EFFECTS OF ANGIOTENSIN II ON PLASMA CONCENTRATIONS OF VASOPRESSIN AND GLUCOCORTICOIDS IN THE CONSCIOUS RABBIT

by

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ABSTRACT

Angiotensin II (AII) can act in rats, dogs, and other animals to increase plasma levels of vasopressin (AVP) and adrenal corticotropic hormone (ACTH) but so far no studies of these effects have been completed using rabbits. Rabbits may serve as excellent subjects for further research in this area. The present experiments were designed to begin development of a conscious rabbit model by determining the responses of plasma AVP and glucocorticoids to increases in plasma AII. Plasma glucocorticoid concentrations were used as an index of ACTH release from the pituitary. Experiments were also conducted to determine whether the responses of AVP and ACTH levels were increased when the pressor effect of AII was negated. Catheters were implanted into the central ear artery and marginal ear vein, and angiotensin II was infused intravenously at rates of 10, 20, or 40 $ng \cdot kg^{-1} \cdot min^{-1}$ for one hour (each dose was given on a different day). Some rabbits receiving 20 or 40 $ng \cdot kg^{-1} \cdot min^{-1}$ infusions of AII also received 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of nitroprusside to counteract the pressor effect of AII. Only infusions of 40 $ng \cdot kg^{-1} \cdot min^{-1}$ of AII combined with nitroprusside caused significant rises in plasma AVP or glucocorticoid concentrations. AVP level rose from 2.6 $\pm$ 0.7 pg/ml to 4.4 $\pm$ 1.3 pg/ml ($p<0.05$, $n=5$) and glucocorticoid levels from 44 $\pm$ 8 ng/ml to 64 $\pm$ 5 ng/ml ($p<0.005$, $n=5$) after 30 minutes of AII + NP infusion. The rise in glucocorticoids was sustained for 60 minutes but the AVP increase was not. Further studies were done to estimate the physiologic range of plasma AII concentrations in the rabbit. A 30% hemorrhage over 30 minutes decreased mean arterial pressure from 73 $\pm$ 4 mm Hg to 63 $\pm$ 2 mm Hg ($p<0.05$, $n=3$) and caused a large increase in plasma AVP levels, but

plasma concentration of AII was not significantly affected. Ten days of a low sodium diet with diuretic injections did cause a mild rise in rabbit plasma AII levels to 67 $\pm$ 31 pg/ml, but this value was lower than effective plasma AII levels for AVP or corticosteroid release. These results indicate that AII is able to stimulate AVP and probably ACTH release in the rabbit. They also support the hypothesis that the pressor effect of AII counteracts stimulatory effects of AII on AVP and ACTH release. This study did not establish that the plasma level of AII found effective for promoting the release of AVP and ACTH is within the physiologic range of the rabbit. The methods developed in this study and the findings mentioned will be important in the design and execution of further experiments in this area using the rabbit.

INTRODUCTION

A growing body of evidence indicates that angiotensin II (AII) stimulates the release of both arginine vasopressin (AVP) and adrenocorticotropic hormone (ACTH) from the mammalian pituitary (for review, see Reid, 1984). The mechanisms, sites of action, and physiologic significance of these effects are the subjects of a considerable amount of ongoing research. Most studies in this area used dogs, rats, and humans, with whole animal studies favoring dogs and most in vitro work done in rats. Despite these efforts, it is still unclear how and where AII has these effects. Also unclear is the importance of these effects in mammalian homeostasis and the relevance of these findings to human pathophysiology.

Until now, rabbits have been neglected as subjects for experiments in this area. The effects of AII on AVP and ACTH release in the rabbit have never been published. The rabbit presents several advantages for more advanced studies in this area. Arterial and venous catheterization is fairly simple and it is possible to infuse substances into the carotid and vertebral arteries (Dickinson & Yu, 1967; Undesser et al., 1985b) as well as the cerebral ventricles (Rosendorff et al., 1970). Sinoaortic denervation, vagotomy, hypophysectomy, and brainstem and hypothalamic lesioning are more advanced procedures that are useful in the study of this area and can be performed easily in the rabbit (Guo et al., 1982; Undesser et al., 1985a). Rabbits are also inexpensive enough to be suitable for in vitro studies of pituitary and other tissues. This advantage permits

direct comparison of results of *in vivo* and *in vitro* studies without concern for interspecies differences.

Although rabbits are promising subjects for advanced studies in this area, the groundwork must first be laid by more basic studies to determine whether the effects of AII in rabbits are similar to those observed in other animals. The purpose of the present study was to examine the effects of elevated plasma AII concentrations on ACTH or AVP release in conscious rabbits. It also investigated the physiologic significance of these effects by estimating the physiologic range of plasma AII levels in rabbits and comparing that range to the AII concentrations produced by exogenous AII administration.

GENERAL BACKGROUND

Angiotensin II is well known as the active product of the renin-angiotensin system (Ganong, 1981; Reid, 1984). Its formation is regulated by the release of a proteolytic enzyme, renin, from the renal juxtaglomerular cells. Renin cleaves the decapeptide, angiotensin I, from its circulating precursor protein globulin, angiotensinogen. Circulating angiotensin I is then rapidly transformed into angiotensin II by an angiotensin converting enzyme in endothelial cells. This active octapeptide has a very short half-life in plasma (less than one minute) and is broken down rapidly into smaller peptide fragments.

The AII-regulating enzyme, renin, is released from the kidney in response to a number of conditions. Hypotension and sodium deprivation cause intrarenal mechanisms to stimulate its release (Ganong, 1981). In addition, extrarenal mechanisms including increases in renal sympathetic nerve activity and circulating catecholamines may also stimulate renin release. As a result, plasma AII levels increase in

response to stress in general as well as specifically to stresses that decrease plasma volume or kidney perfusion pressure.

Angiotensin II acts in several ways to increase or maintain blood pressure and enhance positive fluid balance (Ganong, 1981). Its action on the adrenal cortex to promote aldosterone release increases sodium retention. Its peripheral vasoconstrictor action directly increases systolic and diastolic arterial pressure.

Angiotensin II also acts within the central nervous system to increase blood volume and pressure (Reid, 1984; Phillips, 1987). It increases sympathetic output, causing additional vasoconstriction. It also inhibits parasympathetic activity to the heart, increasing cardiac output. Another well established central effect of AII is its dipsogenic effect, which contributes to positive fluid balance. Finally, the stimulation of AVP and ACTH release by AII is generally regarded as a central effect.

Both AVP and ACTH act to maintain blood volume and pressure. AVP is a hormone released primarily from the posterior pituitary, which strongly promotes renal fluid retention and also has powerful pressor effects (Ganong, 1981). ACTH, an anterior pituitary hormone, increases blood pressure when chronically administered (Whitworth et al., 1981). Its action on the adrenal cortex to increase aldosterone secretion also increases sodium retention (Ganong, 1981). Furthermore, its stimulation of adrenal cortical production and release of corticosteroids is important in the restoration of blood volume after acute hemorrhage (Gann & Pirkle, 1975).

EXPERIMENTAL APPROACHES

Generally, three basic approaches have been used to study the effects of AII on AVP and ACTH in whole animals. (1) Exogenous AII has been either injected by bolus or infused into animals to raise plasma and/or tissue concentrations of AII. (2) Animals have also been subjected to physiologic stresses that cause increased endogenous AII production. (3) Finally, animals with elevated AII levels have been treated with competitive AII antagonists such as saralasin or given inhibitors of converting enzyme to prevent the production of AII. The second and third approaches are often used in studies attempting to assess the physiologic importance of AII's effects in intact animals. The first approach can also evaluate the physiologic significance of the effects of AII by comparing the plasma concentrations of AII during infusions with those occurring naturally in the animal. This requires a knowledge of the physiologic range of plasma AII levels.

Physiologic range is the range of responses of plasma or tissue AII levels to various physiologic states producing extremes in the production of circulating AII. If exogenous infusions of AII have effects only when they produce plasma levels outside the physiologic range, the physiologic importance of these effects is questionable. Since interspecies differences may occur in physiologic AII ranges, the physiologic range for each particular species must be estimated before results of AII infusion experiments in the species can be interpreted accurately.

Increases in plasma levels of AVP and ACTH are generally assumed to be indicative of increases in pituitary release of these hormones. Plasma levels of AVP and ACTH are generally assayed by radioimmunoassay, but levels of plasma glucocorticoids have also been

used as indicators of ACTH release. This method is less direct than radioimmunoassay but may be a more sensitive indicator of the normally pulsatile ACTH output (Wood et al., 1982). However, the method is complicated during AII infusions by the observation that AII can act directly on the adrenals to increase glucocorticoid output (Braverman & Davis, 1973; Reid, 1984).

AII ACTION ON AVP RELEASE

Intravenous infusions of AII producing plasma levels within the physiologic range have been shown to increase plasma levels of AVP in some studies of dogs, humans, and rats (Ramsay et al., 1978; Uhlich et al., 1975; Klingbeil et al., 1986). Other studies have only found this increase with supraphysiologic plasma AII levels (Reid et al., 1982; Usberti et al., 1985; Knepel & Meyer, 1980; Padfield & Morton, 1977). A few studies demonstrate no AVP reaction to even high doses of AII (Cowley et al., 1981; Hammer et al., 1980).

One possible reason for these discrepancies is suggested by evidence that AII produces larger increases in AVP in animals with elevated plasma osmolality. It is well known that increased plasma osmolality is a powerful stimulant of AVP release (Ganong, 1981). The possibility that AII may modulate this reaction was raised by Shimizu et al. (1973) when they found that intracarotid infusions of AII increased the rise in plasma AVP produced by intravenous hypertonic saline infusions in anesthetized dogs. A more recent study found that AII infusion increased AVP responsiveness to intracarotid administration of hypertonic saline in conscious dogs (Wade et al.,

1986). *In vitro* studies using organ culture rat hypothalamo-neurohypophyseal systems also showed that AII plays an important role in modulating AVP release in response to increasing culture medium osmolality (Ishikawa et al., 1980; Sladek et al., 1982). Results such as these have led some researchers to suggest that the variability in the results of intravenous AII infusion experiments may have been related to the fluid status of the animals at the time of experimentation. The lack of information on plasma osmolality in these studies makes these suggestions purely speculative. Nevertheless, it is apparent that further studies of AII's action on AVP must carefully monitor or control the osmolality of the culture medium or the blood.

Site of Action of AII in AVP Release

Many studies have been performed to determine the site of action of AII to increase AVP release. Intracerebral ventricular infusions of AII have been found to consistently produce rises in plasma AVP (Ganong et al., 1982; Keil et al., 1975; Mouw et al., 1971; Fisher & Brown, 1984). This suggests that circulating AII may act directly on the brain to modulate AVP release. Since AII does not cross the blood brain barrier, the circumventricular organs have been considered likely candidates as sites of action. These areas lack a blood brain barrier and are known to possess specific AII receptors (Van Houten et al., 1980; 1983). Furthermore, there is evidence that intraventricular and blood-borne AII may both act on these receptors (Van Houten et al., 1983).

The circumventricular organs are located at sites in both the brainstem and forebrain (Van Houten et al., 1980). Hypothalamic nuclei are especially prominent candidates for sites of action, considering

the importance of hypothalamic factors in the release of anterior pituitary hormones and the extension of hypothalamic neurons into the posterior pituitary. Seeking to differentiate a brainstem from forebrain site of action, Reid et al. (1982) used intravertebral and intracarotid infusions of AII in dogs. They found that plasma AVP is increased in response to supraphysiologic levels of intracarotid AII but not increased during intravertebral infusions. Because carotid blood does not perfuse the brainstem (Reid et al., 1982), these data support the hypothesis that circulating AII acts on forebrain sites to effect a release of AVP.

A leading candidate for a site of action is the subfornical organ. The subfornical organ is a circumventricular organ that is closely connected with hypothalamic nuclei which produce AVP (Phillips, 1987). Lesions of this organ attenuate the AVP response to AII infusion (Thrasher & Keil, 1986). On the other hand, there is also some evidence favoring the neurohypophysis and the organ vasculosum of the lamina terminalis as sites of action (Reid, 1984; Phillips, 1987). For example, AII stimulated AVP release from hypothalamo-neurohypophyseal explants; these explants do not contain the subfornical organ (Sladek et al., 1982). Thus, the exact site or mechanisms by which AII stimulates AVP release has not been definitely established.

Rabbits would be especially good animals for further studies of AII's site of action of AVP release. Their size allows for chronic catheterization with the capacity for multiple blood samples over time. Their size is also advantageous for brain lesion studies, and they are much less expensive than the larger animals used for central nervous system ablation studies.

Physiologic Role of AII in AVP Release

There has been much debate concerning the possibility that endogenous AII may play a role in the regulation of AVP release. Some previously mentioned studies support this possibility. Exogenous AII infusions producing plasma concentrations within the physiologic range have been demonstrated to result in increased AVP concentrations (Ramsey et al., 1978; Uhlich et al., 1975; Klingbell et al., 1986). On the other hand, other studies required supraphysiological levels to induce detectable AVP release (Padfield & Morton, 1977; Knepel & Meyer, 1980; Reid et al., 1982; Usberti et al., 1985).

It is important to emphasize that studies evaluating the physiologic role of AII in the control of AVP release may underestimate the potency of endogenous AII. This is because AII may be acting on AVP release through multiple and sometimes opposing mechanisms. Like renin, AVP release is inversely related to blood pressure, and elevations in arterial pressure suppress vasopressin secretion (Reid, 1984). Since infused AII has a potent pressor effect, it may be acting to inhibit AVP release at the same time it is exerting stimulatory effects. This possibility was investigated by Brooks et al. (1986) with experiments in conscious dogs. These investigators used nitroprusside infusions concurrently with AII infusions to counteract the pressor effects of AII. Physiologic plasma levels of AII produced larger increases in plasma AVP concentrations when the pressor effect of AII was negated.

Another aspect of this study (Brooks et al., 1986) used sodium depletion to chronically elevate endogenous AII levels in dogs. Despite these high plasma AII levels, AVP levels were not different

than sodium replete controls. The infusion of the AII blocker, saralasin, produced a sharp fall in arterial pressure in these animals, emphasizing the importance of endogenous AII in the maintenance of blood pressure in these conditions. Remarkably, this fall in blood pressure had no effect on plasma AVP levels which would be expected to increase. The most likely explanation for this finding was that saralasin also blocked a tonic stimulation of AVP release by AII, counteracting the stimulatory effect of hypotension. If blood pressure was reduced by nitroprusside to levels similar to those produced by saralasin administration, significant increases in plasma AVP levels occurred. These findings support the importance of endogenous AII in the maintenance of AVP levels in the sodium deficient state.

