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Neuroendocrine Factors Mediating Polydipsia Induced by Dietary Electrolyte Depletion

by

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ABSTRACT

Electrolyte depletion in rats, produced by dietary restriction of K alone or K, Na and Cl, is known to cause a polydipsia and a significant impairment of urine concentrating ability. In order to determine whether the increased drinking observed in electrolyte depletion results from activation of the renin-angiotensin system (RAS), young adult male rats were fed a low NaCl/K-free (1.5 mM NaCl/100 g food) diet for a 2 week period during which measurements were made of daily water intakes and urine volumes, plasma osmolality and electrolytes, and PRA and angiotensin I (AI) concentration.

Water intake and urine volume increased beginning on day 2 of the electrolyte deplete diet, peaked by days 4-5, and remained at this level, paralleling the time course of increases in PRA and AI plasma concentrations. Rats maintained on the electrolyte deplete diet were hypokalemic, hypochloremic and had a hypoosmotic plasma with persistently elevated PRA and AI concentrations. The urine concentrating defect (UCD) occurred after the increase in water intake in electrolyte depleted rats, therefore plasma hyperosmolality does not mediate the development of polydipsia. In fact hyperosmolality was not present in these animals. The UCD was corrected within days by allowing the rats access to control rat chow.

Another group of rats received electrolytic lesions of the subfornical organ (SFO), a small midline circumventricular structure in the brain believed to mediate angiotensin-induced thirst. Following lesions of the SFO involving partial to complete ablation of the structure, 24 hr intakes of water were significantly lower than in

sham-operated rats on the same electrolyte deplete diet, but there were no differences in PRA and AI concentration. SFO lesions did not prevent the development of a UCD in the presence of normal or high circulating levels of antidiuretic hormone (ADH).

These results suggest that the effect of the low NaCl/K-free diet is to induce primary polydipsia. Moreover, it seems that the polydipsia results from stimulation of AII receptors at the SFO by high levels of circulating AII.

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1.0 INTRODUCTION

There is a broad interest in the fact that many peptide hormones produce profound effects on behaviour. So called "motivated behaviors" (Mogenson, 1976), such as thermoregulation, feeding, and drinking, have evolved in order to help preserve homeostasis in conjunction with physiological regulators. In the case of fluid and electrolyte homeostasis, a combination of renal, hormonal and behavioural mechanisms contribute to the preservation of homeostasis. Water loss in either the cellular or extracellular fluid compartments of the body initiates drinking, and a simultaneous deficit in both compartments has an additive effect on thirst. Although renal mechanisms are capable of conserving body fluids, they cannot replenish actual water deficits. This can only be achieved by ingestion of fluids via drinking, or via the water content of foodstuffs.

The present study is concerned with elucidating the possible CNS mechanisms mediating polydipsia induced by dietary electrolyte depletion. This section of the thesis will describe in general terms the importance of water intake to the maintenance of body fluid balance. Section 1.3 and 1.4 will discuss the renin-angiotensin system and the renal mechanisms involved in the conservation of body fluids. Section 1.5 reviews the effects of electrolyte depletion on water metabolism. Section 1.6 then describes the importance of the hormone angiotensin II (AII) as a central mediator of extracellular thirst that acts via specific receptor sites in the circumventricular organs of the brain. The introduction is concluded with a general statement of the problem under investigation.

1.1 Cellular Dehydration as a Stimulus for Thirst

Depletion of the cellular fluid compartment is an effective stimulus for inducing thirst. Experimentally, this can occur following water deprivation, administration of membrane impermeable hypertonic solutions, and potassium depletion (Fitzsimons, 1979). In 1937 Gilman demonstrated that dogs drank twice as much water following infusions of hypertonic NaCl, which dehydrates cells, as they did following infusions of equiosmotic urea, which does not. Gilman concluded that cellular dehydration, rather than an increase in cellular osmotic pressure, is the stimulus for thirst. Gilman's pioneering experiment was confirmed by other investigators (Holmes et al.,1950) who found that drinking activity was initiated only by solutions excluded from the cell interior and not by changes in serum Na^+ or Cl^- levels.

