltered (Haywood *et al.*, 1980). The hypothalamus appeared to play a more important role in the acute and chronic hypertensive actions of circulating ANG II. The integrity of the region surrounding the anteroventral third ventricle (AV3V) proved to be essential to ANG II hypertension in the rat (Fink *et al.*, 1982) but not the rabbit (Fink and Mann, 1984). The extent of such lesions is vast, especially in the rat, thus additional lesion studies were performed to systematically determine which nuclei within the AV3V region were critical for hypertension development. This region includes ANG II receptive sites such as the OVLT, and the median preoptic nucleus (MnPO) plus the main fiber bundles that connect the OVLT and an other ANG II receptive site the subfornical organ (SFO). Electrolytic lesion of either the SFO (Bruner *et al.*, 1985), or MnPO (Fink

et al., 1986) failed to prevent ANG II hypertension. Additionally, knife cuts performed to sever the neuronal connection between the SFO and OVLT did not prevent ANG II hypertension (Bruner, 1985). Therefore, individual circumventricular organs within the AV3V region do not appear to be essential to hypertension development.

The AV3V lesioned rat, while maintaining normal fluid balance upon recovery, does not necessarily respond appropriately to challenges of fluid volume expansion or contraction (Bealer *et al.*, 1983). As will be discussed shortly, the ability of ANG II to produce hypertension may require blood volume expansion in response to a sodium enhanced diet. The AV3V lesioned rat may not experience the sodium induced volume expansion, thereby preventing ANG II induced hypertension. This volume-dependency may not be exclusive to ANG II induced hypertension since AV3V lesions retard the development of Dahl salt-sensitive hypertension (Goto *et al.*, 1982) which is also accompanied by volume expansion (Greene *et al.*, 1990). Therefore, the necessity of the AV3V region for ANG II hypertension may not be indicative of a specific interaction of ANG II with this brain area, but rather of the requirement for a normal body fluid volume response to increases in dietary salt intake in the rat.

Angiotensin hypertension did not appear to be dependent on the SFO, OVLT, or MnPO, all sites displaying ANG II receptor labeling after intravenous infusion of radiolabeled ANG II (van Houten *et al.*, 1980) However, prior ablation of the AP prevented maintenance of ANG II hypertension despite the fact that the acute pressor response to the hormone was unchanged (Bruner *et al.*, 1985). These experiments led to the conclusion that as in the dog, circulating ANG II either acts at the AP or gains access to the CNS through the AP to produce chronic hypertension.

ANG II could interact with neuronal cell bodies within the AP. Immunohistochemical studies suggest that cells from the AP predominantly project to either the NTS or the lateral parabrachial nucleus (LPBN) in the pons. Of course the NTS is intricately involved with blood pressure control. However, the LPBN when electrically or chemically stimulated elicits a cardiovascular response (Ward, 1988). The cells associated with terminal fields from the AP in the LPBN also appear to receive afferent innervation from both the NTS and dorsal motor vagal complex (Shapiro and Miselis, 1985). Therefore it follows that the LPBN may be an important integrative center for peripheral input to the CNS in arterial pressure regulation. Thus, it is of interest to determine whether the integrity of the LPBN is essential for hypertension development. Angiotensin hypertension is a good experimental model in which to study the role of ANG II in hypertension. However clinical hypertension is a multifactorial disease. As such, it is important to consider findings in this model and extend them to other more clinically relevant models of hypertension. Two models of experimental hypertension are employed to study two forms of clinical renovascular hypertension. The two-kidney, one-clip (2K,1C) model is associated with large increases in plasma renin activity and plasma ANG II concentrations and is effectively controlled with angiotensin converting enzyme (ACE) inhibitors which prevent the endogenous production of ANG II from ANG I. The one-kidney, one-clip (1K,1C) model is not considered renin-dependent and is not accompanied by increases in PRA. Lesion studies have been performed in order to determine critical brain regions for the development of these two forms of hypertension. In the rat (Haywood *et al.*, 1983) but not the rabbit (Fink and Bryan, 1982) the 2K,1C model is partially dependent on an intact AV3V region. In the rat, lesion studies with the 2K,1C model have produced results similar to those involving ANG II hypertension.

Therefore it would be expected that hypertension putatively dependent on circulating ANG II would also depend on the integrity of the AP. On the other hand the 1K,1C model would presumably be unaffected by prior electrolytic ablation of the AP. Such results would lend support to ANG II-dependency of 2K,1C renovascular hypertension and the involvement of peripheral-central interactions during hypertension.

3. Receptor subtypes

Incubation of brainstem homogenates with ^{125}I-labeled ANG I, both before and after blockade of converting enzyme activity, resulted in the preferential accumulation of the N-terminal heptapeptide ANG (1-7) (Santos *et al.*, 1988). While ANG (1-7) only has weak agonistic pressor and drinking effects (Campagnole-Santos *et al.*, 1989) it has been shown to be equipotent to ANG II in activating neurons in the medullary brainslices of dogs (Barnes *et al.*, 1988). Further, *in vivo* studies demonstrate that microinjection of ANG (1-7) into either the NTS or DMNX produces depressor and bradycardic effects similar to those elicited by ANG II. The binding affinity of ANG (1-7) is similar to ANG II in only some regions of the dorsal medulla (Diz and Ferrario, 1989), however, suggesting subtypes of ANG II receptors. The brainstem appears to play an essential role in ANG II hypertension. If ANG (1-7) is the predominant metabolite of the RAS in the brainstem, ANG (1-7) may be the effector hormone at this level in the brain. Combined with the fact that ANG (1-7) has no peripheral vasoconstrictor actions, chronic iv infusion of ANG (1-7) could uncover a purely neurogenic hypertension by acting in the brainstem at ANG II receptor subtypes responsive to the heptapeptide.

In addition to eliciting actions in the brainstem, ANG (1-7) interacts with the vasopressinergic system (Block *et al.*, 1989; Schiavone *et al.*, 1988). Therefore

ANG (1-7) also may be the effector hormone of the RAS in modulating the release of AVP.

C. Central effects of angiotensin II

Following is a discussion of the actions of circulating ANG II that involve interaction with the central nervous system. For each effect the critical brain regions as identified by lesion studies will be discussed. Further, the possible contribution to ANG II hypertension or other physiologically relevant states will be emphasized.

1.) Angiotensin induced thirst

Many experimental manipulations that induce a state of thirst, as measured by an increase in voluntary water intake, also produce significant increases in circulating ANG II concentrations. These manipulations include caval ligation; subcutaneous injection of either the B-adrenergic receptor agonist, isoproterenol, or polyethylene glycol (Johnson *et al.*, 1981); and water deprivation (Mann *et al.*, 1980; Yamaguchi, 1981). These reports support the belief by many that ANG II plays an important role in the complex behavior of thirst.

It has long been established that icv injection of ANG II into most species tested elicits a profound drinking response (Fitzsimons, 1976 for review). This drinking response is dependent on the ability of the icv injected ANG II to gain access to the anteroventral region of the third ventricle (Hoffman and Phillips, 1976b; Buggy and Johnson, 1978). Further, it has been reported that the integrity of the SFO (Simpson and Routtenberg, 1973; Hoffman and Phillips, 1976a; Simpson, 1981) and/or the OVLT (Landas *et al.*, 1980; Thrasher and Keil, 1987) located in the walls of the third ventricle is(are) essential for the icv ANG II stimulated drinking response. The physiological significance of thirst induced by icv injections of ANG II is unknown, especially since ANG II concentrations in the

CSF are generally negligible (Schelling *et al.*, 1980). However, both the SFO and OVLT are circumventricular organs. Therefore circulating ANG II could access either or both of these brain nuclei to stimulate drinking during physiological states of thirst, or ANG II may be produced or released locally by tissue in or near the SFO or OVLT.

It is fairly certain that ANG II-induced drinking does not contribute to elevations in arterial pressure during ANG II hypertension. Most studies of ANG II hypertension do not report a chronic state of thirst as measured by increases in daily water intake (Brown *et al.*, 1981; Fink *et al.*, 1987; Krieger and Cowley, 1990). Conversely, Trippodo *et al.* (1976) and Hall *et al.* (1978) demonstrated a chronic increase in daily water intake during ANG II-induced hypertension in the dog. Because ANG II is capable of producing chronic hypertension without accompanying elevations in water intake, however, the dipsogenic action of ANG II does not apparently play a significant role in ANG II hypertension. Further, electrolytic ablation of the SFO, necessary for the acute dipsogenic response to circulating ANG II (Simpson and Routtenberg, 1973) does not prevent ANG II hypertension (Bruner *et al.*, 1985).

Although the putative dipsogenic actions of ANG II do not seem important to the hypertensive actions of the hormone, it is of interest under which conditions, if any, ANG II plays a significant role in modulating thirst. The ability of large increases in circulating ANG II to stimulate drinking has been demonstrated in numerous studies. Drinking in response to systemic administration of ANG II has been illustrated in water replete animals, including dogs (Thrasher and Keil, 1987; Fitzsimons *et al.*, 1978), goats (Eriksson *et al.*, 1981) and rats (Fitzsimons and Simons, 1969; Johnson and Schwob, 1975; Hsiao *et al.*, 1977). However, in many studies the doses of ANG II required to elicit a drinking response were relatively

large compared to doses required for other physiological actions of ANG II. Alternatively, the latency of response is not always consistent with a direct action of ANG II. The role of ANG II in the complex behavior of thirst has been extensively examined. Thus far it has not been clearly demonstrated whether or not physiologically relevant increases in ANG II are 1.) capable of eliciting a drinking response as a solitary stimulus; 2.) capable of enhancing the drinking response to various other putative thirst stimuli; or 3.) necessary for the drinking response to various other putative thirst stimuli.

2.) Antidiuretic effect

Arginine vasopressin (AVP) released from the hypothalamo-neurohypophyseal system is known to play a significant role in the maintenance of body fluid and arterial pressure homeostasis. During states such as hemorrhage (Laycock *et al.*, 1979) or dehydration (Trapani *et al.*, 1988) AVP may participate in maintaining circulation by constricting arterial vasculature and stimulating water reabsorption from the renal collecting tubules. Because of these physiological actions, it has been suggested that AVP plays a significant role in the pathogenesis of certain forms of hypertension, including mineralocorticoid (Berecek, 1982; Filep *et al.*, 1987) and renovascular (Lariviere *et al.*, 1988) hypertension in the rat.

Both acute central (Bealer *et al.*, 1979; Breuhaus and Chimoskey, 1990) and systemic (Bonjour and Malvin, 1970; Knepel *et al.*, 1982; Ramsay *et al.*, 1978) administration of ANG II stimulates the release of AVP into the circulation. There is good evidence that AVP plays a significant role in the acute pressor response to icv injected ANG II. First, pretreatment with either a vascular AVP receptor antagonist (Haack and Mohring, 1978) or antibodies to AVP (Hoffman *et al.*, 1979) attenuates the pressor response to acute ivt injections of ANG II. Second, transection of SFO efferents both abolishes the ability of circulating Ang

II to stimulate AVP release (Knepel *et al.*, 1982) and attenuates the acute ANG II pressor response (Lind *et al.*, 1983). Third, the Brattleboro rat, which can not produce AVP, exhibits diminished pressor responses to icv injected ANG II (Hutchinson *et al.*, 1976). These observations lead to the hypothesis that increases in circulating AVP contribute to the maintenance of elevated pressures during chronic ANG II infusion. Further, they suggest one mechanism to explain the central contribution to ANG II hypertension. However, during chronic iv infusion of ANG II in the dog (Cowley *et al.*, 1981) and icv infusion in the rat (Fink *et al.*, 1982) circulating AVP concentrations remained unaltered. Additionally, acute blockade of vascular AVP receptors during ANG II hypertension does not result in a significant fall in arterial pressure during chronic icv (Bruner and Fink, 1986) ANG II infusion. Finally, ablation of the SFO does not prevent ANG II hypertension (Bruner *et al.*, 1985), although it does abolish acute ANG II-stimulated AVP release (Mangiapane *et al.*, 1984). It is unlikely then that circulating AVP plays a significant role in ANG II hypertension.

While AVP does not apparently contribute significantly to ANG II hypertension, it remains of interest whether sustained physiologically relevant increases in circulating AVP alone can produce hypertension. Although AVP has been shown to be an extremely potent constrictor substance of vascular smooth muscle (Altura and Altura, 1977), the dose response to the pressor actions of AVP is very shallow in the animal with intact reflexes (Cowley *et al.*, 1974). It is now apparent that AVP modifies its own pressor response by enhancing reflex input into the central nervous system. That is, circulating AVP facilitates arterial (dog- Cowley *et al.*, 1974; rabbit-Undesser *et al.*, 1985; rat- Peuler *et al.*, 1990) and cardiopulmonary (baboon- Barazanji and Cornish, 1989; rabbit- Gupta *et al.*, 1987; Hasser *et al.*, 1987) reflex inhibition of sympathetic nerve activity. In the rabbit,

the integrity of the area postrema is apparently crucial for the interaction of circulating AVP and reflex control of the circulation (Undesser *et al.*, 1985; Hasser *et al.*, 1987); this also may be true in the rat (Peuler *et al.*, 1990). It is the enhanced withdrawl of renal sympathetic outflow that may be responsible for the escape from the chronic pressor and antidiuretic actions of AVP seen in the servo-controlled dog receiving chronic AVP infusion (Cowley *et al.*, 1984). However, these dogs were maintained on a constant volume intake, therefore the antidiuretic actions of AVP caused volume expansion which was assuredly compensated for by various mechanisms. It has not been clearly demonstrated that AVP infusion alone in the freely drinking animal results in chronic hypertension. Further, it has not been demonstrated whether AVP infusion can produce chronic hypertension in the sino-aortic denervated animal with obvious impairment of reflex control of the circulation. Alternatively, the ability to produce chronic hypertension with AVP in the animal with prior ablation of the area postrema has not been assessed. Such experiments could have clinical relevance as to the role of AVP in hypertension since baroreflex impairment occurs with age (Tanabe and Bunag, 1989) and during hypertension (Andersen *et al.*, 1980; Thames *et al.*, 1981).

3.) Interaction with the autonomic nervous system

The cross-circulation experiments performed by Bickerton and Buckley (1961) first distinguished the direct vasoconstrictor actions of acute increases in circulating ANG II from the indirect, central actions. They demonstrated that the acute central actions of ANG II were abolished with peripheral administration of a sympatholytic agent. Further, it has been shown that the chronic hypertensive actions of intravertebral infusion of ANG II can be prevented by pretreatment with the sympatholytic, reserpine (Sweet *et al.*, 1971) or by continuous iv infusion of an alpha-adrenergic receptor antagonist (Dickinson and Yu, 1967). These

initial observations led to many experiments aimed at determinating the nature of interactions between ANG II and the central autonomic nervous system.

While AVP, as discussed previously, enhances baroreceptor reflex sympathoinhibition, ANG II acts to oppose reflex mediated sympathoinhibition. In all species examined, circulating ANG II attenuates baroreflex induce bradycardia in response to increases in arterial pressure (baboon- Garner *et al.*, 1987; cat- Stein *et al.*, 1984; rabbit- Matsumura *et al.*, 1989; Guo and Abboud, 1984; dog- Lumbers *et al.*, 1979; Brooks and Reid, 1986; rat- Michelini and Bonagamba, 1990). While reflex inhibition of lumbar (Guo and Abboud, 1984) and splenic (Stein *et al.*, 1984) sympathetic activity also are attenuated by circulating ANG II, reflex inhibition of renal sympathetic nerve activity is not affected (Matsumura *et al.*, 1989). These effects are receptor mediated and have been shown to be blocked with saralasin pretreatment (Michelini and Bonagamba, 1990). Additionally treatment with converting enzyme inhibitors or saralasin enhances baroreflex sympathoinhibition in normotensive humans (Ebert, 1985), baboons (Garner *et al.*, 1987), and dogs maintained on a low sodium diet to ensure greater circulating ANG II concentrations (Brooks and Reid, 1986). First, these studies suggest that ANG II is an endogenous modulator of baroreflex control of the circulation. Second, these studies suggest that ANG II opposes reflex mediated sympathoinhibition in a differential manner.

It is believed that an increase in the contribution of sympathetic vasoconstriction to the maintenance of arterial pressure exists during chronic ANG II hypertension. Attempts have been made to demonstrate increased sympathetic nerve activity during chronic ANG II hypertension. Such investigations have produced conflicting results. Carroll *et al.* (1984) reported decreases in renal norepinephrine overflow, as a measure of renal sympathetic nerve activity, in the

chronic (6 day) ANG II hypertensive dog. Conversely, Luft *et al.* (1989) demonstrated in the rat that fourteen day treatment with ANG II resulted in increased splanchnic sympathetic nerve activity, as measured directly. These contradictory results could reflect species variation or a difference in the central control of splanchnic versus renal sympathetic nerve activity. In support of this assumption, it has been demonstrated that infusion of ANG II into the carotid artery of the cat causes a greater increase in splenic than in renal sympathetic nerve activity (Tobey *et al.*, 1983). However, it is possible that the duration of ANG II treatment is important to consider as well.

IV. Sodium Dependency of Angiotensin Hypertension

As previously stated, chronic systemic ANG II administration is not always accompanied by a significant increase in sodium retention despite the antinatriuretic acute actions of the hormone (Trippodo *et al.*, 1976; Brown *et al.*, 1981; Carroll *et al.*, 1984; Fink *et al.*, 1987). However, there is a dependency of ANG II-induced hypertension on sodium intake. That is, the level of hypertension produced by chronic ANG II infusion is directly related to the dietary sodium of the experimental animal (Cowley and McCaa, 1976; Hall *et al.*, 1980). Normally plasma renin activity and hence circulating ANG II concentrations are decreased in response to increases in dietary sodium (Brown *et al.*, 1964; Welch *et al.*, 1987). Consequently increases in dietary sodium are not usually accompanied by increases in arterial pressure. It is in the experimental setting when dietary sodium is increased while plasma ANG II concentrations are maintained by constant infusion that hypertension ensues (Krieger and Cowley, 1990).

A. Volume expansion

The dependency of ANG II hypertension on sodium intake is not due to

increases in plasma sodium. Angiotensin hypertension is not necessarily accompanied by increases in plasma sodium (Hall *et al.*, 1980). Further, when the body fluid volume of the dog is prevented from changing in response to increases in both circulating ANG II and sodium intake, blood pressure does not rise despite a large increase in plasma sodium concentration (Krieger and Cowley, 1990). This study (along with the unpublished control study) illustrates that ANG II hypertension is dependent on an increase in blood volume and cardiac output that accompanies the increase in sodium intake. Therefore, ANG II hypertension appears to require a degree of blood volume expansion but this is secondary to the amount of sodium in the diet and not due to an action of the hormone itself.

B. Prior receptor occupancy

The dependency of the pressor actions of ANG II on volume status of the dog are further illustrated in a series of experiments performed by Cowley and Lohmeier (1978). In these studies they showed that ANG II is a more potent pressor agent in the normovolemic than in the acutely hypovolemic dog. Further, the pressor effect of ANG II is even greater in the volume expanded animal. It is also of interest that plasma sodium alterations in the normovolemic dog did not affect the pressor responsiveness to acute intravenous infusions of ANG II. They conclude that the decreased responsiveness to exogenous administration of ANG II in the volume contracted state is due to prior occupancy of ANG II vascular receptors by greater circulating levels of ANG II. This in not an uncommon theory. Thurston and Laragh (1975) reported that treatment with a converting enzyme inhibitor enhanced the acute pressor response to ANG II in rats maintained on a low sodium diet without altering the response in the rat on a high sodium intake. However, *in vitro* experiments demonstrate that, even using the same perfusion buffer, vascular responsiveness to ANG II is greater in the rabbit

fed a sodium enhanced diet than in normal sodium rabbits (Strewler *et al.*, 1972). Therefore, it is still uncertain whether existing levels of circulating ANG II significantly alter the acute pressor response to ANG II.

A few observations would argue against the theory of prior receptor occupancy by endogenous ANG II to explain the variable sensitivity of the vasculature to the chronic pressor actions of ANG II during alterations in sodium intake. First, ANG II will elicit a chronic hypertensive effect at doses that are acutely subpressor (McCubbin *et al.*, 1965; DeClue *et al.*, 1978). The pressor effect would be expected to be greatest initially (acutely) when the circulating concentrations are lowest. Second, chronic increases in circulating ANG II shift the dose response curves of acute pressor doses of ANG II upward from the responses elicited in the control dog. That is, even though the plasma concentration of ANG II is chronically elevated, acute pressor responses are of equal or greater magnitude as those elicited in the control or recovery period when pre-existing ANG II levels are low (Bean *et al.*, 1979). These studies suggest that a lower degree of prior receptor occupancy does not explain an increase in pressor responsiveness to ANG II accompanying increases in the dietary sodium intake.

C. Angiotensin receptor concentrations

Alterations in dietary sodium have been reported to alter angiotensin receptor concentrations in both the adrenal glomerulosa and smooth muscle. While ANG II receptor concentrations in the vascular and urinary bladder smooth muscle decrease during periods of low sodium intake (Aguilera and Catt, 1981), receptor numbers are increased in the adrenals (Aguilera and Catt, 1981; Ray *et al.*, 1990). Conversely, during periods of increased sodium intake ANG II receptor concentrations are increased in smooth muscle (Aguilera and Catt, 1981) and decreased in the adrenals (Aguilera and Catt, 1981; Ray *et al.*, 1990). These

observations led to the hypothesis that an increase in sodium intake results in a greater responsiveness to ANG II by virtue of increased vascular receptor concentrations. This may in fact be true for the acute actions of ANG II. However, it appears that at least with low or normal sodium intakes, the receptor concentrations are dictated by the levels of circulating ANG II and not sodium intake (Aguilera and Catt, 1983). With increases in sodium intake, when concentrations of circulating ANG II are low, there may be an upregulation of receptor numbers in the vascular smooth muscle. An enhanced pressor response would be expected initially but if elevated circulating ANG II concentrations are maintained, smooth muscle ANG II receptor concentrations would be expected to decline. Indeed, this was demonstrated in the smooth muscle of the urinary bladder with 2-4 day infusion of ANG II in the rat maintained on a normal sodium intake (Aguilera and Catt, 1981). Therefore, the enhanced vascular responsiveness to acute increases in circulating ANG II during periods of increased sodium intake may be due in part to an increased ANG II receptor concentration in the vascular smooth muscle. However, the sodium dependency of chronic ANG II hypertension can not be explained by similar reasoning.

The evidence presented to date indicates that the sodium dependency of ANG II hypertension is not due to an enhanced vascular reactivity due to either lower endogenous ANG II concentrations or increased ANG II vascular receptor concentrations. The ability of a diet enhanced with sodium to cause a slight expansion in body fluid volume and, in this manner sensitize the animal to the actions of circulating ANG II, requires further investigation.

V. Plasma Angiotensin II Concentrations in Hypertension

Hypertension often is categorized by the accompanying level of plasma renin

activity (PRA). Hence, a model of hypertension is considered either renin-dependent (with high PRA) or renin-independent (with normal or low PRA). It is assumed that plasma ANG II concentrations are correlated closely to PRA. In studying ANG II hypertension it is important to use an infusion rate that produces appropriate increases in plasma ANG II concentrations in order to extrapolate results to more clinically relevant forms of hypertension. As previously discussed, the 2-K,1-C renovascular model of hypertension is renin-dependent. During prolonged 2-K,1-C hypertension (5-10 weeks) in the rat, plasma ANG II concentrations reportedly range from 140-180 pg/ml (Morton and Wallace, 1983). Based on evidence from Brown *et al.* (1981) an infusion rate of 10 ng/min should produce plasma ANG II concentrations within this desired range. Achievement of ANG II levels much higher than these may uncover effects of ANG II not operative at the lower levels seen in clinical hypertension. On the other hand, maintenance of ANG II concentrations less than these may underestimate the role of ANG II mechanisms in hypertension. Therefore it is important to confirm the plasma ANG II concentrations resulting from long term ANG II infusion. Such measurements also would aid in comparison of results from other similar experiments.

STATEMENT OF PURPOSE

A central pressor effect of ANG II has for years been implicated in various models of hypertension. The result of the interaction between circulating ANG II and the brain in cardiovascular regulation is the emphasis of the current studies. Specifically, three brain mechanisms potentially involved in ANG II hypertension in the rat will be examined. These three mechanisms are 1.) an increase in water intake, 2.) an increase in circulating AVP, and 3.) increases in sympathetically-mediated vasoconstrictor tone. With regard to the latter, it was hypothesized that initially the arterial baroreceptor reflex partially inhibits full expression of the neurogenic component of ANG II hypertension. The possibility will be assessed that an ANG II receptor subtype selectively sensitive to the ANG II metabolite, ANG (1-7) is responsible for the neurogenic component of ANG II hypertension.

The integrity of the area postrema (AP) in the brainstem is required for ANG II induced hypertension. The AP has dense neural connections with the NTS and lateral parabrachial nucleus (LPBN). In an attempt to determine the neural pathways from the AP that are responsible for ANG II hypertension, the necessity of the LPBN for ANG II hypertension will be examined.

The magnitude of increase in arterial pressure observed with chronic ANG II infusion is much greater than that seen with acute infusion of ANG II at the same rate. The relationship between arterial pressure and plasma ANG II concentrations during both acute and chronic ANG II infusion will be explored. In this manner it will be determined if "ANG II dependence" of arterial pressure level can be predicted from circulating ANG II concentrations.

Finally, two models of hypertension that more closely resemble clinical hypertension (than ANG II infusion) also will be studied. Specifically, the necessity of an intact AP for the development of renin-dependent and renin-independent renovascular hypertension will be determined.

METHODS AND EXPERIMENTAL DESIGN

I. General Experimental Procedures

A. Animals

Male Sprague-Dawley rats weighing 300-350 grams were purchased from either Sasco-King (Madison, WI), or Charles River (Portage, MI) breeding farms. Prior to experimentation rats were housed two per cage with corn-cob bedding and were allowed water and food (Wayne Lab Blox) *ad libitum*. At all times rats were maintained in a light-cycled, temperature controlled room.

B. Surgical Procedures

1. Arterial and venous catherization

Catheters were constructed of polyvinyl chloride (Tygon^R Microbore) tubing with 2.5-5.0 cm silicone rubber tips (Dow Corning Silastic^R). Rats were anesthetized with sodium pentobarbital (60 mg/kg ip) and given atropine sulfate (0.2 mg ip) to prevent bronchial congestion. The top of the head, back of the neck, ventral surface of the neck, and inner left leg were shaven. Small incisions were made in the back of the neck. For experiments requiring two venous catheters, one was tunnelled from an incision on the ventral surface of the neck to the initial incision on the dorsal surface and was then inserted 2.5 cm into the right jugular vein. In all rats an incision was made on the inner left leg from which venous and arterial catheters were tunnelled subcutaneously to the dorsal incision. The femoral vein and artery were exposed and 5.0 cm of catheter was inserted into the vein and 3.5-4.5 cm (depending on the weight of the rat) of catheter was inserted into the artery. The arterial catheter was sutured down to muscle in the leg in such a way to prevent movement of the rat from crimping off flow in the catheter, and the incision was then sutured closed. An incision was made on the top of the head to expose the skull into which three small holes were bored with a 23 g needle. Into each of the

three holes a jeweler's screw was placed for the purpose of securing the dental acrylic onto the skull and holding the spring tether in place. The three catheters were tunnelled from the back of the neck to the top of the skull and the neck incision was closed. The catheters were then threaded through the spring that was attached to a plastic hydraulic swivel. With dental acrylic the spring was secured to the top of the head and the animal was allowed to regain consciousness on a heated pad. The rat was given 20,000 U procaine penicillin G im and placed in a metabolic cage. Unless otherwise noted a sodium solution was given continuously into the femoral vein by use of a Harvard infusion pump, while the jugular vein catheter was filled with 5% dextrose in water and plugged. The arterial catheter was filled with a heparin and sucrose solution and occluded when not in use. Rats were given sodium deficient rat chow and distilled water *ad libitum*. Rats were allowed a minimum of three days to recover from catheter surgery prior to experimentation. All data was obtained from rats without disturbing them in their home cage.

2. Sino-Aortic Denervation

Rats undergoing sino-aortic denervation (SAD) were anesthetized with sodium pentobarbital (60 mg/kg, ip) and given atropine sulfate (0.2 mg, ip). After shaving the ventral surface of the neck a mid-cervical incision was made through which the left carotid sinus was exposed. The internal and external carotid arteries, the occipital artery and the carotid sinus were then stripped of nerve tissue, and the cervical sympathetic and superior laryngeal nerves were severed. The right carotid sinus was then exposed and the procedure was repeated on the right side. The animal was given 20,000 U procaine penicillin G im and allowed to recover from anesthesia prior to return to its cage. A rat was considered an SAD only if the average standard deviation (SD) of three control daily mean arterial pressure measurements were significantly greater (> 1.96 SD)than that of intact rats.

3. Lesion of the area postrema

Surgery for the ablation of the area postrema (AP) was performed in the laboratory of Dr. M.L. Mangiapane in Rochester, NY. Rats were anesthetized with a pentobarbital-chloral hydrate mixture and placed in a stereotaxic device (David Kopf, Tujunga, CA). The AP was exposed on the surface of the medulla, then an anodal current of 700 uA was passed through a tungsten electrode for 9-12 seconds (6.3-8.4 mC). Rats were given 100,000 U procaine penicillin, im and returned to their cages to recover. Upon full recovery, rats were sent to East Lansing, MI for the remainder of the study.

Upon completion of an experiment, rats were anesthetized with pentobarbital (40 mg/kg iv) and perfused with buffered formalin. The brains were removed, coded and sent back to Rochester for histological assessment of lesion extent. Serial frozen sections (30 um thick) were cut through the region of the caudal medulla, slide-mounted, and then stained with cresyl violet (for Nissl substance). The sections were then examined by light microscopy. Only rats exhibiting at least 90% AP ablation with little or no damage to the surrounding tissue were included in the final analysis of data.