The importance of AII in the vasopressin response to hemorrhage has been studied. Hemorrhage is a potent stimulator of AVP release in dogs, and also can stimulate the renin-angiotensin system (Claybaugh & Share, 1972). Mild hemorrhage can increase plasma AVP levels without affecting plasma renin activity (Claybaugh & Share, 1973). During more severe hemorrhages, blockade of the increase in plasma AII by a converting enzyme inhibitor does not affect the AVP response (Morton et al., 1977). Another study used renal blood vessel occlusion to prevent rises in plasma renin activity during hemorrhage (Claybaugh & Share, 1972). The suppression of renin activity had no effect on the response of AVP to hemorrhage. However, this study used anesthetized dogs and circulating AII does not increase AVP release during anesthesia (Claybaugh et al., 1972; Share, 1979).

Another potent stimulator of AVP release is hypotension (Lee et al., 1986). The increase in vasopressin during nitroprusside induced hypotension in conscious dogs is not affected by the intravenous

administration of saralasin (Brooks et al., 1986). Collectively, these studies suggest that AII is not necessary for the AVP response to either hypotension or hypovolemia.

In summary, there is some evidence that AII has a physiologic role in the regulation of AVP release in mammals, especially when plasma AII levels are high. It is not clear what site of action is important for this role. Further studies using rabbits may help to locate this site of action and help to define AII's role in AVP release.

AII ACTION ON ACTH RELEASE

Intravenous, intracarotid, and intracerebroventricular infusions of AII can promote the release of ACTH and/or 11-hydroxycorticosteroids into the circulation of conscious dogs (Ramsay et al., 1978; Maran & Yates, 1977; Reid et al., 1982; Keller-Wood et al., 1986; Raff et al., 1985; Klingbell et al., 1986), rats (Daniels-Severs et al., 1971; Rivier & Vale, 1983; Ganong et al., 1982; Spinedi & Negro-Vilar, 1984) and humans (Rayyis, 1971). The mechanism and site of action for this effect have been investigated in numerous studies. Attention has been focused on the interrelationships between AII and other, more potent corticotropin releasing compounds such as AVP and corticotropin releasing factor (CRF).

Evidence for Direct Pituitary Effects

One possible site of AII action is the anterior pituitary. Maran and Yates (1977) have shown that intrapituitary infusions of AII into conscious dogs stimulate ACTH release. Furthermore, these effects were induced by AII infusion rates which were ineffective when given intravenously.

It also is well established that angiotensin II can act on cultured anterior pituitary cells to promote the release of ACTH (Sobel & Vagnucci, 1982; Sobel, 1983; Gaillard et al., 1981; Aguilera et al., 1983). However, it is much less potent than a number of other substances, most notably CRF and AVP (Hashimoto et al., 1979). This lack of potency requires the use of high concentrations of AII to demonstrate any effect. Only two studies have demonstrated AII effects at concentrations (10^{-9} M) low enough to approach the upper limits of endogenous plasma AII levels in rats (Gaillard et al., 1981; Spinedi & Negro-Vilar, 1984). Some studies have found that AII at concentrations ineffective for ACTH release can potentiate the effect of CRF on this release (Vale et al., 1983; Schoenenberg et al., 1987). Still, these potentiating concentrations were still at least ten times higher than recorded peak plasma concentrations of AII in the rat.

Thus, AII may directly stimulate pituitary ACTH release. However, the high doses of AII required suggest that AII may act primarily by another mechanism, such as through the release of AVP or CRF.

Role of AVP

The observation that AVP and ACTH release are affected similarly by AII infusion suggests the possibility that the release of ACTH may be dependent on increased pituitary levels of AVP. Indeed, AVP is known to have significant corticotropin releasing activity; much more than AII itself (Hashimoto et al., 1979). One recent study in conscious dogs addressed this possibility. Klingbell et al. (1986) found that AII produces larger increases in AVP release in water deprived dogs compared to water replete dogs. This increased response

of AVP was not accompanied by an increased ACTH response. Furthermore, when water replete dogs were infused by a vasopressin receptor (V1) antagonist, the plasma ACTH response to AII was unchanged. Finally, they simultaneously infused AII and AVP into dogs and found no synergy in the two hormones' action on ACTH. These researchers therefore concluded that AVP was not necessary for AII's action on ACTH release.

A study in freely moving Sprague-Dawley rats also showed that the vasopressin antagonist dPTyr (Me) AVP did not effect the action of AII on ACTH (Rivier & Vale, 1983). This lack of importance of AVP was also supported by a study comparing normal and homozygous Brattleboro rats (Spinedi & Negro-Vilar, 1984). Brattleboro rats have extremely low levels of plasma AVP, an inherited diabetes insipidus. Despite this deficiency, their ACTH response to bolus intravenous injections of AII was not different than the response of normal rats. This suggests that AVP release is not an important factor in the stimulation of ACTH release by AII. These researchers also removed and cultured the pituitaries from both strains of rats. Both responded with ACTH release equally well to AII introduced into their media. It was also found that the Brattleboro pituitary cells were far more sensitive to CRF than the normals. This observation raises the possibility that Brattleboro rat cells could be compensating for a deficiency in vasopressin by increasing the sensitivity of the corticotrophs to the other major corticotropin releaser, CRF. This compensation could mask the loss of AVP as one mediator of the ACTH response to AII. However, this possibility is unsupported and entirely speculative. Thus, the weight of the evidence argues against a significant effect of AVP as a mediator between AII and ACTH release.

Role of CRF

Evidence is strong that CRF plays an important role in the action of AII on ACTH. CRF may play two roles. 1) AII may stimulate CRF release, allowing CRF to then exert its own powerful corticotropin releasing activity. 2) CRF may also be a necessary cofactor for a direct AII action on the pituitary.

Studies of rats anesthetized by a method preventing endogenous CRF release showed that subsequent intravenous or intraperitoneal AII infusions were ineffective in raising plasma ACTH levels (Rivier & Vale, 1983; Spinedi & Negro-Vilar, 1984). Simultaneous infusion of CRF with AII in similarly anesthetized rats produced a modest synergism in the release of ACTH (Rivier & Vale, 1983). Another study in freely moving unanesthetized rats showed that anti-CRF serum blocked AII stimulation of ACTH release (Rivier & Vale, 1983).

It is important to consider the previously mentioned study comparing normal and Brattleboro rats (Spinedi & Negro-Vilar, 1984). Brattleboro rats were found to have much larger ACTH release responses to intravenous CRF infusions than normals. This increased sensitivity to CRF was also found in isolated cultured Brattleboro pituitaries. If AII stimulated ACTH release by stimulating CRF release, Brattleboro rats would be expected to have increased sensitivity to AII. However, when AII was administered both <u>in vivo</u> and <u>in vitro</u>, no difference in ACTH responses of normal and Brattleboro rats was observed. These data therefore argue against an AII effect on CRF alone and suggest that AII may act directly on the pituitary and that CRF is a required cofactor. However, it is not known whether CRF release in the Brattleboro rat is blunted, which could counteract increases in pituitary sensitivity.

The possibility that elevated CRF levels are necessary for AII's effects was investigated in conscious dogs by Keller-Wood et al. (1986). Intravenous AII infusion producing physiologic plasma concentrations were found to increase corticosteroid but not ACTH levels in plasma. Coinfusion of CRF with AII was shown to produce a dose-response relationship between AII infusion rate and ACTH or corticosteroid plasma levels. These experiments confirmed that AII and CRF can interact to stimulate ACTH release. They did not, however, define whether CRF was acting as a cofactor or an intermediate for the effect of AII. The possibility remains that AII could both increase the release of CRF and then interact with CRF at the pituitary to increase ACTH output.

Further studies must be done to clearly define the role of CRF in AII stimulation of ACTH release. One possible study is to block endogenous CRF release in an animal and then infuse doses of exogenous CRF to maintain constant plasma CRF levels. ACTH release in response to AII infusions could then be determined. If the ACTH response to AII was blocked, an active role of CRF would be indicated. Findings of continued AII effectiveness would indicate that CRF was a permissive agent for AII's mechanism of action. The methodological difficulty in this experiment is in blocking endogenous CRF without altering the effects of exogenous CRF. This could be done with anesthetics or by surgical ablation of the median eminence. The rabbit would be an excellent subject for studies of this type.

Evidence for Effects on the Central Nervous System

Lending support to the possibility that AII acts by increasing CRF release, studies have indicated that central nervous system receptors

are important sites of action for AII. AII has been found to be effective in raising plasma 11-hydroxycorticoid levels when infused into the cerebral ventricles of dogs (Maran & Yates, 1977; Brooks & Malvin, 1979) and rats (Daniels-Severs et al., 1971; Ganong et al., 1982). Hypophysectomy blocks this response, indicating that ACTH release may be involved. Furthermore, when saralasin (AII blocker) is infused into the cerebral ventricles of sodium deprived dogs, plasma cortisol levels decrease (Brooks & Malvin, 1979). This suggests that endogenous AII acts on receptors in the central nervous system to increase ACTH release through the mediating effect of CRF.

Possible sites of action include the organ vasculosum of the lamina terminalis and the subfornical organ. Both are circumventricular organs bearing AII receptors and both are either within (organ vasculosum of the lamina terminalis) or closely related (subfornical organ) to the hypothalamus. To evaluate the subfornical organ, one group surgically ablated this area in dogs (Thrasher & Keil, 1986). They found that the lesions attenuated ACTH secretion in response to intravenously administered AII.

Another possible central site of AII action on CRF release is the median eminence. It too is a circumventricular organ with AII receptors and it is well known as the site of hypothalamic CRF release into the hypophyseal portal system (Ganong, 1981). Gann (1979) studied the importance of the median eminence in AII stimulation of ACTH release by comparing two groups of anesthetized dogs. One group underwent complete brain removal sparing the areas of the median eminence and pituitary. The other group also underwent brain removal, but this time with removal of the median eminence and posterior

pituitary. The group with intact median eminences and pituitaries had much greater cortisol responses to intravenous AII infusions. Those animals with only the anterior pituitary spared had similar responses to hypophysectomized dogs. It was concluded that the median eminence was necessary for the effect of AII on ACTH.

Since the median eminence is the site of CRF secretion into the portal system, these findings agree with those that found CRF important for AII's actions on ACTH. It remains uncertain whether AII acts directly on the median eminence, on the other circumventricular organs mentioned, or both.

Adrenal Actions of AII on Corticosteroid Release

Some studies have demonstrated that AII infusion can increase corticosteroid levels when ACTH levels are suppressed. Hypohysectomized rats with low basal corticosterone levels experienced an 80% fall in these levels when infused with an AII blocker (saralasin) (Davis & Freeman, 1976). Rabbits treated with dexamethasone to suppress ACTH release had low rates of corticosterone release. In these rabbits, high doses of AII (100 $ng \cdot kg^{-1} \cdot min^{-1}$) had a mild but significant stimulatory effect on corticosterone output (Braverman & Davis, 1973). Anesthetized, nephrectomized, dexamethasone treated dogs were also shown to increase cortisol output in response to AII (Bravo et al., 1975). These data suggest that AII may directly stimulate adrenal corticosteroid release.

There is also evidence both <u>in vitro</u> and <u>in vivo</u> that AII may act on the adrenal cortex synergistically with ACTH to stimulate corticosteroid release. This interaction was studied in conscious dogs pretreated with dexamethasone, which abolished the ACTH and cortisol

response to AII (Brooks et al., 1984). Infusions of ACTH (0.3 $ng \cdot kg^{-1} \cdot min^{-1}$) to increase plasma levels to those noted in control animals failed to reestablish the cortisol response to AII infusion. However, larger doses of ACTH (0.4 $ng \cdot kg^{-1} \cdot min^{-1}$) permitted a dose response effect of AII on corticosteroid levels. This study indicates that ACTH must be increased above resting levels for AII to produce a significant increase in glucocorticoids. It also suggests that infused AII cannot act on the adrenal alone but must also act to increase ACTH release to exert its effects on corticosteroid release.

AII's effect on ACTH release may be a more potent stimulator of corticosterone release than its direct adrenal effect. When dogs are hypophysectomized (Maran & Yates, 1977) or treated with dexamethasone (Brooks et al., 1984) the cortisol response to AII infusion is abolished. Reid et al. (1982) investigated the importance of central versus peripheral effects in conscious dogs. Intracarotid AII infusions were found to stimulate corticosteroid release at far lower doses than intravenously administered AII. However, another study using sodium deprived dogs demonstrated decreases in corticosteroids when an AII blocker (saralasin) was infused intravenously but no decreases when intracarotid or intravertebral saralasin was infused (Brooks & Reid, 1983). This group then concluded that AII's primary effects were peripheral, not central.

In summary, evidence is strong that ACTH is involved with AII induced corticosteroid release. The relative importance of AII's stimulatory effect on ACTH release and interaction with ACTH at the adrenal cortex to release corticosteroids is presently not well established.

Physiologic Significance

Although evidence is strong that AII can cause a release of ACTH from the pituitary, it is less well established that this effect is physiologically relevant. Some studies of intravenous or intracarotid AII infusions in the dog have required supraphysiologic plasma concentrations to induce ACTH or corticosteroid release (Reid et al., 1982). The studies of intracerebral ventricular infusions also raise AII levels in the cerebrospinal fluid far above levels of endogenous AII recorded in animals (Reid, 1977).

As in AVP release, the effect of blood pressure on ACTH complicates the interpretation of AII infusion experiments. ACTH release is inversely related to blood pressure (Gann, 1981; Brooks & Reid, 1986) and the pressor effect of AII may inhibit ACTH release. This inhibition could then counteract more direct stimulation of ACTH release by AII. Brooks & Reid (1986) studied this possibility. Conscious dogs were given infusions of AII simultaneously with sufficient nitroprusside (NP) to maintain arterial pressure near control levels. This infusion resulted in a larger increase in ACTH release. The angiotensin II was infused at a rate maintaining plasma AII concentrations well within the physiologic range. The possibility that the nitroprusside was producing an effect not related to its modulation of blood pressure was tested. A different vasodilator, hydralazine, was used in further experiments to negate the AII induced blood pressure rise. These experiments showed similar results to the AII + NP infusions. Thus, it appears that the pressor effect of exogenous AII does counteract an effect to stimulate ACTH release.

