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Cornette-Finn, Kuuleialoha Marie, Ph.D.

University of Hawaii, 1987

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ADH RESPONSE TO PERIPHERAL AND CENTRAL CORTISOL ADMINISTRATION

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN BIOMEDICAL SCIENCES (PHYSIOLOGY)

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ABSTRACT

Cortisol affects water balance, but whether this effect is mediated through antidiuretic hormone (ADH) is unclear. This study examines the response of plasma ADH (pADH) in two groups of conscious dogs; one received cortisol centrally (ivt) in the third ventricle at 300 ng/min, the other peripherally (iv) at 4.16 μg/kg/min, in 4 states of water balance, i.e., dehydration, normal hydration, 5% NaCl iv infusion (0.05 ml/kg/min), and after a water load (40 ml/kg given iv over 30 min), as compared to control experiments without cortisol. Cortisol, either ivt or iv, had no affect on pADH or plasma osmolality (pOsm) during dehydration or normal hydration. Ivt cortisol infusion caused a progressive decline in plasma cortisol (pCort) while iv cortisol infusion increased pCort (control 2.0 μg%, ivt pCort 0.5 μg%, iv pCort 17 μg%, $P<0.01$). During the 5% NaCl iv infusion, pADH and pOsm increased similarly in both the control and ivt cortisol experiments from 1.0 to 1.9 μU/ml and 295 to 305 mOsm/kg H_2O, respectively ($P<0.01$). The increase in pADH seen with 5% NaCl infusion was delayed in the iv cortisol experiment as compared to the iv control (75 min versus 45 min, $P<0.01$). This delay was also seen in pOsm; 45 min in iv cortisol versus 15 min in iv control ($P<0.01$), indicating that the elevated pCort apparently delays the development of increased pOsm and the subsequent increase in pADH. During a water load, the cumulative urine excreted was 99% of that ingested with iv cortisol

($P<0.05$), 82% in the control, and 70% with ivt cortisol; in all three cases similar decreases in pADH and pOsm occurred. The free water clearance (FWC) was augmented in the iv cortisol infusion and attenuated in the opposite situation of pCort insufficiency which was established during the ivt cortisol infusion. Thus, the present study demonstrates that cortisol has a peripheral effect in that elevated plasma cortisol 1) delays the rise in pOsm during hypertonic saline infusion 2) increases FWC during a water diuresis but 3) does not alter the pADH versus pOsm relationship, therefore 4) affects the abililty to excrete a water load independent of ADH. These data are compatible with a mechanism in which excess cortisol enhances the Na^+ "leak" pathway of the cells by increasing the membrane permeability to Na^+, thereby increasing the osmolar content of the cells.

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CHAPTER I

INTRODUCTION

The first evidence suggesting a function for the pituitary body was presented in 1895 by Oliver and Schafer. Their finding that intravenous injections of pituitary gland extracts resulted in marked increases in blood pressure constituted the beginning of vasopressin research. This pressor effect was confirmed by subsequent investigators and found to be localized in the posterior pituitary lobe (Howell, 1898). An antidiuretic effect of posterior pituitary extracts was reported independently by two physicians, Farini and von den Velden, in 1913 [for ref. see Heller, 1974]. Both noted 1) a strong correlation between the occurrence of diabetes insipidus and diseases or lesions of the pituitary gland, and 2) marked decreases in urine volume when treating patients suffering from diabetes insipidus with injections of posterior pituitary extracts. In addition, posterior pituitary extracts were found to possess other biological activities, such as an oxytocic effect which causes uterine contractions (Dale, 1909), and a milk-ejecting effect (Ott and Scott, 1910; Schafer and Mackenzie, 1911). Not until 1928 were these latter effects demonstrated to be due to one principle, oxytocin, whereas the pressor and antidiuretic effects were found to be associated with the activity of vasopressin [for ref. see Acher and Fromageot, 1957].

Historically, an important paper on the mechanism of the antidiuretic effect of posterior lobe extracts as being an intrarenal

one, was that of Starling and Verney (1924) in which they demonstrated an antidiuretic effect of posterior pituitary extracts on the isolated kidney of the dog. Using a perfused organ system, Verney subsequently demonstrated that the posterior pituitary was required for the production of a normally concentrated urine (Verney, 1926). A quote taken from a symposium of the Colston Society in 1956 entitled "The Neurohypophysis" by Sir Henry Dale best describes the significance of this work (Dale, 1957).

"To me this seemed to be the most direct and convincing evidence till then available, not only of the perfused kidney's requirement, for the production of a normally concentrated urine, of a substance present in an artificial extract of the neurohypophysis, but also, and most convincingly, of the active secretion of that substance from the neurohypophysis, into blood which was circulated through it. In other words, it seemed to be at least as complete a proof of a normal endocrine function for the neurohypophysis as any which had been given, or, indeeed, has since been given, for any other organ."

Others confirmed this finding by demonstrating the development of diabetes insipidus upon removal of the posterior pituitary (Fisher et al., 1935). In 1948, Verney performed the classic and eloquent experiments that characterized the mechanism of the antidiuretic action of vasopressin and its release. He reported that intracarotid infusions in conscious dogs of hypertonic solutions (sufficient to increase the osmolality of blood in the head by as little as 1.8%) increased pituitary antidiuretic substance only when the infusate was

osmotically effective, and postulated the existence of "osmoreceptors" located in the anterior hypothalamus. These studies have remained as the major evidence for the osmoregulation of vasopressin and, largely as a result of them, to this day vasopressin is also known as antidiuretic hormone (ADH). Generally, when the vascular actions of this hormone are being discussed, it is referred to as vasopressin, and it is called antidiuretic hormone (ADH) when its renal actions are involved.

The landmark studies of du Vigneaud and associates provided another cornerstone on which vasopressin research is based. In 1955, just a few years after having identified the structure of oxytocin, they succeeded in isolating and elucidating the structure of arginine-vasopressin from beef pituitaries and lysine-vasopressin from hog pituitaries (du Vigneaud, 1956). They confirmed these nonapeptide structures by synthesizing peptides that were shown to possess the identical biological properties as the naturally occurring principles. This represented the first synthesis of a polypeptide hormone, which has enabled others to develop vast areas of research in neuroendocrinology, including phylogentic and comparative physiology, neuroanatomy, and neurophysiology. It has enabled as well, the development of biochemical agonists and antagonists of polypeptide hormones which aid in the study of molecular and cellular mechanisms of hormone action and metabolism.

Concurrently, the investigations of Bargmann and Scharrer in 1951 provided the basic evidence that the hormones of the neurohypophysis

are synthesized in the cell bodies of magnocellular neurons whose nuclei, the supraoptic nuclei (SON) and the paraventricular nuclei (PVN), lie in the anterior hypothalamus, and whose axons are found in the neurohypophysis. (This finally settled any lingering doubts as to the ability of nerve cells to function as endocrine cells and firmly established the new field of neuroendocrionolgy). These axons do not synapse with either nerves or other cellular elements but terminate in swellings aligned along capillaries, an arrangement ideally suited for hormone transfer directly into the bloodstream. The distinguishing feature of these neurons is the presence of dense granules now known to contain the polypeptide hormones. Vasopressin is stored in these secretory granules along with a carrier protein, neurophysin (Sachs and Takabatake, 1964). Once packed into these membrane-bound granules (then called neurosecretomes, now known as neurosecretory granules), vasopressin is moved via axonal transport through the neurohypophysial tract to its final storage site in the neurohypophysis. This entire anatomical and physiological complex is often referred to as the hypothalamoneurohypophyseal system (HNS).

The axons of these neurosecretory cells have also been found recently to project in a widespread fashion to multiple areas of the central nervous system (CNS) but the majority of the efferent nerve fibers terminate in the posterior pituitary (Sklar and Schrier, 1983). Membrane depolarization in response to electrical impulses generated within the SON and PVN results in the release of ADH, via an exocytotic process, into the systemic circulation. This exocytosis has been shown

to be a calcium-dependent process and is termed "stimulus-secretion coupling" (Douglas and Poisner, 1964; Ishikawa and Schrier, 1983). The work of Sachs and co-workers has shown that the rate of ADH release is a function of both stimulus intensity and duration, and is also dependent on the amount present in the neurohypophysis at the time of stimulation (Sachs et al., 1967). In addition, the biosynthesis and axonal transport of ADH is enhanced when stimulation of ADH occurs, thus maintaining adequate stores of the hormone in the posterior pituitary (Takabatake and Sachs, 1964).

The two major physiological functions of ADH released from the neurohypophysis are: to promote the retention of water via its effect on the renal collecting duct and, to cause vasoconstrtiction of the vascular smooth muscle. The release of ADH in response to hemorrhage has been well documented (Share, 1974) and will be discussed later; however, it was for a long time thought not to be important in the normal regulation of arterial pressure. This was based on the observation that the concentrations of hormone required to affect blood vessels are much greater than those found under basal conditions or with osmotic stimulation (Ginsburg and Brown, 1957). Several early studies did, however, suggest that ADH played a role in cardiovascular regulation. Neurohypophysectomized dogs demonstrated a decreased ability to maintain blood pressure in response to hemorrhage (Frieden and Keller, 1954). ADH, in subpressor doses, has been shown to potentiate the pressor action of catecholamines (Bartelstone and Nasmyth, 1965). In additon, a reduction in blood volume has been found

to be a far more potent stimulus for ADH release than an increase in extracellular fluid osmolality (Share, 1961; Moore, 1971). Since the development of improved methodology for the measurement of small amounts of hormones in biological fluids, evidence has accumulated which indicates that the pressor actions of vasopressin are buffered by the baroreflex arc (Crowley et al., 1974; Montani et al., 1980). In the absence of autonomic reflex control, small changes in ADH, well within the daily physiological range, can exert significant vasoconstrictor effects which increase arterial pressure.

Some indirect evidence has been provided that suggests a central role for ADH in cardiovascular regulation. In addition to the classic magnocellular neurons of the SON and PVN, neurons containing ADH have been found to project from the anterior hypothalamus to centers in the medulla involved in blood pressure regulation (Zimmerman et al., 1982). Thus, ADH appears to be an important hormone involved in normal cardiovascular homeostasis, and has been implicated as a pathogenic factor in some forms of hypertension (Mohring et al., 1977; Share and Croton, 1982).

ADH contributes to the regulation of the volume and composition of body fluids by influencing the handling of water excretion in the kidney. Under the appropriate stimulation, ADH acts upon the kidney to increase tubular water reabsorption (Sawyer, 1974). The site of action in the kidney is the late distal tubule and the collecting duct tubules of the nephron, both physiologically referred to as the collecting duct. By augmenting the water permeability of the collecting duct, the

ADH-increased renal water reabsorption results in the excretion of a small volume of concentrated urine. In the absence of ADH, the dilute urine formed in the ascending loop of Henle is not further changed and large volumes of dilute urine are excreted. The mechanism by which ADH increases the water permeability of the collecting tubule has not been entirely elucidated. It is well established, however, that ADH combines with specific basolateral membrane receptors and that this hormone-receptor complex activates adenyl cyclase which results in the formation and accumulation of cyclic-AMP within the cells (Handler and Orloff, 1971; Orloff and Handler, 1962; 1967). Cyclic-AMP, also known as the "second messenger", acts as the intracellular mediator for this hormone and a number of other hormones as well. The intracellular events that occur after cyclic-AMP formation which produce the changes in water permeability of the apical membrane are still unknown. A cyclic-AMP activated protein kinase has been demonstrated and the involvement of microtubules and microfilaments has been implicated as the link between cyclic-AMP activation and the resulting elicited changes in water permeability (Dousa and Barnes, 1974; Dousa et al., 1977; Taylor et al., 1973). Although uncertainty exists about the intermediate steps, the final events in the action of ADH on responsive epithelia have been clearly shown by using freeze-fracture electron microscopy on sections of resting and ADH-stimulated cells (Hays et al., 1985). Particle aggregates present in the cell cytoplasm are seen to fuse with the luminal (rate-limiting) membrane when ADH is present. These particles are believed to be proteins approximately 80Å in

diameter possessing one or more channels through which water molecules pass. ADH withdrawal results in the rapid disappearance of these particles from the luminal membrane.

The major stimuli for ADH release can be broadly divided into two main categories: osmotic and non-osmotic stimuli. The osmotic regulation of ADH secretion has been found to take place almost entirely within the central nervous system (CNS). "Osmoreceptors", as theorized by Verney, not sodium-sensitive receptors, relay this information to the neurosecretory cells of the SON and PVN (Thrasher et al., 1980a; 1980b). These hypothalamic osmoreceptors are so sensitive that under normal circumstances plasma osmolality does not vary by more than 1 to 2 percent (Robertson, 1985). The osmoreceptor cells, separated from the magnocellular neurons by at least one synapse (Sladek and Joynt, 1979), appear to lie outside the blood-brain barrier and are located in or near the anterior hypothalamus. In the dog, the organum vasculosum of the lateral terminalis (OVLT) seems to be the best candidate for the osmoreceptor site (Thrasher et al., 1982). These osmoreceptors "sense" changes in plasma osmolality and elicit appropriate signals from the SON and PVN. An increase in plasma osmolality, as occurs with dehydration, results in the stimulation of ADH release which reduces renal water loss. A concomitant increase in the thirst mechanism restores the water deficit and water balance. On the other hand, the decrease in plasma osmolality that results after the ingestion of a water load diminishes ADH secretion, and there is an appropriate increase in urinary water excretion which restores the

osmolality of the body fluids back to normal levels. The mechanism by which the osmoreceptor cells operate is unknown but as speculated by Verney it is thought that they undergo cell shrinkage with stimulation and this results in the generation of neural signals to the SON and PVN.

Non-osmotic stimuli for ADH release comprise a very broad range of physiological phenomena, e.g., decreases in ECF volume, hypoxia, pain, nausea, and stress, as well as age, species differences and individual variation. Other stimuli include CNS mediators, such as catecholamines, acetylcholine, angiotensin II, prostaglandins, endogenous opioids and calcium ions, the use of a number of pharmacological agents, surgery, and pathophysiological states (Schrier et al., 1979; Sklar and Schrier, 1983). The major non-osmotic stimuli are those that influence blood volume; a reduction in blood volume stimulates the release of ADH. Two groups of receptors are responsible for this neuroendocrine reflex: the low-pressure system stretch receptors in the wall of the left atrium, and the high-pressure baroreceptors located in the aortic arch and carotid sinuses (Share, 1974). With a small reduction in blood volume, such as 2.6% in the anesthesized dog (Claybaugh and Share, 1973) or 10% in the conscious dog (Henry et al., 1968), the increased ADH secretion is due to a decrease in the firing rate of the atrial receptors. A larger decrease in blood volume, as seen in severe hemorrhage, decreases arterial pressure and the diminished firing rate of the arterial baroreceptors contributes to the resulting increase in ADH release. Conversely, an

increase in blood volume will enhance the activity of both atrial and arterial receptors, resulting in an inhibition of ADH release. These receptor responses are mediated by changes in vagal afferent tone (Share, 1968). It is believed that non-adrenergic neurons located in the dorsal pontine area close by and ventrolateral to the locus coerulus mediate synaptic inputs from the baroreceptos to the ADH-secreting neurons (Yagi et al., 1980).

The two different stimuli for ADH release, osmotic and non-osmotic, are believed to interact by algebraic summation. Baroreceptor and osmoreceptor pathways appear to be anatomically distinct but impinge upon the same neurosecretory cell body where their impulses are processed (Kannan and Yagi, 1978; Schrier et al., 1979). The hypothalamic cells of the SON and PVN integrate this information and the ultimate electrical outflow determining the rate of ADH secretion represents the algebraic sum of the stimuli presented. The interaction between changes in blood volume and plasma osmolality provide an excellent example of this neural integration. The close relationship between plasma ADH concentration and plasma osmolality can be linearly correlated and is used to characteize the osmoregulatory system in terms of its precision, threshold, and sensitivity (Robertson et al., 1976; Moses and Miller, 1971). Changes in blood volume have been shown to re-set the osmoreceptor threshold, without changing sensitivity, in such a way as to counteract the particular type of blood volume disturbance. Hypervolemia, for example, increases the osmotic threshold and shifts this relationship to the right. In the

presence of an expanded blood volume, the tonicity required to increase ADH release is higher, thus allowing the renal compensation for the expanded volume to occur. On the other hand, hypovolemia has the opposite effect and decreases the threshold, shifting this relationship to the left (Robertson and Athar, 1976). Both are examples of appropriate responses to multiple stimuli.

It may be stating the obvious to say that the control of body fluid volume and composition represents the integration of many systems within the body, interacting with each other to affect normal physiological processes. In addition to direct neural control, several hormonal systems are known to influence ADH release; for example, considerable interest has focused on the effect of the renin-angiotensin-aldosterone system. Both in vivo and in vitro studies have found that angiotensin II stimulates the release of ADH (Sklar and Schrier, 1983). In particular, angiotensin II has been shown to potentiate the osmotic stimulation of ADH (Shimizu et al., 1973; Sladek et al., 1982). The physiological significance of this remains controversial.

Evidence also exists suggesting an interrelationship between the glucocorticoids and ADH. Primarily, the observation that patients suffering from Addison's disease or adrenal insufficiency demonstrate an impaired ability to excrete a water load has implicated cortisol in affecting the normal control of urinary water loss along with ADH (Gaunt et al., 1957). In fact, situations of both cortisol excess and insufficiency are characterized by perturbations in water balance. The

possible mechanisms involved have been the subject of debate for many years. Glucocorticoids are thought to either inhibit ADH release from the pituitary or to directly affect the water permeability of the renal collecting duct. The influence of cortisol, the major glucocorticoid, on the release of ADH and its action on the renal handling of water is the focus of this dissertation.

CHAPTER II

LITERATURE REVIEW

The interrelationship between cortisol of the adrenal cortex and neurohypophyseal antidiuretic hormone (ADH) has been the subject of investigation for several decades. Studies have focussed on two main areas of interest. One concerns the impaired water excretion associated with adrenal insufficiency, in which separate roles for mineralocorticoid and glucocorticoid deficiencies have been recently defined. The other area of interest concerns the role ADH plays in the stimulation of adrenocorticotropic hormone (ACTH), and proposes the existence of a negative feedback of glucocorticoids on ADH release in addition to that already established for CRF and ACTH. An excellent review, written in 1957, of the historical development of this topic can be found in a chapter entitled "The adrenal-neurohypophyseal interrelationship" by Gaunt et al. These authors provided a careful description of the early studies that examined the nature of the interrelationship between the neurohypophyseal hormones and those of the adrenal cortex. They suggested possible mechanisms of interaction that are still being investigated. At that time and for many years afterwards, attempts to delineate the adrenal-neurohypophyseal relationship were hampered by the inability to directly measure ADH and, partly as a result, a large volume of literature exists with discrepant results. Therefore, only a fraction of the relevant

literature will be cited; a brief look will be taken at past work with an emphasis placed on more recent observations.

Adrenal Insufficiency and Water Diuresis Defect

The major evidence implicating glucocorticoid involvement in ADH secretion arises from the observation that the normal ability to excrete a water load is impaired in patients and animals with adrenal insufficiency.

Early studies on impairment of water diuresis in adrenal insufficiency. Rowntree and Snell (1931) were the first to note that water was not excreted normally when given to an Addisonian patient [for ref. see Gaunt et al., 1949]. Animal experimentation was first initiated by Silvette and Britton in 1933; they showed that adrenalectomized cats excreted an administered water load at a markedly decreased rate. Subsequent work by numerous investigators confirmed this defect in water excretion in adrenal insufficient dogs, and in both adrenalectomized and hypophysectomized rats; extensive clinical reports were made in primary and secondary hypoadrenalism in man (Gaunt, 1946; Slessor, 1951; Moses et al., 1958).

Efficacy of glucocorticoid treatment. These and other studies showed that replacement therapy using glucocorticoids restored toward normal the ability to excrete a water load. Glucocorticoid replacement was more effective than mineralocorticoid treatment (Gaunt et al., 1949; Oleesky and Stanbury, 1951; Birnie et al., 1950; Garrod and Burston 1952) and both adrenal cortical steroids together were necessary for total correction of body water control. A defect in

water excretion may be due to renal factors such as a decreased filtration rate and/or an increase in tubular reabsorption. According to Slessor (1951), glomerular filtration rate is diminished in adrenal insufficiency and can be improved with deoxycorticosterone acetate (DOCA) administration or salt-maintenance. The diuretic response to a water load, however, is still impaired and it is cortisone (a synthetic glucocorticoid) treatment that corrects this defect.

Inferred elevation of plasma ADH. The complexity of the mechanisms involved in the defect of water excretion in adrenal insufficiency was quickly appreciated. Gaunt and co-workers (1949) were the first to propose that elevated blood levels of ADH were responsible for the defect in water excretion accompanying this syndrome. This was supported by the work of Dingman and Despointes (1960) in which changes in free water excretion were used to infer changes in neurohypophyseal secretion of ADH. Various glucocorticoids were shown to suppress the neurohypophyseal response to intravenous nicotine administration. Since nicotine stimulates ADH release from the neurohypophysis via an acetylcholine-like action on the neurones of the supraoptic nuclei, these authors suggested that glucocorticoids increased free water excretion by inhibiting the hypothalamic control of ADH release. In addition, no effect of glucocorticoids was seen on the renal actions of ADH. Agus and Goldberg (1971) reported additional evidence supporting an elevated ADH concentration. They observed a marked improvement in urinary dilution in patients with anterior hypopituitarism when they were given either hydrocortisone or ethanol,

a known inhibitor of ADH release. Glucocorticoid administration to patients with diabetes insipidus (posterior hypopituitarism) had no effect on solute-free water excretion when ADH was simultaneously infused, thus suggesting that glucocorticoids do not act to alter the renal actions of ADH but rather, like ethanol, act to suppress its release. On the other hand, Lamdin et al. (1956) reported in Addisonian patients a failure of ethanol to provoke a diuresis but that corticosteroid administration corrected both the abnormal water tolerance and also restored the responsiveness to alcohol. They suggested that glucocorticoids act permissively in allowing a normal response to a water load and alcohol ingestion. How this was accomplished remained speculative.

Alternative factors, e.g., GFR and ECF Vol. Numerous studies examined the possiblity of other mechanisms being responsible for this defect in water excretion. Kleeman et al. (1958) and others (Burston and Garrod, 1952) stressed the importance of nonhormonal factors, such as a reduced glomerular filtration rate (GFR), in preventing the diuretic response to water in adrenal insufficiency. Since a decreased GFR has been associated with adrenal insufficiency, they studied the effects of agents known to increase GFR on water loaded adrenal insufficient patients and observed only a partial correction in their water excretion. Hydrocortisone treatment exhibited a marked improvement in the water diuresis; however, this improvement could not be explained entirely by changes in GFR. It was suggested that glucocorticoids allowed for maximal impermeability of the renal

collecting duct in the absence of circulating ADH, and that the absence of glucocorticoids resulted in the back diffusion of water from the lumen into the extracelluar compartment. Observations made by Gill et al. (1962) added another aspect of thought to this problem. Their experiments were designed to determine the effects of extracelluar fluid (ECF) volume expansion on water excretion in the Addisonian patient. It was found that volume maintenance, in the form of intravenous saline infusion, resulted in correcting the water diuresis in a manner comparable to that seen with cortisol treatment. They theorized that the decrease in ECF volume due to adrenal insufficiency induced loss of sodium, mainly due to the lack of mineralocorticoid actions, played a role in the impaired diuresis by stimulating ADH release.

Two main theories evolve from early work. Early attempts to measure plasma ADH concentrations directly used a variety of bioassays with varying results. Both elevated ADH blood levels and non-detectable ADH levels have been reported in adrenal insufficiency. As a result, two main theories have been proposed to explain the mechanisms responsible for the defect in water excretion associated with this disorder. According to one, elevated plasma ADH concentrations, not suppressible by a water load, impair the ability of the Addisonian patient to excrete a water load normally. Since glucocorticoids have been found to correct both the abnormally high ADH levels and the defect in water excretion, it is believed that glucocorticoids suppress ADH secretion. The elevated plasma ADH

concentration is thought to be due to the loss of glucocorticoid inhibiton on its release. According to the other theory, glucocorticoids have a direct effect on the renal tubule. It is thought that glucocorticoids are necessary for the development of maximal impermeability of the distal nephron to water in the absence of circulating ADH, and that ADH is not responsible for the defective water excretion.

The work of Ahmed et al. (1967) and Kleeman et al. (1964), respectively, best typify the studies that support each of these theories. Kleeman et al. compared normal subjects to glucocorticoid-deficient Addisonian patients and studied dogs and rats before and after adrenalectomy. They reported that a similar decrease in plasma ADH concentration occurred in all cases in response to a water load. Despite this fall in plasma ADH concentration, adrenal insufficient man, dog and rat all demonstrated a marked impairment in water diuresis which was seen to improve with cortisone treatment. In contrast, Ahmed et al. measured elevated plasma ADH levels in hydrated patients with Addison's disease. They found both the water diuresis defect and the increased plasma ADH levels were returned to normal within 2 to 3 hours after glucocorticoid administration. This group based the discrepancy of their findings from that of Kleeman and co-workers on the improved extraction and purification methods of plasma employed for the bioassay of ADH.

Many factors contributed to the conflicting results of these and other investigators. As mentioned, the bioassays used were

insensitive, varied between laboratories and were performed with or without extraction procedures. In some cases, the method used to obtain blood samples for ADH measurement induced stress or used anesthetized animals, both procedures that in themselves stimulate ADH release. In addition, the number of subjects used in many of these studies were relatively few, consisting of one or two patients.

Later studies investigating water diuresis defect. The ability to excrete a water load in adrenal insufficiency was found to involve more than just an increase in plasma ADH concentration.

Non-ADH mediated potential effectors. Green et al. (1970), in an attempt to circumvent the controversial bioassay issue, designed an experiment using rats with hereditary hypothalamic diabetes insipidus (DI) which lack ADH (Brattleboro rats). After adrenalectomy, these rats were shown to have a persistent defect in water excretion and it was concluded that an ADH-independent mechanism was also involved. Basing their criteria of a water diuresis on the presence of an increased urine flow and urinary dilution, they compared these parameters in the same animals before and after adrenalectomy and found that simultaneous mineralocorticoid and glucocorticoid administration was required to restore these responses to normal. This may be interpreted as indirect evidence in support of the hypothesis that glucocorticoid deficiency impairs the renal excretion of water by increasing distal nephron water permeability. Although it was not measured, they also suggested that changes in renal hemodynamics could account for the similarity of the defective diuresis seen in the

adrenal insufficient DI rat to that seen in the adrenal insufficient normal rat. It was concluded that ADH was not essential in the manifestation of this defect, although elevated plasma ADH concentrations may participate in the mechanism of this impairment in those individuals capable of producing it.