Wolf (1950) was able to experimentally determine the threshold for thirst resulting from intracellular dehydration by slow intravenous infusion of hypertonic solution that would more accurately parallel the slow rise in plasma osmolality observed under normal physiological conditions. He found that a 1-2% change in blood osmolality would lead to thirst and drinking in dogs and man. Fitzsimons (1961) demonstrated that the volume of water ingested by bilaterally nephrectomized rats given injections of hypertonic solutions was precisely that needed to restore body fluid isotonicity. This provided for a quantitative relationship between cellular dehydration and water intake. The mechanisms controlling urinary water losses via antidiuretic hormone (ADH) and those regulating water intake operate synergistically in most conditions to maintain cellular fluid volumes.

1.2 Extracellular Dehydration as a Stimulus for Thirst

There are mechanisms for ensuring the constancy of the extracellular fluid volume. The mechanisms include those involved in the regulation of fluid exchange at the capillary, controls intrinsic to the circulatory system, such as the adjustment of cardiac output to venous return, the direct effects of arterial pressure on glomerular filtration, cardiovascular reflexes involving the autonomic nervous system, and other vasomotor controls (Fitzsimons, 1972).

However, an actual body fluid deficit can only be corrected by the ingestion of water. A decrease in extracellular fluid volume, either naturally occurring or experimentally induced, is known to stimulate thirst (Fitzsimons, 1972). Experimentally, effective dehydration of ECF can be produced by injecting a hyperoncotic solution (polyethylene glycol, PG) into the peritoneal cavity (Fitzsimons, 1961). The colloid draws fluid from the plasma by a Starling mechanism without a concurrent effect on body fluid osmolality. A functional hypovolemia may be achieved by physically impeding the circulation to specific areas; for example, constriction of the renal arteries leads to drinking (Fitzsimons, 1972).

Baroreceptors in large vessels entering the heart and in the wall of the atria send impulses carried in the vagus nerves that exert an inhibitory effect on thirst neurons. In addition to direct nervous activity from baroreceptors, there are hormonal responses to ECF hypovolemia. The regulatory hormonal system of interest to this study, the renin-angiotensin system, is discussed next.

1.3 The Renin-Angiotensin System

Renin, angiotensin I, II and III, and aldosterone constitute a cascade system which is considered to play a major role in blood pressure homeostasis and in the regulation of fluid and electrolyte homeostasis. The system is vasoconstrictor and antinatriuretic, acting in concert with other homeostatic mechanisms to maintain constant arterial pressure over a wide range of physical activity and sodium intake.

The kidney is the major source of circulating renin, although brain, arteriolar smooth muscle, uterine smooth muscle, placental tissue, submandibular glands, and certain tumors have been shown to produce renin-like enzymes (Oparil et al., 1974). Active renin is an acid protease which cleaves a leucine-valine bond in human angiotensinogen and leucine-leucine bond in angiotensinogen of other mammalian species to form angiotensin I (Tewskbury et al., 1979). Circulating renin substrate arises mainly from the liver. Angiotensin I, the product of renin reaction is a decapeptide with little biological activity. AI is converted to the octapeptide AII, by converting enzyme, dipeptidylcarboxypeptidase, found in vascular endothelium of the lung, kidney and other organs (Oparil et al., 1974). The half-life of circulating AII is short, about 30 seconds, due to rapid degradation by angiotensinases in blood. Removal of the N-terminal aspartic acids results in a heptapeptide, angiotensin III (AIII).

Angiotensin I possesses some biological activity in that it is as potent as AII in stimulating catecholamine production by the adrenal medulla, and may facilitate release of norepinephrine from peripheral

sympathetic neurons (Peach, 1977). Angiotensin II is the major active component of the renin system and has a variety of physiologic actions. Its action is mediated by receptors on vascular smooth muscle to promote vasoconstriction, an effect mediated by calcium. Angiotensin II interacts with the sympathetic nervous system, both centrally by increasing efferent nerve activity to the periphery, and peripherally by increasing release of catecholamines from the adrenal medulla and by enhancing release and blocking reuptake of norepinephrine from peripheral sympathetic neurons (Peach, 1977). The central pressor effect of AII is partly mediated through the area postrema of the caudal medulla which lies outside the blood-brain barrier. Low salt intake decreases the pressor response of AII and high salt diet increases this response (Brunner et al., 1972).