Experiments on sodium depleted dogs were also done using an AII blocker, saralasin (Brooks & Reid, 1986). These studies showed that despite a large drop in blood pressure induced by saralasin infusion, plasma corticosteroid levels remained unchanged. This indicates that AII may well be acting to maintain the output of corticosteroids in this physiologic state. The potency of these animals' ACTH releasing response to hypotension was demonstrated with infusions of nitroprusside. A decrease in blood pressure to similar levels as induced by saralasin was accompanied by large increases in plasma ACTH concentrations. It is unlikely that AII simply uncouples the relationship between blood pressure and ACTH release. When nitroprusside was added to saralasin infusions to lower pressure further, large increases in ACTH again occurred, reconfirming the potency of nitroprusside induced hypotension in saralasin treated animals.

Studies utilizing infusions of saralasin into the cerebral ventricles of sodium deprived dogs have demonstrated significant decreases in plasma corticosteroid concentrations (Brooks & Malvin, 1979). These studies indicate that AII may play a physiologic role in central mechanisms of stimulating glucocorticoid release during sodium deprivation.

Another physiologic state in which AII may influence the adrenal-pituitary axis is that of high renin hypertension. Humans with high renin or renovascular hypertension have been found to have higher morning (0800) cortisol peaks than those with low renin hypertension or normotension (Atlas et al., 1981). Ten days of administration of Captopril (inhibitor of angiotensin converting enzyme) to the high renin subjects not only decreased mean arterial pressure but also

caused cortisol peak levels to fall. Analysis of the data of another study of six patients with essential hypertension (Angeli et al., 1981) showed a correlation between plasma renin activity and cortisol levels. Cortisol concentration then decreased in the three patients with high renin hypertension when treated with Captopril. Experiments in dogs with one clip Goldblatt hypertension showed that chronic activation of the renin angiotensin system produced no changes in plasma corticosteroid levels and that AII blockade with saralasin or Captopril did not decrease corticosteroid concentrations (Ben et al., 1984). This result conflicts with the previous human studies but also raises the possibility that differences in AII actions on corticosteroids may exist between dogs and humans.

Another physiologic state that has been investigated is hemorrhage. Hemorrhage is known to stimulate the renin-angiotensin system (Claybaugh & Share, 1972). Aguilera et al. (1983) investigated the role of angiotensin in the ACTH response to hemorrhage by infusing converting enzyme inhibitor into the cerebral ventricles of conscious rats. This infusion prevented a previously demonstrated increase in plasma ACTH levels during hemorrhage. This indicated the importance of centrally produced AII in this response. If peripherally generated AII was important, the central blockade of converting enzyme would be ineffective. Therefore, although AII may play a role in the ACTH response to hemorrhage, the role of the peripheral renin-angiotensin system is uncertain.

In conclusion, endogenous AII may promote ACTH release in a number of physiologic states, and this action may be important in normal responses to certain physiologic stresses.

Summary

Intravenous, intracarotid, and intracerebroventricular infusions of AII can promote the release of ACTH and/or 11-hydroxycorticosteroids into the circulation of conscious dogs, rats, and humans. The relative importance of three potential sites of AII action have been studied. AII may effect ACTH release by interaction with receptors in the anterior pituitary or areas in the central nervous system that regulate the release of CRF into the hypophyseal portal system. The possibility of direct effects of AII on the adrenal cortex for the release of 11-hydroxycorticosteroids must also be considered.

Rabbits may be excellent subjects for further studies of the mechanism and site of AII's action of ACTH and/or corticosteroid release.

RABBIT STUDIES

The purpose of the present study was to examine the effects of increased plasma AII concentrations on the release of AVP and ACTH from the rabbit pituitary. This project used two different approaches. The first approach involved intravenous infusion of AII into conscious rabbits and addressed the question: Does AII stimulate ACTH and AVP release in rabbits?

The second approach involved subjecting rabbits to physiologic stresses known to raise plasma AII concentrations in other animals. Hemorrhage and sodium depletion were used to assess the range of endogenous AII concentrations that were possible in rabbits. This approach addressed the question: Can rabbit endogenous AII plasma levels rise to the levels found effective in stimulating AVP and/or ACTH release during AII infusions?

METHODS AND MATERIALS

Twenty-seven male New Zealand White rabbits weighing 2.0-4.0 kg were studied. They were housed in individual cages in the OHSU Animal Care Department and fed approximately 1 cup/150 g of Purina rabbit chow (DG-5315, sodium content 0.25-0.50%) daily unless otherwise specified. They also received tap water ad libitum. Rabbits were brought to the lab daily and placed in the stainless steel restrainer boxes used for experiments. They were given at least four days to become familiar with the lab environment before experimentation.

CATHETERIZATION

Arterial catheters were implanted for blood pressure and heart rate monitoring as well as blood sampling. Venous catheters were used for infusion of AII and nitroprusside. The central arteries and marginal veins of the rabbit ears were selected for catheterization. Catheters were introduced in the morning of the day of experimentation and removed immediately upon completion of the experiment.

Arterial catheterization was begun by infusing subcutaneous 1% lidocaine (Elkins-Sinn, Inc.) bilaterally to the central ear artery. This maneuver was mildly irritating to the rabbit but prevented any further pain secondary to the catheterization. After local anesthesia, an 18 or 20 gauge catheter (3.2 cm Quik-Cath [Intravascular Over-the-Needle Teflon catheter], Travenol) was introduced into the vessel and advanced 1 cm toward the base of the ear. It was then flushed with 1-2 ml heparinized saline (heparin, Elkins-Sinn, Inc., 1000 u/ml diluted ten-fold in normal saline; 100 USP units/ml). This flush was easily

seen in the superficial ear vasculature and served as an indication of successful cannulation. The catheter was then secured with cloth tape.

If the first attempt at arterial catheterization failed, a second attempt was made using the opposite ear. If this failed a third attempt was occasionally attempted in the first ear. For further details of catheterization techniques, see Appendix A.

Following arterial catheterization, a 20-gauge catheter was placed in the marginal vein of the opposite ear. Lidocaine (1%) was used for local anesthesia. Catheter placement was aided by the placement of a paper clip on the proximal marginal ear to act as a tourniquet. The catheter (Quik-Cath, Travenol) was inserted in a similar manner to the arterial catheter.

Arterial catheters were connected by tubing (Tygon Mico Bore, I.D. 040) filled with heparinized saline to a pressure transducer (Microswitch, Model 135 PC 05 G1, Honeywell) linked to a Grass (Model 7D) polygraph (Figure 1). Pressure readings were recorded on two channels, one highly damped to reflect mean arterial pressure, and the other minimally damped to monitor pulse amplitude and rate. Pulse rate was recorded by a tachograph. The transducer and polygraph were calibrated before experiments using a water manometer. The internally calibrated tachograph readings were periodically compared with visual counts of inflections of the pressure tracing.

The venous catheter was connected by tubing to a 6 ml syringe filled with infusate. This syringe was placed in an infusion pump (Harvard Model 901) calibrated to deliver 0.080 ml/min.

Rabbits were allowed at least two hours after catheterization before an experiment began. Occasionally, severe vasoconstriction of the ear arteries delayed experiments several hours. The mean time

delay between catheterization and experimentation was 3 hrs and 40 min ± 74 min. This vasoconstriction was sometimes overcome by intra-arterial flushes of 1-2 ml of warm (40°C) saline. This technique was also useful when vasoconstriction during experiments interfered with blood sample withdrawal. When a stable baseline pressure had been maintained for at least thirty minutes, one of the following experiments was begun.

PROTOCOLS

1. AII INFUSION EXPERIMENTS (Figure 2)

These experiments were conducted to determine the dose-response relationship between intravenous AII infusions and plasma levels of AII, AVP and corticosteroids. Experiments used 60-minute infusions of either 0, 10, 20, or 40 $ng \cdot kg^{-1} \cdot min^{-1}$ of AII suspended in a 5% Dextrose in water solution, infused at a rate of 0.08 ml/min. An initial blood sample (4 ml) was withdrawn and replaced with 3.0 ml normal saline and 0.5 ml heparinized normal saline, which also flushed the arterial line. The infusion was then begun. After 30 minutes, the second arterial blood sample (2 ml) was withdrawn and replaced with 2.0 ml normal saline and 0.5 ml heparinized normal saline. Another 30 minutes of infusion passed and a third arterial sample (4 ml) was withdrawn and replaced. Infusion was then halted. After a 30-minute recovery period, a fourth and final arterial sample (4 ml) was withdrawn.

Samples were immediately put on ice. The 4 ml samples were divided with 1.8 ml placed in a tube with EDTA (0.2 ml of 0.3 M EDTA solution) and the remainder placed in a tube with two drops of heparin (1000 USP units/ml). All of the second sample (2 ml) was put in a heparin tube. At the end of the experiment, the samples were

centrifuged at 4°C and the plasma was separated from the red blood cells. Erythrocytes from the heparinized samples were resuspended in normal saline and reinfused into the rabbits. The plasma samples were separated into aliquots and frozen at -20°C for later assay of AII, AVP, and corticosteroid levels. Osmolality was also measured.

2. AII + NITROPRUSSIDE INFUSION EXPERIMENTS (Figure 2)

The purpose of these experiments was to ascertain the effect of AII on ACTH and AVP release independent of AII's pressor effect. Nitroprusside (NP) was infused simultaneously with AII at rates minimizing the elevation of blood pressure during the infusion. One set of experiments combined 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP with 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII. The other set combined 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP with 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII. The simultaneous AII and NP infusions were performed for one hour and blood samples were collected as described above for AII infusions. Solutions were infused at the rate of 0.08 ml/min.

3. NITROPRUSSIDE INFUSION EXPERIMENT

After one rabbit received an infusion of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII plus 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP, it was allowed thirty minutes to recover and an infusion of nitroprusside alone was begun. The nitroprusside infusion rate was increased to a level that produced a 20 mm Hg drop in mean arterial pressure. This rate was calculated to be 60 $\mu g \cdot kg^{-1} \cdot min^{-1}$. When a 20 mm Hg fall was achieved, a 4 ml blood sample was drawn. The infusion was continued at a rate of 60 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for ten more minutes, then a second 4 ml blood sample was drawn.

4. HEMORRHAGE EXPERIMENTS

These experiments were performed to measure the rise in plasma AII levels occurring during a three-step 30% hemorrhage. Estimated blood volume was considered to be 65 ml/kg (Yamazaki & Sagawa, 1985). A catheter was placed in the central ear artery of each rabbit in a manner previously described. This catheter was also connected to the transducer and polygraph apparatus used before. After catheterization, the rabbits were given an hour to recover before experimentation was begun.

Ten minutes before hemorrhage, a control sample of arterial blood (4 ml) was drawn. At the beginning of the first bleed, a second sample of 4 ml was drawn. Blood was then dripped into a graduated tube with a small amount of heparin added. A total of 10% of the estimated blood volume was withdrawn (including the two samples) and the line was then flushed with 0.5 ml heparinized normal saline. Ten minutes after the start of the first hemorrhage, the second bleed was begun. Again, the first 4 ml of blood was placed in sample tubes and the remainder of another 10% of the estimated blood volume was dripped into a larger graduated tube. Ten minutes later this routine was repeated for the third 10% bleed. Ten minutes after the beginning of the third bleed, a final sample of 4 ml was withdrawn. Most bleeds required only two to three minutes although one required six, and another eight, minutes.

Blood samples were drawn and separated in a similar manner to that described in the AII infusion experiments. The samples were centrifuged after the experiment and the heparinized erythrocytes were resuspended in saline and reinfused into the rabbits. Blood volume was also then restored with "non-sample" blood. The five plasma samples

were aliquoted and frozen and later assayed for AII, AVP, and corticosteroid levels.

5. SODIUM DEPRIVATION

This experiment was designed to determine the response of plasma AII levels to chronic sodium deprivation. Blood samples were taken from six rabbits for the measurement of baseline AII, AVP, and corticosteroid concentrations. These rabbits were then fed a low sodium diet (Purina Modified Lab Rabbit Chow with No Added Sodium, sodium content 0.05%, providing 3 mEq sodium per day). On the third day of low sodium diet, 4 mg of furosemide was given intramuscularly to each rabbit and repeated every other day until the ninth day, inclusive. This dose of furosemide per kg body weight was similar to the dose given to dogs during other sodium deprivation experiments (Brooks & Reid, 1986). On the tenth day, a catheter was placed either in an ear artery or vein. Arterial catheterizations allowed blood pressure measurements. Blood samples were collected up to an hour after arterial catheterization but immediately after venous catheterization. Venous blood withdrawal was impossible in one rabbit (#1553) requiring the use of cardiac puncture technique. Anesthesia was induced with 0.6 ml Pentobarbital (64.8 mg/ml, Fort Dodge Laboratories, Inc.). Two 4 ml samples were collected from each rabbit and placed into iced tubes. Each sample was divided in half with 2 ml collected in a tube containing heparin and 2 ml collected in a tube containing EDTA. Samples were then centrifuged and handled as described before. Instead of using part of the heparinized plasma for assay of AVP, this aliquot was used for determination of plasma sodium

content. The remaining plasma was used for measures of plasma AII, glucocorticoids and osmolality.

REUSE OF ANIMALS

After infusion experiments, catheters were removed and animals returned to their quarters. A recovery period of seven days was allowed before further experimentation. During this period, further training was done. Rabbits were often subjects for both AII and AII + NP infusion experiments. None were used for more than four experiments because of increased difficulties with arterial catheterization.

RANDOMIZATION

The infusion protocol for each of the first eighteen experiments was selected by a random die roll from the protocols for 0, 10, 20, and 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII infusions as well as the 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII plus 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP infusion protocol. After fourteen experiments, the protocol for 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII + 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion was added to the other protocols selected at random. In the final fourteen infusion experiments, a protocol was chosen before each rabbit was brought to the lab for catheterization. Details of dates, rabbits used and randomization for each protocol can be found in Appendix B.

ASSAYS

Plasma samples collected in heparinized tubes were assayed for AVP and corticosteroids. AVP was extracted from plasma, dried, frozen, and shipped to another lab (LC Keil, USF, San Francisco, CA) for radioimmunoassay (Keil et al., 1977). Corticosteroid concentrations were measured by competitive binding protein radioassay (Murphy, 1967). The plasma samples anticoagulated with EDTA were assayed for

angiotensin II. AII was extracted from plasma and measured by radioimmunoassay (Reid, 1981; Deschepper & Ganong, 1986).