Effects of salt balance and stress on plasma ADH. In an attempt to settle this controversy, Share and Travis (1970), using a specific and sensitive bioassay for ADH measurement, studied conscious, adrenalectomized dogs maintained on supportive therapy and reported plasma ADH concentrations within normal limits. Withdrawal of steroid maintenance therapy and salt supplementation for 3 days resulted in progressive increases in plasma ADH concentration over the 3 day period. On the other hand, when the salt intake was continued without cortisol administration, no rise in plasma ADH levels was seen. The salt supplement is believed to have prevented the reduction in blood volume associated with its discontinuance. These investigators suggested that other studies may not have controlled for salt intake adequately and this contributed to the variability in results observed. It was concluded that the plasma ADH concentration can increase during adrenal insufficiency and that the stimulus for its release is the reduction in blood volume and pressure rather than the lack of a direct inhibitory action of glucocorticoids on neurohypophyseal ADH release. A subsequent study by Travis and Share (1971) pursued this suggestion further and demonstrated that the acute expansion of ECF volume decreased the elevated plasma ADH concentration in adrenal

insufficiency, again supporting their theory of a decreased volume-pressure stimulus. On the other hand, the acute administration of glucocorticoids was without effect in reducing the plasma ADH concentration; however, in the control experiment receiving vehicle solution, plasma ADH concentration increased seven-fold. They suggested that under certain conditions glucocorticoids can inhibit ADH release by preventing psychogenic stress-stimulated ADH release. It was noted that this effect of glucocorticoids could be a direct inhibition of the central mechanisms that control ADH release or an indirect result of relieving stressful stimuli (Share and Travis, 1971).

Role of mineralocorticoids. Further support of the primary role of sodium balance in the defect in diuresis during adrenal insufficiency was provided by Ufferman and Schrier (1972). Recognizing the need to control some of the many variables inherent in this problem that previous studies omitted, they used conscious, table-trained dogs serving as their own controls, and physiological vs. pharmacological amounts of glucocorticoid hormone replacement together with administering sufficient salt intake to avoid a negative sodium balance and subsequent ECF volume depletion. More importantly, the role of mineralocorticoid deficiency was also examined while in all circumstances measuring GFR. Adrenalectomized dogs treated with physiological doses of glucocorticoids still had an impaired response to a water load, which was corrected by either saline or mineralocorticoid replacement therapy. In addition, no defect in water

excretion or change in GRF was seen when these adrenalectomized animals were maintained, regardless of the steroid(s) withheld, on a high sodium diet. The authors suggested that factors other than or in addition to glucocorticoid deficiency are involved; and that preventing ECF volume depletion, via high sodium intake or mineralocorticoid replacement, corrects the impaired water excretion. Subsequently, Boykin et al. (1979) found elevated radioimmunoassayable levels of ADH and a decreased GFR associated with pure mineralocorticoid deficiency and attributed this elevation in plasma ADH concentration to the decrease in ECF volume secondary to a negative sodium balance.

Improved techniques provide new insights.

RIA assessment for plasma ADH. With the development, of the radioimmunoassay (RIA) by Berson and Yalow (Berson et al., 1956), the increase in specificity and sensitivity for hormone measurement has enabled numerous investigators to define more precisely the role of ADH in adrenal insufficiency, and has settled some of the controversial findings present in the older literature (Schrier et al. 1980; Schrier and Linas, 1980; Raff, 1987). Generally, increased plasma ADH concentrations have been found in clinical and experimental adrenal insufficiency. Seif et al. (1978) measured levels of ADH and neurophysin, the portion of the precursor peptide remaining when ADH is cleaved immediately after release from the neurohypophysis. Both peptides were elevated in the plasma of adrenalectomized rats before and after water loading, which was normalized with glucocorticoid administration. In addition, the pituitary content of ADH and

neurophysin, when compared to sham-operated controls, was significantly lower in the adrenalectomized rats and it was hypothesized that this was indicative of persistent hypersecretion of these materials. Although the site of action of cortisone cannot be ascertained from these data, this and other studies using RIA assessment (Marchetti et al., 1978; Mandell et al., 1980) support the view that ADH is elevated in adrenal insufficiency and is normalized, along with the impaired water excretion, by glucocorticoid administration. Boykin et al. (1978) studied adrenalectomized dogs before and after a water load while on complete steroid replacement therapy and while glucocorticoid-deficient. Withdrawal of the glucocorticoids was associated with abnormal water excretion and elevated plasma ADH levels not suppressible by a water load. These responses were due to a nonvolume-dependent mechanism since no change was seen in body weight, ECF volume, or clearances of inulin (GFR) and PAH (an index used to estimate renal plasma flow or RPF). Changes were seen, however, in cardiac function during glucocorticoid deficiency; the heart rate was higher and the stroke volume was lower, resulting in a decrease in the left atrial receptor firing, a known stimulus for ADH release. Using a similar paradigm in the rat, Linas et al. (1980) again demonstrated elevated plasma ADH concentrations. They also studied DI rats and confirmed the work of Green et al. (1970) by showing that after 14 days of glucocorticoid deficiency, adrenalectomized, mineralocorticoid-replaced Brattleboro rats exhibited an impaired ability to excrete a water load. These two groups concluded that both

ADH-dependent and ADH-independent mechanisms are responsible for the impaired water excretion associated with glucocorticoid deficiency. The non-osmotic ADH release was thought to be associated with systemic hemodynamic stimuli. Since the ADH-independent defect was accompanied by a decrease in cardiac output and RBF, and a significant rise in filtration fraction occurred, a decrease in distal fluid delivery rather than an increase in water permeability was thought to account for the ADH-independent defect in water excretion.

ADH V_1 and V_2 antagonists. Manning, Sawyer, and colleagues have pioneered the development of analogs for ADH this last decade. Both agonistic and antagonistic analogs of ADH have been separately defined for the V_1 and V_2 receptor types associated with ADH (for rev. see Manning and Sawyer, 1985). These peripheral receptors are distinguished by their location and response when stimulated; namely, V_1 receptors located on vascular smooth muscle increase blood pressure when stimulated, and V_2 receptors located in the renal tubule cause the reabsorption of free water when stimulated. Many agonists and antagonists of the vasopressor action (V_1-receptor) of ADH exist with varying degrees of potency and selectivity (Manning and Sawyer, 1985). Although a potent and selective agonist of the antidiuretic action (V_2-receptor) of ADH, dDAVP, has been available for some time, effective antagonists of the hydroosmotic effect were not reported until 1981 (Sawyer et al., 1981). Since then, several more antidiuretic antagonists have been developed (Sawyer and Manning, 1984); however, all exhibit antivasopressor activities as well.

The use of the antagonists of the antidiuretic and vascular effects of ADH has allowed further examination of the role endogenous ADH plays in affecting water excretion and blood pressure maintenance during adrenal insufficiency. Specifically, Ishikawa and Schrier (1982) looked at the effects of the hydroosmotic ADH antagonist [1(β -mercapto-β, β-cyclopentamethylenepropionic acid), 2-O-ethyltyrosine, 4-valine] AVP, [d$(CH_2)_5$Tyr(Et)VAVP], in mineralocorticoid deficient and glucocorticoid deficient adrenalectomized rats. (This inhibitor has been shown to block the antidiuretic effect of ADH without changing GFR, solute excretion, or blood pressure (Ishikawa et al., 1983); it has also been shown to inhibit tritiated-ADH binding to papillary plasma membranes and to block ADH stimulation of adenyl cyclase in papillary collecting ducts (Kim and Schrier, 1982; Kim and Schrier, 1985). These investigators have shown that the antagonism of endogenous ADH resulted in marked improvement in water excretion in both mineralocorticoid and glucocorticoid deficient animals. Although ADH-independent factors may also be present, these and other studies from this laboratory (Ishikawa et al., 1985) provide strong evidence that ADH in the plasma is involved in the impaired water excretion associated with combined glucocorticoid and mineralocorticoid deficiency. Schwartz et al. (1983) studied the effects of the ADH vasopressor antagonist [1-(β -mercapto-β, β-cyclopentamethylenepropionic acid), 2-O-methyltyrosine] AVP, [d$(CH_2)_5$Tyr(Me)AVP], on adrenalectomized dogs before and after withdrawal of steroid treatment. During steroid replacement, the ADH vascular antagonist had no effect

on blood pressure, however, when the plasma ADH concentration was increased after withdrawal of cortisol and DOCA steroid treatment, blockade of the ADH vascular effect caused a marked decrease in blood pressure. The role of ADH in maintaining blood pressure during adrenal insufficiency was attributed to the hypovolemia that develops in this pathophysiological state. Schrier and co-workers further examined the role of ADH in blood pressure maintenance by studying the effects of the ADH vasopressor antagonist in mineralocorticoid deficient and glucocorticoid deficient, adrenalectomized rats (Ishikawa and Schrier, 1984; Ishikawa et al., 1985). They confirmed the data of Schwartz et al. (1983) by demonstrating that ADH is involved in blood pressure maintenance, specifically in mineralocorticoid deficiency but not glucocorticoid deficiency. In addition, mineralocorticoid and not glucocorticoid deficiency was associated with a loss in body weight and blood volume. It was concluded that the elevated ADH levels associated with mineralocorticoid deficiency not only produces water retention but also facilitates the maintenance of blood pressure.

Correlation of findings with neuroanatomical changes. Another approach used to study the adrenal-neurohypophyseal interrelationship entailed correlating changes in pituitary ADH content with the presence or absence of the adrenal gland. Cavellero et al. (1954) were the first to report that pituitary ADH content decreased after adrenalectomy. Gaunt et al. (1957) confirmed this finding and demonstrated that glucocorticoid administration prevented this depletion. They interpreted these findings to suggest that depletion

indicated hypersecretion. They further suggested that glucocorticoids inhibit the release of ADH, and that it is the absence of this glucocorticoid inhibition that accounts for the elevated plasma ADH levels in adrenal insufficiency.

In the 1970's, new advances in histochemical staining techniques were developed, such as immunoperoxidase and immunoflourescence, that have enabled investigators, primarily Zimmerman and colleagues (Zimmerman and Defendi, 1977; Sladek et al., 1980; Zimmerman et al., 1984), to define more precisely the anatomy of the hypothalamoneurohypophyseal system (HNS). In addition to describing a spatial segregation of ADH and oxytocin neurones in the PVN and SON, these investigators have identified some fibers containing ADH and oxytocin that originate in the PVN and project to the portal system of the zona externa of the median eminence. Other neurones have been found that project to extrahypothalamic sites including the forebrain, brain stem and spinal cord, suggesting new roles for these hormones in addition to the traditional ones associated with them in the plasma. In normal rats, very few fibers are seen in the region of the zona externa of the median eminence (Sokol et al., 1976). Stillman et al. (1977) found that adrenalectomy resulted in a marked increase in the immunostaining of ADH fibers in this region and no change in oxytocin fiber immunostaining. This enhanced staining appeared to be due to an increase in the number of fibers visualized, as well as the amount of material contained in the individual fibers. Treatment with dexamethasone (a synthetic glucocorticoid) suppressed the increase in

ADH-staining fibers seen after adrenalectomy. Dehydration (a known stimulus for ADH secretion), on the other hand, had no effect on the immunostaining of the zona externa of the median eminence. The effect of administering varying doses of glucocorticoids and DOCA on this neurosecretory system was also investigated (Silverman et al., 1981). Low doses of corticosterone reduced the amount of staining in comparison to cholesterol-replaced controls. Higher doses completely prevented this increase and resulted in staining comparable to that in intact animals. DOCA produced only a slight inhibition of the response to adrenalectomy. As a result of these findings, it was suggested that ADH release to this region is stimulated by adrenalectomy and that its content in pathways to the zona externa of the median eminence is regulated by glucocorticoids. These anatomical changes following adrenalectomy support the theory that glucocorticoids have a central effect on the synthesis and/or storage of ADH in the hypothalamus in the region of the zona externa. It may also represent an increased release of ADH to this area which is in close proximity to the pituitary portal blood supply.

It is important, perhaps, to be reminded that the previously cited work (Cavellero et al., 1954; Gaunt et al., 1957) in which adrenalectomy was associated with decreased pituitary content, was interpreted by those authors as evidence for increased release and exhaustion of stores due to a lack of glucocorticoid inhibition. On the other hand, the increase in ADH-staining fibers in the zona externa of the median eminence has been interpreted by Stillman et al. (1977)

and Silverman et al. (1981) to mean the same thing, i.e., the increase in ADH in the zona externa is due to the absence of glucocorticoid inhibition and is hypothesized to be associated with an increase in secretion of ADH into the portal system. These types of findings are strictly correlative at best and do not represent the dynamics of the physiology they describe. The interpretation of opposite results to support the same hypothesis points out the importance of measuring plamsa ADH concentrations in these situations.

Thus, many studies confirm an elevated plasma ADH concentration to be present in both mineralocorticoid and glucocorticoid deficiencies. These elevated levels persist despite the presence of hypoosmolality, and thus indicate a non-osmotic release of ADH in association with the impaired water excretion in both glucocorticoid and mineralocorticoid deficient states; however, the suggested mechanisms for the ADH release are different. In the mineralocorticoid deficient state, ECF volume depletion is thought to be the underlying factor responsible for the elevated levels of ADH which contribute to both the impaired water excretion and the maintenance of blood pressure. Also, the changes in systemic and renal hemodynamics associated with hypovolemia decrease the renal capacity to excrete a water load. In contrast, ECF volume depletion is not a cause for the elevated plasma ADH concentration and the impaired water excretion in the glucocorticoid deficient state. Although changes in systemic hemodynamics have been shown to be a factor, a direct effect of glucocorticoids on the central release of ADH into the peripheral circulation remains a possibility.

Interrelationships of Hormonal Factors

Aside from adrenal insufficiency, several lines of evidence in other research areas have implicated glucocorticoids in affecting ADH release. For example, the involvement of ADH in stimulating ACTH secretion suggests the possibility of a negative feedback effect of glucocorticoids on ADH release which may play a role in the overall feedback control of ACTH.

Corticotropin releasing factor (CRF) and ADH. For some time now, it has been known that ADH stimulates the release of ACTH (McCann and Brobeck, 1954). Although certain early studies suggested that ADH may be the principle regulator of CRF activity, it is now generally agreed that ACTH secretion is governed by a CRF distinct from ADH (Yasuda et al., 1982; Rivier and Plotsky, 1986). Regardless of this, a significant role has been established for ADH in participating in the neurohumoral-regulation of ACTH secretion (Rivier and Vale, 1985). This has been substantiated by numerous investigators examining the hypothalamic-pituitary-adrenal axis. Zimmerman and co-workers (1973; 1977), for example, have reported the presence of immunoreactive ADH and its associated neurophysin in neurones found in the zona externa of the median eminence which terminate around the capillaries draining into the portal circulation. They have also reported the concentration of ADH in the hypophyseal portal blood to be 1000-fold higher than that in peripheral blood. Since the dose of ADH necessary to stimulate ACTH secretion is much greater than that required for its antidiuretic activity, the high concentration found in the portal blood might

provide an adequate stimulus for ACTH release from the anterior pituitary. In addition to acting directly on the pituitary to increase ACTH secretion (Yasuda et al., 1978), ADH has also been reported to cause the release of endogeous CRF (Yasuda et al., 1982). With the recent isolation and characterization of the molecular structure of an ovine hypothalamic CRF (Vale et al., 1981, 1983) numerous studies, both in vivo and in vitro, have shown that ADH acts synergistically with hypothalamic CRF (Yates et al., 1971; Gilles et al., 1982; Rivier and Vale, 1983) to increase ACTH release.

Recently, Raff et al. (1985) investigated the relative importance and physiological role of ADH in the control of ACTH release by looking at the pituitary-adrenal axis response to two different types of stimuli, hypotension and CRF infusion, in both intact and neurohypophysectomized dogs. A decrease in blood pressure, 28 mmHg below control values, resulted in significant increases in ADH, ACTH, and cortisol responses that were all reduced by neurohypophysectomy. This suggests that peripheral plasma ADH is involved in the control of ACTH release during hypotension, to a greater extent than are the pituitary portal ADH levels that arise from the zona externa of the median eminence. The CRF infusion was shown to increase ADH release as well as ACTH, in both intact and neurohypophysectomized animals, an interesting and unexpected finding. These authors concluded that neurohypophysectomy decreased the ACTH response to hypotension but not to CRF, and also that CRF stimulated ADH release by an unknown mechanism.

Glucocorticoid effects on ACTH. In addition to the neuroregulation of CRF, ACTH secretion is also influenced peripherally by plasma glucocorticoids. These steroids exert an inhibitory effect on ACTH through a negative feedback mechanism (for rev. see Keller-Wood and Dallman, 1984). In general, the higher the level of cortisol-like steroids, the less ACTH is secreted; this suppression of ACTH by cortisol persists until the cortisol levels return to normal. Conversely, if cortisol levels are subnormal, the levels of ACTH rise and stimulate the adrenal cortex to secrete cortisol until normal blood levels are restored. This represents a classic example of a "servomechanism" or negative feedback system (Williams, 1974). The site(s) of action of glucocorticoid negative feedback in the brain is still being investigated, however, it is generally thought to involve both the anterior pituitary itself and the hypothalamus since glucocorticoids suppress CRF activity as well (Yasuda et al., 1982; Keller-Wood and Dallamn, 1984). Therefore, circulating glucocorticoids may have a neuroregualtory effect by modulating input into the hypothalamus. If this is so, perhaps part of their neuroregulatory action involves the control of ADH by influencing input to the hypothalamic magnocelluar nuclei, the SON and PVN.

Effects of glucocorticoids on ADH. The glucocorticoid negative feedback on ACTH is well established. Since ADH stimulates ACTH release and since both ADH and ACTH respond to similar stimuli, this suggests the possibility that glucocorticoids may also inhibit ADH responses as well.

Water diuresis in normal subjects. In an attempt to explain the improved water diuresis seen with glucocorticoid treatment in adrenal insufficiency, several early studies investigated the effects of glucocorticoids on ADH responses in normal subjects by looking at changes in free water clearance (FWC). Raisz et al. (1957) observed an increase in urine flow during a water diuresis with high doses of glucocorticoids. No changes were seen in GFR or osmolal clearance that could account for this increase in FWC. Since normal subjects were used in this study, it was assumed that plasma ADH levels fell appropriately in response to the water load and resulted in a state of "physiological diabetes insipidus". They interpreted these results to mean that cortisol increased FWC by affecting renal tubular reabsorption and/or permeability to water. This augmentation of the maximal water diuresis was reconfirmed in subsequent studies (Kleeman et al., 1960; Lindeman et al., 1961) in which it was also shown that glucocorticoids did not affect the renal actions of exogenously administered ADH, thus demonstrating that glucocorticoids did not antagonize ADH effects on the renal tubule but perhaps rendered the tubule maximally impermeable instead.

Acute stimuli. Numerous investigators used another approach in studying the effects of glucocorticoids on ADH release. The theory being tested was whether or not the ADH response to a variety of stimuli could be attenuated by glucocorticoid treatment, thus demonstrating an inhibition of ADH by glucocorticoid administration. Dingman and Despointes (1960) reported that glucocorticoids suppressed

the response of ADH to intravenous nicotine stimulation; however, the antidiuretic effects of exogenously administered ADH and of hypertonic saline infusion were not suppressed by glucocorticoids. Since glucocorticoids did not interfere with the renal actions of ADH, it was suggested that glucocorticoids inhibited ADH release and that this neural control was separate from the osmotic control. The use of noxious stimuli in rats, such as mild electric shock, has shown a decrease, following glucocorticoid treatment for 4 hrs, in the response of plasma ADH levels bioassayed (McCann et al., 1958) or no change in the antidiuretic response when given glucocorticoid pretreatment 20-24 hrs prior to the application of a stimulus (deWied and Mirsky, 1959). In fact, the two major types of stimuli used in this approach can be categorized as either osmotic or nonosmotic. The nonosmotic stimuli, in general, entailed using a physiological "stress" of some kind, such as mild electric shock, thigh cuffing, cigarette smoking, hemorrhage or hypoxia. Overall, the response of ADH to these stimuli was attenuated with prior or concurrent glucocorticoid treatment (for rev. see Raff, 1987). Under these conditions, the attenuation of an increase in ADH release by glucocorticoid administration may be due to a direct effect of glucocorticoids on the neurohypopheseal control of ADH release and/or to the effects glucocorticoids have in relieving the unknown entity of stimuli referred to as "stress". A look at the role of glucocorticoid inhibition of the ADH response to an osmotic stimulus perhaps would be more informative since this approach eliminates the "stress" factor present with most nonosmotic stimuli.

Osmotic stimuli. Using such an approach, Moses (1963) reported a study that examined pituitary depletion in response to the stimulus of dehydration of bioassayed ADH in untreated, DOCA, or methylprednisolone treated rats. The depletion of pituitary ADH content, after 48 hr of water deprivation, was shown to be significantly less in the glucocorticoid treated group, whereas DOCA had little effect. Three mechanisms were postulated in an attempt to explain these findings: 1) perhaps glucocorticoids increased ADH synthesis, 2) glucocorticoids may act directly on the brain to inhibit ADH release, or 3) the osmotic stimulus to the neurohypophysis was decreased due to peripheral effects of glucocorticoids on renal function. The reaccumulation rate of pituitary ADH content was unaltered with steroid treatment thus suggesting that an augmentation of ADH synthesis was not responsible for the decrease in pituitary depletion. Plasma osmolalities provided some insight into the mechanism responsible for the effect of glucocorticoids on the pituitary ADH content. As expected, the plasma osmolality in all groups increased after 48 hrs of dehydration, however, the plasma osmolality of the methylprednisolone treated group did not increase as much as the untreated controls. The glucocorticoid treated animals had a greater urine volume associated with an increased solute and sodium excretion during the 48 hr dehydration. This was thought to account for the lesser rise in plasma osmolality observed in this group. It must be noted, however, that the urine osmolality during these experiments was not affected by cortisol treatment. As a result of

this lower plasma osmolality, the regression line describing the pituitary ADH content vs. plasma osmolality relationship was not affected by glucocorticoid treatment. If glucocorticoids had an effect on ADH release in response to an osmotic stimulus, this relationship was expected to have been changed. Since no effect of steroid treatment was seen on plasma volume, measured with I-131 serum albumin, this was concluded to be in support of a renal effect of glucocorticoids rather than a central effect to inhibit ADH release.

Subsequently, a study by Aubry et al. (1965) using changes in FWC as an index of ADH release, reportedly found an effect of cortisol on the release of ADH. They studied the effect of cortisol administration, both chronic for 2 days, and acutely during the hypertonic saline infusion, on the osmotic threshold for ADH release in normal hydrated subjects. The osmotic threshold, in this case, is defined as being the plasma osmolality at which water-loaded subjects first respond to an intravenous infusion of hypertonic saline with a statistically significant fall in FWC. This drop in FWC was assumed to be the point at which ADH is first released. Under chronic glucocorticoid adminstration, the plasma osmolality obtained after water loading, prior to the hypertonic saline infusion, was significantly higher in the cortisol treated group by 4.63 mOsm/kg. Therefore, although the time required for the hypertonic saline infusion to reach the osmotic threshold, and the increment in plasma osmolality needed to stimulate ADH release, was not changed, the osmotic threshold in these experiments was found to be elevated by 3.72

mOsm/kg with cortisol administration. Similarly, during the acute administration of cortisol along with the hypertonic saline infusion, the osmotic threshold was increased significantly by 2.57 mOsm/kg, but was also associated with a significantly prolonged duration of hypertonic saline infusion necessary to achieve similar degrees of hypertonicity. Since it was not shown to alter the renal response to ADH, cortisol was thought to delay the release of ADH until a higher plasma osmolality had been reached, although the mechanism of action was unclear.

In an attempt to uncover this mechanism, Streeten et al. (1981), from the same laboratory, again used the osmotic threshold as an index of ADH responsiveness. They thought that cortisol may directly inhibit the osmotically stimulated release of ADH and investigated this possibility by performing repeated experiments in three conscious rhesus monkeys. Using stereotaxically placed cannulae, they introduced cortisol crystals directly into the supraoptic nuclei and studied the response of the osmotic threshold to hypertonic saline infusion. The aniamls were water loaded with 20-25 ml/kg tap water via a gastrostomy tube in order to produce a constant diuresis, at which time the hypertonic saline infusion was started. Again, the initial release of ADH was inferred by a statistically significant drop in FWC. Cortisol did not affect the absolute level of the osmotic threshold in these experiments. It was 301.8±1.9 mOsm/kg in the control experiments and 301.7±2.2 mOsm/kg in the cortisol experiments. A greater hypertonic saline load, however, was required to reach the same osmotic threshold

in the cortisol treated animals due to a significant lowering of plasma osmolality in response to hydration, i.e., prior to the hypertonic saline infusion, the mean plasma osmolaity in the control experiment after hydration was 291.5 mOsm/kg, whereas the administration of cortisol crystals into the SON one half to 1 hr before water loading resulted in a plasma osmolality of 279.3 mOsm/kg at the start of the hypertonic saline. Therefore, in the cortisol experiments a larger volume of the hypertonic saline was infused over a longer period of time before a drop in FWC, indicating the release of ADH, was observed. These authors could not explain the greater drop observed in plasma osmolality after water loading in the cortisol-treated experiments and concluded, "Whatever the exact explanation might be, it is clear that minute amounts of cortisol acting on the supraoptic nuclei do increase the magnitude of the osmotic load needed to stimulate vasopressin release in the conscious rhesus monkey."