In the kidney, AII decreases renal blood flow, particularly in the outer cortex. In the presence of prostaglandin synthesis inhibition, AII consistently decreases inner cortical and medullary blood flow (reviewed in Peach, 1977), but does not appear to be important in renal autoregulation. An important physiologic effect of AII is the stimulation of aldosterone production in the adrenal zona glomerulosa by binding receptors on the membrane. Through aldosterone, the renin system contributes to changes in extracellular fluid volume and potassium homeostasis. Aldosterone works at the distal nephron by stimulating an active pump located at the pericapillary membrane to transfer potassium from the blood into the tubular cell. The transport involves a Na-K-ATPase pump, such that aldosterone also effects an increase in tubular sodium reabsorption. AII also stimulates the posterior pituitary to release antidiuretic hormone (Malvin, 1971;

Bonjour et al., 1970; Haack et al., 1978). Angiotensin II is a potent dipsogen and may play an important role in extracellular fluid volume (ECFV) homeostasis (Kucharczyk and Mogenson 1975 and 1977).

AIII, in sharp contrast to its efficacy in promoting aldosterone secretion (Peach and Chiu, 1974), is a relatively poor dipsogen and pressor agent presumably because the heptapeptide does not stimulate AII biosynthesis (Evered and Fitzsimons, 1981). However, recent studies in the pig (Mutter, Lemoine, Tsang and Kucharczyk, 1984) and gerbil (Wright et al., 1984) have shown that, at least in these species, AIII can induce water intake when given centrally or systemically. As well, AIII has been found to have a primary effect in stimulating salt appetite in the pig (Mutter et al., 1984).

Renin release regulates the level of activity of the renin-angiotensin cascade system. There is a great deal of literature devoted to the mechanisms which regulate renin release. Renin is produced and secreted by juxtaglomerular (JG) cells, which are differentiated smooth muscle cells located in the renal afferent arteriole. There are four major mechanisms which regulate renin release. These include the baroreceptors located in the renal afferent arteriole, the sympathetic nervous system, the macula densa, and negative feedback of AII. Agents such as prostaglandins and calcium may mediate some of these mechanisms.

Goldblatt et al. (1934) in their pioneer experiments, induced hypertension by constriction of the renal artery in the dog and postulated that renal ischemia promoted the release of a pressor substance into the circulation. The substance was renin. Tobian et al. (1959) suggested that renal baroreceptors in the afferent arteriole

'stretch' in response to renal perfusion pressure, signaling the release of renin. The presence of baroreceptors was confirmed using the nonfiltering, denervated kidney model, which responded to hemorrhage and suprarenal aortic constriction by increasing renin release (Blaine et al., 1971). The role of the macula densa in control of renin release was suggested by Goormoghtigh (1945). It has been demonstrated that chloride as well as sodium transport into the macula densa may be the signal controlling renin release (Opava-Stitzer and Martinez-Maldonado, 1976).

Sympathetic nerve endings surround JG cells, which contain β-adrenergic receptors on their membranes. When the renal nerves are stimulated without changing renal blood flow, glomerular filtration, or sodium excretion, an increase in renin release occurs which is specifically blocked by propranol (Taher et al., 1978). Hence, β-adrenergic stimulation of renin release occurs without evoking baroreceptors or macula densa signals. AII suppresses renin secretion by a direct effect on the JG cell. This represents a means of 'feedback regulation' of renin release. The inhibitory effect of AII can be blocked by specific AII antagonists (Keeton et al., 1980). Potassium also plays a role in renin release, an effect that is probably manifested through the macula densa mechanism (Linas, 1981). Vasopressin suppresses renin release (Bunag et al., 1967).

1.4 Urine Concentrating Ability

Urine concentrating ability is influenced by both neuroendocrine and intrarenal factors. The renal mechanisms for a maximally concentrated urine require the distal tubules and collecting ducts to respond adequately to ADH so as to allow water reabsorption to occur.

The medullary thick ascending limb of Henle may also respond to ADH to increase NaCl reabsorption (Hall et al. 1980; Herbert et al., 1981; Greger, 1981; Knepper et al., 1983). Furthermore, the hypertonicity of the medulla must be maintained. This involves adequate sodium chloride delivery to the loops of Henle, normal rates of sodium chloride transport out of the thin and thick segments of the ascending limbs, low medullary blood flow, delivery of adequate amounts of urea to the medulla, and its subsequent reabsorption by urea permeable collecting ducts. Finally, there must be low rates of fluid delivery to inner medullary collecting ducts, where maximum concentration of urine occurs.