Plasma sodium was measured by a Nova 1 Sodium/Potassium Analyzer (Nova Biomedical). Plasma osmolality was measured by a freezing point osmometer (Advanced Digimatic Osmometer, Model 3D2, Advanced Instruments Co).

ANALYSIS OF DATA

In AII or AII + NP infusion experiments, rabbits with high (>100 ng/ml) baseline corticosteroid levels were excluded from the statistical analysis. Rabbits with baseline corticosteroid levels below 100 ng/ml were not excluded, even if subsequent levels rose above 100 ng/ml. It was noticed in preliminary experiments that rabbits which appeared more agitated and had higher baseline blood pressure also tended to have glucocorticoid levels above 100 ng/ml. This value is comparable with the elevation in glucocorticoid levels in other rabbits exposed acutely to handling stress (Redgate et al., 1981). Therefore, this criteria was used to exclude rabbits that presumably were undergoing a stress reaction to the preparation of the experiment, since this reaction possibly could mask an effect of AII on glucocorticoid levels.

The mean blood pressure value given for each experimental period was determined by calculating the average value of the polygraph tracing over the 20-minute period preceding the blood sample. Mean heart rate values were determined in a similar manner.

Cardiovascular and endocrine responses to angiotensin II infusion or hemorrhage were statistically evaluated with one-way analysis of

variance for repeated measures (Winer, 1971). If changes over the course of experiments were statistically significant, a post hoc Duncan's multiple-range test (Winer, 1971) was used to assess which values differed from the control value.

The increases in blood pressure produced by 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII infusions were compared with those produced by 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII + 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP infusions with an unpaired t test (Winer, 1971). A similar comparison was made between 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII infusions and 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII + 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP infusions.

Results of sodium deprivation experiments required different analysis. Sodium depleted AII values were compared with the sodium replete values of the same rabbits along with control values of sodium replete rabbits used for other experiments. An unpaired t test (Winer, 1971) was used. Sodium depleted corticosteroid values were compared to controls with a paired t test (Winer, 1971). Plasma sodium values for sodium depleted rabbits were compared to values for different, sodium replete control rabbits with an unpaired t test (Winer, 1971).

Values in figures and tables were presented as means plus or minus standard error ($\pm$ SE).

RESULTS

Thirty-one AII or AII + NP infusion experiments were performed. High baseline plasma glucocorticoid levels (>100 ng/ml) excluded nine experiments from analysis. These nine also tended to have elevated baseline blood pressures and plasma AVP levels. Mean arterial pressure for control periods of rejected experiments was 80 ± 3 mm Hg compared to the value of 71 ± 2 mm Hg for control periods of included experiments ($p<0.05$, unpaired t test). The mean control AVP level of excluded experiments was 17 ± 5 pg/ml whereas included rabbits had a level of 6 ± 3 pg/ml ($p=0.05$).

ANGIOTENSIN II INFUSION EXPERIMENTS

1. Effects on Plasma AII Concentration (Table 1)

Plasma AII concentration did not rise from baseline (41 ± 15 pg/ml) levels during or after 60-minute infusions of the 5% dextrose in water vehicle ($p>0.10$, $n=3$). AII infusions of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ increased plasma AII levels in all experiments, but this was followed by an elevated recovery level (176 pg/ml) in one rabbit. This resulted in a large recovery standard error and $p>0.05$ in one-way analysis of variance. However, the AII concentration during infusion (131 ± 2 pg/ml) was much larger than the control level of 25 ± 3 pg/ml ($p<0.001$, $n=3$) when compared by a paired t test. When 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII infusions were accompanied by 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of nitroprusside, plasma AII rose significantly ($p<0.001$) to levels not different from levels achieved by infusions of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ of AII alone ($p>0.10$, unpaired t test). Forty $ng \cdot kg^{-1} \cdot min^{-1}$ infusions of AII also raised plasma AII levels when administered either alone (191 ± 35 pg/ml,

$p<0.005$) or in combination with nitroprusside 194 ± 22 pg/ml, $p<0.005$). These two mean peak values were not different ($p>0.10$, unpaired t test). In all AII infusions except 20 $ng \cdot kg^{-1} \cdot min^{-1}$, recovery plasma AII concentrations were similar to preinfusion controls.

2. Effects on blood pressure (Tables 2 and 3)

When the 5% dextrose in water vehicle was infused alone, arterial pressure did not change significantly. Each dose of AII produced increases in arterial blood pressure. When nitroprusside was infused simultaneously with either 20 or 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII, arterial pressure did not change significantly from control values.

Figure 3 compares the pressor effects of AII infusions with those of AII + NP infusions. AII infusion of 40 $ng \cdot kg^{-1} \cdot min^{-1}$ resulted in a mean pressor response significantly greater than the response to 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII + 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP infusions ($p<0.05$, unpaired t test).

3. Effects on heart rate (Table 4 and 5)

Heart rate was not significantly affected by AII infusion either alone or in combination with nitroprusside infusion. However, during the recovery periods following 20 and 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII infusions, heart rate was elevated above control levels ($p<0.05$).

4. Effects on plasma corticosteroid concentration (Tables 6 and 7)

Infusions of AII alone had no significant effects on plasma glucocorticoid concentration (Table 6 and Figures 4 and 5). Plasma glucocorticoid concentration was unaltered by simultaneous infusions of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII and 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP. However, 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII + 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP infusions produced a 50% rise in mean

glucocorticoid level ($p<0.005$) which was sustained through the infusions. Glucocorticoids then returned to near the control concentration during the post-infusion recovery period (Table 7 and Figure 5).

5. Effects on plasma AVP concentration and osmolality

Infusions of AII alone had no significant effects on plasma AVP levels (Table 8). Simultaneous infusion of 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII and 3-6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ NP did result in a small but significant increase in AVP ($p<0.05$) (Table 9 and Figure 6). This increase did not persist and by the end of the 60-minute infusion, AVP levels had fallen significantly to near control levels, where they remained after the recovery period. 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII + NP infusions had no effect on AVP levels (Table 9). Baseline osmolalities did not differ between protocol groups and no changes were found in mean osmolalities over the time course of any protocol group (Table 11). The mean baseline osmolality for all groups was 279 $\pm$ 5 mOsm/kg.

NITROPRUSSIDE INFUSION EXPERIMENT

Nitroprusside was infused into one rabbit at a rate of 30-60 $\mu g \cdot kg^{-1} \cdot min^{-1}$ which produced a transient fall in mean arterial pressure from 62 to 42 mm Hg, over the course of 5 minutes. A blood sample was then taken (requiring 1.4 minutes) and when the arterial line was returned to the pressure transducer, mean pressure returned to 55 mm Hg. The mean pressure then rose gradually over the next ten minutes to 58 mm Hg immediately before the second sample was drawn. Plasma AII concentration rose from a preinfusion level of 56 pg/ml to 340 pg/ml in the first sample and 330 pg/ml in the second. Plasma corticosteroid

concentration rose from 64 ng/ml to 76 and 81 ng/ml, respectively. AVP levels were not measured.

HEMORRHAGE EXPERIMENTS

Table 10 illustrates the effects of hemorrhage on blood pressures, heart rate, and plasma AII, corticosteroid, and AVP concentrations. Mean blood pressure was not significantly lower than control levels until after the third hemorrhage when it decreased from 73 ± 4 to 63 ± 2 mm Hg ($p<0.05$). Heart rate and plasma AII levels did not change significantly during the hemorrhage.

Corticosteroid levels began at 60 ± 8 ng/ml but fell to 58 ± 7 ng/ml after the first 10% bleed. The value after the final bleed was 69 ± 8 ng/ml. One-way analysis of variance repeated over time indicated that values changed over the course of the experiment. However, Duncans multiple range test indicated that the value after 10% hemorrhage was significantly different than the final corticosteroid value ($p<0.05$). However, no corticosteroid levels significantly differed from the control value.

AVP levels rose precipitously after the third 10% hemorrhage ($p<0.01$). Two of the three animals measured had final AVP plasma levels higher than the upper range of the assay (62.5 pg/ml). Both of these animals were therefore assigned a value of 62.5 pg/ml for purposes of data analysis.

SODIUM DEPRIVATION EXPERIMENTS

Sodium deprivation plus diuretic injections increased the mean plasma AII level to 67 ± 31 pg/ml (n=6), a value that was significantly different than the level of 30 ± 4 pg/ml (n=26) found in sodium replete

controls ($p<0.05$, unpaired t test). The rabbit that was bled while anesthetized had a much higher AII level (221 pg/ml) than the other five sodium deprived rabbits.

Plasma sodium concentration was lower in sodium depleted rabbits (138.6 $\pm$ 1.2 mM, n=6) than sodium replete controls (142.1 $\pm$ 0.4 mM, n=9, $p<0.05$, unpaired t test). One sodium deprived rabbit had far lower levels (132.6 mM) than the others. This was the same rabbit with extremely elevated AII levels (221 pg/ml).

Corticosteroids were not altered by sodium deprivation with control values of 47 $\pm$ 10 pg/ml (n=6) in sodium replete rabbits compared to 42 $\pm$ 6 pg/ml (n=6) when the rabbits were depleted ($p>0.10$, paired t test).

DISCUSSION

This study served three purposes. The first was to develop the conscious rabbit as a model for investigation of the actions of angiotensin II (AII) on the release of adrenocorticotropic hormone (ACTH) and arginine vasopressin (AVP) from the pituitary. The second purpose was to compare the actions of AII in the rabbit with those previously discovered in other animals, such as the rat and dog. Finally, this study sought to determine whether the actions of AII on ACTH and AVP release were physiologically significant in the rabbit.

A major difficulty encountered during this study was overcoming the tendency for ACTH and corticosteroid levels to increase due to incidental stresses of experimentation. Corticosteroids are elevated in rabbits during such diverse situations as exposure to a new environment, handling, and venipuncture (Redgate et al., 1981; Fenske et al., 1982). It was therefore uncertain at the outset of this project whether rabbits could be studied within a few hours of catheterization.

A major finding of this study was that if properly handled and trained, conscious rabbits are suitable for acute studies of ACTH release. Although 28% of infusion experiments were rejected due to elevated baseline corticosteroid levels, the incidence of rejection fell markedly as refinements in training and experimental technique were implemented.

Because of the small number of rabbits included in each experimental group, further findings of this study must be regarded as preliminary, and more work will be necessary before they can be solidly established. Despite this, some of the results reported in this study

reached high levels of significance; it is expected that these findings will be confirmed in the future.

The second major finding of this study was that AII infusions can induce rises in plasma corticosteroid and AVP levels in rabbits. However, this effect could only be demonstrated when the highest dose of AII (40 $ng \cdot kg^{-1} \cdot min^{-1}$) was infused and its pressor effect was negated by concurrent nitroprusside coinfusion. This suggests that the pressor effect of AII may inhibit AVP and ACTH release, counteracting other stimulatory effects of AII. The enhancement of these actions of AII by nitroprusside agrees with findings of similar experiments in dogs (Brooks & Reid, 1986; Brooks et al., 1986). Still, the infusion rate necessary for these effects in rabbits was much higher than the rate found effective for both effects in dogs (10 $ng \cdot kg^{-1} \cdot min^{-1}$).

It is conceivable that nitroprusside promotes AVP and corticosteroid secretion through a mechanism other than by decreasing blood pressure. The fact that plasma AVP and corticosteroid levels were unaltered by infusion of nitroprusside with 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII argues against a direct action of nitroprusside on AVP or ACTH release. Evidence in dogs shows that the vasodilator hydralazine, when infused simultaneously with AII, is as effective as nitroprusside in stimulating AVP and ACTH (Brooks & Reid, 1986; Brooks et al., 1986). Brooks and colleagues also reported that sinoaortic baroreceptor denervation eliminated the effects of nitroprusside infusion on AVP and ACTH. This is further evidence that the action of nitroprusside on AVP and ACTH release is mediated by its effect on blood pressure alone. However, another mechanism of nitroprusside action cannot be decisively ruled out by these studies.

One interpretation of the finding that AII infusions can increase plasma corticosteroid levels is that AII stimulates the release of ACTH from the pituitary since corticosteroid levels are generally considered to be good indicators of pituitary ACTH release (Reid, 1984). However, the possibility of a direct stimulatory effect of AII on the adrenal cortex cannot be ignored. This effect could cause corticosteroid levels to rise independently from ACTH release. A study of Braverman and Davis (1973) showed that AII infusions increase corticosteroid output in adrenal veins of dexamethasone-treated rabbits. Since dexamethasone suppresses ACTH release, this was evidence of a direct effect of AII on the adrenal cortex. It should be noted that this effect was seen with 100 $ng \cdot kg^{-1} \cdot min^{-1}$ AII infusion rates; in the present study the highest rate used was 40 $ng \cdot kg^{-1} \cdot min^{-1}$. However, the Braverman and Davis study also found that the glucocorticoid release response to ACTH infusion was blunted by dexamethasone, raising the possibility that dexamethasone was acting at the adrenal cortex to inhibit glucocorticoid release. This could explain why high doses were needed to stimulate an increase in glucocorticoid output in their experiments. The finding of a direct adrenal effect does not preclude the possibility that AII also increases ACTH release. Resolution of this issue awaits studies measuring ACTH release more directly in the rabbit.

This study presents evidence that AII, when infused at a rate similar to that used in dogs, produces lower levels of plasma AII. Infusions of 40 $ng \cdot kg^{-1} \cdot min^{-1}$ AII in rabbits were found to raise plasma AII concentrations to 190 pg/ml. This value is similar to that found in dogs during infusions of only 10 $ng \cdot kg^{-1} \cdot min^{-1}$. Infusion rates of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ AII produced even lower plasma AII concentrations in

rabbits. Therefore, evidence exists that a given dose of AII is much less effective in raising plasma AII levels in rabbits than in dogs. It also accounts for the necessity of using larger doses of AII in rabbits than dogs to produce measurable rises in plasma AVP and ACTH levels.

Although AII is apparently able to increase plasma levels of AVP and glucocorticoids in the rabbit, it remains uncertain that the plasma levels of AII required for these effects are within the physiologic range. Two attempts were made to increase endogenous AII levels above those found effective during AII infusion.

