Title
Cardiovascular and endocrine actions of vasopressin

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Publication Date
1983

Peer reviewed|Thesis/dissertation

CARDIOVASCULAR AND ENDOCRINE ACTIONS OF VASOPRESSIN

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ENDOCRINOLOGY

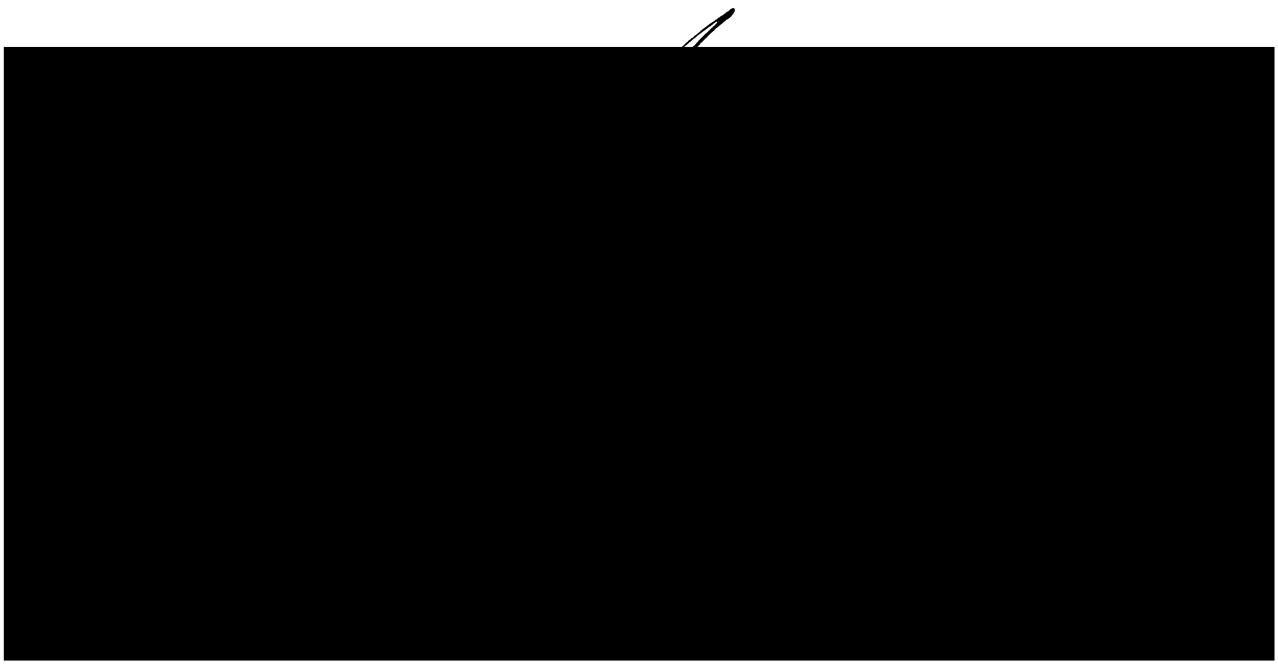
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



DEDICATED TO

My parents

Max Schwartz 1912-1979 Roza Drytter Schwartz

whose inspiration has always made me
strive beyond my own expectations

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ACKNOWLEDGEMENTS

The studies described in this dissertation were performed under the supervision of Dr. Ian Reid. I am grateful for his interest, encouragement and patience, all of which seemed limitless, during the course of these studies. I am grateful to the other members of the dissertation committee, Dr. Mary Dallman and Dr. W.F. Ganong, for their guidance in the planning, execution, and now, presentation of these studies. I would also like to thank their postdoctoral fellows and graduate students for the hand they were always willing to lend. My thanks are extended to Dr. G.A. Bentley for his help in reviewing this dissertation.

The expert asisstance of Ms. Helen Hughes, Ms. Shirley Chi, Ms. Judy Maselli, Dr. Lanny Keil and Ms. Jeanette Shinsako in performing radioimmunoassays is gratefully appreciated. Ms. Annette Lowe is also due thanks for her help in preparing some of the figures.

I thank Dr. Maurice Manning of the Medical College of Ohio for generously supplying the vasopressin analogs used in these studies.

I was aided in performing many experiments by Dr. Orlando Morales, Ms. Carol Ott, Dr. Virginia Brooks and Mr. Roy Shackelford, for which I owe a debt of thanks.

The contribution of Patent Fund grants for research is gratefully acknowledged, as is financial support in the form of a Regents Fellowship and an Earl C. Anthony Fellowship.

I would also like to thank the other members of the faculty and staff of UCSF whose assistance and instruction have been invaluable. In this regard, I owe a particular debt of gratitude to Dr. Richard Morrish, Dr. Ralph Kellogg, Dr. Julien Hoffman and Dr. James Roberts.

Finally, I thank my family, without whose love, support and encouragement this work might have never been accomplished.

ABSTRACT

Experiments were performed in dogs to investigate the role of endogenous vasopressin (VP) in cardiovascular regulation, and to characterize the receptors which mediate the inhibition of renin secretion, the stimulation of corticosteroid secretion and the increase in sodium excretion produced by vasopressin.

The role of endogenous VP in cardiovascular regulation was assessed in conscious dogs with analogs of VP which are specific antagonists of the vasoconstrictor activity of VP. In water-replete dogs, VP blockade had no effect on mean arterial pressure (MAP), heart rate (HR) or plasma renin activity (PRA). In 48h water-deprived dogs, VP blockade increased HR from 85±6 to 134±15 beats/min ($P<0.001$), cardiac output from 2.0±0.1 to 3.1±0.1 l/min ($P<0.005$) and PRA from 12.4±2.2 to 25.9±3.4 ng/ml/3h ($P<0.001$) and decreased total peripheral resistance from 46.6±3.1 to 26.9±3.1 U ($P<0.001$). MAP did not change significantly. The results suggested that the increases in HR, cardiac output and PRA were reflex responses to decreased peripheral resistance. When the same dogs were again deprived of water and pretreated with the β-adrenoceptor antagonist propranolol, the HR and PRA responses to VP blockade were attenuated and MAP decreased from 103±2 to 91±3 mm Hg ($P<0.001$). In dogs with adrenal insufficiency, plasma

vasopressin concentration increased 4-fold and VP blockade decreased MAP by 22±5 mm Hg ($P<0.001$). The role of VP in blood pressure maintenance during nonhypotensive hemorrhage was investigated using a 15 min, 1 ml/kg/min hemorrhage. The hemorrhage itself had no effect on MAP or HR, but PRA increased from 3.8±0.9 to 10.8±3.1 ng/ml/3h ($P<0.005$). In the presence of VP blockade, the same hemorrhage decreased MAP from the control value of 96±2 to 64±7 mm Hg ($P<0.005$), increased HR from 71±10 to 130±23 beats/min ($P<0.05$) and increased PRA from 7.1±0.8 to 30.3±6.7 ng/ml/3h ($P<0.005$). These results indicate that endogenous vasopressin plays an important role in the maintenance of blood pressure during water deprivation, adrenal insufficiency and nonhypotensive hemorrhage.

The receptors which mediate the effects of VP on renin and corticosteroid secretion and sodium excretion were characterized by comparing the responses to exogenous vasopressin, with those elicited by VP analogs with selective antidiuretic and vasoconstrictor properties. VP, a selective agonist of the vasoconstrictor activity of VP (PheOrnOT), and a selective agonist of the antidiuretic activity of VP (DDAVP) were tested, each in a dose of (1 ng/kg/min). VP was also infused following treatment with a selective antagonist of the vasoconstrictor activity of VP. These procedures were performed both in conscious dogs and anesthetized water-loaded dogs.

In conscious dogs, VP and PheOrnOT decreased plasma renin activity (PRA) from 4.4±1.1 to 2.4±0.8 ($P<0.05$) and from 5.5±1.1 to 2.7±0.2 ($P<0.001$) ng/ml/3h, respectively. DDAVP had no effect on PRA. The antagonist blocked the PRA response to vasopressin. In anesthetized water-loaded dogs, the results were different. VP and DDAVP decreased PRA from 11.9±4.7 to 3.8±1.7 ($P<0.05$) and from 13.5±4.6 to 7.0±2.0 ($P<0.05$) ng/ml/3h, respectively. PheOrnOT had no effect on PRA. The vasoconstrictor selective antagonist did not block the PRA response to vasopressin, and PRA decreased from 5.9±1.8 to 2.9±1.6 ng/ml/3h ($P<0.001$).

In conscious dogs, VP and PheOrnOT increased plasma corticosteroid concentration from 1.0±0.2 to 2.2±0.2 ($P<0.001$) and from 1.1±0.1 to 2.9±0.9 ($P<0.005$) μg/dl, respectively. The response to VP was blocked by the antagonist. The antidiuretic selective agonist had no effect on plasma corticosteroid concentration.

In anesthetized water-loaded dogs, 1.9±0.5 mEq sodium/kidney was excreted during a 60 min vehicle infusion. VP significantly increased the amount of sodium excreted during the infusion to 6.5±1.9 mEq/kidney ($P<0.05$). The effect of VP was blocked by the antagonist. Neither the DDAVP nor PheOrnOT increased sodium excretion.

These results indicate that antidiuretic- or vasoconstrictor-type receptors can mediate the inhibition of renin secretion by

vasopressin, and that the receptors which mediate the stimulation of corticosteroid secretion are the same as, or closely resemble vasoconstrictor-type receptors. They also suggest, when considered along with other published data, that the natriuretic effect of vasopressin is mediated by a third type of VP receptor, distinct from those which mediate the antidiuretic and vasoconstrictor effects.

GENERAL INTRODUCTION

For many years the sole physiological action of vasopressin was thought to be an effect on water permeability of the distal segments of nephrons, hence the name, antidiuretic hormone (ADH) (62). It had been known from earlier observations that injections of vasopressin *in vivo* can elevate arterial pressure (78) but, because this was thought to occur only at plasma vasopressin concentrations too high to be considered physiological, the significance of the pressor action was questioned (62). Recent development of research tools such as radioactive microspheres, sensitive radioimmunoassays and selective vasopressin antagonists, has made it possible to determine the hemodynamic actions of vasopressin at low plasma concentrations, and this has led to a reassessment of the role of vasopressin in cardiovascular regulation.

A number of other important actions of vasopressin were observed in the decades following the discovery of its antidiuretic and vasopressor functions. These include stimulating ACTH and corticosteroid secretion (50, 73), increasing sodium excretion (16, 46, 83, 119) and inhibiting renin secretion (52). While it is known that two distinct types of receptors mediate the antidiuretic and vasoconstrictor actions of vasopressin, and these

have been extensively studied, much less is known about the receptors which mediate the other actions. Current studies of the nature of the vasopressin receptors which mediate its various actions also owe a great deal to the development of sensitive assays, and of vasopressin analogs with selective antidiuretic and vasoconstrictor properties.

Two basic questions are posed in this dissertation. The first is whether vasopressin plays a role in blood pressure and hemodynamic control, and under what conditions this occurs. The second is what are the natures of the receptors which mediate the renin, corticotropic and natriuretic responses to vasopressin. In the introduction section which follows, previous research, which provided the background for the studies performed is briefly reviewed.

INTRODUCTION

CONTROL OF VASOPRESSIN SECRETION

In order to discuss the cardiovascular and endocrine actions of vasopressin it is also necessary to review briefly the physiology of vasopressin secretion and its antidiuretic action. Because dogs were the experimental animal used in the present studies, the values presented in this section will be obtained in dogs, whenever possible.

Vasopressin and its neurophysin are synthesized in certain cell bodies of the hypothalamus, particularly in the paraventricular and supraoptic nuclei (13). Both are synthesized as a common precursor preprohormone (60). The two peptides are processed in the golgi apparatus and packaged into secretory vesicles bound to each other. The vesicles are transported down the axons to the nerve terminals, which are located in the posterior pituitary, and stored there as secretory granules (18). Vasopressin and neurophysin are released by exocytosis into the circulation in response to changes in plasma osmolality, blood volume and blood pressure, and in response to several other stimuli.

Plasma osmolality: Secretion of vasopressin can be stimulated by increasing the osmolality of body fluids (12). Therefore, it has been proposed that there are "osmoreceptors" which are sensitive to the total concentration of certain plasma solutes such as sodium and, by definition, are able to transmit this information to the pituitary which responds by adjusting the secretion of vasopressin (97, 100). Although the precise location of the osmoreceptors has yet to be identified, it has been proposed to be in the anterior hypothalamus (97, 108). Recently, Thrasher et al. (113) provided evidence for an osmoreceptor in the organum vasculosum of the lamina terminalis. Other osmoreceptors have also been identified. For example, there is even evidence for extracerebral osmoreceptors, such as hepatic osmoreceptors (12).

In dogs, plasma osmolality normally ranges from 280-305 mOsm/kg (107). Robertson and associates (98) and Quillen and Cowley (87) have examined the relationship between plasma osmolality and plasma vasopressin concentration in dogs over the normal range of osmolalty. The data from both groups indicate that vasopressin is secreted at rates to maintain a steady basal plasma concentration at all plasma osmolality values below an osmotic threshold. Plasma vasopressin concentration increases linearly as plasma osmolality increases, above the threshold. Wade et al. (117) correlated jugular plasma osmolality with plasma

vasopressin concentration, and found a similar relationship. Weitzman and Fisher (122), who used sheep, observed the relationship between osmolality and vasopressin to be exponential. Nonetheless, in the physiological range of osmolality, there is little functional difference betweeen the two models (108). The system is very sensitive to small changes in osmolality. For example, using the data of Quillen and Cowley (87), a 1% change in plasma osolality will change the concentration of vasopressin by 0.6 pg/ml, which will markedly change renal water reabsorption (see below). Their data also indicate that over the range from 280 to 305 mOsm/kg the corresponding plasma vasopressin concentrations would be expected to range between 0.6-5.8 pg/ml.

Blood volume: Reduction in blood volume is also a potent stimulus to vasopressin secretion. It has been observed in humans and dogs that the relationship between reduced blood volume and plasma vasopressin concentration is exponential (23, 97). Claybaugh and Share (23) subjected anesthetized dogs to slow continuous hemorrhages (0.28 and 0.42 ml/kg/min) and reported a significant elevation in plasma vasopressin with a blood loss of as little as 2.1 ml/kg. Pullan et al. (86) observed a similar situation in conscious dogs, where a decrease of 5% of blood volume increased plasma vasopressin from a control concentration of 6.6 to 7.6

pg/ml. The exponential nature of the dose response to hemorrhage occurs even in the absence of any decrease in arterial pressure (23), itself a potent stimulus to vasopressin secretion. The vasopressin response to a continuous, slow, nonhypotensive hemorrhage in conscious dogs was also measured in the present study.

Recently Quillen and Cowley (87) described the interaction between osmotic and volume control of vasopressin secretion in dogs. They measured plasma vasopressin concentrations in dogs during infusions of hypoosmotic and hyperosmotic solutions to produce curves which describe the relationship between plasma osmolality and vasopressin concentration. This was performed in normovolemic dogs, dogs made hypovolemic by hemorrhage and in dogs made hypervolemic by autotransfusion. Compared to normovolemic dogs, the osmotic threshold for vasopressin secretion was lower in hypovolemic dogs and higher in hypervolemic dogs. In addition, the average slope of the curves was increased by hypovolemia and decreased by hypervolemia.

Arterial pressure: There is also an exponential relationship between plasma vasopressin secretion and decreased arterial pressure (97, 99, 124). Data from studies performed in humans indicate that significant increases in plasma vasopressin occur with blood pressure decreases of only 5% (97). It bears noting that, because of the transitions humans make between upright and horizontal positions, a 5% reduction in arterial pressure may be a more commonly occurring physiological event in humans than in quadrupedal species.

Other influences: Among the other known physiological stimuli to vasopressin secretion are certain types of stress and pain (73), emesis (97), and hypoxia (88). Angiotensin II has been reported to stimulate vasopressin secretion (77, 89, 92).

Corticosteroids have been observed to exert an inhibitory effect on vasopressin secretion (73). More recent evidence for this is the observation that an increase in the number of vasopressinergic neurons in the brain is seen following adrenalectomy and this is decreased by treatment with the synthetic glucocorticoid dexamethasone (19).

Various pharmacological agents are known to stimulate or inhibit vasopressin secretion. For purposes of this dissertation, only the barbiturate anesthetics need be discussed, as these are most commonly used in experiments requiring anesthetized animals. While there is some indication that barbiturates stimulate vasopressin secretion (33), there have also been studies in which barbiturate anesthesia did not significantly elevate plasma vasopressin concentration (88). However, because barbiturates inhibit respiratory and circulatory reflexes (39, 131), and hypoxia and hypotension are known stimuli to vasopressin secretion, secondary effects might be responsible for increased vasopressin secretion. In addition, experiments are often performed in anesthetized animals because certain surgical procedures are required for the experiments, and surgical stress is a very potent stimulus to vasopressin secretion. Thus, for purposes of interpreting data from experiments in anesthetized animals, it should be noted that plasma vasopressin might be elevated by effects of anesthesia or surgery. Data from experiments in anesthetized animals which deal with the cardiovascular actions of vasopressin must also be interpreted carefully because of the interference by barbiturates with many cardiovascular reflexes (39, 131).

ANTIDIURETIC ACTION OF VASOPRESSIN

Vasopressin increases water reabsorption from nephrons by increasing water permeability of collecting tubules (48). Vasopressin increases hydraulic conductivity (Lp) ten-fold or more in rabbit outer medullary and cortical collecting tubules, even more in inner medullary and papillary collecting tubules. However, in antidiuresis so much water is reabsorbed by the cortical and outer medullary segments that little further water reabsorption takes place beyond these segments (48). Small increments in plasma vasopressin induce marked increases in water reabsorption. According to Robertson et al. (99, 100), in the range of plasma vasopressin concentrations from 0.5 to 5 pg/ml urine osmolality increases from less than 100 to greater than 1000 mOsm/kg in humans. In dogs, it appears that the maximum antidiuretic effect of vasopressin occurs at plasma concentrations of approximately 10 pg/ml (Ian Reid, personal communication).

CARDIOVASCULAR ACTIONS OF VASOPRESSIN

The plasma vasopressin concentration required to elevate arterial pressure in intact animals is higher than that required for its maximum antidiuretic effect (68, 76) and this has been used as an important argument against any role for vasopressin in hemodynamic regulation. However, recent evidence indicates that many other actions of vasopressin do occur at concentrations less than those required to maximally concentrate urine, and there is evidence of complex cardiovascular and endocrine interactions at low vasopressin concentrations. These actions and interactions are the subject of this section.

Vasoconstrictor activity of vasopressin in vitro: Vasopressin is a potent vasoconstrictor agent. Altura and Altura (6) have demonstrated, by comparison of the dose-response relationships of several vasoconstrictor agents on rat aortic strips and mesenteric arterioles, that vasopressin is more potent than either angiotensin II or norepinephrine in contracting some types of vessels. Additionally they have demonstrated that vasopressin contracts venules and precapillary sphincters. Recently, Penit et al. (82) reported that vasopressin contracts cultured rat aortic smooth muscle cells at concentrations as low as 10 pM (approximately 10 pg/ml).

Cardiovascular actions of vasopressin in vivo: Recent observations indicate that infusions of vasopressin, resulting in plasma concentrations in the range associated with its antidiuretic activity, also exert cardiovascular and endocrine actions in intact animals.

The most immediate consequence of constriction of a blood vessel is increased resistance to flow through that vessel. In the whole animal this is reflected by the calculated total peripheral resistence (TPR). Montani and associates (76) infused vasopressin into intact conscious dogs at a rate which increased plasma vasopressin levels from 1.9 to 3.9 pg/ml and recorded a significant increase in TPR.

Another significant change noted by Montani et al. with the same infusion rate was a decrease in cardiac output. Heart rate was also decreased, but the change was not significant until a higher infusion rate was used which increased plasma vasopressin to approximately 7 pg/ml (76). Baroreceptor denervation abolished the effects on cardiac output and heart rate at these infusion rates, suggesting that they are baroreceptor-mediated reflexes (25, 76).

The effect of vasopressin on cardiac function however, involves more than a simple baroreflex. The bradycardia caused by elevating blood pressure to a given level with vasopressin is

greater than that caused with other vasoconstrictors, such as phenylephrine, methoxamine or angiotensin II (41, 66). While increases in arterial pressure produced by vasopressin or phenylephrine cause similar increases in baroreceptor discharge, vasopressin causes greater increases in cardiac vagal efferent activity (66). Such findings suggest a central action of vasopressin in the control of cardiac activity. This conclusion is supported by the finding of Liard and associates (64) that intravertebral vasopressin infusions elicit greater decreases in cardiac output than intravenous infusions which result in the same plasma vasopressin concentration. Therefore, it appears that the vasoconstrictor effect of vasopressin is buffered by its effects on heart rate and cardiac output, which are mediated by the baroreceptors, and a central effect of vasopressin which potentiates the baroreflex response.

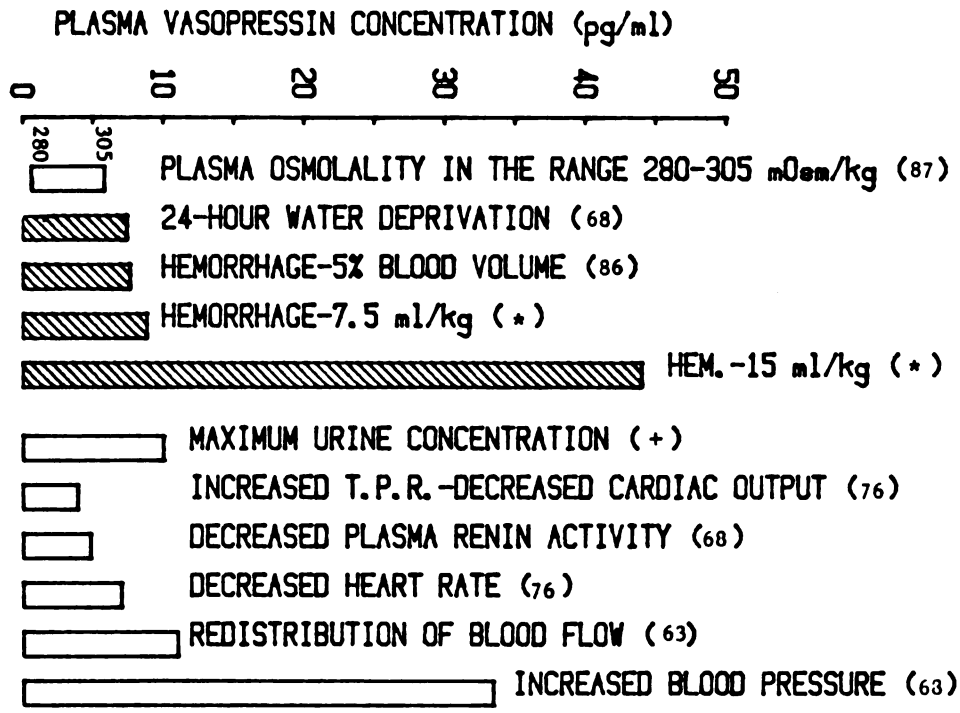
The vasoconstrictor action of vasopressin is also buffered by its effect on the secretion of renin. Malayan et al. (68) infused vasopressin into conscious intact dogs at a rate which increased the plasma vasopressin concentration from 2.7 to 4.8 pg/ml and observed that plasma renin activity decreased by 19%. By decreasing the production of angiotensin II, the pressor action of vasopressin is buffered.