4. Lesion of the lateral parabrachial nucleus

Surgery for ablation of the lateral parabrachial nucleus in the dorsolateral pons was performed in the laboratory of Dr. J.R. Haywood at the University of Texas in San Antonio. Rats were anesthetized with chloral hydrate and placed in a stereotaxic apparatus for placement of an electrode into the dorsolateral pons. The skull was exposed between lambda and bregma and opened at a point 9.3 mm posterior to bregma and 2.4 mm lateral from the midsagittal suture. A stainless steel electrode was lowered 5.9 mm ventral to dura, and a Grass lesion instrument was used to pass a 1.6 mA current for 4 seconds. Lesions were made bilaterally. Sham lesions were produced in a similar manner, except that the electrode was lowered 5.7 mm ventral from dura, therefore lying dorsal to the target area, and no

current was passed. The rats were allowed to recover from surgery (at least two weeks) and sent to Michigan State University for the remainder of the experiment. At the end of an experiment, the formalin-perfused brains of rats were coded and sent back, coded, to San Antonio for determination of lesion extent. Coronal sections were cut on a freezing microtome (40 um thick) throughout the rostral caudal extent of the LPBN, slide-mounted, and stained with cresyl violet. Brains were then examined under light microscopy. Data from lesioned rats were included only if the extent of the lesion included the ventrolateral region of the LPBN and extended throughout most of the rostral-caudal extent of the nucleus bilaterally. Nuclei typically damaged by this lesion included the extreme, external, dorsal, and ventral LPBN subnuclei, Kolliker-Fuse, the lateral tip of the medial parabrachial nucleus, the spinocerebellar tract, and the dorsal edge of the motor root of the trigeminal nerve. The rostral most portion of the LPBN was not ablated in any rat.

C. Hemodynamic measurements

Arterial blood pressure (MAP) was measured by connecting the exposed arterial catheter to a Statham P50 pressure transducer in turn attached to a Grass polygraph for the measurement of pulsatile pressure. Heart rate (HR) was counted directly from these tracings.

In sino-aortic denervated rats blood pressure was measured for 20 minutes using a data aquisition program on an Apple II Plus computer which receives an analog signal from the output channel on the polygraph. This program sampled pressure every ten seconds and then averaged 120 samples and calculated the mean and standard deviation (SD) of the mean. This latter value provided a measure of pressure lability.

D. Fluid and Electrolyte Balance

Water intake (WI) was determined by use of calibrated drinking tubes plus addition of the fluid infused daily (5 ml) iv. The volume of urine output (UO) was

obtained by 24 hour collection into calibrated beakers. Water balance (WB) then was determined by subtraction of these two parameters. Sodium intake was controlled through daily infusion since rats were only allowed sodium deficient chow. Unless otherwise indicated rats received either 6 mEq (high intake), 2 mEq (normal intake), or 1 mEq (low intake) Na^+ per 24 hour period. A sample of urine was collected daily for subsequent determination of sodium (UNaV) and potassium (UKV)excretion using a Beckman E2A electrolyte analyzer. The product of urine volume and urine sodium concentration yielded total urine sodium excretion. The sodium balance was calculated from subtracting UNaV from the constant sodium intake (ie. 1, 2, or 6 mEq Na+).

E. Plasma Assays

Blood samples were taken from the arterial catheter without disturbing the rat. Unless otherwise specified samples were taken over heparin into a 1 c.c. syringe, immediately spun in a microcentrifuge, and the plasma drawn off and frozen.

1. Osmolality

Plasma osmolality (P_{osmol}) was determined using triplicate samples of 50 ul each of plasma by freezing point depression using a micro-osmometer.

2. Sodium and potassium

Plasma sodium and potassium (P_{Na} and P_K) concentrations were determined using a Beckman E2A electrolyte analyzer. A sample size of 50 ul was required.

3. Blood urea nitrogen

Blood urea nitrogen (BUN) was determined using a colorimetric assay kit (Sigma) which required 10 ul plasma per sample.

4. Angiotensin II

Plasma angiotensin II concentration was measured by radio-immunoassay. The extraction and assay procedures used were similar to those described by

Nicholls *et. al.* (1976). ***Extraction.*** Blood samples were drawn with 0.125 M EDTA and 0.025 M o-phenanthroline and spun at 4^{o} C. A 0.4 ml plasma aliquot was placed into 0.8 ml ethanol (absolute). Samples were thoroughly mixed, then spun in a centrifuge for ten minutes (3000 rpm) and the supernatant was decanted into a clean labelled tube. Pellets were then reconstituted with 0.4 ml ethanol and respun and decanted into the first tube of supernatant. The samples were taken to dryness under a stream of air in a water bath at 40^{o} C, capped and stored frozen until assayed. ***Assay.*** The frozen samples were reconstituted with 0.2 ml assay buffer (0.05 molar Tris, 0.3% BSA, 0.2% neomycin sulphate, pH 7.4). Duplicate samples were assayed by separation into two aliquots of 0.1 ml reconstituted sample. Ang II assay was performed using ^{125}I-labeled ANG II (DuPont-NEN, Boston MA; final concentration 200,000 cpm/ml assay buffer) and ANG II antiserum (Arnel, New York, NY; final dilution 1/5000). Samples were incubated for 24 hours at 4^{o} C. Dextran coated charcoal was used for separation, with a ten minute spin in a centrifuge at 4^{o} C (3000 g); the bound fraction (supernatant) was then counted using a Micromedic Plus Automatic Gamma counter.

5. Arginine vasopressin

Plasma vasopressin levels (pAVP) were measured on samples (1 ml each) drawn over EDTA and extracted with acetone. The assay was performed by radioimmunoassay, previously described (Larose *et al.*, 1985), in the laboratory of Dr. Keith Lookingland in our department.

6. Renin activity

Plasma renin activity (PRA) was determined in samples taken over EDTA and stored at -40^{o}C. The assay itself was performed using procedures previously described (Blair *et al.*, 1976) in the laboratory of Dr. Martha Blair in Rochester, NY.

F. <u>Acute Responses to Vasoactive Drugs</u>

Some protocols require the determination of acute responses to either ganglionic blockade using hexamethonium (20 mg/kg iv), a V1-receptor vasopressin antagonist {[B-mercapto-B,B-cyclopentamethylenepropionyl^{1}, O-ME-Tyr^{2},Arg^{8}] vasopressin (Manning Compound); 10 ug/kg iv}, sodium nitroprusside (12 ug/min), or the ANG II receptor antagonist (partial agonist), saralasin (1 ug/min). In each case the MAP and HR of the animal were allowed to come to a stable resting value prior to the injection of the drug given in the jugular venous catheter, either as a bolus (hexamethonium and vasopressin antagonist), or a continuous infusion (nitroprusside and saralasin). Five minutes after the drug was given (15 min for saralasin) the MAP and HR were again determined and the changes in both parameters were regarded as the response.

G. Statistics

The majority of experiments were designed for mixed factorial analysis of variance with repeated measures in two or more groups of rats distinguished by one characteristic (one factor repeated, one factor independent). Others were analyzed by a one-way analysis of variance for repeated measures. Post-hoc testing for specific differences between means were performed using Dunnett's test or the "protected" least significant difference test for comparisons of independent means. Homogeneity of variance was routinely tested using the F-max test. Data not normally distributed was examined using the non-parametric signed ranks test or tests of independence based on the chi-squared distribution. A p-value of less than 0.05 was considered statistically significant. All data was expressed as mean and standard error of the mean.

II. Experimental Protocols

A. Angiotensin II and Drinking Behavior

1. Chronic iv ANG II infusion

a. Normal sodium diet

Six rats underwent surgery for catheter implantation and were placed in metabolic cages. They were maintained on normal rat chow (0.18 mEq Na+/g). All rats received intravenous infusion of 5% dextrose solution via Harvard infusion pumps. A total of 5 ml of solution was delivered over each twenty-four hour period. After a three day post-surgical recovery period, daily MAP, HR, WI, and UO were measured for the next fifteen days. During the fifteen day experiment, the first three days were considered a control period during which dextrose alone was intravenously infused. For the next nine days ANG II was added to the dextrose infusate such that each rat received stepped doses of 10, 30, and 60 ng/min for three days at each dose. Three days of recovery followed during which rats again received only dextrose.

b. Low sodium diet

Six rats underwent similar surgical and experimental procedures as described above. However, these rats were maintained on sodium deficient rat chow (Teklad) to prevent sodium-dependent hypertension from developing during ANG II infusion.

c. Acute drinking responses

In addition to daily water intake measurements in the above experiments, the intake of water during the initial two hours of each infusion (dextrose, 10, 30, and 60 ng/min ANG II) was determined. These were recorded as the acute drinking response to various doses of ANG II.

2. ANG II and dehydration

Six rats were maintained on normal rat chow *ad libitum* following catheter implantation and allowed three days to recover from surgery. On the third day drinking bottles were removed and a 24 hour period of dehydration followed. After 24 hours rats were given back calibrated drinking bottles and simultaneously given

an intravenous infusion of ANG II at a concentration of either 10, 20, 30 or 40 ng/min for 60 minutes at a rate of 3.5 ul/min. During the one hour, water bottle measurements were recorded at 10 minute intervals to determine milliliters of water consumed. Rats were allowed two days to return to fluid balance and once again deprived of water for 24 hours; drinking to a second dose of ANG II was then determined. Rats remained in the experiment until each dose had been tested in a random order with two days recovery allowed between doses. These results were then compared to responses elicited by the same rats similarly dehydrated but receiving only a dextrose infusion during the one hour rehydration period.

3. ANG II and thirst associated with an osmotic stimulus

Chronically instrumented rats were subjected to four, thirty minute infusion protocols. Individual infusions were separated by at least 24 hours and were administered in random order to each of 10 rats. The four infusates used were isotonic saline (0.15 M NaCl), isotonic saline with Ang II, given at a rate of 10 ng/min, a hypertonic saline solution (1.5 M NaCl) and hypertonic saline with Ang II, again so that a dose of 10 ng/min was administered. All solutions were administered at a rate of 3.5 ul/min so that a total of 0.105 ml were delivered in 30 min. At the time of an individual infusion, the water bottle reading was noted and a blood sample (0.8 ml) was taken for measurement of control pOsm. A syringe filled with the infusate was placed on a Harvard infusion pump and connected to the exposed venous cathether for direct iv administration. Each solution was infused for 30 min during which time a water bottle reading was obtained every 10 min. Further, the time when the rat first drank from the water bottle during the 30 min (i.e. latency to drink) was noted and a blood sample was immediately drawn for pOsm measurements. If a rat failed to drink during the infusion period, a blood sample was taken at the end of the 30 min infusion.

4. ANG II and thirst associated with acute hypotension

Eight chronically instrumented rats were individually subjected to three infusion protocols each separated by a minimum of 24 hours and administered in a random order. At the time of an infusion procedure, control MAP and HR were obtained and further noted at 10 min intervals throughout the infusion and again upon recovery. Water bottle readings were observed at the time of MAP and HR recordings. A syringe filled with infusate was placed on a Harvard infusion pump and connected to the exposed venous catheter for direct intravenous drug administration. One protocol consisted of the rat receiving nitroprusside at a rate of 12 ug/min for 30 min; this rate was determined to consistently lower mean pressure between 20 and 30 mmHg. In a second protocol, nitroprusside was infused to lower pressure with immediate addition of ANG II infused from a second pump at a rate of 10 ng/min for 30 min. A connector at the venous catheter was fashioned in a T-shape to allow the simultaneous infusion of the two solutions into the rat for the half hour. The final protocol consisted of a bolus injection of enalapril (2 mg/kg iv) given ten minutes prior to a thirty minute nitroprusside infusion (12 ug/min) to prevent endogenous production of ANG II in response to lowering of arterial pressure. This dose of enalapril was previously determined to prevent the pressor response to an bolus injection of ANG I (50 ng) iv.

B. Chronic Intravenous Vasopressin Infusion

1. Intact rats

Thirty rats underwent chronic catheter implantation surgery and were each housed in a standard steel metabolic cage. The rats were allowed free access to distilled water from calibrated drinking tubes and to sodium deficient rat chow (0.002 meq Na+, 0.3 meq K+/g). The venous catheter was connected to a syringe-type Harvard infusion pump which delivered 5 ml of a NaCl solution via continuous intravenous infusion, such that 1 meq Na+ was infused daily. Ampicillin (10 mg) was given twice a day by iv injection.

After allowing the rat three days of recovery in the metabolic cages the experimental procedures were begun. Three days of control measurements were followed by a ten day AVP infusion period (saline, 0.2 ng/kg/min or 2.0 ng/kg/min) during which fresh AVP was added to the 1 meq NaCl infusate daily. Three recovery days followed, during which time rats received only the salt solution. Every morning (8-11 a.m.) throughout the 16 day protocol MAP, HR, WI, UO, and UNaV were measured.

In addition to daily measurements, the change in MAP and HR in response to a bolus injection of hexamethonium (20 mg/kg iv) was determined on two occasions during the AVP infusion period and once during both the control and recovery periods in some of the rats. Changes in MAP and HR in response to a V_1-receptor antagonist (Manning compound; 10 ug/kg iv) were examined twice during chronic AVP infusion in half of the rats. This dose of AVP antagonist previously was shown to inhibit the acute pressor response to exogenous infusion of AVP up to 3 mU/min (approximately 30 ng/kg/min) (Bruner and Fink, 1986). Blood samples (0.8 ml) were taken twice during the AVP infusion period and once during both the control and recovery periods for analysis of pNa, pK, pOsm, and BUN.

2. Area postrema ablated rats

The above experiment was performed in twenty rats that had previously undergone surgery for electrolytic ablation of the area postrema (APX). Rats were separated into three groups. Five rats received saline alone, five received AVP at a rate of 0.2 ng/kg/min and 10 received 2.0 ng/kg/min AVP during the ten day experimental period.

3. Sino-aortic denervated rats

The above experiment was performed in SAD rats. Two groups of five each received either saline alone or AVP at a rate of 2.0 ng/kg/min during the ten day experimental period. In addition to daily MAP the standard deviation from the

mean MAP was recorded. Data from 5 other rats were disallowed for not meeting criteria previously set for inclusion as an SAD.

C. Ganglion Blockade During Angiotensin Hypertension

1. Intact rats

Ten control rats (CON) received no surgical intervention prior to catheter implantation. The rats were allowed free access to distilled water from calibrated drinking tubes and to sodium deficient rat chow (Teklad Lab Diets, Madison, WI; 0.002 meq Na+, 0.3 meq K+/g). The femoral venous catheter was connected to a syringe-type Harvard infusion pump which delivered a NaCl solution via continuous infusion, such that 6 meq Na+ (5 ml) were delivered daily. Health of the rat was maintained by administration of tobramycin sulphate (Lilly, Indianapolis, IN; 1 mg i.v. twice daily) and ticarcillin (25 mg i.v. twice daily) during post-surgical recovery and as needed throughout the experiment.

After allowing the rat three days of recovery in the metabolic cages the experimental procedures were begun. Three days of control measurements were followed by a fourteen day ANG II infusion period (10 ng/min) during which fresh ANG II was added to the 6 meq NaCl infusate daily. Five recovery days followed, during which time rats again received only the salt solution. Every morning (8-11 a.m.) throughout the 22 day protocol MAP, HR, WI, UO, UNaV, and UKV were recorded.

In addition to daily measurements, the change in MAP and HR 5 min after a bolus injection of hexamethonium (20 mg/kg i.v.) was determined on three occasions during the ANG II infusion period and once during both the control and recovery periods. Changes in MAP and HR in response to a 5 min iv infusion of sodium nitroprusside (12 ug/min) also were obtained three times during chronic ANG II infusion and once each during control and recovery periods.

2. Sino-aortic denervated rats

Ten SAD rats were catheterized and studied in the above experimental protocol. In addition to daily measurement of MAP the standard deviation from the mean MAP also was recorded. The data from 4 other rats were disallowed for not meeting criteria previously set for inclusion as an SAD.

D. Circulating Angiotensin (1-7)

1. Acute iv ANG (1-7) pressor effect

The acute (5 minute) pressor response to iv ANG (1-7) was determined in conscious rats with chronic indwelling catheters. Three doses were administered (10, 30 and 100 ng/min). These responses were compared to responses previously elicited by ANG II (10 and 30 ng/min) in similarly prepared rats.

2. Chronic iv ANG (1-7) infusion

Fifteen rats underwent surgery for chronic catheter implantation and were individually housed in metabolic cages. The rats were allowed distilled water and sodium deficient rat chow *ad libitum*. Through the venous catheter they received a continuous infusion of sodium solution that delivered 6 mEq Na+ daily. Following three days of control infusion, ANG (1-7) was added to the infusate such that the hormone was administered at a rate of 10 ng/min (n=9) or 30 ng/min (n=6) for ten days. Three recovery days followed. Throughout the sixteen day protocol MAP, HR, WI, UO, UNaV, and UKV were recorded daily.

In addition to daily measurements blood samples were obtained once each during control and ANG (1-7) infusion periods for the measurement of pAVP. Twice during the ten day ANG (1-7) infusion, changes in MAP and HR in response to the V_1-AVP receptor antagonist (10 ug/mg iv) were assessed.

E. Area Postrema and Renovascular Hypertension

1. 2-Kidney, 1-clip hypertension

Rats were catheterized, placed into individual metabolic cages and allowed three days for recovery. Half the rats had previously undergone surgery for

electrolytic ablation of the area postrema (Lesioned-L); the other half underwent a sham lesion operation (Sham-lesioned-S). They were allowed free access to distilled water and standard rat chow. The protocol included three days of control measurements. On the third day rats were briefly anesthetized with methohexital (Brevital^{R} sodium, 10 mg/kg iv) and through a midabdominal incision the left kidney and renal artery were exposed. At this time a silver clip (0.20 or 0.25 mm) was placed around the renal artery to reduce blood flow to the kidney (Clipped-C). The incision was then sutured closed and the rat was allowed to recover prior to return to its cage. In ten rats the kidney and renal artery were exposed but a clip was not placed on the artery prior to closure of the incision (sham clipped-S). Therefore, four groups were studied: 5 sham-lesioned, sham-clipped (SS); 5 AP-lesioned, sham-clipped (LS); 10 sham-lesioned, clipped (SC); and 10 AP-lesioned, clipped (LC).

The protocol consisted of 3 control days and 14 days post-clip application. Throughout the experiment, daily MAP, HR, WI and UO were recorded. In addition to daily measurements the change in MAP in response to a 15 minute saralasin infusion (1 ug/min iv) was recorded on day 2 of the control period and on days 3 and 10 after clip application. Further, arterial blood samples were obtained on the first day of the control period and on days 7 and 14 after clip placement. Subsequently the plasma from these samples were analyzed for PRA.

2. 1-Kidney, 1-clip hypertension

Sham-lesioned (S) and AP-lesioned (L) rats underwent surgery for the removal of the right kidney. This procedure entailed the exposure of the right kidney through a midflank incision in the pentobarbital (50 mg/kg, ip) anesthetized rat. The renal artery and vein were ligated with suture silk and the vessels were severed proximal to the kidney which was then removed. The incision was then sutured and the animal was allowed one week to recover. Rats were then

catheterized, placed in metabolic cages and allowed three days recovery. The ensuing protocol was similar to that used in the 2K-1C hypertension experiment. After three days of control measurements the left renal artery was clipped (C) or sham-clipped (S). Therefore four groups of rats were studied: 5 sham-lesioned, sham-clipped (SS); 5 AP-lesioned, sham-clipped (LS); 9 sham-lesioned, clipped (SC); and 10 AP-lesioned, clipped (LC). As in the above experiment MAP, HR, WI, and UO were recorded daily. The change in MAP in response to saralasin infusion (1 ug/min iv), and PRA, were each measured once during control and twice post clip surgery following the protocol of the previous experiment.

F. Lateral Parabrachial Nucleus and Angiotensin Hypertension

Rats that had previously undergone surgery for the electrolytic ablation of the lateral parabrachial nucleus (LPBN) or a sham operation were catheterized and placed into individual metabolic cages. Rats were allowed distilled water and sodium deficient chow *ad libitum*. A sodium solution was continuously delivered iv to ensure a daily sodium intake of 6 mEq. Three days of post-surgical recovery were allowed prior to commencement of the sixteen day protocol. Three control days were followed by ten days during which rats received ANG II at a rate of 10 ng/min and subsequently by three recovery days. Throughout the protocol, daily MAP, HR, WI, UO, UNaV, and UKV were recorded.

In addition to daily measurements the changes in MAP and HR in response to hexamethonium (20 mg/kg iv) and V_1-AVP receptor antagonist (10 ug/kg iv) (separated by 24 hours) were recorded once each during control and recovery periods, and twice during chronic ANG II infusion.

G. Plasma Angiotensin II Concentrations

1. Acute iv ANG II infusion

Chronically instrumented rats were allowed at least four days post surgical recovery at which time a blood sample (1 ml) was drawn from the arterial catheter.

Plasma was collected and extracted as described for analysis of plasma ANG II concentrations (pANG). The red blood cells were given back to the rat, and a one hour iv infusion was begun. At the end of the 60 min infusion period a second blood sample was taken. Each of fourteen rats received a one hour saline infusion alone and at least one of three doses of Ang II (10, 30 or 60 ng/min). Each one hour experiment was separated by at least two days.

2. Chronic ANG II infusion

Ten rats underwent surgery for chronic catheter implantation and were maintained on a daily sodium intake of 6 mEq by intravenous infusion. After four days post-surgical recovery, a resting MAP and HR were obtained and a 1 ml blood sample was taken for subsequent analysis pANG. ANG II was then added to the sodium infusate to be delivered at a rate of 10 ng/min. After one hour MAP and HR were again recorded and a second blood sample was obtained. ANG II infusion was continued for the next four days at the same rate during which time MAP, HR, and pANG were measured on days 1 and 4. On the fourth day, the ANG II dose was increased three-fold to 30 ng/min and MAP, HR, and pANG were measured three days later. All three parameters were then measured after two days of recovery from ANG II treatment.

RESULTS

I. Angiotensin II and Drinking Behavior

A. Chronic iv ANG II infusion

1. Normal sodium diet

Three day infusions of three stepped doses of ANG II caused a dose-dependent increase in resting MAP when compared to MAP measured during the control period in rats maintained on a normal sodium intake. These results are illustrated in Figure 1. Heart rate was significantly elevated only on the last day of ANG II infusion and the first day of recovery when compared to values obtained during the control period. While water balance (daily UO subtracted from daily WI) was significantly decreased in response to infusion of the two highest doses of ANG II, WI remained unaltered throughout the fifteen day protocol. Chronic infusion of ANG II at three doses that produce significant hypertension in the rat thus failed to produce a chronic increase in water intake measured on a daily basis.

2. Low sodium diet

Hypertension may have acted to inhibit the ability of chronic elevations of ANG II to stimulate drinking. Rats maintained on the sodium deficient diet did not experience reliable increases in resting MAP. These data are depicted in Figure 2. Significantly elevated pressures were observed during the three day infusion of ANG II at the middle dose of 30 ng/min and only on the first day of ANG II infusion at the highest rate. The pressures obtained during these infusions, however, were not as great as those obtained in rats on the normal sodium diet. Rats experienced bradycardia on the last day of infusion of ANG II at 30 ng/min and the first day of the 60 ng/min ANG II infusion. Water balance was significantly decreased from control only on the first day of infusion of ANG II at the middle rate. Chronic infusion of ANG II at three stepped doses did not produce chronic

Figure 1. Effect of chronic iv ANG II infusion at three stepped doses (10, 30, and 60 ng/min) on fluid balance and hemodynamic parameters in rats maintained on a normal sodium intake. Water intake (WI), water balance (WB), mean arterial pressure (MAP), and heart rate (HR) measured daily during a fifteen day protocol (three control, nine ANG II infusion, and three recovery days). The respective treatment is indicated on top of each bracketed three day period. Data exhibiting statistically significant differences from control values, at a level of $p < 0.05$, are indicated by asterisks. $N=6$. Bars indicate standard errors for individual comparisons over time.

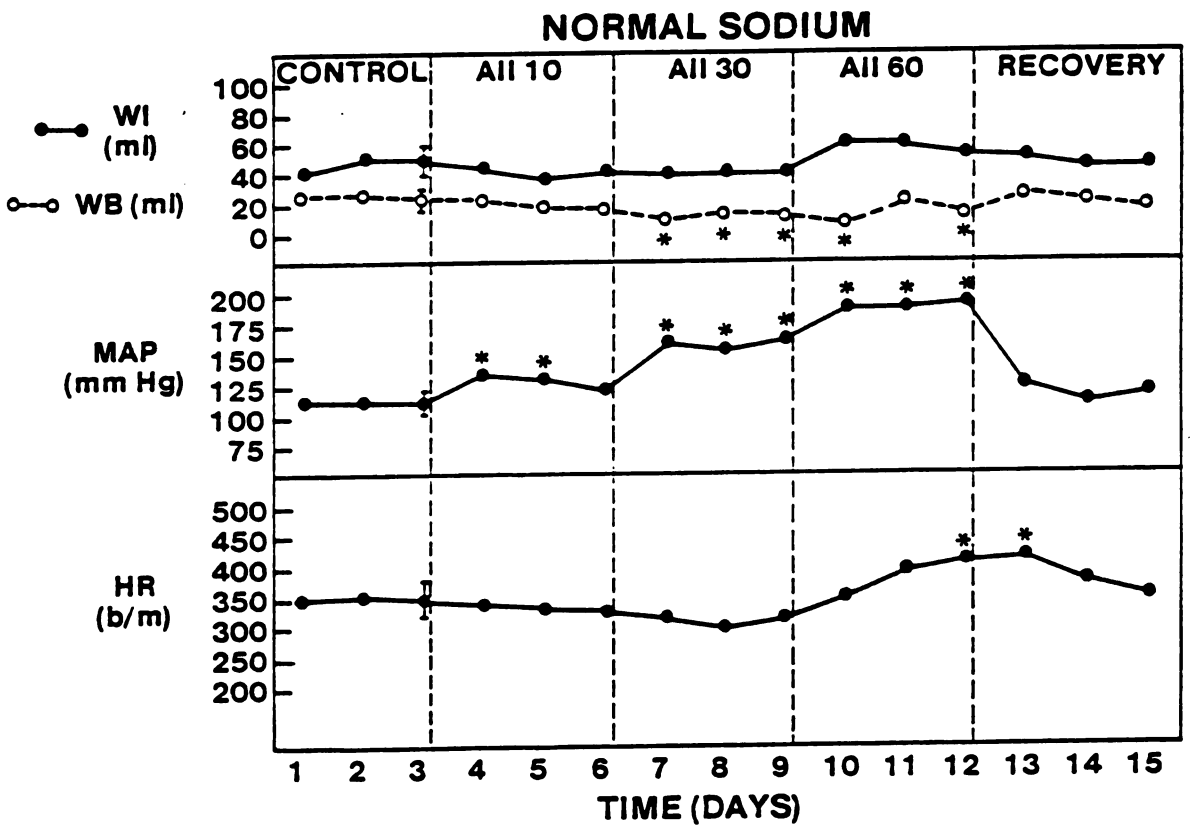
NORMAL SODIUM
CONTROL
All 10
All 30
All 60
RECOVERY
WI (ml)
WB (ml)
MAP (mm Hg)
HR (b/m)
100
80
60
40
20
0
200
175
150
125
100
75
500
450
400
350
300
250
200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (DAYS)

Figure 1

Figure 2. Effect of chronic iv ANG II infusion at three stepped doses on fluid balance and hemodynamic parameters in rats maintained on a low sodium diet. Daily WI, WB, MAP, and HR averages from six rats maintained on a sodium deficient diet while receiving three day infusions of Ang II at stepped doses with three day control and recovery periods.

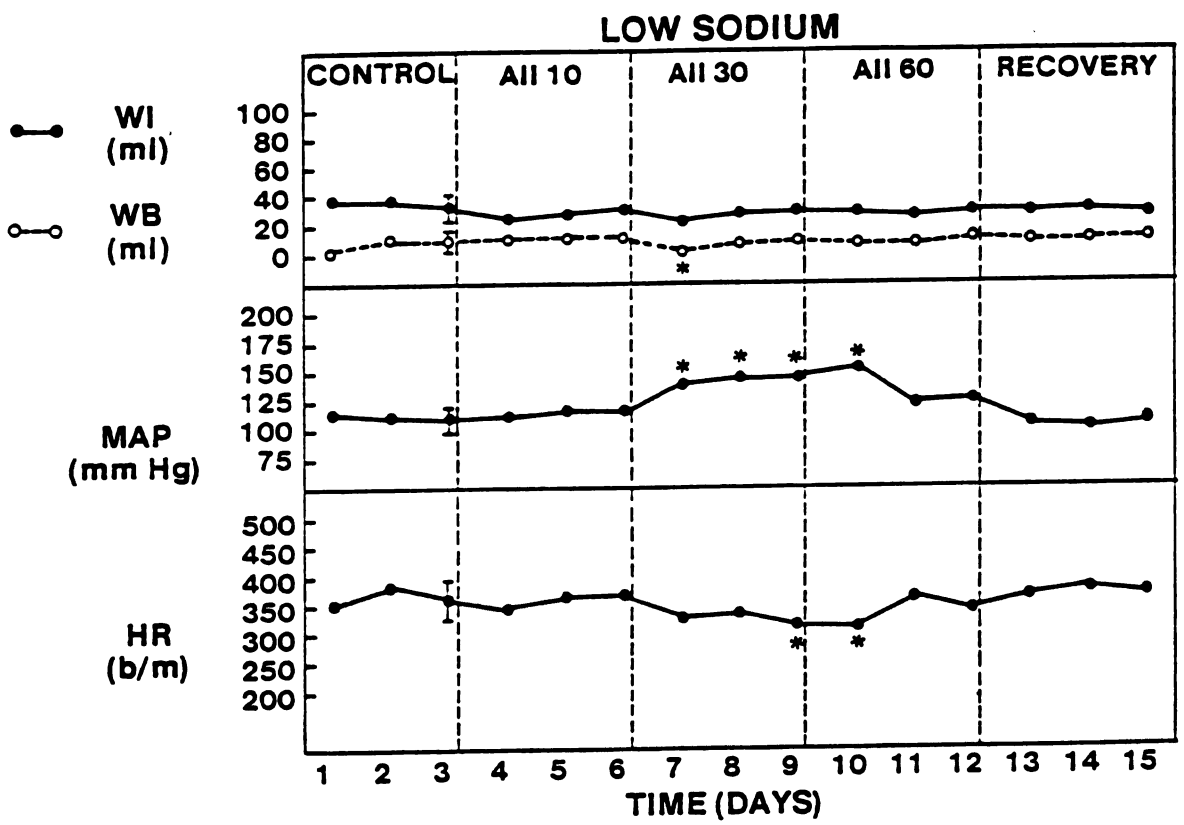
LOW SODIUM
CONTROL
All 10
All 30
All 60
RECOVERY
WI (ml)
WB (ml)
MAP (mm Hg)
HR (b/m)
100
80
60
40
20
0
200
175
150
125
100
75
500
450
400
350
300
250
200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (DAYS)

Figure 2

increases in daily WI even in these rats maintained on a sodium deficient diet to minimize associated increases in arterial pressure.