Having previously shown that CRF stimulates ADH release (Raff et al., 1985), these investigators examined the effects of CRF infusion with hypertonic saline stimulation on ADH release, and looked at possible blockade of these responses with prior glucocorticoid (dexamethasone) treatment (Raff et al., 1986). Simultaneous administration of CRF and hypertonic saline produced an increase in ADH release, as measured by RIA, that was significantly larger than the sum of CRF alone and NaCl alone. Since ACTH was also measured and not shown to have an osmotically induced augmentation to CRF, the effect on ADH was thought to occur at sites in the brain distinct from those that

control ACTH release. The administration of dexamethasone inhibited the response of ACTH and ADH to the CRF and/or NaCl infusion. Interestingly, dexamethasone pretreatment resulted in elevated control plasma osmolalities. Since glucocorticoids have an established negative feedback effect on ACTH release, the ACTH inhibition was expected. Although the CRF inhibiton by dexamethasone and the subsequent absence of its stimulatory effect on ADH release was proposed as a possible mechanism, the inhibition of the ADH response was thought to demonstrate that glucocorticoids attenuate the osmotic stimulation of ADH release. Several sites of action were suggested: possibly the magnocellular neurones themselves, the hypothalamus or the posterior pituitary.

In summary, aside from exerting a negative feedback effect on ACTH release, glucocorticoids may also inhibit ADH release as part of the overall pituitary-adrenal axis interrelationship. Many studies have examined the effect of glucocorticoids on the response of ADH to various stimuli. The studies using nonosmotic stimuli are confounded with the presence of a physiological stress factor also known to stimulate ADH release and known to be relieved by glucocorticoids. If an attenuation in the ADH response is observed under these conditions, the effect of the glucocorticoids could be a part of its ability to reduce the stress-induced ADH release rather than a direct effect of glucocorticoids on neurohypophyseal ADH regulation. The influence of glucocorticoids on the osmotic stimulation of ADH release provides an approach devoid of these problems. Of the studies done using this

approach, variable results have been obtained. Glucocorticoids have been reported to increase the osmotic threshold for ADH release, suggesting a central inhibition, whereas glucocorticoids have also been shown not to change the ADH response to hyperosmolality. In only the most recent study was the plasma ADH concentration measured. More carefully controlled studies, in which plasma ADH concentration is measured, are required if these inconsistencies are to be resolved.

Statement of Problem

It is known that cortisol, as well as ADH, also influences water balance. This influence is best described by the effects of cortisol on a water diuresis. An excess of cortisol has been found to augment free water clearance (FWC), while the opposite situation of cortisol insufficiency inhibits a water diuresis. Although the mechanism responsible for this effect of cortisol on the renal handling of water has been extensively studied, it remains unclear.

Two main theories have been proposed to explain the effect of cortisol on FWC. The first hypothesis suggests that cortisol directly inhibits ADH release; while the second proposes that cortisol has a direct effect on the renal tubule, rendering the collecting duct maximally impermeable to water.

A frequently cited study (Aubry et al., 1965) reported that peripheral cortisol administration increased the osmotic threshold for ADH release, thereby altering the plasma ADH versus plasma osmolality relationship by shifting it to the right. Another study by Streeten et al. (1981) examined the effect of central cortisol administration and found no effect on the osmotic threshold for ADH release. These conflicting reports were both hampered by the indirect assessment of ADH, since changes in FWC were used as an index of ADH release in both cases.

The present study was done in an attempt to resolve this controversy. Experimental protocols were designed to evaluate the acute effects of cortisol on ADH release, comparing central and

peripheral cortisol administration while directly measuring plasma ADH and plasma cortisol concentrations, during several different osmotic states in the conscious dog. Specifically, these questions were addressed:

1) Is ADH release inhibited by cortisol?

2) If so, is this due to a peripheral or central effect of cortisol?

3) Does cortisol alter the plasma ADH versus plasma osmolality relationship?

4) Lastly, during a water load, are the renal responses to cortisol excess and insufficiency mediated by ADH?

CHAPTER III

MATERIALS AND METHODS

Studies were conducted in conscious, table-trained dogs of either sex weighing between 10 and 27 kgs. The dogs were fed Purina High Protein Dog Chow daily and allowed free access to water. The dogs were housed and otherwise used in AAALAC certified facilities and all procedures were conducted utilizing protocols approved by an animal use committee. Experiments were conducted within the guidelines set forth by the NIH Guide for the Care and Use of Laboratory Animals.

Surgical Preparation

All surgery was performed in a sterile, air-conditioned surgical area. The dogs were sedated with a mixture of 0.1 mg/kg Acepromazine (Fort Dodge Laboratories, Inc.; Fort Dodge, Iowa) and 0.09 mg/kg atropine (Eli Lily Co; Indianapolis, Indiana) and anesthetized with 0.9 mg/kg thiopental sodium (Pentothal; Abbott Laboratories; Chicago, Illinois) for presurgical preparation. During surgery, anesthesia was maintained by tracheal tube administration of 1/2-2% methoxyflurane gas (Penthrane; Abbott Laboratories; Chicago, Illinois).

Each dog was prepared prior to experimentation by surgically creating an exteriorized carotid artery loop and allowed at least 3 weeks recovery. The dogs were divided into two groups: one in which they were given intravenous (iv) infusions of cortisol, and the other in which cortisol was administered intracerebroventricularly (ivt). The latter group, therefore, underwent an additional surgical

procedure. A stainless steel cannula was stereotaxically placed into the third cerebral ventricle of the brain. A similar technique has been used previously by Reid and Ramsay (1975) and by Thrasher et al. (1980b).

Cannulation of the third ventricle was done with a stereotaxically mounted drill used to make a hole in the skull. The cannula consisted of a 20-gauge, 3-1/2 inch spinal needle (Tru-Fit; Travenol Laboratories; Deerfield, Illinois) 60 mm in length, minus the luer plug and stilette. The cannula was advanced approximately 33 mm below the dorsal surface of the brain, or when evidence of cerebral spinal fluid (CSF) backflow was observed. Its position was confirmed by fluoroscopy using a 0.3 ml injection of 60% meglumine iothalamate (Conray; Mallinckrodt; St. Louis, Missouri). The cannula was carefully bent into an L-shape and anchored down with methyl methacrylate (Orthodontic resin; L.D. Caulk Co.; Milford, Delaware). Sterile tygon tubing, connected with an adapter specially designed in our laboratory for injection, was attached to the metal cannula and buried subcutaneously behind the neck. The void volume of this entire cannula and injection port was determined to be 150 μls. This completely subdermal injection port, accessible by syringe and needle, eliminated the use of a protective jacket or harness and reduced the chance of infection from that seen with the otherwise routinely used exteriorized injection ports. Cannulae prepared in this fashion can be kept patent for several months with no apparent discomfort to the animals (Hadick and Cornette, 1982).

Three weeks following surgery, a thirst test (ivt injection of angiotensin II [Beckman; Palo Alto, California] 150 ng in 150 μl) was performed to ensure patency, location, and the normal established physiological response. Since the central administration of angiotensin II elicits a strong dipsogenic response, increases blood pressure and plasma ADH (Fitzsimons, 1980), it was used at the end of each ivt experiment to verify proper catheterization of the subdermal injection port as well as patency of the third ventricle cannula.

Table 1 shows the coordinates used in the placement of the third ventricle cannula in seventeen dogs, and Figure 1 is a representative radiograph showing the final placement of a cannula in the third ventricle. The cannula tip lies just anterior to the massa intermedia and can be seen to open into the ventral portion of the third ventricle which is outlined by contrast media.

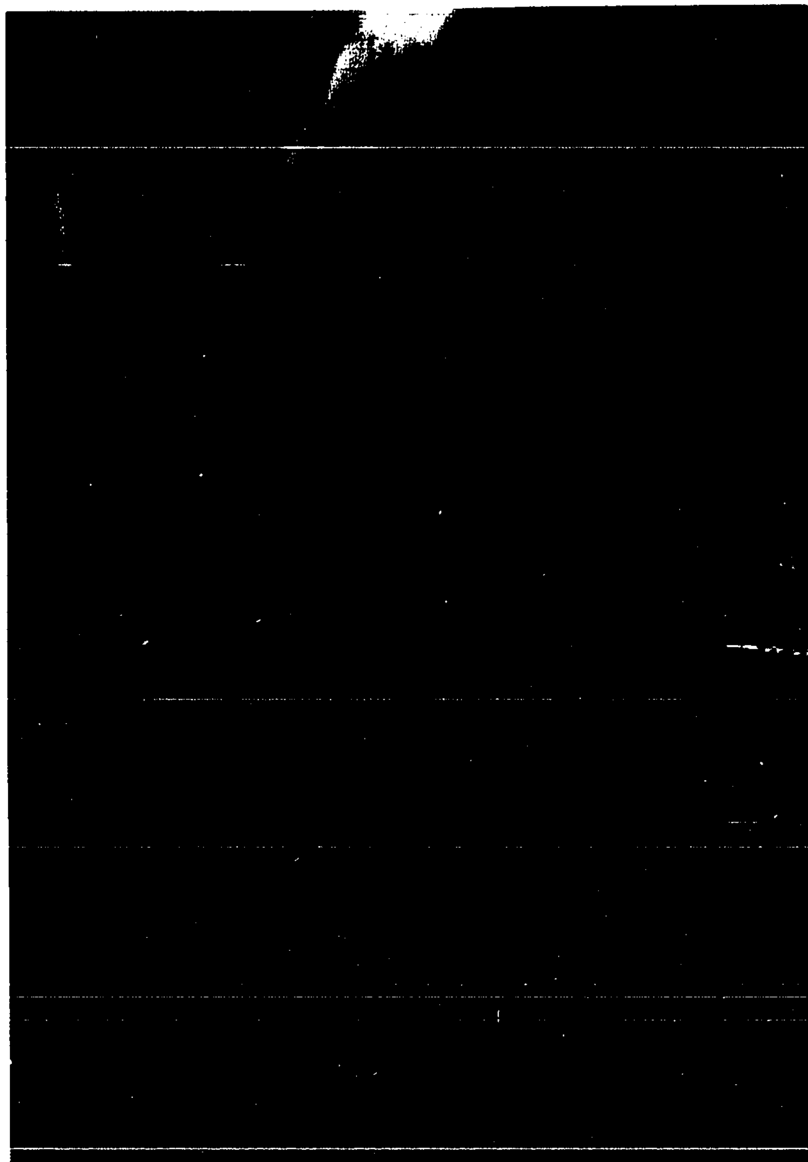
Experimental Protocols

The dogs were divided into two groups: those surgically prepared with a chronic intracerebroventricular cannula (the ivt group) received centrally administered cortisol, and the other group (the iv group) received cortisol peripherally through an intravenous catheter. Each group of dogs was subjected to six experimental protocols. In protocol 1, the dogs were dehydrated for 48 hr and received infusions of cortisol; protocol 2 was the corresponding control experiment in which the dogs were infused with saline or in the case of the ivt group, infused with artificial CSF. In protocol 3, the dogs were given cortisol and then subjected to an intravenous 5% sodium chloride

Table 1: Coordinates used for third ventricular cannula placement.

Sex	Wt(kg)	Distance Anterior to Ear Bar (mm)	Skull Thickness (mm)	Total Cannula Depth (mm)
M	15.89	24.0	10.5	42
M	31.78	23.0	11.0	45
F	15.89	23.0	4.0	38
M	18.16	24.5	5.5	37
F	11.80	22.5	6.0	40
F	19.98	24.5	12.0	45
M	20.43	24.5	14.0	45
M	21.34	23.5	18.5	42
F	18.16	24.5	10.0	42
F	16.34	29.0	7.0	43
M	22.70	24.0	19.0	56
M	15.89	23.0	10.5	44
F	15.89	25.5	9.0	43
F	12.26	24.5	7.0	40
F	27.24	24.0	12.0	44
F	20.43	26.0	7.5	40
M	19.52	27.0	9.0	42

Figure 1: Radiograph demonstrating placement of third ventricle cannula and subdermal injection port.



infusion which produced a gradual increase in plasma osmolality; protocol 4 was the corresponding control experiment in which the dogs were infused with saline or artificial CSF followed by the 5% sodium chloride iv infusion. In protocol 5, the dogs were normally hydrated and given cortisol; while in protocol 6, the appropriate control experiment was run with vehicle instead of cortisol during normal hydration. In addition to these experiments, the ivt group underwent three more protocols in which an intravenous water load was given with and without cortisol. The cortisol was administered centrally (ivt) or peripherally (iv). Each protocol is described in further detail below.

The composition of the artificial CSF used for the central experiments in the ivt group is shown in Table 2 and was adapted from Thrasher et al. 1980b. The artificial CSF was made using sterile, bottled pyrogen-free water, USP (Cutter Laboratories Inc.; Berkeley, California). The sodium bicarbonate was added to the stock solution on the morning of the experiment. After being filtered through a 0.22 µm millipore filter (Millipore Corp.; Bedford, Massachusetts) to insure sterility, the artificial CSF was then incubated at 37 °C and gassed with 95% O_2 and 5% CO_2 for approximately 10 to 20 min.

On the morning of the experiment, the dogs were weighed, placed on a table and the exteriorized carotid artery was cannulated using a 20-gauge, 1-1/4 inch catheter (Cathalon IV; Critikon; Tampa, Florida). This cannula was used for the removal of blood samples and for contiuous measurement of mean arterial blood pressure (MABP) and heart rate (HR) using a Statham P23DP pressure transducer and a Beckman R511A

Table 2: Composition of artificial cerebral spinal fluid (CSF) used in the central ivt experiments.

SOLUTE	mM
Na	150
K	3
Cl	130
HCO_3	25
Ca	0.6
Mg	0.9
PO_4	0.3

Measured values: pH = 7.45

pCO_2 = 30 mmHg

mOsm/kg = 290

recorder. The two cephalic veins were cannulated with similar catheters for the administration of solutions and the injection of replacement saline (0.9% sodium chloride, injection, USP; Travenol Laboratories; Deerfield, Illinois) used to replace the volume taken for blood samples. When necessary, hypertonic saline solutions were used for replacement to ensure that the plasma osmolality of the dogs was maintained at the time of sampling and during the experimental time course, as was required for the dehydration and hypertonic sodium chloride infusion experiments. The experimental protocols were conducted in a random fashion, except for the water loading experiments. These were done as a last series in random order. At least 2 to 3 weeks were allowed between experiments in all series.

In the ivt group, the skin over the subdermal injection port connected to the third ventricle cannula was shaved and surgically scrubbed. Sterile technique was used to cannulate this port, and the void volume of 150 μl was immediately administered.

The water loading experiments required two additional catheters. Another iv catheter was placed into the saphenous vein using an 18-gauge, 2-1/2 inch catheter (Longdwell; Becton-Dickinson; Rutherford, New Jersey) to administer the 40 ml/kg body wt "water" load of 2.5% dextrose (5% Dextrose, diluted to half strength; Travenol Laboratories; Deerfield, Illinois). This water load was administered over a 30 min period using a tubing pump by Sage Instruments, Model 375A. In addition, the bladder of these dogs was also catheterized using a Foley

catheter (C.R.Bard Inc.; Murray Hill, New Jersey) for the controlled collections of urine at 10 min intervals.

For the purposes of this work, the six protocols and additional water loading experiments will be divided into four experimental series. This scheme is summarized in Table 3.

For each protocol in series I, II and III in both the iv-group and ivt-group,(with one exception) at least one dog underwent an additional procedure at the conclusion of the experiment. The dog was immediately anesthetized with a 4% sodium thiamylal solution to effect (Suritol; Park Davis Co.; Morris Plains, New Jersey) using approximately 17.5 mg/kg body wt. A cisternal CSF sample was obtained via a spinal needle placed into the cisterna magna. The CSF sample of approximately 1 ml was then frozen and saved for subsequent analysis of cortisol. The one exception is the ivt-group of the dehydration protocols in series I. In this case, no CSF samples were taken.

Series I. The response of ADH to central and peripheral administration of cortisol during dehydration.

The dogs in both groups, the iv group and the ivt group, were dehydrated by removal of their drinking water for 48 hr prior to the morning of experimentation. After cannulation, a PRE-sample of 5 ml of blood was taken for immediate measurement of plasma osmolality. A corresponding hyperosmotic saline solution was made for simultaneous replacement during each blood sample withdrawal to maintain osmotic and volume balance throughout the experiment. The dogs were then allowed 1 hr to get accustomed to their surroundings. Then at 15 min before

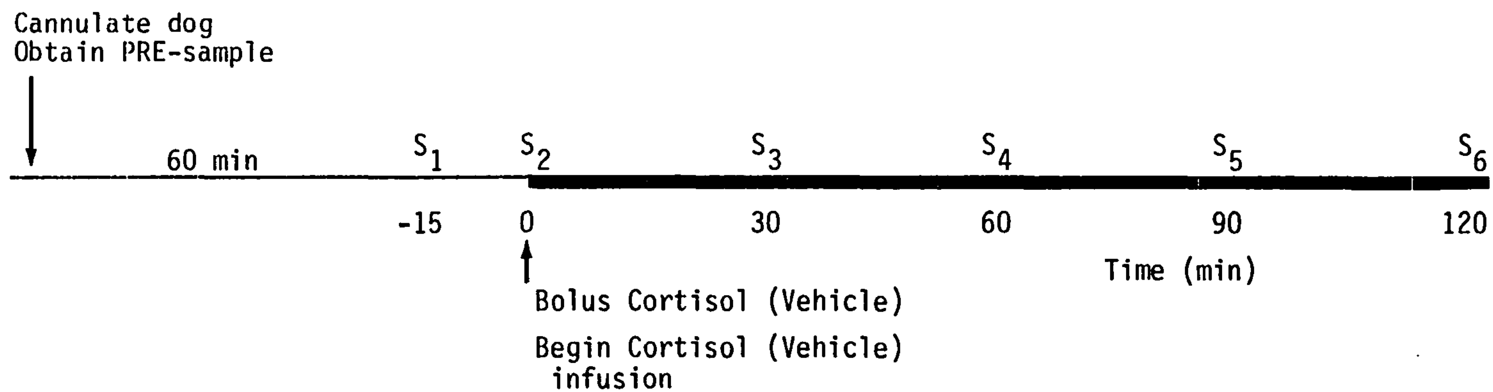
Table 3: Summary of experimental protocols and division into experimental series.

	PROTOCOL	EXPERIMENT
Series I	1	Dehydration + Cortisol
	2	Dehydration control
Series II	3	5% NaCl + Cortisol
	4	5% NaCl control
Series III	5	Normal hydration + Cortisol
	6	Normal hydration conrtol
Series IV	7	Water loading control
	8	Water loading + Cortisol ivt
	9	Water loading + Cortisol iv

time 0, a control blood sample was taken followed by another 15 min later at time 0. Depending on the protocol, cortisol or saline infusion was begun and blood samples were taken every 30 min for 2 hr.

The ivt group received 300 ng/min cortisol (Hydrocortisone; Sigma Chemical Co.; St. Louis, Missouri) dissolved in 95% ethanol at 13.6 μl/min using a Harvard Apparatus syringe pump with tubing connected to the injection port of the third ventricle cannula. The 120 min blood sample was followed by an angiotensin II-thirst test. This entailed injecting into the third ventricle 150 μl of 100 ng/ 100 μl angiotensin II dissolved in artificial CSF and observing the increase in MABP and dipsogenic drive associated with central angiotensin II administration in conscious dogs. An additional blood sample was taken 5 min after angiotensin II administration in order to observe the associated increase in plasma ADH concentration, thus indicating successful cannulation of the subdermal injection port and patency of the third ventricle cannula. The control experiment used vehicle in artificial CSF as the infusate.

The iv group was given an iv bolus of 1 mg/kg body wt cortisol (Solu-Cortel; hydrocortisone sodium succinate; Upjohn; Kalamazoo, Michigan) in 5 ml saline immediately after the second control blood sample at time 0. The cortisol was then infused iv at 4.16 μg/kg/min for 2 hr using a Harvard Apparatus syringe pump set to deliver 13.6 μl/min. A similar protocol was followed but without cortisol for the corresponding control experiment. The experimental protocol for series I is illustrated in Figure 2.



Infusion rates: Cortisol i.v. @ 4.16 μg/kg/min

Cortisol ivt @ 300 ng/min ending with AII test

S denotes blood sampling

Figure 2: Series I experimental protocol.

Series II. The response of ADH to an acute osmotic load during central and peripheral cortisol administration.

After cannulation, a PRE-sample was taken for immediate measurement of plasma osmolality, and an aliquot frozen and saved for later measurement of plasma cortisol. Immediately following cannulation, the cortisol infusion was initiated. The infusion rates, dosages and onset of cortisol used were identical to those used in the dehydration protocols of series I, namely, 13.6 μl/min delivering 300 ng/min was infused intraventricularly into the ivt group, and 4.16 μg/kg/min at the same infusion rate of 13.6 μl/min was infused into the iv group. In the iv group, cortisol infusion was preceded by a bolus of 1 mg/kg body wt cortisol. After the 1-hr control period, control blood samples were taken at time -15 min and again at time 0. Immediately following this the 5% NaCl was infused at a rate of 0.05 ml/kg/min into the cephalic vein using the Sage pump. Blood samples were taken every 15 min for 75 min and then 30 min later at time 105 min. It should be noted that the volume of isosmotic replacement saline used at each blood sampling period equalled: the sample volume minus the volume infused over the time interval. The corresponding control experiments utilized artificial CSF without cortisol in the ivt group, and vehicle saline infusion in the iv group, at the same infusion rates. This was followed by the 5% NaCl infusion with exactly the same time course and sampling periods. Again, the ivt group received the angiotensin II-thirst test and subsequent blood sample withdrawal at the conclusion of the 105 min 5% NaCl infusion.

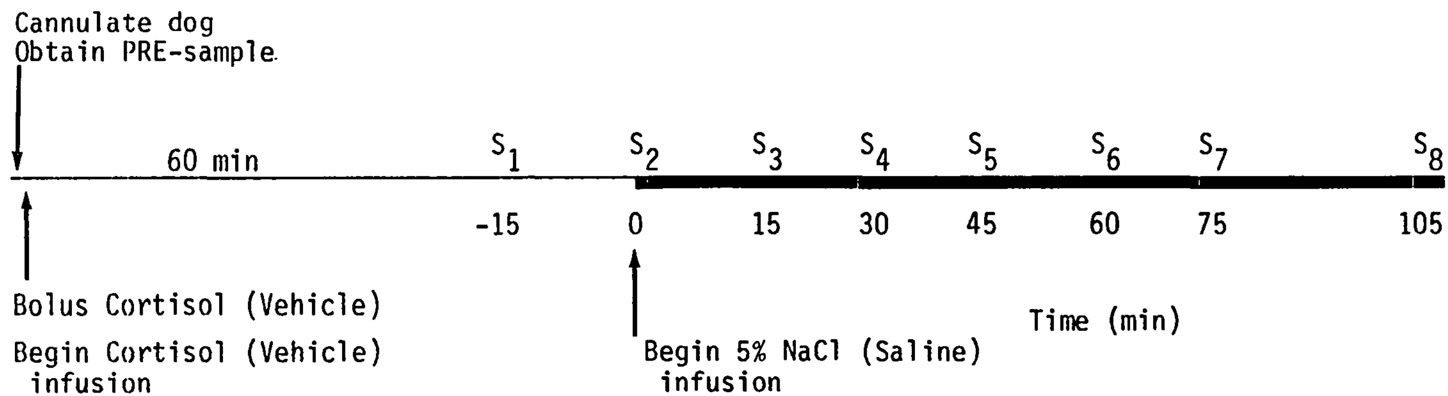
Series III. Effects of centrally and peripherally administered cortisol on the release of ADH without influence of an osmotic challenge.

In order to serve as both a control experiment looking at the effect of cortisol alone, and as a comparative control study with respect to the 5% NaCl infusion experiments, these two protocols were identical to those in series II except that 0.9% saline substituted the 5% NaCl infusion. Figure 3 illustrates the experimental design for both series II and series III.

Series IV. Renal function and ADH regulation in water-loaded dogs during central and peripheral cortisol administration.

After cannulation of the exteriorized carotid artery, both cephalic veins, a saphenous vein, the bladder and the subdermal injection port to the third ventricle cannula, a PRE-sample of 5 ml of blood was taken for the immediate measurement of plasma osmolality, with an aliquot frozen and saved for cortisol assessment. Depending on the protocol, artificial CSF containing vehicle (protocol 7) or cortisol (protocol 8) was immediately administered into the third ventricle. This was done by slowly injecting 300 μl of the test solution into the injection port. The first 150 μl accounted for the void volume of the system, and therefore the second 150 μl injected into the third ventricle introduced the test solution. The concentration of cortisol used was 22.1 μg/ml.

A one hour control period was allowed, and then at time -15 min and again at time 0 control blood samples were taken. At time -30 min the



Infusion rates: 5% NaCl @ 0.05 ml/kg/min

Cortisol i.v. @ 4.16 μg/kg/min

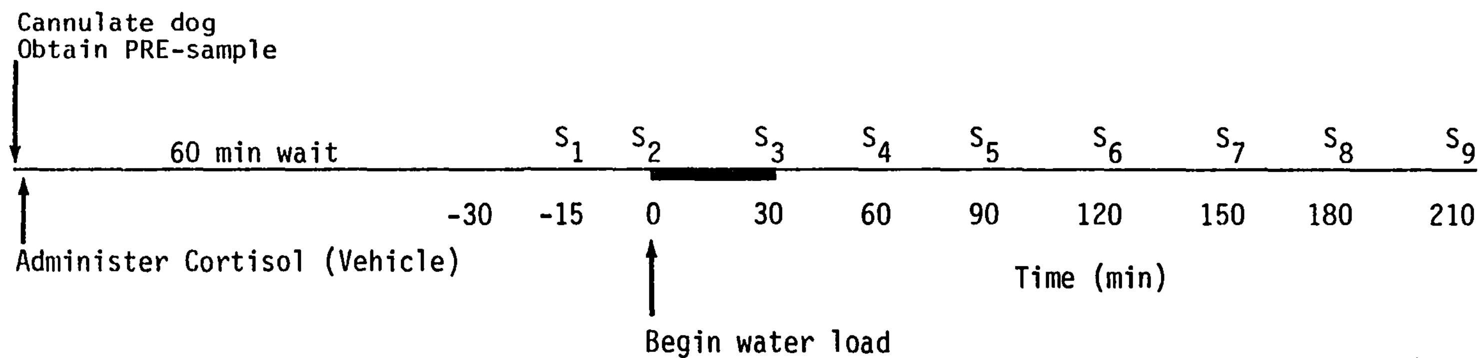
Cortisol ivt @ 300 ng/min ending with AII test

S denotes blood sampling

Figure 3: Series II and III experimental protocol.

control urine collections were begun and collections continued for every 10 min throughout the experiment. Immediately after collecting the second control blood sample and the third control urine sample, at time 0 min, another 150 μl of test solution was injected slowly into the third ventricle, and the water load infusion was initiated. The water load consisted of 2.5% dextrose administered at 40 ml/kg body wt into the saphenous vein over a 30 min time period using the Sage pump. The test solution in 150 μl was injected into the third ventricle every hour from the start of the water loading. Blood samples were taken every 30 min for 3-1/2 hr. Meanwhile, the urine collections proceeded at 10 min intervals, being careful to obtain complete bladder emptying by expressing it with air and applying mild pressure to the abdominal area. Once again, the conclusion of the experiment was followed by the angiotensin II-thirst test and subsequent blood sampling.