The vasoconstriction caused by vasopressin is not uniform throughout all vascular beds (41) and the result is the redistribution of blood flow. This was recently demonstrated by Liard and coworkers (63). Infusion of vasopressin in conscious dogs, which increased plasma vasopressin concentration to 11 pg/ml, and decreased cardiac output by 13%, significantly decreased blood flow to skeletal muscle, myocardium, skin and brain, while maintaining flow to other organs such as kidneys, adrenals, testes and bone. A similar pattern was observed in dogs deprived of water for 48h to increase endogenous vasopressin except that the decreases in cutaneous and brain blood flows were not significant Since vasopressin and angiotensin II exhibit different potencies in constricting different vascular beds (41), the effect of vasopressin in redistributing blood flow may be due in part to its action to decrease angiotensin II formation.

As noted above, the plasma vasopressin concentration required to elevate arterial pressure is comparatively high. Malayan et al. (68) observed a significant increase in blood pressure in conscious dogs only when vasopressin was infused at a rate which increased plasma vasopressin concentration to 33.5 pg/ml. The reason for such high concentrations is the baroreceptor-mediated reflexes induced by vasopressin. When the baroflexes are eliminated by baroreceptor denervation, vasopressin increases

arterial pressure at very low concentrations (25, 76). For example, Montani and coworkers (76) reported that, in baroreceptor-denervated dogs, all doses of vasopressin which increased TPR also increased mean arterial pressure. Thus, the net effect of vasopressin on blood pressure is the total of a number of cardiovascular actions which tend both to decrease as well as increase pressure.

The reported thresholds for the cardiovascular actions of vasopressin *in vivo* are summarized in Fig. 1. They are compared with the plasma vasopressin concentrations associated with a number of physiological stimuli.



* Present Study
\+ Ian Reid, personal communication

Figure 1. Comparison of plasma vasopressin concentrations observed in conscious dogs under several physiological circumstances, with observed threshold concentrations required for renal, endocrine and cardiovascular responses.

QUESTIONS ASKED IN THIS DISSERTATION

The studies of this dissertation posed a number of specific questions. It is appropriate to define these questions at this point and to review the research which prompted them.

1A. Does vasopressin play a role in cardiovascular function in conscious dogs:

i) in the resting water-replete state?

ii) during water deprivation?

iii) during nonhypotensive hemorrhage?

1B. What is the quantitative effect of adrenal insufficiency on plasma angiotensin II and vasopressin concentrations, and does vasopressin play a role in blood pressure regulation in this state?

2. What is the nature of the vasopressin receptors which mediate:

i) the inhibition of renin secretion?

ii) the stimulation of ACTH and corticosteroid secretion?

iii) the increase of sodium excretion?

Role of vasopressin at rest: Vasopressin is such a potent vasoconstrictor that it seems possible that it might play a small role in blood pressure regulation at plasma concentrations observed at rest. However, resting blood pressure in man and in animals unable to secrete vasopressin is normal (24, 61)

Role of vasopressin during water deprivation: Circumstantial evidence, such as that presented in Fig. 1, suggests that vasopressin might play a role in blood pressure regulation during water deprivation. The threshold plasma vasopressin concentrations for some cardiovascular and endocrine effects were found to be lower than the plasma vasopressin concentration observed during 24h water restriction, and this suggested a role for vasopressin in cardiovascular regulation during water deprivation. A more direct method for evaluating the role of vasopressin involves the use of specific antagonists of the vasoconstrictor activity of vasopressin (description below). While the present studies in conscious dogs were underway, two studies utilizing antagonists in rats were reported; one in anesthetized rats following 30-40h water deprivation (8) and one in conscious rats following 24h water deprivation (4). In both cases, blockade of vasopressin decreased blood pressure.

Role of vasopressin during nonhypotensive hemorrhage: As discussed above, blood loss is a potent stimulus to vasopressin secretion. It has been demonstrated that administration of exogenous vasopressin in doses that increase plasma vasopressin concentration to levels seen during hemorrhage, can increase blood pressure in normovolemic reserpinized anesthetized dogs (102), anesthetized dogs whose vagi and sinus nerves had been cut (102) and in conscious intact dogs (68, 112).

More direct evidence that vasopressin plays a role in blood pressure maintenance was observed in hemorrhage studies in animals unable to secrete vasopressin. For example, it has been demonstrated that hypophysectomized dogs have an impaired ability to maintain arterial pressure following hemorrhage (26, 32). Other investigators have reported that rats with hereditary diabetes insipidus are less able to maintain blood pressure during blood loss, than matched control animals (61, 129, 130). When vasopressin was administered to rats with diabetes insipidus, the ability to maintain blood pressure was increased (61).

A vasopressin antagonist had also been used to study the role of vasopressin in blood pressure regulation during hemorrhage. Cowley et al. (26) observed that blockade of the vasoconstrictor activity of vasopressin, in anesthetized, spinal-areflexic, nephrectomized dogs subjected to a hypotensive hemorrhage,

resulted in a similar degree of impaired blood pressure regulation as in hypophysectomized animals.

Role of vasopressin during adrenal insufficiency: Adrenal insufficiency is associated with conflicting stimuli to vasopressin secretion. On one hand, the absence of glucocorticoids, decreased plasma volume (22), decreased arterial pressure (96) and increased plasma renin activity (95), all might be expected to increase vasopressin secretion. On the other hand, mineralocorticoid deficiency greatly increases the loss of sodium in the urine, and there is decreased plasma osmolality (15). Bioassays by different investigators of plasma vasopressin during adrenal insufficiency have yielded conflicting results (2, 3, 55, 110). Radioimmunoassay studies have demonstrated increased plasma vasopressin in glucocorticoid-deficient rats (65, 70), but the combined effects of mineralocorticoid- and glucocorticoid-deficiences on plasma vasopressin concentration had not been measured with radioimmunoassay.

Three indirect lines of evidence suggest that vasopressin plays a role in blood pressure regulation during adrenal insufficiency. First, adrenal insufficiency is a hypovolemic state,and thus resembles water deprivation and hemorrhage. Second, it is associated with increased total peripheral resistance (96, 121), which is a known effect of vasopressin (76) and one by which vasopressin could act to increase pressure. Finally, glucocorticoid-deficiency causes a decreased response to norepinephrine and epinephrine in blood vessels (33). In this way, it resembles pharmacological sympathectomy in which vasopressin helps maintain blood pressure (34, 42).

Related to both vasopressin secretion and blood pressure regulation is the question of the effect of adrenal insufficiency on plasma angiotensin II (Ang II) concentration. Plasma renin activity has been reported to increase with adrenal insufficiency despite decreased angiotensinogen production (95) and the Ang II thus generated might increase vasopressin secretion (77, 89, 92). Ang II has also been demonstrated to maintain blood pressure in adrenal insufficiency (11).

Renin, corticotropic, and natriuretic effects of vasopressin: In addition to its antidiuretic and vasoconstrictor actions, vasopressin exerts a number of other effects. The scope of its actions on the secretion of renin, ACTH, 11-hydroxycorticosteroids and the excretion of sodium have been extensively studied and some current concepts regarding these actions are briefly reviewed in this section.

The receptors which mediate the antidiuretic and vasoconstrictor effects of vasopressin have been studied and characterized. For example, it is known that the antidiuretic action must be mediated by different receptors than those which mediate the the vasoconstrictor effect because it is possible to synthesize vasopressin analogs which have either selective antidiuretic or selective vasoconstrictor activity (72). It has also been demonstrated that antidiuretic type receptors are coupled to adenylate cyclase (13) whereas vasoconstrictor type receptors are not (31). The receptors which mediate the other actions of vasopressin have not been as extensively studied, and there are conflicting reports regarding the nature of the receptors which mediate the renin, corticotropic and natriuretic responses to vasopressin. These actions could be mediated by antidiuretic- or vasoconstrictor-type receptors, or by different receptor types.

Action of vasopressin on renin secretion: Vasopressin at low physiological concentrations inhibits renin secretion (28, 52, 68). Despite the uniformity of opinion on tha action of vasopressin, there is conflicting evidence regarding the type of receptor which mediates it. Malayan and Reid (69) concluded that the renin inhibitory action was mediated by antidiuretic-type receptors because an analog of vasopressin with selective antidiuretic activity, 1-deamino-4-threonine-8-D-arginine-vasopressin, suppressed plasma renin activity (PRA) in anesthetized dogs as effectively as did vasopressin. Similarly, Joppich and Weber (51) reported that the selective antidiuretic agonist 1-deamino,8-D-arginine-vasopressin (DDAVP) suppressed PRA in infants and young children. On the other hand, Johnson et al. (49) found that DDAVP did not decrease PRA in conscious dogs at a dose which duplicated the antidiuretic effect of a dose of vasopressin which suppressed PRA.

Action of vasopressin on ACTH and corticosteroid secretion: It has been known for three decades that vasopressin can stimulate the secretion of ACTH (50, 73), yet its physiological role in this regard is only currently being investigated. In the past, there has been evidence for and against vasopressin being a (or the) corticotropin releasing hormone (CRH) which regulates ACTH

secretion. On one hand, there were observations that vasopressin and ACTH are released together under some stressful conditions, that glucocorticoids inhibit the secretion of both ACTH and vasopressin, and that posterior lobectomized animals and rats with diabetes insipidus resulting from hypothalamic lesions show decreased corticosteroid responses to neurogenic stress (50, 73). In addition, it has been proposed that vasopressin acts in the brain to stimulate the release of other CRHs because intrahypothalamic injections of vasopressin result in the release of more ACTH than injections of the same dose into the anterior pituitary (40). On the other hand, there is evidence against vasopressin being a CRH. For example, it has been observed that rats with hereditary diabetes insipidus secrete ACTH (albeit less than normal rats), and there are conditions when ACTH release is increased in the absence of increased vasopressin secretion. In addition, the increase in plasma corticosteroid concentration caused by administration of vasopressin could be due to a direct effect on the adrenal rather than on the pituitary (though this was observed only with large doses of vasopressin) (73).

Recently Vale and coworkers (114) have isolated a polypeptide with CRH activity from sheep hypothalami (oCRF). This discovery and subsequent experiments with CRF have led to a re-evaluation of the role of vasopressin in the control of ACTH secretion and a

modified picture has emerged. Gillies et al. (35) have reported that ACTH secretion can be stimulated from pituitaries *in vivo* with either vasopressin or CRF. They add, however, that although CRF is more potent than vasopressin, the ACTH dose-response to CRF is not as steep as that to an extract of median eminence. Moreover, it had been previously observed by Gillies and Lowry (36) and Koch et al. (57) that treatment of median eminence extract with an antiserum to vasopressin decreases its capacity to stimulate ACTH secretion . The most recent observations, then, suggest that overall control of ACTH secretion may be regualated by a number of factors including vasopressin, the CRF peptide and possibly other unidentified elements extractable from median eminence (35, 50).

Exogenously administered intravenous vasopressin has been used to study its corticotropic activity (5, 68), and this activity can be seen during infusions that result in physiological plasma vasopressin levels (68).

As is the case with renin, there are conflicting results from *in vivo* and *in vitro* studies of the vasopressin receptor which mediates the corticotropic response. In a number of a studies, a correlation has been found between pressor and corticotropic activity (5, 74, 81), though this conclusion has not reached by others (7, 9, 73, 85).

Action of vasopressin on sodium excretion: Vasopressin has been shown to increase renal excretion of sodium in a number of species (16, 46, 83, 119). The natriuretic action of the neurohypophyseal hormones and a number of analogs has been examined in a number of studies. While the results are varied, they can generally be classified as falling into one of two groups, those that indicate that natriuretic activity is independent of antidiuretic and vasoconstrictor activity (20, 21, 43, 67, 83) and those that specifically indicate that it is not (119).

Typical of studies in the former group is that of Chan and du Vignaud (21), in which neurohypophyseal analogs which lacked or blocked the antidiuretic and vasoconstrictor activities of vasopressin caused natriuresis when administered to rats undergoing water diuresis. More recently Chan (20) produced a number of structure-function correlations that suggested that the receptor which mediates the natriuretic response might optimally recognize a molecule similar to oxytocin. There is now evidence that suggests less of a correlation between oxytocic and natriuretic activity. Malayan and Reid (69) infused an analog which has been reported to have less than 4% the oxytocic activity of vasopressin (71), and it increased sodium excretion.

Walter et al. (119) have also studied the natriuretic activities of vasopressin, oxytocin and a number of analogs, some of which were used by Chan as well. In direct contrast to the studies of Chan, they reported that only vasopressin and oxytocin, which had similar effects on urine flow at the doses used, increased sodium excretion to a greater extent than that observed during infusion of the carrier vehicle alone. It bears noting however, that under the conditions of the experiment, both hormones increased urine flow.

APPROACH

The questions asked in this dissertation were addressed from two perspectives. In one, the actions of endogenous vasopressin were examined by observing hemodynamic, cardiac and endocrine responses to pharmacological blockade of the vasoconstrictor activity of the hormone (Antagonist Studies in Conscious Dogs). In the other, the receptors which mediate some of the actions of vasopressin were characterized by measuring the effects of a number of vasopressin analogs, with selective antidiuretic and vasoconstrictor properties on plasma renin activity, plasma corticosteroid concentration and urinary sodium excretion, and comparing them to the responses to vasopressin (Analog Studies).

The question of whether endogenous vasopressin plays a role in the regulation of cardiovascular function in conscious dogs was studied from the first perspective. The reactions to vasopressin blockade were examined in dogs with low (water-replete), moderate (water-deprived) and relatively high (hemorrhage) plasma vasopressin concentrations.

The experiments were designed to provide continuous heart rate and arterial pressure measurements, before and after administration of a specific antagonist of the vasoconstrictor activity of vasopressin. Since it can be assumed that any changes which might occur in heart rate or arterial pressure as a result of vasopressin blockade will be opposite in direction to those of endogenous vasopressin, a role could thus be assigned to vasopressin in having maintained the function. Cardiac output was measured to enable calculation of total peripheral resistance and to determine the role of vasopressin in regulating resistance and flow. Plasma renin activity was regularly measured to assess the impact of vasopressin on the renin-angiotensin system, another important blood pressure regulating mechanism.

The quantitative effects of adrenal insufficiency on plasma angiotensin II and vasopressin concentrations were studied in adrenalectomized dogs by measuring the concentration of the hormones as adrenal insufficiency developed. The role of

vasopressin in the maintenance of blood pressure during adrenal insuficiency was determined by using a vasopressin antagonist in experiments similar to those described above.

The nature of the vasopressin receptors which mediate the renin, corticotropic and natriuretic responses was studied from both perspectives.

The Analog Study experiments were designed to measure the changes produced by a number of vasopressin analogs in plasma renin activity, plasma ACTH and corticosteroid concentrations and urinary sodium excretion, and to compare them to the responses produced by vasopressin. The analogs were selective vasoconstrictor and antidiuretic agonists and a selective antagonist of the vasoconstrictor action of vasopressin. The renin response provides an example of how the results were analyzed. If a renin response to the vasoconstrictor agonist is similar to that produced by vasopressin, this can be interpreted as evidence that the receptor which mediates the renin response is of the vasoconstrictor type. The same applies to the antidiuretic agonist. Furthermore, the finding that the vasoconstrictor antagonist blocked the renin response to vasopressin would constitute evidence that renin inhibition is mediated by vasoconstrictor-type receptors.

There were other possibilities as well. For example, an absence of a response (renin, corticotropic or natriuretic) to both agonists would suggest a third type of receptor, that is unlike both the antidiuretic and vasoconstrictor types. A partial response to an agonist or partial blockade with the antagonist suggests a receptor similar to, but not identical with, the corresponding receptor. Alternatively, these results might suggest that the agonist is only a partial agonist or that the antagonist possesses weak intrinsic agonist activity, as for example, does the angiotensin II antagonist, saralasin.

The oxytocic properties of the analogs were noted as well. With the exception of the vasoconstrictor antagonist, which has been observed to block oxytocin receptors, none of the analogs had any oxytocic properties.

The receptors which mediate the inhibition of renin secretion were characterized by studying the effects on plasma renin activity of the analogs in conscious and anesthetized dogs. The two groups were studied because anesthesia and surgery are known to affect the secretion of vasopressin and renin (52). These receptors were also studied in the Antagonist Studies in Conscious Dogs by observing the effects of vasoconstrictor receptor blockade on plasma renin activity in water-deprived dogs and in dogs during hemorrhage.

The receptors which mediate the corticotropic effects were studied by examining the changes in plasma corticosteroid concentration caused by the analogs. This was restricted to conscious dogs because the surgery associated with the experiments in anesthetized dogs introduces a varible stress response (73) and because anesthesia may alter the corticosteroid response to vasopressin (30). These receptors were also studied by observing the effect of vasoconstrictor receptor blockade in conscious dogs during hemorrhage.

Since vasopressin increases corticosteroid secretion by stimulating ACTH secretion and by acting directly at the adrenal (73), the measurement of plasma corticosteroid concentration represents the sum of both actions. Plasma ACTH was measured in two experiments to determine the extent of the contribution at the pituitary.

The receptors responsible for the natriuretic effect were studied by examining the effects of the analogs on urinary sodium excretion rate in anesthetized dogs undergoing a water diuresis. The water diuresis was required to eliminate the effects of endogenous vasopressin, and because the natriuretic response is more pronounced during water diuresis (83).

The analog studies were also designed to assess the vasopressor and antidiuretic activities of the agonists, and to determine whether the antagonist blocked the vasoconstrictor and antidiuretic activities of vasopressin.

VASOPRESSIN ANALOGS

The analogs of vasopressin used in these studies are described in this section. Modification or replacement of certain amino acids in the vasopressin molecule can selectively enhance or diminish antidiuretic or vasoconstrictor activity. The structure-activity relations of various parts of the vasopressin molecule have been extensively studied and reported elsewhere (10, 84, 118, 119). Here, some examples of amino acid modifications and substitutions, which resulted in the analogs used in these studies, and their effects on vasopressin activity are discussed.

Substitution of the L-arginine in position 8 with the D-isomer greatly reduces vasoconstrictor activity, so that 8-D-arginine vasopressin is a selective antidiuretic agent (71, 127). Deamination of the N-terminal cysteine also increases antidiuretic activity, so that 1-deamino-arginine-vasopressin also has enhanced antidiuretic to pressor activity (45). When the two modifications are combined, the result is 1-deamino-8-D-arginine-vasopressin (DDAVP, Fig. 2) a powerful selective antidiuretic agonist (116, 128). This peptide was used in the present study.

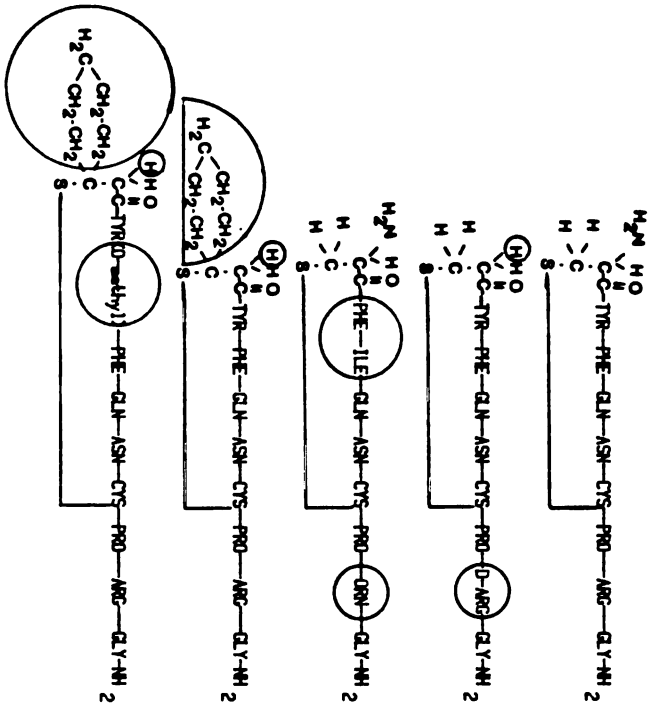


Figure 2. Structures of vasopressin and the analogs used in these studies. Modifications or substitutions of vasopressin are noted by circles.

A basic L-amino acid in position 8 is an essential requirement for vasoconstrictor activity. Oxytocin, which has a leucine in position 8, has very little vasoconstrictor (or antidiuretic) activity. Replacement of the leucine with the basic amino acid ornithine, and substitution of phenylalanine for tyrosine in position 2, results in a vasopressin analog with vasoconstrictor and very little antidiuretic activity. 2-Phenylalanine-8-ornithine-oxytocin (44) (PheOrnOT, Fig. 2) was the selective vasoconstrictor agonist used in the present study.

While deamination of the N-terminal cysteine of vasopressin enhances its antidiuretic activity, a combination of N-terminal deamination and replacement of the beta methylene hydrogens with aliphatic side chains greatly reduces antidiuretic activity. Kruszynski et al. (59) have demonstrated that one other result of those two modifications is to turn vasopressin into an antagonist of its own vasoconstrictor response. For example deamination of the N-terminal cysteine plus replacement of both beta methylene hydrogens with a single cyclopentamethylene ring results in the compound [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin, (Fig. 2). This compound has less than 0.01% of the antidiuretic activity of vasopressin and is an extremely potent antagonist of its vasoconstrictor activity. It is abbreviated $d(CH_2)_5$AVP, and, along with [1-(β-mercapto-β,β-

cyclopentamethylenepropionic acid),2-(O-methyl)tyrosine]arginine vasopressin, abbreviated d(CH_2)$_5$MeTyrAVP, was employed in the present study for its antagonist property.

Very little of the other pharmacology of these compounds has been investigated. Because many of the properties of these analogs have been assessed in rats under conditions which may vary from those employed in the present studies, the vasoconstrictor and antidiuretic properties of the compounds were investigated in the dogs, as was the ability of the antagonists to block the vasoconstrictor or antidiuretic responses to exogenous vasopressin. The specific doses and routes and modes of administration of the analogs are described in the following sections.

ANTAGONST STUDIES IN CONSCIOUS DOGS

MATERIALS AND METHODS

These experiments were performed in mongrel dogs of both sexes, weighing between 11 and 19 kg. Unless otherwise noted, the dogs had free access to water and standard dog food (Purina Dog Chow, Ralston Purina Co., St. Louis, MO). All surgical preparation was completed at least one week prior to experimentation. The surgery was performed under sterile conditions. The anesthesia used was 0.5 mg/kg acepromazine (Ayerst, New York City) sc, followed 30 min later by 12 mg/kg sodium thiopental (Pentothal, Abbott Laboratories, North Chicago, IL) iv. In most cases surgery was limited to insertion of polyvinyl catheters into the descending aorta and inferior vena cava via a femoral artery and vein. All catheters were tunnelled subcutaneously to a point between the shoulders, where they were exteriorized and protected by a vest. Following surgery, the dogs were injected with 600,000 U penicillin G, which was repeated daily if any sign of post-operative infection was present.