3. Acute drinking responses

The acute effects on WI of infusion of ANG II at doses unable to produce chronic increases in daily WI are illustrated in Figure 3. Two hour infusions of ANG II at rates of 10, 30, and 60 ng/min failed to elicit significantly greater WI than a dextrose infusion of similar duration. ANG II at a rate of 60 ng/min failed to produce a drinking response in 5 of the rats maintained on normal sodium intake, however one rat drank a large quantity of water during this two hour period; thus average water intake in this group was increased, but not to a statistically significant level.

B. Angiotensin II and dehydration

The drinking responses of dehydrated rats receiving ANG II at doses of 10, 20, 30, and 40 ng/min or dextrose during a one hour rehydration period are illustrated in Figure 4. In each graph the response elicited during ANG II infusion is compared to the response by the same rats receiving dextrose during a separate rehydration period. Rats drank similar quantities, and at similar rates, upon rehydration whether receiving ANG II or dextrose.

C. Angiotensin II and thirst associated with an osmotic stimulus

The drinking responses elicited by rats receiving infusions of hypertonic saline, and isotonic saline, each with and without addition of ANG II, are illustrated in Figure 5. As measured by water intake during a thirty minute infusion period, hypertonic saline with ANG II (10 ng/min) produced a similar degree of thirst to that caused by hypertonic saline alone. Fewer rats (6 of 10) drank during the thirty minute infusion of ANG II plus hypertonic saline than rats given only hypertonic saline (9 of 10), but this difference was not statistically significant. ANG II (10 ng/min) infused with isotonic saline was ineffective in eliciting a reliable drinking

Figure 3. Acute effect on water intake of infusion of ANG II. WI measured during the first 2 hours of Ang II infusions of 3 doses (10, 30, and 60 ng/min, iv) or an infusion of dextrose (CON) in rats maintained on either a normal or low sodium diet. One rat of 6 in the normal salt group drank a large quantity of water during the first two hours of Ang II at the rate of 60 ng/min.

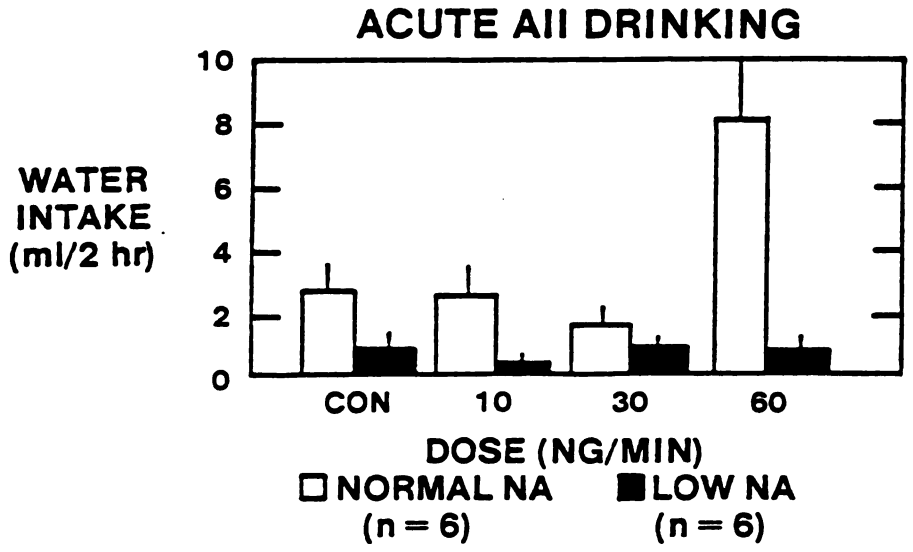
ACUTE AII DRINKING
WATER INTAKE (ml/2 hr)
10
8
6
4
2
0
CON
10
30
60
DOSE (NG/MIN)
NORMAL NA (n = 6)
LOW NA (n = 6)

Figure 3

Figure 4. Effect of ANG II on water intake in dehydrated rats. Cumulative water intake measured every ten minutes during a one hour period coinciding with the return of water bottles to rats dehydrated for 24 hours. Two groups represented in each graph depict data from the same rats receiving an infusion of dextrose (CONTROL), or receiving an infusion of Ang II at four different doses indicated during the one hour rehydration period. Vertical lines represent standard error for between groups comparison.

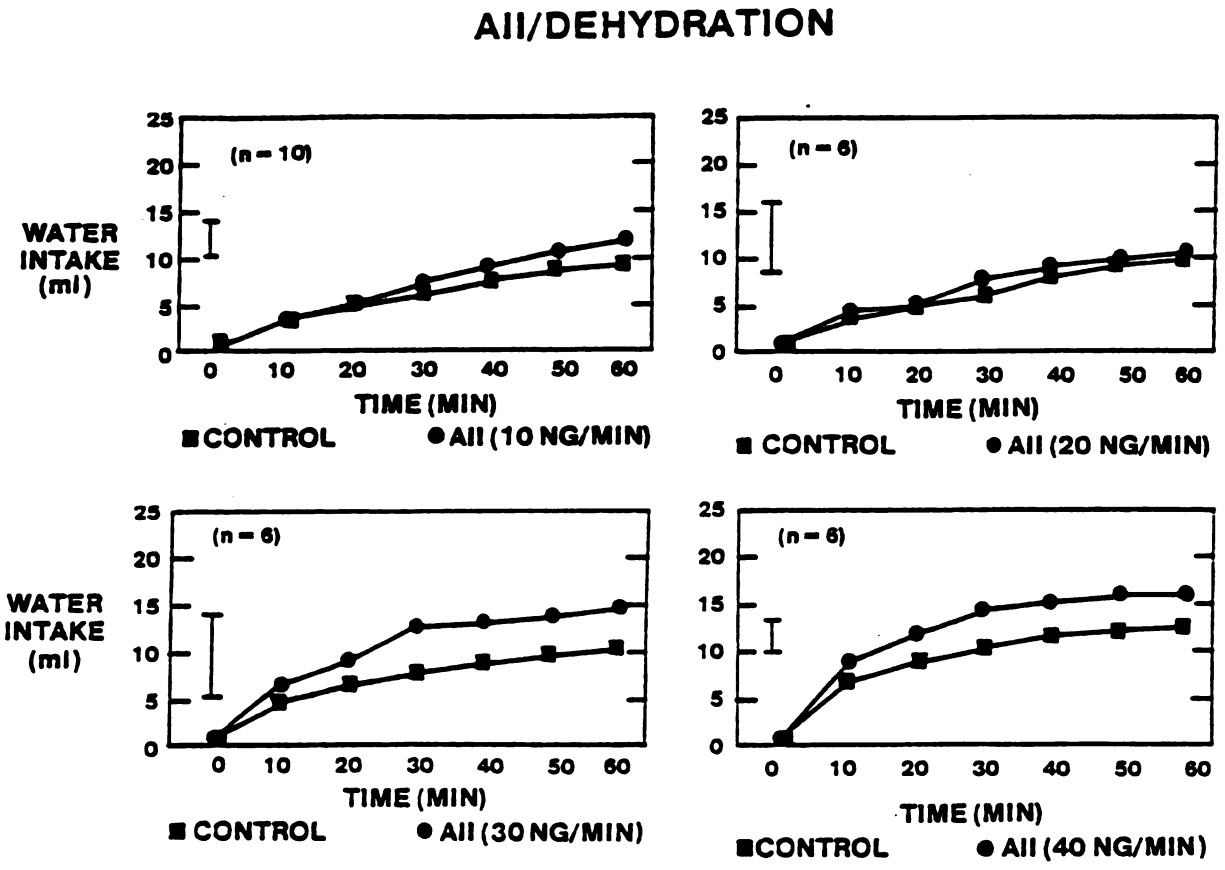
AII/DEHYDRATION
WATER INTAKE (ml)
(n = 10)
TIME (MIN)
CONTROL
AII (10 NG/MIN)
(n = 6)
TIME (MIN)
CONTROL
AII (20 NG/MIN)
WATER INTAKE (ml)
(n = 6)
TIME (MIN)
CONTROL
AII (30 NG/MIN)
(n = 6)
TIME (MIN)
CONTROL
AII (40 NG/MIN)

Figure 4

response (4 of 10) compared to responses produced by isotonic saline alone (2 of 10).

Table 1 depicts plasma osmolalities of samples obtained prior to infusion (CONTROL) and either at the onset of drinking or at cessation of infusion if the rat failed to drink. Further, the average latency to drink during each infusion protocol is given. Data from rats receiving the isotonic saline infusions are broken down to compare the plasma osmolalities of rats that did drink to those that did not drink during the 30 min infusion. The onset of drinking appears to correspond with an increase in plasma osmolality whether rats were receiving an isotonic or hypertonic infusion. Further, only a small increase in plasma osmolality (i.e. 2-5 mOsm) is required to prompt drinking. No statistically significant differences in plasma osmolality at drinking, or latency to drink, existed between groups receiving ANG II and their respective controls.

D. Angiotensin II and thirst associated with acute hypotension

Figure 6 illustrates both the MAP and drinking responses to nitroprusside infusion given alone, after prior treatment with an ACE inhibitor or with simultaneous infusion of ANG II (10 ng/min). Mean arterial pressure was similar during control and recovery periods for each protocol, and hypotensive responses were no different during each infusion. Lowering blood pressure with nitroprusside provided a reliable stimulus to drink with neither the number of responders nor the volume drank significantly altered by either addition of ANG II or by prevention of the endogenous production of the hormone with ACE inhibition.

II. Chronic Intravenous Vasopressin Infusion

A. Saline control

Rats maintained on the 1 mEq sodium solution alone for the sixteen day protocol experienced few statistically detectable changes in measured parameters. The

Figure 5. Effect of ANG II on thirst produced by a hyperosmotic stimulus. Cumulative water intake measured every ten minutes during thirty minute infusions of isotonic or hypertonic saline with and without Ang II (10ng/min). Numbers above values at thirty minutes represent the fraction of rats that drank to respective stimuli during the thirty minute infusions.

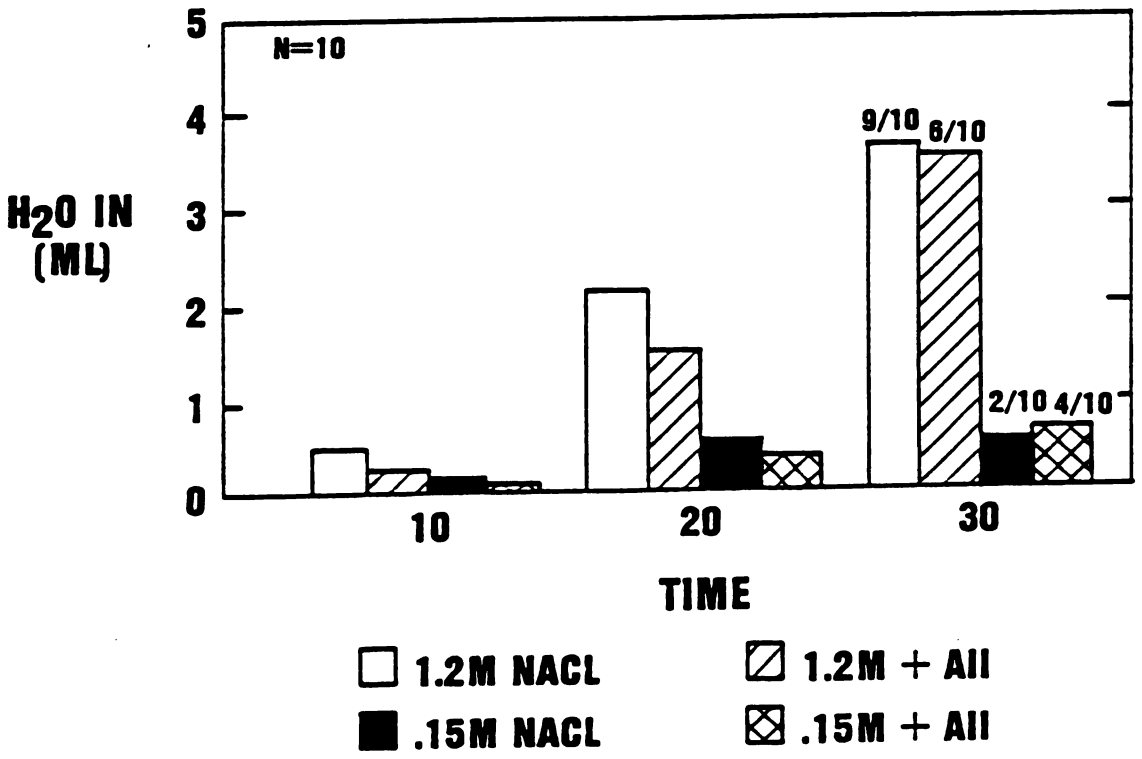
N=10
9/10
6/10
2/10
4/10
5
4
3
2
1
0
H2O IN (ML)
10
20
30
TIME
1.2M NACL
.15M NACL
1.2M + All
.15M + All

Figure 5

Table 1. Effect of hypertonic saline and ANG II infusion on plasma osmolality. The pOsm was measured in plasma samples obtained prior to each half hour infusion and at the onset of drinking during the infusion, or at the end of the half hour if the rat did not drink. The average latency to drink to each stimulus is also depicted. Data from the isotonic infusions are expressed separating data obtained from rats that drank (upper figures) from those that failed to drink (lower figures) during the half hour infusion.

N=10	$[OSM]_p$ CONTROL	$[OSM]_p$ DRINKING ONSET	LATENCY TO DRINK (MIN)
1.2M NaCl	292±1.8	297±1.7	12.5±1.9
1.2M NaCl + All(10ng/min)	293±1.5	297±2.7	9.1±2.6
.15 M NaCl	294±3.0	296±3.1	17.7±6.7
	293±2.4	292±0.9	———
.15M NaCl + All(10ng/min)	290±3.0	292±3.8	19.0±2.7
	293±2.8	293±3.2	———

Table 1

Figure 6. Effect of ANG II on hypotension induced thirst. Mean arterial pressure (left axis) measured over the forty minute protocol (30 minute infusion + 10 minute recovery), and cumulative water intake (right axis; bar graph) measured every ten minutes during the thirty minute infusion period. Fractions above 30 minute water intake values represent the fraction of rats that drank to the respective infusion protocols.

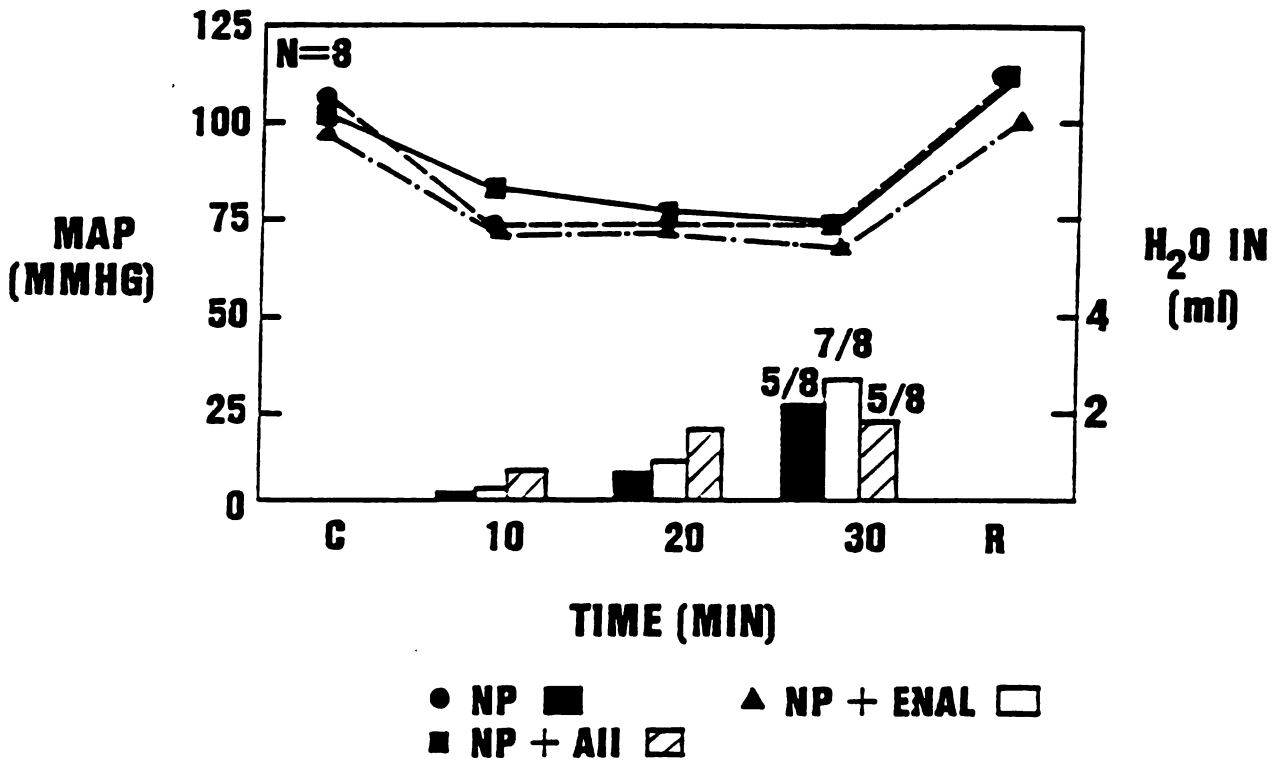
125
100
75
50
25
0
N=8
MAP
(MMHG)
H_2O IN
(ml)
4
2
7/8
5/8
5/8
C
10
20
30
R
TIME (MIN)
● NP ■
▲ NP + ENAL □
■ NP + AII ▨

Figure 6

average standard deviation in daily blood pressure of the SAD group during the control period was calculated to be 12.01 $\pm$ 0.90, and was not significantly altered during the remainder of the protocol. Data from CONTROL, APX and SAD are illustrated together and therefore will be discussed as such. Figure 7a illustrates that MAP in CONTROL, APX, and SAD rats did not significantly change during the control experiment. Heart rate tended to increase slightly in all three groups, occasionally reaching statistical significance, especially in the SAD group. No group experienced any significant changes in WI, UO or WB as depicted in Figure 7b. Further, no alterations in pOsm, pNa, pK, BUN, or daily UNaV were observed; these data are presented in Figures 7c and 7d. For most parameters measured (except WB), there existed no detectable difference between the SAD and CONTROL rats, however, WB, MAP, HR and pK values were often lower in the APX rats compared to the CONTROL group throughout the control experiment. A tendency for APX rats to exhibit low MAP and HR when on normal salt intake has been observed in this laboratory (unpublished results), but the reason for these effects is not clear.

B. AVP 0.2 ng/kg/min

Vasopressin infused at a rate of 0.2 ng/kg/min for ten days had no effect on daily MAP or HR in either APX or CONTROL rats as seen in Figure 8a. Chronic, ten day infusion of AVP (0.2 ng/kg/min) did, however, cause a parallel decrease in both daily WI and UO compared to levels obtained during the three day control period. Both the CONTROL and APX rats exhibited similar decreases in both WI and UO; these responses were not different from each other and were sustained throughout the ten day infusion period. Upon cessation of AVP infusion, daily WI and UO returned to control levels. Water balance remained unchanged throughout the experiment in both groups of rats. These data are illustrated in Figure 8b. Effects of AVP (0.2 ng/kg/min) on pOsm, pNa, pK, BUN, and daily UNaV are presented

in Figures 8c and 8d. Ten day low dose AVP infusion in CONTROL and APX rats induced no significant change in any of these five parameters from levels observed during the control period.

C. AVP 2.0 ng/kg/min

The results of ten day infusion of AVP at the rate of 2.0 ng/kg/min in CONTROL, APX, and SAD rats are shown in Figures 9a-d. The average standard deviation of daily mean arterial pressure during the control period was 10.92 $\pm$ 0.62 for the SAD group, and was unaltered during AVP infusion or recovery. Daily MAP and HR were not altered by chronic AVP infusion at this higher dose. However, daily WI and UO decreased significantly from control values. Vasopressin at this rate significantly decreased WI and UO in all three groups and these changes were sustained until AVP treatment ceased. During the three day recovery period daily WI and UO returned to control levels. Water balance was not altered from control during ten day infusion of AVP at this rate in either group of rats. As illustrated in Figures 9c and 9d, pOSM, pNa, pK, and BUN were unaffected by chronic AVP infusion at this rate in CONTROL and SAD rats. A significant decrease in pNa+ was observed in APX rats. Thus some degree of water retention appears to occur in APX rats during AVP infusion; the significance of this finding is uncertain. A transient natriuresis was observed in all groups of rats the first day of AVP infusion. With continued infusion UNaV returned to control levels and did not vary from these levels during the remainder of the study.

The changes in MAP and HR in response to V-1 AVP receptor antagonist (10 ug/kg), given on days 5 and 10 during the ten day experimental infusion period, were measured in CONTROL rats receiving various amounts of AVP. Rats receiving either dose of AVP responded similarly to V-1 receptor antagonism, compared to rats receiving only the 1 mEq Na+ solution; no significant cardiovascular effects of the antagonist were observed under any of these conditions

Figure 7a. Daily mean arterial pressure and heart rate during control infusion experiment in intact, sino-aortic denervated (SAD) and AP-lesioned (APX) rats. MAP and HR in rats maintained on a daily 1 mEq sodium intake. Bar on control data represents the SEM for within groups comparisons. * indicates data significantly different from three day control period. Bracketed data indicates the ten day experimental infusion period. In these rats control solution was infused throughout the entire protocol (3 control, 10 experimental, and 2 recovery days).

CONTROL

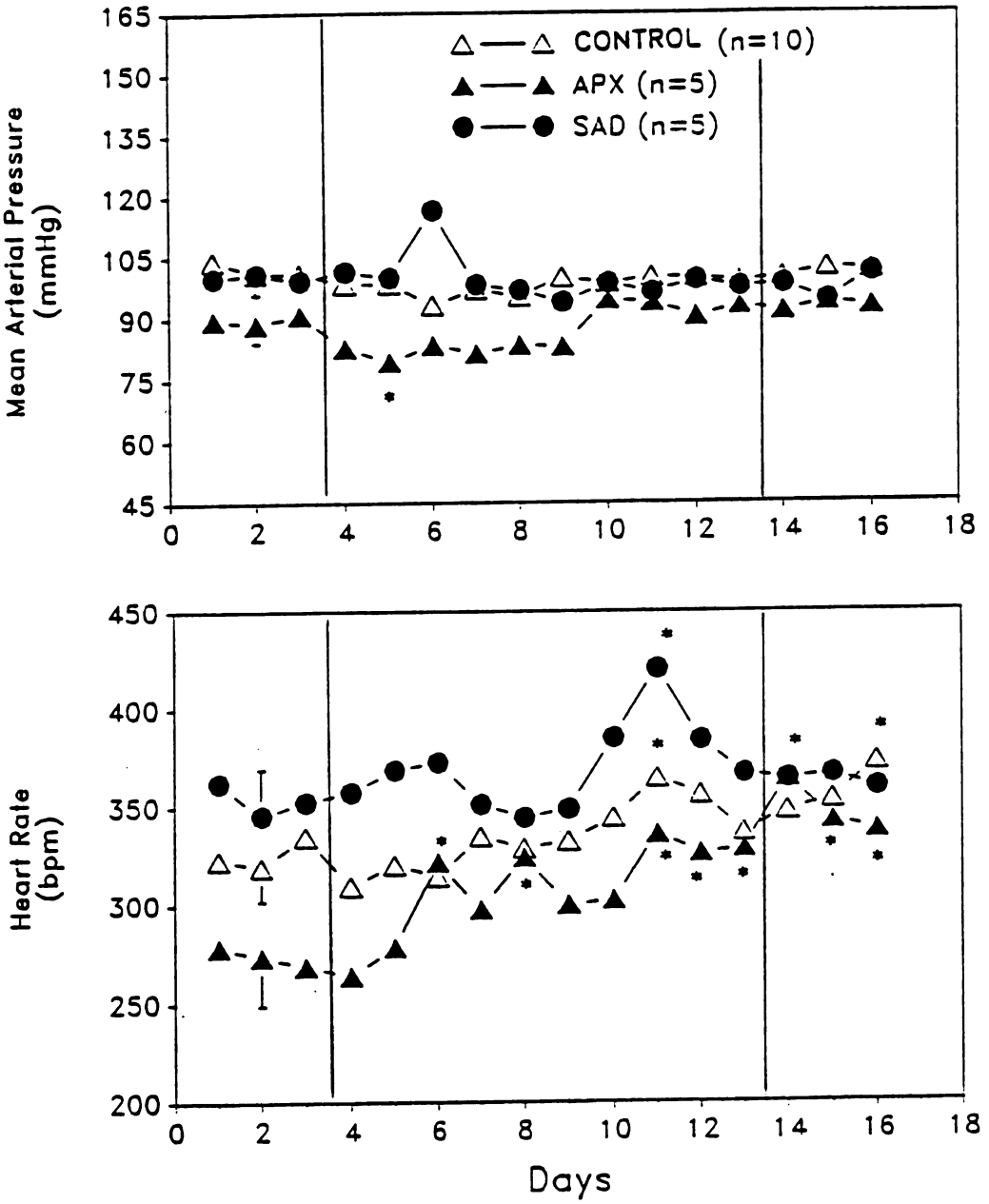

Figure 7a

Figure 7b. Fluid balance in rats maintained on control infusion. UO, WI, and WB in CONTROL, APX and SAD rats throughout the 16 day protocol. See Figure 7a for further explanation.

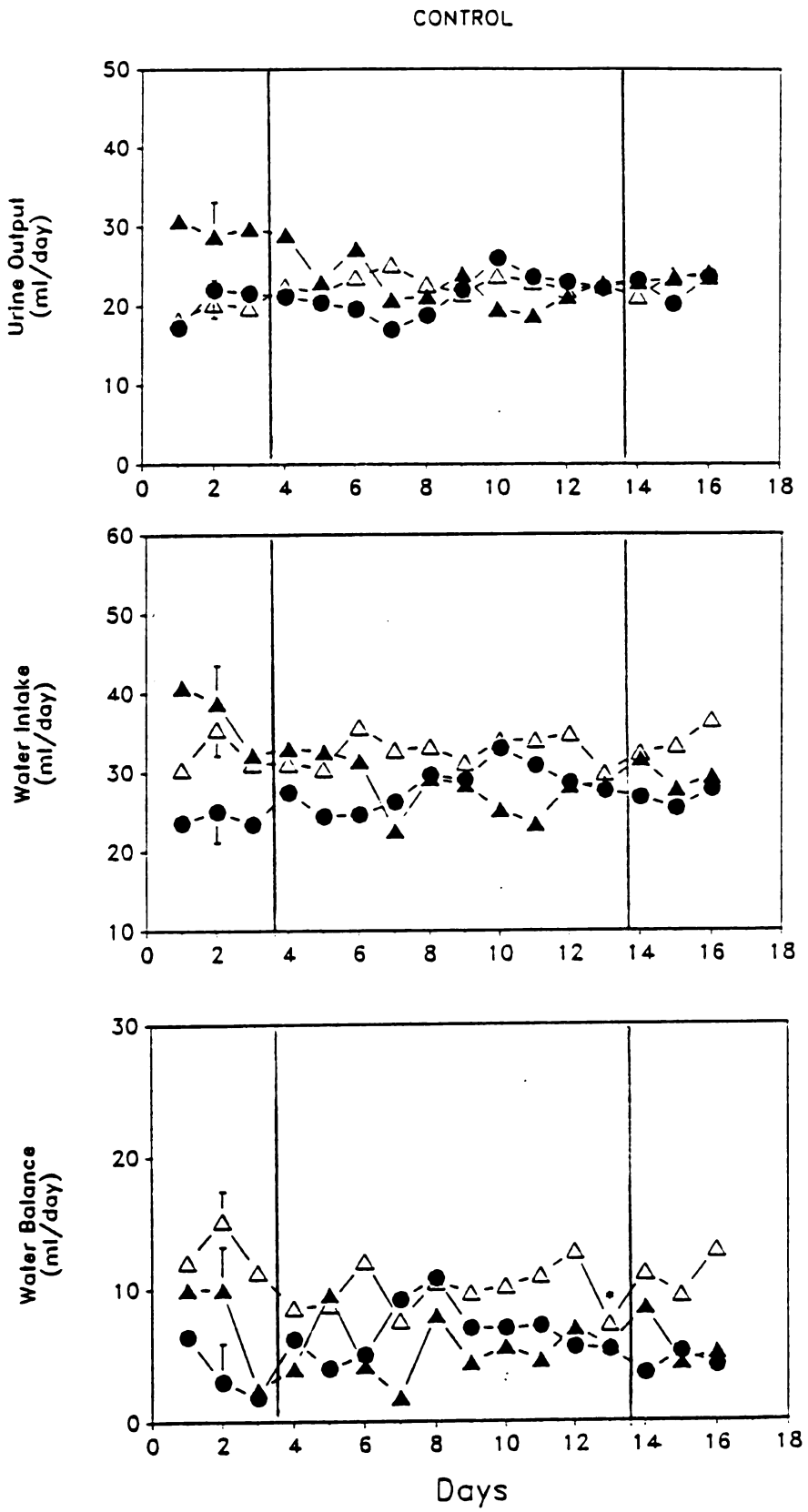
CONTROL
Urine Output (ml/day)
Water Intake (ml/day)
Water Balance (ml/day)
Days

Figure 7b

Figure 7c. Plasma electrolytes in rats maintained on control infusion. pOsm, pNa, and pK measured in plasma taken once during control (C) and recovery (R) and three times during experimental (A) periods from rats maintained on 1 mEq sodium intake. Crosses (+) indicate a significant difference from the value found in sham rats at the same time point.