The final protocol in this series, protocol 9, was done in the same group of dogs as that of protocols 7 and 8 above, with cortisol administered peripherally into a cephalic vein. The dose of cortisol used was a 1 mg/kg body wt bolus given in 5 ml, immediately following the PRE- blood sample withdrawal. Infusion was then begun at 4.16 μg/kg/min on the Harvard pump set to deliver 13.6 μl/min. A comparison of central cortisol administration can therefore be made with that of peripheral cortisol administration during a water load using the two-way analysis of variance for repeated measures in the same subjects. Figure 4 shows the experimental design utilized in this last series of experiments.



Water load i.v. @ 40 ml/kg 2.5% Dextrose over 30 min

Cortisol i.v. bolus 1 mg/kg followed by 4.16 μg/kg/min

Cortisol ivt 3.31 g in 150 l administered at times -75 min, 0, 60, 120 and 180 min

S denotes blood sampling

Urine samples collected every 10 min from time -30 to 210 min

Figure 4: Series IV experimental protocol.

Sample Handling

Blood samples of 15 ml each were taken and placed into heparinized vacutainer test tubes containing 286 USP units of heparin (Becton-Dickinson; Rutherford, New Jersey). Aliquots were also taken for measurement of hematocrit levels in heparinized microcapillary tubes. The heparinized test tubes were immediately placed on ice and at the end of the experiment were centrifuged at 1,100 x g for 20 min at 4 °C. The plasma supernatant was then drawn off and dispensed into appropriate tubes for subsequent analyses. At least 5 to 7 ml of plasma were taken and treated with 0.1 ml of 1N HCl per ml plasma, and extracted on the day of the experiment for later radioimmunoassay of ADH concentration. Two aliquots of untreated plasma were taken for the determination of plasma osmolality, sodium, potassium, creatinine, plasma cortisol, and aldosterone. All samples were frozen and stored at -5 °C until the desired measurements were made.

Urine specimens were measured for total volume and two aliquots of 1 to 4 ml each were taken during the experiment and immediately placed on ice. These samples were frozen and stored for later measurement of osmolality, sodium, potassium and creatinine.

All CSF samples taken were immediately frozen and analyzed later for osmolality and cortisol concentration.

Sample Analyses

Plasma, urine and CSF samples were analyzed for osmolality. Two methods were used for the plasma osmolality determinations: a vapor-pressure osmometer (Wescor, Model 5100B) and a freezing-point

depression osmometer (Advanced Instruments Model 3DII). Both methods have excellent correlation and possess a ± 3 mOsm/kg H_2O precision. The urine samples and corresponding plasma samples in series IV experiments were all assessed for osmolalities using the freezing-point depression osmometer due to problems encountered with the vapor-pressure osmometer. All samples were measured for sodium and potassium by a flame photometer (Instrumentation Laboratories, Model 643). Hematocrit was determined in duplicate by the microcapillary method using an International Equipment Co. microcapillary centrifuge and reader. In the experiments of series IV, plasma creatinine was measured on the Beckman Astra 4, Model 1057, while urine creatinine concentration was determined using the colorimetric method of the Jaffe's reaction. The creatinine forms a red compound with the picric acid reagent in alkaline solution which was measured using a Gilford spectrophotometer, Model 2400S.

Several radioimmunoassays were used to determine hormone concentrations. Plasma and CSF cortisol concentrations were measured using Clinical Assays Gamma-coat I-125 cortisol RIA kits (Division of Travenol Laboratories; Cambridge, Massachusetts). Intra- and inter-assay coefficients of variability were 7.8% and 7.9%, respectively. Plasma aldosterone concentration was measured with a radioimmunoassay kit from Damon Diagnostics (Needham, Massachusetts). The coefficient of variability within-assay was 8.2% and 13.9% between assays.

Assay of ADH

Antidiuretic hormone concentration was determined by a radioimmunoassay developed in this laboratory. The plasma for ADH measurement was first extracted using a modification of the octadecyl silane extraction method of LaRochelle et al. (1980). Cartridges containing octadecylsilica (Sep-Pak C-18; Water Associates; Milford, Massachusetts) were primed by passing 5 ml methanol, 5 ml 8M urea and 10 ml distilled water before the application of sample. Plasma acidified with 1N HCl (0.1 ml per ml plasma) was passed through the cartridge slowly. The cartridge was then washed with 10 ml distilled water followed by 10 ml 4% acetic acid solution. The eluate, consisting of 10 ml 40% ethanol - 4% acetic acid solution, was collected in test tubes coated with assay buffer, dried under vacuum using an Evapomix evaporator (Buchler Instruments, Inc.; Fort Lee, New Jersey), and reconstituted in 0.5 ml of assay buffer. The assay buffer consisted of 0.33% NaCl and 0.1% bovine serum albumin (BSA) in 0.1M sodium phosphate buffer at pH 7.2. The samples were then frozen and stored for later radioimmunoassay assessment. The mean recovery of added hormone to plasma samples was 90%.

Antibody production to arginine-vasopressin was accomplished using the method of Skowsky and Fisher (1972). A conjugate of either lysine-vasopressin (LVP) or arginine-vasopressin (AVP) and thyroglobulin was injected subcutaneously into rabbits at 2 wk intervals. The rabbits were bled after a minimum of 3-booster series. The serum was heated to 56°C for 30 min to destroy the complement and

the titer checked. This was continued for several months as the titer usually improved with further booster series. The antisera used for the ADH determinations was raised against the AVP conjugate and had a cross-reactivity of 1.10% with oxytocin. Antisera were diluted to final concentrations in the radioimmunoassay by 1:400,000. This dilution produced between 30 to 47% binding of I-125 labeled AVP in the presence of no standard, i.e., Bo/T values.

The iodination of arginine-vasopressin was done using a modification of the lactoperoxidase method of Thorell and Johansson (1971). A 1 x 10-cm DEAE anion exchange column was used to separate the unreacted free iodide from the iodinated hormone. Aliquots of 1 ml were collected and the peak activity tube was then further purified by passing it through a 1 x 10-cm Sephadex G-25-40 column. The 1 ml aliquots from this second purification step that were used in the assay consisted of the two tubes after the peak activity tube. This aliquot of purified iodinated hormone was diluted to give approximately 3,000 counts/min in 50 µl and used in assays. The use of I-125 AVP supplied by New England Nuclear (Boston, Massachusetts) was recently employed and showed excellent correlation with the iodinated hormone made in our laboratory.

The radioiummunoassay procedure used was similar to those described by Skowsky et al. (1974) and Miller and Moses (1972). The buffer system consisted of 0.33% NaCl and 0.1% BSA in 0.1M sodium phosphate at pH 7.2. The standard curves for the assay were generated in duplicate using arginine-vasopressin (V-0377, Synthetic; Sigma; St. Louis,

Missouri) as the reference standard. The standard curves generated with this standard and the U.S.P. Pharmacopea reference standard are identical on all doses tested. Control samples were run at the start and end of each assay. The lower limit of sensitivity of the assay was 0.5 μU/ml. The final volume of the assay tube was 0.5 ml. Separation of the antibody-bound I-125 ADH from free I-125 ADH was accomplished using a BSA-coated charcoal suspension (0.3 ml of 0.8% Norit A charcoal and 0.16% BSA in assay buffer). Plasma samples were assayed at 3 doses and in duplicate. Both free and bound counts were employed in the calculations of the standard curves and unknown samples using a logit versus log-dose transformation to obtain the linear regression curve. The calculations were performed on a Wang 2200B computer and are fully described by Rodbard et al. (1969). The intra-assay and inter-assay coefficients of variability are 5.7% and 21.4%, respectively.

Calculations and Statistics

The formulae related to renal function used in this study are derived and explained in Vander (1975) and Rose (1977).

(1) Urine flow rate ($\dot{V}$) = ml/min.

(2) Creatinine clearance (Ccr): Used as a closely estimated measurement of glomerular filtration rate: ml/min.

$Ccr = Ucr \times \dot{V}/Pcr$, Pcr = plasma creatinine concentration, mg%

Ucr = urine creatinine concentration, mg%

(3) Osmolar clearance (Cosm): The volume of water required to make urinary solute concentration equal to plasma osmolality, also regarded as the isotonic fraction of urine volume.

$$Cosm = Uosm \times \dot{V}/Posm,\quad Posm = \text{plasma osmolality, mOsm/kg}$$
$$Uosm = \text{urine osmolality, mOsm/kg}$$

(4) Free water clearance (FWC): The net gain or loss of water in addition to that of the osmolar clearance.

$$FWC = \dot{V} - Cosm$$

(5) Urinary excretion rate of electrolytes: mEq/min.

$$U[z] \times \dot{V},\quad U[z] = \text{urinary concentration of } z\text{:}$$
$$z = Na,\ K,\ osm$$
$$\dot{V} = \text{urine flow rate, ml/min}$$

Statistical analyses were performed using an analysis of variance for repeated measures in the same subjects. This was followed by the Duncan's multiple range test to determine differences within and between different experimental protocols (Zar, 1974; Winer, 1971). The level of statistical significance was set at P 0.05.

CHAPTER IV

RESULTS

Series I

The response of ADH to central and peripheral cortisol administration during dehydration.

The i.v. infusion of cortisol into dehydrated dogs caused the plasma cortisol concentration to rise significantly ($P<0.01$) from a mean value of 3.7 μg% to a plateau mean of 26.9 μg%. This elevation of plasma cortisol peaked at 33.4 μg% within 30 min after the start of the infusion and remained significantly greater than both the pre-infusion control values as well as the time controls throughout the experiment (Fig. 5). Forty-eight hours of dehydration resulted in basal plasma ADH levels between 7.28 and 7.83 μU/ml with correspondingly high plasma osmolalities between 315 and 317 mOsm/kg H_2O (Fig. 6). As can be seen, plasma ADH did not change during the 2-hr i.v. cortisol infusion (Fig. 6). Plasma osmolality declined slightly with time ($P<0.05$) in both groups. Plasma sodium and plasma aldosterone concentrations remained constant (Table 4). The plasma potassium concentration and hematocrit, however, decreased significantly after infusion of either vehicle or cortisol (Table 4); this is probably due to blood sampling and subsequent replacement with saline. Both mean arterial blood pressure (MABP) and heart rate (HR) remained virtually unchanged throughout the experiment.

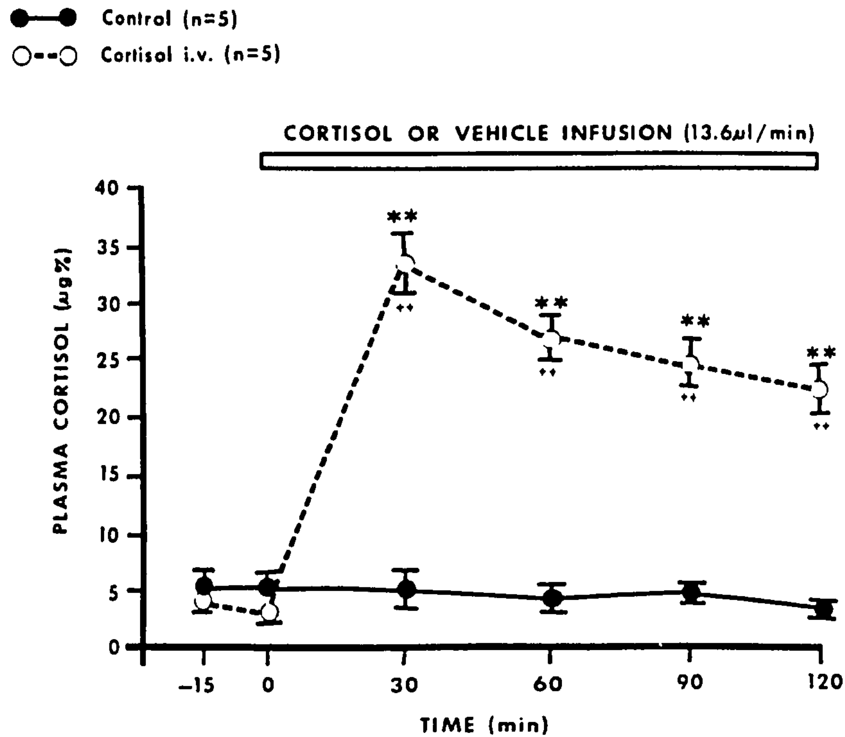


Figure 5: Effect of i.v. cortisol infusion on plasma cortisol concentration during the dehydration protocols of Series I. Vertical lines indicate ± SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

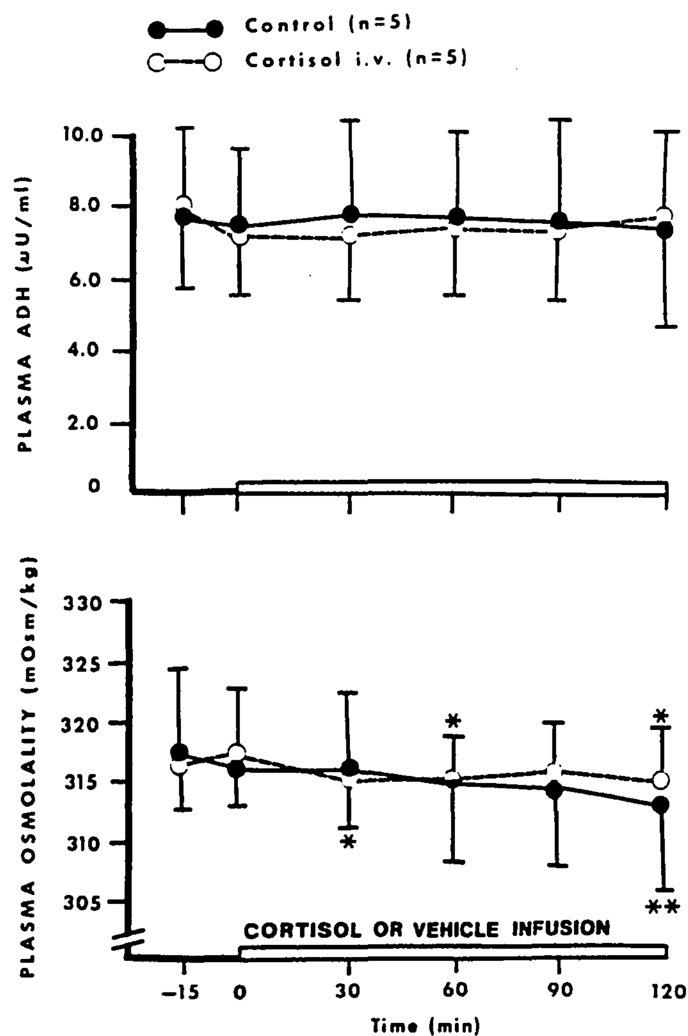


Figure 6: Effect of i.v. cortisol infusion on plasma ADH concentration and plasma osmolality during the dehydration protocols of Series I. Vertical lines indicate ±SEM. Significantly different from initial values at time 0, * $P<0.05$, ** $P<0.01$. No significant difference between groups.

Table 4: Plasma sodium, potassium, and aldosterone concentrations, mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct) in time control experiments (V) and during i.v. infusion of cortisol (C) in the dehydration protocols of Series I. Mean ± SEM. (n=5).

		TIME (min)					
		-15	0	30	60	90	120
plasma sodium	V	156 ± 3	156 ± 3	155 ± 3	156 ± 3	156 ± 3	156 ± 3
(mEq/l)	C	157 ± 4	157 ± 4	156 ± 3	156 ± 3	157 ± 4	155 ± 3
plasma potassium	V	4.3 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.1 ± 0.1**	4.0 ± 0.2**	3.9 ± 0.2**
(mEq/l)	C	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1*	4.1 ± 0.1**	4.0 ± 0.1**
plasma aldosterone	V	72 ± 6	69 ± 6	60 ± 7	57 ± 9	55 ± 7	47 ± 7
(pg/ml)	C	70 ± 13	60 ± 8	77 ± 9	68 ± 11	63 ± 10	68 ± 10
MABP	V	100 ± 7	99 ± 7	99 ± 7	95 ± 5	102 ± 9	100 ± 6
(mm Hg)	C	97 ± 5	95 ± 4	93 ± 6	94 ± 5	94 ± 3	95 ± 4
HR	V	48 ± 3	50 ± 4	47 ± 3	47 ± 3	51 ± 5	47 ± 4
(beats/min)	C	45 ± 4	47 ± 5	47 ± 5	46 ± 6	46 ± 5	46 ± 4
Hct	V	42 ± 3	42 ± 3	41 ± 3*	39 ± 3**	40 ± 3**	39 ± 3**
(% pkd cells)	C	43 ± 3*	41 ± 3	42 ± 3	41 ± 3	40 ± 3	40 ± 3*

* P<0.05, ** P<0.01 when compared to time 0 min values.

The ivt administration of cortisol or vehicle during dehydration resulted in a slight, statistically insignificant, decline in plasma cortisol concentration from pre-infusion values of about 4.0 μg% to 2.5 μg% at the 90-min sample (Fig. 7). However, the data show no significant differences over time or between groups. Similarly, plasma ADH concentration and plasma osmolality remained constant in both groups with averages of 5.70 μU/ml and 318 mOsm/kg H_2O, respectively (Fig. 8). The central infusion of cortisol did not change plasma ADH levels during the 2-hr experimental time course. Plasma sodium and plasma aldosterone concentrations, and MABP and HR showed no significant changes (Table 5). Again, plasma potassium concentration and hematocrit decreased significantly independent of vehicle or cortisol infusion (Table 5).

Series II

The response of ADH to an acute osmotic load during central and peripheral cortisol administration.

The administration of cortisol or vehicle was begun 75 min prior to the hypertonic (5%) saline infusion. The plasma cortisol concentration during i.v. cortisol infusion increased significantly ($P<0.01$) and remained elevated throughout the experiment (Fig.9). The pre-infusion mean value of 2.63 μg% increased to a mean plateau of 17.2 μg%. Hypertonic saline infusion caused a significant increase ($P<0.05$) in plasma osmolality in the control experiment within 15 min after it was started. In contrast, during the i.v. cortisol infusion, the same osmotic load did not cause a significant increase in plasma

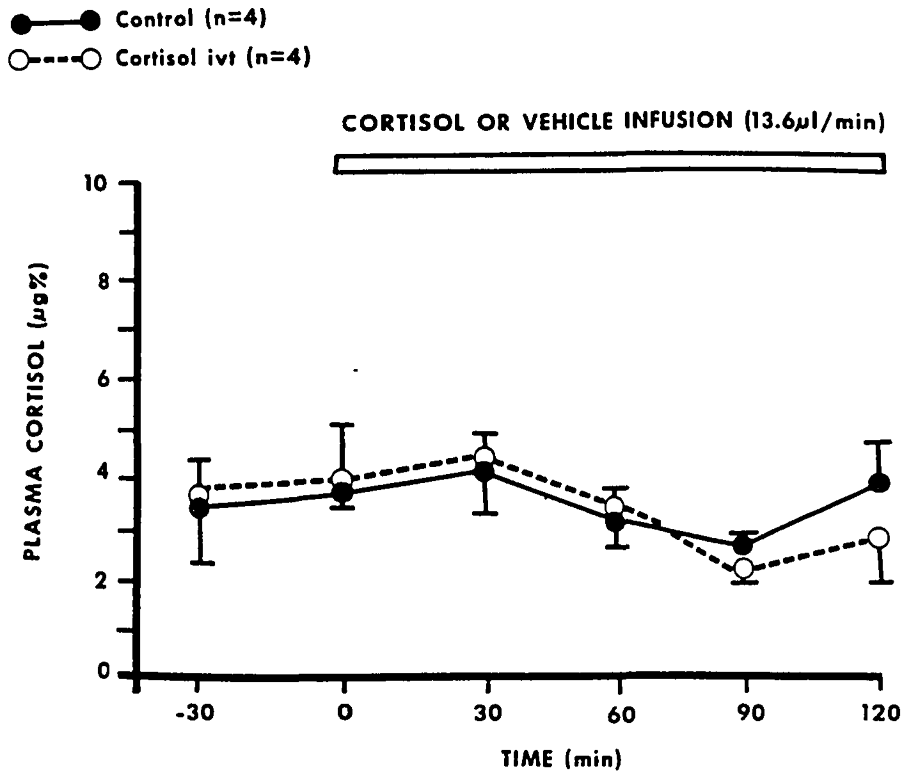


Figure 7: Effect of ivt cortisol infusion on plasma cortisol concentration during the dehydration protocols of Series I. Vertical lines indicate ±SEM. No significant differences were seen within or between groups.

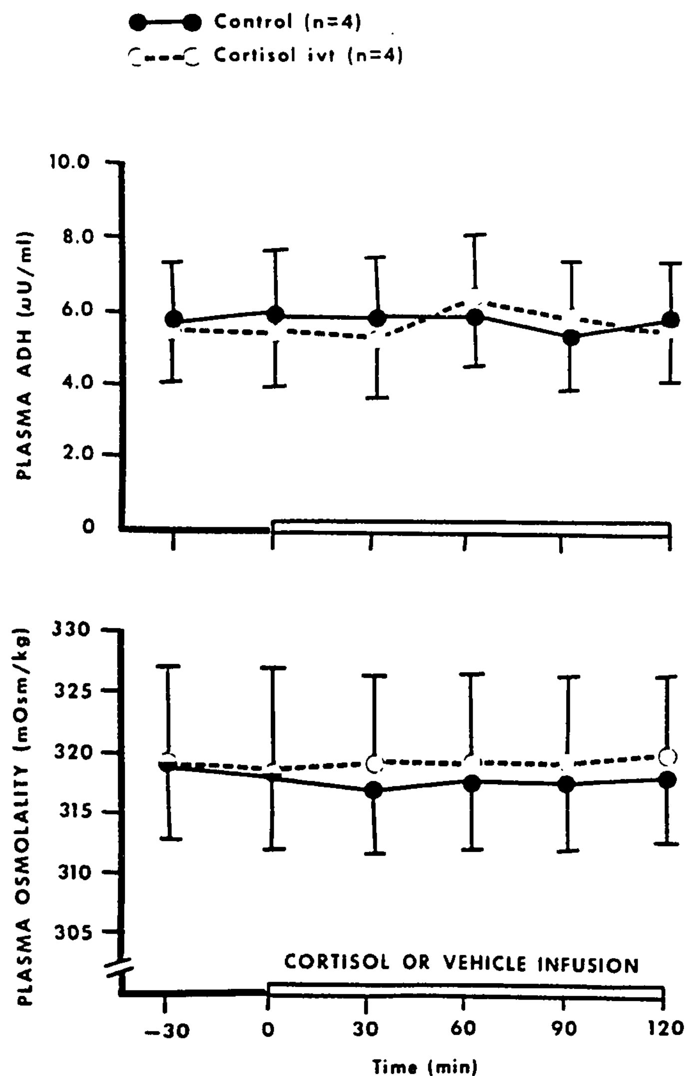


Figure 8: Effect of ivt cortisol infusion on plasma ADH concentration and plasma osmolality during the dehydration protocols of Series I. Vertical lines indicate ± SEM. No significant differences were seen within or between groups.

Table 5: Plasma sodium, potassium, and aldosterone concentrations, mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct) following ivt infusions of artificial CSF (V) or cortisol (C) in the dehydration protocols of Series I. Mean ± SEM. (n=4).