The accumulation of urea in the medulla is of importance for several reasons. It accounts for approximately 50% of the medullary interstitium against which urine is concentrated and it may drive passive NaCl reabsorption from the thin ascending limb of Henle (Kokko et al., 1972; Kawamura et al., 1976; Imai et al., 1974).. Urea delivered to the papillary collecting duct comes from the glomerular filtrate, urea secretion by the pars recta (Kawamura et al., 1976), and ascending limb (Imai et al., 1974).

To date, evidence supports the counter-current multiplication system as the most likely explanation of urine concentrating mechanisms. Active Cl transport was implicated in the thick ascending limb of Henle (TAL) as the primary driving force in the counter-current multiplication system responsible for urinary concentration and dilution (Burg et al., 1973; Rocha et al., 1973). There is recent evidence that the TAL actually transports Cl actively in co-transport with primary Na transport (Herbert et al., 1981; Greger, 1981; Knepper et al., 1983) This co-transport also involves the K ion (Greger et al., 1981).

Secondary active Cl transport is driven by the electrochemical gradient for Na from the lumen to the cell interior. This depends on the baso-lateral Na-K-ATPase for removing Na from the cell to keep the gradient intact.

1.5 The Effects of Electrolyte Depletion on Water Metabolism

The development of potassium depletion affects water and electrolyte homeostasis through central as well as renal mechanisms. The water-losing disorder characterized by polydipsia, polyuria and a renal concentrating defect in hypokalemia has been recogenized for several decades (Relman et al., 1958). Changes in renal hemodynamics and medullary solute concentration, have been postulated as possible mediators of the concentrating defect characteristic of potassium depletion (reviewed in Berl, 1980). A decrease in medullary blood flow has been shown which could not effect a 'washout' of medullary solute content (Whinnery et al., 1979; Peterson, 1984). Antidiuretic hormone release appears to be normal or elevated as a consequence of hyperosmolality in potassium-deficient rats (Paller and Linas, 1983) and the impairment in urinary concentrating ability seems unrelated to circulating levels of ADH (Berl et al., 1977).

Data from clearance studies in potassium depleted rats suggest that both impaired concentrating and diluting ability are the result of reduced Na transport by the TAL into the medullary interstitium (Eknoyan et al., 1970). A striking impairment in TAL function has been demonstrated in potassium depleted rats by single nephron perfusion, and measurement of solute concentration in the TAL fluid presented to the early distal site (Gutsche et al., 1984). This impairment can be reversed when by systemic infusion of potassium suggesting that net NaCl

reabsorption by the TAL is impaired because decreases in peritubular K concentration limit Na^+ efflux across the peritubular membrane by decreasing the activity of the Na-K-ATPase pump (Gutsche et al., 1984).

Smith and Lasater (Berl, 1980) noted that dogs increased their water intake soon after being placed on a potassium deficient diet. Brokaw (1953) made similar observations in rats and also found that water turnover was further augmented by removal of sodium from the potassium deficient diet. Berl et al. (1977) were able to demonstrate that the increase in water intake precedes the development of a concentrating defect in potassium depletion, and that the polyuria of this disorder is largely a consequence of the polydipsia.

The fact that increased water intake precedes a defect in water conservation suggests that the stimulus to thirst is not hyperosmolality but rather of a nonosmolar nature, which has lead to attempts to assess some of the potential nonosmolar factors (Berl, 1980). Since potassium depletion is characterized by persistently elevated plasma renin activity (PRA) (Davis et al., 1976; Linas, 1981), and angiotensin II is a potent dipsogen (Fitzsimons, 1976), it is possible that this is the mechanism responsible for the polydipsia. In this regard, studies employing converting enzyme inhibitor (SQ14225) have been inconclusive, since the agent itself appears to cause an increase in water turnover in normokalemic rats (Berl, 1980; Schiffrin et al., 1982). Therefore, although potassium depletion is characterized by a primary polydipsia, its mechanism remains unresolved.