During experiments the dogs were allowed to stand freely with movement restricted by a cloth sling. Mean and pulsatile blood pressure was continuously monitored with a Grass polygraph (Grass Instruments, Quincy, MA). Heart rate was monitored with a

tachograph, triggered by the blood pressure pulse wave. Seven ml samples of arterial blood were drawn from the catheter, as indicated in the protocols, for various measurements, and the volume withdrawn was replaced with sterile isotonic saline. A 2.7 ml aliquot of blood was added to 0.3 ml 0.3 M EDTA in a chilled centrifuge tube for the measurement of plasma renin activity, and the remainder was added to another chilled heparinized centrifuge tube.

The two antagonists of the vasoconstrictor activity of vasopressin used in these studies, $d(CH_2)_5$AVP and $d(CH_2)_5$MeTyrAVP, were described above. They were kindly provided by Dr. Maurice Manning of the Medical College of Ohio. They produced quantitatively similar effects in the studies and were therefore used interchangeably.

Protocol 1-A. Vasopressin blockade in water-replete dogs. The effects of vasopressin blockade were studied in five water-replete dogs. Control blood pressure and heart rate measurements were made and then a single 10 µg/kg dose of either of the two antagonists was injected intravenously. Blood samples for analysis of PRA were collected immediately before injection of the antagonist and 5, 15, and 30 min later.

Protocol 1-B. Vasopressin blockade following water deprivation. In seven dogs, control measurements of blood pressure and heart rate were made and a blood sample for plasma osmolality was collected. The dogs were then deprived of water for 48h. Twenty-four and 48h later, an additional blood sample was drawn and plasma osmolality determined. The increase in plasma osmolality was taken as an index of the degree of dehydration of the animals. After 48h water deprivation, the effects of vasopressin were studied as described in protocol 1-A.

Protocol 1-C. Vasopressin blockade in water-deprived dogs pretreated with propranolol. Five dogs were deprived of water for 48h as described in protocol 1-B. A blood sample was drawn, and then the β-adrenoceptor blocker propranolol (Inderal, Ayerst Laboratories, New York, NY) was administered iv in a dose of 0.5 mg/kg, infused over 5 min. Twenty min later, the effects of vasopressin blockade were studied as described in protocols 1-A and 1-B. The effects of vasopressin blockade following propranolol were also studied in three water-replete dogs.

Protocol 1-D. Vasopressin blockade in water-deprived dogs--cardiac output measurements. Three dogs of similar weight were prepared for the measurement of cardiac output by the thermodilution technique. In these dogs the femoral artery catheter also contained a thermistor and the venous cannula was inserted into the right atrium via the jugular vein. Protocol 1-B was performed. Duplicate measurements of cardiac output were made by thermodilution immediately prior to collection of blood samples, following the method of Warren and Ledingham (1974), modified for measurements in dogs.

Protocol 2-A. Hemorrhage. Four female dogs were used in the hemorrhage protocols. The surgical preparation of these dogs included insertion of a second aortic catheter to permit simultaneous arterial hemorrhage and continuous measurement of arterial pressure. An arterial blood sample for PRA and plasma concentrations of vasopressin and 11-hydoxycorticosteroids (CORTS) was drawn, and then 3 ml sterile isotonic saline was injected iv as a vehicle control for drug injections (see below). Three min later, 1000 U heparin was injected iv. Another blood sample was drawn at 5 min, and then a 15 min arterial hemorrhage was begun at 1 ml/kg/min using a Masterflex peristaltic pump (Cole-Parmer, Chicago, IL). The blood was collected into a sterile Blood-Pak

collection bag (Fenwall Laboratories, Deerfield IL) from which the anticoagulant had been drained and which had been rinsed with sterile isotonic saline. The collected blood was kept at 37 C. Blood samples for analysis were drawn at midhemorrhage and again at the end of the hemorrhage. Fifteen min after the end of the hemorrhage, blood was again drawn for analysis, and the blood which had been withdrawn was reinfused via the venous catheter.

Protocol 2-B Hemorrhage following vasopressin blockade. This protocol was identical to protocol 2-A, except that a vasopressin antagonist was injected at the beginning of the experiment instead of saline. Plasma vasopressin was not measured because the antagonist cross-reacted with the antibody used in the RIA. In two experiments, $d(CH_2)_5$AVP was injected; in the other two, $d(CH_2)_5$MeTyrAVP was used. The antagonists were injected iv in a dose of 10 µg/kg. In two dogs, protocol 2-A was performed before protocol 2-B; in the other two dogs the order was reversed. The protocols were performed on different days.

Adrenalectomy and adrenal insufficiecy. The study of the roles of vasopressin and angiotensin II in blood pressure regulation during adrenal insufficiency necessitated additional surgical preparation of the dogs. Five were adrenalectomized in two stages via flank incisions. After adrenalectomy, the dogs were maintaintained on im injections of cortisol (Solu-Cortef, Upjohn, Kalamazoo, MI; 7.5 mg/day) and deoxycorticosterone acetate (Doca acetate, Organon, West Orange, NJ; 0.5 mg/day).

Steroid treatment was discontinued no sooner than one week after adrenalectomy and the dogs were allowed to develop adrenal insufficiency. Daily blood samples were drawn for the measurement of plasma sodium and potassium, ang II and vasopressin concentrations. Body weight and arterial pressure were also measured. The dogs were judged to have developed adrenal insufficiency when plasma sodium had decreased by at least 4 mEq/l and plasma potassium had increased by at least 1.5 mEq/l. The role of vasopressin in maintaining blood pressure was studied according to the following two protocols:

Protocol 3-A. Vasopressin blockade in dogs with adrenal insufficiency. The effects of vasopressin blockade were studied in four adrenalectomized dogs, three of which had been injected with their last steroid treatment 96-100h before the experiment and one which had received its last steroid treatment 120h before the experiment. Control measurements were taken for 15 min, and then a single 10 μg/kg dose of $d(CH_2)_5$MeTyrAVP was injected iv. Blood pressure and heart rate were monitored continuously for the next 35 min. At the end of this period, the dogs were treated with cortisol and DOCA.

Protocol 3-B. Vasopressin blockade in steroid-maintained dogs. Protocol 3-A was performed in three adrenalectomized dogs while they were being maintained on the steroid regimen, either before steroid withdrawal or at least one week after resumption of the steroid treatment. A pressor dose of vasopressin (Peninsula Laboratories, San Carlos, CA; 1.0 ng/kg/min iv) was infused in the same dogs 30 min after the injection of the antagonist, to test the effect of the antagonist on the hypertensive effects of vasopressin.

Plasma renin activity (PRA) was measured by radioimmunoassay of the angiotensin I generated during a 3h incubation period (ng/ml/3h) (94, 111). The lowest detectable quantity of angiotensin I with this assay is 0.015 ng, and the intra- and interassay coefficients of variation for this assay are 7.9% and 15.9%, respectively. Plasma vasopressin and angiotensin II concentrations were measured by RIA, after the peptides had been extracted on bentonite from the plasma, eluted and dried. Recovery of vasopressin with this procedure is 62%, and recovery of angiotensin II is 63%. Vasopressin was measured using the method of Keil and Severs (53) (intra- and interassay coefficients of variation = 6.3% and 7.0%, respectively, lowest detectable amount of vasopressin = 0.075 pg). Ang II was assayed according to the method of Reid (91) (interassay coefficient of variation is 6.9%, interassay coefficient is 11.6%). The lowest detectable quantity of angiotensin II with this procedure is 2.5 pg. Plasma 11-hydroxy corticosteroid concentrations (CORTS) were measured in unextracted plasma, where indicated, by RIA (1). Commercially available antibody and tracer was used. The lowest detectable amount of corticosteroids with this assay is 0.01 µg, and the intra- and interassay coefficients of variation are 13.5% and 14.5%, respectively. Plasma osmolality was measured by freezing point depression with a Fiske osmometer (Fiske, Uxbridge, MA;

measurements are reproducible within 1.0%) and sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratories, Lexington MA; measurements are reproducible within 0.5%).

All results are expressed as the mean±s.e.m. Statistical evaluation of the data was performed by one way analysis of variance for repeated measures (123). Additional tests including two way analysis of variance and Dunnett's t-test were applied where appropriate and are noted in the results section.

RESULTS

Vasopressin blockade in water-replete dogs. Injection of the vasopressin antagonists (10μg/kg iv) caused no significant changes in blood pressure, heart rate or plasma renin activity from the control values of 102±3 mm Hg, 82±9 beats/min and 6.2±1.2 ng/ml/3h, respectively (Fig. 3).

Vasopressin blockade following water deprivation. During the 48h period of water deprivation, plasma osmolality increased from 293±1 to 315±2 mosmol/kg ($P<0.001$). In these animals, vasopressin blockade caused a marked increase in heart rate from 85±6 to 134±15 beats/min 15 min following the injection ($P<0.001$); heart rate was still 41 beats/min above control 30 min after the

injection (Fig. 4). Vasopressin blockade also increased PRA from 12.4±2.2 to 25.9±3.4 ng/ml/3h ($P<0.001$)(Fig. 4). Mean arterial pressure did not change significantly throughout the experiment (Fig. 4).

Vasopressin blockade in water-deprived dogs pretreated with propranolol. Propranolol pretreatment attenuated the heart rate and renin secretory responses to vasopressin blockade in water-deprived dogs. Under these conditions, heart rate increased from 82±12 to only 104±13 beats/min following injection of the antagonist ($P<0.001$) and plasma renin activity did not change significantly (Fig. 5). Following pretreatment with propranolol, vasopressin blockade resulted in a decrease in blood pressure from 103±2 to 91±3 mmHg ($P<0.001$). Propranolol itself had no statistically significant effects on arterial pressure, heart rate or plasma renin activity in the water-deprived dogs. However, it bears noting that PRA decreased in 4 of 5 dogs. Vasopressin blockade following propranolol in water-replete dogs had no significant effects on blood pressure (98±4 to 102±4 mmHg) or heart rate (70±5 to 78±3).

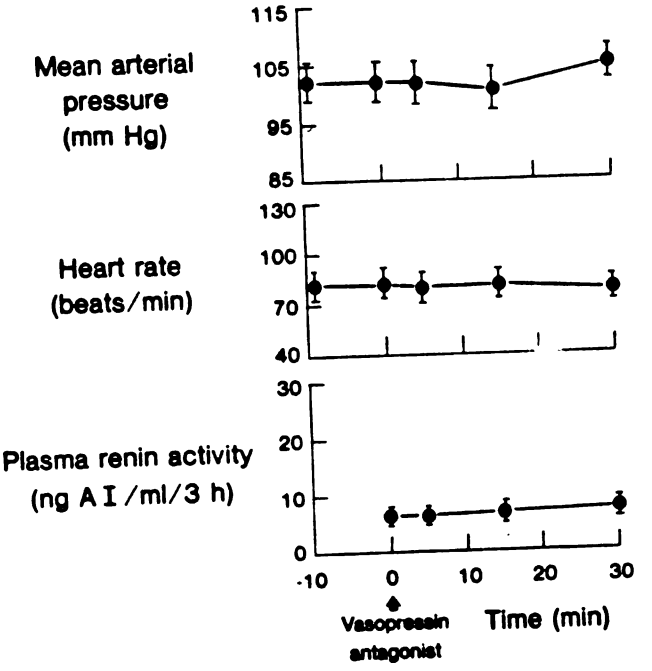


Figure 3. Effects of vasopressin blockade in water-replete dogs. N = 5. Each point represents mean±SE.

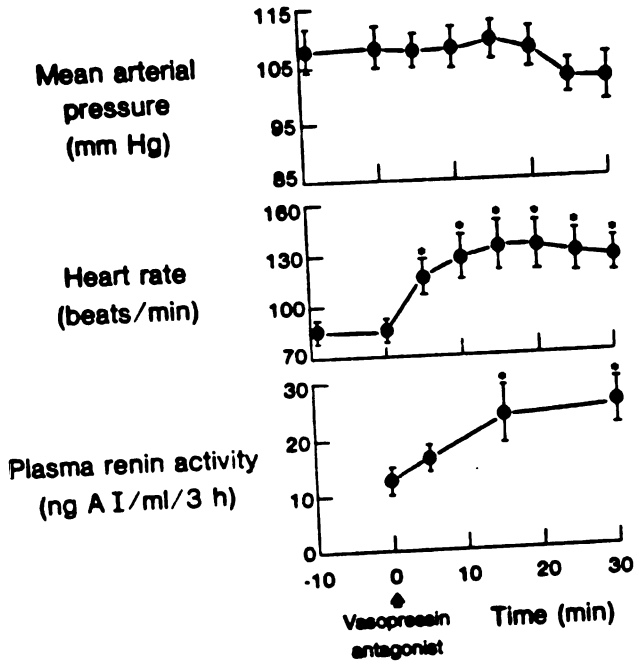


Figure 4. Effects of vasopressin blockade following water deprivation. N = 7. Each point represents mean±SE. * P<0.05 compared to control by Dunnett's t-test.

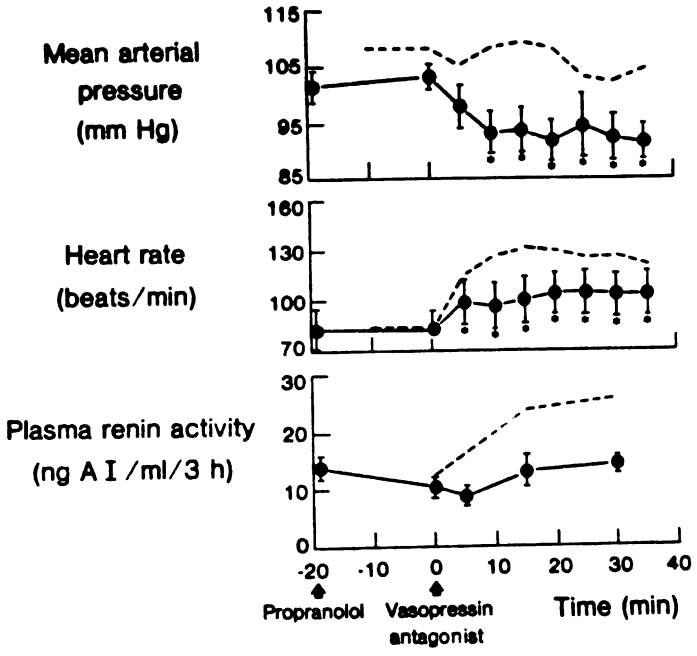


Figure 5. Effects of vasopressin blockade in water-deprived dogs pretreated with propranolol. N = 5. Each point represents mean±SE. Dashed lines show response to vasopressin blockade in the absence of propranolol (from Fig. 4). *P<0.05 compared to control by Dunnett's t-test.

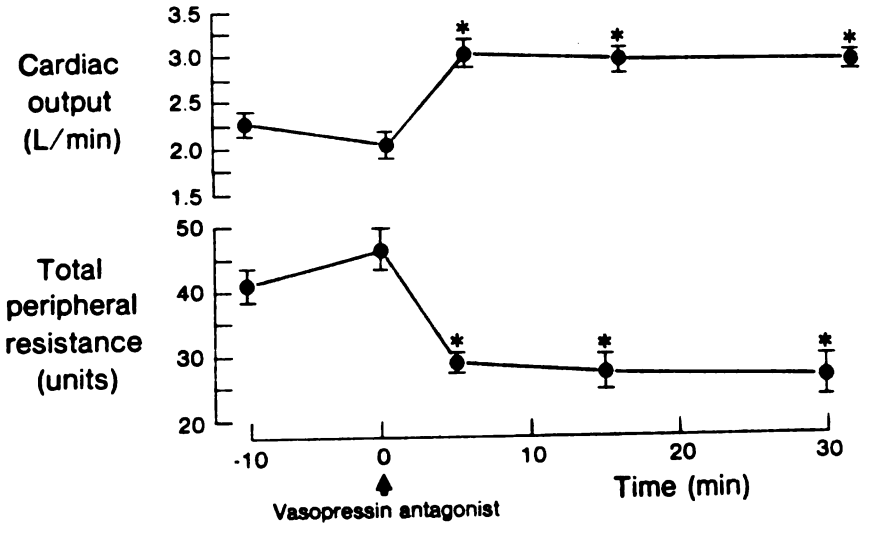


Figure 6. Effects of vasopressin blockade on cardiac output and total peripheral resistance in water-deprived dogs. N = 3. Each point represents mean±SE. *P<0.05 compared to control by Dunnett's t-test.

Vasopressin blockade in water-deprived dogs--cardiac output measurements. In this group of water-deprived dogs, vasopressin blockade caused a marked increase in cardiac output from 2.0±0.1 to 3.1±0.1 l/min ($P<0.005$)(Fig. 6). Calculated total peripheral resistance decreased from 46.6±3.1 to 26.9±3.1 Units ($P<0.001$). Blood pressure did not change significantly.

Hemorrhage. There was a significant increase in plasma vasopressin concentration from 6.7±2.7 to 8.8±3.7 pg/ml after 7.5 ml/kg blood had been withdrawn, and to 44.0±16.6 after 15 ml/kg had been withdrawn (Fig. 7). Over the course of the hemorrhage, there was no significant change in blood pressure (Fig. 8) or heart rate (Fig. 9). Transient fluctuations in mean arterial pressure did not exceed 7 mm Hg nor 30 sec duration. Plasma renin activity increased from 3.8±0.9 to 10.8±3.1 ng/ml/3h at the end of hemorrhage ($P<0.005$) and was 9.0±2.9 ng/ml/3h immediately before blood reinfusion (Fig. 10). Plasma CORTS concentration increased from 1.5±0.8 to 8.6±2.0 µg/dl ($P<0.001$)(Fig. 11).

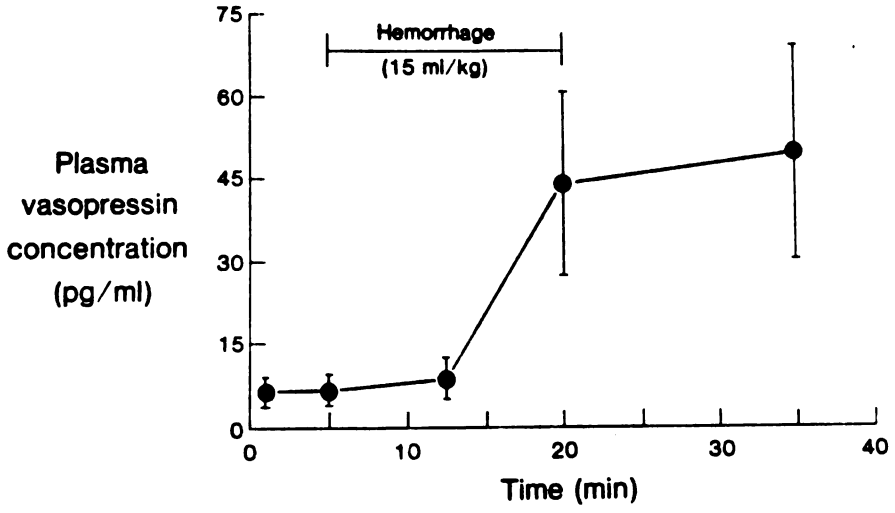


Figure 7. Effect of hemorrhage on plasma vasopressin concentration. N = 4. Each point represents mean±SE.

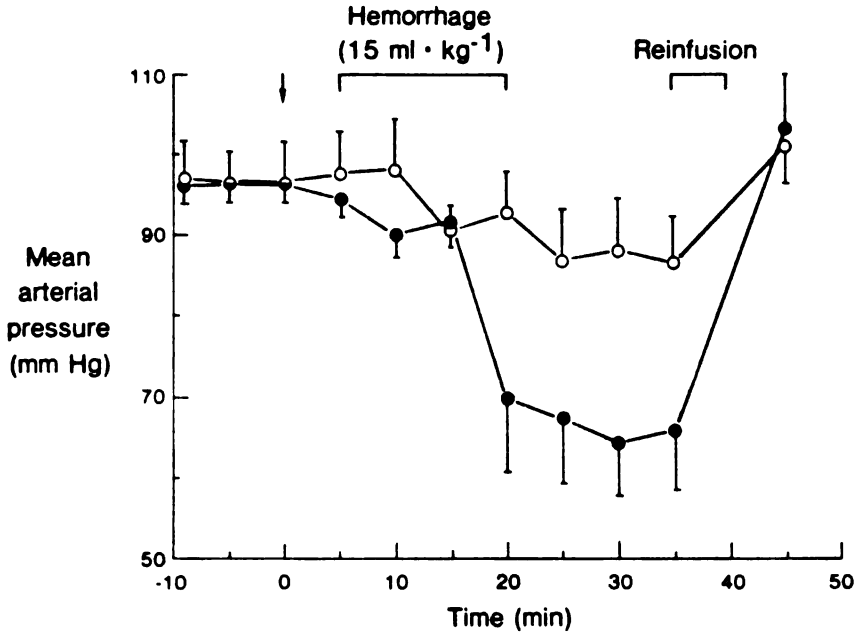


Figure 8. Effect of hemorrhage on mean arterial pressure in the absence ⊖ and presence ● of vasopressin blockade. N = 4 in both groups. The antagonist or saline was injected at time 0 as indicated by the arrow. Each point represents mean±SE.

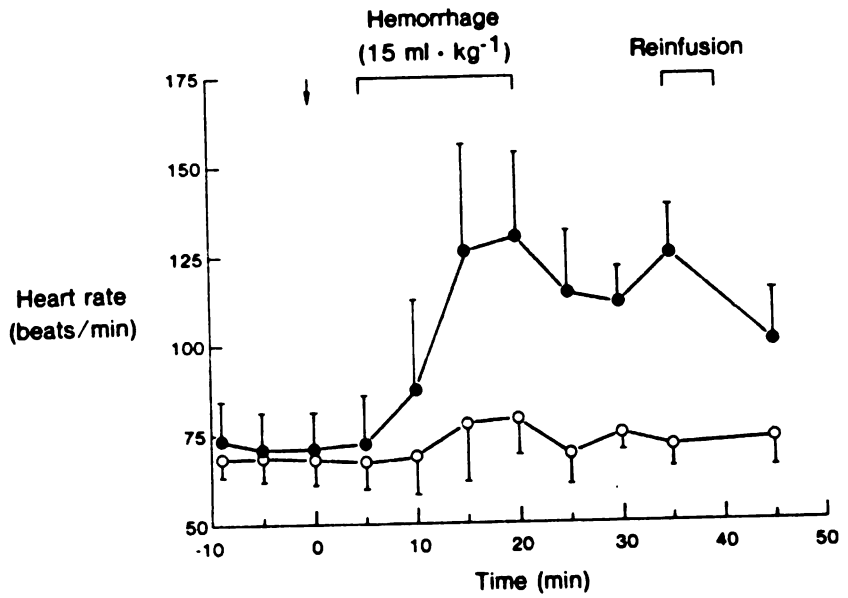


Figure 9. Effect of hemorrhage on heart rate in the absence -○- and presence -●- of vasopressin blockade. N = 4 in both groups. The antagonist or saline was injected at time 0 as indicated by the arrow. Each point represents mean±SE.