CONTROL

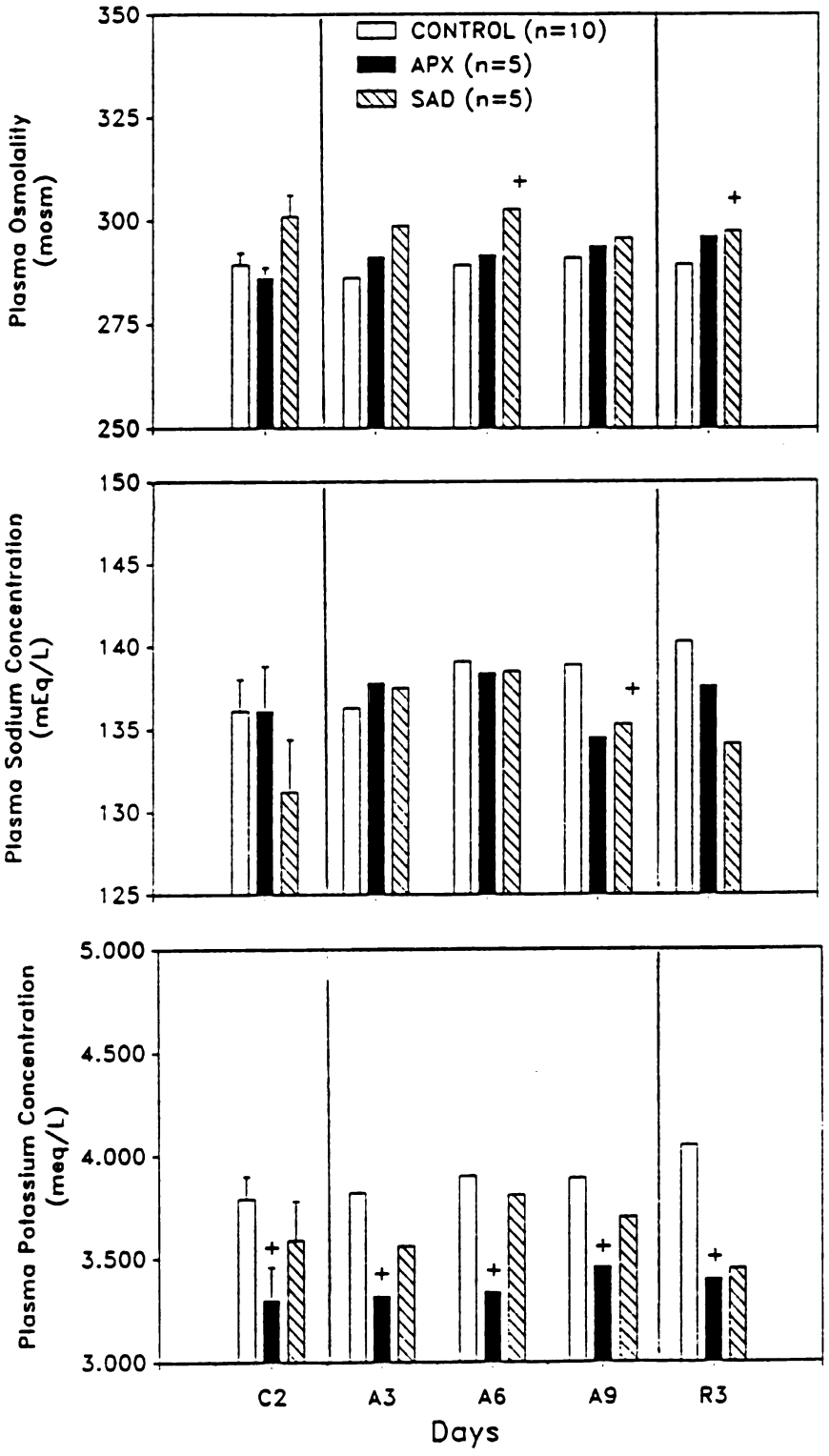

Figure 7c

Figure 7d. Blood urea nitrogen and urinary sodium excretion during control infusion experiment. See Figures 7a and 7c for further explanation.

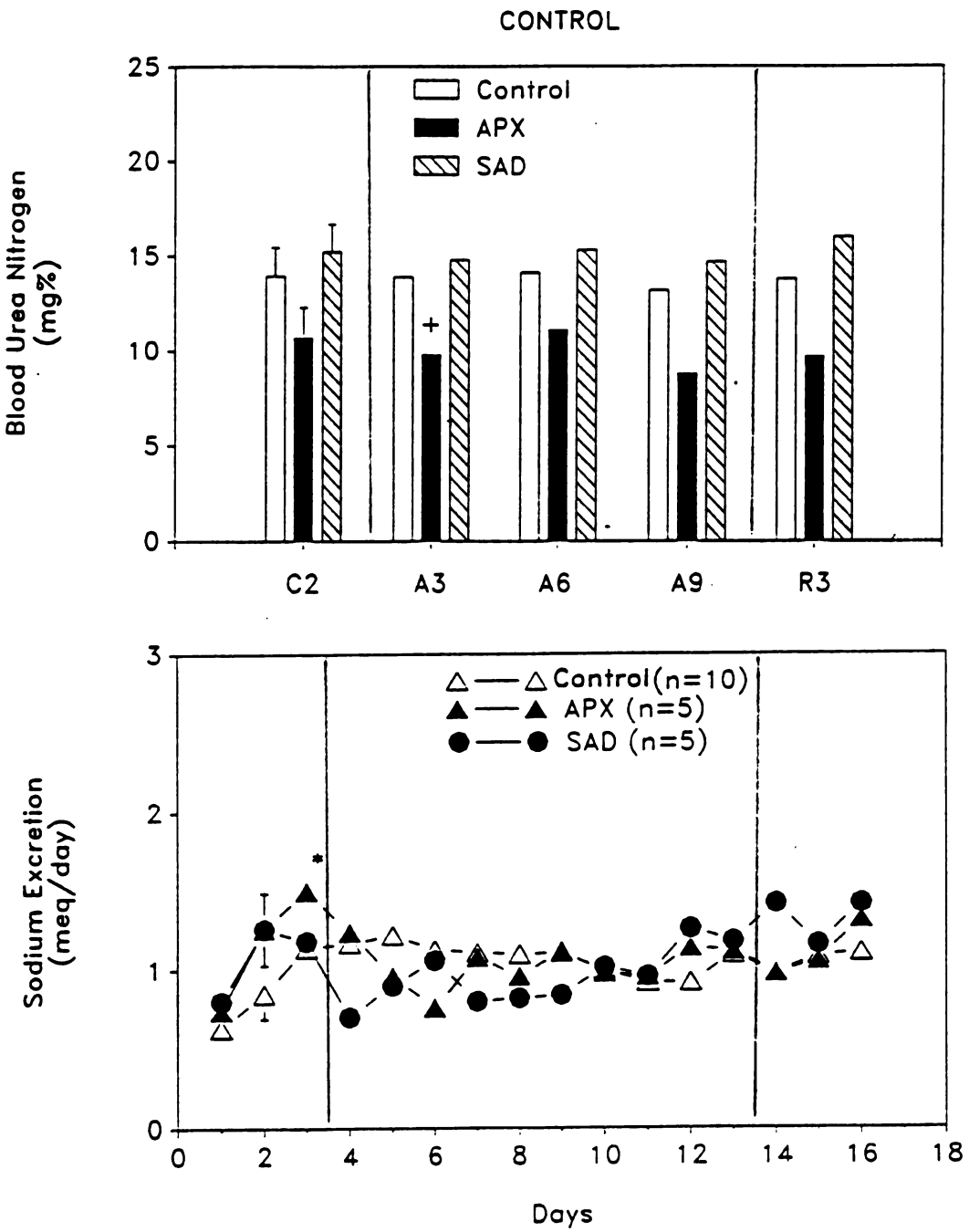
CONTROL
Blood Urea Nitrogen (mg%)
25
20
15
10
5
0
Control
APX
SAD
+
C2
A3
A6
A9
R3
Sodium Excretion (meq/day)
3
2
1
0
Control(n=10)
APX (n=5)
SAD (n=5)
*
0
2
4
6
8
10
12
14
16
18
Days

Figure 7d

Figure 8a. Effect of low dose (0.2 ng/kg/min) AVP on mean arterial pressure and heart rate in Control and APX rats. Daily MAP and HR measured during the sixteen day protocol (3 control, 10 AVP infusion, and 3 recovery days). AVP infusion period indicated by bracketed data. Bars on control period data indicate SEM used for within groups comparisons.

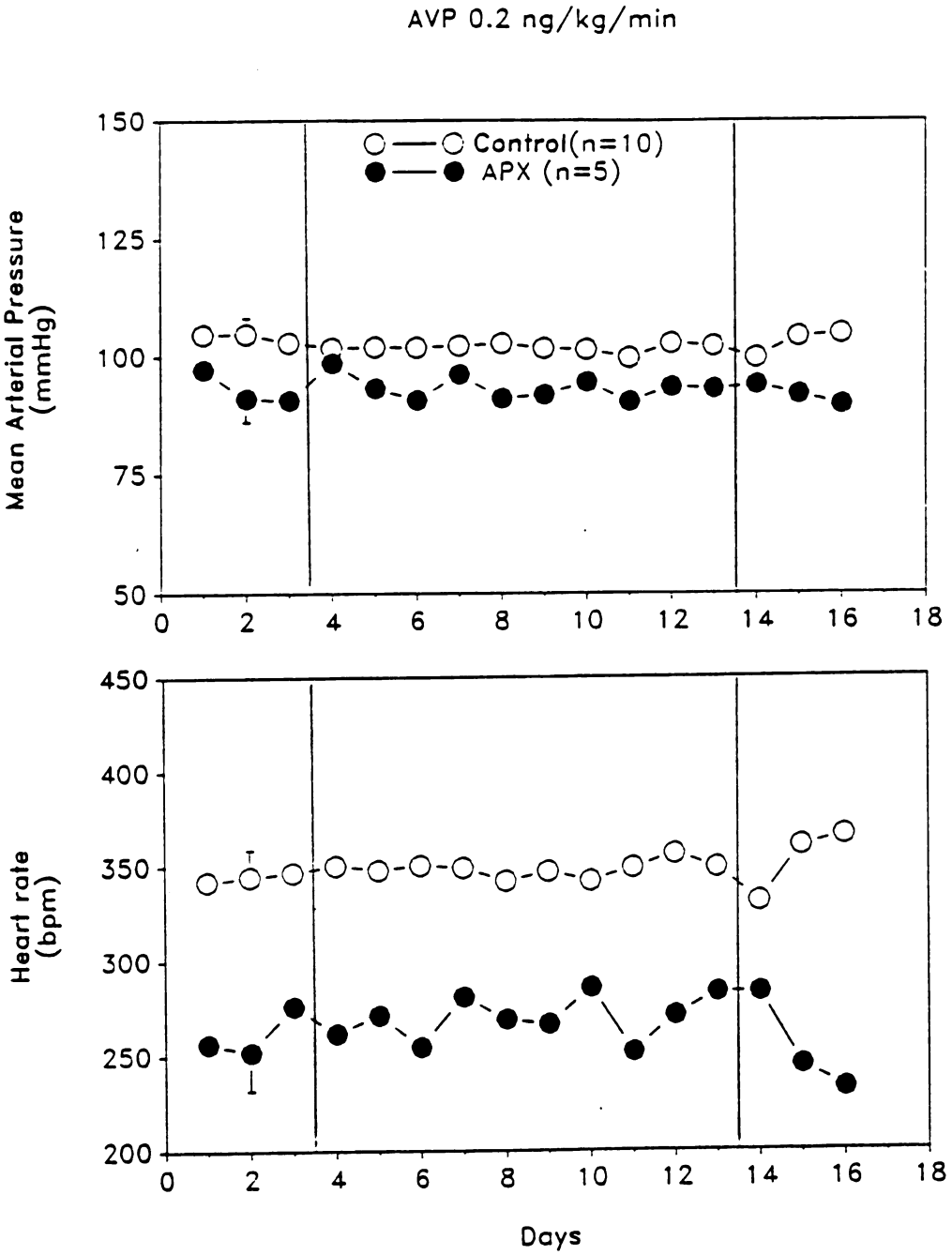
AVP 0.2 ng/kg/min
Control(n=10)
APX (n=5)
Mean Arterial Pressure (mmHg)
150
125
100
75
50
0
2
4
6
8
10
12
14
16
18
Heart rate (bpm)
450
400
350
300
250
200
Days

Figure 8a

Figure 8b. Effect of chronic low dose AVP infusion on fluid balance in the intact and AP-lesioned rat. UO, WI, and WB measured daily throughout the sixteen day protocol. See Figure 8a for further explanation. An * indicates data statistically different from control period data.

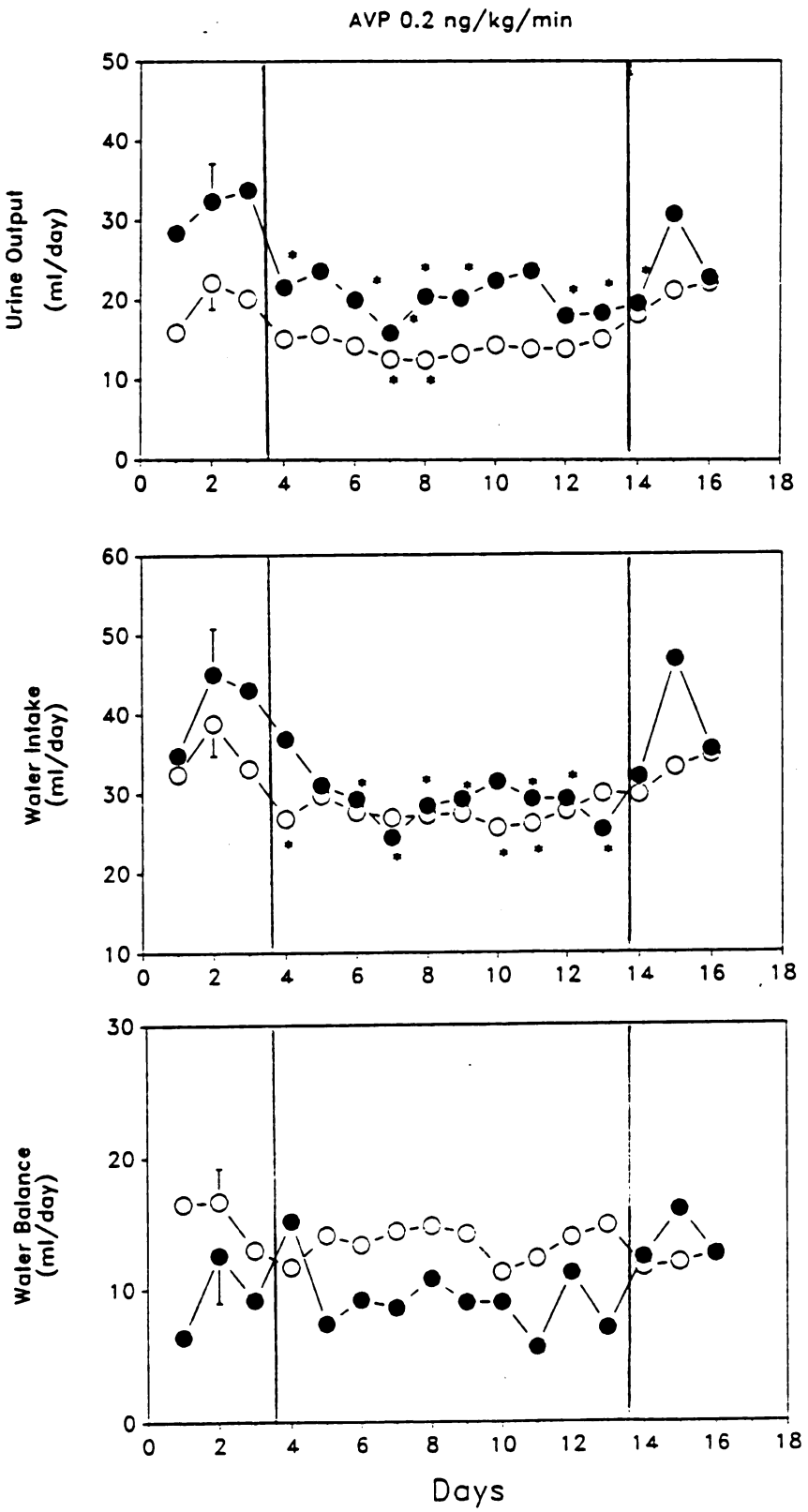
AVP 0.2 ng/kg/min
Urine Output (ml/day)
Water Intake (ml/day)
Water Balance (ml/day)
Days

Figure 8b

Figure 8c. Plasma electrolytes during low dose AVP infusion. pOsm, pNa, and pK measured once during control (C) and recovery (R) and three times during AVP infusion periods (A).

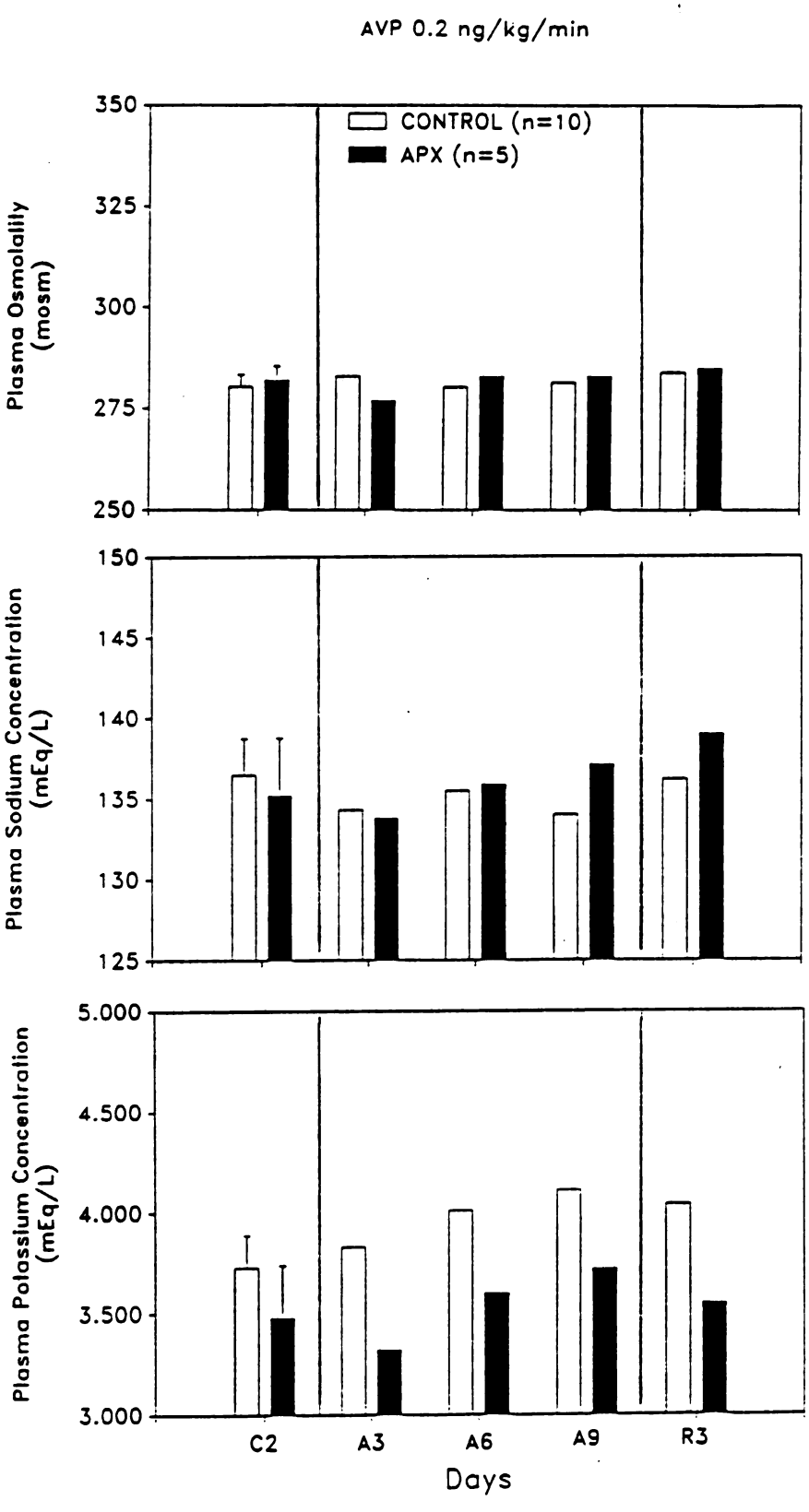
AVP 0.2 ng/kg/min
CONTROL (n=10)
APX (n=5)
Plasma Osmolality (mosm)
350
325
300
275
250
Plasma Sodium Concentration (mEq/L)
150
145
140
135
130
125
Plasma Potassium Concentration (mEq/L)
5.000
4.500
4.000
3.500
3.000
C2
A3
A6
A9
R3
Days

Figure 8c

Figure 8d. Blood urea nitrogen and urinary sodium excretion during chronic low dose AVP infusion. See figures 8a and 8c for further explanation.

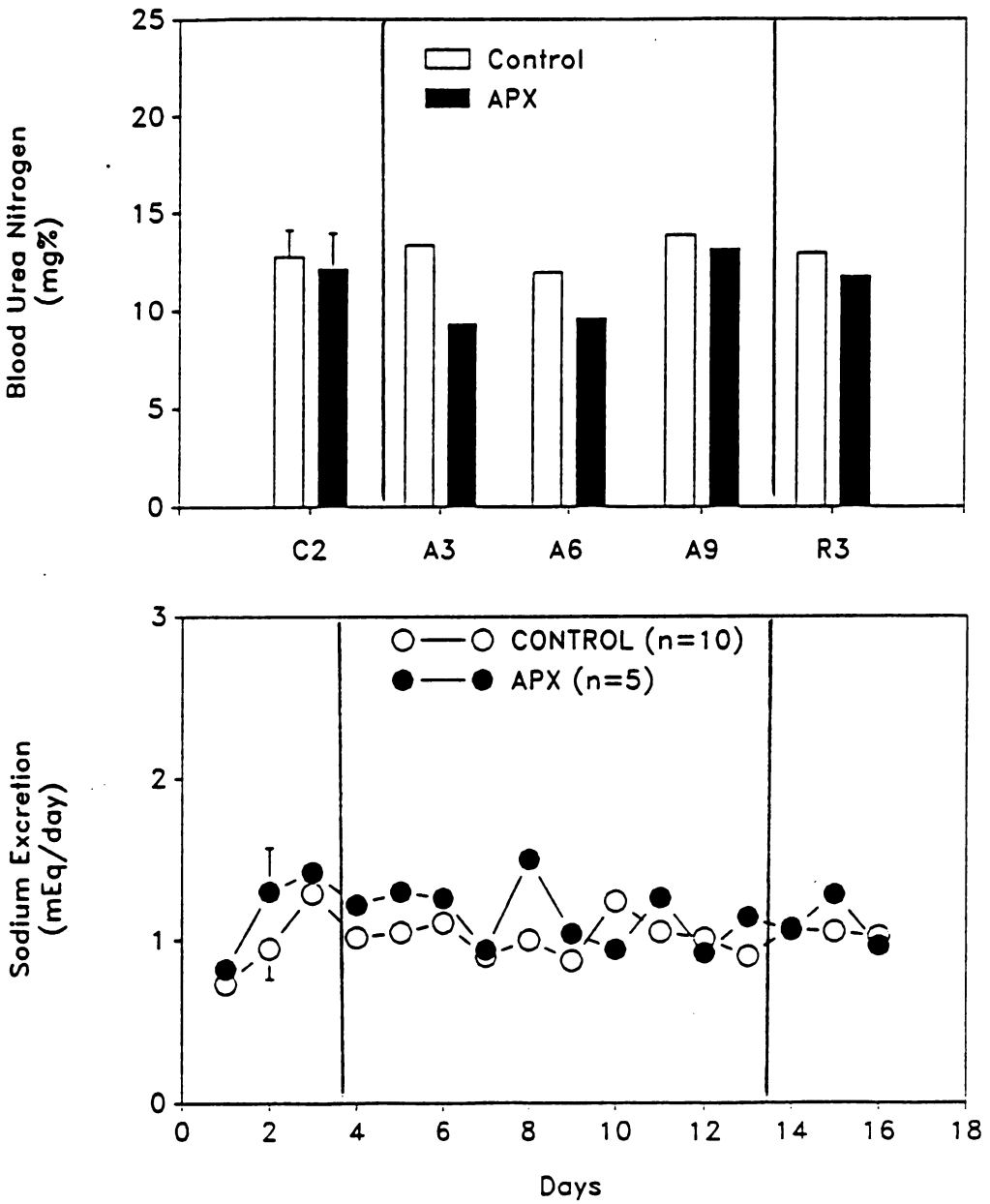
Blood Urea Nitrogen (mg%)
25
20
15
10
5
0
Control
APX
C2
A3
A6
A9
R3
Sodium Excretion (mEq/day)
3
2
1
0
CONTROL (n=10)
APX (n=5)
0
2
4
6
8
10
12
14
16
18
Days

Figure 8d

Figure 9a. Effect of chronic high dose (2.0 ng/kg/min) AVP infusion on mean arterial pressure and heart rate in intact, sino-aortic denervated and AP-lesioned rats. Daily MAP and HR measured during sixteen day protocol. AVP treatment period indicated by data between brackets. An * indicates data significantly different from control period data.

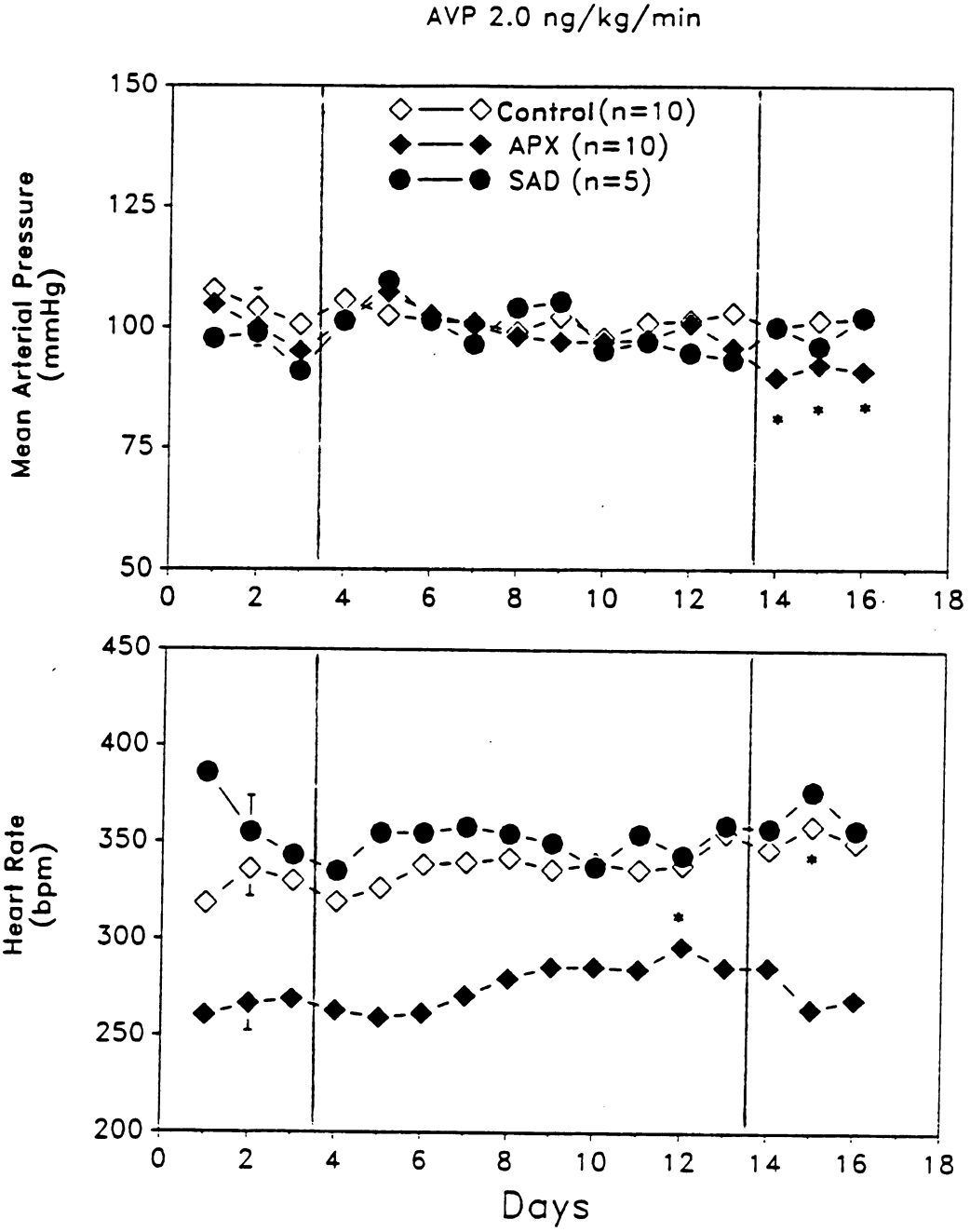
AVP 2.0 ng/kg/min
Control (n=10)
APX (n=10)
SAD (n=5)
Mean Arterial Pressure (mmHg)
150
125
100
75
50
0
2
4
6
8
10
12
14
16
18
Heart Rate (bpm)
450
400
350
300
250
200
Days

Figure 9a

Figure 9b. Fluid balance in response to chronic AVP infusion in intact, sino-aortic denervated and AP-lesioned rats. Daily UO, WI, and WB in rats receiving high dose AVP during the 10 day experiemental period. See figure 9a for further details.