1.6 Angiotensin and Central Thirst Mechanisms

The current view is that thirst results from a depletion of either cellular or extracellular compartments of the body (Fitzsimons, 1972). Thirst is believed to arise following depletion of cellular water as a result of activation of specialized cells, osmoreceptors or possibly sodium receptors located in the brain. The drinking induced by extracellular volume depletion appears to be mediated by the production of elevated levels of circulating angiotensin II and perhaps by activation of vascular baroreceptors. Therefore, by employing a variety of manipulations that induce fluid deficits in these compartments investigators can evaluate various thirst control mechanisms.

Some of the effects of AII mediated via the brain are: i) a neurogenic pressor effect; ii) increased adrenocorticotrophic hormone secretion; iii) increased antidiuretic hormone secretion; and iv) evoke water ingestion. These central effects of AII involve synergistic responses, both behavioural and physiological, directed at maintenance of extracellular fluid homeostasis.

The first evidence implicating renal renin in thirst was the observation that ligation of the inferior vena cava in the abdomen just below the liver was a less effective stimulus for drinking in the nephrectomized rat than in either the normal rat or the rat made anuric by ureteric ligation (Fitzsimons, 1964). Since most of the known physiological effects of renin are mediated by AII (Peart, 1976) investigators examined the effects of AII on drinking. Short-latency and vigorous drinking behaviour could be induced by injecting small quantities of angiotensin into the anterior hypothalamus and preoptic region in the rat (Epstein et al., 1970). Renin and the precursors of

AII are also effective, but by use of specific antagonists of the various reactions that lead to the formation of angiotensin II, it has been shown that their effects are mediated through angiotensin II (Epstein, 1978; Fitzsimons et al., 1978).

Since AII exerts its effects by way of interaction with brain receptors, the question of where such effects occur becomes important. Sufficient evidence for inferring a locus of angiotensin-receptive neurons concerned with behaviour requires agreement of evidence from different experimental techniques. Circulating AII must meet the criteria of accessibility, meaning that the blood-borne hormone must be accessible to neurons presumed to be affected by it. There are structures collectively termed the circumventricular organs, in regions of the brain that possess a reduced, or complete absence of the blood-brain barrier. These structures include the area postrema, the neurohypophysis, the median eminence, the organum vasculosum laminae terminalis, and the subfornical organ. These are regions which possess vascular specializations that allow rapid equilibration of blood-borne peptide hormones with the interstitial fluid. Largely for this latter reason, the possible action of AII at these structures have been evaluated. Direct evidence for AII dipsogenic receptor localization is obtained from studies on animals where drinking behaviour is used as the experimentally dependent variable.

Experiments (Epstein, 1978; Swanson et al., 1973) have demonstrated that intracranial AII was more effective than was intravenous AII in eliciting drinking. This indicated a central site of action of AII for the effect. Johnson and Epstein (1975) reported that extrahypothalamic application of AII produced dipsogenesis only

following diffusion of the injected substance to unknown sites of action bordering the cerebral ventricles. One structure suggested to be involved in drinking control in the rodent, is the subfornical organ (SFO) (Simpson et al., 1972). The SFO is a small nodule of neuro-gliovascular tissue, situated in the midline on the rostal wall of the third ventricle, near the point where the choroid plexes of the lateral ventricles join together. Three regions are identified in the rat SFO (Dellman et al., 1976; Phillips et al., 1974): a rostral region dominated by nerve fibres interspersed with relatively few neurons and glia; the central region occupying the largest area of the SFO and containing most of the neuronal perikarya and glia; and the caudal region dominated by nerve fibres. Efferent projections from many SFO neurons terminate within the diencephalon, and in particular in the organum vasculosum and the supraoptic nucleus (Miselis et al., 1977; Shapiro et al., 1978; Renaud et al., 1983). The latter efferent projection may participate in the release of vasopressin following activation of AII receptors in the SFO (Renaud et al., 1983).