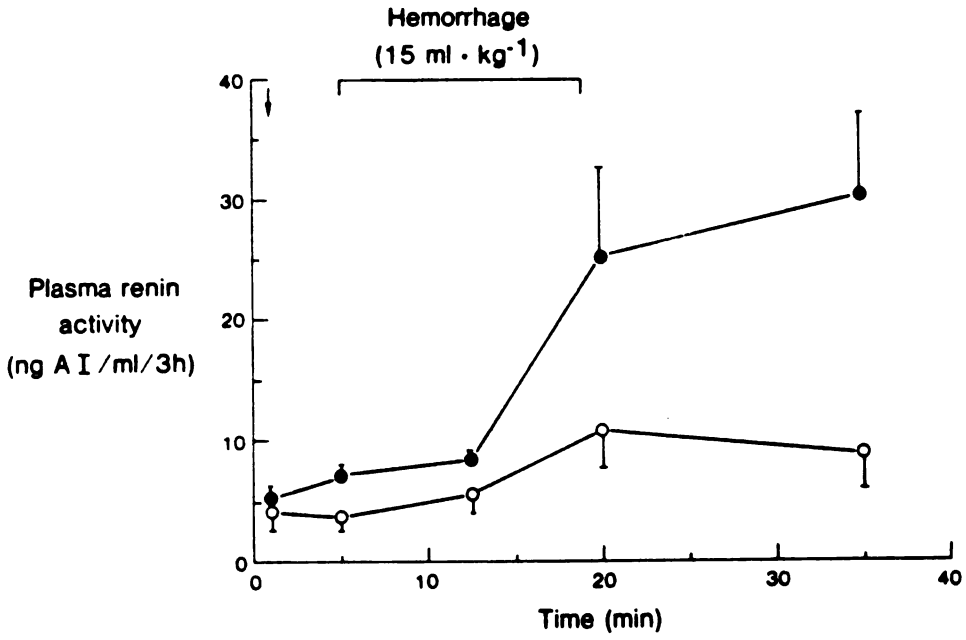


Figure 10. Effect of hemorrhage on plasma renin activity in the absence ⊖ and presence ● of vasopressin blockade. N = 4 in both groups. The antagonist or saline was injected at time 0 as indicated by the arrow. Each point represents mean±SE.

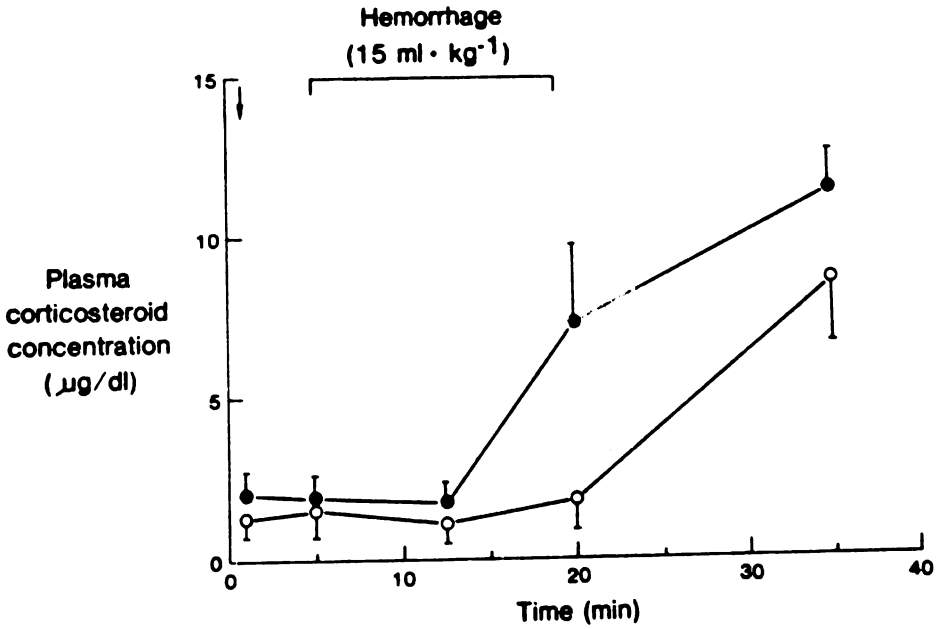


Figure 11. Effect of hemorrhage on plasma corticosteroid concentration in the absence -O- and presence -●- of vasopressin blockade. N = 4 in both groups. The antagonist or saline was injected at time 0 as indicated by the arrow. Each point represents mean±SE.

Hemorrhage following vasopressin blockade. Vasopressin blockade markedly altered the cardiovascular and endocrine responses to hemorrhage (Figs. 8-11). During the hemorrhage, mean arterial pressure decreased from 96±2 to 64±7 mmHg ($P<0.001$). Heart rate increased from 71±10 to 130±23 beats/min ($P<0.05$) and remained above 110 beats/min until the blood was reinfused. Plasma renin activity increased from 7.1±0.8 to 30.3±6.7 ng/ml/3h ($P<0.005$) and plasma CORTS concentration increased from 1.9±0.7 to 11.4±1.2 μg/dl ($P<0.001$). Two way analysis of variance for repeated measures revealed that the blood pressure ($P<0.05$), heart rate ($P<0.05$), PRA ($P<0.01$) and CORTS ($P<0.05$) responses to hemorrhage were significantly greater in the presence of vasopressin blockade.

Effects of adrenal insufficiency. Adrenal insufficiency caused marked increases in plasma vasopressin and angiotensin II concentrations. Vasopressin concentration increased 4-fold from 4.0±1.7 to 15.0±3.6 pg/ml ($P<0.05$) in adrenalectomized dogs during four days without steroids (Fig. 12); in one dog from which steroids had been withheld for five days, plasma vasopressin concentration reached 125.0 pg/ml. Angiotensin II concentration increased 3-fold from 121.1±44.3 to 352.0±19.7 pg/ml ($P<0.001$) over the four day period (Fig. 13). The corresponding changes in

body weight and plasma sodium and potassium concentrations are summarized in Table 1. Mean arterial pressure decreased from 98±1 to 83±6 mmHg over the four days, but this change was not statistically significant ($P>0.05$), probably because of the small number of dogs.

Vasopressin blockade in dogs with adrenal insufficiency. Blockade of the vasoconstrictor action of vasopressin caused a marked and rapid decrease in mean arterial pressure (Fig. 14). Ten min after the injection, blood pressure had decreased by 22±5 mmHg ($P<0.001$). Heart rate increased from 146±6 to 165±15 beats/min, but the response varied from dog to dog, and the change was not statistically significant.

Vasopressin blockade in dogs maintained on steroids. Injection of the antagonist caused no changes in blood pressure or heart rate when the dogs were maintained on steroids. Infusion of a pressor dose of synthetic vasopressin 30 min after injection of the antagonist had no effect on blood pressure.

TABLE 1. Effects of adrenal insufficiency on body weight, plasma sodium, and potassium concentrations and blood pressure

	Time (days)					P
	0	1	2	3	4	
BW (kg)	14.4 ± 1.0	14.3 ± 1.0	14.3 ± 1.0	14.0 ± 1.0	13.7 ± 0.9	<0.05
Plasma Na (meq/liter)	140.4 ± 0.2	142.7 ± 1.4	139.4 ± 1.4	136.7 ± 2.3	132.7 ± 2.0	<0.0001
Plasma K (meq/liter)	3.90 ± 0.34	4.21 ± 0.44	4.61 ± 0.4	5.09 ± 0.44	5.28 ± 0.29	<0.0001
Mean arterial pressure (mm Hg)	98 ± 1	97 ± 5	85 ± 6	88 ± 5	83 ± 6	>0.1

Conscious adrenalectomized dogs were maintained on 7.5 mg cortisol/day and 0.5 mg DOCA/day im until day 0. Measurements on day 0 were made 1.5 h after the final steroid treatment. Values are expressed as mean ± SEM.

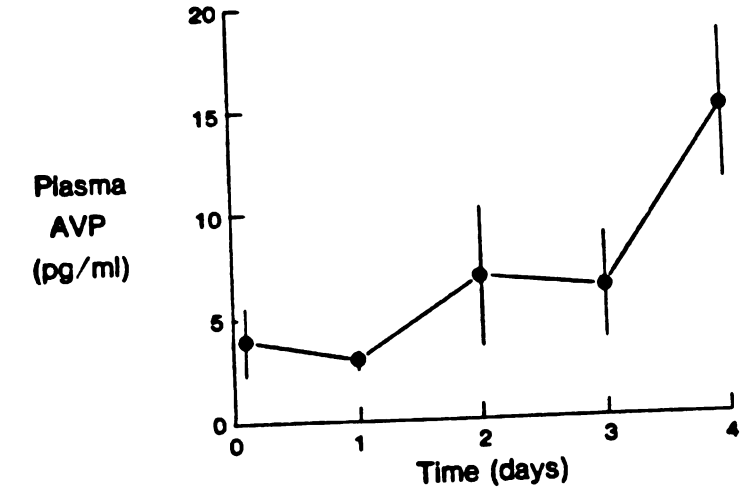


Figure 12. Effect of adrenal insufficiency on plasma vasopressin concentration (AVP). N = 4. Time after withdrawal of steroid treatment in conscious adrenalectomized dogs is plotted on the abscissa. Blood on day 0 was drawn 1.5h after the final steroid treatment. Each point represents mean±SE.

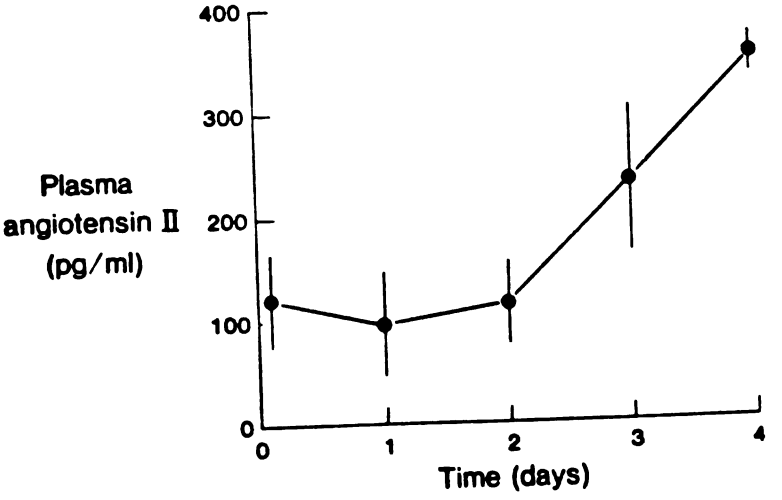


Figure 13. Effect of adrenal insufficiency on plasma angiotensin II concentration. N = 4. Time after withdrawal of steroid treatment in conscious adrenalectomized dogs is plotted on the abscissa. Blood on day 0 was drawn 1.5h after the final steroid treatment. Each point represents mean±SE.

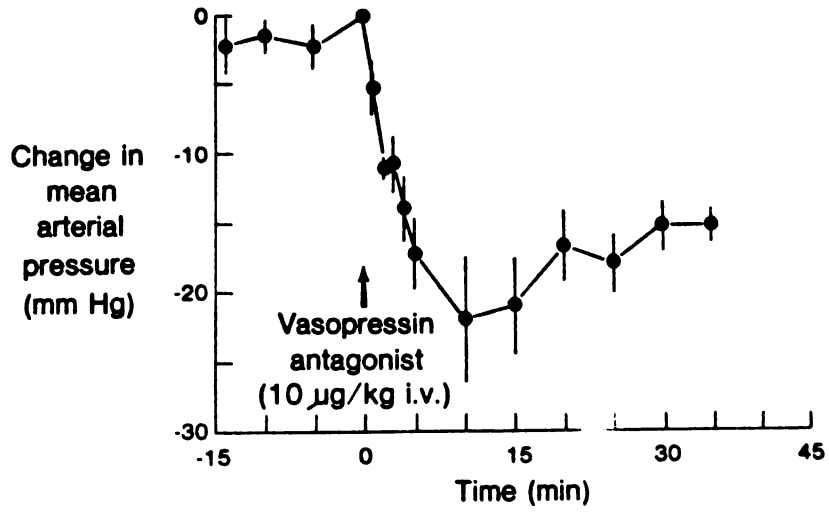


Figure 14. Effect of vasopressin blockade on blood pressure in dogs with adrenal insufficiency. N = 4. Each point represents mean±SE.

ANALOG STUDIES

MATERIALS AND METHODS

These experiments were performed in dogs of both sexes, weighing between 14 and 29 kg. The dogs were fed a standard diet (Purina Dog Chow, Ralston Purina Co., St. Louis, MO) which provided approximately 75 mEq sodium per day.

The peptides used in these studies were arginine vasopressin, AVP (Peninsula laboratories, San Carlos, CA), and the structural analogs which were described above (Fig. 2). The selective vasoconstrictor agonist, 2-phenylalanine-8-ornithine-oxytocin, PheOrnOT, and the selective vasoconstrictor antagonist, $d(CH_2)_5$MeTyrAVP, were kindly provided by Dr. Maurice Manning of the Medical College of Ohio. The selective antidiuretic agonist used was DDAVP (Ferring, New York City). All peptides were dissolved in sterile isotonic saline (0.45% NaCl for use in anesthetized water-loaded dogs). Solutions of the agonist peptides were infused at 0.5 ml/min; the antagonist was injected in a volume of approximately 1 ml and washed in with 2 ml saline.

In all experiments, mean and pulsatile arterial blood pressure and heart rate were continuously recorded with a Grass polygraph (Grass Instruments, Quincy, MA).

Samples of arterial blood were drawn (volume=7ml), and replaced by an equal volume of sterile isotonic saline (0.45% NaCl for use in anesthetized water-loaded dogs). A 2.7 ml aliquot of the blood was added to 0.3 ml 0.3 M EDTA in a chilled centrifuge tube for determination of PRA and the reminder was put into a chilled heparinized centrifuge tube for other measurements.

STUDIES IN CONSCIOUS DOGS

Eight dogs were used in these experiments, and were prepared according to the procedures described above for the Antagonist Studies in Conscious Dogs. The experiments were performed according to the following protocols, in random order with a minimum 48h between any experiments performed in the same dog.

Protocol 4-A. Vasopressin infusion. The cardiovascular and endocrine responses to infusion of vasopressin were studied in six dogs. An iv infusion of sterile isotonic saline was commenced. After 10 min, an additional 3 ml bolus of saline was injected as a control for antagonist experiments (protocol 4-D). After a total 20 min, the saline infusion was replaced by an infusion of vasopressin at 1.0 ng/kg/min for the following 60 min. This was followed in turn by resumption of the saline infusion for an additional 40 min. Control blood samples for determination of

PRA, CORTS and ACTH were drawn at 0, 10 and 20 min. Blood samples were drawn again at 40, 60 and 80 min (20, 40 and 60 min into the vasopressin infusion) and a recovery sample was drawn at 120 min.

Protocol 4-B. DDAVP infusion. In six dogs the effects of DDAVP (1.0 ng/kg/min) were studied as described in protocol 4-A, substituting DDAVP for vasopressin. Plasma was analyzed for PRA and CORTS only.

Protocol 4-C. PheOrnOT infusion. The vasoconstrictor selective analog was infused at 1.0 ng/kg/min in five dogs as described in protocol 4-A, substituting PheOrnOT for vasopressin.

Protocol 4-D. Vasopressin infusion in the presence of the vasoconstrictor antagonist. In six dogs vasopressin was infused as described in protocol 4-A and 10 μg/kg of $d(CH_2)_5$MeTyrAVP was injected iv at 10 min instead of saline. Plasma was analyzed for PRA and CORTS only.

Protocol 4-E. Time Control. Five dogs were subjected to the experimental protocol as described above except that only saline was infused for the 120 min.

STUDIES IN ANESTHETIZED WATER-LOADED DOGS

The dogs were fasted for 18h. They were treated with acepromazine (Ayerst, New York City, 0.5 mg/kg sc) to prolong the duration of action of the anesthetic, and were anesthetized 30 min later with pentobarbital (Fort Dodge Laboratories, Fort Dodge IA, 15 mg/kg iv). Anesthesia was maintained with additional iv pentobarbital as required. During surgery and experiments, body temperature was maintained at 37 C with a water blanket. Polyethylene catheters were inserted into the thoracic aorta and vena cava via a leg artery and vein. An intracath (Deseret Pharmaceutical, Sandy, UT) was inserted percutaneously into a cephalic vein. The right ureter was cannulated with polyethylene tubing through either a flank or midline incision for urine collection. All incisions were closed with wound clips. To hydrate the dogs, a solution of 2.5% dextrose in distilled water was infused iv at approximately 7 ml/min and continued throughout the entire experiment. Experiments were begun following establishment of a steady state water diuresis. Only one experiment was performed in each dog, according to the protocols below, which were designed to duplicate those followed in conscious dogs:

Protocol 5-A. Vasopressin infusion. In five dogs, 0.45% NaCl was infused iv (0.5 ml/min) for 20 min. After 10 min, an additional 3 ml bolus of 0.45% NaCl was injected iv as a control for experiments with the antagonist. At 20 min, the saline infusion was replaced by a vasopressin infusion (1.0 ng/kg/min) for 60 min, which was in turn replaced by resumption of the saline infusion for 40 min. Ten minute urine collections were made throughout the experiment, beginning with the period from -10 min to 0 min. Blood samples for PRA and plasma osmolality determinations were drawn at 0, 20, 40, 60, 80 and 120 min.

Protocol 5-B. DDAVP infusion. In five dogs protocol 5-A was performed, with the substitution of DDAVP (1.0 ng/kg/min) for vasopressin.

Protocol 5-C. PheOrnOT infusion. In five dogs protocol 5-A was performed, with the substitution of PheOrnOT (1.0 ng/kg/min) for vasopressin.

Protocol 5-D. Vasopressin infusion in the presence of the vasoconstrictor antagonist. In five dogs vasopressin was infused as described in protocol 5-A and 10 µg/kg $d(CH_2)_5$MeTyrAVP was injected at 10 min instead of saline.

An analog of vasopressin, which had been observed to be an antagonist of the antidiuretic response in rats, was also tested in anesthetized water-loaded dogs. It did not block the antidiuretic activity of vasopressin and further studies with this compound were discontinued.

Protocol 5-E. Time Control. Five dogs were subjected to identical anesthetic, surgical and experimental procedures as described above, but only 0.45% NaCl was infused for the 120 min experimental period. This protocol is important because the effects of acepromazine and pentobarbital in combination were not known, and thus it was necessary to be able to differentiate between any effects of the anesthesia and the effects of the peptides.

PRA, CORTS and plasma and urinary osmolality and electrolyte concentrations were measured according to the procedures described above in the Antagonist Studies in Conscious Dogs. Plasma ACTH concentration was measured in extracts of plasma samples (recovery = 60-90%), where indicated, by RIA (24). The intra- and interassay coefficients of variation for the ACTH assay are 8% and 19%, respectively. The lowest detectable amount of ACTH with this procedure is 8.0 pg.

All results are expressed as mean±s.e.m. statistical evaluation of the data was performed by one- and two way analysis of variance for repeated measures and Duncan's multiple range test (123).

RESULTS

CONSCIOUS DOGS

Vasopressin. Infusion of vasopressin in conscious dogs decreased PRA from 4.4±1.1 to 2.4±0.8 ng/ml/3h ($P<0.05$)(Fig. 15). Mean arterial pressure increased from 106±2 to 115±3 mmHg ($P<0.05$) and heart rate decreased from 81±6 to 56±6 beats/min ($P<0.001$). These changes reversed following cessation of the infusion.

CORTS increased from the control value of 1.0±0.2 µg/dl as a result of the vasopressin infusion ($P<0.001$). The peak concentration measured was 2.2±0.2 µg/dl after 20 min (Fig. 16). There was no statistically significant change in plasma ACTH concentration from the control value of 44±7 pg/ml, but the ACTH response to the vasopressin infusion parallelled the CORTS response (Fig. 16).

DDAVP. In contrast to the responses to vasopressin, infusion of DDAVP at the same rate did not change PRA, mean arterial pressure, heart rate or CORTS significantly from the control values of

3.9±0.5 ng/ml/3h (Fig. 17), 102±4 mmHg (Fig. 17), 70±3 beats/min (Fig. 17) or 1.2±0.2 µg/dl respectively.

PheOrnOT. The cardiovascular and endocrine responses to PheOrnOT in conscious dogs closely resembled the responses to vasopressin. PRA decreased from 5.5±1.1 to 2.7±0.2 ng/ml/3h ($P<0.001$)(Fig. 18). Blood pressure increased from 112±4 to 128±6 mmHg ($P<0.001$) and heart rate decreased from 69±3 to 47±4 beats/min ($P<0.001$).

Infusion of PheOrnOT also increased plasma CORTS from 1.1±0.1 to 2.9±0.9 µg/dl ($P<0.005$). By the end of the infusion however, plasma CORTS was only 1.6±0.4 µg/dl and 40 min later was 0.8±0.2 µg/dl (Fig. 19). The decrease in plasma ACTH was significant (control value = 53±8 pg/ml)($P<0.05$) and appears to be responsible for the decrease of CORTS rather than the initial increase (Fig. 19).