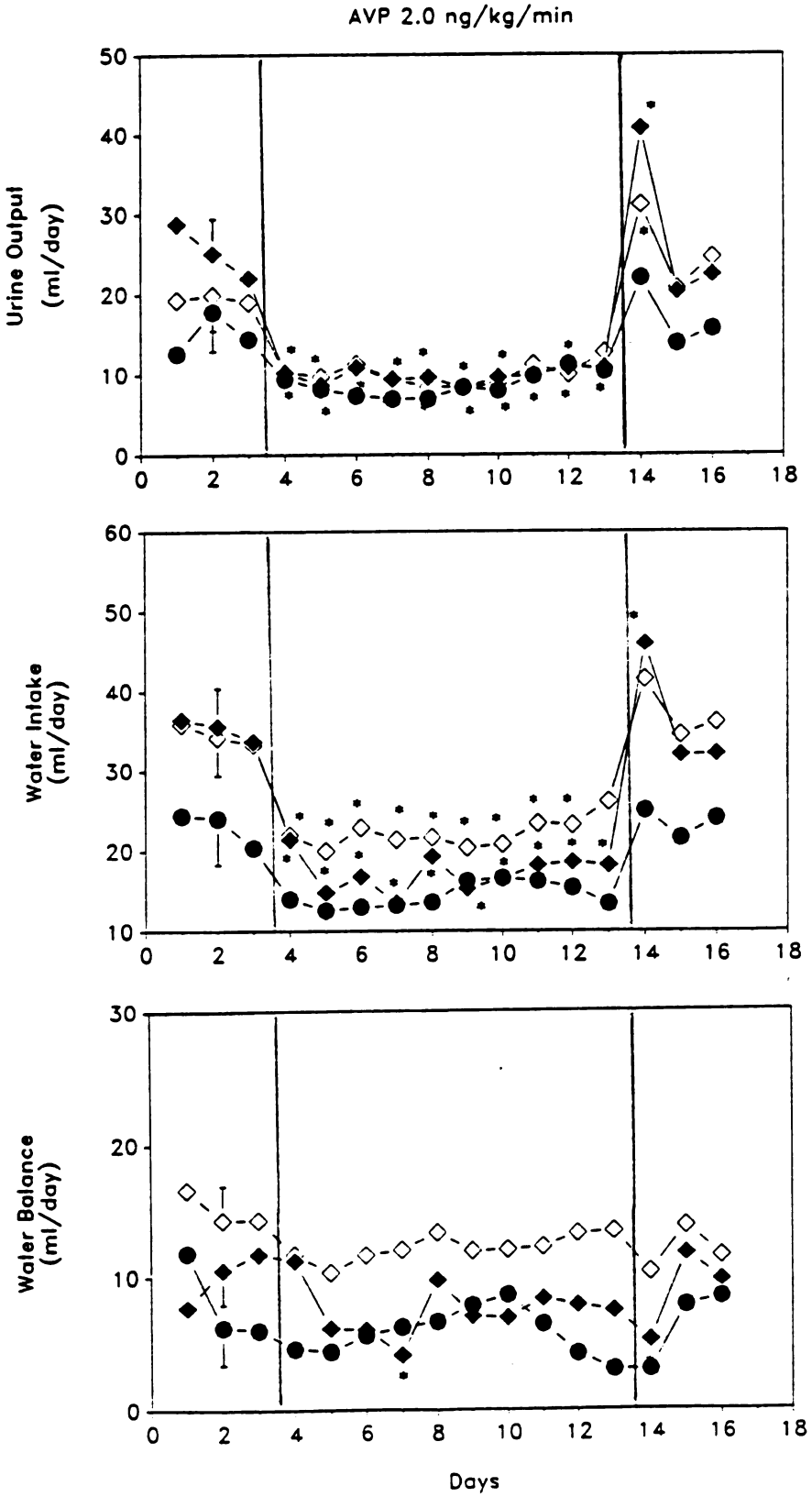
AVP 2.0 ng/kg/min
Urine Output (ml/day)
Water Intake (ml/day)
Water Balance (ml/day)
Days

Figure 9b

Figure 9c. Plasma electrolytes during high dose AVP infusion in rats. pOsm, pNa, and pK measured in plasma obtained before (C), during (A), and after (R) AVP infusion. + indicates data significantly different from value in CONTROL rats at the same time point.

AVP 2.0 ng/kg/min

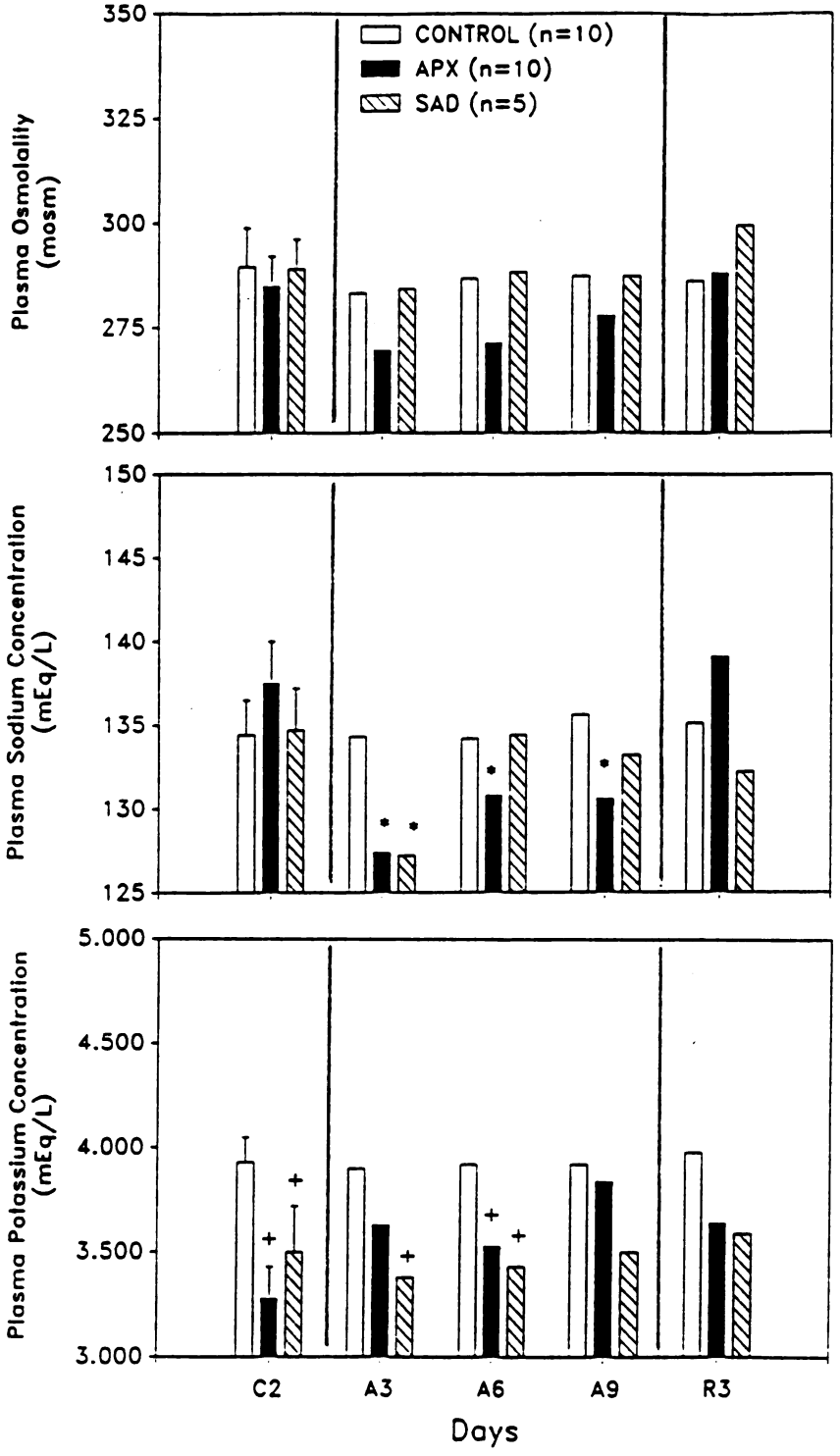

Figure 9c

Figure 9d. Blood urea nitrogen and urinary sodium excretion in rats receiving high dose AVP for ten days. See figures 9a and 9c for further details.

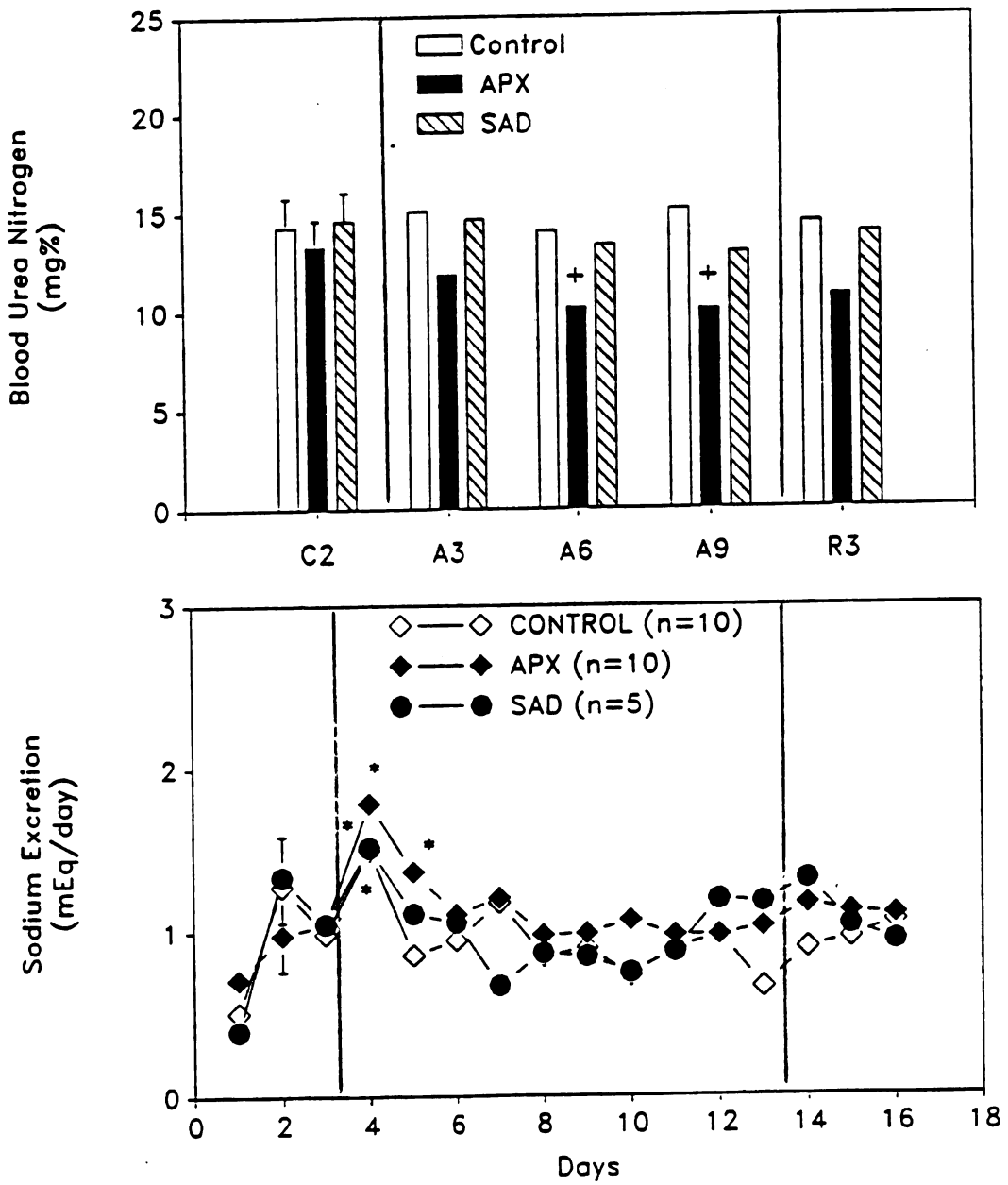
Blood Urea Nitrogen (mg%)
25
20
15
10
5
0
Control
APX
SAD
+
+
C2
A3
A6
A9
R3
Sodium Excretion (mEq/day)
3
2
1
0
CONTROL (n=10)
APX (n=10)
SAD (n=5)
0
2
4
6
8
10
12
14
16
18
Days

Figure 9d

(data not shown).

Changes in MAP and HR in response to ganglionic blockade by bolus injection of hexamethonium (20 mg/kg i.v.) are illustrated in Figure 10. Depressor responses to ganglionic blockade in all groups of rats receiving the sodium solution alone were not altered during the sixteen day experiment. The depressor responses elicited in the CONTROL rats were not different from those elicited in either the APX or SAD animals. CONTROL, APX, and SAD rats receiving either dose of AVP (0.2 or 2.0 ng/kg/min) also did not exhibit significantly altered depressor responses to ganglionic blockade during chronic AVP infusion compared to responses obtained during control or recovery periods.

III. Ganglion Blockade During Angiotensin Hypertension

Daily MAP and HR measured in rats maintained on a 6 mEq daily sodium intake during a 22 day protocol (3 control, 14 ANG II infusion, and 5 recovery days) are shown in Figure 11. In both SAD and CON rats MAP was significantly elevated throughout ANG II infusion compared to MAP measured during the control period. In the CON group MAP exhibited 2 plateau stages during ANG II infusion (i.e. a second pressure plateau reached on day 12). However, pressure in the SAD group reached a plateau by the first 24 hours of ANG II treatment and no further change in pressure occurred within the ANG II infusion period. Daily standard deviation (SD) of MAP was not changed by ANG II in CON or SAD rats. In the CON rats the average SD during the control period was 6.6 versus 6.7 during the ANG II infusion period. Similarly, in the SAD rats the average SD during the control period was 18.2 versus 20.6 during ANG II treatment.

Daily HR was significantly reduced from control much of the first week of ANG II infusion in both groups of rats but much more so in the CON group. In both groups HR returned to control levels by the second week of ANG II infusion and

Figure 10. Depressor responses to ganglion blockade in rats receiving chronic saline (control) or AVP infusion. Change in MAP in response to hexamethonium during control (C), AVP infusion (A) and recovery (R) in CONTROL, sino-aortic denervated and AP-lesioned rats.

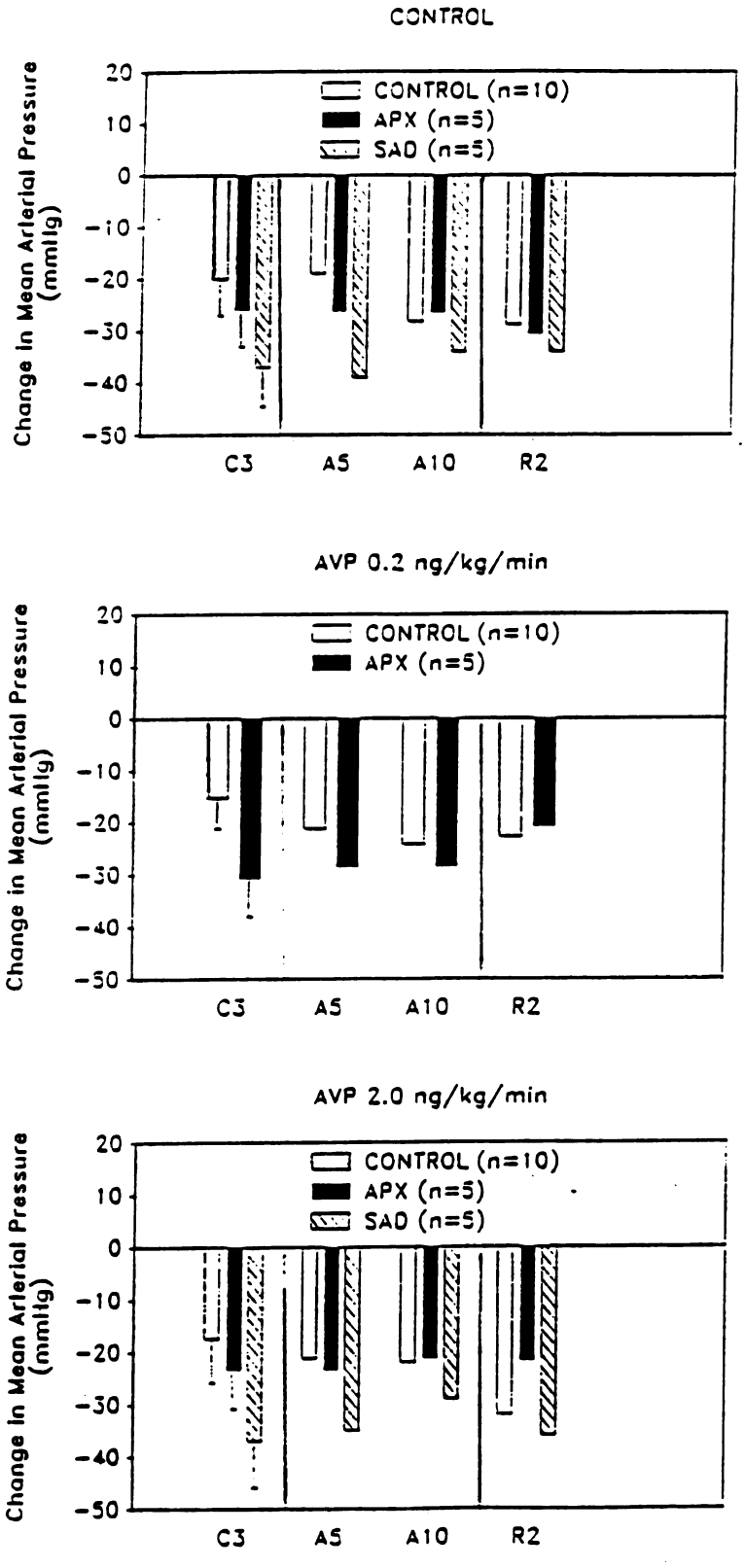
CONTROL
Change in Mean Arterial Pressure (mmHg)
20
10
0
-10
-20
-30
-40
-50
CONTROL (n=10)
APX (n=5)
SAD (n=5)
C3
A5
A10
R2
AVP 0.2 ng/kg/min
Change in Mean Arterial Pressure (mmHg)
CONTROL (n=10)
APX (n=5)
AVP 2.0 ng/kg/min
Change in Mean Arterial Pressure (mmHg)
CONTROL (n=10)
APX (n=5)
SAD (n=5)

Figure 10

Figure 11. Effect of chronic iv infusion of ANG II (10 ng/min) on hemodynamic parameters in intact and sino-aortic denervated rats. Daily MAP and HR measured during control, ANG II infusion (denoted by bracketed data) and recovery in CON and SAD rats. All MAP data points from both groups during ANG II infusion were significantly greater than respective control data. An * indicates data point statistically different from control data for HR and all subsequent figures. Bars on day 2 data points represent S.E.M. for comparisons of within group differences (i.e. over time).

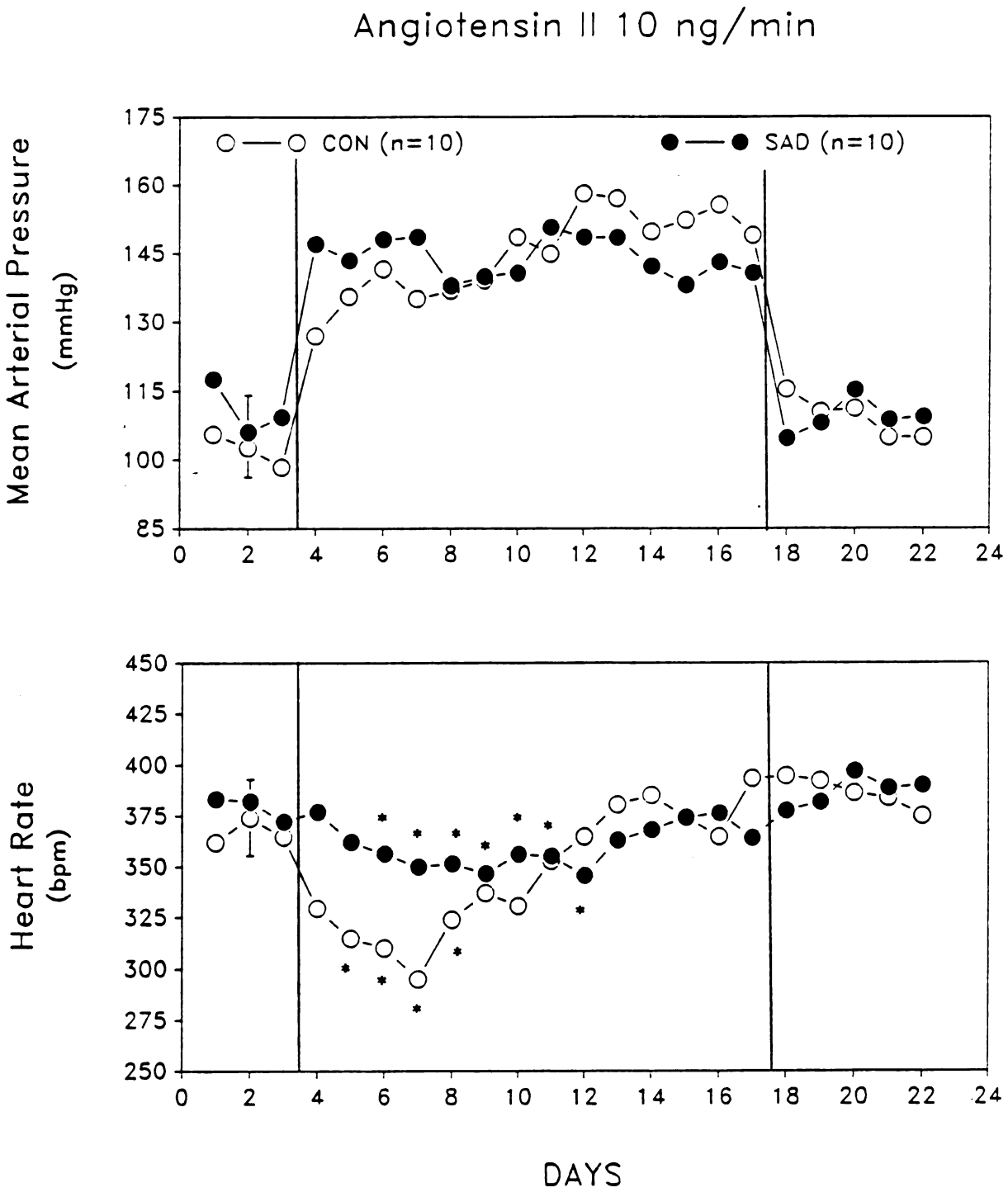
Angiotensin II 10 ng/min
Mean Arterial Pressure
(mmHg)
CON (n=10)
SAD (n=10)
175
160
145
130
115
100
85
0
2
4
6
8
10
12
14
16
18
20
22
24
Heart Rate
(bpm)
450
425
400
375
350
325
300
275
250
DAYS

Figure 11

was not altered during recovery.

Daily WI, UO and UNaV are illustrated in Figure 12. In the CON group none of these daily variables changed during ANG II infusion or upon recovery. However, SAD rats drank and urinated greater volumes from the last days of ANG II treatment through the recovery period. Daily UNaV did not change in the SAD group.

The depressor responses to hexamethonium and nitroprusside are illustrated in Figure 13. The top graph represents the responses elicited from CON rats during control, ANG II infusion, and recovery. CON rats responded similarly during control and recovery to both depressor agents. However, during chronic ANG II infusion nitroprusside elicited a greater depressor response throughout. Ganglion blockade produced significantly greater depressor responses only during the later stages (day 13) of ANG II infusion in these rats.

The lower graph represents data obtained from the SAD rats. All depressor responses in the SAD group were greater that those elicited in the CON group, reflecting the loss of reflex buffering of pressure. Depressor responses produced with nitroprusside were significantly greater than control period responses throughout ANG II infusion and were similar to control period responses during recovery. Unlike CON rats, the depressor responses with ganglion blockade were significantly greater than control period values at all times assessed during ANG II infusion in the SAD rats. and not different from the control period response during recovery.

Figure 14 represents the arterial pressure after ganglion blockade and thus illustrates the non-autonomic component of arterial pressure at various time points throughout control, ANG II infusion and recovery periods. It is evident that the non-autonomic component of pressure significantly increased during ANG II infusion in the CON rats and returned to control levels during recovery. However,

Figure 12. Effect of chronic iv ANG II infusion on fluid balance in intact and sino-aortic denervated rats. Daily WI, UO, and UNaV measured in CON and SAD rats during the 22 day protocol. Symbols explained in FIG. 11.

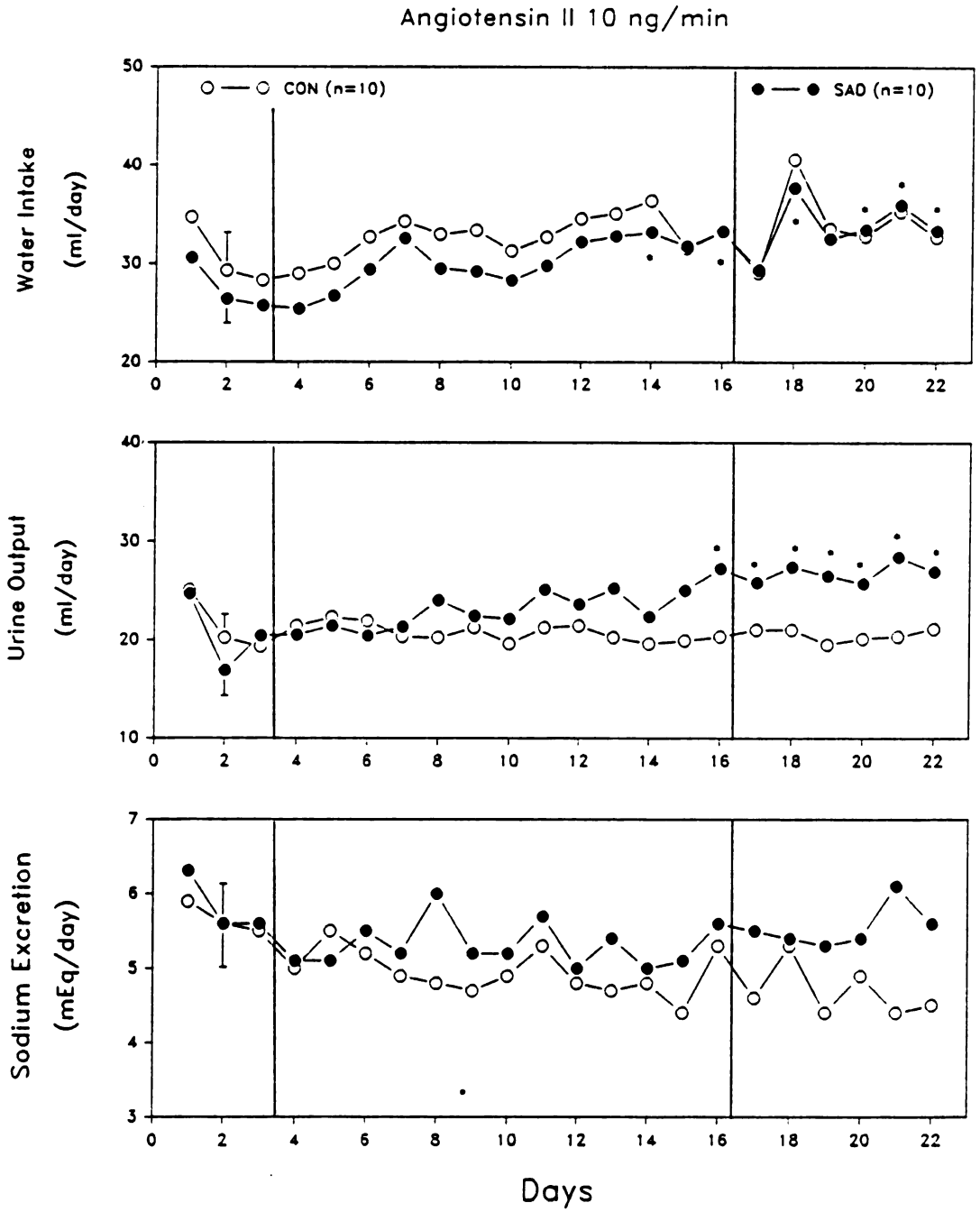
Angiotensin II 10 ng/min
CON (n=10)
SAD (n=10)
Water Intake (ml/day)
Urine Output (ml/day)
Sodium Excretion (mEq/day)
Days

Figure 12

Figure 13. Changes in mean arterial pressure in response to hexamethonium (HEX) and sodium nitroprusside (SNP) infusion during control (C), ANG II infusion (A), and recovery (R) periods represented as means and individual group S.E.M.s. Numerals on abscissa indicate days during respective period that responses were elicited. HEX and SNP challenges were conducted 24 hours apart. Top and bottom graphs illustrate data from CON and SAD rats respectively.

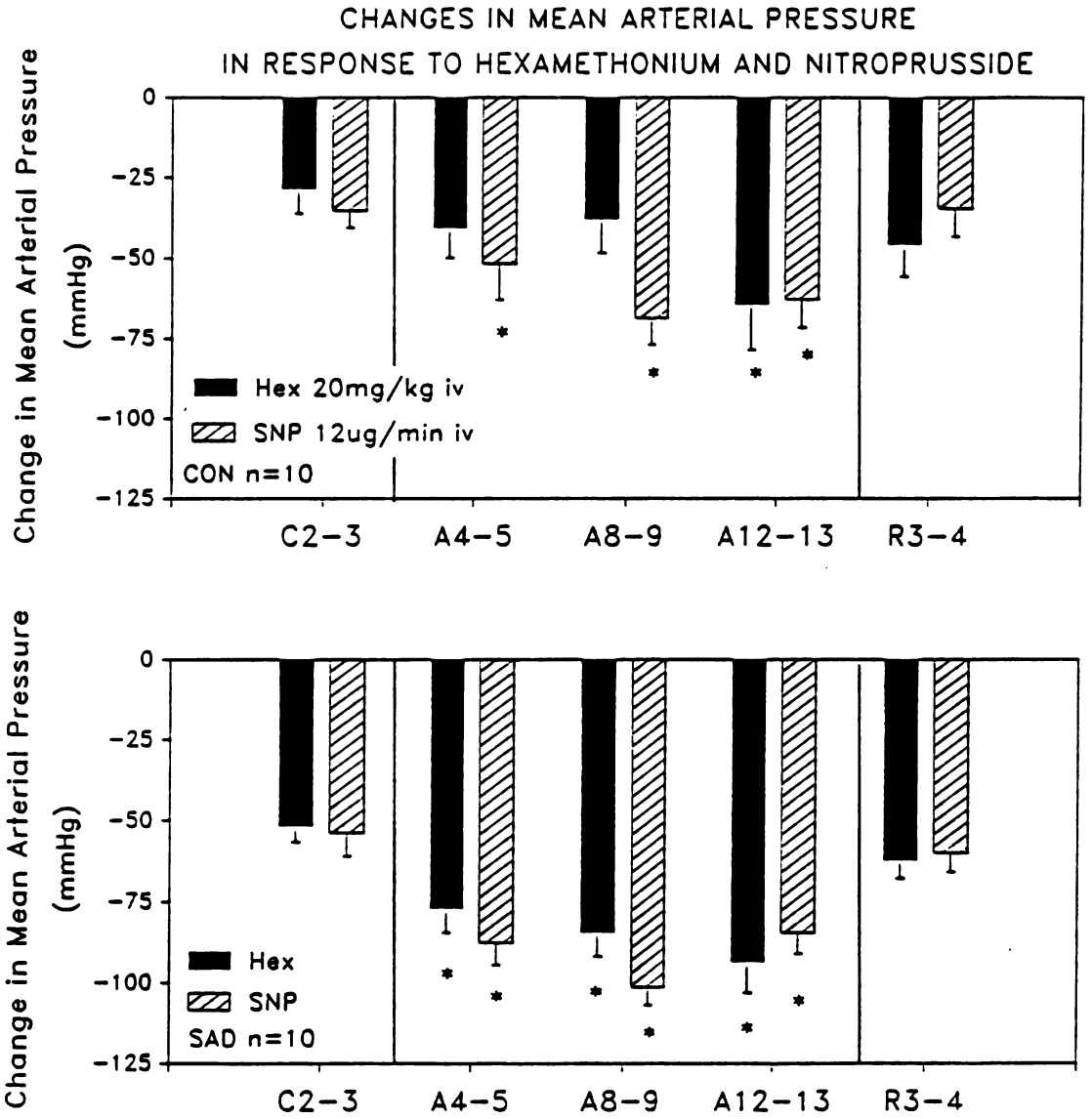
CHANGES IN MEAN ARTERIAL PRESSURE
IN RESPONSE TO HEXAMETHONIUM AND NITROPRUSSIDE
Change in Mean Arterial Pressure (mmHg)
0
-25
-50
-75
-100
-125
Hex 20mg/kg iv
SNP 12ug/min iv
CON n=10
C2-3
A4-5
A8-9
A12-13
R3-4
Change in Mean Arterial Pressure (mmHg)
Hex
SNP
SAD n=10

Figure 13

Figure 14. Non-autonomic component of arterial pressure before, during, and after chronic iv ANG II infusion in intact and sino-aortic denervated rats. MAP after bolus iv injecton of hexamethonium during control (C), ANG II infusion (A) and recovery (R). The day on which hexamethonium was given during ANG II infusion is indicated by the accompanying numeral on the abscissa. Data significantly different from control value indicated by an *.