The SFO possesses fenestrated capillaries surrounded by wide perivascular spaces, characteristics believed to be responsible for the absence of the blood-brain barrier. Many circulating molecules that are normally barred from the brain thus may gain rapid access to the SFO (Broadwell et al., 1976) and influence its function. Early reports of involvement of the SFO in AII drinking indicated that injection at the SFO of low doses of the hormone provoked short-latency drinking in rats (Simpson et al., 1973). Lesions of the SFO reduced drinking elicited by large doses of AII administered systemically (Abdelaal et al., 1974; Kucharczyk et al., 1976; Simpson et al., 1975). Experiments have

confirmed that the SFO does possess receptors for the dipsogenic action of AII, involving intracranial injections of AII directly into the SFO provoking short-latency dose dependent drinking (Simpson et al., 1978). The threshold dose for AII drinking at the SFO was lower than for any other locus that was tested, including the preoptic area, lateral ventricle, dorsal or rostroventral third ventricle, and ventral fornical commissure. Additional studies in rats indicated that the effect on drinking of SFO lesions is permanent and selective for models of extracellular dehydration (Simpson et al., 1978). Drinking to cellular dehydration was unaltered following these lesions.

The criteria of pharmacological specificity for localization of AII dipsogenic receptors has also been evaluated for the SFO. Intracranial infusion of saralasin acetate, a specific competitive antagonist of AII, reduced or prevented the drinking elicited by intravenous infusions of low doses of AII (Simpson et al., 1978). Maximal suppression of elicited drinking was observed if the antagonist was applied directly to the SFO and not to adjacent tissue or ventricles. The effect of SFO infusions of saralasin was specific for AII drinking in that the treatment had no effect on drinking to osmotic stimuli (Simpson et al., 1978).

The SFO thus satisfies some important criteria for localization of receptors for the dipsogenic action of circulating AII. The structure lacks the blood-brain barrier, and circulating AII is accessible to neurons located there. Second, direct injection of low doses of AII produced short-latency drinking at the SFO, a site sensitive to AII. Selective lesions at the SFO specifically and permanently eliminated drinking to intravenous infusions of AII.

Fourth, antagonism of AII at the SFO prevented drinking to AII, showing that the action of AII at the SFO possesses pharmacological specificity.

The tissue bordering the optic recess of the third ventricle, including the organum vaculosum laminae terminalis (OVLT) may also be a site of AII action. The OVLT, like the SFO, has fenestrated capillaries. Lesions centered in the region of the optic recess produce dramatic changes in body fluid homeostasis (Fitzsimons et al., 1978; Buggy et al., 1977). Such destruction includes damage to the OVLT, the median preoptic region, and adjacent parenchyma and ependyma. The OVLT has been localized as the tissue responsible for the deficits in drinking to AII applied intraventricularly or systemically following such lesions. Anatomical data describing the efferent pathways of the SFO indicate that its neurons project to and through this region (Miselis et al., 1977; Shapiro et al., 1978). This suggests that the deficits in AII drinking produced by the diencephalic lesions may be due in part to destruction of efferents of the SFO.

Current literature suggests that two of the circumventricular organs, the SFO and OVLT, are sites of dipsogenic action of AII, and that they subserve AII drinking in different manners. The subfornical organ (SFO) is one central site at which a complete analysis of angiotensin II (AII) dipsogenic efficacy has been accomplished. This does not preclude a dipsogenic action of AII at other loci.

1.7 The Present Study - Statement of Purpose

The data presented suggests the possibility that the increased water intake produced by potassium depletion may be due to stimulation of AII receptors in the SFO by high levels of circulating hormone. The objectives of this study are:

1) to define more closely the role of the renin-angiotensin system in dietary electrolyte depletion (defined earlier as restriction of K alone or K, Na and Cl) induced polydipsia; and

2) to determine whether ablation of CNS structures (SFO) involved in angiotensin induced water intake will prevent the development of polydipsia induced by a K-free diet and by a low salt diet (defined as a low NaCl/K-free diet).

2.0 GENERAL METHODS

Methods common to all experiments in this study are described in this section. Procedures and techniques specific to individual experiments are described in Section 3.

2.1 Animals, Care and Diet

Male Sprague-Dawley rats (Charles River, Montreal, P.Q. and from a colony at the University of Ottawa) weighing 175-225 g at the beginning of the study were used. They were housed in individual, wire mesh, metabolic cages in a temperature controlled room on a 14 h:10 h light-dark cycle and allowed ad libitum access to food and distilled water. At the start of the study, animals were randomly divided into groups.