Vasopressin following the vasoconstrictor antagonist. The PRA, blood pressure and heart rate responses to vasopressin in the presence and absence of the antagonist are compared in Fig. 20. The blocker completely eliminated the changes in pressure, heart rate and CORTS (control CORTS = 1.8±0.8; after 20 min infusion = 1.8±0.5; at end of infusion = 2.1±0.7 µg/dl) caused by vasopressin. It blocked the decrease in PRA; in fact in the

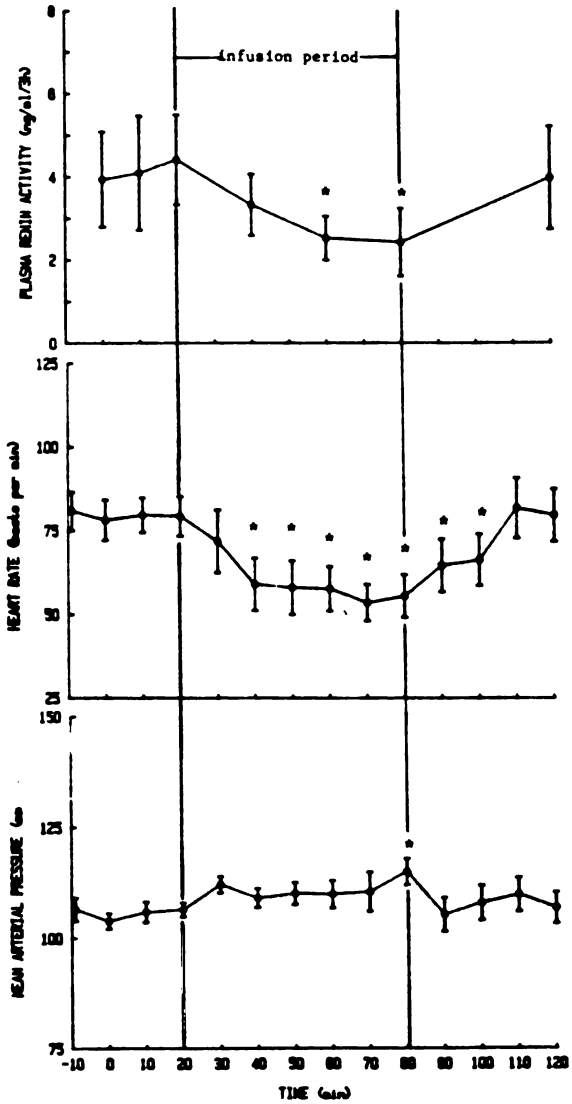


Figure 15. Effects of vasopressin infusion (1ng/kg/min) in conscious dogs. N = 6. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

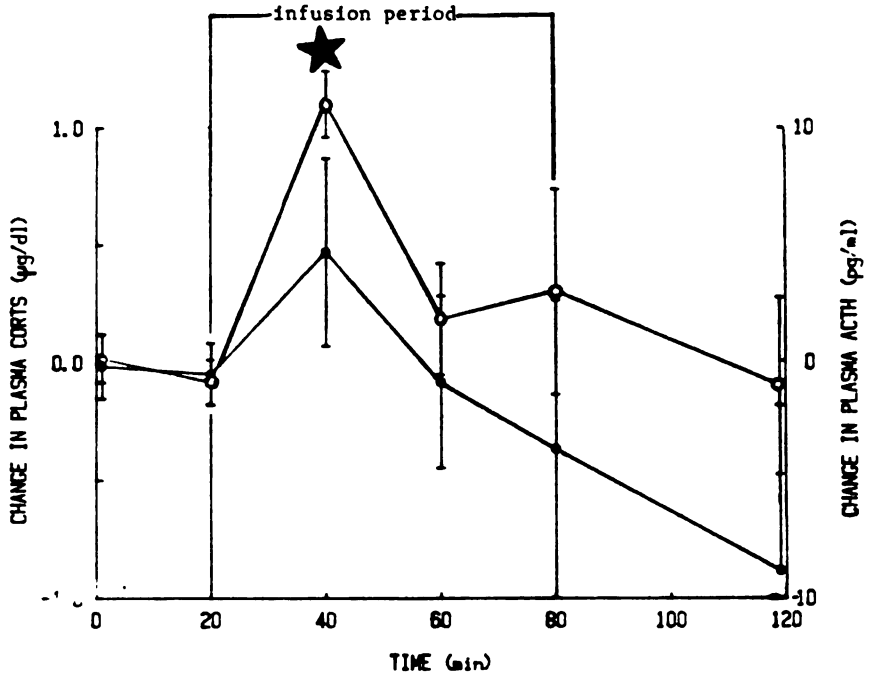


Figure 16. Effects of vasopressin infusion (1ng/kg/min) on plasma corticosteroid -O- and ACTH -●- concentrations. N = 6. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

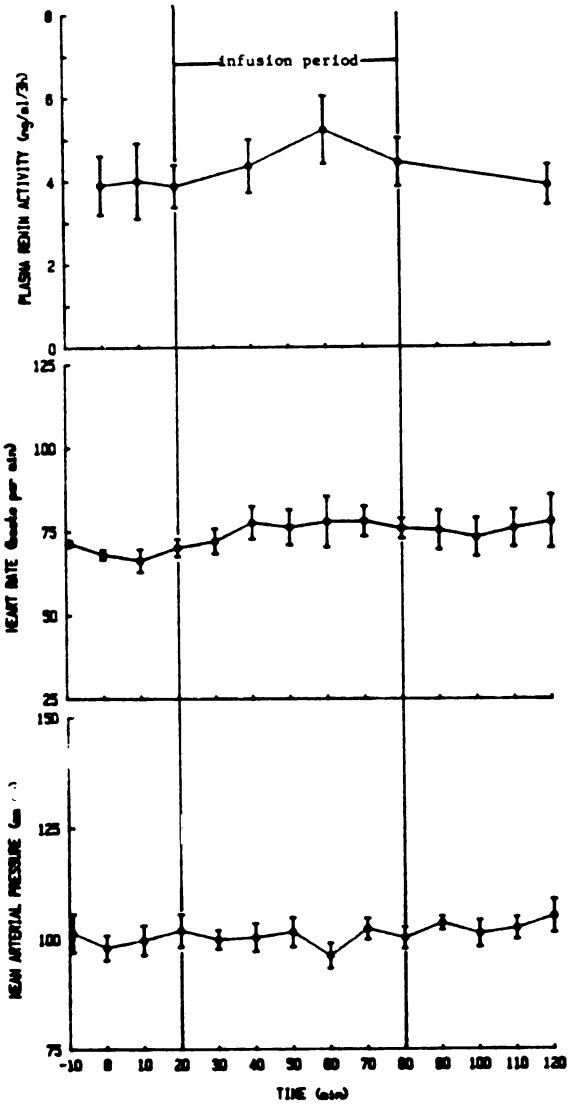


Figure 17. Effects of DDAVP infusion (1ng/kg/min) in conscious dogs. N = 6. Each point represents mean±SE.

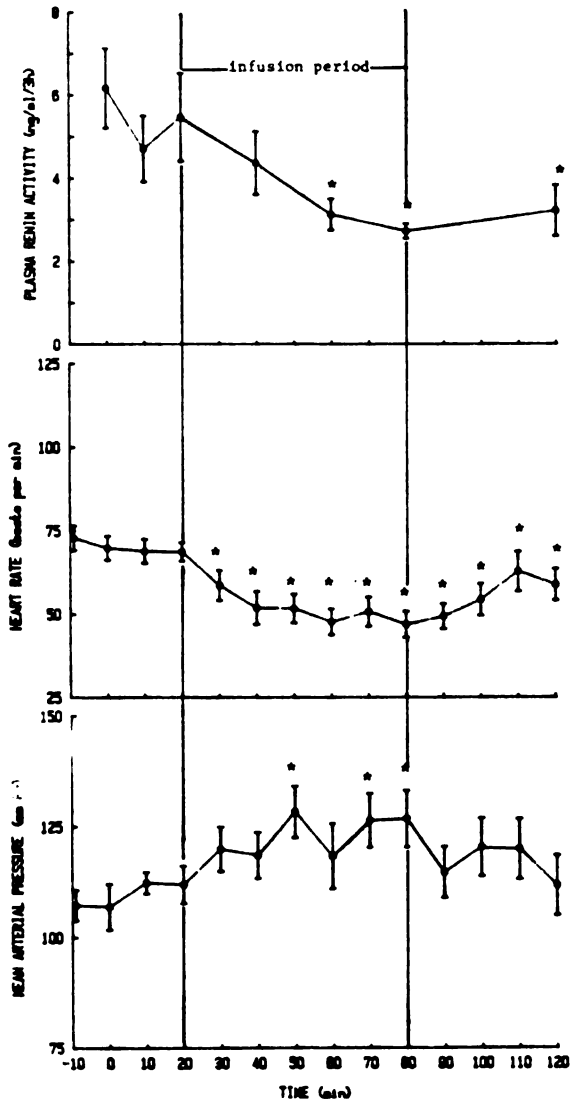


Figure 18. Effects of PheOrnOT infusion (1ng/kg/min) in conscious dogs. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

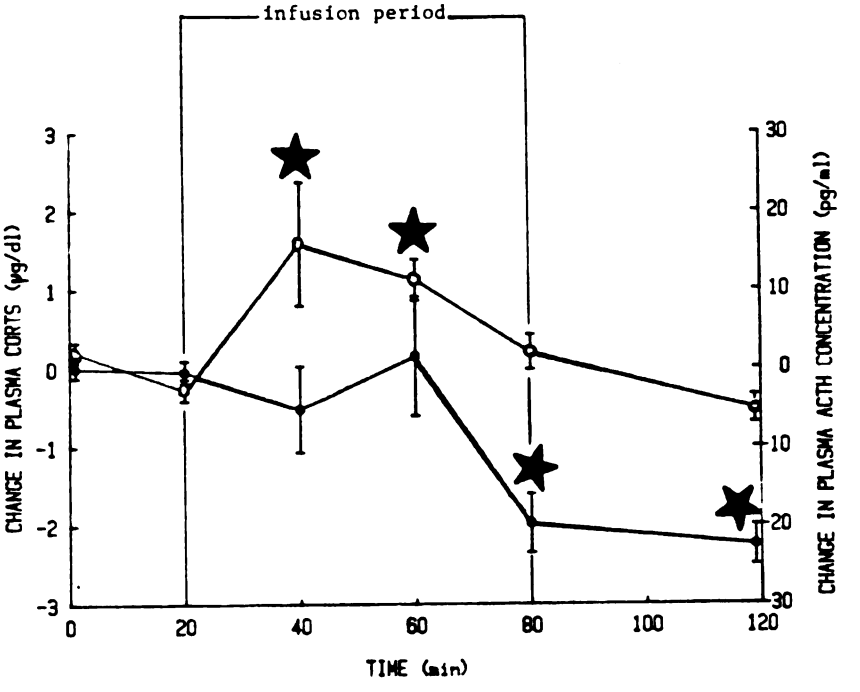


Figure 19. Effects of PheOrnOT infusion (1ng/kg/min) on plasma corticosteroid -O- and ACTH -●- concentrations. N = 5 for CORTS; N = 4 for ACTH. Each point represents mean±SE. * P< 0.05 compared to control by Duncan's multiple range test.

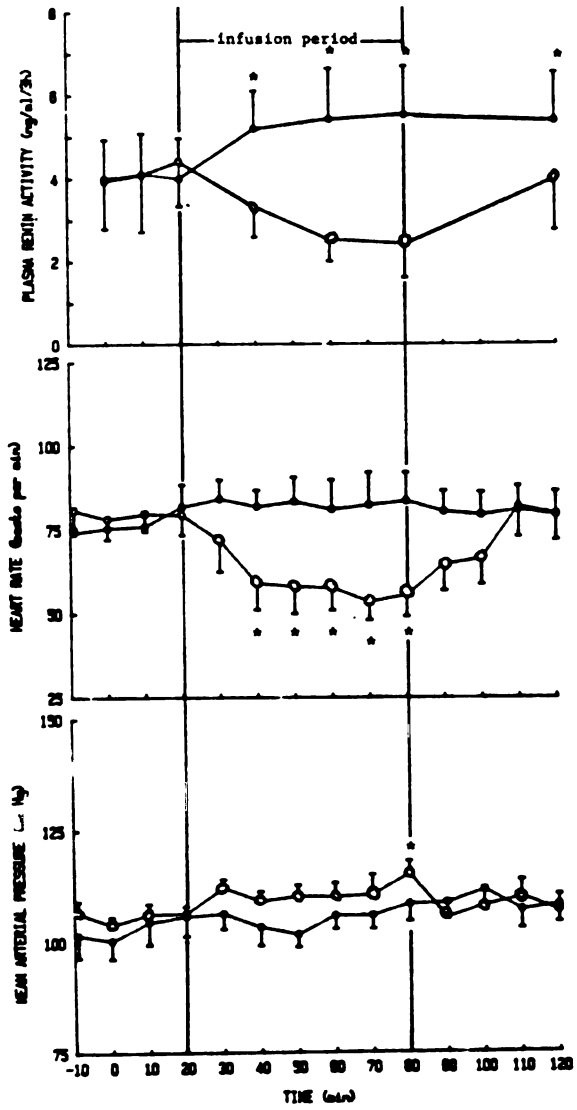


Figure 20. Effects of vasopressin infusion (1ng/kg/min) in conscious dogs in the absence (-○-, from Fig. 15) and presence -●- of vasoconstrictor blockade. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

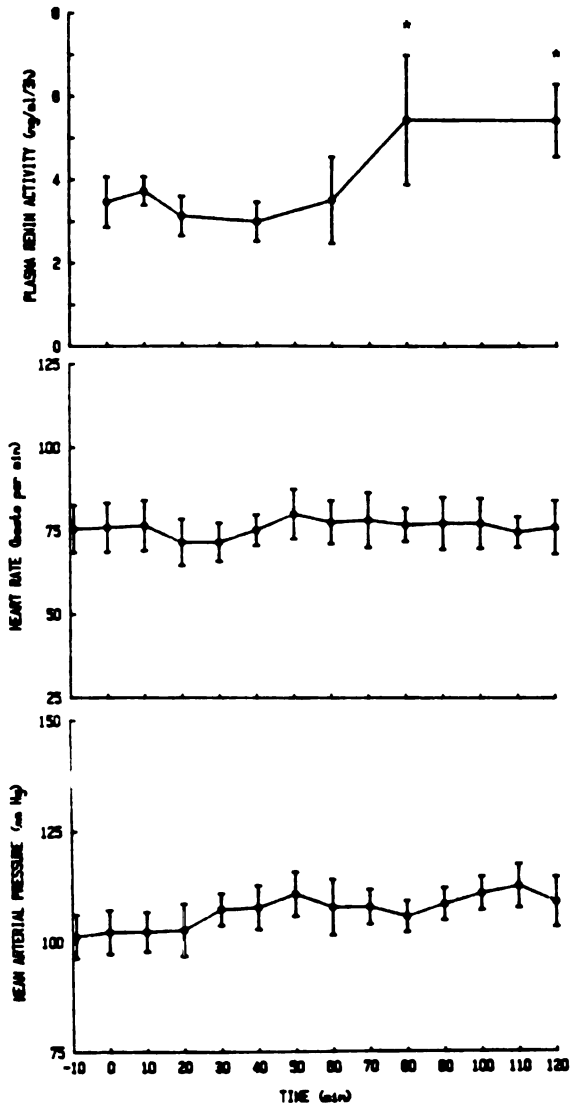


Figure 21. Effects of isotonic saline infusion in conscious dogs during 120 min experimental protocol. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

presence of $d(CH_2)_5$MeTyrAVP and vasopressin, PRA increased from 4.0±1.0 to 5.5±1.2 ng/ml/3h (P<0.01), which is very similar to the observations made in the time control (see below).

Time control. There were no significant changes in mean arterial pressure, heart rate or plasma CORTS in dogs subjected to infusion of sterile isotonic saline for 120 min; only a tendency for all variables to drift slowly upward. Plasma renin activity increased from 3.5±0.5 to 5.4±0.9 over 120 min (P<0.05) (Fig. 21).

ANESTHETIZED, WATER-LOADED DOGS

Vasopressin. Infusion of vasopressin in anesthetized water-loaded dogs decreased PRA from 11.9+4.7 to 3.8+1.7 ng/ml/3h (P<0.05)(Fig. 22). Mean arterial pressure increased from 134±9 to 158±12 mmHg (P<0.001) and heart rate decreased from 159±12 to 105±11 beats/min (P<0.001)(Fig. 22). There was a marked decrease in urine volume and increase in osmolality (Table 2). Free water clearance decreased from 2.87±0.50 to -0.9±0.19 ml/min/kidney (P<0.001) (Fig. 23). Sodium excretion increased from 28.3±10.6 to 123.0±13.8 µEq/min (P<0.001)(Fig. 24). All these changes reversed following cessation of the infusion.

DDAVP. In contrast to its effect in conscious dogs, DDAVP decreased PRA from 13.5±4.6 to 7.0±2.0 ng/ml/3h in anesthetized water-loaded dogs ($P<0.05$)(Fig. 25). There were no significant changes in blood pressure or heart rate (Fig. 25). Urine flow decreased in association with a marked increase in osmolality (Table 2), so free water clearance decreased from 3.72±0.25 to -0.62±0.14 ml/min/kidney (Fig. 23). Sodium excretion did not change significantly (Fig. 24).

PheOrnOT. In anesthetized water-loaded dogs, infusion of PheOrnOT increased mean arterial pressure from 124±11 to 145±15 mmHg ($P<0.001$) and decreased heart rate from 124±12 to 106±12 beats/min $P<0.005$)(Fig. 26). These changes reversed after cessation of the infusion. In contrast to its effect in conscious dogs, PheOrnOT did not decrease PRA in anesthetized animals (Fig. 26). This peptide also had no effect on urine flow or osmolality (Table 2) or on free water clearance (Fig. 23). Infusion was associated with a small increase in sodium excretion (Fig. 24). However the increase in sodium excretion was not significantly different from that which occurred during infusion of the NaCl vehicle (Fig. 27).

Vasopressin following the vasoconstrictor antagonist. The responses to vasopressin in the presence and absence of the vasoconstrictor antagonist are compared in Fig. 28. As in conscious dogs, the antagonist completely blocked the arterial pressure and heart rate responses to vasopressin. Heart rate actually increased significantly ($P<0.001$) in the final 20 min of the experiment, but the reasons for this are not clear. However, in contrast to results in with conscious dogs, the antagonist failed to block the PRA response in anesthetized water-loaded dogs, and vasopressin decreased PRA from 5.9 ± 1.8 to 2.9 ± 1.6 ng/ml/3h ($P<0.001$). The antagonist also failed to alter the antidiuretic responses to vasopressin. Urine flow decresed and osmolality increased markedly (Table 2). Free water clearance decreased from 2.74 ± 0.29 to -0.63 ± 0.04 ml/min/kidney (Fig. 23).

The antagonist blocked the natriuretic response to vasopressin, so that there was no significant difference in sodium excretion between that observed with vasopressin following $d(CH_2)_5$MeTyrAVP and that observed during infusion of 0.45% NaCl alone (Fig. 24; Fig. 27).

Time control in anesthetized dogs. The combination of anesthesia, surgery and water loading caused increases in mean arterial pressure, heart rate and plasma renin activity. This is reflected in the elevated control values observed in anesthetized water-loaded dogs compared to values obtained in the conscious dogs. The 0.45% NaCl vehicle infusion following anesthesia and surgery had no effect on blood pressure, heart rate, urine flow rate or osmolality, or free water clearance. There was a slow upward drift of sodium excretion during the course of the experiment ($P<0.001$)(Fig. 24).

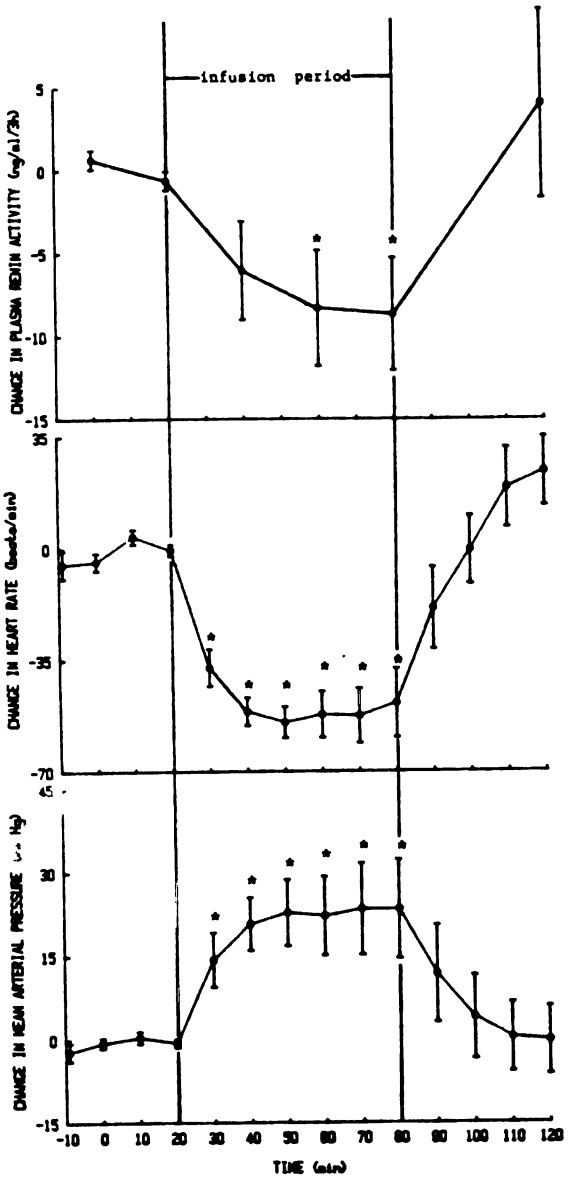


Figure 22. Effects of vasopressin infusion (1ng/kg/min) in anesthetized water-loaded dogs. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

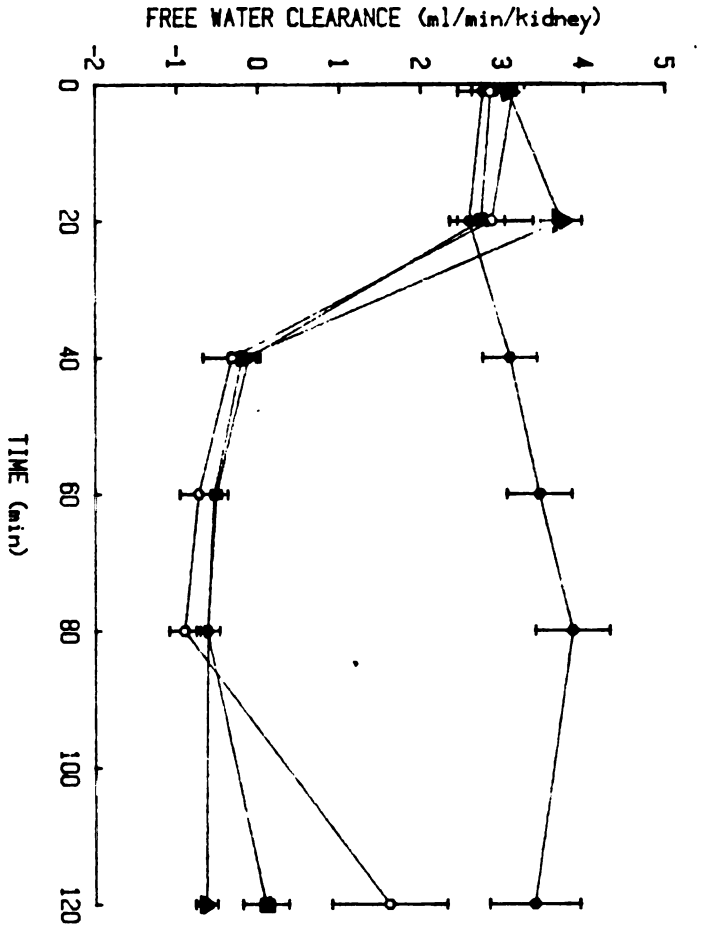


Figure 23. Effects of vasopressin -O-, DDAVP -▲-, PheOrnOT -●- (all 1ng/kg/min infusions) and vasopressin in the presence of d(CH2)5MeTyrAVP (10 µg/kg) -■- on free water clearance in anesthetized water-loaded dogs. N = 5 in all groups. Each point represents mean±SE.