The first attempt utilized a hypotensive hemorrhage of 30% of the rabbit's estimated blood volume. This hemorrhage produced no discernable increase in plasma AII levels, despite a 10 mm Hg drop in arterial blood pressure and a precipitous rise in AVP levels. This finding is consistent with a study in dogs (Claybaugh & Share, 1973) that demonstrated that hemorrhage can increase plasma AVP levels without affecting plasma renin activity. However, the present hemorrhage was more severe than hemorrhage rates in the dog which were effective in increasing both AVP and AII levels. This study also conflicts with results of a study in conscious rabbits which showed that a 20% hemorrhage raised plasma renin activity almost three-fold (Bartley & Anderson, 1984).

The second attempt to raise endogenous plasma AII levels utilized a ten-day sodium deprivation regimen. Sodium deprivation is an effective stimulus of the renin-angiotensin system in dogs (Brooks & Reid, 1983), rats (Semple, 1980), and rabbits (Braverman & Davis, 1973). In the latter study, 4-6 days of a sodium deficient diet plus

daily diuretic injections raised plasma renin activity in rabbits five-fold. The results of the present study were less impressive, with ten days of sodium deprivation producing a milder, although significant rise in plasma AII levels. Comparisons of daily sodium intake of the two rabbit groups may explain the difference in these findings. The sodium content of the low sodium rabbit chow used in the present experiment was 22 mEq/kg compared to a level of 7 mEq/kg in the chow used by Braverman and Davis (1973). Further experiments are needed with more severe sodium restriction to raise endogenous plasma AII levels maximally.

Supporting a physiologic role of AII in the release of AVP and ACTH in rabbits is the comparison of effective AII levels in rabbits (191 pg/ml) with those produced endogenously during sodium deprivation in the dog (172 pg/ml) and during hemorrhage in rats (500-1000 pg/ml). In addition, two of the rabbits in this study were found to have very high plasma AII levels. One sodium depleted rabbit required anesthesia for a cardiac puncture; it had a plasma AII level of 210 pg/ml. The other rabbit, given nitroprusside alone, experienced a 20 mm Hg fall in mean arterial pressure; it had plasma AII levels of 340 pg/ml. These preliminary data indicate that not only is AII effective in increasing AVP and corticosteroid release with plasma levels similar to endogenous levels seen in other animals, but the rabbit is also capable of producing these levels as well. Nevertheless, it is possible that these high rabbit AII plasma levels cannot be induced by physiological stimuli.

Cardiovascular responses to AII were observed in this study. the pressor response to AII infusion was similar to other published results in rabbits (Bartley & Anderson, 1984). It is notable that AII

Infusions did not significantly depress heart rates despite increases in mean arterial pressure. When the higher dose AII infusions ended and blood pressure fell to near control levels, heart rate increased markedly. A likely explanation is that sustained elevation in arterial pressure raised the baroreflex set point, resulting in reflex increases in heart rate when blood pressure fell to normal levels. Rapid arterial baroreceptor resetting has been observed after only 15 minutes of pressure elevation in rabbits (Dorward et al., 1982).

SUMMARY AND CONCLUSIONS

It was found that intraveous infusions of AII could stimulate rises in plasma AVP and glucocorticoid concentrations in the conscious rabbit. These effects of AII could only be demonstrated when nitroprusside was infused simultaneously with the highest AII infusion rate. This rate induced plasma AII levels that were similar to levels found to be effective for the stimulation of AVP and ACTH release in the dog.

Another finding was that for a given rate of AII infusion, plasma AII levels rise far less in rabbits than in dogs. This necessitated higher infusion rates in rabbits to reach the same effective plasma levels.

Attempts to raise endogenous AII levels in conscious rabbits by hemorrhage and sodium deprivation were only mildly successful. Neither procedure increased plasma AII concentration levels to levels similar to those found effective for AVP and glucocorticoid release.

It is therefore concluded that AII may increase the release of AVP and ACTH from the rabbit pituitary when its pressor effect is negated. This effect occurs when plasma levels are within the physiologic range of other animals. However, this study neither supports nor disproves the hypothesis that these effects of AII are physiologically relevant in the rabbit. These findings will be an important contribution to further studies of AII induction of AVP and ACTH release in conscious rabbits.

REFERENCES

Aguilera G, CC Chiueh, MK Mohan, KJ Catt: Role of angiotensin II in the regulation of ACTH secretion. Endocrinology 112A:90, 1983 [abstract]

Angeli A, B Orlandi, P Paccotti, R Tabasso, C Tamagnone, G Lavezzaro: Pituitary-adrenocortical function in patients during treatment with the angiotensin-converting enzyme inhibitor captopril. Clin Endocrinol 15:555-565, 1981

Altas SA, DB Case, JE Sealey, JH Laragh: Relationship between plasma renin and cortisol in hypertensive patients. Clin Sci 61:265s-268s, 1981

Bartley P, W Anderson: Prostaglandins and the renal responses to haemorrhage, angiotensin II and methoxamine in conscious rabbits. Clin Exp Pharm Physiol 11:71-80, 1984

Ben LK, J Maselli, LC Keil, IA Reid: Role of the renin-angiotensin system in the control of vasopressin and ACTH secretion during the development of renal hypertension in dogs. Hypertension 6:35-41, 1984

Braverman B, J Davis: Adrenal steroid secretion in the rabbit: Sodium depletion, angiotensin II, and ACTH. Am J Physiol 225:1306-1310, 1973

Bravo EL, MC Khosla, FM Bumpus: Vascular and adrenocortical responses to a specific antagonist of angiotensin II. Am J Physiol 228:110-114, 1975

Brooks VL, L Daneshvar, IA Reid: Mechanism of the rise in plasma

corticosteroids after intravenous angiotensin II infusion in conscious dogs. Fed Proc 43:717, 1984 [abstract]

Brooks VL, LC Keil, IA Reid: Role of the renin-angiotensin system in the control of vasopressin secretion in conscious dogs. Circ Res 58:829-838, 1986

Brooks VL, RL Malvin: An intracerebral, physiologic role for angiotensin: Effects of central blockade. Fed Proc 38:2272-2275, 1979

Brooks VL, IA Reid: Effects of blockade of brain and angiotensin II receptors in conscious, sodium-deprived dogs. Am J Physiol 245:R881-R887, 1983

Brooks VL, IA Reid: Interaction between angiotensin II and the baroreceptor reflex in the control of adrenocorticotropic hormone secretion and heart rate in conscious dogs. Circ Res 58:816-828, 1986

Claybaugh JR, L Share: Role of renin-angiotensin system in the vasopressin response to hemorrhage. Endocrinology 90:453-460, 1972

Claybaugh JR, L Share: Vasopressin, renin and cardiovascular responses to continuous slow hemorrhage. Am J Physiol 224:519-523, 1973

Claybaugh JR, L Share, K Shimizu: The inability of infusions of angiotensin to elevate plasma vasopressin concentration in the anesthetized dog. Endocrinology 90:1647-1652, 1972

Cowley AW, Jr, SJ Switzer, MM Skelton: Vasopressin, fluid, and electrolyte response to chronic angiotensin II infusion. Am J Physiol 240:R130-R138, 1981

Daniels-Severs A, E Ogden, J Vernikos-Danellis: Effects of centrally

administered angiotensin II in the unanesthetized rat. Physiol Behav 7:785-787, 1971

Davis JO, RH Freeman: The use of angiotensin II blockade to study adrenal steroid secretion. Fed Proc 35:2508-2511, 1976

Deschepper CF, WF Ganong: Interference of eluates from octadecy/cartridges with an angiotensin II radioassay. Peptides 1:365-367, 1986

Dickinson CJ, R Yu: Mechanisms involved in the progressive pressor response to very small amounts of angiotensin in conscious rabbits. Circ Res 20-21 (Suppl II):157-163, 1967

Dorward PK, MC Andresen, SL Burke, JR Oliver, PI Korner: Rapid resetting of the aortic baroreceptors in the rabbit and its implications for short-term and longer term reflex control. Circ Res 50:428-439, 1982

Fenske M, E Fuchs, B Probst: Corticosteroid, catecholamine and glucose levels in rabbits after repeated exposure to a novel environment or administration of (1-24) ACTH or insulin. Life Sci 31:127-132, 1982

Fisher LA, MR Brown: Corticotropin-releasing factor and angiotensin II: Comparison of CNS actions to influence neuroendocrine and cardiovascular function. Brain Res 296:41-47, 1984

Gaillard RC, A Grossman, G Gillies, LH Rees, GM Besser: Angiotensin II stimulates the release of ACTH from dispersed rat anterior pituitary cells. Clin Endocrinol 15:573-578, 1981

Gann DS: Cortisol secretion after hemorrhage: Multiple mechanisms. Nephron 23:119-124, 1979

Gann DS, MF Dallman, WC Engeland: Reflex control and modulation of ACTH and corticosteroids. Int Rev Physiol 24:157-199, 1981

Gann DS, JC Pirkle: Role of cortisol in the restitution of blood volume after hemorrhage. Am J Surg 130:565-569, 1975

Ganong WF: Review of Medical Physiology. Lange Medical Publications, Los Altos, CA, pp 178-197 and 364-370, 1981

Ganong WF, J Shinsako, IA Reid, LC Keil, DL Hoffman, EA Zimmerman: Role of vasopressin in the renin and ACTH responses to intraventricular angiotensin II. Ann NY Acad Sci 394:619-624, 1982

Guo GB, MD Thames, FM Abboud: Differential baroreflex control of heart rate and vascular resistance in rabbits: Relative role of carotid, aortic, and cardiopulmonary baroreceptors. Circ Res 50:554-565, 1982

Hammer M, K Olgaard, S Madsen: The inability of angiotensin II infusion to raise plasma vasopressin levels in haemodialysis patients. Acta Endocrinol 95:422-426, 1980

Hashimoto K, S Yunoki, H Hosogi, J Takahara, T Ofuji: Specificity of cultured anterior pituitary cells in detecting corticotropin releasing factor(s): The effect of biologically active peptides and neurotransmitter substances on ACTH release in pituitary cell cultures. Acta Med Okayama 33:81-90, 1979

Ishikawa S-E, T Saito, S Yoshida: The effect of osmotic pressure and angiotensin II on arginine vasopressin release from guinea pig hypothalamo-neurohypophyseal complex in organ culture. Endocrinology 106:1571-1578, 1980

Keil LC, J Summy-Long, WB Severs: Release of vasopressin by angiotensin II. Endocrinology 96:1063-1065, 1975

Keller-Wood M, B Kimura, J Shinsako, MI Phillips: Interaction between

CRF and angiotensin II in control of ACTH and adrenal steroids. Am J Physiol 250:R396-R402, 1986

Klingbell CK, LC Keil, D Chang, IA Reid: Role of vasopressin in stimulation of ACTH secretion by angiotensin II in conscious dogs. Am J Physiol 251:E52-E57, 1986

Knepel W, DK Meyer: Role of the renin-angiotensin system in isoprenaline-induced vasopressin release. J Cardiovasc Pharmacol 2:815-824, 1980

Laragh J, J Sealey: The renin-angiotensin-aldosterone hormonal system and regulation of sodium, potassium and blood pressure homeostasis. In: Handbook of Physiology, Section 8: Renal Physiology, edited by J Orloff and R Berliner. American Physiological Society: Washington, DC, p 849, 1973

Lee M, TN Thrasher, LC Keil, DJ Ramsay: Cardiac receptors, vasopressin, and corticosteroid release during arterial hypotension in dogs. Am J Physiol 251:R614-R620, 1986

Maran JW, FE Yates: Cortisol secretion during intrapituitary infusion of angiotensin II in conscious dogs. Am J Physiol 233:E273-E285, 1977

Morton JJ, PF Semple, IM Ledingham, B Stuart, MA Tehrani, AR Garcia, G McGarrity: Effect of angiotensin-converting enzyme inhibitor (SQ 20881) on the plasma concentration of angiotensin I, angiotensin II, and arginine vasopressin in the dog during hemorrhagic shock. Circ Res 41:301-308, 1977

Mouw D, JP Bonjour, RL Malvin, A Vander: Central action of angiotensin in stimulating ADH release. Am J Physiol 220:239-242, 1971

Murphy BEP: Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of

various steroids in body fluids by competitive protein-binding radioassay. J Clin Endocr 27:973-990, 1967

Padfield PL, JJ Morton: Effects of angiotensin II on arginine-vasopressin in physiological and pathological situations in man. J Endocrinol 74:251-259, 1977

Phillips MI: Functions of angiotensin in the central nervous system [review]. Ann Rev Physiol 49:413-435, 1987

Raff H, J Shinsako, CE Wade, LC Keil, MF Dallman: Acute volume expansion decreases adrenocortical sensitivity to ACTH and angiotensin II. Am J Physiol 249:R611-R616, 1985

Ramsay DJ, LC Keil, MC Sharpe, J Shinsako: Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion. Am J Physiol 234:R66-R71, 1978

Rayyis SS, R Horton: Effect of angiotensin II on adrenal and pituitary function in man. J Clin Endocrinol 32:539-546, 1971

Redgate ES, RR Fox, FH Taylor: Strain and age effects immobilization stress in Jax rabbits. Proc Soc Exp Biol Med 166:442-448, 1981

Reid IA: Is there a brain renin-angiotensin system? Circ Res 41:147-153, 1977

Reid IA: The renin angiotensin system. In: Hypertensin Research: Methods and Models, edited by RM Radzialowski. Dekker: New York, pp 101-137, 1981

Reid IA: Actions of angiotensin II on the brain: Mechanisms and physiologic role [editorial review]. Am J Physiol 246:F533-F543, 1984

Reid IA, VL Brooks, CD Rudolph, LC Keil: Analysis of the actions of

angiotensin on the central nervous system of conscious dogs. Am J Physiol 243:R82-R91, 1982

Rivier C, W Vale: Effect of angiotensin II on ACTH release in vivo: Role of corticotropin-releasing factor. Regul Pept 7:253-258, 1983

Rosendorff C, RD Lowe, H Lavery, WI Cranston: Cardiovascular effects of angiotensin mediated by the central nervous system of the rabbit. Cardiovasc Res 4:36-43, 1970

Schoenenberg P, P Kehrer, AF Muller, RC Gaillard: Angiotensin II potentiates corticotropin releasing activity of CRF^{41} in rat anterior pituitary cells: Mechanism of action. Neuroendocrinology 45:86-90, 1987

Semple PF: The effects of hemorrhage and sodium depletion on plasma concentrations of angiotensin II and [des-Asp^1] angiotensin II in the rat. Endocrinology 107:771-773, 1980

Share L: Interrelations between vasopressin and the renin-angiotensin system. Fed Proc 38:2267-2271, 1979