		TIME (min)					
		-15	0	30	60	90	120
plasma sodium	V	163 ± 3	164 ± 2	163 ± 3	163 ± 4	163 ± 2	163 ± 3
(mEq/l)	C	167 ± 4	167 ± 4	165 ± 2	166 ± 4	168 ± 4	164 ± 3
plasma potassium	V	4.7 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.4 ± 0.1*	4.2 ± 0.1**	4.1 ± 0.1**
(mEq/l)	C	4.6 ± 0.2	4.6 ± 0.1	4.4 ± 0.1	4.4 ± 0.1*	4.2 ± 0.2**	4.0 ± 0.1**
plasma aldosterone	V	94 ± 15	96 ± 12	95 ± 10	73 ± 10	103 ± 13	91 ± 18
(pg/ml)	C	107 ± 24	103 ± 19	116 ± 33	105 ± 28	90 ± 23	104 ± 24
MABP	V	92 ± 4	94 ± 4	97 ± 6	98 ± 6	95 ± 6	94 ± 4
(mm Hg)	C	88 ± 2	89 ± 4	91 ± 7	92 ± 6	90 ± 6	90 ± 5
HR	V	53 ± 5	54 ± 7	56 ± 9	59 ± 7	56 ± 7	57 ± 8
(beats/min)	C	42 ± 4 +	43 ± 4 +	45 ± 6 +	44 ± 5 +	51 ± 8	50 ± 8
Hct	V	43 ± 3	43 ± 3	42 ± 3	42 ± 3	41 ± 3**	40 ± 3**
(% pkd cells)	C	41 ± 1++	41 ± 1++	40 ± 1++	39 ± 1** ++	39 ± 1** ++	38 ± 1** ++

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

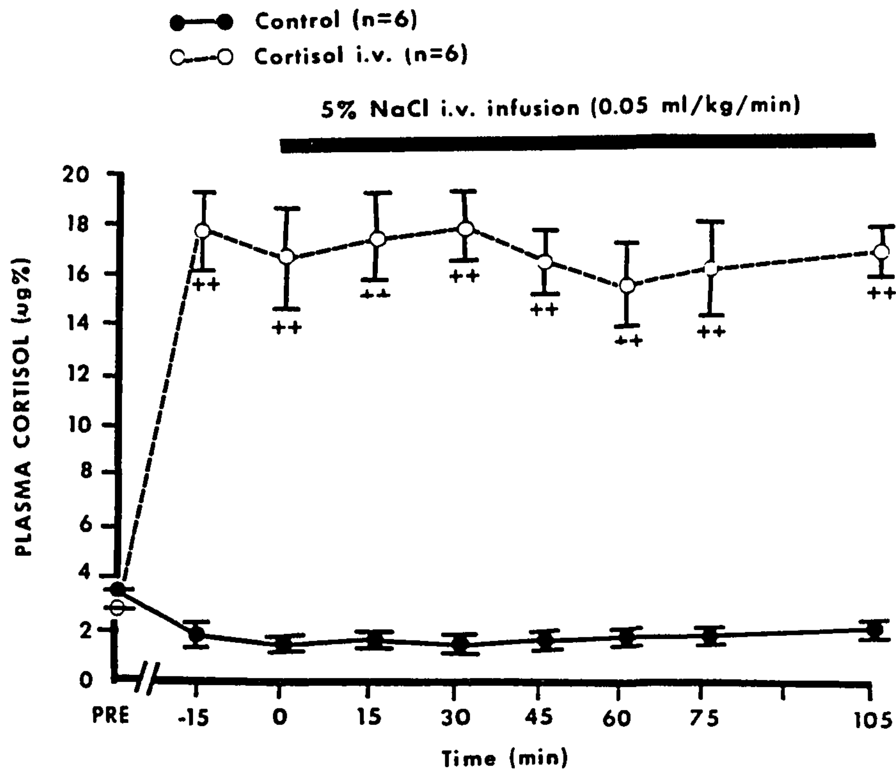


Figure 9: Effect of i.v. cortisol administration on plasma cortisol concentration during i.v. hypertonic (5%) NaCl infusion (Series II). Vertical lines indicate ±SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

osmolality ($P<0.01$) until 45 min after the onset of hypertonic saline infusion (Fig. 10). This delay resulted in differences in plasma osmolality between the two experiments throughout the first 75 minutes of the protocol. However, plasma osmolality increased from control values of 298 to about 308 mOsm/kg H_2O in both experiments. The plasma ADH concentration changes followed a similar pattern. In the control experiment, plasma ADH increased significantly ($P<0.01$) at 45 min, whereas in the i.v. cortisol experiment plasma ADH did not increase significantly ($P<0.05$) until 75 min after the onset of hypertonic saline infusion (Fig. 10). The difference between plasma ADH concentrations in the two experiments was significant at 60 min ($P<0.05$) and 75 min ($P<0.01$). The increase in plasma ADH concentration in the control experiment was from 1.31 to 3.11 µU/ml, and 1.18 to 2.97 µU/ml in the i.v. cortisol experiment.

Linear regression analysis indicated a positive correlation between plasma ADH concentration and plasma osmolality during the hypertonic saline infusion in both control and i.v. cortisol experiments (Table 6, Fig. 11). The lines generated for each experiment were obtained using plasma osmolalities that were associated with an increase of at least 20% in plasma ADH concentration from the previous sample. This allowed for the elimination of the flat portion of this relationship at the lower plasma osmolality values, a characteristic of the osmotic control of ADH release. The regression line describing this relationship in the control experiment,

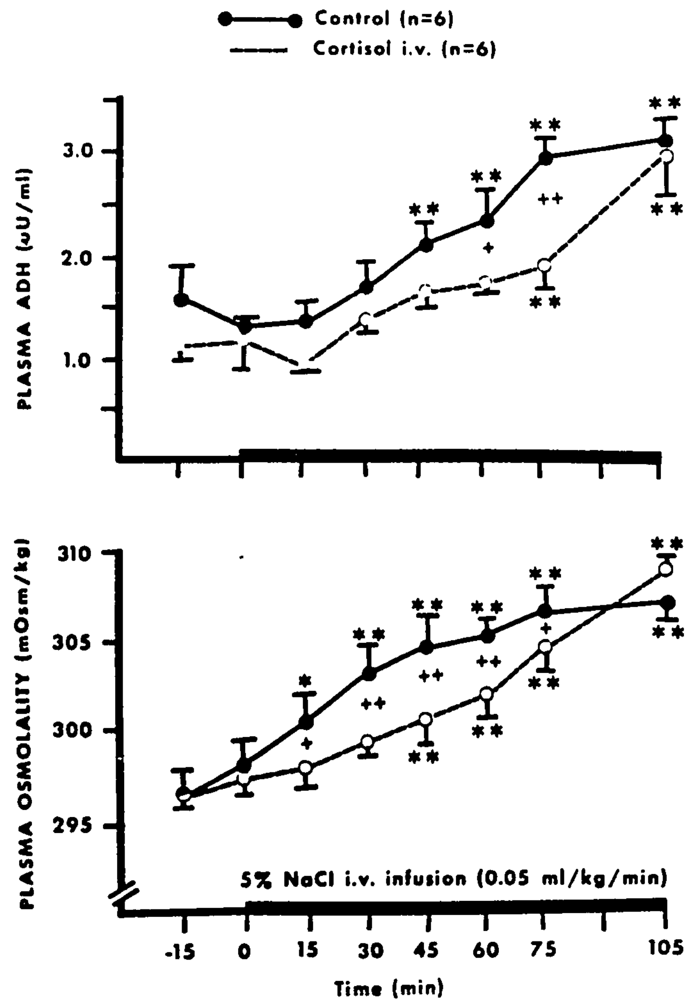


Figure 10: Changes with time in plasma ADH concentration and plasma osmolality during i.v. hypertonic (5%) NaCl infusion with and without i.v. cortisol administration (Series II). Vertical lines indicate ± SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

Table 6: The intercepts (mOsm/kg H_2O), slopes (μU/ml ADH)/(mOsm/kg H_2O) and correlation coefficients (r) determined by linear regression analysis within individual dogs for plasma ADH concentration and plasma osmolality during hypertonic saline infusion with and without i.v. cortisol administration (Series II).

DOG	X-intercept		Slope		Correlation (r)	
	Control	Cortisol i.v.	Control	Cortisol i.v.	Control	Cortisol i.v.
Hawkeye	300.4	298.6	0.473	0.330	0.967	0.981
Happy	293.1	290.4	0.174	0.111	0.680	0.812
Tabby	300.2	282.9	0.476	0.078	0.776	0.806
Betty	290.8	289.9	0.131	0.159	0.936	0.751
Gertrude	299.4	288.3	0.287	0.120	0.769	0.833
Maria	289.2	284.9	0.233	0.124	0.839	0.883
Mean	295.5	289.2	0.296	0.154		
±SEM	2.1	2.2	0.060	0.034		

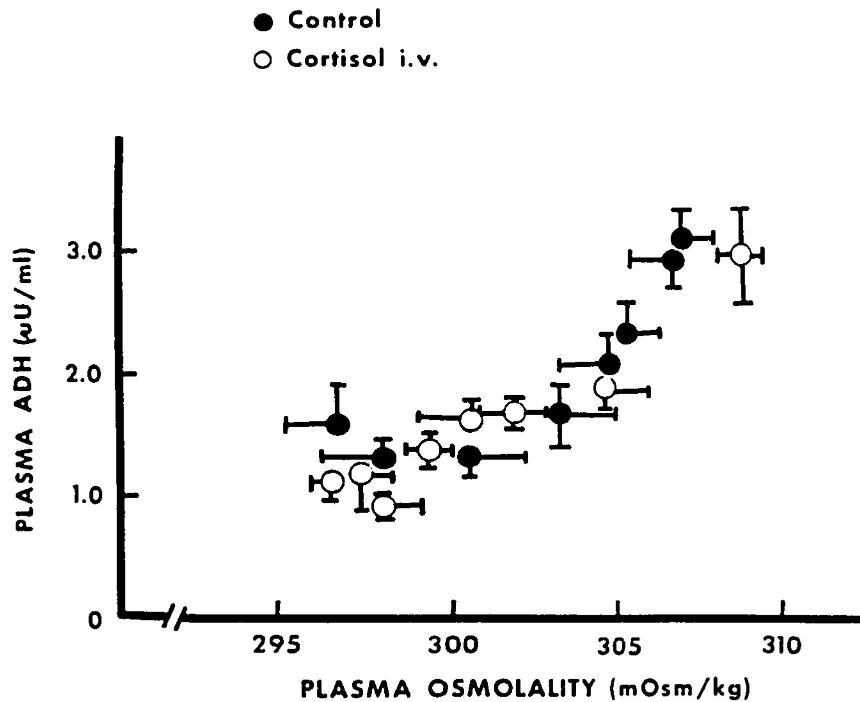


Figure 11: Relationship between plasma osmolality (mOsm/kg H_2O) and plasma ADH concentration (μU/ml) with (○) and without (●) i.v. cortisol administration in normally hydrated dogs (Series II). The points represent mean osmolalities versus mean plasma ADH concentrations at each blood sampling time period.

pADH = 0.296(pOSM - 295.5), was compared to that obtained for the i.v. cortisol experiment, pADH = 0.154(pOSM - 289.2), using a paired t-test. No statisically significant difference was seen between the slopes or x-intercepts of these lines. Caution must be exercised, however, when interpreting calculated x-intercepts. These values lie beyond the measured values and assume a linear relationship down to the osmotic threshold. Because of the splay that exists (see Discussion), calculations of the intercepts cannot be considered accurate determinations of the osmotic threshold. Although the average of the slopes for the i.v. cortisol experiment was less than that of the control experiment, suggesting a decrease in sensivitity, it was not statistically significant.

Hypertonic saline infusion caused a significant rise ($P<0.01$) in the plasma sodium concentration, a fall in the plasma aldosterone concentration ($P<0.05$), as well as a fall in the plasma potassium concentration ($P<0.01$) and hematocrit ($P<0.01$), (independent of vehicle or cortisol infusion experiment) (Table 7). In addition, HR decreased ($P<0.05$) while MABP remained unchanged (Table 7).

Centrally administered cortisol (ivt) resulted in a progressive decrease in plasma cortisol concentration. This finding has not previously been demonstrated and is most likely due to a central negative feedback effect of cortisol on ACTH (see Discussion). The plasma cortisol concentration in the ivt cortisol experiment after 75 min of infusion was at a mean value of 1.42 µg% when the hypertonic saline infusion was begun, and decreased significantly ($P<0.01$) to near

Table 7: Effect of vehicle (V) and i.v. cortisol (C) administration during i.v. hypertonic (5%) NaCl infusion on plasma sodium (pNa+), plasma potassium (pK+), and plasma aldosterone (pAldo) concentrations, mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct). (Series II) Mean ± SEM. (n=6)

		TIME (min)							
		-15	0	15	30	45	60	75	105
pNa+ (mEq/l)	V	148±2	149±2	151±2	152±1*	151±1	153±1**	155±1**	154±1**
	C	150±1	151±2	152±2	152±2	153±2*	154±2**	154±2**	158±2**
pK+ (mEq/l)	V	4.4±0.1	4.4±0.1	4.3±0.1	4.3±0.1	4.2±0.1*	4.2±0.1**	4.2±0.1**	4.2±0.1**
	C	4.6±0.1	4.4±0.1	4.3±0.1*	4.2±0.1** +	4.1±0.1**	4.2±0.1**	4.0±0.1** +	4.1±0.0**
pAldo (pg/ml)	V	30±8	34±9	28±6	26±5	24±5	22±4*	26±4	22±3*
	C	52±2**	38±5	37±2	40±5 ++	39±3 ++	36±5 ++	33±4	40±5**
MABP (mm Hg)	V	98±4	96±4	95±3	94±3	93±3	98±4	97±3	100±4
	C	94±2	95±3	94±2	90±3	91±3	94±3	93±3	97±2
HR (beats/min)	V	51±4	50±4	47±3	46±4	39±2**	42±3*	43±2	41±1**
	C	50±6	46±4	49±6	43±5	43±3	44±4	44±4	39±3*
HCT (% pkd cells)	V	38±1	37±1	35±1**	34±1**	33±1**	32±1*	31±1**	31±1**
	C	39±1	38±0.5	37±0.3**	35±0.5** ++	34±0.4**	34±0.5** ++	32±0.5**	31±0.5**

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

non-detectable levels of 0.24 μg% by the end of the experiment (Fig. 12). In the control experiment, plasma cortisol concentration increased slightly at time 30 and 45 min but remained otherwise unchanged with a mean value of 2.0 μg%. This resulted in significant differences ($P<0.01$) between the ivt cortisol experiment and the control experiment, beginning at time 15 min and continuing throughout. The plasma osmolalities, on the other hand, increased similarly in both experiments in response to hypertonic saline infusion (Fig. 13). This increase was significant ($P<0.05$) in both experiments within 15 min after the onset of hypertonic saline infusion. Plasma osmolality increased from a starting mean of 295 mOsm/kg H_2O to a mean of 306 mOsm/kg H_2O at the end of the protocol. The plasma ADH concentration changes were also similar in the two experiments with a significant increase ($P<0.01$) detected after 75 min of hypertonic saline infusion (Fig. 13). No statistically significant differences were seen in plasma ADH concentration between the two experiments. Hypertonic saline infusion increased the plasma ADH concentration in the control experiment from 0.99 to 1.98 μU/ml, and in the ivt cortisol experiment these values increased from 1.21 to 2.40 μU/ml over the time course of the experiment.

The linear correlation between plasma ADH concentration and plasma osmolality during hypertonic saline infusion in the ivt cortisol experiment is shown in Table 8 and illustrated in Figure 14. The regression line for the control experiment is:

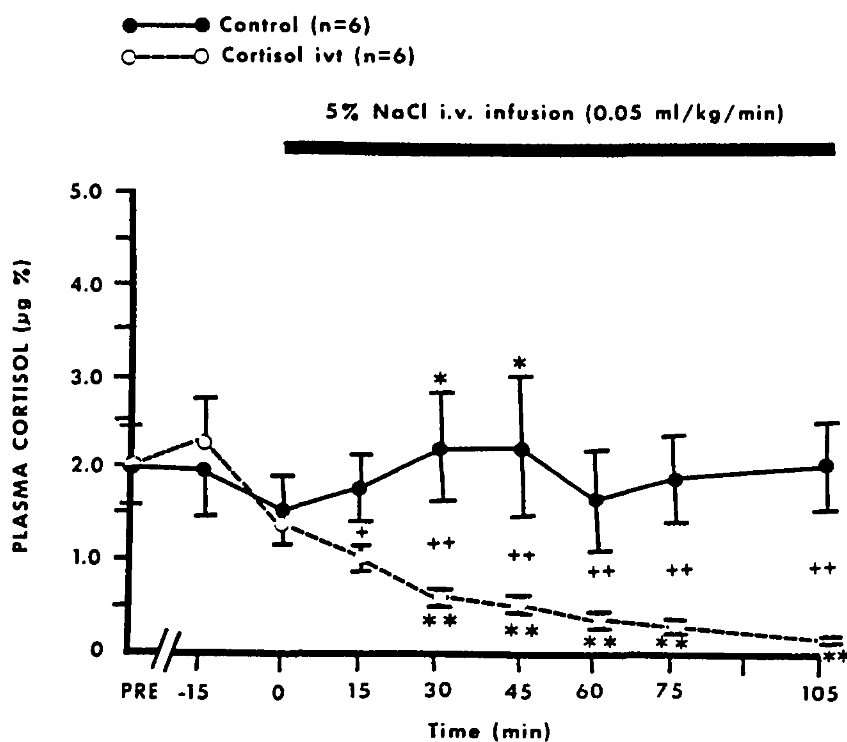


Figure 12: Effect of ivt cortisol administration on plasma cortisol concentration during i.v. hypertonic (5%) NaCl infusion (Series II). Vertical lines indicate ±SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

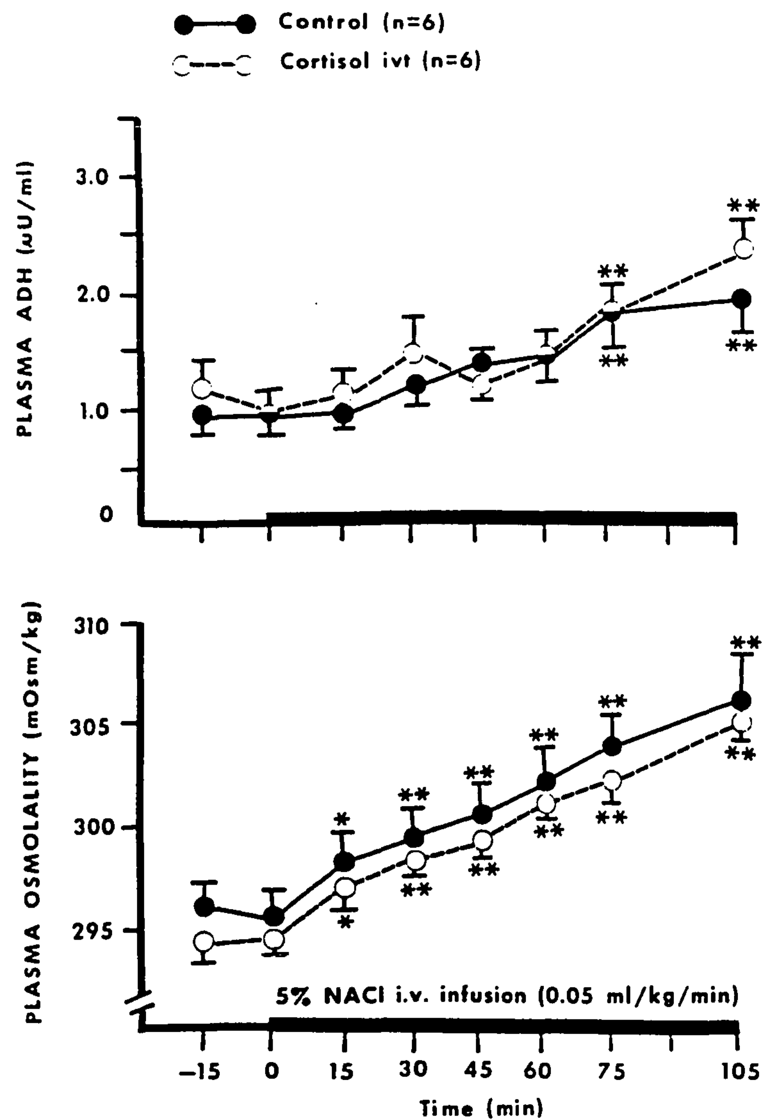


Figure 13: Changes with time in plasma ADH concentration and plasma osmolality during i.v. hypertonic (5%) NaCl infusion with and without ivt cortisol administration (Series II). Vertical lines indicate ±SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

Table 8: The intercepts (mOsm/kg H_2O), slopes (μU ADH/ml)/(mOsm/kg H_2O) and correlation coefficients (r) determined by linear regression analysis within individual dogs for plasma ADH concentration and plasma osmolality during hypertonic saline infusion with and without ivt cortisol administration (Series II).

	X-intercept		Slope		Correlation (r)	
DOG	Control	Cortisol ivt	Control	Cortisol ivt	Control	Cortisol ivt
Quincy	286.4	276.4	0.101	0.077	0.987	0.700
Goblin	288.3	290.0	0.150	0.188	0.866	0.747
Patches	261.9	254.4	0.024	0.033	0.594	0.177
Pam	279.9	291.6	0.055	0.190	0.450	0.953
Nick	296.9	292.6	0.187	0.208	0.919	0.794
Shaggy	289.2	300.9	0.243	0.211	0.927	0.914
Mean	283.8	284.3	0.127	0.151		
±SEM	4.9	6.8	0.034	0.062		

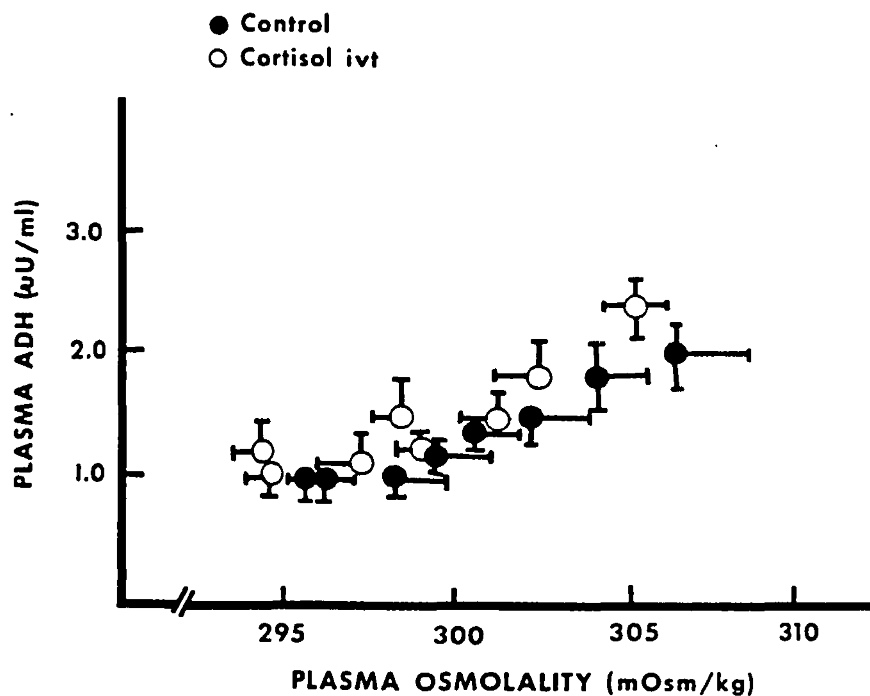


Figure 14: Relationship between plasma osmolality (mOsm/kg H_2O) and plasma ADH concentration (µU/ml) with (O) and without (●) ivt cortisol administration in normally hydrated dogs (Series II). The points represent mean osmolalities versus mean plasma ADH concentrations at each blood sampling time period.

pADH = 0.127(pOSM - 283.8) and is: pADH = 0.151(pOSM - 284.3) for the ivt cortisol experiment. Again, these lines were calculated using plasma osmolalities associated with at least a 20% increase in plasma ADH concentration from the previous sample. There was no statistically significant difference between these two lines (paired t-test), with the slopes and x-intercepts being similar.

The plasma sodium concentration significantly increased ($P<0.01$) during the hypertonic saline infusion (Table 9). Also, plasma aldosterone concentration decreased slightly with respect to time but not significantly, whereas the plasma potassium concentration and hematocrit decreased significantly ($P<0.01$). MABP and HR remained unchanged (Table 9).

Series III

Effects of centrally and peripherally administered cortisol on the release of ADH without influence of an osmotic challenge.

Aside from serving as time controls for the hypertonic saline infusion experiments, the infusion of isotonic saline with and without cortisol provides information about the effect of cortisol on the release of ADH in the absence of any osmotic stimuli.

The i.v. infusion of cortisol in this series of experiments (Fig. 15) resulted in the same pattern of plasma cortisol concentration as seen in the hypertonic saline infusion series (Fig. 9). Plasma cortisol concentration increased significantly ($P<0.01$) from a pre-infusion control mean of 2.53 μg% to a mean plateau of 15.4 μg% (Fig. 15). The i.v. infusion of cortisol with isotonic saline had no

Table 9: Effect of vehicle (V) and ivt cortisol (C) administration during i.v. hypertonic (5%) NaCl infusion on plasma sodium (pNa+), plasma potassium (pK+), and plasma aldosterone (pAldo) concentrations, mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct). (Series II) Mean ± SEM. (n=6)

		TIME (min)							
		-15	0	15	30	45	60	75	105
pNa+ (mEq/l)	V	149±2	146±3	148±2	149±3*	150±2*	152±2**	153±2**	155±2**
	C	146±1	147±1	148±1	150±1**	150±1**	150±1**+	152±1**	152±1**+
pK+ (mEq/l)	V	4.5±0.1	4.4±0.1	4.4±0.1	4.4±0.1	4.3±0.1	4.4±0.1	4.2±0.1*	4.2±0.2*
	C	4.5±0.1	4.4±0.1	4.4±0.1	4.4±0.1	4.3±0.1*	4.2±0.0**	4.2±0.1*	4.1±0.1**
pAldo (pg/ml)	V	38±11	38±12	33±10	34±10	33±11	33±9	33±12	32±12
	C	34±13	38±13	40±18	32±17	29±12	32±17	30±13	26±10
MABP (mm Hg)	V	94±4	94±3	92±2	93±4	94±3	92±2	90±3	92±2
	C	92±5	89±4	90±4	90±3	91±2	92±4	92±3	93±2
HR (beats/min)	V	40±3	40±5	40±4	39±4	39±3	39±3	40±4	39±3
	C	40±3	37±2	36±2	35±2	36±2	37±3	36±2	37±2
HCT (% pkd cells)	V	38±1	38±1	37±1	36±1**	36±1**	35±1**	34±1**	33±1**
	C	37±1++	36±1++	35±1++	35±1**++	34±1**++	33±1**++	33±1**++	33±1**

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

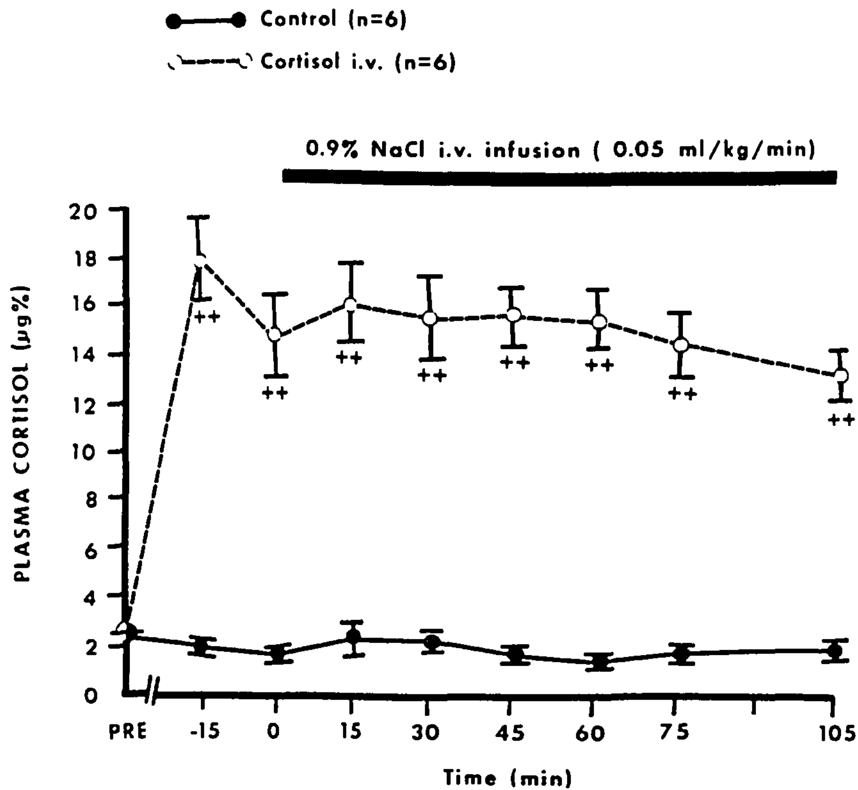


Figure 15: Effect of i.v. cortisol administration on plasma cortisol concentration during normal saline infusion (Series III). Vertical lines indicate ± SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

significant effects on either plasma osmolality or the plasma ADH concentration (Fig. 16). Similarly, the plasma sodium concentration and plasma aldosterone concentration did not change significantly, but the plasma potassium concentration and hematocrit fell significantly (Table 10). No changes were seen in MABP and HR (Table 10).