Experimental animals with SFO-lesions, or sham-operation rats were operated on using sterile surgical techniques, and were allowed a minimum of 7-days post-surgical recovery before the study began. Water intakes and urine outputs (± 1.0 g), as well as body weights (± 1.0 g), were measured at the same time each day. The study began when body weights were equal to or surpassed pre-surgical levels.

Tables 1 and 2 list the chemical composition of the three synthetic diets used in this study. The electrolyte content is expressed in mmoles per 100 g food and the chemicals used are expressed in g per 1.0 kg of diet.

Table 1

Electrolyte content (mmoles/100 g food) of rat diets used in the present study

	Control	K-free Diet	Low NaCl/K-free diet
Dietary electrolytes			
Sodium (Na)	16.3	16.3	1.5
Potassium (K)	27.47	0	0
Chloride (Cl)	16.3	16.3	1.5
Magnesium (Mg)	19.37	14.72	11.13
Phosphorous (P)	9.14	9.35	8.60
Calcium (Ca)	14.2	14.23	14.23
Ca/P ratio	1.56	1.52	1.65

Table 2

Chemical composition of rat diets used in the present study

	Control	K-free diet	Low NaCl/K-free diet
Chemical ingredients			
Sodium Phosphate Monobasic ($NaH_2PO_4.H_2O$)	-	12.9	-
Sodium Chloride (NaCl)	9.5	4.07	0.877
Potassium Phosphate Dibasic (K_2HPO_4)	15.93	-	-
Potassium Citrate Monohydrate (K citrate)	9.93	-	-
Magnesium Phosphate dibasic-trihydrate ($MgHPO_4\ 3H_2O$)	-	-	14.994
Magnesium Chloride ($Mg\ Cl_2.6H_2O$)	-	9.59	-
Magnesium Carbonate n-hydrate ($4\ MgCO_3.Mg(OH)_2nH_2O$)	8.35	4.31	1.09
Calcium Carbonate ($Ca\ CO_3$)	14.25	14.24	14.24
Teklad electrolyte free diet (Teklad test charts, Madison, Wisc.)	942.04	954.9	968.8
Total weight	1 kg	1 kg	1 kg

- values expressed in units of grams/kg food

2.2 Blood Sampling and Analysis

i) Cardiac Puncture: Animals were anesthetized with halothane and the heart was located by palpating the chest wall. A 26 gauge needle, attached to a 3 ml syringe, was inserted into the heart through the chest wall. A 1-2.0 ml blood sample was withdrawn of which 0.5 ml was immediately transferred to a chilled heparinized Eppendorf tube, and the remainder to a chilled test tube containing EDTA. The animals were returned to their cages to recover.

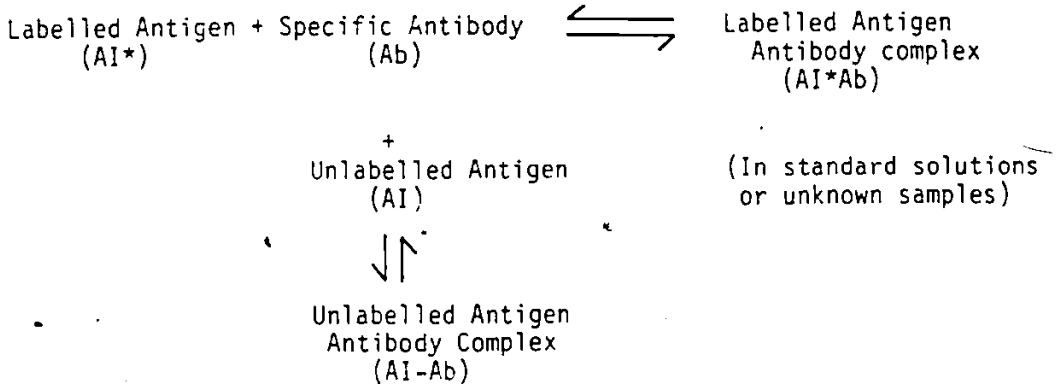
ii) Analysis: The blood samples were kept on ice for approximately 30 to 90 minutes until aliquoted for analysis. The whole blood in the heparinized Eppendorf tubes was centrifuged in an Eppendorf Centrifuge 3200 C (Brinkmann Instruments, Rexdale, Ontario) to separate the plasma from the cells. The plasma was used for analysis of osmolality and electrolytes (Na^+, K^+ and Cl^-). Plasma osmolality was measured in 8 µl duplicate samples on a Wescor 5100 CXR Vapour Pressure Osmometer (Johns Scientific) or 50 µl duplicate samples in a Precision uOsmette Model 5004 freezing point depression osmometer (Precision System, Inc., Sudbury, Massachusetts). Plasma Cl (10 µl) was measured coulometrically using a Radiometer CMT 10 Chloride Titrator (Radiometer, Copenhagen). Plasma Na and K were measured by flame emission spectroscopy using a IL Model 443 Flame Photometer (Instrumentation Laboratory Inc., Lexington, Ma.) with lithium as the internal standard. Plasma electrolytes were also measured in duplicate.