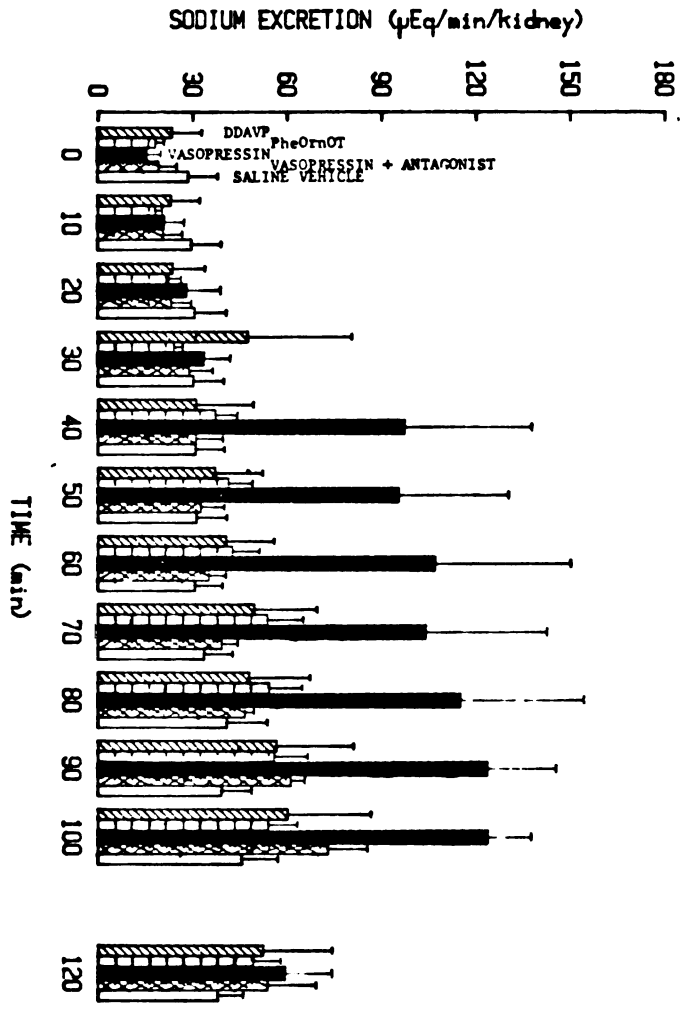


Figure 24. Effects of vasopressin ■ ,DDAVP ▨ ,PheOrnOT ▤ ,vasopressin in the presence of $d(CH_2)_5$MeTyrAVP ⊠ ,and vehicle infusion □ on rate of sodium excretion in anesthetized water-loaded dogs. N = 5 in all groups. Each bar represents mean±SE.

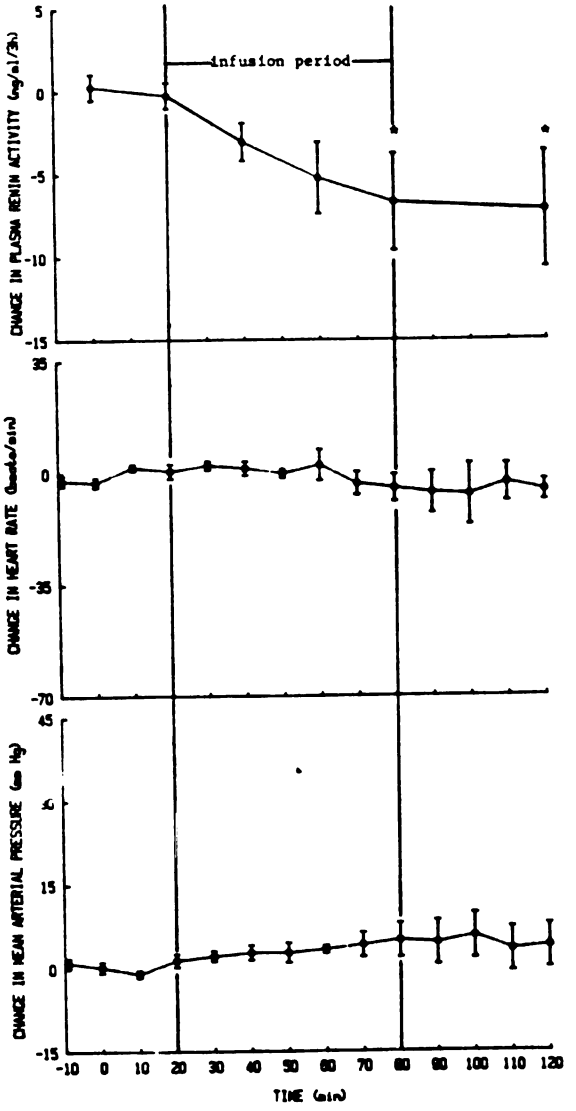


Figure 25. Effects of DDAVP infusion (1ng/kg/min) in anesthetized water-loaded dogs. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

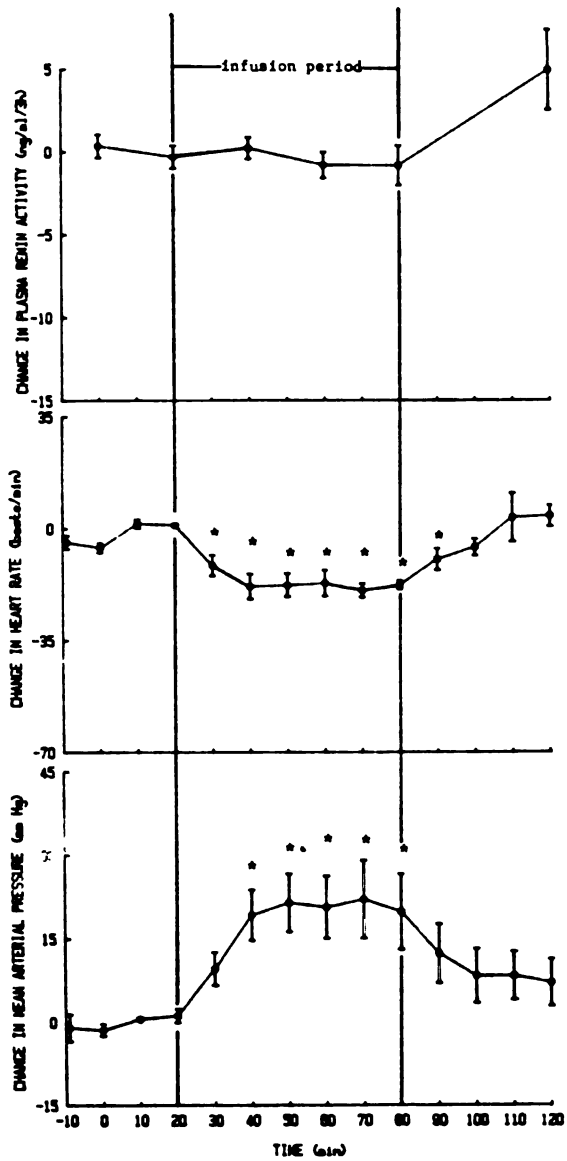


Figure 26. Effects of PheOrnOT infusion (1ng/kg/min) in anesthetized water-loaded dogs. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

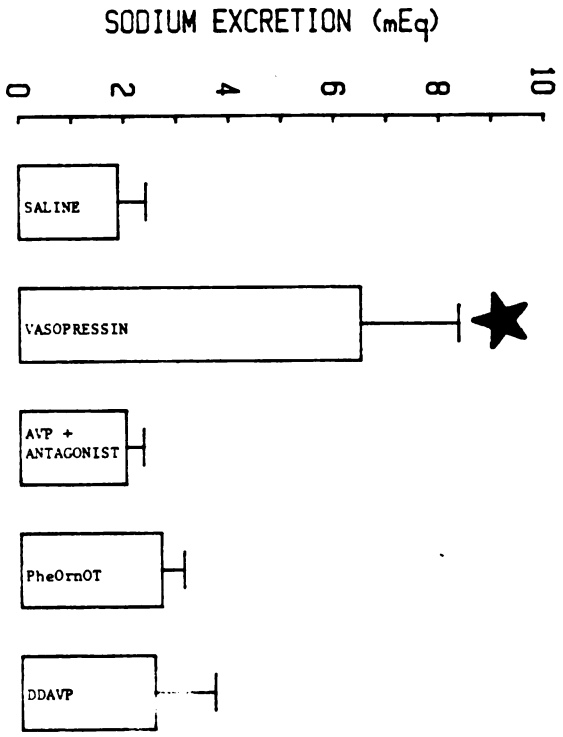


Figure 27. Effect of peptides and saline vehicle on total amount of sodium excreted during 60 min infusion period. N = 6 in vasopressin group; N = 5 in other groups. Each bar represents mean±SE. * indicates significant difference ($P<0.05$ by Duncan's multiple range test) between vasopressin and all other groups.

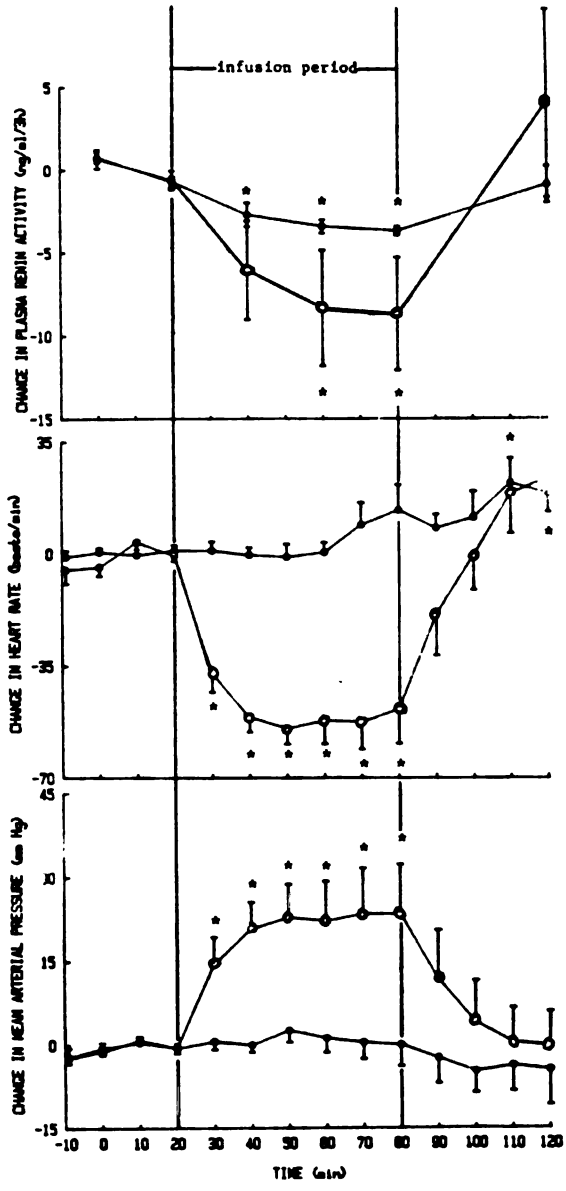


Figure 28. Effects of vasopressin infusion (1ng/kg/min) in anesthetized water-loaded dogs in the absence (-○-, from Fig. 21) and presence -●- of vasoconstrictor blockade. N = 5. Each point represents mean±SE. * P<0.05 compared to control by Duncan's multiple range test.

TABLE 2. Effects of vasopressin, DDAVP, PheOrnOT, and vasopressin in the presence of $d(CH_2)_5$MeTyrAVP on urine flow and osmolality in anesthetized water-loaded dogs.

	TIME (min)												
FLOW ml/min/kidney	0	10	20	30	40	50	60	70	80	90	100	110	120
TREATMENT:													
Vasopressin	3.85	3.88	3.77	2.93	1.26	0.90	0.89	0.79	0.83	0.83	1.11	1.52	2.74
	0.05	0.32	0.42	0.57	0.46	0.39	0.45	0.36	0.35	0.16	0.23	0.40	0.68
DDAVP	3.71	4.30	4.42	3.00	0.35	0.27	0.28	0.32	0.31	0.38	0.42	0.42	0.39
	0.10	0.25	0.34	0.30	0.08	0.07	0.07	0.10	0.11	0.13	0.15	0.15	0.12
PheOrnOT	3.54	3.26	3.27	3.40	4.04	4.47	4.48	4.90	4.93	4.78	4.60	4.51	4.38
	0.32	0.25	0.35	0.28	0.35	0.42	0.44	0.46	0.55	0.50	0.71	0.73	0.60
Vasopressin+ $d(CH_2)_5$MeTyrAVP	3.65	3.85	3.56	2.66	0.50	0.26	0.24	0.25	0.28	0.36	0.53	0.89	1.17
	0.25	0.34	0.34	0.37	0.20	0.06	0.06	0.06	0.07	0.09	0.20	0.24	0.44
OSMOLALITY mOsm/kg													
Vasopressin	50	60	74	98	479	519	665	672	661	602	467	380	143
	2	10	22	27	153	143	164	124	111	93	100	146	45
DDAVP	48	39	40	94	377	718	886	935	945	930	906	854	874
	12	8	6	47	103	132	187	233	245	258	250	239	227
PheOrnOT	60	63	75	67	64	62	63	62	60	61	62	68	65
	8	6	10	8	6	4	5	5	3	1	6	12	10
Vasopressin+ $d(CH_2)_5$MeTyrAVP	58	56	62	83	435	927	960	1018	1001	927	704	452	289
	5	6	6	14	91	231	161	182	193	194	168	103	42

Values are expressed as $\frac{\text{mean}}{\text{se}}$ for 10 min collection period ending at time indicated.

DISCUSSION

ANALOGS

The peptides used in this study were assessed for antidiuretic and vasoconstrictor activity in dogs. The vasoconstrictor antagonist, $d(CH_2)_5$MeTyrAVP, completely blocked the arterial pressure and heart rate responses to exogenous vasopressin, both in conscious and anesthetized dogs, but left the antidiuretic response intact. Neither $d(CH_2)_5$MeTyrAVP nor $d(CH_2)_5$AVP had any effect on blood pressure or heart rate in conscious dogs with low circulating vasopressin levels. When injected into dogs whose endogenous vasopressin secretion had been stimulated, the effects were opposite to those caused by infusion of vasopressin. This is in agreement with the findings of others who used similar antagonists in rats whose plasma vasopressin concentrations had been suppressed and in rats with elevated vasopressin levels (4, 8). It has also been shown that these compounds are specific antagonists of vasopressin and do not block the pressor responses to either angiotensin II or norepinephrine (4, 79). These analogs blocked the pressor response to vasopressin for at least one hour. Other workers have also reported a long duration of action following single injection of these compounds (79). Therefore the antagonists were very well suited to the purpose of vasoconstrictor blockade in these studies.

DDAVP was at least as potent an antidiuretic agent as vasopressin, as has been observed in rats (128), dogs (116) and man (101), but only one dose was tested and this probably exerted maximum antidiuretic activity. The longer duration of action was consistent with observations made on its half-life in rats (106), dogs (116) and man (101). The absence of any heart rate or blood pressure response to DDAVP in the present study is also in agreement with previous observations (101, 128).

The selective vasoconstrictor agonist, PheOrnOT, exhibited no antidiuretic activity in the present study and this agrees with reports that it is only about 1/700 as potent an antidiuretic agent as vasopressin (44). PheOrnOT was a potent pressor agent in these studies. It appeared to be slightly less potent than vasopressin in anesthetized dogs, in agreement with data obtained in the anesthetized rat pressor assay (44). On the other hand, in conscious dogs, PheOrnOT was at least as potent a pressor agent as vasopressin. The reason for the difference in relative potencies in anesthetized versus conscious dogs is not clear.

DOES VASOPRESSIN PLAY A ROLE IN CARDIOVASCULAR REGULATION

The present studies provide direct evidence for a role for vasopressin in blood pressure maintenance during water deprivation, nonhypotensive hemorrhage and adrenal insufficiency. This is based on the cardiovascular responses to selective blockade of the vasoconstrictor activity of vasopressin during these conditions in conscious dogs.

Water-Replete Dogs. Vasopressin blockade in dogs in normal water balance produced no effects on mean arterial pressure, heart rate or plasma renin activity. This is consistent with the findings of Aisenbrey et al. (4) in rats. It is also consistent with the observation that there is no significant difference between the resting blood pressures in normal rats and those unable to secrete vasopressin (61, 130). Taken together, these results indicate that vasopressin probably does not contribute to blood pressure regulation in resting animals in normal water balance.

Water-Deprived Dogs. The present studies indicate that when the plasma concentration of vasopressin is elevated during water deprivation, vasopressin takes on an increased role in the maintenance of blood pressure. In 48h water-deprived conscious dogs, vasopressin blockade did not change mean arterial pressure

but did cause a marked tachycardia and increase in plasma renin activity. In two studies in water-deprived rats, vasopressin blockade was observed to decrease blood pressure. However, in one of the studies (8) the rats were anesthetized, and in the other (4) vasopressin blockade was performed shortly after surgery under anesthesia. The increased vasopressin secretion due to surgical stress or anesthesia may have caused the contribution of vasopressin in blood pressure maintenance to be overestimated in those studies. In a more recent study in conscious water deprived rats, Rockhold et al. (103) observed no change in blood pressure with vasopressin blockade.

One explanation for the present results is that the vasopressin antagonists blocked the vasoconstriction produced by endogenous vasopressin, and that the increases in heart rate and PRA were reflex responses to help maintain blood pressure. This is a likely explanation because it is known that some of the actions of vasopressin on heart rate, cardiac output and renin secretion buffer its vasoconstrictor effect. The buffering actions must be at least in part baroreceptor-mediated reflexes because in baroreceptor-denervated dogs, heart rate and cardiac output do not change with small increases in plasma vasopressin concentrations and blood pressure is increased (25, 76). It has not been demonstrated whether the renin response to vasopressin is mediated

by baroreceptors, but the results of the present study shed light on this possibility and this will be discused below.

To test the hypothesis that reflex increases in heart rate and PRA acted to maintain blood pressure during vasopressin blockade in the present study, the experiment was repeated in five of the same dogs following pretreatment with propranolol. The rationale was that propranolol, in a dose at which it is a pure β-adrenoceptor blocking drug (38), would inhibit relexes mediated by β-adrenoceptors, and thus uncover any decreases in total peripheral resistance. In water-deprived dogs, propranolol itself did not change arterial pressure or heart rate. Propranolol decreased PRA in 4 of 5 water-deprived dogs before injection of the antagonist, but this decrease was not statistically significant. However, propranolol completely eliminated the increase in PRA produced by vasopressin blockade and significantly reduced the tachycardia. With at least a portion of the sympathetic reflexes attenuated by propranolol, vasopressin blockade significantly decreased blood pressure. A recent study in spinally transsected dogs subjected to 36h water deprivation is in excellent agreement with these findings. Mikami et al. (75) injected $d(CH_2)_5$MeTyrAVP and observed no change in blood pressure, unless the renin-angiotensin system had been disrupted by pretreatment with captopril.

To further test the hypothesis that the heart rate and plasma renin activity responses were reflex responses to decreased vascular resistance, the hemodynamic effects of vasopressin blockade were studied in water-deprived dogs. These studies confirmed that the antagonists decreased total peripheral resistance. A decrease in total peripheral resistance has also been observed in rats (4). Taken together, these data indicate that vasopressin plays an important role in blood pressure maintenance during periods of water deprivation, and does so by contributing to the total peripheral resistance. When the vasoconstrictor action of vasopressin is blocked, cardiac output and renin secretion increase as compensatory mechanisms to maintain pressure.

Nonhypotensive Hemorrhage. The results from the hemorrhage experiments also demonstrate that vasopressin plays a role in maintaining blood pressure. A 1ml/kg/min hemorrhage over 15 min was used in the present studies because this stimulus has previously been observed to cause a large increase in plasma vasopressin levels without any change in arterial pressure (90). The plasma vasopressin concentration achieved by hemorrhage in the present study is comparable to that observed by Reid (90).

The hemorrhage itself did not change blood pressure. However, in the presence of the blockade of vasopressin, the hemorrhage decreased mean arterial pressure by an average 32 mmHg. This finding agrees with that of Cowley et al. (26), who showed that the ability of dogs to restore arterial pressure following hypotensive hemorrhage is impaired by pharmacological vasopressin blockade to the same extent as by hypophysectomy. In contrast to those studies, the dogs in the present study were conscious and not stressed by surgery, so that plasma vasopressin concentrations before the hemorrhage were normal, and all the reflex mechanisms were intact. More recent studies, using $d(CH_2)_5$MeTyrAVP in rats and sheep, have confirmed the findings of the present study. Zerbe et al. (129, 130) reported that the antagonist impaired the ability of rats to recover from hypotensive hemorrhage; Rose and Kelly (104) reported that administration of the antagonist increased the hypotensive response to a given hemorrhage in fetal sheep.

Vasopressin blockade also altered other responses to hemorrhage in the present study. The hemorrhage itself was not associated with any change in heart rate. In the presence of vasopressin blockade, it caused a marked tachycardia, which, as with vasopressin blockade in water-deprived dogs, was presumably a reflex response. PRA was increased by hemorrhage, in the

presence and absence of vasopressin blockade. However, in the presence of the antagonist the increase in PRA was significantly greater. The changes in PRA were also presumably reflex responses to hypovolemia, and the additional increase observed with vasopressin blockade may have resulted from a decrease in renal perfusion pressure (28). Alternatively, it may have also been due to blockade of the inhibitory effect of vasopressin on renin secretion (28, 52, present results). Regardless of the cause of the heart rate and renin responses, it is likely that the large increases in heart rate and PRA observed during hemorrhage in the presence of the antagonist prevented blood pressure from falling even more than it did. Thus, the contribution of endogenous vasopressin to arterial pressure regulation may have been underestimated in this study.

Adrenal Insufficiency. Testing whether vasopressin plays a role in blood pressure regulation during adrenal insufficiency was potentially more complex than the experiments assessing the roles of vasopressin during water deprivation and hemorrhage. Unlike the latter hypovolemic conditions, there was conflicting evidence regarding vasopressin secretion during adrenal insufficiency. For this reason, plasma vasopressin was measured by RIA during adrenal insufficiency in these studies, and found to increase. This

agrees with some bioassay studies (2, 3, 110) and RIA studies in glucocorticoid-deficient dogs (14), mineralocorticoid-deficient dogs (15) and glucocorticoid-deficient rats (65, 70).

Angiotensin II has been observed to help maintain arterial pressure in animals with adrenal insufficiency (11) and to stimulate vasopressin secretion (77, 89, 92). The effect of adrenal insufficiency on plasma ang II concentration had not been investigated, and it was of interest to measure plasma ang II levels during adrenal insufficiency. The high plasma ang II concentration observed while the animals were on steroids may indicate that the replacement regime was incomplete. Nonetheless, the increase which followed withdrawal of steroids indicates a strong ang II response to adrenal insufficiency, much like the PRA response (95).