The ivt infusion of cortisol was associated with changes in plasma cortisol concentration similar to those seen before (Figs. 17 & 12, respectively). Initial plasma levels of a mean of 1.43 μg% progressively decreased ($P<0.01$) to a mean of 0.40 μg% at the end of the experiment. No effect was seen on plasma osmolality or plasma ADH concentration during ivt cortisol infusion (Fig. 18). Plasma sodium and aldosterone concentrations, MABP and HR were unchanged but plasma potassium concentration and hematocrit fell (Table 11).

CSF Cortisol Concentrations

For each protocol in Series I, II, and III, in both the i.v.-group and ivt-group (with one exception being the dehydration protocols) a CSF sample was taken at the end of the experiment from at least one dog. The results comparing CSF cortisol concentrations with corresponding plasma cortisol concentrations are shown in Table 12. In all cases, the control experiments were without detectable cortisol levels in the CSF. Although small amounts were detected in the CSF during i.v. cortisol infusion, the levels in plasma were near 10-fold greater. On the other hand, ivt cortisol administration yielded CSF cortisol concentrations of about 23.0 μg% with corresponding plasma levels too low to detect. Also, since plasma aldosterone concentration

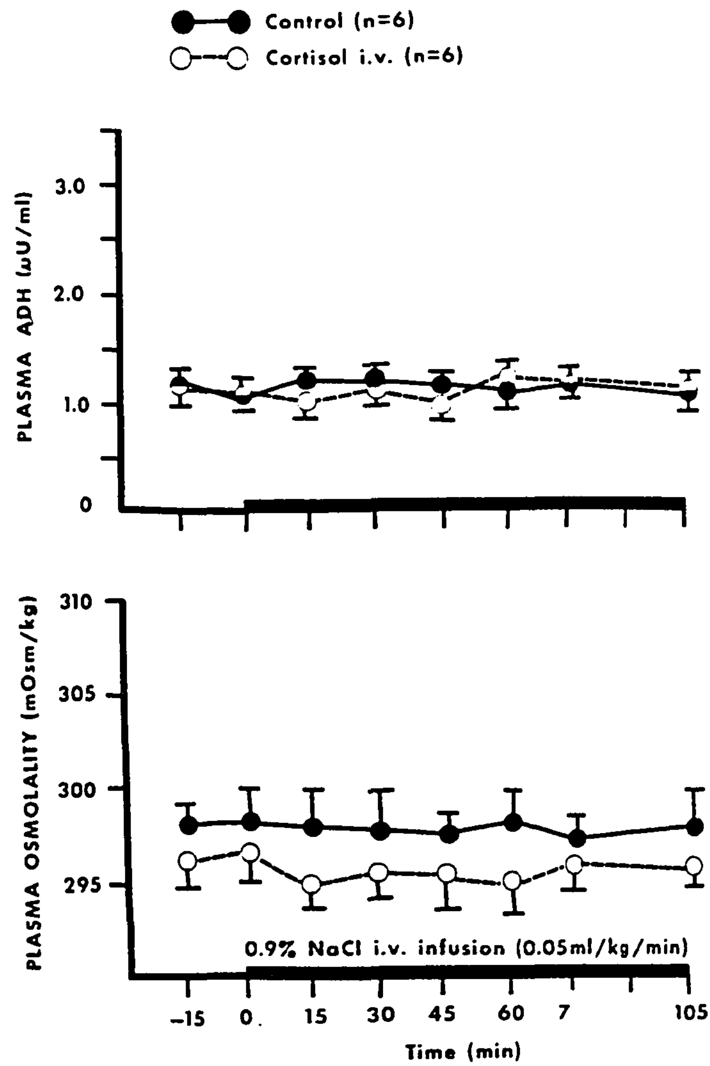


Figure 16: Plasma ADH concentration and plasma osmolality following i.v. cortisol administration during the normal saline infusion protocols of Series III. Vertical lines indicate ±SEM. No significant differences were seen within or between groups.

Table 10: Effect of vehicle (V) and i.v. cortisol (C) administration during normal saline (0.9% NaCl) infusion on plasma sodium (pNa+), plasma potassium (pK+), and plasma aldosterone (pAldo) concentrations, mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct). (Series III) Mean ± SEM. (n=6)

		TIME (min)							
		-15	0	15	30	45	60	75	105
pNa+ (mEq/l)	V	148±2	149±2	148±2	148±2	148±2	148±2	148±2	148±2
	C	147±2	147±2	147±2	146±1	146±2	146±1	146±1	146±1
pK+ (mEq/l)	V	4.6±0.1	4.6±0.1	4.5±0.1	4.5±0.1	4.4±0.1	4.4±0.1**	4.3±0.1**	4.3±0.1**
	C	4.4±0.1	4.4±0.0	4.3±0.0+	4.2±0.1*+	4.2±0.1**++	4.2±0.1**	4.1±0.1**++	4.1±0.1**++
pAldo (pg/ml)	V	48±12	45±10	42±8	46±10	46±10	40±9	40±8	55±8
	C	52±7	47±6	40±2	46±3	39±4	41±4	34±3	38±7
MABP (mm Hg)	V	91±6	88±9	87±8	89±6	89±8	88±6	87±6	88±7
	C	87±4	87±4	91±4	91±5	88±4	89±4	87±3	86±3
HR (beats/min)	V	47±6	43±6	44±6	44±6	45±6	42±5	44±6	44±6
	C	44±5	46±6	45±6	44±7	42±4	41±5	40±4	40±4
HCT (% pkd cells)	V	37±2	36±2	35±2	35±2	35±2*	34±2**	34±2**	33±1**
	C	36±2	36±1	35±1	35±1	34±1**	34±1**	33±1**	32±1**

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

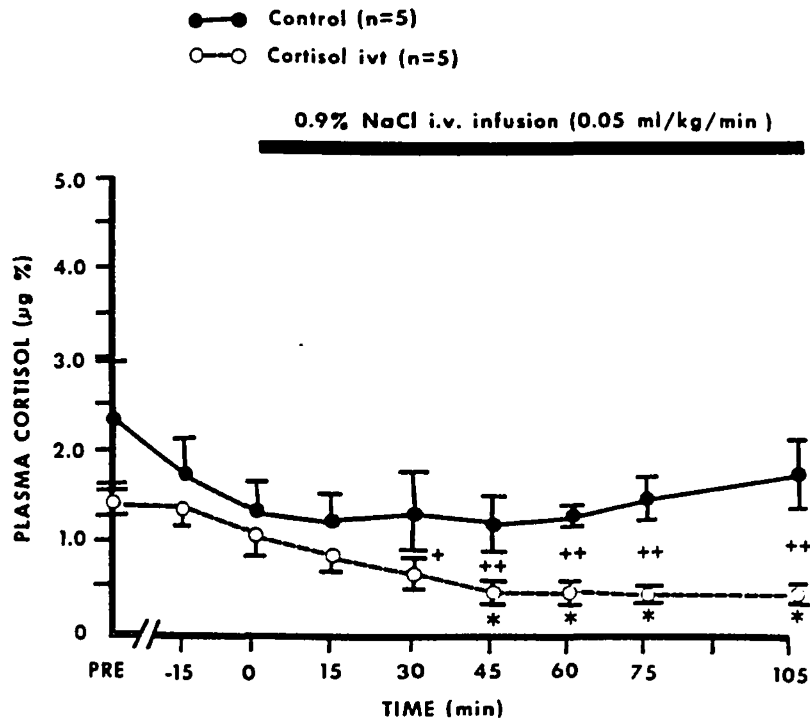


Figure 17: Effect of ivt cortisol administration on plasma cortisol concentration during normal saline infusion (Series III). Vertical lines indicate ± SEM. Statistically significant differences from initial observations within groups are shown by asterisks (*), differences between groups are shown by pluses (+). *,+ $P<0.05$; **,++ $P<0.01$.

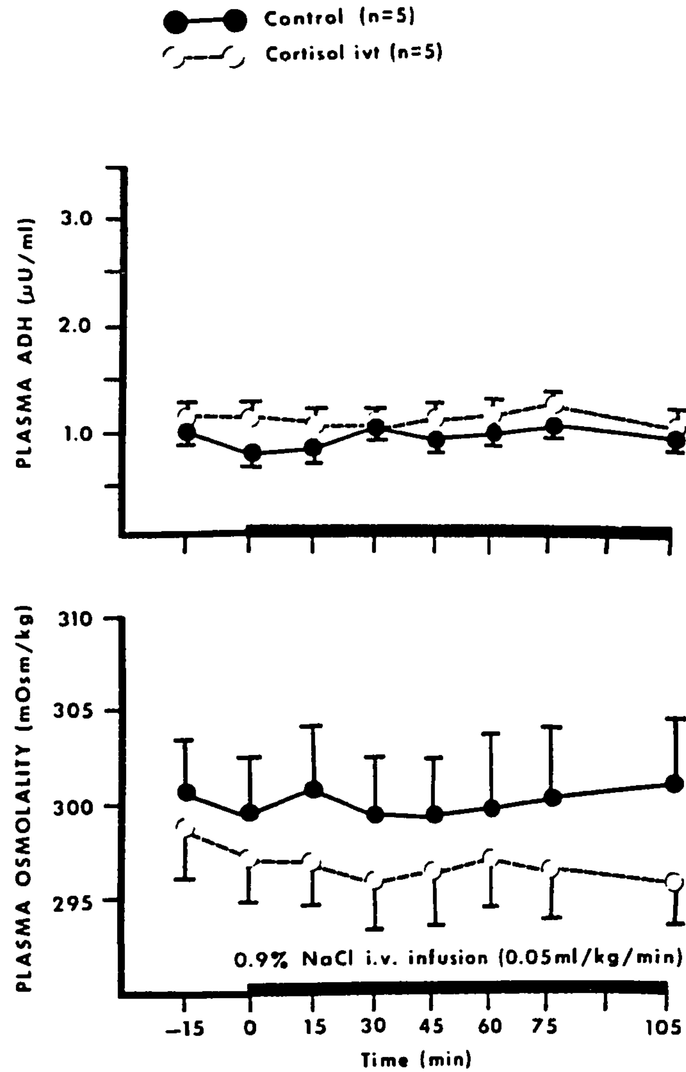


Figure 18: Plasma ADH concentration and plasma osmolality following ivt cortisol administration during the normal saline infusion protocols of Series III. Vertical lines indicate ± SEM. No significant differences were seen within or between groups.

Table 11: Effect of vehicle (V) and ivt cortisol (C) administration during normal saline (0.9% NaCl) infusion on plasma sodium (pNa+), plasma potassium (pK+), and plasma aldosterone (pAldo) concentrations, mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct). (Series III) Mean ± SEM. (n=5)

		TIME (min)							
		-15	0	15	30	45	60	75	105
pNa+ (mEq/l)	V	149±2	149±2	149±2	150±2	149±2	150±2	150±2	150±2
	C	144±1++	145±1++	145±1++	144±1++	143±2++	143±2++	143±1++	144±1++
pK+ (mEq/l)	V	4.6±0.1	4.5±0.1	4.5±0.1	4.4±0.1	4.4±0.1*	4.3±0.1**	4.3±0.1**	4.2±0.1**
	C	4.5±0.2	4.5±0.1	4.2±0.1	4.4±0.1	4.4±0.1	4.3±0.2**	4.2±0.2**	4.2±0.1**
pAldo (pg/ml)	V	66±13	51±10	53±12	53±14	46±11	45±11	50±12	48±10
	C	98±25	92±30	80±24	68±16	67±12	66±16	69±14	66±16
MABP (mm Hg)	V	93±4	95±5	96±5	97±6	96±5	97±6	94±5	95±5
	C	91±3	93±3	91±3	91±4	90±2	88±2	88±2	89±1
HR (beats/ min)	V	49±7	47±7	50±8	47±7	50±7	52±9	49±8	50±8
	C	37±7++	37±1++	35±2++	34±1++	32±1*++	34±1++	33±2++	33±1++
HCT (% pkd cells)	V	37±2	37±2	36±2	36±2*	36±1*	35±1**	35±1**	34±2**
	C	36±1++	36±1++	35±1++	34±1**++	34±1**++	33±1**++	33±1**++	33±1**++

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

Table 12: Comparison of CSF cortisol and corresponding plasma cortisol concentrations for Series I, II, and III.

Protocol Name	CSF Cortisol Concentration (μg%)	Plasma Cortisol Concentration (μg%)	
i.v. cortisol group			
Dehydration control	too low*	3.02	(n=2)
Dehydration & Cortisol i.v.	2.54	20.10	(n=1)
5% NaCl control	too low*	3.14	(n=2)
5% NaCl & Cortisol i.v.	1.82	16.60	(n=2)
0.9% NaCl control	too low*	1.58	(n=2)
0.9% NaCl & Cortisol i.v.	1.10	12.80	(n=3)
ivt cortisol group			
5% NaCl control	too low*	2.48	(n=2)
5% NaCl & Cortisol ivt	25.02	too low*	(n=1)
0.9% NaCl control	too low*	1.82	(n=2)
0.9% NaCl & Cortisol ivt	21.11	too low*	(n=1)

*Below minimum detectability of 0.3 μg%.

was unaffected, the results indicate that a model for selective plasma cortisol insufficiency may be obtained acutely with the ivt administration of cortisol.

Series IV

Renal function and ADH regulation in water loaded dogs during central and peripheral cortisol administration.

The effect of peripheral and central cortisol administration on the renal handling of a water load was investigated. The administration of cortisol was begun 90 min prior to the water load of 40 ml/kg B.Wt. The mean plasma cortisol concentrations achieved during the water loading protocols are shown in Fig. 19. Central (ivt) cortisol administration again produced a progressive decline in the plasma cortisol concentration to near the minimum detectability of the assay (see Figs. 12, 17 & 19). The pre-infusion mean of 4.8 μg% decreased approximately 10-fold to a mean of 0.45 μg% ($P<0.01$). Peripheral (i.v.) cortisol infusion elevated the plasma cortisol concentration from a mean of 2.50 μg% to a plateau mean of 12.0 μg% ($P<0.01$). The time control values remained constant at a mean of 2.0 μg%.

In all three experiments, the water load produced a marked decrease ($P<0.01$) in plasma osmolality (Fig. 20). The mean plasma osmolality of 301 mOsm/kg H_2O decreased to a minimum mean value of 283 mOsm/kg H_2O. There was no significant difference between experiments until 30 min after the water load was administered (t=60 min), at which time the i.v. cortisol experiment plasma osmolalities

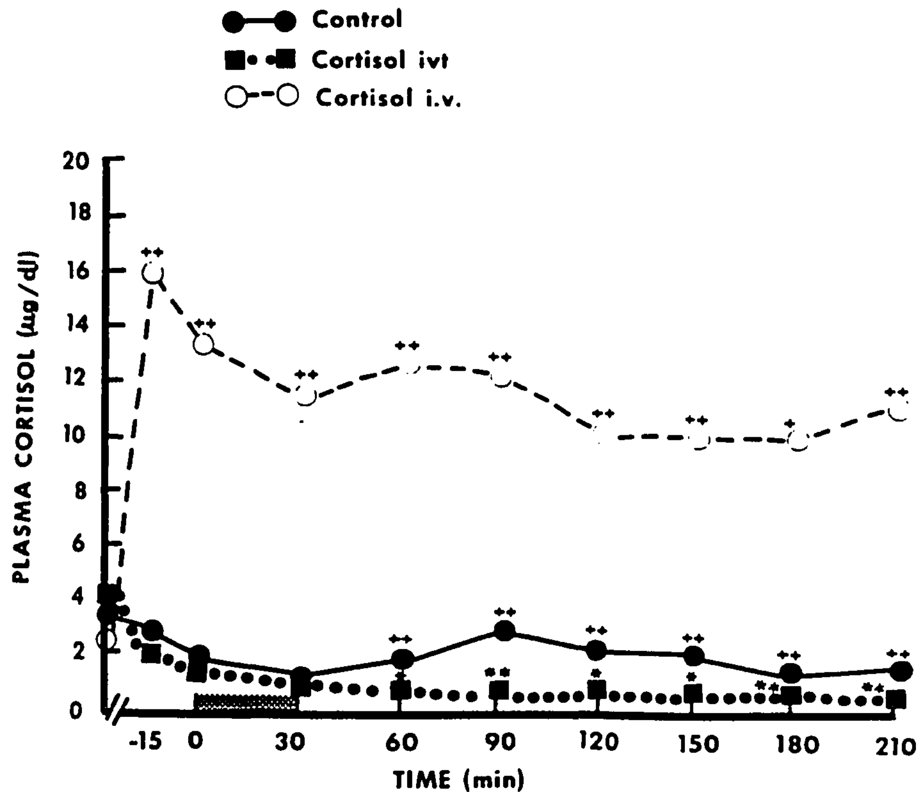


Figure 19: Time course of plasma cortisol concentration after a water load, during the control experiment (●), ivt cortisol (■), and i.v. cortisol (○) administration. The mean values and the results of statistical comparisons within and between groups are given. Asterisks (*) represent differences within each group, as compared to time zero. Pluses (+) represent differences between groups, as compared to the control experiment. *,+ $P<0.05$; **,++ $P<0.01$. (n=5)

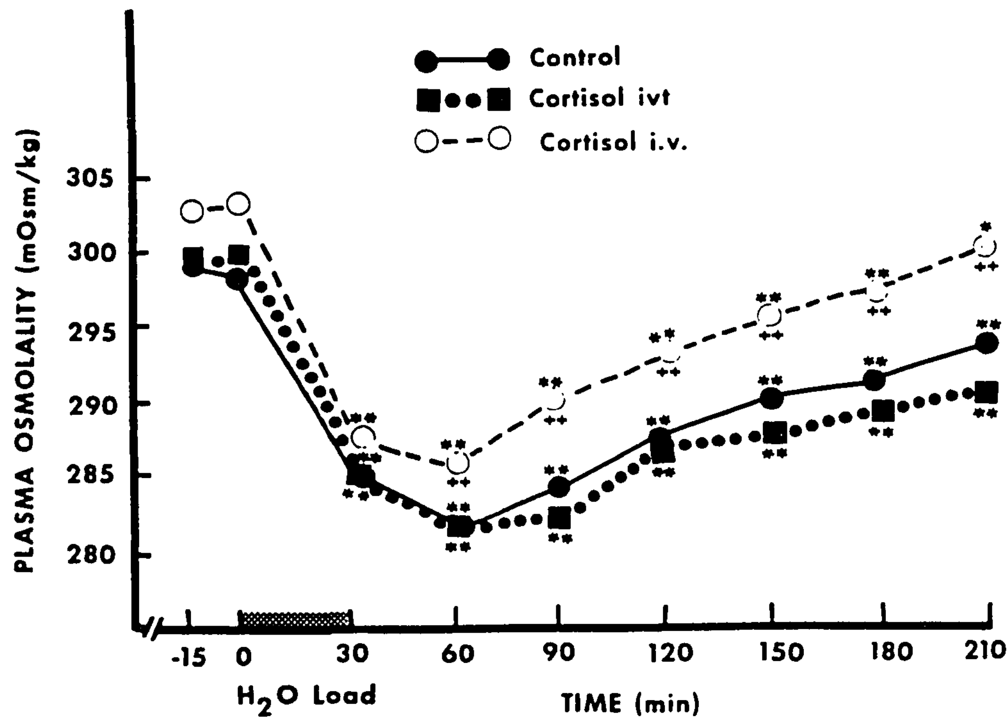


Figure 20: Time course of plasma osmolality after a water load, during the control experiment (●), ivt cortisol (■), and i.v. cortisol (○) administration. The mean values and the results of statistical comparisons within and between groups are given. Asterisks (*) represent differences within each group, as compared to time zero. Pluses (+) represent differences between groups, as compared to the control experiment. *,+ $P<0.05$; **,++ $P<0.01$. (n=5)

were significantly above ($P<0.01$) those of the control, and the plasma osmolalities of the ivt cortisol experiment were below those of the controls (n.s.). This difference persisted throughout the remainder of the protocol. In all cases, the plasma osmolality exhibited a progressive increase back towards basal values.

The plasma ADH concentration fell during all three experiments in response to the water load, but this was only significant ($P<0.05$) in the control experiment at t=60 min (Fig.21). A one-way analysis of variance on the pooled data, however, demonstrates a significant decrease ($P<0.05$) for periods 30 through 180 min. The plasma ADH concentration decreased approximately 50% in all experiments, from a mean of 1.0 μU/ml to 0.52 μU/ml ADH. There was no detectable difference in the response of plasma ADH concentration to an administered water load between any of the three experiments.

The linear regression analysis indicated a positive correlation between plasma ADH concentration and plasma osmolality during the water load experiments (Table 13 and Figure 22). The regression lines are as follows: for the control experiment, pADH = 0.028(pOSM - 272.4); for the ivt cortisol experiment, pADH = 0.025(pOsm - 242.1); and for the i.v. cortisol experiment, pADH = 0.017(pOSM - 229.1). Using an overall analysis of variance for slopes, no difference was seen between the slopes of these lines although the average of the slopes for the i.v. cortisol protocol was less than that of the control experiment.

The patterns in plasma sodium and potassium concentration, plasma proteins, and hematocrit resemble that seen in plasma osmolality

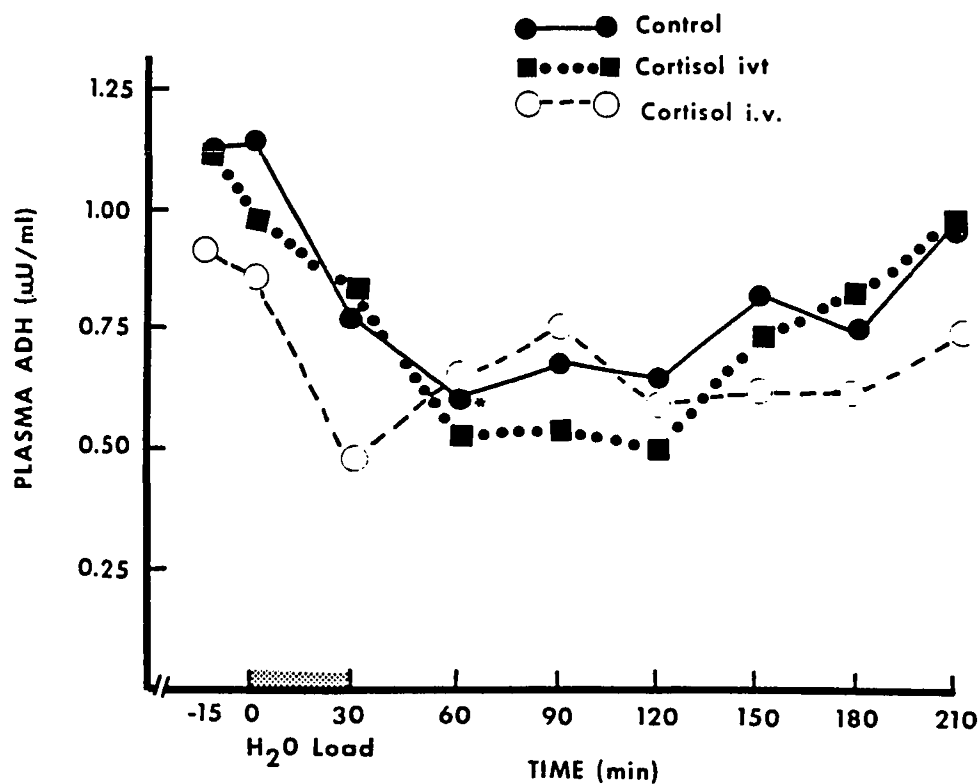


Figure 21: Time course of plasma ADH concentration after a water load, during the control experiment (●), ivt cortisol (■), and i.v. cortisol (O) administration. The mean values and the results of statistical comparisons within and between groups are given. There was no significant difference between groups. The control experiment demonstrated a significant ($P<0.05$) decrease at 60 min when compared to time zero. (n=5)

Table 13: The intercepts (mOsm/kg H_2O), slopes (μU ADH/ml)/(mOsm/kg H_2O) and correlation coefficients (r) determined by linear regression analysis within individual dogs for plasma ADH concentration and plasma osmolality during the water load experimental protocols of Series IV.