Shimizu K, L Share, JR Claybaugh: Potentiation by AII of the vasopressin response to an increasing plasma osmolality. Endocrinology 93:42-50, 1973

Sladek CD, ML Blair, DJ Ramsay: Further studies on the role of angiotensin in the osmotic control of vasopressin release by the organ-cultured rat hypothalamo-neurohypophyseal system. Endocrinology 111:599-607, 1982

Sobel DO: Characterization of angiotensin-mediated ACTH release. Neuroendocrinology 36:249-253, 1983

Sobel D, A Vagnucci: Angiotensin II mediated ACTH release in rat pituitary cell culture. Life Sci 30:1281-1286, 1982

Spinedi E, A Negro-Vilar: Angiotensin II increases ACTH release in the absence of endogenous arginine-vasopressin. Life Sci 34:721-729, 1984

Thrasher TN, LC Keil: Effect of subfornical organ ablation on secretion of arginine vasopressin and adrenocorticotrophic hormone in response to angiotensin II in conscious dogs. Fed Proc 45:166, 1986 [abstract]

Uhlich E, P Weber, J Eigler, U Groschel-Stewart: Angiotensin stimulated AVP-release in humans. Klin Wochenschr 53:177-180, 1975

Undesser KP, EM Hasser, JR Haywood, AK Johnson, VS Bishop: Interactions of vasopressin with the area postema in arterial baroreflex function in conscious rabbits. Circ Res 56:410-417, 1985a

Undesser KP, P Jing-Yun, MP Lynn, VS Bishop: Baroreflex control of sympathetic nerve activity after elevations of pressure in conscious rabbits. Am J Physiol 248:H827-H834, 1985b

Usberti M, S Federico, G Di Minno, B Ungaro, G Ardillo, C Pecoraro, B Cianciaruso, A Cerbone, F Cirillo, M Pannain, A Gargiulo, V Andreucci: Effects of angiotensin II on plasma ADH, prostaglandin synthesis, and water excretion in normal humans. Am J Physiol 248:F254-F259, 1985

Vale W, J Vaughan, M Smith, G Yamamoto, J Rivier, C Rivier: Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophyseal peptides, and other substances on cultured corticotropic cells. Endocrinology 113:1121-1131, 1983

Van Houten M, EL Schiffrin, JFE Mann, BI Posner, R Boucher:

Radioautographic localization of specific binding sites for blood-borne angiotensin II in the rat brain. Brain Res 186:480-485, 1980

Van Houten M, ML Mangiapane, IA Reid, WF Ganong: [sar^1, Ala^8] angiotensin II in cerebrospinal fluid blocks the binding of blood-borne [^{125}I] angiotensin II to the circumventricular organs. Neuroscience 10:1421-1426, 1983

Wade CE, LC Keil, DJ Ramsay: Effects of sodium depletion and angiotensin II on osmotic regulation of vasopressin. Am J Physiol 250:R287-R291, 1986

Whitworth JA, D Saines, R Thatcher, A Butkus, BA Scoggins, JP Coghlan: Blood pressure, renal and metabolic effects of ACTH in normotensive man. Clin Sci 61:269s-272s, 1981

Winer BJ: Statistical Principles in Experimental Design. McGraw, New York, 1971

Wood CE, J Shinsako, MF Dallman: Comparison of canine corticosteroid responses to mean and phasic increases in ACTH. Am J Physiol 242:E102-E108, 1982

Yamazaki T, K Sagawa: Hypotension 1.5 min after 10% hemorrhage permits evaluation of rabbit's baroreflex. Am J Physiol 249:H450-H456, 1985

Table 1: EFFECT OF AII INFUSION ON PLASMA AII CONCENTRATION (pg/ml)

INFUSION	SAMPLE C	SAMPLE E_2	SAMPLE R	ONE WAY ANALYSIS OF VARIANCE p	ONE WAY ANALYSIS OF VARIANCE n
AII 20ng·kg^{-1}·min^{-1}	25±3	131±2	83±47	>.05	3
AII 20ng·kg^{-1}·min^{-1} + 3μg·kg^{-1}·min^{-1} NP	35±7	149±13*	42±8	<.001	4
AII 40ng·kg^{-1}·min^{-1}	40±24	191±35*	35±12	<.005	3
AII 40ng·kg^{-1}·min^{-1} + 3–6μg·kg^{-1}·min^{-1} NP	22±6	194±22*	24±5	<.001	5

AII=Angiotensin II; NP=Nitroprusside; C=Control; E_2 sample drawn after 60min of AII infusion; R=Recovery(90min). * different than control value–p<.001. Sample values are means±SE.

Table 2: EFFECT OF AII INFUSION ON BLOOD PRESSURE (mm Hg)

	SAMPLE				ONE WAY ANALYSIS OF VARIANCE		
AII DOSE	C	E_1	E_2	R	p	F	n
0 (Control)	65	64	71	75			2
10ng·kg^{-1}·min^{-1}	85	93	97	78			1
20ng·kg^{-1}·min^{-1}	75±6	88±10*	85±8*	71±4	<.05	8.582	3
40ng·kg^{-1}·min^{-1}	72±4	98±3*	99±6*	77±4	<.001	62.55	3

C=Control; E_1=30min; E_2=60min; R=Recovery(90min). * different than control value–p≤0.05; * different than control value–p<.001. AII=Angiotensin II. Sample values are means±SE.

Table 3: EFFECTS OF AII & NP INFUSION ON BLOOD PRESSURE (mm Hg)

DOSE		SAMPLE C	E_1	E_2	R	ONE WAY ANALYSIS OF VARIANCE p	F	n
$20ng \cdot kg^{-1} \cdot min^{-1}$	AII							
$3\mu g \cdot kg^{\pm 1} \cdot min^{-1}$	NP	73±5	71±6	73±8	72±5	>.10	.208	3
$40ng \cdot kg^{-1} \cdot min^{-1}$	AII							
$3-6\mu g \cdot kg^{+-1} \cdot min^{-1}$	NP	63±6	68±8	69±7	65±4	>.10	.592	5

C=Control; E_1=30min; E_2=60min; R=Recovery(90min); AII=Angiotensin II; NP=Nitroprusside Sample values are means±SE.

Table 4: EFFECT OF All INFUSION ON HEART RATE (bpm)

All DOSE	SAMPLE C	E_1	E_2	R	ONE WAY ANALYSIS OF VARIANCE p	F	n
0 (Control)	242±13	246±10	245±8	252±9	>.10	.3521	3
20ng·kg^{-1}·min^{-1}	225±12	210±9	240±20	280±20‡	<.05	10.87	3
40ng·kg^{-1}·min^{-1}	220±37	205±34	230±48	275±38*	<.05	5.401	3

C=Control; E_1=30min; E_2=60min; R=Recovery(90min); All=Angiotensin II; bpm=beats per minute. ‡ different than control value—p<0.01; * different than control value—p<0.05. Sample values are means±SE.

Table 5: EFFECT OF AII & NP INFUSION ON HEART RATE (bpm)

DOSE		SAMPLE C	E_1	E_2	R	ONE WAY ANALYSIS OF VARIANCE p	F	n
$20ng \cdot kg^{-1} \cdot min^{-1}$	AII							
+ $3\mu g \cdot kg^{-1} \cdot min^{-1}$	NP	228±15	243±4	252±3	255±10	>.05	3.942	3
$40ng \cdot kg^{-1} \cdot min^{-1}$	AII							
+ $3-6\mu g \cdot kg^{-1} \cdot min^{-1}$	NP	217±14	238±19	231±16	255±21	>.05	3.346	5

*C=Control; E_1=30min; E_2=60min; R=Recovery(90min). AII=Angiotensin II; NP=Nitroprusside; bpm=beats per minute.

Table 6: EFFECT OF AII INFUSION ON PLASMA GLUCOCORTICOID CONC. (ng/ml)

	SAMPLE				ONE WAY ANALYSIS OF VARIANCE		
AII DOSE	C	E_1	E_2	R	p	F	n
0 (Control)	49±10	62±13	74±19	76±21	>.10	0.914	4
10ng·kg^{-1}·min^{-1}	51	64	64	51			2
20ng·kg^{-1}·min^{-1}	53±19	51±13	42±16	45±16	>.10	0.251	3
40ng·kg^{-1}·min^{-1}	69±12	80±13	81±12	87±17	>.05	3.474	4

Sample values are means±SE. *C=Control; E_1 30min; E_2=60min; R=Recovery (90min). AII=Angiotensin II.

Table 7: EFFECT OF AII & NP INFUSION ON PLASMA GLUCOCORTICOID CONCENTRATION (ng/ml)

DOSE		SAMPLE C	E_1	E_2	R	ONE WAY ANALYSIS OF VARIANCE p	F	n
$20ng \cdot kg^{-1} \cdot min^{-1}$ + $3\mu g \cdot kg^{-1} \cdot min^{-1}$	AII							
	NP	70±10	73±10	74±8	59±13	>.10	.901	4
$40ng \cdot kg^{-1} \cdot min^{-1}$ ± $3-6\mu g \cdot kg^{-1} \cdot min^{-1}$	AII							
	NP	44±8	64±5**	66±6**	44±12	<.005	7.26	5

Sample values are means±SE. C=Control; E_1=30min; E_2=60min; R=Recovery (90min). AII=Angiotensin II; NP=Nitroprusside. **different than control level—p<0.01.

Table 8: EFFECTS OF AII INFUSION ON PLASMA VASOPRESSIN CONC. (pg/ml)

	SAMPLE				ONE WAY ANALYSIS OF VARIANCE	
AII DOSE	C	E_1	E_2	R	p	n
0 $ng \cdot kg^{-1} \cdot min^{-1}$	2.4±.7	3.9±.9	2.9±1.0	1.8±.8	>.10	3
10$ng \cdot kg^{-1} \cdot min^{-1}$	11.2	7.7	3.7	3.7		2
20$ng \cdot kg^{-1} \cdot min^{-1}$	1.8±1.2	2.8±1.1	3.8±1.6	9.2±8.1	>.10	3
40$ng \cdot kg^{-1} \cdot min^{-1}$	14.5±11.7	6.0±3.0	4.4±1.2	9.5±6.0	>.10	4

Sample values are means±SE. C=Control; E_1=30min; E_2=60min; R=Recovery(90min); AII=Angiotensin II.

Table 9: EFFECTS OF AII & NP INFUSION ON PLASMA VASOPRESSIN CONC. (pg/ml)

	SAMPLE				ONE WAY ANALYSIS OF VARIANCE	
AII DOSE	C	E_1	E_2	R	p	n
$20ng \cdot kg^{-1} \cdot min^{-1}$ AII						
$3\mu g \cdot kg^{-1} \cdot min^{-1}$ NP	7.0±2.8	7.3±2.5	7.9±3.4	3.2±.9	>.05	4
$40ng \cdot kg^{-1} \cdot min^{-1}$ AII						
+ $3-6\mu g \cdot kg^{-1} \cdot min^{-1}$ NP	2.6±.7	4.4±1.3*	3.0±.6	2.7±.7	<.05	5

Sample values are means±SE. C=Control; E_1=30min; E_2=60min; R=Recovery(90min); AII=Angiotensin II; NP=Nitroprusside. * different than control value—p<.05.

Table 10: EFFECTS OF HEMORRHAGE ON BP, HR, PLASMA AII, CORTICOSTEROID AND AVP CONCENTRATION

	SAMPLE				ONE WAY ANALYSIS OF VARIANCE	
	C	H3	H4	H5	p	n
AMNT. HEMORRHAGED (% EBV)		10%	20%	30%		
BP (mm Hg)	73±4	69±4	67±2	63±2*	<.05	3
HR (bpm)	237±4	254±15	292±16	296±19	>.05	3
AII (pg/ml)	24±5	31±8	42±16	47±15	>.05	4
Corts (ng/ml)	60±8	58±7	67±10	69±8	<.05	4
AVP (pg/ml)	4.0±.6	3.8±.6	15±3	49±13	<.01	3

C=Control; EBV=Estimated blood volume; BP=Blood pressure; HR=Heart rate; bpm=Beats per minute; AII=Angiotensin II; Corts=Corticosteroids; AVP=Arginine vasopressin. Samples H3–H5 taken after % EBV removed. * different than control value–p<.05. Sample values are means ±SE.

Table 11: PLASMA OSMOLALITY (mOsm/kg) DURING All INFUSIONS

		SAMPLE				
		C	E_1	E_2	R	n
0 $ng \cdot kg^{-1} \cdot min^{-1}$	All	277	276±2	275±2	278±2	3
10$ng \cdot kg^{-1} \cdot min^{-1}$	All	281	281	282	283	2
20$ng \cdot kg^{-1} \cdot min^{-1}$	All	278±2	278±2	278±2	281±2	3
40$ng \cdot kg^{-1} \cdot min^{-1}$	All	276±4	277±3	276±3	278±3	4
20$ng \cdot kg^{-1} \cdot min^{-1}$ + 3$\mu g \cdot kg^{-1} \cdot min^{-1}$	All NP	280±2	280±1	281±1	284±1	4
40$ng \cdot kg^{-1} \cdot min^{-1}$ + 3–6$\mu g \cdot kg^{-1} \cdot min^{-1}$	All NP	276±3	276±3	278±4	280±3	4

See tables 1–10 for abbreviations

FIGURE 1: Experimental Set Up.