In this study, vasopressin blockade had no effect on blood pressure or heart rate in adrenalectomized dogs maintained on cortisol and DOCA. However, in dogs made adrenally insufficient by withdrawal of steroid treatment, vasopressin blockade caused a marked decrease in arterial pressure. Blood pressure was stable during the experiment and did not decrease until after the antagonist was injected. Thus, the decrease in arterial pressure was due to vasopressin blockade. The action of vasopressin in maintaining blood pressure has been subsequently observed in

mineralocorticoid-deficient rats by Ishikawa and Schrier (47) where vasopressin blockade decreased arterial pressure by 5 mmHg.

The sum of evidence therefore, indicates that vasopressin secretion increases during adrenal insufficiency and the elevated circulating vasopressin acts to maintain blood pressure by causing vasoconstriction.

NATURE OF THE RECEPTORS WHICH MEDIATE THE RENIN, CORTICOTROPIC AND NATRIURETIC RESPONSES TO VASOPRESSIN

Inhibition of Renin Secretion. In conscious dogs, the vasoconstrictor selective agonist,PheOrnOT, suppressed PRA as effectively as vasopressin, whereas DDAVP, the antidiuretic selective agonist, had no effect. These observations suggest that the receptor which mediates suppression of renin secretion in conscious dogs is of the vasoconstrictor type. This conclusion is reinforced by the observations made during selective blockade of the receptors which mediate the vasoconstrictor action of vasopressin in conscious dogs. The vasoconstrictor antagonist completely abolished the decrease in PRA caused by infusion of exogenous vasopressin. As described above, the antagonist also increased PRA in conscious dogs during water deprivation and hemorrhage.

These findings are consistent with those of Johnson et al. (49) who reported that acute administration of DDAVP in conscious dogs, at a dose which duplicated the antidiuretic effect of a dose of vasopressin, did not decrease PRA, while vasopressin did. The data also agree with the observations of Konrads et al. (58) that DDAVP did not decrease renin output in the unstimulated isolated perfused rat kidney, although it was effective in reducing renin release stimulated by isoproterenol. On the other hand, Joppich and Webber (51) observed that DDAVP decreased PRA in newborn infants and young children. The reason for this difference is not clear. However, the inhibition of renin secretion in the human study was accompanied by a natriuresis, whereas Johnson et al. observed no change in sodium excretion with DDAVP in dogs.

Quite a different pattern of results was obtained in anesthetized water-loaded dogs. Vasopressin and DDAVP decreased PRA, while PheOrnOT did not alter renin secretion. In addition, the vasoconstrictor selective antagonist, which left the antidiuretic response intact, also left the renin response intact. These results indicate that in anesthetized water-loaded dogs, the inhibition of renin secretion is mediated by receptors of the antidiuretic type, rather than of the vasoconstrictor type. These data agree with those of Malayan and Reid (69) who reported that a vasopressin analog with selective antidiuretic activity,

1-deamino-[4-threonine, 8-D-arginine]-vasopressin, decreased PRA as effectively as vasopressin in anesthetized water-loaded dogs.

Taken together, these results indicate that vasopressin can inhibit the secretion of renin by acting on both types of receptors, antidiuretic and vasoconstrictor. Although the studies were primarily designed to characterize the receptors which mediate this inhibition, the results permit some conclusions to be drawn regarding the mechanism of the inhibitory effect.

In the present studies vasopressin and PheOrnOT decreased PRA in conscious dogs, but they also increased mean arterial pressure. It is known that increased blood pressure can decrease renin secretion via intrarenal mechanisms (28), while actions which result in decreased sympathetic nervous system activity can decrease renin secretion (93). The former mechanism was proposed by Vandongen (115) to explain the inhibition of renin secretion by vasopressin in the isolated perfused rat kidney. The latter mechanism might help to explain how low doses of vasopressin can decrease PRA in the absence of a pressor effect (68). This mechanism could also explain the renin response to blockade of endogenous vasopressin in conscious dogs. The increase in PRA occurred in the absence of any change in mean arterial pressure, and was itself blocked by the β-adrenoceptor antagonist propranolol, which indicates that the inhibition of renin

secretion by endogenous vasopressin was mediated by the sympathetic nervous system and was possibly a reflex response to increased vascular resistance. The greater increase in PRA during hemorrhage in the presence of vasopressin blockade may also have been a sympathetically mediated reflex, but it occurred simultaneously with a decrease in blood pressure so a decrease in renal perfusion pressure is a more likely explanation.

The mechanisms mediated by vasoconstrictor type receptors did not operate in anesthetized water-loaded dogs. One possible explanation is that because the control mean arterial pressure in the anesthetized dogs (132 mmHg) was as high as the maximum pressure observed during peptide infusions in conscious dogs (128 mmHg-during infusion of PheOrnOT), so the baroreflex-mediated contribution to suppression of renin secretion may have been occurring even before infusion of the pressor peptides. Alternatively, anesthesia may have interfered with neurally mediated reflexes (39, 131).

In anesthetized water-loaded dogs, vasopressin inhibited renin secretion by way of antidiuretic type receptors. It is possible that suppression of renin secretion in this case was secondary to volume expansion. This has been proposed to be responsible for the inhibition of renin secretion during long term administration of vasopressin in dogs (126) and humans (37).

On the other hand, the dogs in the present study were greatly volume expanded before infusion of the peptides and it is questionable whether the renal water reabsorption activity of vasopressin and DDAVP could cause sufficient further volume expansion to result in acute inhibition of renin secretion. The dextrose in the infusate is metabolized so quickly that effectively free water is being infused, and this is evenly distributed through all aqueous fluid compartments. Khokhar et al. (54) measured a decrease in PRA in human subjects during acute infusion of vasopressin. They observed a decrease in plasma protein concentration and packed cell volume. From this, they concluded that vasopressin suppressed renin secretion by increasing plasma volume at the expense of extravascular fluid volume. If this conclusion is true, then one possible mode of action for vasopressin in the present studies is by mobilizing the extra volume provided by water loading. This possibility certainly requires further testing.

Another question which must be addressed is whether volume expansion could reflexly decrease renin secretion in anesthetized water-loaded dogs. The possibility that pressure reflexes are disrupted in this state was mentioned above. Future studies to test the possibility that renin suppression in anesthetized water-loaded dogs is secondary to volume expansion should provide for

measurements of pressure at the low pressure baroreceptors and studies where the dextrose infusion is stopped during infusion of the experimental peptides.

It has been suggested that vasopressin and analogs with antidiuretic activity may inhibit renin secretion by increasing the sodium load at the macula densa (69). The present data argue against this possibility because the inhibition of renin secretion did not parallel the natriuretic activity of the peptides and because the antagonist blocked the natriuretic response to vasopressin, but left the renin response intact. In addition, Shade et al. (109) demonstrated that vasopressin inhibits renin secretion in the nonfiltering kidney, where the macula densa mechanism does not operate.

A direct effect of vasopressin on renin secreting cells might also contribute to renin inhibition (17, 80, Morris, Reid and Ganong, unpublished observations). However, two groups failed to see any effect of vasopressin on renin secretion in vitro (56, 105). The present results do not argue for or against a direct action of vasopressin on juxtaglomerular cells. They do suggest that any direct inhibition, mediated by receptors which bind PheOrnOT or DDAVP, is subject to being overridden or obscured by other influences (such as increased blood pressure) in whole animal studies because neither peptide decreased renin secretion both in conscious and in anesthetized water-loaded dogs.

In summary, the present data indicate that vasopressin can inhibit renin secretion by acting on antidiuretic or vasoconstrictor type receptors, depending on the state of the animal. The data are consistent with a number of possible mechanisms which could mediate the inhibition of renin secretion, but further work is required to definitively establish which is involved .

Stimulation of ACTH and Corticosteroid Secretion. In the present studies the plasma concentration of 11-hydroxycorticosteroids (CORTS) was used to measure "corticotropic" activity. This includes the effects of vasopressin to increase ACTH secretion (57, 73), and to stimulate corticosteroid secretion directly at the adrenal (73). Plasma ACTH concentration was measured in two experiments to try to determine the contribution of the effect of vasopressin at the pituitary.

An increase in plasma concentration was interpreted to have resulted from increased secretion. However, a decrease in the rate of metabolism of ACTH or corticosteroids could also increase plasma concentrations.

In the present peptide infusion studies, vasopressin and the pressor selective analog, PheOrnOT, increased plasma corticosteroid concentration, whereas the antidiuretic selective agonist

did not. This suggests that the receptors which stimulate ACTH/CORTS secretion in conscious dogs are of the vasoconstrictor type. This conclusion is reinforced by the observation that the vasoconstrictor selective antagonist blocked the CORTS response to vasopressin. These data are in excellent agreement with those obtained with vasopressin analogs in vitro (81), and in anesthetized rats (5). While the combination of morphine and pentobarbital anesthesia is part of standard procedure in assaying CRF activity, it bears noting that there may be an altered corticosteroid response to vasopressin in animals treated with pentobarbital and morphine compared to conscious animals (30). Therefore it was important to study the CORTS response in conscious animals.

In a number of other analog studies, no correlation between pressor activity and the secretion of ACTH or CORTS has been observed (7, 9, 73, 85). While the reasons for the differences between these and the present studies are not readily apparent, a number of factors must be considered. Most important are the possible interactions among vasopressin, other corticotropin-releasing factors, and corticosteroids (57). For example, in incubations in vitro, vasopressin alone does not stimulate corticotrophs as well as median eminence extracts, but it very clearly contributes to the activity of the extracts (36).

Glucocorticoids exert negative feedback at both the hypothalamus and pituitary (57). Thus, animals, and possibly tissues obtained from animals, with different background levels of CORTS or corticotropin-releasing factors would be expected to react differently to vasopressin and analogs.

The increases in CORTS, observed with infusion of vasopressin and PheOrnOT in the present studies, did not persist throughout the period of the infusions. CORTS were increased in the first plasma sample drawn during the vasopressin infusion (20 min following commencement) and in the first two drawn during the PheOrnOT infusion (20 min and 40 min). It is possible that the maximum CORTS response occurred sooner than 20 min into the infusion. Aizawa et al. (5) observed the ACTH response to vasopressin injection in anesthetized rats to peak at 5 min. The return of CORTS to control values during the peptide infusions may have been a result of negative fast feedback (50). Alternatively, increases in blood pressure may have caused the reversal of the CORTS response. Wood et al. (125) have demonstrated that decreased blood pressure increases ACTH secretion. With regard to increased blood pressure, Raff et al. (88) proposed that the difference between the thresholds for chemoreceptor activity and ACTH secretion observed during hypoxia might be due to inhibition of ACTH secretion by increased blood pressure.

The ACTH responses to the vasopressin and PheOrnOT infusions also suggest the involvement of negative feedback at the pituitary or an inhibitory effect on ACTH secretion of increased blood pressure. There was a significant decrease in plasma ACTH concentration by the end of the PheOrnOT infusion by which time there had been a threefold increase in CORTS for 40 min and a sustained increase in mean arterial pressure. The changes in plasma ACTH concentration during the vasopressin infusion were not statistically significant, However the pattern suggests that vasopressin acted to stimulate ACTH, and a plasma sample drawn earlier in the infusion might have permitted a determination of whether the increase in CORTS was due to ACTH secretion.

Taken together, these studies suggest that while vasopressin can increase CORTS by an action on vasoconstrictor-type receptors, this action, at the plasma vasopressin concentration achieved with a 1 ng/kg/min infusion, may not be as potent as other factors in determining the ultimate plasma concentration of ACTH or corticosteroids.

The receptors which mediate the corticotropic response to vasopressin were also studied by vasopressin blockade during hemorrhage. The fact that the CORTS response to hemorrhage was not decreased, but rather increased, in the presence of vasoconstrictor blockade would argue against a role for

vasoconstrictor type receptors in ACTH secretion. However, there is evidence that vasopressin is not a required corticotropin-releasing factor during hemorrhage, because posterior lobectomized rats secrete as much ACTH during exsanguination as do intact rats (29). The ACTH response to hemorrhage may have also been mediated by decreases in blood pressure. Transient decreases in arterial pressure may have contributed to increased ACTH secretion in the absence of the vasopressin blockade. Another stimulus to ACTH secretion may have been a decrease in central venous pressure, which was not measured in the present studies. The increased CORTS response observed in the presence of the blockade was most likely a response to the stress of hypotension.

In summary, the studies on the corticotropic activity of vasopressin and analogs in conscious dogs indicate that the action is mediated by receptors which more closely resemble those that mediate the vasoconstrictor action of vasopressin than those that mediate the antidiuretic action. Vasopressin is only one of several factors involved in ACTH and coticosteroid secretion, and the present studies emphasize the need to take account of other factors, such as negative feedback and blood pressure changes, when assessing the role of vasopressin.

Natriuresis. In agreement with previous studies (16, 119), infusion of vasopressin in anesthetized water-loaded dogs produced a natriuresis. In contrast, DDAVP and PheOrnOT had little effect on sodium excretion despite the fact that the antidiuretic and vasopressor responses produced were similar to those of vasopressin. This suggests that the natriuresis was not a result of either antidiuretic activity or vasoconstrictor activity. It was therefore of considerable interest that the vasoconstrictor antagonist reduced the natriuretic response to vasopressin to the level observed during the time control.

One explanation of these results is that the natriuresis requires both antidiuretic and vasoconstrictor activity. This is not likely because in other studies, analogs of vasopressin which lacked one or both activities were observed to increase sodium excretion (20, 21, 43, 67, 69).

A more likely possibility is that the natriuresis is mediated by a third type of receptor. Chan (20) proposed that such a receptor might optimally recognize the oxytocin molecule. Since $d(CH_2)_5$MeTyrAVP is also a potent antagonist of oxytocic activity (59), the results of the present study are consistent with this hypothesis. On the other hand, Malayan and Reid (69) produced a natriuresis in anesthetized dogs with a vasopressin analog which has no more oxytocic activity than PheOrnOT or DDAVP, and much

less oxytocic activity than vasopressin (71).

Therefore, the natriuretic action of vasopressin is probably mediated by a type of receptor, unlike those which mediate the antidiuretic and vasoconstrictor actions. Further experiments are required to more closely characterize the nature of this receptor.

CONCLUSIONS

A number of conclusions can be drawn from the present studies:

1) Vasopressin plays an important role in the maintenance of arterial pressure in dogs. When levels of endogenous vasopressin are elevated during water deprivation and nonhypotensive hemorrhage, vasopressin acts to maintain blood pressure by contributing to total peripheral resistance.

2) During adrenal insufficiency, there is increased vasopressin secretion, and vasopressin helps to maintain blood pressure during adrenal insufficiency by its vasoconstrictor action.

3) Inhibition of renin secretion by vasopressin can be mediated by vasoconstrictor- or antidiuretic-type receptors. In the conscious state the action is mediated exclusively by vasoconstrictor-type receptors, but in the anesthetized water-loaded state, it is mediated by antidiuretic-type receptors.

4) The corticotropic response to vasopressin in conscious dogs is mediated by vasoconstrictor-type receptors.

5) The natriuretic response to vasopressin is probably mediated by a receptor distinct from those that mediate the antidiuretic or vasoconstrictor responses.

REFERENCES

1. Abraham, G.E., F.S. Manlimos, and R. Garza. Radioimuunoassay of steroids. In: Handbook of Radioimmunoassay, edited by G.E. Abraham. New York: Dekker, 1977, p. 591-565.

2. Agus, Z.S., and M. Goldberg. Role of antidiuretic hormone in the abnormal water diuresis of anterior hypopituitarism in man. J. Clin. Invest. 50: 1478-1489, 1971.

3. Ahmed, A.B.J., B.C. George, C. Gonzalez-Auvert, and J.F. Dingman. Increased plasma arginine vasopressin in clinical adrenocortical insufficiency and its inhibition by glucosteroids. J. Clin. Invest. 46: 111-123, 1967.

4. Aisenbrey, G.A., W.A. Handelman, P. Arnold, M. Manning, and R.W. Schrier. Vascular effects of arginine vasopressin during fluid deprivation in the rat. J. Clin. Invest. 67: 961-968, 1981.

5. Aizawa, T., N. Yasuda, M.A. Greer, and W.H. Sawyer. In vivo adrenocorticotropin-releasing activity of neurohypophyseal hormones and their analogs. Endocrinology 110: 98-104, 1982.

6. Altura, B.M., and B.T. Altura. Vascular smooth muscle and neurohypophseal hormones. Federation Proc. 36: 1853-1860, 1977.

7. Andersson, K-E., B. Arner, P. Hedner, and J.L. Mulder. Effects of 8-lysine-vasopressin and synthetic analogs on release of ACTH. Acta Endocrinologica 69: 640-648, 1972.

8. Andrews, C.E. Jr., and B.M. Brenner. Relative contributions of arginine vasopressin and Angiotensin II to maintenance of systemic arterial pressure in the anesthetized water-deprived rat. Circ. Res. 48: 254-258, 1981.

9. Arimura, A., A.V. Schally, and C.Y. Bowers. Corticotropin releasing activity of lysine vasopressin analogs. Endocrinology 84: 579-583, 1969.

10. Berde, B., and Boissonnas, R.A. Basic pharmacological properties of synthetic analogues and homologues of the neurohypophyseal hormones. In: Handbook of Experimental Pharmacology, edited by B. Berde, vol. 23, 1968.

11. Berns, A.S., R.G. Pluss, A.L. Erickson, R.J. Anderson, K.M. McDonald, and R.W. Schrier. Renin-angiotensin system and cardiovascular homeostasis in adrenal insufficiency. Am. J. Physiol. 233: F509-F513, 1977.

12. Bie, P. Osmoreceptors, vasopressin, and control of renal water excretion. Physiol. Rev. 60: 961-1048, 1980.

13. Bisset, G.W. Neurohypophyseal hormones. In: Peptide Hormones, edited by J.A. Parsons, University Park Press, Baltimore, 1976, p. 145-174.

14. Boykin, J., A. de Torrente, A. Erickson, G. Robertson, and R.W. Schrier. Role of vasopressin in impaired water excretion of glucocorticoid deficiency. J. Clin. Invest. 62: 738-744, 1978.

15. Boykin, J., A. de Torrente, G.L. Robertson, A. Erickson, and R.W. Schrier. Persistent plasma vasopressin levels in the hypoosmolar state associated with mineralocorticoid deficiency. Mineral Electrolyte Metab. 2: 310-315, 1979.

16. Brooks, F.P., and M. Pickford. The effect of posterior pituitary hormones on the excretion of electroyltes, in dogs. J. Physiol. (Lond) 142: 468-493.

17. Brooks, V.L., K.W. Cho, R.L. Malvin, and B.J. Cohen. Central and hormonal regulation of renin release by baboon kidneys. Proc. Soc. Exp. Biol. Med. 165: 147-150.

18. Brownstein, M.J., J.T. Russel, and H. Gainer. Biosynthesis of posterior pituitary hormones. In: Frontiers in Neuroendocrinology, vol. 7, edited by W.F. Ganong and L. Martini, New York, Raven, 1982, p. 31-43.

19. Burlet, A., M. Chateau, and P. Czernikow. Infundibular localization of vasopressin, oxytocin and neurophysins in the rat: its relationship with corticotrope function. Brain Res. 168: 275-286, 1979.

20. Chan, W.Y. An investigation of the natriuretic, antidiuretic and oxytocic actions of the neurohypophysial hormones and related peptides: delineation of separate mechanisms of action and assessment of molecular requirements. J. Pharmacol. Exp. Ther. 196: 746-757, 1976.

21. Chan, W.Y., and V. du Vigneaud. Natriuretic, diuretic and anti-arginine-vasopressin (ADH) effects of two analogs of oxytocin: [4-leucine]-oxytocin and [2,4-diisoleucine]-oxytocin. J. Pharmacol. Exp. Ther. 174: 541-549, 1970.

22. Clark, A.P.W., R.A. Cleghorn, J.K.W. Ferguson, and J.L.A. Fowler. Factors concerned in the circulatory failure of adrenal insufficiency. J. Clin. Invest. 26: 359-363, 1947.

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

24. Coggins, C.H., and A. Leaf. Diabetes Insipidus. Am. J. Med. 42: 807-813, 1967.

25. Cowley, A.W., E. Monos, and A.C. Guyton. Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial pressure in the dog. Circ. Res. 34: 505-514, 1974.

26. Cowley, A.W. Jr., S.J. Switzer, and M.M. Guinn. Evidence and quantification of the vasopressin arterial control system in the dog. Circ. Res. 46: 58-67, 1980.

27. Dallman, M.F., D. DeManicor, and J. Shinsako. Diminished corticotrope capacity to release ACTH during sustained stimulation: the twenty four hours after bilateral adrenalectomy in the rat. Endocrinology 95: 67-73, 1974.

28. Davis, J.O., and Freeman, R.H. Mechanisms regulating renin release. Physiol. Rev. 56: 1-56, 1976.

29. De Wied, D. The significance of the antidiuretic hormone in the release mechanism of corticotropin. Endocrinology 68: 956-970, 1960.

30. De Wied, D, P.R. Bouman, and P.G. Smelik. The effect of a lipide extract from the posterior hypothalamus and of pitressin on the release of ACTH from the pituitary gland. Endocrinology 62: 605-613, 1958.

31. Eisen, V. How vasopressin produces antidiuresis. Trends in Pharmacol. Sci. 4: 152-153, 1983.

32. Frieden, J., and A.D. Keller. Decreased resistance to hemorrhage in neurohypophysectomized dogs. Circ. Res. 11: 214-220, 1954.

33. Ganong, W.F. Review of Medical Physiology, Los Altos, CA, Lange, 1983.

34. Gavras, H., P. Hatzinikolaou, W.G. North, M. Bresnahan, and I. Gavras. Interaction of the sympathetic nervous system with vasopressin and renin in the maintenance of blood pressure. Hypertension 4: 400-405, 1982.

35. Gillies, G.E., E.A. Linton, and P.J. Lowry. Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299: 355-357, 1982.

36. Gillies, G.E., and P.J. Lowry. Corticotropin-releasing hormone and its vasopressin component. In: Frontiers in Neuroendocrinology, vol. 7, edited by W.F. Ganong and L. Martini, New York, Raven, 1982, p. 45-75.

37. Goodwin, F.J., J.C.G. Ledingham, and J.H. Laragh. The effect of prolonged administration of vasopressin and oxytocin on renin, aldosterone and sodium balance in normal man. Clin. Sci. 39: 641-651, 1970.

38. Harry, J.D., C.T. Kappagoda, R.J. Linden, H.M. Snow. Action of propranolol on the dog heart. Cardiovascular Res. 7: 729-739, 1973.

39. Harvey, S.C. Hypnotics and sedatives: the barbiturates. In: The Pharmacological Basis of Therapeutics, fifth edition, edited by L.S. Goodman and A. Gilman, New York, Macmillan, 1975, p. 102-123.

40. Hedge, G.A., M.B. Yates, R. Marcus, and F.E. Yates. Site of action of vasopressin causing corticotropin release. Endocrinology 79: 328-340, 1966.

41. Heyndrickx, G.R., D.H. Boettchner, and S.F. Vatner. Effects of angiotensin, vasopressin, and methoxamine on cardiac function and blood flow distribution in conscious dogs. Am. J. Physiol. 231: 1579-1587, 1976.