	X-intercept			Slope			Correlation (r)		
DOG	Control	ivt Cort	i.v. Cort	Control	ivt Cort	i.v. Cort	Control	ivt Cort	i.v. Cort
Nick	267.7	234.0	275.7	0.057	0.009	0.016	0.759	0.469	0.740
Joni	250.9	222.5	194.4	0.025	0.014	0.009	0.716	0.345	0.785
Scott	208.1	264.8	131.3	0.006	0.067	0.005	0.298	0.751	0.107
Betty	279.8	251.2	272.2	0.062	0.028	0.029	0.863	0.371	0.895
Sandy	355.4	238.0	272.6	-0.010	0.007	0.028	0.234	0.286	0.610
Mean	272.4	242.1	229.2	0.028	0.025	0.017			
±SEM	24.0	7.3	28.9	0.014	0.011	0.005			

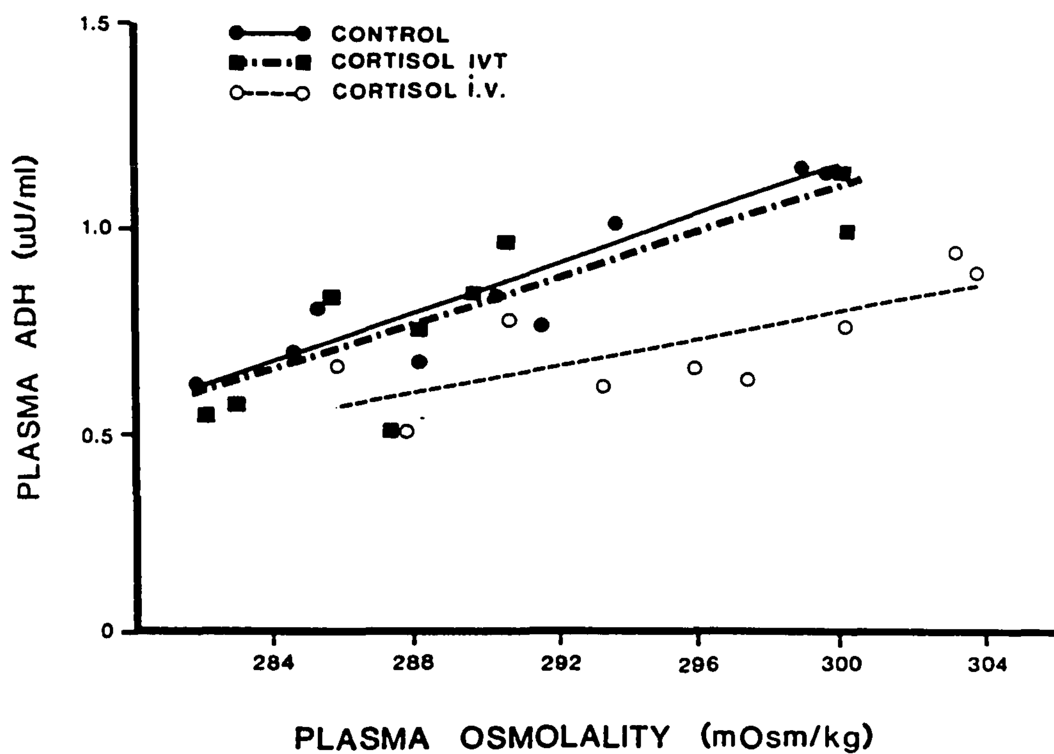


Figure 22: Relationship between plasma osmolality (mOsm/kg H_2O) and plasma ADH concentration (μU/ml) during the control (●), ivt cortsiol (■), and i.v. cortisol (O) administration experiments in the water load protocols of Series IV. The points represent mean plasma osmolalities versus mean plasma ADH concentrations at each blood sampling time period.

(Table 14). Although all values fell to a similar minimum, the i.v. cortisol experimental values remained above those of the control and ivt experiments throughout the rest of the protocol, and thus ended the experiments closest to the pre-water load control values. Plasma aldosterone concentration in the control and ivt experiments increased transiently, although not significantly, in response to the water load (Table 15). MABP and HR, in all cases, significantly increased after administration of the water load (t=30 min), but quickly returned towards basal values by 60 or 90 min (Table 15).

Effects of peripheral and central cortisol administration on renal water excretion and renal function: The water load of 40 ml/kg B.Wt. administered in each experiment resulted in significant increases in urine flow rate (Fig. 23). The i.v. cortisol infusion experiment resulted in a significant increase in urine flow rate from 0.52 ml/min to 2.68 ml/min at t=30 min, while the control and ivt cortisol experiments significantly increased from 0.62 ml/min to 3.16 ml/min at t=60 min. In fact, there was a significant difference between the i.v. cortisol experiment (6.84 ml/min) and the controls (4.5 ml/min) at t=90 min. This difference is reflected in the cumulative urine excreted during each experiment (Fig. 24). At the end of the 3-1/2 hr time course, the i.v. cortisol experimental animals excreted 99 ± 6% of the administered water load, in contrast to 82 ± 8% in the controls, and 70 ± 9% in the ivt cortisol experimental animals. A significant difference was detected when comparing the percent of excreted water in the i.v. cortisol infusion to the controls ($P<0.05$), and to the ivt

Table 14: Concentrations of plasma sodium (pNa+), plasma potassium (pK+), plasma proteins (PP) and hematocrit (Hct) during the water load experiments of Series IV. Mean ± SEM. (n=5)

		TIME (min)								
		-15	0	30	60	90	120	150	180	210
pNa+ (mEq/l)	con	147±2	146±2	133±1**	140±1**	141±1**	142±1**	143±1**	144±1*	145±1
	ivt	145±1	145±1	133±1**	137±1** ++	139±1**	141±1**	141±1** ++	142±1** +	142±2** ++
	i.v.	147±1	147±2	134±2**	140±2**	142±2**	144±2** +	145±2*	145±2	146±2
pk+ (mEq/l)	con	4.3±0.2	4.3±0.2	3.9±0.2**	4.0±0.2**	4.1±0.2**	4.1±0.1*	4.0±0.2**	4.0±0.1**	3.9±0.2**
	ivt	4.3±0.1	4.3±0.1	3.9±0.1**	4.0±0.1**	4.2±0.1*	4.2±0.1*	4.1±0.1**	4.1±0.1**	4.0±0.2**
	i.v.	4.4±0.1	4.3±0.1	3.9±0.1**	4.2±0.1* +	4.2±0.1	4.2±0.2	4.1±0.1*	4.1±0.1*	4.0±0.1**
PP (g/dl)	con	7.4±0.2	7.3±0.2	6.2±0.3**	6.8±0.3**	6.9±0.3**	6.9±0.3**	6.9±0.3**	6.9±0.2**	7.0±0.2**
	ivt	7.4±0.3	7.3±0.3	6.4±0.4** +	6.8±0.3**	6.9±0.3**	7.0±0.3**	6.9±0.3**	6.9±0.3**	6.9±0.3**
	i.v.	7.5±0.2	7.4±0.2	6.4±0.2**	7.0±0.2**	7.1±0.1**	7.2±0.2* ++	7.2±0.2 ++	7.2±0.1* +	7.2±0.2*
Hct (%pkd cells)	con	38±1	38±1	32±1**	35±1**	36±1*	35±1**	35±1**	35±1**	34±1**
	ivt	36±1	36±1 +	31±1**	33±1** +	34±1	34±1	34±1**	34±2*	33±1**
	i.v.	41±2	41±3 ++	36±2** ++	40±2 ++	39±2* ++	39±2* ++	39±2* ++	38±2** ++	38±2** ++

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

Table 15: Plasma aldosterone concentration (pAldo), mean arterial blood pressure (MABP), and heart rate (HR) during the water load experiments of Series IV. Mean ± SEM. (n=5)

		TIME (min)								
		-15	0	30	60	90	120	150	180	210
pAldo (pg/ml)	con	32±14	34±21	29±12	32±12	45±19	40±18	36±16	31±12	30±14
	ivt	24±9	25±8	22±13	19±8 ++	25±8 ++	25±10 +	30±13	23±9	25±12
	i.v.	31±13	30±13	24±11	28±12	29±13 +	21±9 ++	19±8 ++	25±14	21±10
MABP (mm Hg)	con	102±5	102±5	115±4 **	105±5	108±4	106±3	108±6	104±4	99±3
	ivt	103±6	105±6	115±6 *	108±6	105±4	103±6	105±6	102±6	104±7
	i.v.	99±4	103±5	112±4 *	105±3	100±4	103±6	99±5	100±5	98±3
HR (beats/ min)	con	48±4	50±3	57±7 *	45±4	44±5	46±6	50±6	46±3	42±6
	ivt	47±5	46±6	55±6	45±6	44±4	46±4	45±4	41±4	46±4
	i.v.	44±4	46±5	51±7	47±4	46±6	44±6	44±5	45±4	42±5

*, + P<0.05; **, ++ P<0.01. Asterisks (*) compare to time 0 min, pluses (+) are between groups.

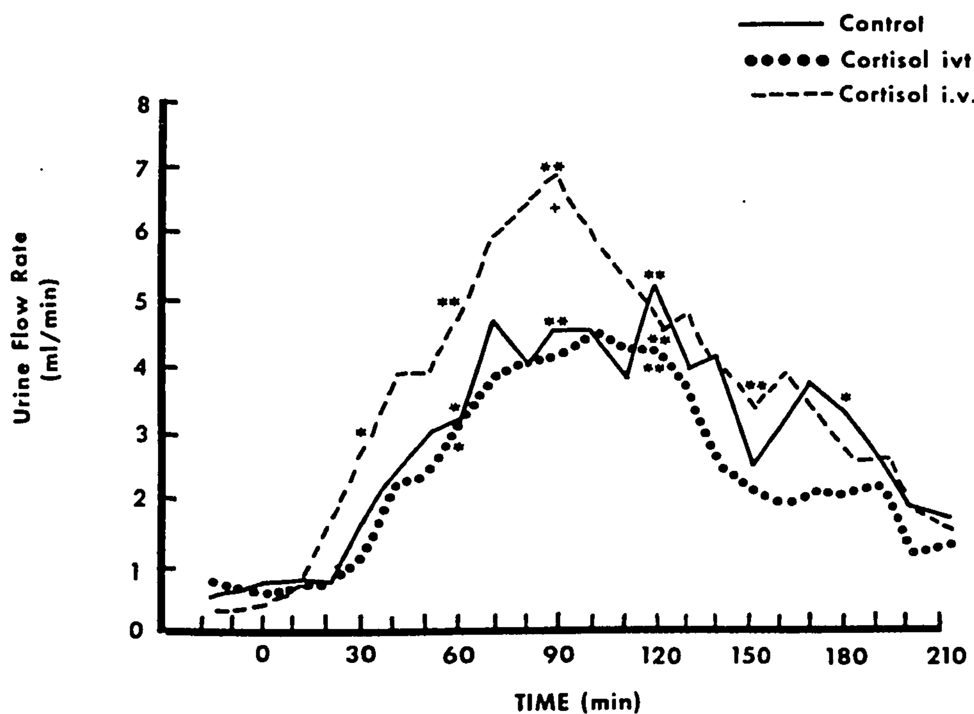


Figure 23: Results of urine flow rate (ml/min) in response to a water load during the control, ivt cortisol, and i.v. cortisol infusion experiments. The mean values and statisitical comparisons are given. Asterisks (*) represent differences within each group, as compared to time zero. Pluses (+) represent differences between groups, as compared to the control experiment. *,+ P<0.05; **,++ P<0.01. (n=5)

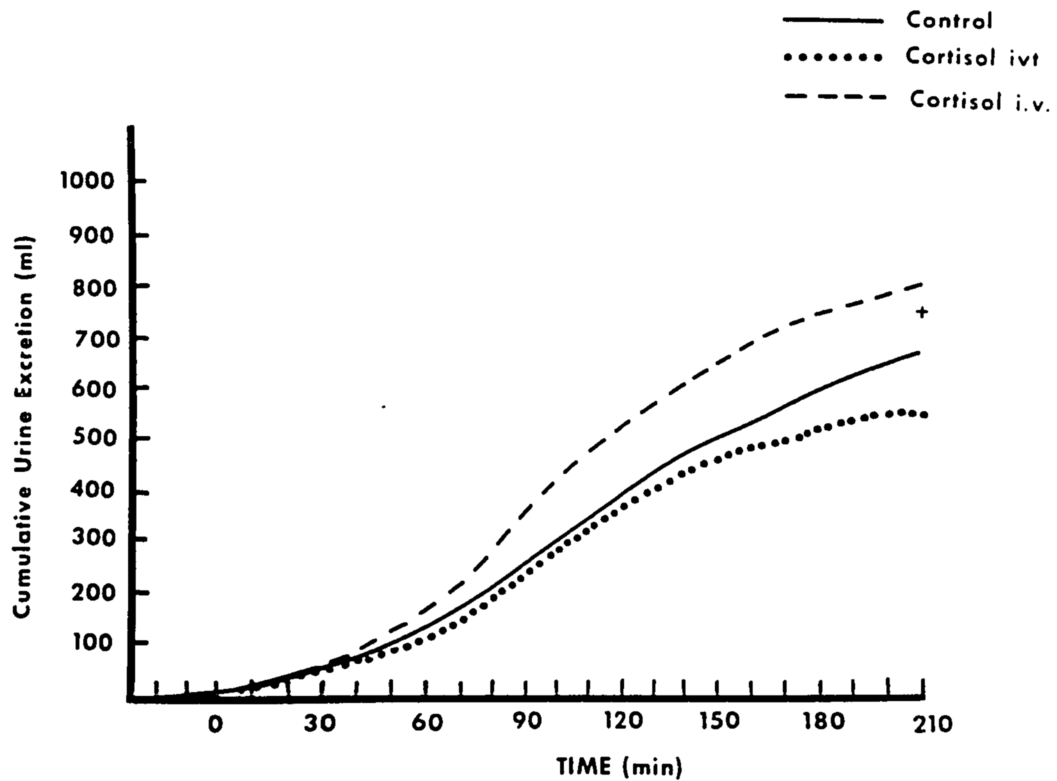


Figure 24: Excretion of water load over the experimental time course in the control, ivt cortisol, and i.v. cortisol protocols. The mean values are given. There was a significant difference (P<0.05) at the conclusion of the experiment between the control and i.v. cortisol experiments. (n=5)

cortisol experiments ($P<0.01$). The urinary diluting ability, or minimum urine osmolality, was similar in all experiments (Fig. 25). The pre-water load mean value of 1179 mOsm/kg H_2O significantly decreased ($P<0.01$) to a minimum mean value of 98 mOsm/kg H_2O, regardless of the protocol.

During the water diuresis, creatinine clearance, an index of glomerular filtration rate (GFR), did not change over time or between protocols (Fig. 26). Although it increased slightly at t=30 min in the control and i.v. cortisol experiments, this was not significant. Creatinine clearance (Ccr) was at control rates thereafter in each experiment. The renal clearance of solutes, or osmolal clearance, increased significantly a t=30 min in all cases ($P<0.01$) (Fig. 27). This change was transient and returned to basal values by t=60 min. The osmolal clearance (Cosm) then remained at control values throughout the remainder of the study.

The urinary excretion of sodium and potassium was corrected for changes in urinary creatinine excretion and is expressed as mEq(Na^+ or K^+)/mg creatinine (Tables 16 & 17). The renal excretion of both sodium and potassium increased slightly during the water diuresis at t=30 min and returned to basal values by t=60 min. No statistically significant changes were seen over time or between groups.

Free water clearance (CH_2O) was calculated for each experiment to determine what effect cortisol may have had on the renal excretion of solute-free water in response to an administered water load. In all of the protocols, CH_2O increased significantly (Fig. 28). At t=90 min,

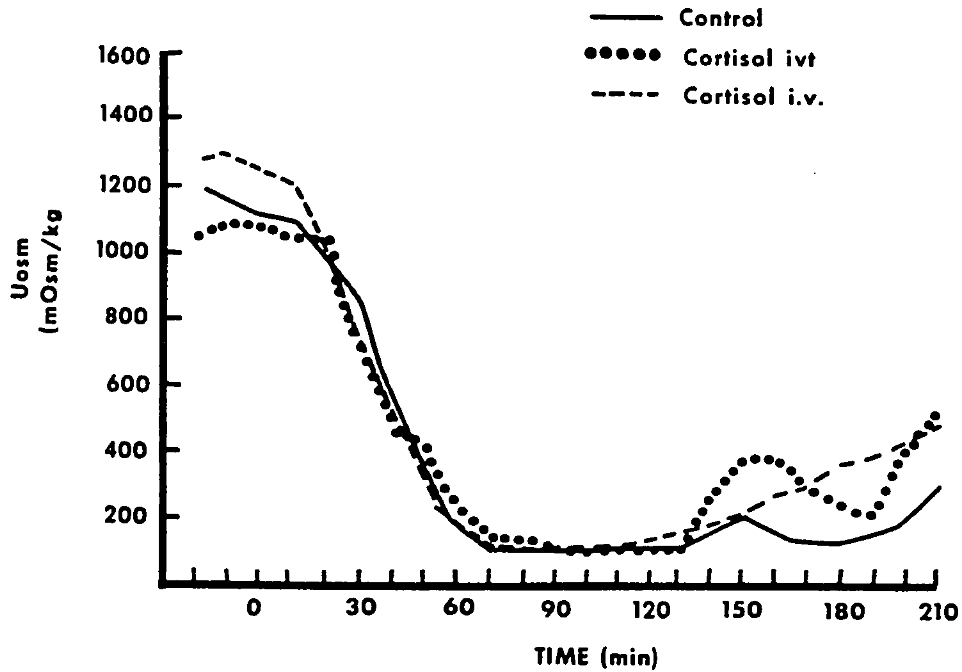


Figure 25: Urinary dilution as indicated by changes in urine osmolality (Uosm) in response to a water load is shown for the control, ivt cortisol, and i.v. cortisol protocols. The mean values are given. In all cases, Uosm fell significantly to similar values (P<0.01) but there was no significant difference between groups. (n=5)

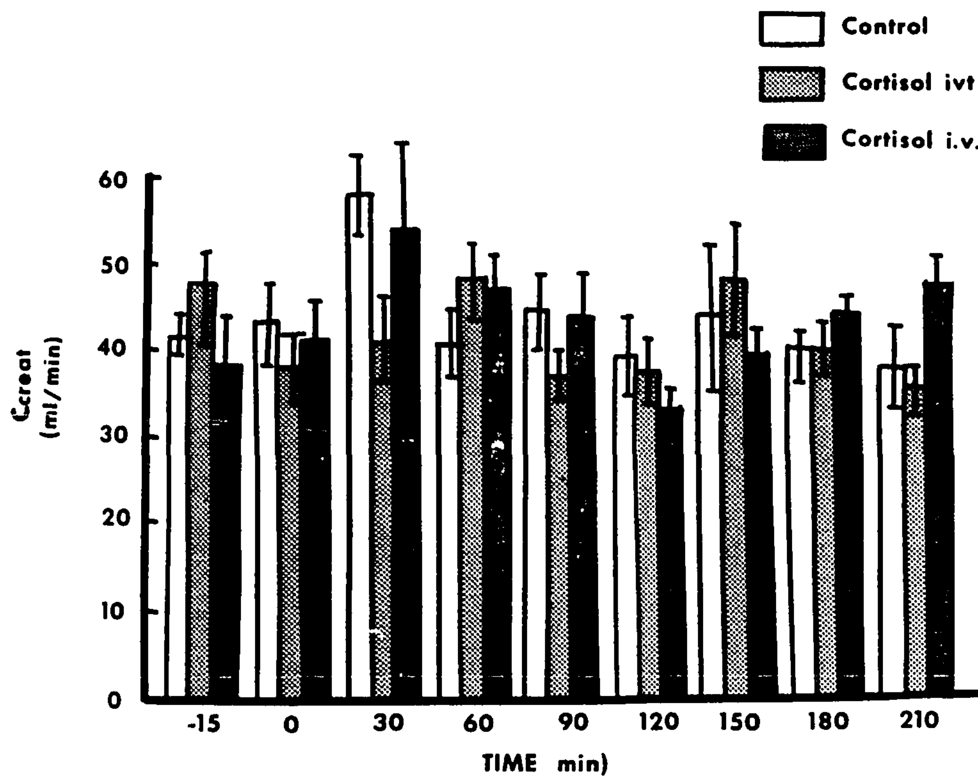


Figure 26: Creatinine clearance (ml/min) during water loading in the control, ivt cortisol, and i.v. cortisol experiments. Vertical lines indicate ± SEM. No significant differences were seen within or between groups. (n=5)

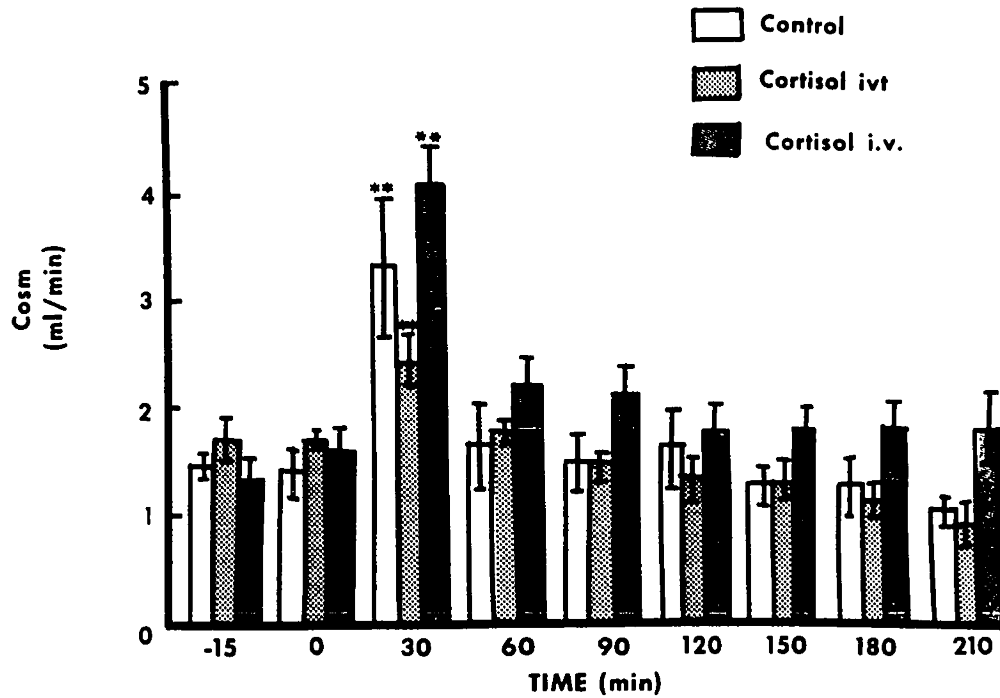


Figure 27: Osmolal clearance (ml/min) during water loading in the control, ivt cortisol, and i.v. cortisol experiments. Vertical lines indicate ± SEM. In all cases, a significant difference (**, $P<0.01$) compared to time zero was observed at t=30 min. There was no significant difference between groups. (n=5)

Table 16: Urinary sodium excretion corrected for changes in urinary creatinine excretion during the water load experiments of Series IV. No statistically significant differences were seen within or between groups. Mean ± SEM. (n=5)

Urine Na^+/Urine Creatinine (mEq/mg)

Time (min)	Control	Cortisol ivt	Cortisol i.v.
-20	0.24 ± 0.08	0.24 ± 0.05	0.18 ± 0.04
-10	0.23 ± 0.07	0.22 ± 0.05	0.21 ± 0.04
0	0.25 ± 0.08	0.18 ± 0.04	0.20 ± 0.04
10	0.28 ± 0.10	0.23 ± 0.05	0.23 ± 0.06
20	0.28 ± 0.07	0.28 ± 0.06	0.28 ± 0.08
30	0.37 ± 0.16	0.39 ± 0.10	0.48 ± 0.22
40	0.42 ± 0.21	0.40 ± 0.09	0.39 ± 0.20
50	0.24 ± 0.08	0.28 ± 0.08	0.30 ± 0.06
60	0.16 ± 0.05	0.17 ± 0.04	0.21 ± 0.05
70	0.16 ± 0.04	0.20 ± 0.07	0.27 ± 0.08
80	0.20 ± 0.04	0.27 ± 0.07	0.23 ± 0.07
90	0.18 ± 0.03	0.22 ± 0.04	0.20 ± 0.04
100	0.21 ± 0.04	0.21 ± 0.03	0.24 ± 0.05
110	0.20 ± 0.04	0.19 ± 0.03	0.26 ± 0.06
120	0.20 ± 0.03	0.20 ± 0.04	0.26 ± 0.06
130	0.16 ± 0.03	0.21 ± 0.03	0.26 ± 0.04
140	0.17 ± 0.04	0.18 ± 0.02	0.23 ± 0.05
150	0.16 ± 0.04	0.21 ± 0.04	0.25 ± 0.06
160	0.16 ± 0.03	0.20 ± 0.04	0.25 ± 0.05
170	0.15 ± 0.03	0.17 ± 0.03	0.25 ± 0.04
180	0.15 ± 0.02	0.11 ± 0.02	0.22 ± 0.04
190	0.18 ± 0.03	0.16 ± 0.03	0.24 ± 0.03
200	0.17 ± 0.03	0.16 ± 0.04	0.23 ± 0.05
210	0.13 ± 0.02	0.17 ± 0.04	0.22 ± 0.03

Table 17: Urinary potassium excretion corrected for changes in urinary creatinine excretion during the water load experiments of Series IV. No statistically significant differences were seen within or between groups. Mean ± SEM. (n=5)

Urine K^+/Urine Creatinine (mEq/mg)

Time (min)	Control	Cortisol ivt	Cortisol i.v.
-20	0.09 ± 0.01	0.14 ± 0.03	0.13 ± 0.02
10	0.08 ± 0.01	0.13 ± 0.02	0.15 ± 0.02
0	0.09 ± 0.01	0.11 ± 0.02	0.15 ± 0.02
10	0.10 ± 0.01	0.11 ± 0.02	0.16 ± 0.02
20	0.11 ± 0.01	0.15 ± 0.02	0.17 ± 0.02
30	0.10 ± 0.01	0.11 ± 0.02	0.17 ± 0.03
40	0.07 ± 0.01	0.08 ± 0.02	0.12 ± 0.01
50	0.05 ± 0.02	0.06 ± 0.02	0.10 ± 0.01
60	0.05 ± 0.02	0.04 ± 0.02	0.09 ± 0.01
70	0.06 ± 0.02	0.06 ± 0.03	0.13 ± 0.01
80	0.06 ± 0.02	0.08 ± 0.03	0.13 ± 0.02
90	0.05 ± 0.02	0.06 ± 0.03	0.11 ± 0.02
100	0.07 ± 0.02	0.05 ± 0.02	0.11 ± 0.01
110	0.06 ± 0.02	0.05 ± 0.02	0.12 ± 0.01
120	0.07 ± 0.03	0.05 ± 0.02	0.14 ± 0.02
130	0.06 ± 0.02	0.05 ± 0.02	0.12 ± 0.00
140	0.07 ± 0.02	0.05 ± 0.02	0.11 ± 0.01
150	0.06 ± 0.02	0.05 ± 0.02	0.11 ± 0.01
160	0.07 ± 0.02	0.05 ± 0.02	0.11 ± 0.01
170	0.07 ± 0.02	0.05 ± 0.02	0.09 ± 0.02
180	0.07 ± 0.03	0.03 ± 0.02	0.10 ± 0.01
190	0.07 ± 0.03	0.05 ± 0.02	0.10 ± 0.01
200	0.07 ± 0.02	0.06 ± 0.02	0.10 ± 0.00
210	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.01

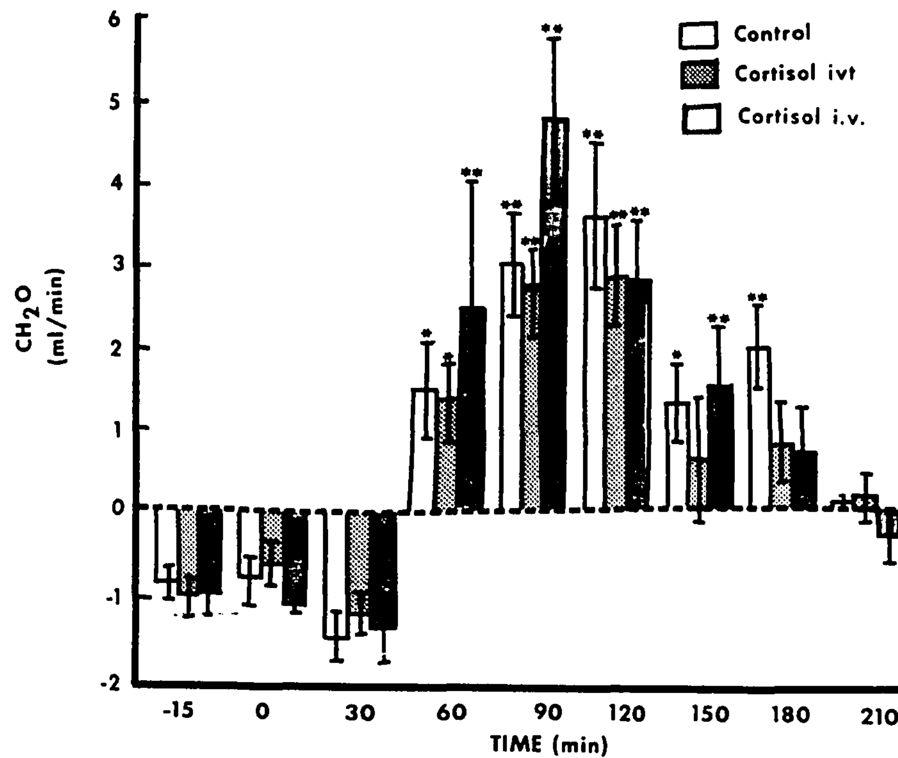


Figure 28: Solute-free water clearance (CH_2O) in response to a water load during the control, ivt cortisol, and i.v. cortisol experiments. Vertical lines indicate ±SEM. Significant increases compared to time zero were seen (*, $P<0.05$; **, $P<0.01$). (n=5)

the CH_2O for the i.v. cortisol experiment was 4.75 ml/min. Although it was not significantly different from the control value of 3.01 ml/min, it did differ ($P<0.05$) from the ivt cortisol experiment value of 2.66 ml/min. This elevated CH_2O persisted until most of the water load was excreted by the end of the experiment at t=210 min.