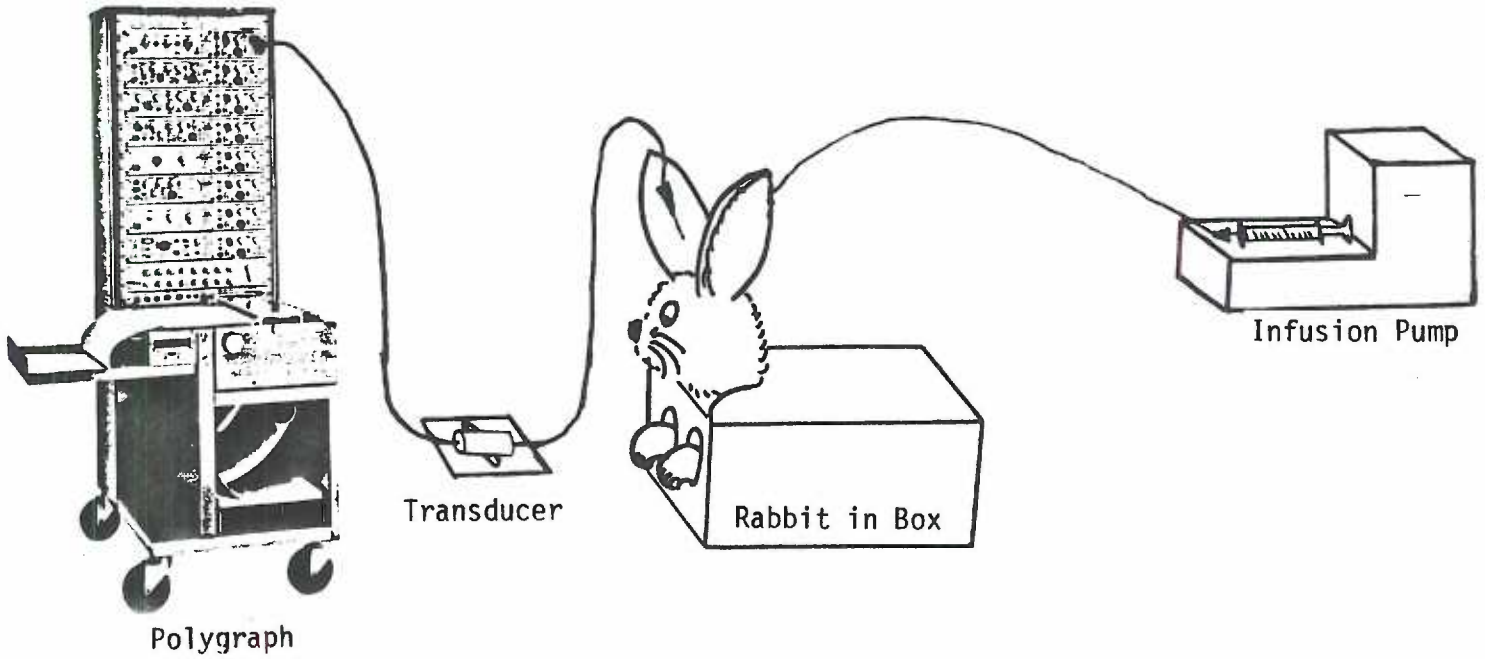

Infusion Pump
Rabbit in Box
Transducer
Polygraph

FIGURE 2: AII Infusion Protocol. AII = angiotensin II, NP = nitroprusside, AVP = vasopressin, CORTS = glucocorticoids. AVP, AII, and CORTS refer to assays applied to plasma samples above.

ANGIOTENSIN II INFUSION PROTOCOL

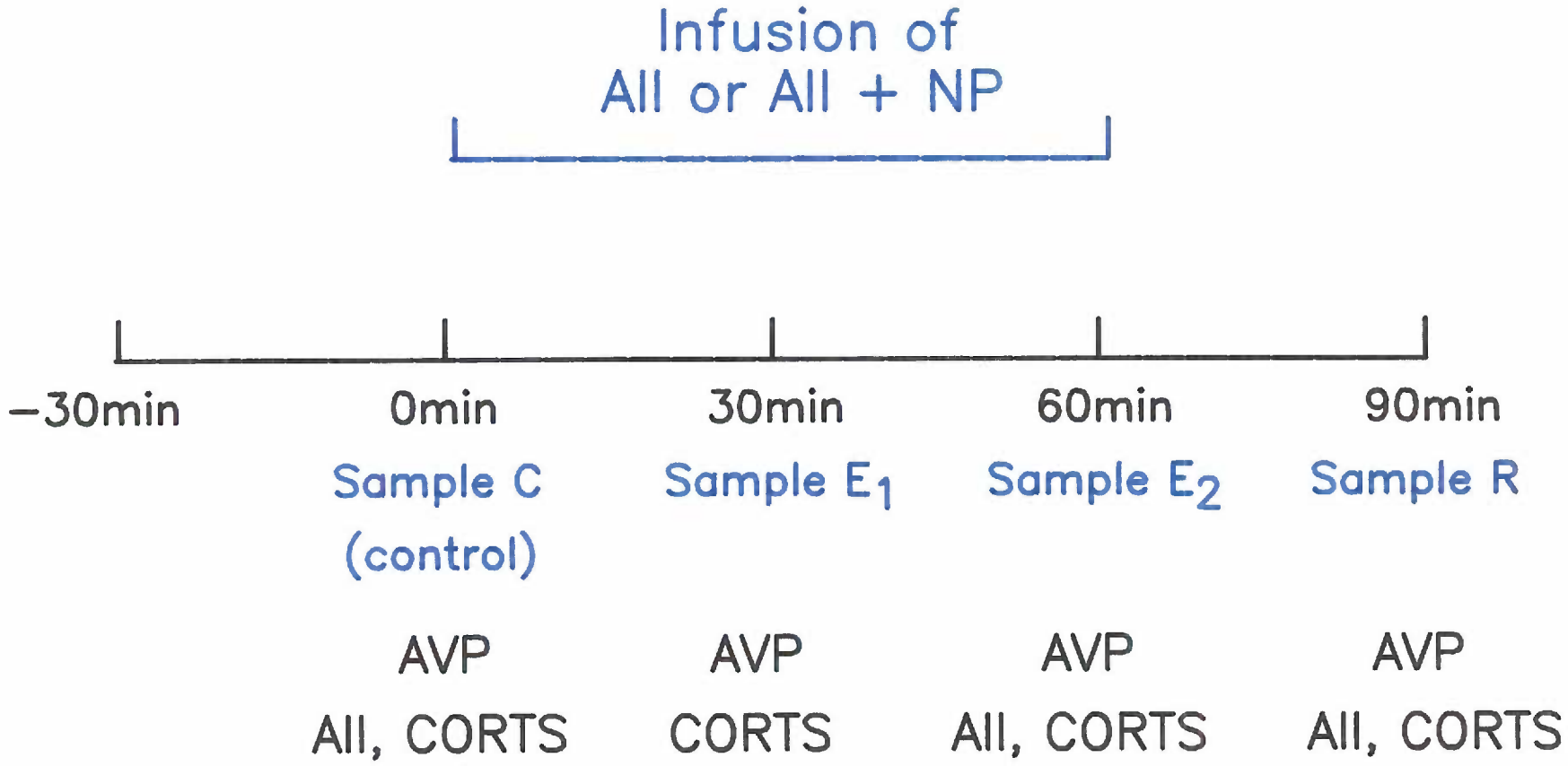


FIGURE 3: The change (Δ) in mean arterial pressure from control (mean ± SE) during infusion of two doses of angiotensin II (AII) either alone or in combination with nitroprusside. * indicates that blood pressure responses to infusions 3 and 4 differed significantly ($p<0.05$, unpaired t test).

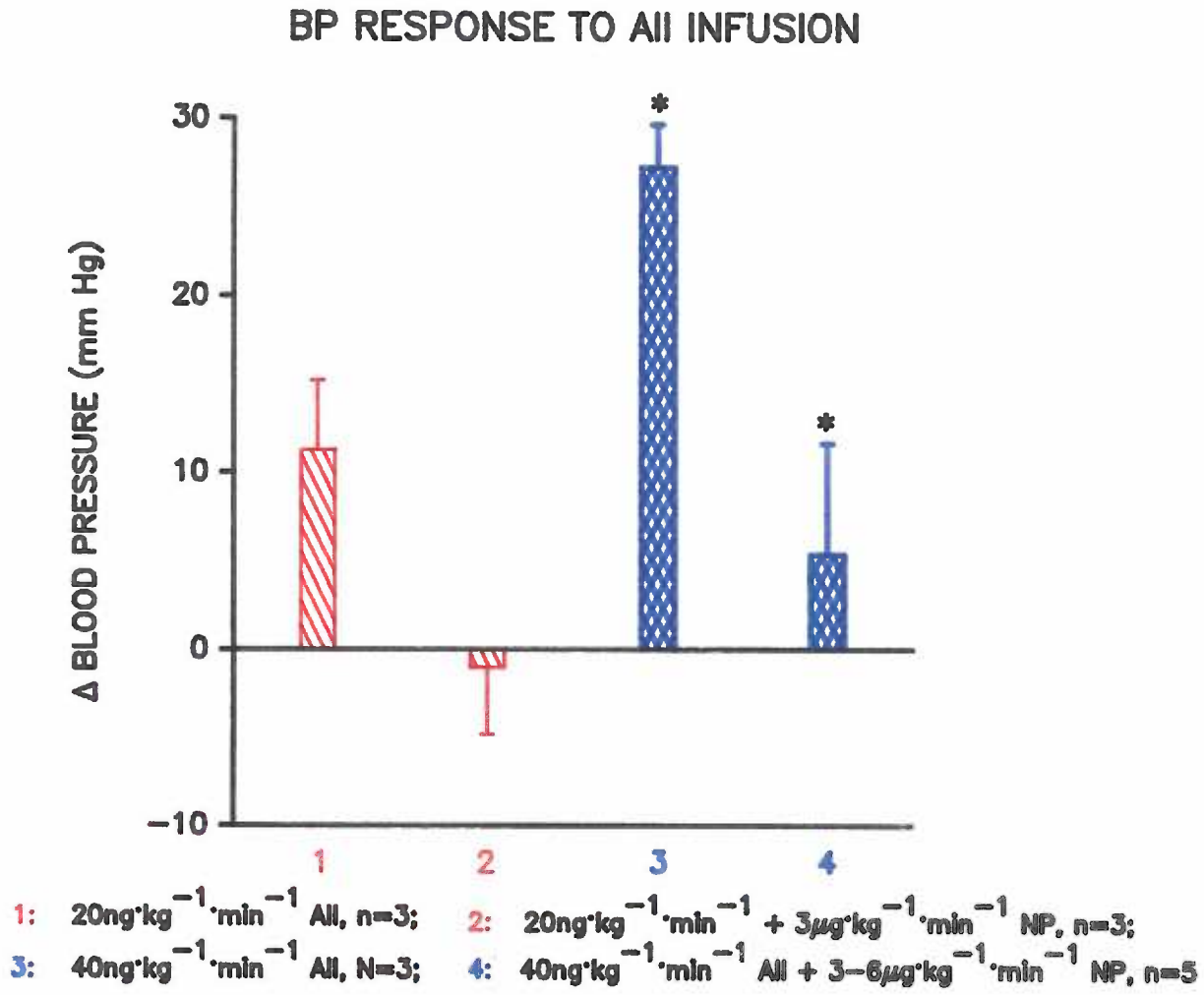

BP RESPONSE TO AII INFUSION
Δ BLOOD PRESSURE (mm Hg)
30
20
10
0
−10
*
*
1
2
3
4
1: 20ng·kg−1·min−1 AII, n=3;
2: 20ng·kg−1·min−1 + 3μg·kg−1·min−1 NP, n=3;
3: 40ng·kg−1·min−1 AII, N=3;
4: 40ng·kg−1·min−1 AII + 3–6μg·kg−1·min−1 NP, n=5

FIGURE 4: Effect of 20 $ng \cdot kg^{-1} \cdot min^{-1}$ angiotensin II (AII) infusion either alone or in combination with 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ nitroprusside (NP) on plasma corticosteroid concentration. Values are means ± SE.

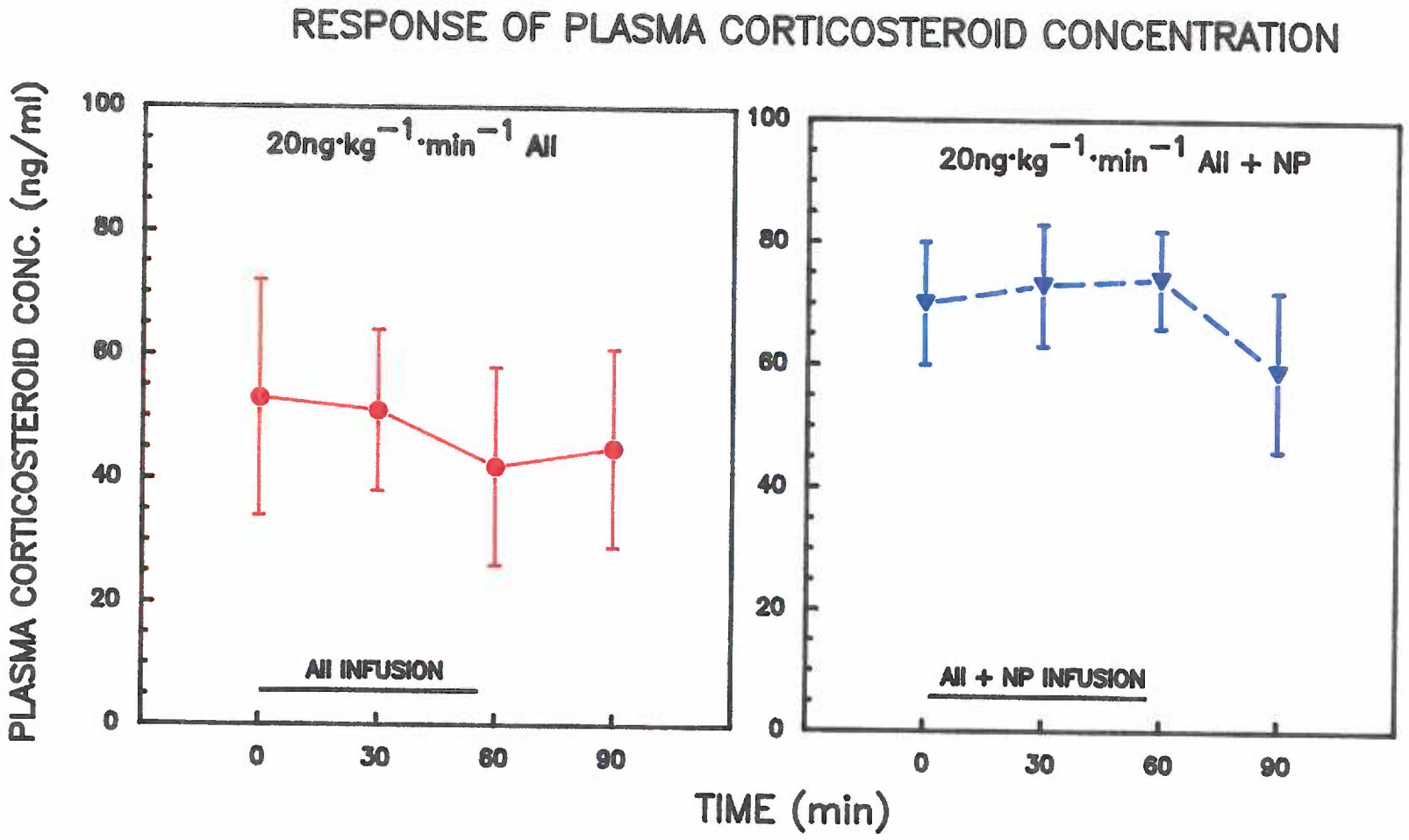

RESPONSE OF PLASMA CORTICOSTEROID CONCENTRATION
PLASMA CORTICOSTEROID CONC. (ng/ml)
100
80
60
40
20
0
20ng·kg⁻¹·min⁻¹ AII
AII INFUSION
20ng·kg⁻¹·min⁻¹ AII + NP
AII + NP INFUSION
0
30
60
90
TIME (min)

FIGURE 5: Effect of 40 $ng \cdot kg^{-1} \cdot min^{-1}$ angiotensin II (AII) infusion either alone or in combination with nitroprusside (NP) on plasma corticosteroid concentration. Values are means ± SE. * indicates values significantly different than controls ($p<0.005$, $n=5$).