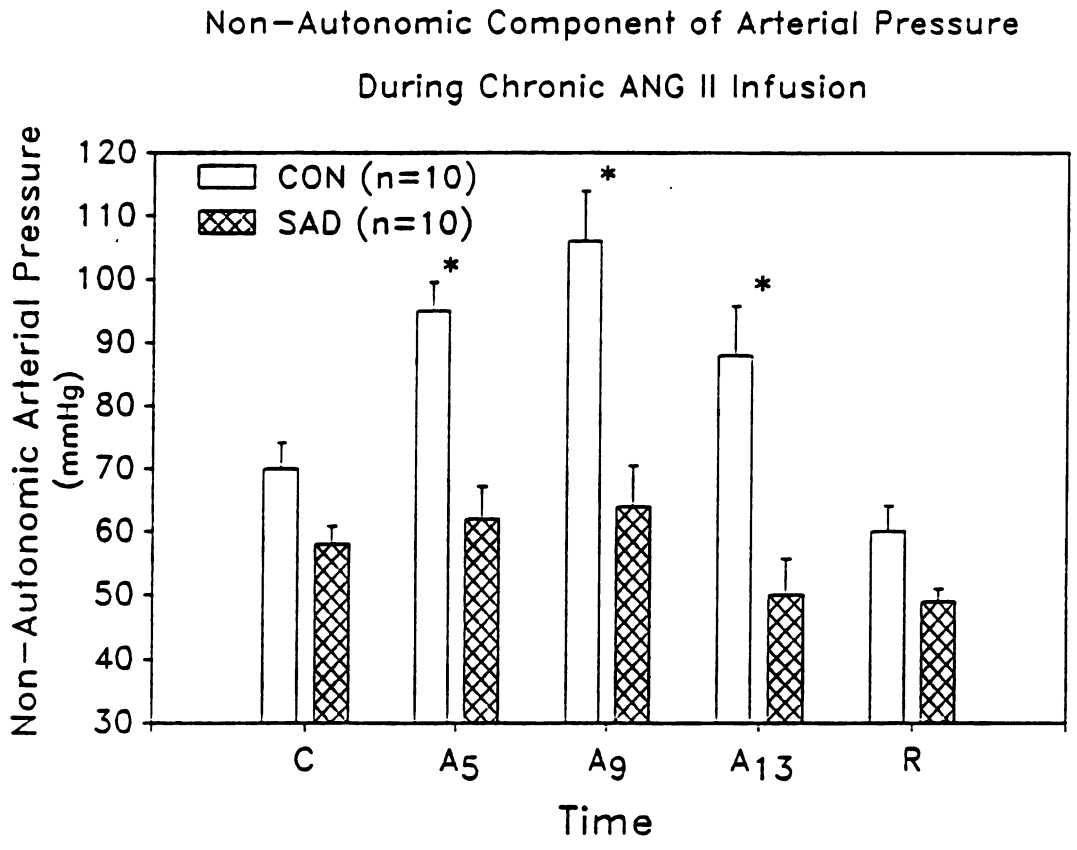
Non-Autonomic Component of Arterial Pressure
During Chronic ANG II Infusion
Non-Autonomic Arterial Pressure (mmHg)
120
110
100
90
80
70
60
50
40
30
CON (n=10)
SAD (n=10)
*
*
*
C
A5
A9
A13
R
Time

Figure 14

in the SAD rat chronic ANG II infusion was not accompanied by an increased contribution of non-autonomic mechanisms to the level of arterial pressure.

IV. Circulating Angiotensin (1-7)

A. Acute iv ANG (1-7) pressor effect

The pressor responses to acute 5 minute iv infusion of ANG (1-7) at 3 doses (10, 30, and 100 ng/min) are illustrated in Figure 15. These responses are compared to those elicited by ANG II infusions of the two lowest doses. These results confirm the findings that unlike ANG II, ANG (1-7) does not possess direct vasoconstrictive actions.

B. Chronic iv ANG (1-7) infusion

Figure 16 illustrates the daily MAP and HR measured during a 16 day protocol (3 control, 10 ANG (1-7) infusion, and 3 recovery) in rats maintained on a fixed 6 mEq sodium intake. These results are illustrated with results of a similar protocol in which a separate group of rats received ANG II (10 ng/min) for 10 days for comparison. ANG II clearly produced hypertension throughout the infusion period while ANG (1-7) treatment failed to alter daily MAP. HR remained unchanged as well.

In support of the ability of ANG (1-7) to stimulate AVP release, pAVP was increased from 1.62 $\pm$ 0.38 pg/ml during the control period to 2.17 $\pm$ 0.16 pg/ml after ten days of infusion of the higher (30 ng/min) dose. Low dose ANG (1-7) infusion (10 ng/min) did not result in increases in circulating AVP (from 2.55 $\pm$ 0.38 pg/ml to 2.39 $\pm$ 0.26 pg/ml). Further, as illustrated in Figure 17, UO is decreased during ANG (1-7) infusion, attaining statistical significance with the lower dose. WI followed a similar pattern. However iv injection of an acute vascular AVP antagonist produced no significant change in arterial pressure during chronic ANG (1-7) infusion (data not shown). UNaV was unaltered during these experiments (data not shown).

Figure 15. Pressor responses to acute iv infusion of ANG (1-7). Changes in MAP after five minute infusions of ANG (1-7) at three doses (10, 30, and 100 ng/min; n= 13). Responses to ANG II (n= 13) are illustrated for comparison at the two lower doses.

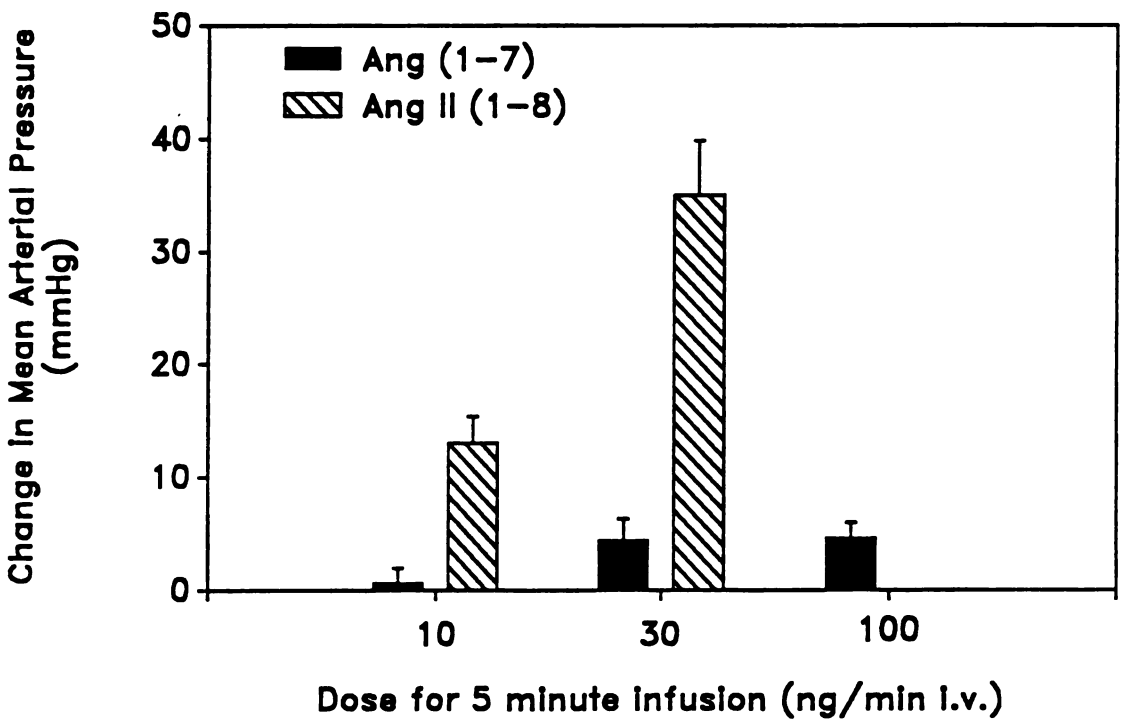
Change in Mean Arterial Pressure (mmHg)
50
40
30
20
10
0
Ang (1–7)
Ang II (1–8)
10
30
100
Dose for 5 minute infusion (ng/min I.v.)

Figure 15

Figure 16. Effect of chronic iv ANG (1-7) infusion on hemodynamic parameters in the rat. Daily MAP and HR during 3-day control, 10-day experimental, and 3-day recovery periods. ANG (1-7) was infused for 10 days indicated by the bracketed data at two doses (10 and 30 ng/min). Data are illustrated with MAP response to similar protocol in separate rats receiving ANG II (10 ng/min) during the experimental period for comparison. All data points during ANG II infusion are statistically increased compared to control period values. At no time during ANG (1-7) infusion were MAP or HR statistically different from their respective control period values.

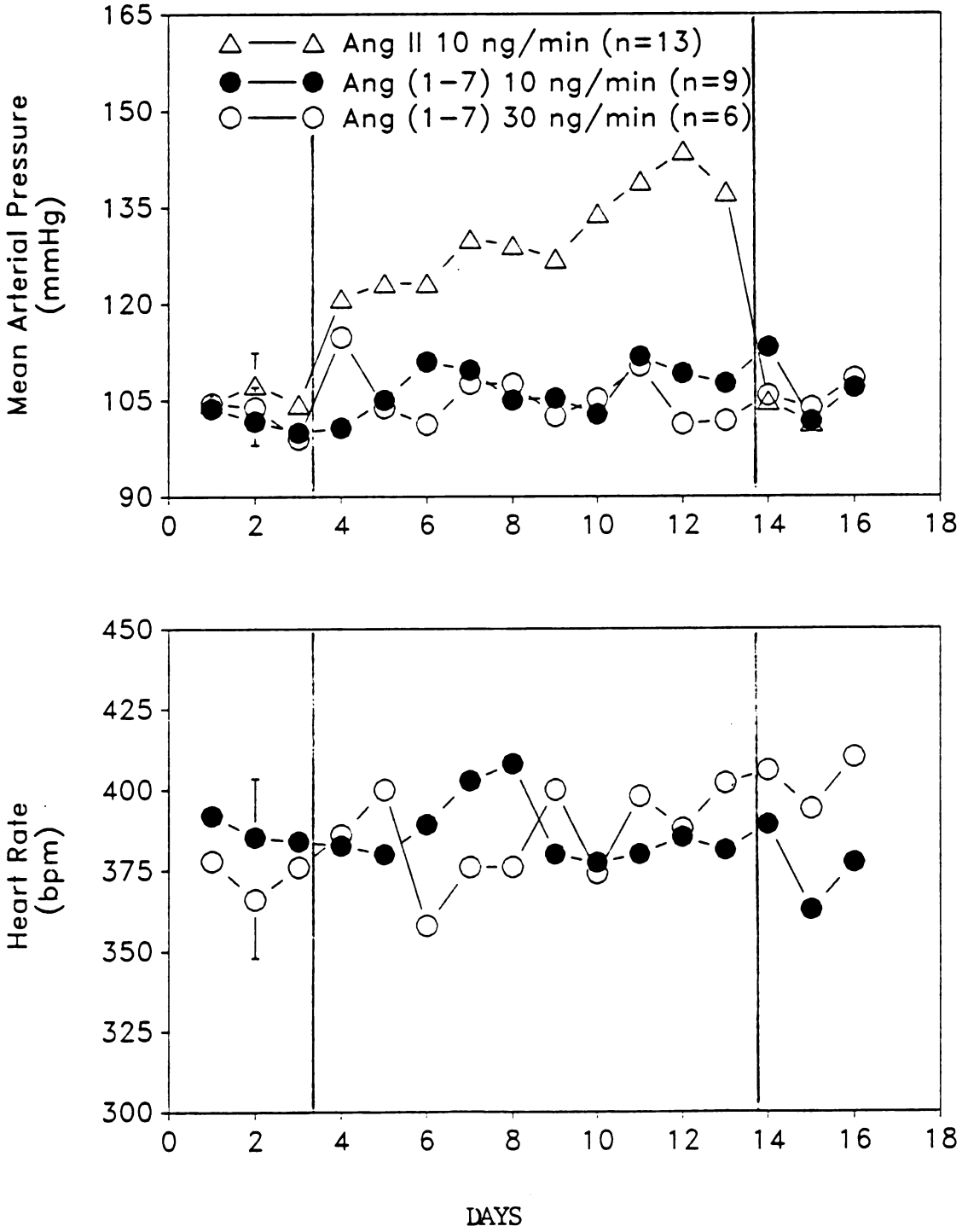
Ang II 10 ng/min (n=13)
Ang (1-7) 10 ng/min (n=9)
Ang (1-7) 30 ng/min (n=6)
Mean Arterial Pressure (mmHg)
165
150
135
120
105
90
Heart Rate (bpm)
450
425
400
375
350
325
300
0
2
4
6
8
10
12
14
16
18
DAYS

Figure 16

Figure 17. Effect of chronic iv infusion of ANG (1-7) on fluid balance in the rat. Daily WI and UO measured during a sixteen day protocol. An * indicates data significantly different from control period values. See Figure 16 for further details.

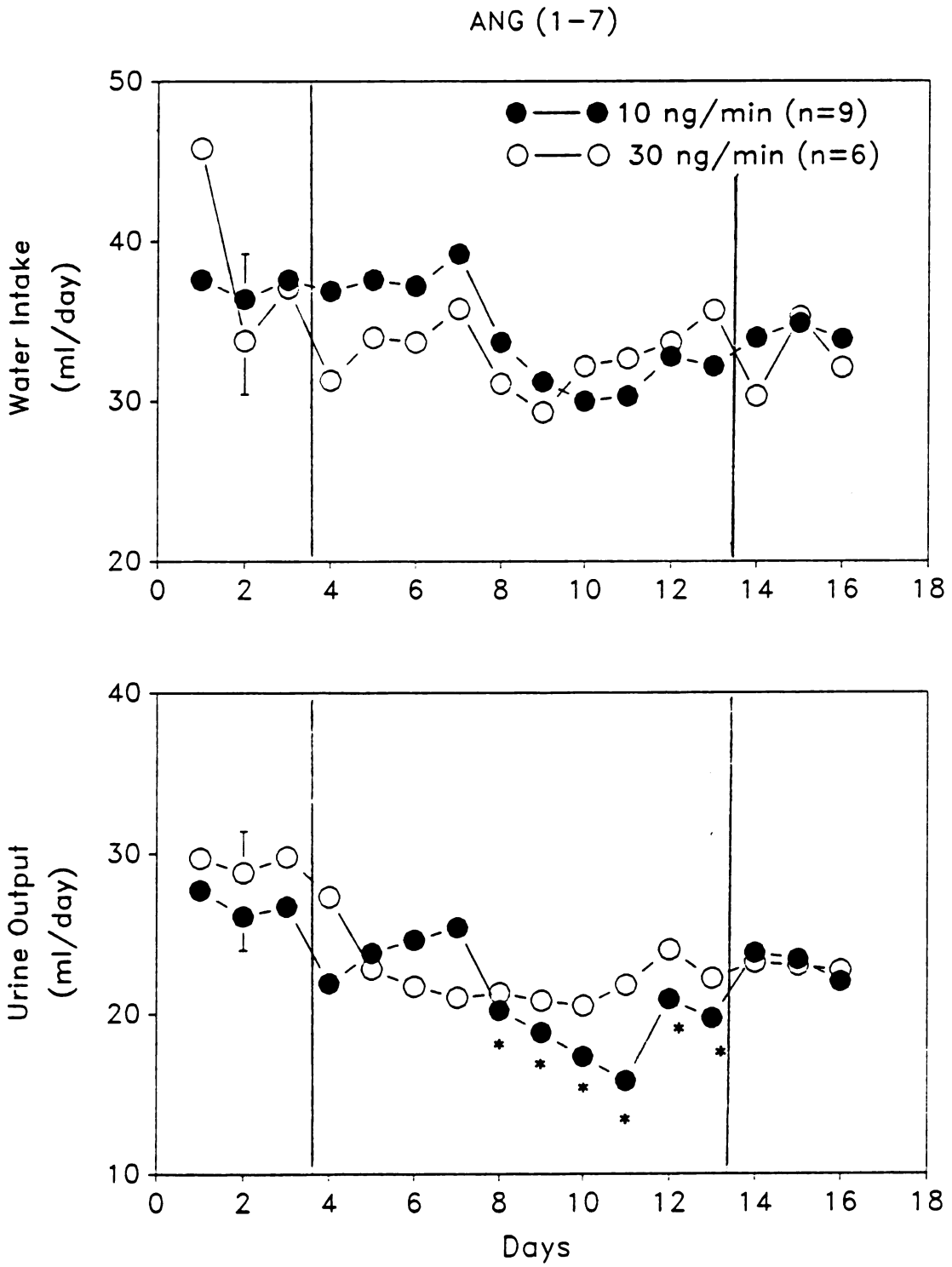
ANG (1-7)
10 ng/min (n=9)
30 ng/min (n=6)
Water Intake (ml/day)
Urine Output (ml/day)
Days
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18

Figure 17

V. Area Postrema and Renovascular Hypertension

A. Two-kidney, 1-clip hypertension

Chronic reduction of blood flow to the left kidney by placement of a silver clip on the renal artery resulted in a prolonged hypertensive state in sham-lesioned rats (SC) as seen in Figure 18. In contrast sham-clipped (SS,LS) and clipped AP-lesioned (LC) rats did not experience prolonged increases in daily MAP. The LC rats did become hypertensive during the initial days following renal artery constriction but were normotensive by the fifth day.

Figure 18 also illustrates the PRA measured once during control and twice after sham-clip or clip surgery. There were no changes in PRA from control after the sham-clip surgery in the SS group or after clip surgery in the LC group. However, PRA in the LC group was greater during control compared to the other groups of rats. These results may have been due to the lesioned rats requiring longer post-surgical recovery periods prior to full return of appetite, and thus sodium intake, than the sham rats. PRA decreased in the LS group following sham-clip surgery and was significantly increased in the SC rats one week after clip surgery. Although PRA was equal in the LC and SC rats after fourteen days of reduced renal blood flow, blood pressure was elevated only in the SC group at this time point.

The changes in MAP in response to fifteen minute saralasin infusion are shown on the bottom graph of Figure 18. All groups responded similarly during the control period. However only the SC group exhibited significant depressor responses during saralasin infusion after renal artery constriction.

B. One-kidney, 1-clip hypertension

Chronic reduction of blood flow to the left kidney resulted in chronic hypertension in the uninephectomized rat with or without an intact AP. These results are illustrated in Figure 19. Rats that underwent sham-clip surgery did not experience any alterations in daily MAP throughout the experiment.

Figure 18. Area postrema and 2-K, 1-C hypertension. The top graph illustrates daily MAP obtained during three control days and fourteen days post sham-clip (S) or clip (C) surgery in sham-lesioned (S) and AP-lesioned (L) rats. SS= sham-lesioned, sham-clipped. LS= AP-lesioned, sham-clipped. SC= sham-lesioned, clipped. LC= AP-lesioned, clipped. PRA and the arterial pressure response to saralasin infusion were measured once during control (C) and twice after sham-clip or clip surgery (P). Numbers below data points on abscissa indicate day during period data was obtained. An * indicates data significantly different from control period data.

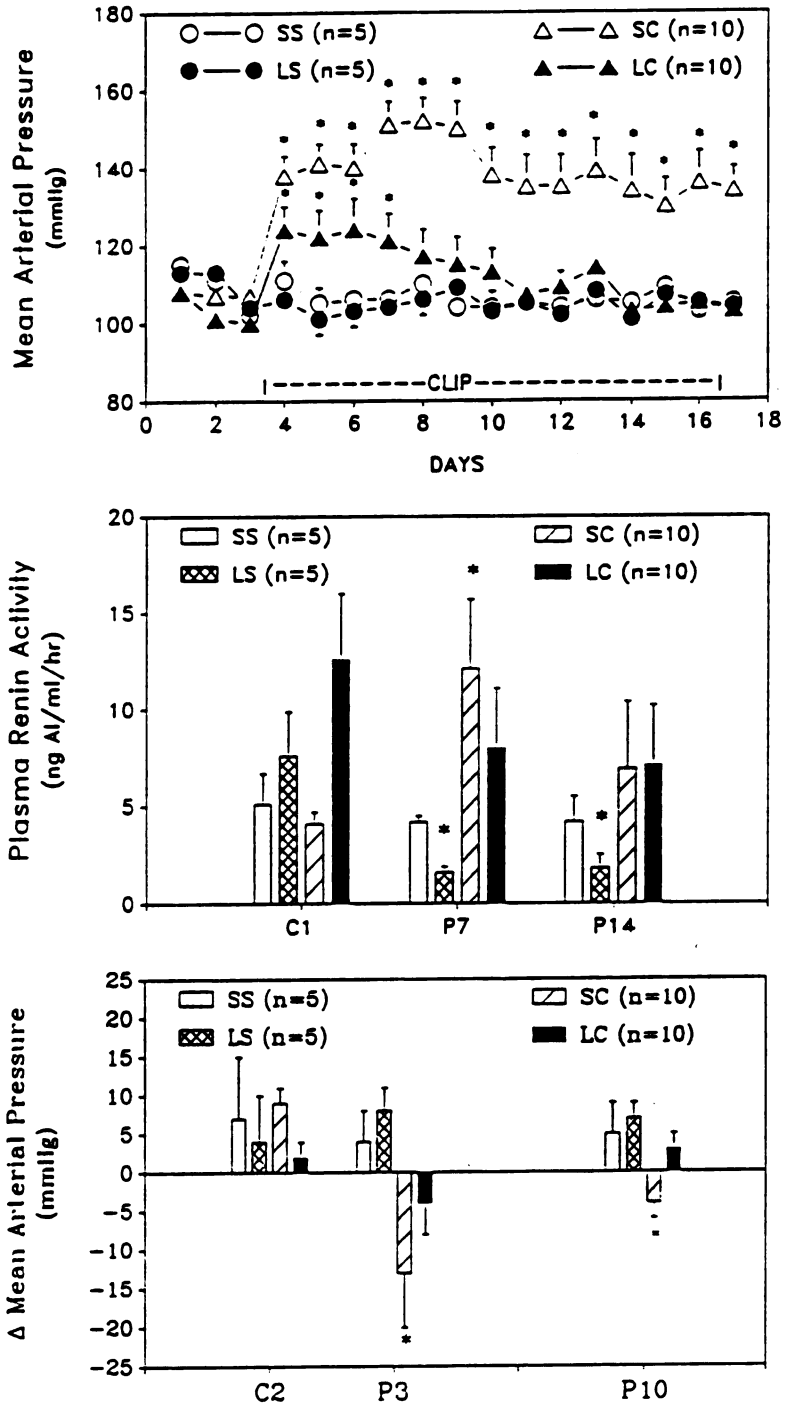
Mean Arterial Pressure (mmHg)
SS (n=5)
LS (n=5)
SC (n=10)
LC (n=10)
CLIP
DAYS
Plasma Renin Activity (ng AI/ml/hr)
C1
P7
P14
Δ Mean Arterial Pressure (mmHg)
C2
P3
P10

Figure 18

Figure 19. Area postrema and 1-K, 1-C hypertension. Daily MAP measured during three control days and fourteen days post sham-clip or clip surgery is illustrated in the top graph. PRA and arterial pressure responses to saralasin infusion are depicted in the lower two graphs. See Figure 18 for more details.

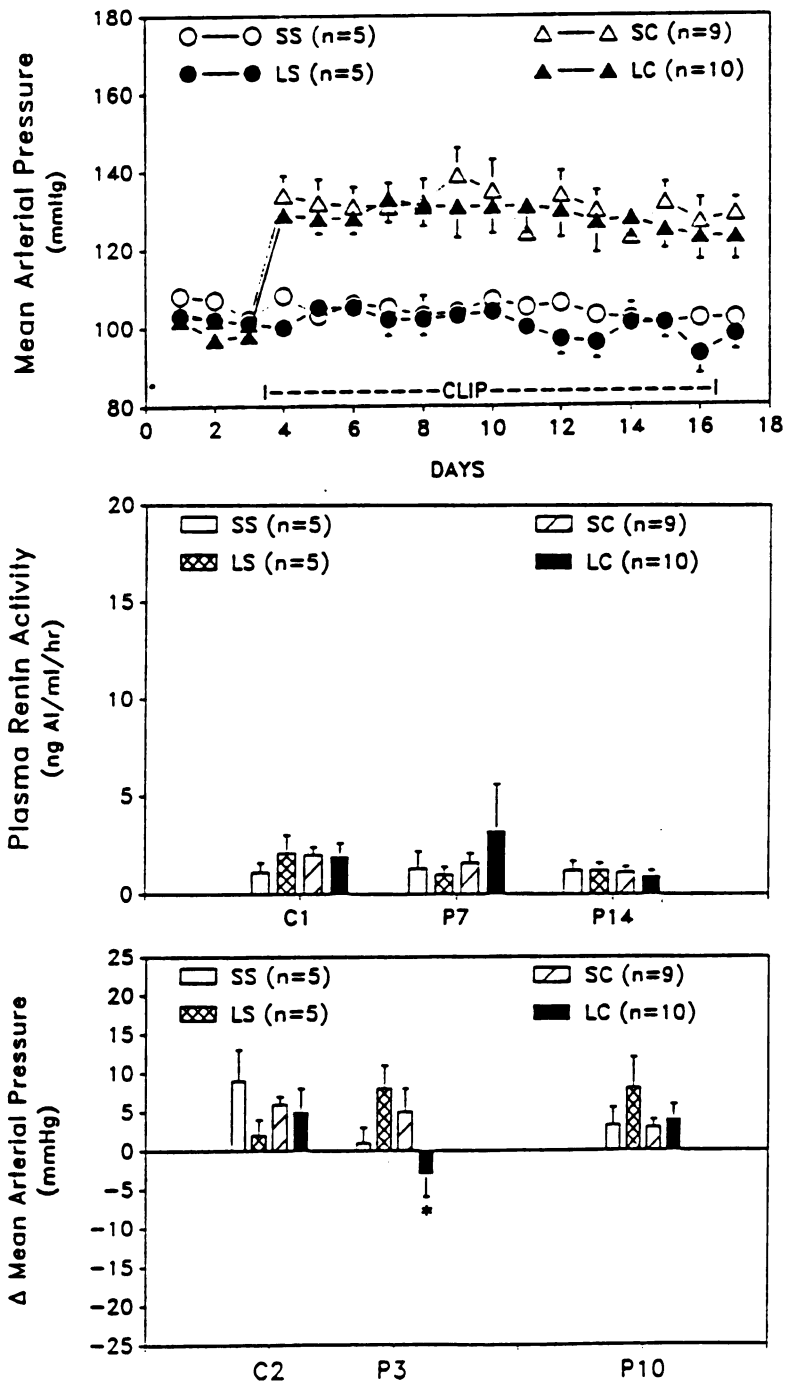
Mean Arterial Pressure (mmHg)
SS (n=5)
SC (n=9)
LS (n=5)
LC (n=10)
CLIP
DAYS
Plasma Renin Activity (ng AI/ml/hr)
C1
P7
P14
Δ Mean Arterial Pressure (mmHg)
C2
P3
P10

Figure 19

Unsurprisingly PRA was not altered by sham-clip or clip surgery in the uninephrectomized rats. Further there were no differences between groups throughout the experiment.

In response to saralasin infusion only the LC group exhibited a significantly different response after clip-surgery. In contrast to a small pressor response during the control period, a slight depressor response was observed three days after clip surgery in the AP lesioned rat (LC).

VI. Lateral Parabrachial Nucleus and Angiotensin Hypertension

Ten day ANG II infusion (10 ng/min) resulted in chronic hypertension in the sham-lesioned rats maintained on a 6 mEq sodium intake. These results are shown in Figure 20. In contrast to the SHAM rats, MAP was significantly elevated on only the first three days of ANG II infusion in the LPBN lesioned rats. Although bradycardia was observed during the initial days of ANG II infusion in the SHAM rats, HR tended to increase during the final days of ANG II treatment and recovery in both groups. There were no statistically significant differences between groups or within groups throughout the experiment with regard to WI or UO (data not shown). However, SHAM rats did retain sodium as evidenced by decreased daily UNaV during both the ANG II infusion period and recovery compared to the control period (data not shown). There were no differences in UNaV between groups.

Figure 21 depicts the area common to all lesions, the parabrachial region believed to be critically involved in the rise in blood pressure induced by systemic infusion of ANG II.

Changes in MAP in response to hexamethonium (20 mg/kg iv) during control, recovery, and ANG II infusion periods in sham-opertated and LPBN-lesioned rats are illustrated in Table 2. In the SHAM group the depressor response to ganglion

Figure 20. Lateral parabrachial nucleus and angiotensin hypertension. Daily MAP and HR obtained during sixteen day protocol [3-control, 10-ANG II infusion (10 ng/min) and 3-recovery] are depicted here. Rats were sham-lesioned (SHAM) or LPBN-lesioned (LPBN) several weeks prior to catheterization surgery. An * indicates data significantly different from control period data. An + indicates data points for which statistically significant differences exist between SHAM and LPBN rats.