Summary

Series I, II, and III: The administration of cortisol either centrally or peripherally did not affect plasma ADH levels in the dehydrated state (Series I). The increase in plasma ADH concentration in response to osmotic stimulation (hypertonic saline infusion) was significantly delayed during i.v. cortisol administration as compared to controls, however, this was a result of a significant delay in the rise of plasma osmolality associated with the administration of the same osmotic load (Series II). The plasma cortisol levels obtained during i.v. cortisol infusion in this series of experiments were aproximately 10-fold higher than those of the control experiment. The linear regression analysis indicated a positive correlation between the plasma ADH concentration and plasma osmolality. Although the average of the slopes was less in the i.v. cortisol experiments, it was not statistically significant. On the other hand, the increases in plasma ADH concentration and plasma osmolality during osmotic stimulation were similar in both the central cortisol (ivt) administration experiments and the corresponding control experiments (Series II). In this protocol, the plasma cortisol concentration decreased to near non-detectable levels as opposed to a CSF cortisol concentration of

approximately 20 μg%. With similar patterns in plasma and CSF cortisol concentrations as compared to Series II, no changes in plasma ADH concentration or plasma osmolality were seen during central or peripheral cortisol administration in the isotonic saline infusion experiments of Series III.

Series IV: Plasma constituents: The water load resulted in marked decreases in plasma osmolality for the control, ivt cortisol and i.v. cortisol experimental protocols. In each case, the plasma osmolality fell to a similar minimum value, however, during the progressive increase back towards basal values, the i.v. cortisol experiment plasma osmolalities were significantly above those of the control experiment while the plasma osmolalities of the ivt cortisol experiment were below those of the control experiment. Both trends persisted throughout the remainder of the protocol.

Plasma ADH concentration fell similarly in all three experiments and did not differ between groups. The linear correlation between plasma ADH concentration and plasma osmolality resulted in slopes for the regression lines that were similar for both the control and ivt cortisol experiments. Again, i.v. cortisol administration resulted in an average value for the slopes that was less than that of the control experiment, and although close to being significant, this difference was not statistically different.

Renal function during the water load protocols: The peripheral administration of cortisol increased urine flow rate above that of the control experiment and resulted in a significantly higher cumulative

urine excretion as compared to the controls, whereas the ivt cortisol experiment was not statistically different from the control experiment. The urinary diluting capacity, or minimum urine osmolality, was similar in all three protocols. In addition, no significant change was seen in creatinine clearance over time or between groups.

An apparent pressure diuresis was evident in response to the water load because the increase at t=30 min in osmolal clearance (which was accompanied by small increases in urinary sodium and potassium excretion at t=30 min) was associated also at this time period with and increase in MABP and HR. In all cases, these values returned to basal levels by t=60 min.

The urinary excretion of solute-free water was markedly increased in all protocols, however, it was greater in the i.v. cortisol experiment at t=90 min as compared to controls. This increase in free water clearance seen in the i.v. experiment accounts for the difference obtained in the cumulative urine excretion, where the i.v. cortisol administration was significantly higher than the control or ivt cortisol experiments.

Conclusions

Over all, the data do not support a direct action of cortisol on ADH release. Thus, cortisol infusions, either i.v. or ivt, did not decrease the elevated levels of ADH accompanying dehydration. Cortisol, again either i.v. or ivt, did not significantly alter the plasma ADH - plasma osmolality relationship either in response to hypertonic saline or a water load. Finally, if cortisol was altering

the renal excretion of water via a direct effect on ADH release, it would seem reasonable that the central administration of cortisol would have produced renal responses in the same direction from control values as did the peripheral administration. Instead, the opposite was more frequently observed.

CHAPTER V

DISCUSSION

The present studies describe the effects of the acute administration of cortisol, either centrally or peripherally, on the plasma ADH concentration in normal conscious dogs, during 4 different osmotic situations or states of osmolality: dehydration, euhydration, hyperosmolality via hypertonic saline infusion, and hypoosmolality via water load administration. The effect of cortisol on the relationship between plasma osmolality and plasma ADH concentration was determined and a direct, central effect of cortisol was compared to peripheral administration. Although a substantial literature base exists that suggests a possible inhibitory effect of glucocorticoids on ADH release (Gaunt et al., 1957; Agus and Goldberg, 1971; Seif et al., 1978; Schrier et al., 1980; Silverman et al., 1981; Raff 1987), the results of the present study indicate that cortisol has no effect on influencing the plasma ADH concentration when administered either centrally or peripherally.

Static Osmotic States

Several lines of evidence suggest a direct effect of glucocorticoids on the release of ADH (Schrier et al., 1980; Silverman et al., 1981; Raff 1987), however, in the present study no effect of cortisol was seen, either with central or peripheral administration, when plasma ADH concentration was elevated by 48 hrs of water deprivation (Figs. 6 & 8). Likewise, the acute administration of

cortisol had no effect on the basal plasma ADH levels during normal hydration (Figs. 16 & 18). Previous work done to investigate the relationship between glucocorticoids and ADH during basal conditions in normal subjects is limited. Two studies examining the effect of glucocorticoids on ADH release under these circumstances have been reported with disparate results.

Moses (1963) examined in the rat, the effects of glucocorticoids on ADH release induced by the stimulus of 48 hr dehydration and reported that the depletion of pituitary ADH content was significantly reduced by the administration of a synthetic glucocorticoid, methylprednisolone, during the 2 days of dehydration. This was also accompanied, however, by a change in plasma osmolality. The plasma osmolality after dehydration in the methylprednisolone-treated rats was less than the untreated controls and as a result, no significant difference was seen between the pituitary ADH concentration vs. plasma osmolality relationship. In other words, methylprednisolone did not demonstrate a direct inhibitory action on ADH release and acted instead to lessen the increase in plasma osmolality due to dehydration. The reduction in plasma osmolality resulted in a decrease in the secretion of ADH and therefore, a decrease in pituitary ADH depletion.

In direct contrast, and with no apparent explanation given, Raff et al. (1986) reported that 1 day of dexamethasone treatment in dogs resulted in a significant elevation of plasma osmolality. This prior dexamethasone treatment was also reported to attenuate the response of

ADH to CRF and hypertonic NaCl infusion, thereby suggesting that glucocorticoids inhibit ADH release.

The interpretation of the present study, in light of these previous findings must include some explanation of the differences in experimental protocols, i.e., the difference in results from the present study may relate to the different time interval of glucocorticoid administration. The time interval of glucocorticoid treatment in the present study was more acute with cortisol being administered for 2 hrs, as opposed to the chronic administration for 1 to 2 days, before experimentation. Therefore, this study cannot directly support or refute the contrasting data of the two studies described. In this acute setting, no change in plasma osmolality was observed with cortisol administration. Therefore, this protocol focused strictly on the effect of glucocorticoids on plasma ADH concentration without the occurrence of differences in plasma osmolality. The time frame of the acute glucocorticoid exposure in the present study can be considered adequate for the elicitation of responses to occur (1) since appropriate responses were observed as in the water load experimental protocols (see discussion - Water load administration) in regards to changes in FWC, and also, (2) since central cortisol administration was shown to inhibit ACTH release and decrease plasma cortisol levels well within the time course of the experiment.

Like the study by Moses (1963), the present data do not support a glucocorticoid inhibition on basal ADH release. This was also found in another study done under different conditions. Travis and Share (1971)

studied the effect of acute glucocorticoid administration on the increased plasma ADH concentration associated with adrenal insufficiency and found no effect of glucocorticoids on plasma ADH levels when given 1 hr before blood sampling. After 2 hrs, however, glucocorticoid treatment prevented the rise in plasma ADH concentration that was observed in the vehicle control experiment, which they interpreted as an indication of perhaps relief of glucocorticoids on a stress related response.

Induced Hypertonicity

In the present study, the increase in plasma ADH concentration in response to hypertonic saline infusion was significantly delayed during acute i.v. cortisol administration, however, this was a result of a delayed increase in plasma osmolality associated with the cortisol administration (Fig. 10). This effect of cortisol on plasma osmolality is indirect support of the work by Moses (1963), who found that cortisol treatment decreased the rise in plasma osmolality due to dehydration. Therefore, no effect was seen on the plasma ADH concentration vs. plasma osmolality relationship with cortisol treatment (Fig. 11) in either of these studies. Similarly, using changes in FWC as an index of ADH release, Dingman and Despointes (1960) reported no effect of glucocorticoids on the antidiuresis due to hypertonic saline infusion. In contrast, Aubry et al. (1965) found that both the acute and chronic administration of cortisol elevated the osmotic threshold for ADH release by as little as 2.57 mOsm/kg. The osmotic threshold was defined as the plasma osmolality at which

hydrated normal subjects first responded to an i.v. infusion of hypertonic saline with a significant fall in FWC. Although the present study did observe a delayed increase in plasma ADH concentration during i.v. cortisol administration, this was due to a delayed increase in plasma osmolality, while receiving the same osmotic load, and not due to a direct effect of cortisol on ADH release. Thus, the relationship between plasma osmolality and plasma ADH concentration was not altered in the present studies by pretreatment with cortisol. It must be noted that the study of Aubry et al. may be said to have inherent pitfalls in its design. The assessment of ADH release was performed by observing changes in FWC. In order to accomplish this, prior to the hypertonic saline infusion, the subjects were administered an oral water load of 20 ml/kg B.Wt. (approx. 3% of B.Wt.) which was maintained by replacing voided urine with equal volumes of ingested tap water. Clearly, these subjects were somewhat volume expanded prior to the start of the hypertonic saline infusion which is further exasperated by the latter. Since volume expansion has been shown to shift the plasma ADH concentration vs. plasma osmolality relationship to the right (Robertson and Athar, 1976), this does not constitute a normal setting and instead results in a situation which already has influenced the response of the osmoreceptor with hypervolemia.

When a hypertonic solution is added to the ECF, the plasma osmolality increases and causes the movement of water out of the cells into the extracellular compartment. This osmosis will cease when the ECF becomes diluted sufficiently to equal the osmolal concentration of

the intracellular fluid. In addition, it has been proposed that a stable cell volume depends on a balance between "pump"-mediated active transport of Na^+ and K^+ and the diffusive "leaks" of Na^+ and K^+ down their respective electrochemical gradients, which is otherwise known as the pump-leak hypothesis of steady-state cell volume regulation (Siebens, 1985). An extension of this hypothesis maintains that a volume controlling mechanism exists that regulates the cell volume by controlling additional or modified "leak" pathways for Na^+ and K^+. These volume regulatory leaks are thought to result in changes in cellular ion content (i.e., osmolar content) that enable shrunken or enlarged cells to return toward their original volume.

Intravenous cortisol administration was shown to delay the increase in plasma osmolality during the infusion of hypertonic saline. As previously observed, (Moses, 1963; Aubry et al., 1965; Streeten et al., 1981; Raff et al., 1986) it is apparent in the present study that, independent of actions on ADH, cortisol is affecting water and/or electrolyte (e.g. Na^+) movements in the body, thereby producing this delayed increase in plasma osmolality, which in turn delayed the increase in plasma ADH concentration. Several possible mechanisms can be postulated to explain this effect. Among these are: (1) cortisol augments water movement out of the cells and this results in the delayed rise in plasma osmolality, (2) cortisol influences the "leak" pathways for Na^+ and K^+ thereby increasing the cell osmolar content, (3) cortisol reduces the Na^+-K^+-ATPase activity resulting in an increase in Na^+ movement into the cells, or (4) cortisol increases the

renal excretion of Na^+ out of the body. In vitro studies on anuran membranes have demonstrated that glucocorticoids enhance ADH-induced water movement but have no effect alone (Handler et al., 1969; Zusman et al., 1978). In addition, glucocorticoids have been shown to enhance renal Na^+-K^+-ATPase activity rather than inhibit it (Rayson and Edelman, 1982). The response of the anuran membrane (an ADH-mediated membrane model) may not reflect that of the cell membranes in general, throughout the body; therefore, an effect of cortisol on water movement, aside from the ADH-mediated effect, may exist. The observations on in vitro preparations of the Na^+-K^+-ATPase activity would suggest that the latter two possibilities mentioned above are not probable, since enhanced Na^+-K^+-ATPase activity results in exclusion of Na^+ from cells and renal Na^+ retention. Responses of the "leak" pathways for Na^+ to cortisol remain a possibility; however, this author is unaware of previous studies investigating this subject or suggesting this possibility.

The osmotic secretion of ADH has been found to be dependent on both the absolute level of plasma osmolality and also on the rate of change in plasma osmolality (Robertson et al., 1976). This effect is not noticed when plasma osmolality changes at 2% or less per hr but becomes apparent with an exaggerated plasma ADH response when changes in plasma osmolality approach 5.5% per hr. Under these conditions, the greater rise in plasma ADH concentration per unit increment in plasma osmolality appears to be exaggerated by an amount roughly proportional to the fractional increase in the stimulation rate.

In the present study, the infusion of hypertonic saline was similar to that used by Aubry et al. (1965) being set at 0.05 ml/kg/min of 5% NaCl. However, unlike Aubry et al. a significant difference in the rate of rise in plasma osmolality occurred between the control experiment and the i.v. cortisol administration. The control experiment slightly exceeded the point of no-rate effect (2%) at 2.9% /hr, however, i.v. cortisol infusion lowered it to 1.9% /hr (the infusion rate was controlled by the use of an infusion pump). The effect of this difference in the rate of rise of plasma osmolality would be to produce a greater rise in plasma ADH concentration in the control experiments and possibly cause a change in the plasma ADH concentration vs. plasma osmolaity relationship which would bias the results toward a suggested inhibitory effect of glucocorticoids on ADH release. Despite this difference, the plasma ADH concentration vs. plasma osmolality relationship was not altered. On the other hand, Aubry et al. also slightly exceeded the point of no-rate effect but they were unable to detect a difference in the rate of rise of plasma osmolality with or without glucocorticoid. As a result, these authors suggested that cortisol somehow increased the osmolal content of the osmoreceptors and thereby increased the osmotic threshold for ADH release. In contrast, the observations of the present study suggest that cortisol may have increased the osmolar content of all cells which therefore caused a delay in the development of hypertonicity in the plasma.

Cortisol was administered into the third cerebral ventricle in order to identify a central effect of cortisol. The central administration of cortisol was without effect on the increase in plasma ADH concentration during hypertonic saline infusion (Fig. 13). Streeten et al. (1981) reported similar findings when they introduced cortisol crystals into the SON in conscious rhesus monkeys. In their study, although cortisol did not affect the absolute level of the osmotic threshold, the plasma osmolality was significantly less in the cortisol experiments than the controls during the water diuresis. Consequently, more hypertonic saline was required to reach the osmotic threshold. These authors were unable to explain the effect cortisol crystals placed into the SON had on the plasma osmolality after hydration. In the present study, plasma ADH concentration was measured directly and therefore the need to induce a water diuresis in order to monitor changes in FWC was not necessary. As a result, the animals in the present study were not hydrated prior to inducing the hypertonic stimulus and began the experiment from a normal starting point. Cortisol administered into the third ventricle did not change the plasma osmolality at the start of the hypertonic saline infusion or affect the rate of rise of plasma osmolality, therefore, no influence of cortisol on plasma osmolality occurred to obscure these findings (Fig. 13). Also, since no effect was seen on the plasma ADH concentration, it can be said that central administration of cortisol did not inhibit neurohypophyseal ADH release in response to a hypertonic stimulus. Although this is in basic agreement with the only

previous study (Streeten et al., 1981), the results of the present study provide more direct evidence against an ADH-mediated response since plasma ADH levels were measured and not inferred from the point of plasma osmolality at which the diuresis induced by a water load can be inhibited by a saline load. In addition, their experimental findings were based on observations from 3 monkeys.

Among the more interesting observations obtained in the present study was that the central administration of cortisol resulted in diminished plasma cortisol concentrations toward the minimum detectability of the RIA assay employed for cortisol assessment. During ivt cortisol infusion, the measurements made of CSF cortisol concentration revealed levels of cortisol in the CSF that were comparable to those obtained in the plasma during i.v. cortisol infusion (Table 12) and were sufficient to inhibit peripheral cortisol levels. Previous studies have shown glucocorticoids to inhibit ACTH release at the level of the hypothalamus, as well as at the pituitary itself (Keller-Wood and Dallman, 1984). In addition, glucocorticoids have been shown to decrease the hypothalamic CRF content (Sayers and Portanova, 1975). Since elevated CSF cortisol resulted in decreased plasma cortisol levels, it would seem that a central inhibition of ACTH by ivt cortisol administration occurred. Although this demonstrates that cortisol is working centrally to inhibit ACTH release, cortisol administration was without effect on ADH release. By having measured both the plasma cortisol and plasma ADH concentrations, this is the first report that has shown that concurrent with central cortisol

induction of a negative feedback effect on ACTH, ADH release was not affected.

Water Load Administration

Since plasma aldosterone concentrations were shown not to be affected, the virtual elimination of peripheral cortisol levels by the central administration of cortisol provides a new approach with which to investigate the acute effects of isolated glucocorticoid deficiency without concomitant decreases in mineralocorticoid activity. That is, in the present experiments, central administration of cortisol reduced cortisol but not aldosterone in the circulating plasma. Therefore, the effects of centrally infused cortisol resulting in selective (peripheral) glucocorticoid deficiency, and the opposite situation of cortisol excess via i.v. cortisol administration on the ability to excrete a water load was studied, while measuring concomitant responses of plasma ADH concentration and renal function.

The results of the present study confirm those of others in that the ability to excrete a water load was diminished in glucocorticoid deficiency (Gaunt et al., 1957; Schrier et al., 1980) and was augmented in situations of glucocorticoid excess (Raisz et al., 1957; Kleeman et al., 1960; Lindeman et al., 1961). After administration of the water load (40 ml/kg), the percentage of water excreted by the end of the protocol was 99% in the i.v. cortisol experiment, 82% in the controls, and 70% in the ivt cortisol experiment (Fig. 24), with the i.v. cortisol experiment being significantly different from the control experiment ($P<0.05$) and from the ivt cortisol experiment ($P<0.01$). The

urinary diluting ability or minimum urine osmolality was similar in all three experiments (Fig. 25). The effect on FWC (Fig. 28), however, was not due to a direct effect of cortisol on neurohypophyseal ADH release since plasma ADH concentrations (Fig. 21) were seen to fall similarly in all three experiments. It was also quite evident that the central administration of cortisol had no effect on ADH release but produced a renal response to the administered water load that was qualitatively opposite to and significantly different from the i.v. cortisol experiment, resembling a response like that of a glucocorticoid deficent state. This new finding confirms that the effect of cortisol on FWC is determined by the plasma cortisol levels, and demonstrates that this effect on FWC is _not_ an ADH-mediated effect.

The augmentation in FWC by i.v. cortisol administration (or attentuation by ivt cortisol) occurred without a change in renal hemodynamics such as GRF (Ccreat), or renal clearance of solutes, i.e., osmolal clearance (Cosm), thus confirming the observations of others (Raisz et al., 1957; Kleeman et al., 1960; Lindeman et al., 1961). As a result, the return towards basal levels of plasma osmolality in the i.v. cortisol experiment was faster than that of the control and ivt experiments (Fig. 20) and therefore the plasma osmolality in the i.v. cortisol experiment was higher during the water diuresis. This agrees with the findings of Aubry et al. (1965) who obtained an increased plasma osmolality after water loading and before the hypertonic saline infusion following prior glucocorticoid administration for 2 days.

Since the effect of cortisol on FWC in the present study occurred without changes in GFR, solute excretion, or in contrast to a previous hypothesis, without being related to changes in ADH concentration, this supports the hypothesis that glucocorticoids influence the renal tubular handling of water and that the mechanism involved deals with the increased formation of free water. In addition, since the present study did not hold plasma cortisol constant, this effect may involve a concentration dependent modulation by cortisol. How this is accomplished remains speculative. One hypothesis proposes that cortisol antagonizes ADH effects on the renal tubule, however, several studies have demonstrated that glucocorticoids have no effect on the antidiuretic action of ADH (Dingman and Despointes, 1960; Kleeman et al., 1960; Lindeman et al., 1961; Aubry et al., 1965). Another hypothesis has suggested that cortisol administration decreases the collecting duct epithelium permeability to water, i.e., renders it maximally impermeable to water. Although this has been postulated by several investigators (Raisz et al., 1957; Kleeman et al., 1960), in vitro studies do not support this hypothesis. Perfusion studies on the isolated collecting ducts from glucocorticoid deficient animals have not shown an increase in water permeability due to the absence of glucocorticoids (Rayson et al., 1978; Schwartz et al., 1978). Studies in anuran membranes have consistently demonstated that glucocorticoids enhance rather than inhibit water movement (Handler et al., 1969; Zusman et al., 1978). Although the mechanism of this peripheral effect of cortisol cannot be ascertained by this study, an alternative

hypothesis independent of ADH involvement and the glucocorticoid induced impermeability of the collecting ducts can be offered. The present results are compatible with a mechanism in which excess cortisol impairs proximal tubule reabsorption thus resulting in the increased delivery of fluid to the distal tubule. This in itself can increase the possible amount of free water excreted. Thus, in the three experimental protocols of the water load experiments, all three had similar plasma ADH concentrations and similar minimum urine osmolalities, but the cortisol i.v. experiments reflected a greater excretion of free water. Furthermore, this is also compatible with the role of cortisol in enhancing the Na^+ "leak" pathway thereby increasing proximal tubular cell osmolar content and subsequently decreasing proximal tubule reabsorption of sodium. Since water movement occurs secondary to sodium in this tubular segment, this would decrease proximal tubule reabsorption.

Summary

The summary of these findings include: (1) The renal response to exogenous cortisol administration was an increase in free water excretion as previously reported, and (2) in the latter experiment, during a water load, where ivt cortisol administration rendered a peripheral cortisol insufficiency, the renal response, again consistent with previous reports, was a decrease in free water loss. However, the findings of the present study, by having measured plasma ADH concentration and plasma cortisol concentration and studying renal function, provide direct evidence that these are not ADH-mediated

responses. As a result, an explanation is provided, in agreement with observations in Brattleboro (D.I.) rats where ADH is absent, that a defect in water diuresis still persists and is due to an ADH-independent mechanism. In addition, (3) cortisol administration, either centrally or peripherally, did not affect ADH levels during basal conditions or when elevated with dehydration. (4) The plasma ADH concentration vs. plasma osmolality relationship was unaltered with cortisol administration, either centally or peripherally, during both induced hypertonicity and hypoosmolality. (5) The ivt administration of cortisol did demonstrate a central effect by inhibiting ACTH release and provided a model of peripheral glucocorticoid deficiency with which to study this further, however, no effect was seen on ADH release. The mechanism of action explaining these results cannot be fully deduced from these findings. Clearly, further experiments on the effect of cortisol on the Na^+-leak pathways are warranted.

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