42. Houck, P.C., M.J. Fiksen-Olsen, S.L. Britton, and J.C. Romero. Role of angiotensin and vasopressin on blood pressure of ganglionic blocked dogs. Am. J. Physiol. 244: H115-H120, 1983.

43. Hruby, V.J., G. Flouret, and V. du Vigneaud. The synthesis and some pharmacological properties of [4-L-isoleucine]-oxytocin and [4-L-leucine]-oxytocin. J. Biol. Chem. 244: 3890-3894, 1969.

44. Huguenin, R.L. Synthese de la Phe^{2}-Orn^{8}-vasopressine et de la Phe^{2}-Orn^{8}-oxytocine, deux analogues de la vasopressine doues d'une activite pressorique selective. Helv. Chim. Acta 47: 1934-1941, 1964.

45. Huguenin, R.L., and R.A. Boissonnas. Synthese de la desamino^{1}-Arg^{8}-vasopressine et de la desamino^{1}-Phe^{2}-Arg^{8}-vasopressine, deux analogues possedant une activite antidiuretic plus elevee et plus selective que celle des vasopressines naturelles. Helv. Chim. Acta 49: 695-705, 1966.

46. Humphreys, M.H., R.M. Friedler, and L.E. Earley. Natriuresis produced by vasopressin or hemorrhage during water diuresis in the dog. Am J. Physiol. 219: 658-665, 1970.

47. Ishikawa, S., and R.W. Schrier. Role of AVP, norepinephrine and angiotensin II in maintenance of blood pressure in mineralocorticoid and glucocorticoid deficient rats. Proceedings, third international conference on the neurohypophysis, in press.

48. Jamieson, R.L. Urine concentration and dilution. In: The Kidney, second edition, edited by B.M. Brenner and F.C. Rector, 1981, p. 495-550.

49. Johnson, M.D., L.B. Kinter, and R. Beeuwkes. Effects of AVP and dDAVP on plasma renin activity and electrolyte excretion in conscious dogs. Am. J. Physiol. 236: F66-F70, 1979.

50. Jones, M.T., and B. Gilham. Corticotropin secretion. In: Synthesis and Release of Adenhypophyseal Hormones, edited by M. Jutisz and K.W. McKerns, New York, Plenum, 1980, p. 587-615.

51. Joppich, R., and P. Weber. Effects of ADH on the activity and function of the renin-angiotensin-aldosterone system in infants and children. Europ. J. Pediat. 122: 303-308, 1976.

52. Keeton, T.K., and W.B. Campbell. The pharmacologic alteration of renin release. Pharmacol. Rev. 32: 81-227, 1980.

53. Keil, L.C., and W.B. Severs. Reduction in plasma vasopressin levels of dehydrated rats following acute stress. Endocrinology 100: 30-38, 1977.

54. Khokhar, A.M., J.D.H. Slater, M.L. Forsling, and N.N. Payne. Effect of vasopressin on plasma volume and renin releae in man. Clin. Sci. Mol. Med. 50: 415-424, 1976.

55. Kleeman, C.R., J.W. Czaczkes, and R. Cutler. Mechanism of impaired water excretion in adrenal and pituitary insufficiency IV. J. Clin. Invest. 43: 1641-1648, 1964.

56. Knepel, W., W. Reimann, and D. Nutto. On the mechanism of the vaopressin-induced inhibition of renin release. Horm. Metab. Res. 14: 157-160, 1982.

57. Koch, B., B. Lutz-Bucher, and C. Mialhe. Interactions of glucocorticoids, CRF, and vasopressin in the regulation of ACTH secretion. In: Synthesis and Release of Adenohypophyseal Hormones, edited by M. Jutisz and K.W. McKerns, New York, Plenum, 1980, p. 561-585.

58. Konrads, A., K.G. Hofbauer, U. Werner, and F. Gross. Effects of vasopressin and its deamino-D-arginine analogue on renin release in the isolated perfused rat kidney. Pfluger's Arch. 377: 81-85, 1978.

59. Kruszynski, M., B. Lammek, M. Manning, J. Seto, J. Haldar, and W.H. Sawyer. [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid),2-(O-methyl)tyrosine]arginine vasopressin and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine vasopressin, two highly potent antagonists of the vasopressor response to arginine vasopressin. J. Med. Chem. 23: 364-368, 1980.

60. Land, H., G. Schutz, H. Schmale, and D. Richter. Neucleotide sequence of cloned cDNA encoding bovine arginine vasopressin-neurophysin II precursor. Nature 295: 299-303, 1982.

61. Laycock, J.F., W. Penn, D.G. Shirley, and S.J. Walter. The role of vasopressin in blood pressure immediately following acute haemorrhage in the rat. J. Physiol. (Lond) 296: 267-275, 1979.

62. Leaf, A., and C.H. Coggins. The neurohypophysis. In: Textbook of Endicrinology, edited by R.H. Williams, Philadelphia, W.B. Saunders, 1974, p. 80-94.

63. Liard, J.F., O. Deriaz, P. Schelling, and M. Thibonnier. Cardiac output during vasopressin infusion or dehydration in conscious dogs. Am. J. Physiol. 243: H663-H669, 1982.

64. Liard, J.F., O. Deriaz, M. Tschopp, and J. Schoun. Cardiovascular effects of vasopressin infused into the vertebral circulation of conscious dogs. Clin. Sci. 61: 345-347, 1981.

65. Linas, S.L., T. Berl, G.L. Robertson, G.A. Aisenbrey, R.W. Schrier, and R.J. Anderson. Role of vasopressin in the impaired water excretion of glucocorticoid deficiency. Kidney Int. 18: 58-67, 1980.

66. Lumbers, E.R., and E.K. Potter. The effects of vasoactive peptides on the carotid cardiac baroreflex. Clin. Exp. Pharmacol. Physiol. suppl. 7: 45-49, 1982.

67. Machova, A., and K. Jost. Comparison of natriuretic action of carba-analogues of deaminooxytocin and [4-leucine, 8-arginine]-vasotocin in rats. Endocrinologia Experimentalis 9: 269-277, 1975.

68. Malayan, S.A., D.J. Ramsay, L.C. Keil, and I.A. Reid. Effects of increases in plasma vasopressin concentration on plasma renin activity, blood pressure, heart rate, and plasma corticosteroid concentration in conscious dogs. Endocrinology 107: 1899-1904, 1980.

69. Malayan, S.A., and I.A. Reid. Effects of a nonpressor analog of vasopressin on plasma renin activity and salt and water excretion in water-loaded anesthetized dogs. Life Sci. 31: 2757-2763, 1982.

70. Mandell, I.N., R.A. De Fronzo, G.L. Robertson, and J.N. Forrest Jr. Role of plasma arginine vasopressin in the impaired water diuresis of isolated glucocorticoid deficiency in the rat. Kidney Int. 17: 186-195, 1980.

71. Manning, M., L. Balaspiri, J. Mohring, J. Haldar, and W.H. Sawyer. Synthesis and some pharmacological properties of deamino[4-threonine,8-D-arginine]vasopressin and deamino[8-D-arginine]vasopressin, highly potent and specific antidiuretic peptides, and [8-D-arginine]vasopressin and deamino-arginine-vasopressin. J. Med. Chem. 19: 842-845, 1976.

72. Manning, M., and W.H. Sawyer. Antagonists of the vasopressor and antidiuretic responses to arginine vasopressin. Ann. Int. Med. 96: 520-521, 1982.

73. Martini, L. Neurohypophysis and anterior pituitary activity. In: The Pituitary Gland, edited by G.W. Harris and B.T. Donovan, Berkeley, University of California Press, 1966, vol. 3, p. 535-577.

74. McCann, S.M. The ACTH-releasing activity of extracts of the posterior lobe of the pituitary in vivo. Endocrinology 60: 664-676, 1957.

75. Mikami, H., A.T. Kosgolov, and C.M. Ferrario. Blood pressure regulation in chronic spinal dogs. Physiologist 25: 329 (abstr.), 1982.

76. Montani, J.P., J.F. Liard, J. Schoun, and J. Mohring. Hemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. Circ. Res. 47: 346-355, 1980.

77. Mouw, D., J-P. Bonjour, R.L. Malvin, and A. Vander. Central action of angiotensin in stimulating ADH release. Am. J. Physiol. 220: 239-242, 1971.

78. Oliver, G., and E.A. Shafer. On the physiological action of extracts of the pituitary body and certain other glandular organs. J. Physiol. (Lond.) 18: 277-279, 1895.

79. Pang, C.C.Y., and K.M. Leighton. Prolonged inhibition of pressor response to vasopressin by a potent specific antagonist, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid) 2-(O-methyl)tyrosine]arginine-vasopressin. Can. J. Physiol. Pharmacol. 59: 1008-1012, 1981.

80. Park, C.S., D.S. Han, and J.C.S. Fray. Calcium in the control of renin secretion: Ca^{2+} influx as an inhibitory signal. Am. J. Physiol. 240: F70-F74, 1981.

81. Pearlmutter, A.F., E. Rapino, and M. Saffran. A semi-automated in vitro assay for CRF: activities of peptides relate to oxytocin and vasopressin. Neuroendocrinology 15: 106-119, 1974.

82. Penit, J., M. Faure, and S. Jard. Vasopressin and angiotensin II receptors in rat aortic smooth muscle cells in culture. Am. J. Physiol. 244: E72-E82, 1983.

83. Peters, G., and F. Roch-Ramel. Renal effects of posterior pituitary peptides and their derivatives. In: International Encyclopedia of Pharmacology and Therapeutics, section 41, edited by H. Heller and B.T. Pickering, New York, Pergammon, 1970, p. 229-278.

84. Pickering, B.T. Aspects of the relationships between chemical structure and biological activity of the neurohypophyseal hormones and their synthetic structural analogs. In: International Encyclopedia of Pharmacology and Therapeutics, section 41, edited by H. Heller and B.T. Pickering, New York, Pergammon, 1970, p. 81-110.

85. Portanova, R., and G. Sayers. Isolated pituitary cells: CRF-like activity of neurohypophysial and related polypeptides. Proc. Soc. Exp. Biol. Med. 143: 661-666, 1973.

86. Pullan, P.T., C.I. Johnston, W.P. Anderson, and P.I. Korner. Plasma vasopressin in blood pressure homeostasis and experimental renal hypertension. Am. J. Physiol. 239: H81-H87, 1980.

87. Quillen, E.W. Jr., and A.W. Cowley Jr. Influence of volume changes on osmolality-vasopressin relationships in conscious dogs. Am. J. Physiol. 244: H73-H79, 1983.

88. Raff, H., J. Shinsako, L.C. Keil, and M. Dallman. Vasopressin, ACTH, and corticosteroids during hypercapnia and graded hypoxia in dogs. Am. J. Physiol. 244: E453-E458, 1983.

89. Ramsay, D.J., L.C. Keil, M.C. Sharpe, and J. Shinsako. Angiotensin II infusion increases vasopressin, ACTH, and 11-hydoxycorticosteroid secretion. Am. J. Physiol. 234: R66-R71, 1978.

90. Reid, I.A. Interactions between the renin-angiotensin system and the brain. Adv. Exp. Med. Biol. 130: 257-288, 1980.

91. Reid, I.A. The renin-angiotensin system. In: Hypertension Research, edited by F.M. Radzialowski, New York, Dekker, 1982, p. 101-137.

92. Reid, I.A., V.L. Brooks, C.D. Rudolph, and L.C. Keil. Analysis of the actions of angiotensin on the central nervous system of conscious dogs. Am. J. Physiol. 243: R82-R91, 1982.

93. Reid, I.A., B.J. Morris, and W.F. Ganong. The renin-angiotensin system. Ann. Rev. Physiol. 40: 377-410, 1978.

94. Reid, I.A., J.R. Stockigt, A. Goldfien, and W.F. Ganong. Stimulation of renin secretion in dogs by theophylline. Eur. J. Pharmacol. 17: 325-332, 1972.

95. Reid, I.A., W.H. Tu, K. Otsuka, T.A. Assaykeen, and W.F. Ganong. Studies concerning the regulation and importance of plasma angiotensinogen concentration in the dog. Endocrinology 93: 107-114, 1973.

96. Reidenburg, M.M., E.A. Ohler, R.W. Sevy, and C. Harakal. Hemodynamic changes in adrenalectomized dogs. Endocrinology 72: 918-923, 1963.

97. Robertson, G.L. The regulation of vasopressin function in health and disease. Rec. Prog. Horm. Res. 23: 333-385, 1977.

98. Robertson, G.L., S. Athar, and R.S. Shelton. Osmotic control of vasopressin function. In: Disturbances in Body Fluid Osmolality, edited by T.E. Andreoli, J. Grantham, and F.C. Rector, Washington, American Physiological Society, 1977, p. 125-148.

99. Robertson, G.L., E.A. Mahr, S. Athar, and T. Sinha. Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J. Clin. Invest. 52: 2340-2352, 1973.

100. Robertson, G.L., R.L. Shelton, and S. Athar. The osmoregulation of vasopressin. Kidney Int. 10: 25-37, 1976.

101. Robinson, A.G. DDAVP in the treatment of central diabetes insipidus. New Engl. J. Med. 294: 507-511, 1976.

102. Rocha e Silva, M., and M. Rosenberg. The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. J. Physiol. (Lond.) 202: 535-557, 1969.

103. Rockhold, R.W., J.T. Crofton, D.P. Brooks, and L. Share. Lack of vascular response to vasopressin (AVP) antagonist following water deprivation in the rat. Federation Proc. 42: 986 (Abstr.), 1983.

104. Rose, J.C., and R.T. Kelly. Vasopressin and fetal blood pressure. Endocrinology 110: 321 A (Abstr.), 1982.

105. Rosset, E., and R. Veyrat. Effect of vasopressin (ADH), aldosterone, norepinephrine (NE), angiotensin I (AI) and II (AII) on renin release (RR) by human kidney (HK) slices in vitro. Acta Endocrinologica 155: 179, 1971.

106. Sawyer, W.H., M. Acosta, and M. Manning. Structural changes in the arginine-vasopressin molecule that prolong its antidiuretic action. Endocrinology 95: 140-149, 1974.

107. Schalm, O.W. Veterinary Hematology, second edition, Philadelphia, Lea and Febiger, 1965.

108. Schrier, R.W., T. Berl, and R.J. Anderson. Osmotic and nonosmotic control of vasopressin release. Am. J. Physiol. 236: F321-F332, 1979.

109. Shade, R.E., J.O. Davis, J.A. Johnson, R.W. Gottshall, and W.S. Spielman. Mechanism of action of angiotensin II and antidiuretic hormone on renin secretion. Am. J. Physiol. 224: 926-929, 1973.

110. Share, L., and R.H. Travis. Plasma vasopressin concentration in the adrenally insufficient dog. Endocrinology 86: 196-201, 1970.

111. Stockigt, J.R., R.D. Collins, and E.G. Biglieri. Determination of plasma renin secretion by angiotensin I immunoassay. Circ. Res. 28-29 suppl. 2: 175-191, 1971.

112. Szczpanska-Sadowska, E. Hemodynamic effects of a moderate increase of the plasma vasopressin concentrations in conscious dogs. Pfluger's Arch. 338: 313-322, 1973.

113. Thrasher, T.N., L.C. Keil, and D.J. Ramsay. Lesions of the organum vasculosum of the lamina terminalis (OVLT) attenuate osmotically-induced drinking and vasopressin secretion in the dog. Endocrinology 110: 1837-1839, 1982.

114. Vale, W., J. Spiess, C. Rivier, and J. Rivier. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science 213: 1394-1397, 1981.

115. Vandongen, R. Inhibition of renin secretion in the isolated rat kidney by antidiuretic hormone. Clin. Sci. Mol. Med. 49: 73-76, 1975.

116. Vavra, K., A Machova, J.H. Holocek, M. Cort, M. Zaoral, and F. Sorm. Effect of a synthetic analogue of vasopressin in animals and patients with diabetes insipidus. Lancet 1: 948-952, 1968.

117. Wade, C.E., P. Bie, L.C. Keil, and D.J. Ramsay. Osmotic control of vasopressin in the dog. Am. J. Physiol. 243: E287-E292, 1982.

118. Walter, R., J. Rudinger, and I.L. Schwartz. Chemistry and structure-activity relations of the antidiuretic hormones. Am. J. Med. 42: 653-677, 1967.

119. Walter, R., C.W. Smith, P.K. Mehta, S. Boonjarern, J.A.L. Arruda, and N.A. Kurtzman. Conformational considerations of vasopressin as a guide to development of biological probes and therapeutic agents. In: Disturbances in Body Fluid Osmolality, edited by T.E. Andreoli, J. Grantham, and F.C. Rector, Washington, American Physiological Society, 1977, p. 1-36.

120. Warren, D.J., and J.G.G. Ledingham. Cardiac output in the conscious rabbit: an analysis of the thermodilution technique. J. Appl. Physiol. 36: 246-251, 1974.

121. Webb, W.R., I.U. Degerli, J.D. Hardy, and M. Unal. Cardiovascular responses in adrenal insufficiency. Surgery 58: 273-282, 1965.

122. Weitzman, R.E., and D.A. Fisher. Log linear relationship between plasma arginine vasopressin and plasma osmolality. Am. J. Physiol. 233: E37-E40, 1977.

123. Winer, B.J. Statistical Principles in Experimental Design, second edition, New York, McGraw-Hill, 1971.

124. Wood, C.E., L.C. Keil, and A.M. Rudolph. Hormonal and hemodynamic responses to vena caval constriction. Am. J. Physiol. 243: E278-E286, 1982.

125. Wood, C.E., J. Shinsako, L.C. Keil, D.J. Ramsay, and M.F. Dallman. Hormonal and hemodynamic responses to 15 ml/kg hemorrhage in conscious dogs: responses correlate to body temperature. Proc. Soc. Exp. Biol. Med. 167: 15-19, 1981.

126. Young, D.B., and R.E. McCaa. Lack of prolonged effect of antidiuretic hormone on the renin-aldosterone system in the dog. Clin. Exp. Pharmacol. Physiol. 8: 267-271, 1981.

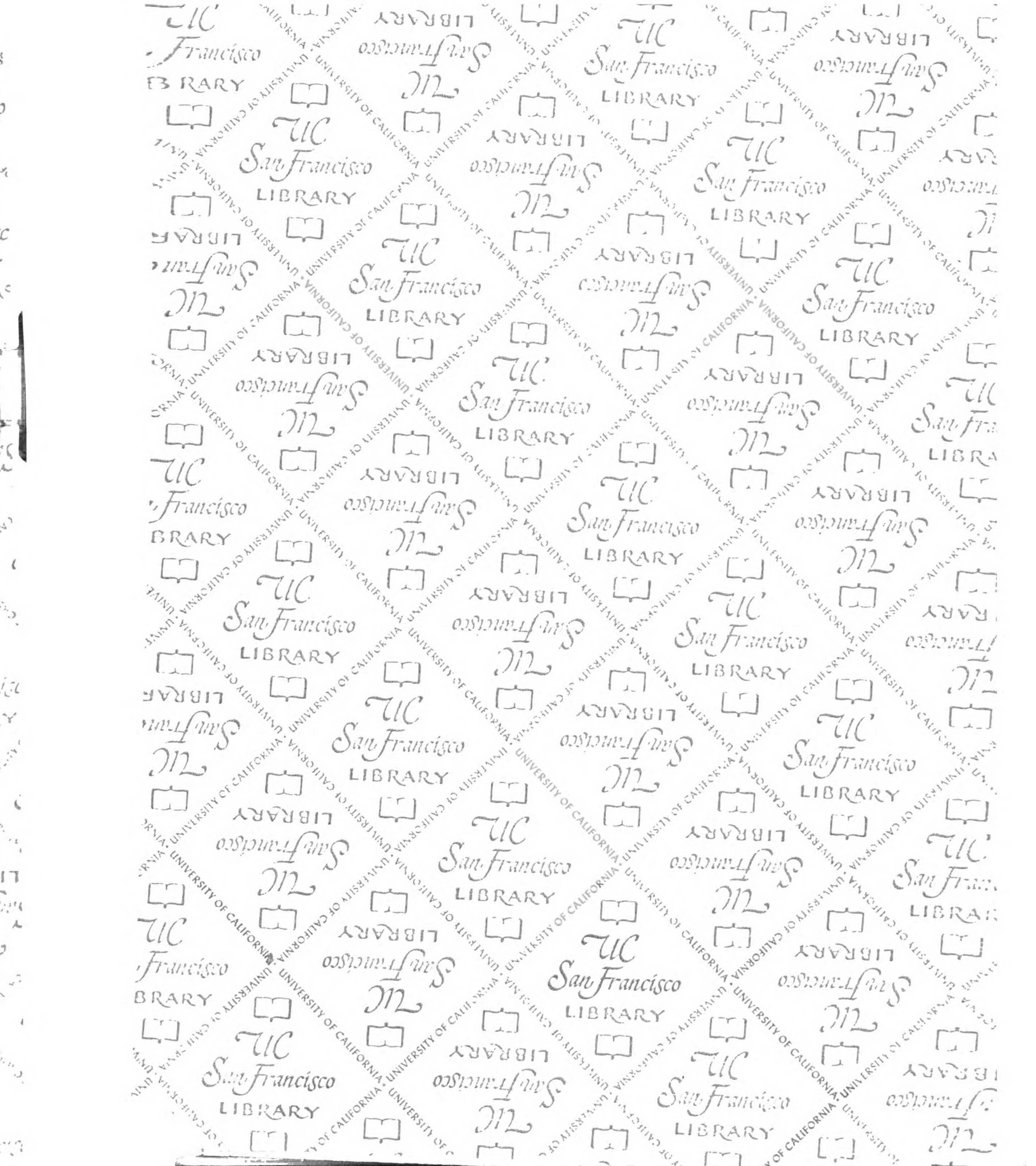
127. Zaoral, M., J. Kolc, and F. Sorm. Synthesis of D-Arg^{8}- and D-Lys^{8}-vasopressin. Colln. Czechoslov. Chem. Commun. 31: 382-383, 1966.

128. Zaoral, M., J. Kolc, and F. Sorm. Amino acids and peptides LXXI. Synthesis of 1-deamino-8-γ-amino butyrine-vasopressin, 1-deamino-8-D-lysine-vasopressin and 1-deamino-8-D-arginine-vasopressin. Colln. Czechoslov. Chem. Commun. 32: 1250-1257, 1967.

129. Zerbe, R.L., M.A. Bayorh, and G. Feuerstein. Vasopressin: an essential pressor factor for blood pressure recovery following hemorrhage. Peptides 3: 509-514, 1982.

130. Zerbe, R.L., G. Feuerstein, D.K. Meyer, and I.J. Kopin. Cardiovascular, sympathetic, and renin-angiotensin system responses to hemorrhage in vasopressin-deficient rats. Endocrinology 111: 608-613.

131. Zimpfer, M.W., T. Manders, A.C. Barger, and S.F. Vatner. Pentobarbital alters compensatory neural and humoral mechanisms in response to hemorrhage. Am. J. Physiol. 243: H713-H721, 1982.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