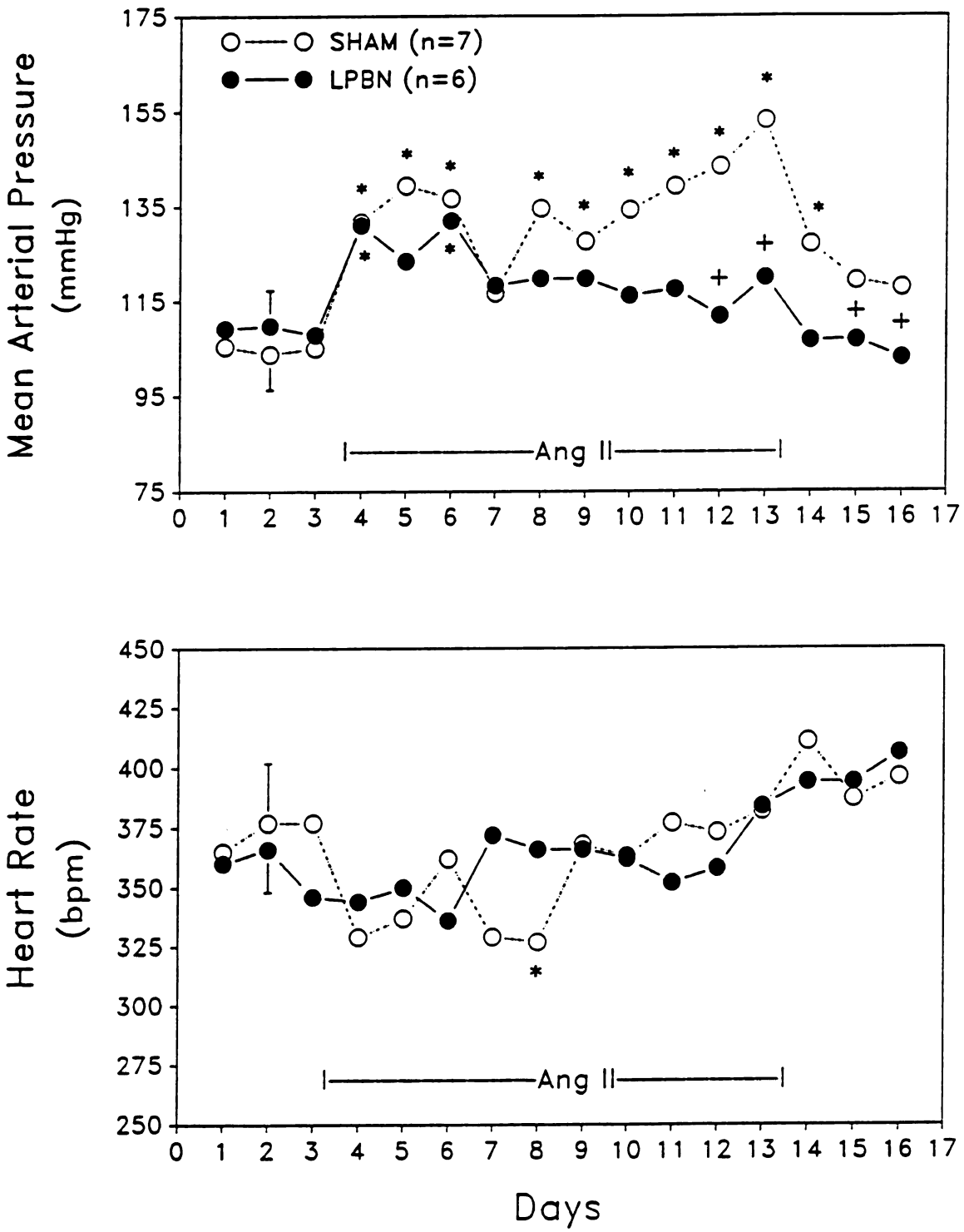
Mean Arterial Pressure (mmHg)
SHAM (n=7)
LPBN (n=6)
Ang II
Heart Rate (bpm)
Ang II
Days

Figure 20

Table 2: Change in mean arterial pressure with hexamethonium (20 mg/kg iv) in sham-lesioned (SHAM) and LPBN-lesioned (LPBN) rats receiving chronic ANG II infusion. Responses were obtained on the first day during control (C1), on days 4 and 8 during ANG II infusion (A4 and A8), and on day 2 during recovery (R2). Data reported as mean change in MAP (mmHg) and standard error of the mean at each time point. An * indicates data significantly different from data obtained during the control period.

Table 2

CHANGE IN MEAN ARTERIAL PRESSURE WITH GANGLION BLOCKADE
(mmHg)

	C1	A4	A8	R2
SHAM	-12.86	-31.28	-47.71*	-31.86
n=7	± 4.78	± 7.94	±18.03	± 6.03
LPBN	-12.50	-33.17	-21.83	-25.50
n=6	± 5.36	±10.14	± 5.63	± 7.19

Figure 21. Reconstruction of the area common to all lesions redrawn from Paxinos and Watson (1986). Sections are taken from -9.2 (A), -9.3 (B), -9.7 (C), and -9.8 (D) from bregma. bc, brachium conjunctivum; d, dorsal lateral parabrachial nucleus; e, external lateral parabrachial nucleus; kf, Kolliker-Fuse nucleus; Mo5, motor nucleus of the trigeminal nerve; v, ventral lateral parabrachial nucleus.

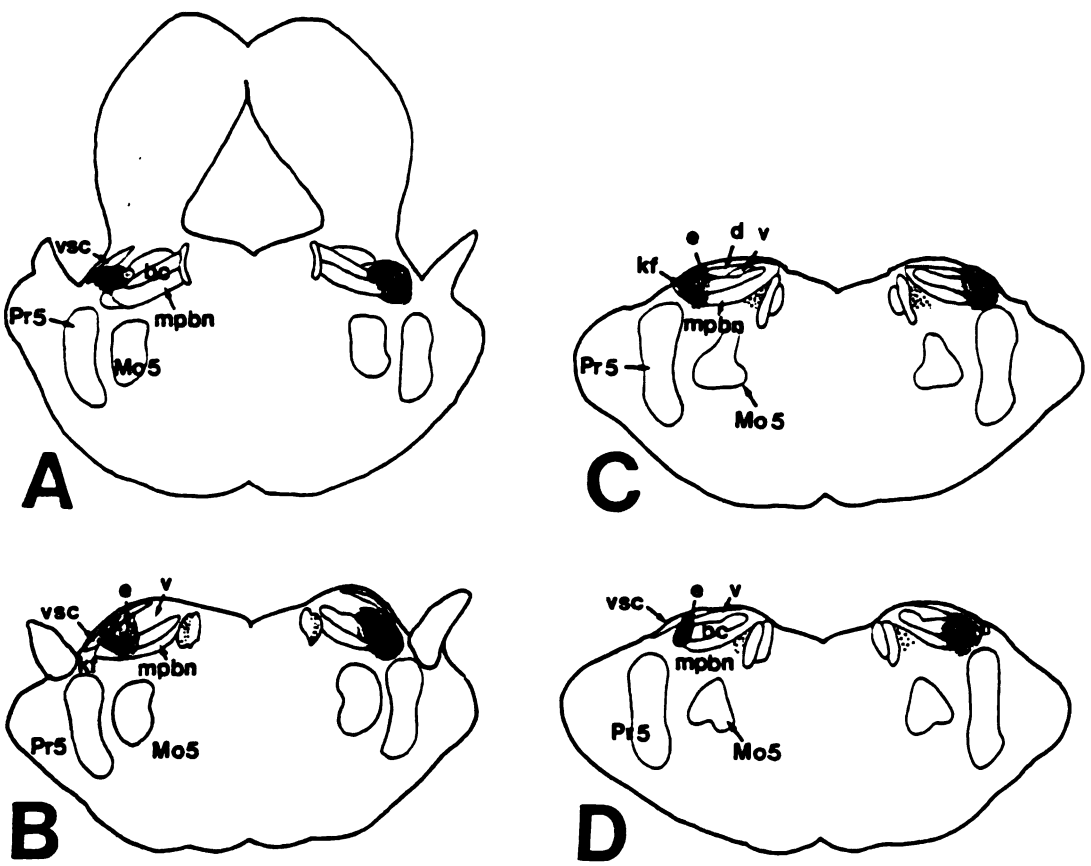
vsc
bc
mpbn
Pr5
Mo5
A
d
v
kf
mpbn
Pr5
Mo5
C
v
vsc
kf
mpbn
Pr5
Mo5
B
v
vsc
bc
mpbn
Pr5
Mo5
D

Figure 21

blockade was significantly greater by day 8 of ANG II infusion than the responses obtained during the control period. The depressor response to hexamethonium in the LPBN group was unchanged throughout the 16 day experiment.

The change in MAP in response to a bolus iv injection of V_1-AVP receptor antagonist was negligible in both the SHAM and LPBN group during the control (1.6 $\pm$ 3.1 vs. -1.2 $\pm$ 2.0 mmHg, respectively) and the recovery (-3.0 $\pm$ 2.1 vs. 3.2 $\pm$ 1.6, respectively) periods. Further, the response to the AVP antagonist, determined on days 5 and 9 during chronic infusion of ANG II, was no different from those elicited during the control period in either group (SHAM-- -2 $\pm$ 1.8, -0.3 $\pm$ 1.4; LPBN-- 0.2 $\pm$ 4.5, 1.0 $\pm$ 2.6).

VII. Plasma Angiotensin II Concentrations

A. Acute iv ANG II infusion

The plasma Ang II concentrations (pANG) resulting from 1 hour hormone infusions are illustrated in Table 3. One hour intravenous infusion of saline alone did not significantly alter pANG. However, one hour infusion of all three doses of ANG II resulted in dose-dependent increases in pANG. There were no differences in body weight between groups of rats. No rat receiving any dose of ANG II in this experiment was observed drinking during the infusion period.

B. Chronic iv ANG II infusion

Figure 22 illustrates MAP, HR, and pANG measured during the 10 day protocol. MAP and pANG were the same during control and recovery. During ANG II infusion at 10 ng/min MAP and pANG were significantly elevated from control levels after one hour and remained elevated for the next four days. While pressure continued to increase, pANG actually tended to decrease with continued infusion at the same rate. MAP after 4 days of ANG II infusion was significantly

Table 3. Plasma ANG II concentrations during acute iv ANG II infusion. pANG (mean ± S.E.M. pg/ml) before (C) and after 1 hr intravenous infusion of ang II at rates of 0, 10, 30, and 60 ng/min. Rat body weight and number of animals per group are listed below respective infusion rates.

Table 3

Ang II Infusion Rate (ng/min)

	0		10		30		60	
	C	1hr	C	1hr	C	1hr	C	1hr
[Ang II]p (pg/ml)	93.0 ±21.0	49.7 ±7.8	52.1 ±7.4	179.9 ±40.3	71.3 ±29.2	663.8 ±265.5	69.5 ±26.8	1538.3 ±492.6
Weight (g)	398±19		410±14		365±11		404±36	
n=	14		11		5		6	

greater than after one hour of ANG II infusion, while pANG was not different. When the infusion rate was increased three-fold for three days, MAP was not statistically different from MAP measured after four days of infusion of the lower dose of ANG II. However, pANG was doubled and was significantly elevated from pANG at all time points during ANG II infusion at the rate of 10 ng/min.

Heart rate did not change from control with ANG II infusion at either dose, but did significantly increase upon recovery from ANG II infusion.

Figure 22. Plasma ANG II concentrations, arterial pressure and heart rate before, during, and after acute and chronic iv ANG II infusion. MAP (line graph), pANG (bar graph) and HR measured during control (C), after sixty minutes (60'), one day (A1) and four days (A4) of ANG II infusion at 10 ng/min, and three days (A3) at 30 ng/min and after two days of recovery (R2). * indicates data significantly different from CON data point. a indicates data significantly different from 60' data point. b indicates data significantly different from A1 data point. c indicates data significantly different from A4 data point.

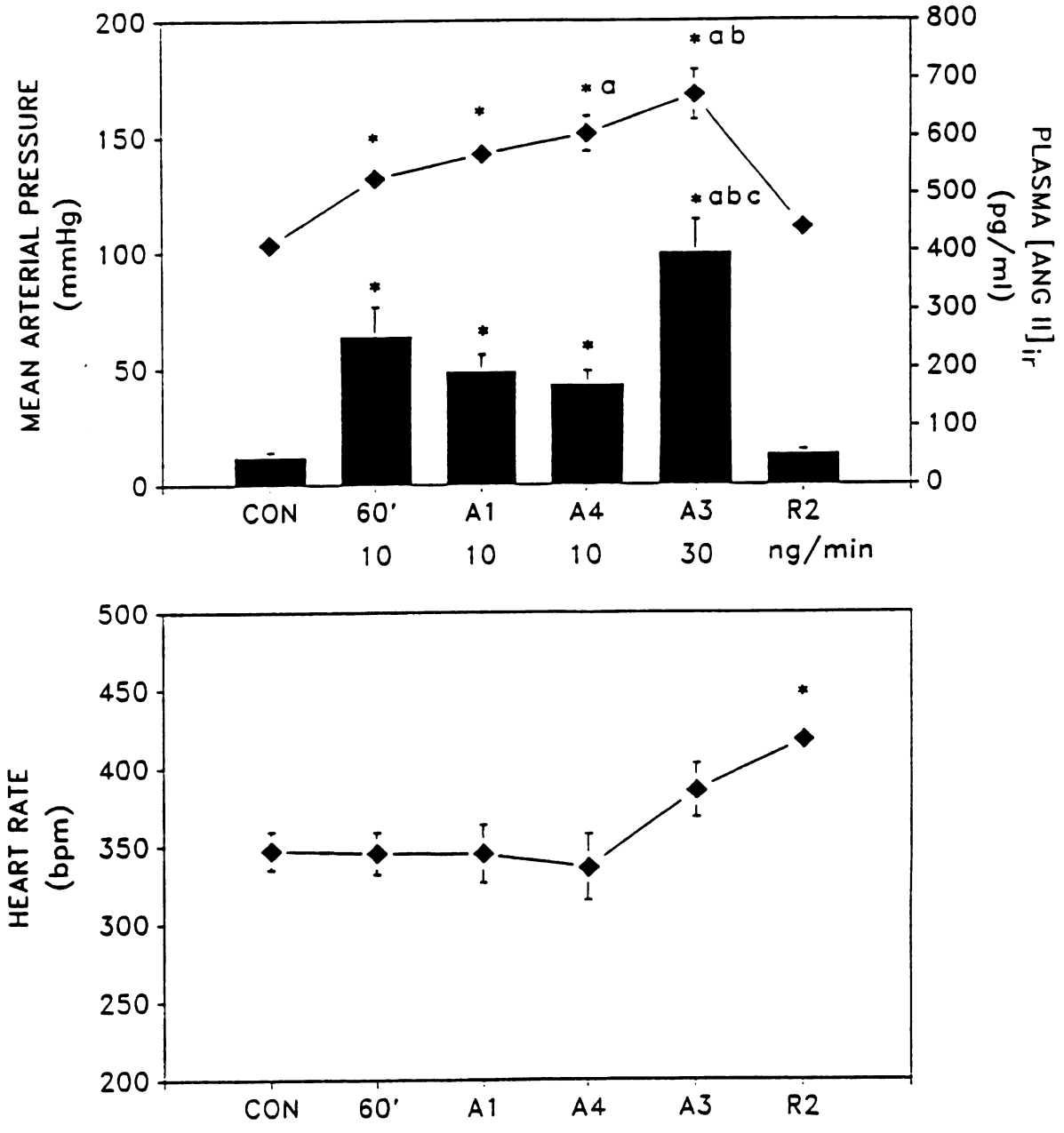
MEAN ARTERIAL PRESSURE (mmHg)
PLASMA [ANG II]ir (pg/ml)
200
150
100
50
0
800
700
600
500
400
300
200
100
0
*ab
*a
*abc
CON
60'
A1
A4
A3
R2
10
10
10
30
ng/min
HEART RATE (bpm)
500
450
400
350
300
250
200
CON
60'
A1
A4
A3
R2

Figure 22

DISCUSSION

Direct vasoconstriction produced by ANG II is likely the most important pressor mechanism during acute intravenous infusion of the hormone (fast pressor effect). This effect is partially responsible for the long term hypertensive actions of ANG II as well, but other mechanisms clearly contribute to maintain elevated arterial pressure. Many other mechanisms have been proposed to explain the slow pressor effect of ANG II, as previously discussed. Chronic sodium retention accompanied by water retention and volume expansion is one mechanism by which ANG II has been proposed to chronically raise arterial pressure (Krieger *et al.*, 1990). However, in the current studies, as well as others (Brown *et al.*, 1981; Fink *et al.*, 1987; Kanagy *et al.*,1990) a sodium or fluid retentive state was not observed in connection with chronic increases in circulating ANG II in rats. In these studies water balance and sodium balance were assessed only over a twenty-four hour period and an earlier effect may have been missed. Clearly, however, the overall daily volume status was unchanged in the rats receiving ANG II. Angiotensin-induced hypertension is a sodium-dependent model of hypertension: the level of blood pressure is directly related to the sodium intake of the animal (Cowley *et al.*, 1976; DeClue *et al.*, 1978). Thus, sodium must be playing a role in the hypertension, but this role cannot be explained simply by ANG II-induced sodium retention leading to chronic volume expansion.

Circulating ANG II is a powerful secretagogue of aldosterone, which when chronically administered, is itself a pressor hormone. It has been proposed that increase levels of circulating aldosterone could contribute to the chronic hypertensive actions of ANG II (Cowley *et al.*, 1976; Kanagy *et al.*, 1990). However, this effect of circulating ANG II is not maintained since ANG II

hypertension is not accompanied by chronic increases in circulating aldosterone (Cowley *et al.*, 1976; Kanagy *et al.*, 1990). Therefore, increased blood levels of aldosterone do not contribute to the chronic pressor actions of circulating ANG II.

It has previously been shown that the chronic, but not acute, pressor actions of ANG II in the rat are dependent on the integrity of the AP (AP; Fink *et al.*, 1987). This observation supports the hypothesis that interactions of circulating ANG II with the central nervous system act to increase arterial pressure. It was hypothesized that the interaction of circulating ANG II and the central nervous system results in an increase in at least one of the following:

1.) Water intake

2.) Circulating AVP

3.) Neurogenic vasoconstrictor tone

Primary objectives of the present work included the investigation of the involvement of these three mechanisms in ANG II hypertension. Further, studies were proposed to assess the importance of receptor subtypes and neuronal projections from the area postrema in ANG II hypertension. Finally the importance of the AP in the development of renin-dependent and independent renovascular hypertension was examined.

I. Circulating Angiotensin II and Drinking Behavior in the rat

Intracerebral ventricular injection of ANG II produces a profound drinking response that is well documented (reviewed in Fitsimons, 1976). This central action of ANG II is one mechanism by which ANG II hypertension might be explained. An increase in water intake could result in volume expansion and thereby contribute to elevated arterial pressure during ANG II infusion. However, the contribution to hypertension of this effect of circulating ANG II remains

controversial (Trippodo *et al*, 1976; Brown *et al.*, 1981; Carroll *et al.*, 1984; Bruner *et al.*, 1985; Fink *et al.*, 1986). Therefore, it was the purpose of the current studies to examine the ability of physiological increases in circulating ANG II to elicit a drinking response alone, or to enhance the drinking responses produced by other manipulations that reportedly result in a state of thirst.

Previous studies have shown that chronic infusion of Ang II produces a sustained increase in arterial pressure without altering daily WI in rats (Brown *et al.*, 1981; Bruner *et al.*, 1985; Fink *et al.*, 1986). Similarly, Carroll *et. al.* (1984) reported that Ang II-induced hypertension in the dog was not accompanied by an increase in daily WI. Conversely, Trippodo *et. al.* (1976) reported that WI is significantly increased during chronic Ang II infusion in the dog. While there may exist species differences in the drinking responses to chronic Ang II infusion, this difference also may be accounted for by differences in doses of Ang II employed. For example, the doses of Ang II necessary to produce chronic hypertension in the rat are relatively low compared to those doses reported to acutely stimulate drinking in that species (Mann *et al.*, 1987, review). Therefore, the drinking response (daily WI) to chronic iv infusion of ANG II at dose similar to those reported to produce acute drinking responses was evaluated.

The lowest dose of Ang II employed in this experiment (10 ng/min; approximately 30 ng/kg/min) was described by Mann *et. al.* (1980) as the rate necessary to raise circulating levels of Ang II to approximately 300 pg/ml; this is just above the putative dipsogenic threshold in the rat. The next highest rate of infusion (30 ng/min) has been reported to produce circulating levels of Ang II approximately 650 pg/ml, much greater than those seen after 48 hours of water deprivation (approximately 400 pg/ml). The highest infusion rate used (60 ng/min) was described by Mann as greater than that required to elevate Ang II

levels to those seen in malignant renal hypertension. The infusion experiments performed in this lab resulted in very similar plasma ANG II concentrations to those reported by Mann *et. al.* (1980) using similar infusion rates. These rates of infusion were chosen for our experiments to chronically produce both physiological as well as supraphysiological increases in circulating levels of ANG II.

Nine day intravenous infusion of Ang II (3 day infusions of the three stepped doses), however, did not produce a chronic state of thirst, as measured by daily WI, in rats maintained on a normal sodium diet. The results from this first experiment clearly support data previously observed in this laboratory (Bruner *et al.*, 1985; Fink *et al.*, 1986; Fink *et al.*, 1987) and that obtained by others (Brown *et al.*, 1981). Specifically, chronic infusion of Ang II was characterized by a sustained increase in MAP without an accompanying change in daily water intake. We conclude from this experiment that chronic increases in circulating Ang II to both physiological as well as supraphysiological levels, while producing sustained increases in MAP, are alone incapable of altering daily water intake in the rat.

As stated previously, doses similar to those employed in these chronic infusions have been reported to produce acute drinking responses in the rat (reviewed by Mann *et al.*, 1987). While Hsiao *et. al.* (1977) found the lowest rate of infusion used in this study (approximately 30 ng/kg/min) to be dipsogenic when acutely infused in the rat for one hour, Robinson and Evered (1977) and Phillips, *et. al.* (1985) have shown higher rates of infusion insufficient to elicit an acute drinking response in either rats (Robinson and Evered, 1977) or humans (Phillips *et al.*, 1985). To assess the acute drinking response to Ang II over a range of doses, WI was recorded during the first two hours of infusion of each dose (10, 30, and 60 ng/min) of Ang II. The results of this study indicate that, under these experimental conditions, rats do not drink more during acute increases in

circulating Ang II than during a two hour dextrose infusion. The remaining experiments were performed in order to determine under which conditions, if any, rats will drink to acute increases in circulating Ang II.

One important difference between the study by Hsiao, *et. al.* (1977) and studies by Robinson and Evered (1977), is that the former completed all experiments within three days after catheter implantation surgery, whereas the latter study allowed at least one week for surgical recovery prior to experimental manipulation. Physiological changes accompanying surgical trauma (e.g. blood loss, volume depletion, steroid secretion) may enhance the ability of high doses of Ang II to elicit drinking in rats. Therefore, the current studies were all performed in rats after at least three days (but generally much longer) post-surgical recovery.

An important aspect of drinking studies is the time frame of the experiment. A thirty minute infusion would appear to be an appropriate time period to evaluate the dipsogenic effect of Ang II. First, plasma levels of Ang II have reached a plateau. Mann *et. al.* (1980) showed that plasma levels of Ang II were no different after fifteen minutes than after one hour of intravenous infusion of Ang II at various rates. Further, since Ang II has multiple actions which influence body water and electrolyte composition it is possible that a drinking response seen after thirty minutes of infusion is due to an indirect effect of Ang II. Drinking following dehydration also occurs predominantly within the first thirty minutes after access to water is restored (DiNicolantonio and Mendelsohn, 1981). Therefore, all acute infusion experiments were performed for either thirty or sixty minutes with water intake recordings made every ten minutes.

Hsiao *et. al.* (1977) suggested that rats may be hesitant to drink with the investigator present in the laboratory. To test the hypothesis that chronically instrumented rats may not drink even when "thirsty" in the presence of the

investigator rats were initially water deprived for 24 hours prior to a one hour Ang II infusion. The results illustrate that while rats drank reliably after water deprivation even with the investigator present, infusion of Ang II at various rates concurrent with rehydration did not enhance the drinking response compared to dehydrated rats receiving dextrose infusion. That is, even when rats were "primed" to drink by dehydration, Ang II failed to produce greater thirst as determined by the failure of Ang II-treated rats to drink more water than vehicle-treated rats during rehydration.

It is possible that the endogenous levels of Ang II produced by 24 hour dehydration were sufficient to maximally influence the drinking response. Therefore, additional Ang II would be ineffective in enhancing the drinking response to dehydration. Although plasma Ang II levels are increased during dehydration, it remains controversial whether or not endogenous production of Ang II is necessary for the drinking response to dehydration or even influences the drinking response to this stimulus (Severs *et al.*, 1977; Hoffman *et al.*, 1978; Barney *et al.*, 1980; Lee *et al.*, 1981; Yamaguchi, 1981; DiNicolantonio, 1984). The next experiment therefore was performed to test the same hypothesis using hypertonic saline infusion, a model of cellular dehydration not accompanied by an endogenous increase in circulating Ang II. It was hypothesized that rats receiving hypertonic saline would drink more while also receiving Ang II than rats receiving hypertonic saline alone. It also has been suggested that Ang II lowers the osmotic threshold to drink (Andersson, 1978). Therefore, a second hypothesis tested in this experiment was that Ang II-treated rats would drink during hypertonic saline infusion at lower plasma osmolalities than rats treated with only hypertonic saline.

Previously it has been shown that a small change in plasma osmolality is sufficient to produce thirst in humans receiving hypertonic saline infusions

(Phillips *et al.*, 1985a). Further, this thirst is due to an increase in osmolality rather than specifically an increase in plasma sodium concentration (Wood *et al.*, 1977; Erikkson *et al.*, 1981; Zerbe and Robertson, 1983). In the current experiment hypertonic saline infusion provided a reliable stimulus to drink in chronically instrumented rats, similar to 24 hour water deprivation. At the onset of drinking, plasma osmolalities were routinely higher than in preinfusion samples and only a small but significant increase (approximately 2-5 mOsm) was sufficient to elicit a drinking response.

In the current experiment Ang II was added to hypertonic saline in order to assess the ability of Ang II to further stimulate drinking in a rat primed to drink by cellular dehydration. The rate of infusion of Ang II chosen has been described as that required to raise circulating levels of Ang II above the putative dipsogenic threshold in the rat (Hsiao *et al.*, 1977; Mann *et al.*, 1980) which is well above levels seen after 24 hours of water deprivation in that species (Mann *et al.*, 1980). Addition of Ang II to the infusate, however, did not alter the drinking response (volume or latency) to acute cellular dehydration. Further, Ang II did not alter the level of plasma osmolality required to elicit drinking. From this study, therefore, we conclude that even when rats are "primed" to drink with hypertonic saline infusion, Ang II does not prompt the rat to drink a greater quantity of water, nor does the hormone lower the osmotic threshold to initiate drinking in response to cellular dehydration.

It has been proposed that the pressor action of Ang II may inhibit a dipsogenic action of the hormone (Robinson and Evered, 1987). It is possible that Ang II is ineffective in producing increases in WI, both acutely and chronically, simply because arterial pressure is elevated. Robinson and Evered (1987) have shown that when pressure was initially increased with Ang II infusion and then returned

to control levels with various depressor agents, rats drank more than rats whose pressures remained elevated during Ang II infusion. They concluded that normalizing pressure uncovered the dipsogenic effects inhibited by the pressor response produced by Ang II. A control experiment not performed, however, was assessment of the drinking response to normalizing pressure initially elevated with an alternate pressor agent such as phenylephrine. Further, they do not report WI at various time points during infusion, only after the entire time period of ninety minutes. As previously mentioned, the time course is important since Ang II may induce thirst by indirect mechanisms and it is the direct stimulation of drinking that is the phenomenon of interest. The lowest rate of infusion in their study was 16.7 ng/min (approximately 85 ng/kg/min in 200 g rats employed). This rate, on a weight basis, would produce very high physiological levels of circulating Ang II [approximately 650 pg/ml (Mann *et al.*, 1980)]. In the current study, two experiments were performed to test the hypothesis that elevated arterial pressure inhibits both chronic and acute Ang II stimulated drinking.

Ang II-induced hypertension is sensitive to sodium intake, therefore, rats were maintained on a sodium deficient diet and given three day infusions of Ang II at three stepped doses. Chronic infusion of Ang II in doses chosen to produce both physiological and supraphysiological increases in circulating Ang II failed to stimulate a chronic increase in WI in rats maintained on the sodium deficient diet. This was despite the fact that arterial pressure remained relatively normal throughout Ang II infusion. Therefore, we conclude that even when arterial pressure is not chronically elevated, Ang II does not stimulate chronic increases in WI in the rat.

A second experiment was performed to test the hypothesis that acute increases in MAP inhibit the acute dipsogenic effect of Ang II. A thirty minute period of

hypotension produced by nitroprusside infusion provided a reliable stimulus to drink. Further, hypotension-induced drinking was not dependent on reflex-mediated generation of Ang II since pretreatment with enalapril failed to alter the drinking response to nitroprusside. We chose a rate of Ang II infusion that would produce circulating Ang II in the same range as those observed during physiological states associated with hypotension and drinking, e.g. hemorrhage (Russel *et al.*, 1974). However, intravenous infusion of Ang II during hypotension failed to further stimulate drinking in the rat.

The results from this experiment indicate that endogenous production of Ang II is not necessary for the drinking response to a short-term hypotensive stimulus. Further, it does not appear that a drinking response to short-term (30 minute) physiological increases in circulating levels of Ang II is masked by a pressor action of the hormone.

Based on these results we conclude that circulating Ang II at physiologically relevant levels does not alone stimulate drinking in the rat. Further, Ang II does not enhance the drinking response of rats to water deprivation, cellular dehydration or hypotension. Finally, chronic increases in circulating Ang II alone do not alter the daily water intake of the rat. Therefore, increases in water intake do not contribute to ANG II hypertension.

II. Circulating Vasopressin and Arterial Pressure

A second effect of acute icv administration of ANG II is the release of AVP (Bealer *et al.*, 1979; Breuhaus and Chimoskey, 1990). Circulating AVP has been shown to contribute to the increase in arterial pressure associated with acute central injections of ANG II (Haack and Mohring, 1978; Hoffman *et al.*, 1979). It would not be surprising if increases in circulating AVP alone were capable of

producing hypertension, or were a contributing factor in ANG II hypertension, due to both its actions as an antidiuretic and vasoconstrictor hormone. Indeed, reports that AVP antagonists lower arterial pressure in other models of experimental hypertension (DOCA-salt-- Mohring *et al.*, 1977; 2-K, 1-C-- Mohring *et al.*, 1978) suggest that circulating AVP contributes significantly to maintenance of elevated MAP in certain models of hypertension. Contradictory results were obtained by others however, in response to acute AVP receptor antagonism (DOCA-salt-- Rabito *et al.*, 1981; Filep *et al.*, 1985; 2-K, 1-C-- Filep *et al.*, 1987; SHR-- Filep and Fejes-Toth, 1986) as well as chronic AVP receptor blockade in the SHR (Sladek *et al.*, 1987). Thus controversy remains over the role of AVP as a hypertensive agent. To address this issue in ANG II hypertension two approaches were taken in the current experiments. First, the contribution of circulating AVP to ANG II hypertension was evaluated by the resulting depressor responses elicited by acute iv injection of a vascular V_1-AVP receptor antagonist during chronic ANG II infusion. Second, a series of experiments were designed to evaluate the ability of chronic physiological increases in circulating AVP to produce hypertension.