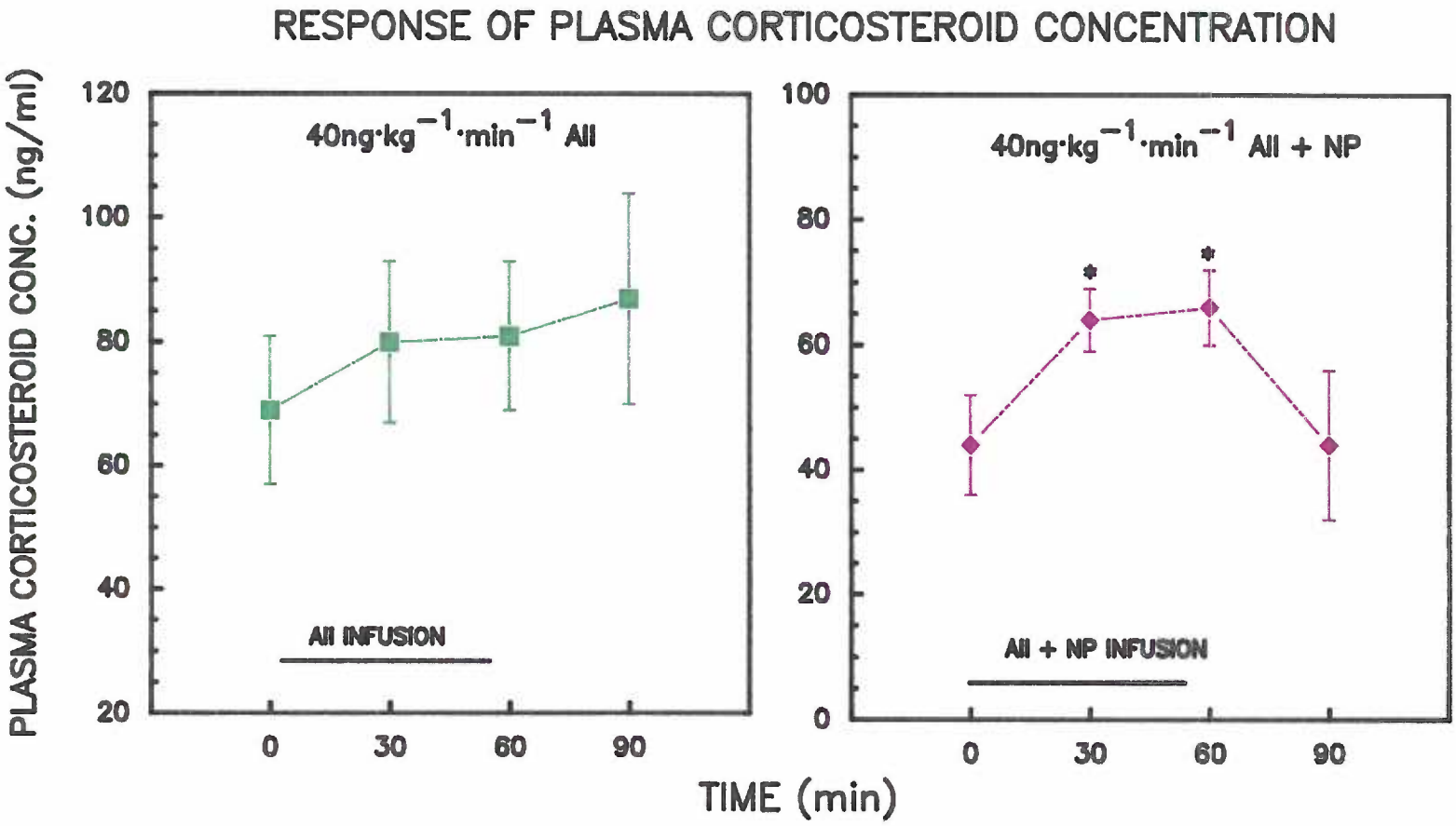
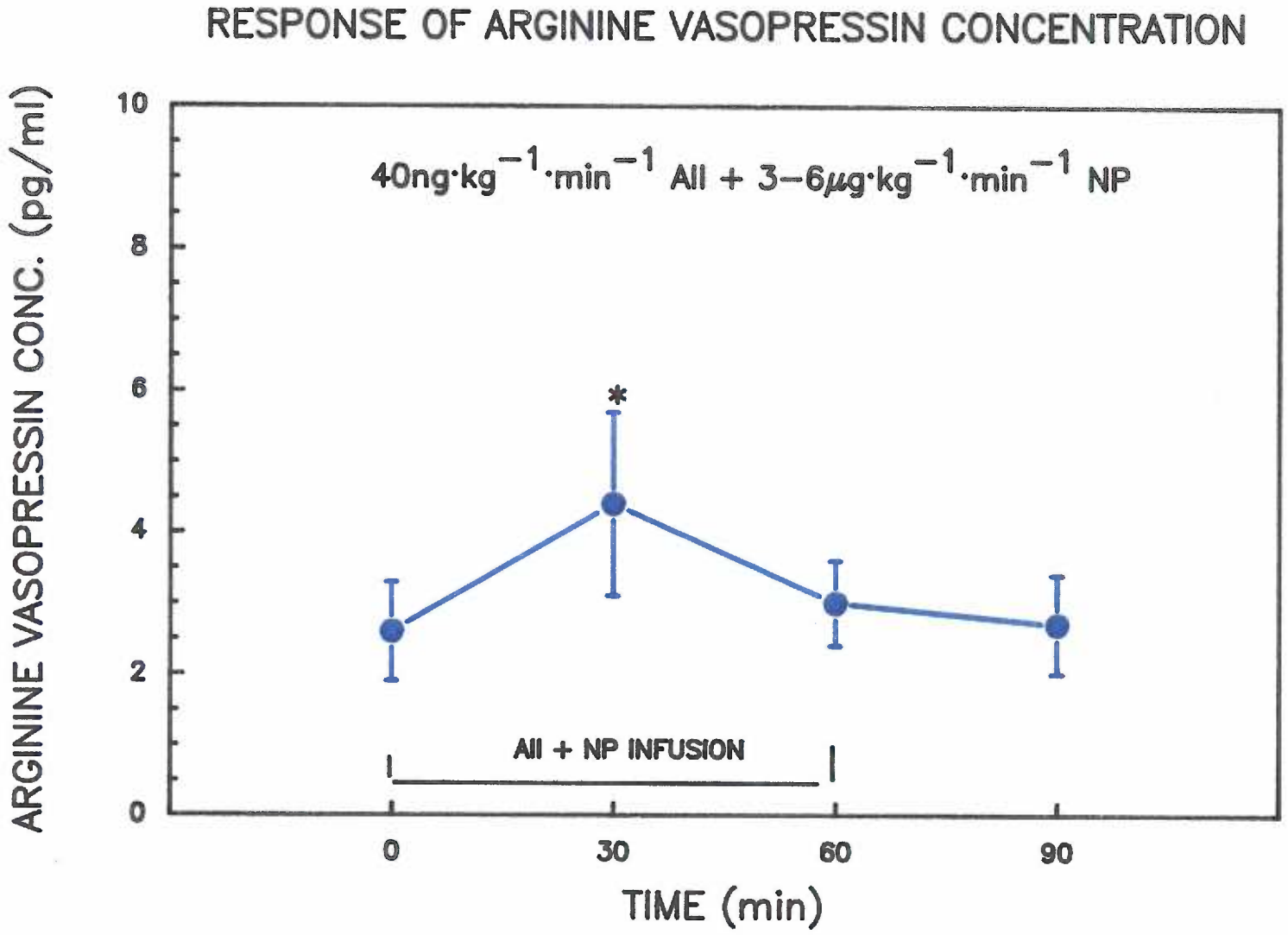

RESPONSE OF PLASMA CORTICOSTEROID CONCENTRATION
PLASMA CORTICOSTEROID CONC. (ng/ml)
120
100
80
60
40
20
40ng·kg⁻¹·min⁻¹ AII
AII INFUSION
0
30
60
90
100
80
60
40
20
0
40ng·kg⁻¹·min⁻¹ AII + NP
*
*
AII + NP INFUSION
0
30
60
90
TIME (min)

FIGURE 6: Effect of 40 $ng \cdot kg^{-1} \cdot min^{-1}$ angiotensin II (AII) + 3 µg/kg/min nitroprusside (NP) infusion on plasma AVP concentration. Values are means ± SE. * indicates value significantly different than control ($p<0.05$, $n=5$).


RESPONSE OF ARGININE VASOPRESSIN CONCENTRATION
ARGININE VASOPRESSIN CONC. (pg/ml)
10
8
6
4
2
0
40ng·kg⁻¹·min⁻¹ AII + 3–6μg·kg⁻¹·min⁻¹ NP
*
AII + NP INFUSION
0
30
60
90
TIME (min)

APPENDIX A: ARTERIAL CATHETERIZATION TECHNIQUES

Suggestions for implanting 18 or 20 gauge catheters (Quik-Cath, Intravascular Over-the-Needle Catheter, Travenol) into the central ear artery of the rabbit.

1. Shave ears the day before the catheterization.
2. Attempt arterial catheterization before venous catheterization when both are needed. Arterial catheterization has a much higher failure rate.
3. Infuse lidocaine without epinephrine subcutaneously bilaterally to the catheterization site.
4. Avoid over use of lidocaine since it tends to vasoconstrict ears in local areas of subcutaneous infusion.
5. Use a 25-gauge needle to infuse lidocaine.
6. Avoid puncturing small but visible blood vessels lateral to central artery. Hemotoma formation also is associated with central artery constriction.
7. Prepare heparinized saline filled tubing and heparinized saline flush solutions before catheterization. Also prepare cloth tape strips for securing the catheter.
8. 18-gauge catheters seem to perform better in providing consistent blood pressure readings but 20-gauge may be necessary for catheterization of small or somewhat constricted vessels.
9. Attempt to maximize arterial vasodilation immediately before inserting the catheter. This can be done by gently stroking the ear along the course of the artery or applying slight pressure with thumb and forefinger on both sides of

the ear and sliding along the course of the artery toward the base of the ear, emptying the artery. When fingers are removed in the latter maneuver, the artery often refills to a larger diameter.

10. Another technique for increasing arterial diameter is that of flicking the ear several times, which usually causes transient arterial engorgement. However, this is short-lived and the maneuver tends to irritate the rabbit.
11. The catheter should be inserted into the skin bevel up directly over the artery. Apply traction to the skin with the thumb and forefinger of the opposite hand.
12. When the catheter enters the arterial lumen, a flashback of blood will occur into the transparent chamber of the catheter needle. At this time the angle of the catheter should be adjusted slightly upward so that the needle does not pass through the opposite side of the vessel.
13. The catheter assembly should then be advanced into the vessel until the shoulders of the teflon catheter have entered the vessel (1-2 mm). The teflon catheter may then be advanced over the needle approximately 3 mm, burying the point. Then both the needle and catheter should be advanced another centimeter into the vessel for improved security. If the catheter is in the lumen, it should advance easily.
14. Immediately after removing the needle, flush catheter with heparinized normal saline and secure with cloth tape.

15. Venous catheterization may be done in a similar manner as arterial. Usually unilateral administration of subcutaneous local anesthesia is sufficient.

APPENDIX B: EXPERIMENTAL DATES AND RANDOMIZATION

PROTOCOL: CONTROL INFUSIONS (5% DEXTROSE IN WATER)

DATE	RABBIT	RANDOMIZED	EXCLUDED
8/12/86	873	*	*
10/3/86	931	*	
11/14/86	102	*	*
12/23/86	912	*	
12/29/86	228	*	
1/5/87	443	*	*
4/8/87	690		
5/4/87	711		*

PROTOCOL: 10 NG·KG^{-1}·MIN^{-1} ANGIOTENSIN II INFUSIONS

DATE	RABBIT	RANDOMIZED	EXCLUDED
11/13/86	952	*	
12/19/86	951	*	

PROTOCOL: 20 NG·KG^{-1}·MIN^{-1} ANGIOTENSIN II INFUSIONS

DATE	RABBIT	RANDOMIZED	EXCLUDED
9/2/86	311	*	*
11/18/86	951	*	*
1/2/87	446	*	
1/13/87	443	*	
1/19/87	445	*	

PROTOCOL: 40 NG·KG^{-1}·MIN^{-1} ANGIOTENSIN II INFUSIONS

DATE	RABBIT	RANDOMIZED	EXCLUDED
1/20/87	449		
1/21/87	443		*
1/30/87	449		
4/24/87	776		
4/25/87	711		

APPENDIX B, CONTINUED.

PROTOCOL: 20 NG·KG^{-1}·MIN^{-1} ANGIOTENSIN II PLUS 3 MICROGRAMS·KG^{-1}·MIN^{-1} NITROPRUSSIDE INFUSIONS

DATE	RABBIT	RANDOMIZED	EXCLUDED
11/11/86	951	*	*
12/7/86	952	*	
12/16/86	101	*	*
1/15/87	449	*	
5/5/87	736		
5/7/87	738		

PROTOCOL: 40 NG·KG^{-1}·MIN^{-1} ANGIOTENSIN II PLUS 3-6 MICROGRAMS·KG^{-1}·MIN^{-1} NITROPRUSSIDE INFUSIONS

DATE	RABBIT	RANDOMIZED	EXCLUDED
1/12/87	251	*	*
1/22/87	448		
1/27/87	449		
3/27/87	690		
4/15/87	779		
4/20/87	780		