Previous reports illustrate that, unlike acute infusion (Bonjour *et al.*, 1970; Knepel *et al.*, 1982; Ramsay, 1978), chronic infusion of ANG II does not result in increases in circulating AVP (Cowley *et al.*, 1981). These results do not preclude entirely a role for AVP in ANG II hypertension. However, during ten day ANG II infusion iv injection of V_1-AVP receptor antagonist did not result in a significant depressor response. Taken together, these results strongly suggest that circulating AVP does not contribute to elevations to arterial pressure produced by chronic ANG II infusion.

The next series of experiments were designed to determine directly the ability of AVP to produce chronic hypertension, and thereby predict a role for the

circulating hormone in other forms of hypertension. It has been demonstrated that hypertension in the dog is maintained during AVP infusion for only a few days before renal escape from the antidiuretic actions occur (Cowley *et al.*, 1984; Hall *et al.*, 1986). These results were obtained from dogs maintained on a fixed fluid intake. Volume expansion and elevated MAP were observed until the antidiuretic action of the hormone was overcome. In contrast, chronic iv infusion of AVP did not produce hypertension in the dog in which fluid balance was maintained constant by servo-control of body weight throughout the experiment (Cowley *et al.*, 1984). Conversely, systemic pressure rose drastically and antidiuresis was maintained throughout AVP infusion when renal perfusion pressure was servo-controlled and prevented from increasing (Hall *et al.*, 1986). Many of these dogs however experienced large volume expansion and pulmonary edema. The hypothesis that chronic increases in circulating AVP result in hypertension in the freely drinking animal had not been tested until the present experiment. Confirmation of this hypothesis would lend support for a major role of AVP in hypertension.

The choice of infusion rates employed in the current experiments (0.2 and 2.0 ng/kg/min) were based on evidence that these rates produce plasma AVP levels 1.4 and 4.2 times the normal euvolemic levels in conscious rats (Osborn *et al.*, 1987). Plasma AVP concentrations during benign experimental and clinical hypertension are reportedly 1.5-4.0 times those seen in normotensive controls (Crofton *et al.*, 1978; Mohring *et al.*, 1979; Crofton *et al.*, 1980; Matsuguchi *et al.*, 1981; Mohring *et al.*, 1981; Cowley *et al.*, 1985; Os *et al.*, 1986; Ribeiro *et al.*, 1986). Further, preliminary studies suggested that no further decrease in daily UO occurred with infusion rates greater than the highest reported dose (2.0 ng/kg/min). However, despite maximal antidiuresis maintained throughout AVP

infusion, a chronic hypertensive effect was not observed.

Several compensatory mechanisms can be implicated to exlain the inability of AVP alone to produce hypertension. First, AVP fails to produce volume expansion despite chronic reductions in UO. This is due to accompanying decreases in water intake in either the freely drinking rat or the servo-controlled dog (Cowley *et al.*, 1984). Therefore, as long as the animal is allowed to regulate its own water intake, or fluid balance is artificially maintained, volume dependent hypertension is not induced with AVP infusion.

Increases in total peripheral resistance produced by direct vascular effects of AVP (V_1-receptor stimulation) may not result in elevated MAP due to a compensatory decrease in cardiac output. Indeed, acute infusion of AVP results in increases in total peripheral resistance accompanied by decreases in cardiac output (Montani *et al.*, 1980; Osborn *et al.*, 1987). With prolonged iv infusion of AVP however, cardiac output returns to pre-infusion levels (Liard, 1987; Tipayamontri, 1987). Therefore, a compensatory decrease in cardiac output is not likely the mechanism by which a chronic hypertensive effect of AVP is prevented.

Acute infusion of AVP (2.0 ng/kg/min) produces approximately a 10-20 mmHg increase in arterial pressure. Tachyphylaxis to the direct vasoconstrictor actions of AVP might explain normotension experienced during prolonged AVP infusion at the same rate. Failure of blockade of selective V_1-AVP receptors to produce a significant depressor response during chronic AVP infusion in the current experiments supports this hypothesis of diminished vascular responsiveness to AVP. Similar experiments performed in dogs after 2 day AVP infusions indicate that acute injection of a V_1-receptor antagonist also resulted in no change in MAP (Liard, 1987). However, while MAP was unchanged, total peripheral resistance decreased and cardiac output increased in response to AVP antagonist treatment.

These results do not support the hypothesis that tachyphylaxis to the vasoconstrictor actions of AVP prevent chronic AVP -induced hypertension.

The acute pressor effect of AVP is compensated for by the arterial baroreflex. In fact, as previously discussed, acute increases in circulating AVP enhances arterial and cardiopulmonary baroreflex sympathoinhibition in the dog (Cowley *et al.*, 1974), rabbit (Undesser *et al.*, 1985; Gupta *et al.*, 1987; Hasser *et al.*, 1987), man (Aylward *et al.*, 1986; Ebert *et al.*, 1986), baboon (Baraznji and Cornish, 1989), and rat (Peuler *et al.*, 1990). However, there is some evidence that AVP facilitation of arterial baroreflexes may not be operative in the rat (Webb *et al.*, 1986; Osborn *et al.*, 1987). It is currently believed that the site of interaction between AVP and the baroreflexes is in the brainstem. Specifically the integrity of the area postrema appears to be critical for AVP facilitation of reflex inhibition of heart rate and sympathetic nerve activity in the dog (Michelini *et al.*, 1986; Applegate *et al.*, 1987) and rabbits (Undesser *et al.*, Bishop *et al.*, 1987). Clearly, interruption of baroreflexes by either deafferentation, neuroablation or ganglion blockade results in much greater pressor responses to acute injections of AVP (Cowley *et al.*, 1974; Undesser *et al.*, 1985, Osborn *et al.*, 1987). In addressing the possible long term hypertensive effects of AVP it is possible that such manipulations could uncover AVP-induced hypertension.

We initially hypothesized that if chronic baroreflex-mediated sympathoinhibition was occurring during AVP infusion, SAD rats would become hypertensive during such infusions. However, interruption of the arterial baroreflex by deafferentation failed to render rats susceptible to chronic AVP-induced hypertension. In this experiment cardiopulmonary reflex mechanisms remained intact and could have sufficiently compensated for AVP induced changes in arterial pressure. As it is impossible to produce chronic selective

cardiopulmonary deafferentation, an alternative approach was taken to address the possibilty that enhanced cardiopulmonary reflex activity prevents AVP hypertension. Acute facilitation of cardiopulmonary sympathoinhibition apparently requires an intact AP (Applegate *et al.*, 1987). It was hypothesized then that AVP infusion in rats with prior electrolytic ablation of the AP would result in hypertension. However, similar to both intact and SAD rats, APX rats failed to exhibit elevated MAP during ten day AVP infusion.

The change in MAP in response to ganglion blockade often is used as an index of the autonomic contribution to resting arterial pressure. We hypothesized that depressor responses to ganglion blockade during AVP infusion would be diminished in the intact rats. Further, sino-aortic denervation or AP ablation were expected to reverse AVP mediated sympathoinhibition, and greater depressor responses to hexamethonium were expected in SAD and APX rats during AVP treatment. However, it was demonstrated that responses to acute ganglion blockade were unchanged during AVP infusion in the intact rats. Similarly, Trapani *et al.* (1988) reported that baroreflex function was unaltered despite chronic increases in AVP due to water deprivation in the rabbit. Finally, the current experiments demonstrate that SAD and APX rats respond to hexamethonium similarly to intact rats, in that no change in the depressor response occurred during AVP infusion. Thus, these results strongly argue against chronic AVP-mediated sympathoinhibition in the rat.

It is unclear how the acute hypertensive and sympathoinhibitory actions of AVP are compensated for during prolonged elevations of the hormone. It is clear, however, that physiological elevations in circulating AVP alone are incapable of producing hypertension in rats allowed to regulate their fluid intake. Further, removal of sino-aortic baroreflex or the area postrema does not change the

cardiovascular response to chronic infusion of AVP in rats. These results would not support a major role for AVP in hypertension even during states of impaired reflex function such as seen with old age.

III. Autonomic Contribution to Angiotensin Hypertension

In a final attempt to determine the efferent mechanism involved in elevated arterial pressure during ANG II infusion, we hypothesized that the interaction between circulating ANG II and the AP ultimately results in an increase in sympathetically-mediated vascular smooth muscle tone. Therefore, one primary objective of the current experiments was to examine the role of the autonomic nervous system in the slow pressor actions of circulating ANG II. To this end the contribution of neurogenic vasoconstriction to the elevated arterial pressure observed during chronic systemic ANG II administration was determined. Further, the influence of arterial baroreceptor reflex sympathoinhibition on the development and maintenance of ANG II hypertension was examined. In the current experiments a slowly developing increase in neurogenic vasoconstrictor tone, as assessed by greater depressor responses to ganglion blockade, was demonstrated in rats chronically receiving ANG II intravenously. Although the depressor response to ganglionic blockade was interpreted here to indicate inhibition of neurogenic vasoconstriction, it is possible that the fall in pressure after hexamethonium resulted to some degree from a decrease in cardiac output. There is good reason, however, to suggest that a decrease in cardiac output does not significantly contribute to the depressor response to hexamethonium, during ANG II hypertension. Cardiac output in the rat is primarily determined by the rate of cardiac contraction; and after hexamethonium HR is unchanged in both normal rats and in rats receiving chronic ANG II infusion. Nonetheless, direct

measurement of cardiac output would be required to prove that the hexamethonium depressor response was due exclusively to inhibition of neurogenic vasoconstrictor activity. The depressor response to nitroprusside was determined throughout the protocol as a non-autonomic vasodilator control. It was hypothesized that a greater depressor response could be elicited with any depressor agent throughout ANG II infusion simply due to greater resting arterial pressure. We found, however, that while nitroprusside did indeed elicit depressor responses whose magnitude was highly correlated to resting arterial pressure, ganglion blockade produced greater depressor responses only in the later stages of chronic ANG II infusion despite pressure being elevated throughout ANG II treatment. Ganglion blockade, however, has the limitation of not being able to reveal the source of the increased autonomic influence on arterial blood pressure. For instance, it is not possible to distinguish between an increased sympathetic nerve activity and an increased sensitivity of the vascular smooth muscle to similar or even decreased neuronal norepinephrine release; both are among the proposed interactions of circulating ANG II with the sympathetic nervous system (Westfall *et al.*, 1977). Luft *et. al.* (1989) though have demonstrated that chronic (fourteen day) ANG II-induced hypertension in rats is accompanied by an increased electroneurographically recorded splanchnic sympathetic nerve activity. These findings suggest that the increased neurogenic vasoconstrictor tone indicated by the present results is at least partially due to an actual increase in sympathetic nerve activity. While the results reported by Luft *et. al.* (1989) agree with our findings, others have shown a decrease in renal norepinephrine overflow (Carrol *et al.*,1984), indicative of decreased renal sympathetic nerve activity, during ANG II hypertension in dogs. However, in the overflow studies, measurements were made after only six days of ANG II treatment, at a time in the current experiment when a

greater depressor response to hexamethonium also was not observed. The duration of ANG II treatment thus may be a factor in the discrepancy between these findings. It has previously been shown that circulating ANG II has the ability to differentially affect splenic and renal nerve activity (Tobey *et al.*, 1983). While acute administration of ANG II into the cerebral arteries results in an increase in splenic nerve activity, renal nerve activity remains unaltered. Therefore, it is also possible that during ANG II hypertension there is an increase in sympathetic vasoconstrictor tone in some vascular beds while renal sympathetic nerve activity is decreased or unchanged.

Chronic sino-aortic denervation did not alter the magnitude of the slow pressor effect of ANG II compared to that elicited in the intact rat. However, two important observations were made with regard to ANG II and the arterial baroreceptor reflex. The first finding confirms data previously reported (Cowley *et al.*, 1976) that the arterial baroreflex partially inhibits the initial rise in arterial pressure during chronic ANG II infusion. That is, the maximal MAP is obtained by the first day of ANG II treatment in the SAD rat, whereas in the intact rat MAP continues to rise for days. Furthermore, an augmented depressor response to ganglion blockade was elicited at the first time point tested, and at all subsequent time points, during ANG II infusion in the SAD rats. This apparent increase in neurogenic vasoconstriction was not observed in the baroreflex intact rats (CON) until day thirteen of ANG II treatment. These data provide strong evidence then that the full expression of the neurogenic actions of ANG II are observable only in SAD animals, or during periods of infusion sufficiently long to allow substantial resetting of the baroreflex. The second finding was that ANG II hypertension in the SAD rats, unlike the CON rats, was not accompanied by an increase in a contribution of non-autonomic mechanisms to maintaining resting arterial

pressure. As stated previously, the direct vasoconstrictor actions of ANG II is one non-autonomic mechanism believed to contribute to the increase in arterial pressure during ANG II hypertension. In the intact (CON) rat chronic ANG II infusion is characterized by an increase in arterial pressure due to non-autonomic factors, one component of which could be direct vasoconstriction. It is notable in this regard that the magnitude of increase in non-autonomic blood pressure control during chronic ANG II infusion is similar to the magnitude of the acute pressor effects of the hormone (postulated here to be due mainly to direct vasoconstriction). On the other hand, during ANG II infusion in SAD rats the resulting rise in arterial pressure appears to be strictly autonomic in origin. Besides indicating the powerful ability of the baroreflex to mask the sympathoexcitatory actions of circulating ANG II, the results in SAD rats also demonstrate that the non-neural cardiovascular actions of ANG II are altered by SAD. Understanding the nature of this alteration would require further investigation.

Angiotensin infusion in SAD rats suggests that involvement of peripheral mechanisms (non-autonomic) may not be necessary for chronic hypertension development. Therefore, selective stimulation of central ANG II receptors (presumably in the AP) by circulating hormone should result in hypertension. The heptapeptide angiotensin metabolite ANG (1-7) has been hypothesized to selectively stimulate central ANG II receptors. It was previously shown that ANG (1-7) is the predominant metabolite of ANG I in the brainstem of the dog. Further, ANG (1-7) has potent physiological actions in the brain; for example, it is equipotent to ANG II with regard to stimulating release of AVP from the rat hypothalamo-neurohypophyseal system. ANG (1-7) does not stimulate vascular ANG II receptors, however, as acute iv infusion of ANG (1-7) fails to produce a

pressor response (Tonnaer *et al.*, 1983; current results). The experiments reported here then were designed to determine whether a chronic iv infusion of ANG (1-7) would selectively activate putative brain receptors involved in the neurogenic vasoconstriction caused by ANG II, and thereby produce hypertension. However, chronic infusion of ANG (1-7) failed to elicit a sustained elevation in arterial pressure. This finding doses not support the hypothesis that selective stimulation of brain angiotensin receptors is alone able to cause chronic hypertension. This could be explained by a failure of ANG (1-7) to gain access to brain receptors.

Chronic infusion of ANG (1-7) was accompanied, however, by small but significant increases in circulating AVP concentrations. Although these results were not consistent, decreases in daily urine volume were regularly observed. These findings suggest that chronic increases in AVP results from prolonged stimulation of receptors responsive to ANG (1-7). It is likely then that ANG (1-7) does have physiologically relevant actions within the central nervous system. These actions, however, do not appear to influence arterial pressure regulation. Circulating ANG II thus may interact in the brain with receptors distinct from those activated by the ANG (1-7) metabolite.

IV. Lateral Parabrachial Nucleus and Angiotensin Hypertension

It is currently believed that circulating ANG II requires the integrity of the AP to produce hypertension (Fink *et al.*, 1987). The nature of the interaction between the hormone and brain tissue is unknown. However, the current experiments indicate that the ultimate result of this interaction is an increase in neurogenic vasoconstrictor tone. It is possible that ANG II acts directly on neurons in the AP, or requires intact AP cells to gain access to other brain regions, most notably the nucleus of the solitary tract (NTS). One means by which to approach this issue

was to determine the importance of brain nuclei exhibiting connectivity with the AP in ANG II hypertension. The two predominant regions which receive neuronal projections from the AP (as well as send projections to the AP) are the NTS and lateral parabrachial nucleus (LPBN) located in the pons. We hypothesized that the connection between the LPBN and AP was important to the induction of ANG II hypertension.

Electrolytic lesion of the LPBN did not permanently alter resting arterial pressure in the rat. However, prior electrolytic lesion of the LPBN did prevent ANG II-induced hypertension in much the same manner as did AP ablation. That is arterial pressure was increased during the first few days of ANG II infusion (presumably due to direct vasoconstriction), but long-term hypertension was prevented by LPBN lesion. Further, the depressor response to ganglion blockade in the hypertensive sham-lesioned rat was significantly greater after the first week of ANG II infusion, similar to the results from our earlier fourteen day ANG II infusion study. In contrast, the depressor response to hexamethonium was unchanged throughout the protocol in the LPBN-lesioned rat. These results, while not conclusive, suggest that the neuronal connection between the AP and LPBN is critical to the development of ANG II hypertension. Questions still remain however. For instance, the LPBN may be critical for ANG II hypertension independent of AP input. The LPBN does send and receive neuronal projections to and from the NTS, as well as other regions of the brain believed to be involved in blood pressure control (Ward, 1988). Nonetheless, it remains a strong possibility that the neurogenic component of ANG II hypertension is reliant on an interaction between the AP and the LPBN.

V. Plasma Angiotensin II Concentrations and Hypertension

The efficacy of angiotensin receptor antagonists in lowering arterial pressure in SHR acutely (hours) when given icv (Phillips *et al.*, 1979; McDonald *et al.*, 1980) suggests renin or ANG II dependency in a model of hypertension clearly exhibiting normal plasma ANG II concentrations. The inability of acute iv infusion of ANG II receptor antagonists, such as saralasin, to lower MAP in the SHR is consistent with blood levels of ANG II being too low to exert a direct vascular effect in this model (Phillips *et al.*, 1979). On the other hand, chronic treatment with ACE inhibitors has been shown to lower blood pressure in SHR and not normotensive controls (Nazarali *et al.*, 1989; Lo *et al.*, 1990). Because plasma ANG II levels are normal in the SHR, it has been suggested that converting enzyme inhibitors lower blood pressure by mechanisms independent of their ability to prevent endogenous ANG II production, such as increasing circulating bradykinin levels. However, recently developed nonpeptidic ANG II receptor antagonists, which unlike ANG II analogues (i.e. saralasin, sarthran) have no agonist properties (Wong *et al.*, 1979a), acutely and chronically lower arterial pressure when given systemically to SHR (Koepke *et al.*, 1990; Wong *et al.*, 1990). In addition, it has been reported recently that immunization against renin effectively lowered arterial pressure in the SHR (Lo *et al.*, 1990). Essential hypertension in humans has long been effectively managed with ACE inhibitors and more recently the use of renin inhibitors also appears to be efficacious (Boger *et al.*, 1990). The ability of drugs that inhibit the renin-angiotensin system to effectively lower arterial pressure in a clearly renin-independent (as defined by normal PRA and plasma ANG II concentrations) model of hypertension implicates other ANG II mechanisms (hypothesized here to be primarily neurogenic) in the pathogenesis of hypertension. In this regard, it is

interesting that ablation of the AP attenuates both ANG II infusion (Fink *et al.*, 1987) and SHR (Mangiapane *et al.*, 1989) models of hypertension. Taken together, these findings may warrant a change in the definition of renin dependency in several forms of hypertension. Plasma ANG II concentrations or PRA alone may not be indicative of the importance of ANG II in the pathogenesis of hypertension.

One aim of the current experiments was to evaluate the relationship between MAP and plasma ANG II concentrations with both acute (1 hour) and chronic (several days) intravenous infusion of ANG II (clearly an angiotensin dependent model of hypertension). Acute intravenous infusion of ANG II produced significant increases in both MAP and plasma ANG II concentrations. With chronic infusion, however, MAP continued to increase while plasma ANG II levels tended to decrease. Others have reported similar results in both the rat (Brown *et al.*, 1981) and dog (Bean *et al.*, 1979). Further, when the dose of chronic intravenously administered ANG II was tripled, the plasma levels increased significantly without producing an additional significant increase in arterial pressure. The acute pressor responses to these two doses of ANG II, 10 and 30 ng/min i.v., are clearly dose-dependent (Fink *et al.*, 1987), while the chronic pressor response to various doses of ANG II produces a much less steep dose response relationship. Therefore a small sustained increase in circulating ANG II can alone produce chronic increases in arterial pressure of a magnitude substantially greater than the acute pressor responses elicited by equivalent plasma ANG II levels. Failure to consider this important fact may have led many investigators to underestimate the contribution of circulating ANG II to various forms of hypertension, since blood concentrations of ANG II in hypertensive individuals are rarely found to be in the range required to produce significant

acute vasoconstriction. The results from the current studies indicate that the neurogenic mechanisms involved in angiotensin hypertension may require substantially lower levels of circulating hormone than the direct vascular effects. Thus, categorizing hypertensive models as to renin dependency based on PRA or circulating ANG II concentrations alone may be inaccurate.

VI. Area Postrema and Renovascular Hypertension

The two models of renovascular hypertension studied in the current experiments, 2-K,1-C and 1-K,1C, are classically referred to as renin-dependent and renin-independent, respectively. Unilateral renal artery constriction in the rat with both kidneys intact results in a concomitant rise in arterial pressure and PRA. Treatment with a converting enzyme inhibitor prevents hypertension development (Bengis *et al.*, 1978; Freeman *et al.*, 1979; Deforrest *et al.*, 1984). Further, chronic infusion of a nonpeptide ANG II antagonist effectively lowers blood pressure in rats with pre-existing 2-K,1-C hypertension (Wong *et al.*, 1989; Wong *et al.*, 1990). In contrast, renal artery constriction in the uninephrectomized rat results in hypertension without an accompanying increase in PRA. Chronic treatment with an ACE inhibitor fails to lower pressure in rats with pre-existing 1-K,1-C hypertension (Bengis *et al.*, 1978; Freeman *et al.*, 1979). The results of the current experiment support a role for ANG II in 2-K,1-C but not in 1-K,1-C hypertension. Specifically, 2-K,1-C hypertension, similar to ANG II hypertension (Fink *et al.*, 1987), requires an intact AP, whereas 1-K,1-C hypertension does not.

The infusion rate used in the current experiments to produce ANG II hypertension (10 ng/min) resulted in plasma concentrations of ANG II (approximately 150 pg/ml) similar to those reported in chronic (> 4 weeks) 2-K,1-C hypertension (Morton and Wallace, 1983). However, despite chronic increases in arterial pressure in sham-lesioned, clipped rats, PRA was not consistently

elevated. These results are consistent with results reported by Morton and Wallace (1983). Specifically, they report that early (1-4 weeks) 2-K,1-C hypertension is associated with only a 1-2 day period during which PRA is elevated. Following this 2 day period hypertension is accompanied by normal PRA until the fourth week following renal artery constriction. Taken together, these results indicate that hypertension, while dependent on an intact renin-angiotensin system, may not necessarily be associated with measurable elevations in circulating ANG II. Some hypertensive mechanisms of ANG II may be operative at these near-normal plasma ANG II levels. Because 2-K,1-C hypertension is also dependent on an intact AP, as shown in the current studies, these mechanisms are likely neurogenic in nature. This hypothesis is supported by evidence that depressor responses to ganglion blockade are greater in rats 12-40 days after coarctation of the descending aorta, which results in hypertension very similar to 2-K,1-C hypertension, than in normotensive control rats (Bellini *et al.*, 1979). Ganglion blockade in rats during the early phase of hypertension (5 days) elicited depressor responses not different than those elicited in normotensive controls. These data demonstrate then that similar to ANG II hypertension, 2-K,1-C renovascular hypertension is associated with a slowly developing increase in neurogenic vasoconstrictor tone that is dependent on the integrity of the AP.

Clearly ablation of the AP does not significantly lower resting arterial pressure under control conditions. Neither does it prevent 1-K,1-C hypertension development. These results indicate that the prevention of hypertension by AP ablation is selective for ANG II dependent hypertension, and does not simply reflect a nonspecific effect of the lesion.

SUMMARY

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

Hypothesis 1. Chronic infusion of ANG II produces chronic increases in daily water intake, which play an important role in the observed elevations in arterial pressure. It was demonstrated that chronic ANG II-hypertension was not accompanied by increases in daily water intake. In addition, it was shown that chronic infusion of ANG II in the rat maintained on low sodium intake (to prevent hypertension development) did not result in increases in daily water intake. Further, acute increases in circulating ANG II did not enhance osmotic, dehydration, or hypotension-induced water intake. Results also indicated that blockade of endogenous ANG II production did not reduce the volume of water intake stimulated by acute periods of hypotension.

Hypothesis 2.) Actions of ANG II-stimulated increases in circulating AVP contribute to ANG II-hypertension. These studies demonstrated that acute blockade of vascular V_1-AVP receptors did not produce a depressor response in ANG II-hypertensive rats. Chronic increases in circulating AVP alone did not result in elevated arterial pressure. Additionally, these experiments illustrated the inability of chronic AVP infusion to produce hypertension in the baroreceptor impaired, SAD rat or the APX rat.

Hypothesis 3.) A component of the increase in arterial pressure observed during chronic ANG II infusion is due to an increase in sympathetically-mediated vasoconstrictor tone. In these experiments ganglionic blockade resulted in a greater depressor response during chronic ANG II infusion than during the control period. In addition, significantly greater depressor responses to ganglionic blockade (compared to responses elicited during the control period) were exhibited in the

SAD rat much sooner during ANG II infusion than were exhibited in the control rat. A pressure plateau was achieved much sooner during ANG II infusion in the SAD than in the control rat as well.

Hypothesis 4.) The neurogenic component of ANG II hypertension is due to stimulation of ANG II receptor subtypes sensitive to stimulation of ANG II metabolite, ANG (1-7). It was demonstrated here that chronic increases in circulating ANG (1-7) did not result in hypertension similar to that produced by chronic infusion of ANG II.

Hypothesis 5.) The maintenance of chronic ANG II hypertension is dependent on the integrity of the LPBN which receives a major neuronal projection from the AP. Chronic ANG II infusion, in rats that previously had undergone surgery for the electrolytic ablation of the LPBN, failed to result in hypertension.

Hypothesis 6.) The integrity of the AP is critical to development of ANG II-dependent but not ANG II-independent renovascular hypertension. Prior electrolytic ablation of the AP prevented the development of 2-K,1-C but not 1-K,1-C renovascular hypertension.

Hypothesis 7.) Chronic ANG II infusion produces a gradual rise in arterial pressure while plasma concentrations of the hormone reach a plateau much more acutely. Intravenous infusion of ANG II produced both an acute increase in arterial pressure and pANG. However, with continued infusion, arterial pressure increased further while pANG actually decreased.

CONCLUSIONS

The salient features of ANG II-induced hypertension thus far reported from many years of investigation of the model are as follows: 1.) Hypertension development is dependent on sodium intake (Cowley *et al.*, 1976; DeClue *et al.*, 1978). 2.) Hypertension development requires a pre-existing volume expansion, presumably the result of a large daily intake of sodium (Krieger and Cowley, 1990). 3.) Volume expansion, which is accompanied by decreases in circulating ANG II, alone is not sufficient to cause hypertension; i.e. high salt intake alone results in the same degree of volume expansion as does high salt intake plus chronic ANG II infusion, but without the accompanying elevations in arterial pressure. 4.) Hypertension development requires an intact area postrema (Fink *et al.*, 1987). 5.) Hypertension development requires integrity of the lateral parabrachial nucleus (current studies). 6.) ANG II hypertension is not produced by stimulation of receptors responsive to ANG (1-7) stimulation (current studies). 7.) There is a slowly developing increase in neurogenic vasoconstrictor tone during ANG II hypertension (current studies). In addition, there is evidence that the ANG II receptors in the brainstem are localized on sensory afferent terminals (including arterial and cardiopulmonary baroreceptors; Diz *et al.*, 1986; Healy *et al.*, 1986) Further, ANG II is known to impair baroreflex function (Severs *et al.*, 1966; Guo and Abboud, 1984; Stein *et al.*, 1984; Brooks and Reid, 1986; Garner *et al.*, 1987; Matsumura *et al.*, 1989; Michelini and Bonagamba, 1990). Considered together these observations suggest the following integrated hypothesis regarding the mechanism of chronic ANG II-induced hypertension. Circulating ANG II causes direct vasoconstriction only at relatively high plasma concentrations. At

lower plasma concentrations ANG II acts on brainstem circuits including the AP and LPBN, probably by inhibiting cardiopulmonary baroreceptor afferent pathways, to increase the average level of sympathetic nervous system activity. This postulate implies that the degree of neurogenic vasoconstriction caused by ANG II depends importantly on the level of activity in these afferent pathways. The strong dependence of ANG II-induced hypertension on increased sodium intake and volume expansion may be related then to the ability of volume expansion to increase cardiopulmonary afferent activity, thereby providing the necessary neural substrate for the sympathoexcitatory actions of ANG II. High salt intake and volume expansion do not normally cause hypertension because endogenous ANG II levels are suppressed by volume expansion; thus, cardiopulmonary and arterial baroreflex sympathoinhibition are unopposed by ANG II, and volume stimulated increases in cardiac output lead to an appropriate fall in total peripheral resistance.

Renin-dependent renovascular hypertension is accompanied by a slowly developing increase in sympathetically mediated vasoconstrictor tone (Bellini *et al.*, 1979). Further, 2-K,1-C hypertension is dependent on the integrity of the AP (current studies). These findings provide good evidence that the above mentioned hypothesis may apply to more clinically relevant renovascular hypertension as well.

An important implication of this hypothesis is that circulating ANG II at concentrations well below the direct vasoconstrictor threshold exerts powerful cardiovascular effects not revealed by the traditional approach of assessing the acute hemodynamic effects of administering converting enzyme and renin inhibitors, or angiotensin receptor antagonists. Thus in previous studies the role of circulating ANG II in the pathogenesis of hypertension may have been substantially underestimated.

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