The whole blood in the test tubes containing EDTA was centrifuged in a Damon/IEC HN-SII Centrifuge (Needham, Ma.) at 4° C. The plasma was aliquoted into micro-centrifuge tubes and stored at -20° C until radioimmunoassay (RIA) analyses for plasma renin activity (PRA), and

angiotensin I (AI) concentration could be carried out. Plasma renin activity and angiotensin I concentration were measured by RIA using a kit from New England Nuclear (North Billerica, Ma.). Under the conditions of this assay, the accumulation of AI is favoured by allowing the endogenous renin and substrate to react in the presence of reagents which inhibit both plasma converting enzyme and proteolysis by angiotensinases.

Samples for RIA analyses were collected into EDTA containing tubes as described earlier and were stored at -20° C until analyzed. Prior to the RIA procedures, the samples were thawed at 4° C and extracted. Once extracted, the samples were counted for 10 min on a LKB Walloc 1282 compugamma Universal Gamma Counter.

The amount of generated AI is measured by radioimmunoassay (RIA). The basic principle of RIA involves competition between radioactive and non-radioactive antigen for a fixed number of antibody binding sites.



If increasing amounts of non-radioactive Ag (ie. standards or unknowns) and a fixed amount of tracer are allowed to react with a constant and limiting amount of antibody, a decreasing quantity of

radioactive antigen is bound to the antibody. The separation of bound from free antigen is accomplished by differential absorption of the free material onto activated charcoal and the concentration of Ab-bound Ag in the supernatant is determined by gamma counting.

The relationship of bound to added can be expressed as a standard curve from which values of the unknowns may be obtained by interpolation. Plasma renin activity is expressed as ng/ml/hr of angiotensin I generated.

For detailed information on the reagents and step by step procedure of the assay for analysis of plasma samples, the reader is referred to the Angiotensin I ^{125}Iodine Radiommunoassay Kit Cat. No. NEA-026, 022 Instruction Manual.

(iii) Abdominal Aorta - Terminal Sample: All animals were anaesthetized with pentobarbitol sodium (25-35 mg/kg, i.p.) and a 6-8 cm midline abdominal incision was made. The intestines were reflected to one side and a section of the aorta was isolated. An 18 gauge needle, attached to a heparinized 3 ml syringe, was inserted into the abdominal aorta and a 1.5 ml blood sample was withdrawn for analysis of plasma electrolytes and osmolality. The 3 ml syringe was quickly substituted with a 3 ml syringe containing ethylene diamine tetra-acetate (EDTA) for analysis of PRA and AI concentration. The blood samples were processed as previously described. This method of taking blood was replaced by the cardiac puncture procedure (section 2.2(i)) so as to standardize the method of collecting repeated blood samples from control and experimental animals.

2.3 Surgical Procedures - Stereotaxic lesions of the SFO

In the rat the SFO is a relatively small structure projecting into the lumen of the third ventricle at the intersection between the two lateral and the third ventricles. It lies approximately 2 mm above the dorsal edge of the anterior commissure, and its stereotaxic coordinates are (Pelligrino and Cushman, 1967): frontal 0.0 to -0.4, and dorsoventral -4.5 to -4.75 with respect to bregma and cortex, respectively.

Standard stereotaxic procedures were used in Innovar-Vet-anesthetized rats (I.M. dosage is 0.13 ml/kg) by passing a 1.0 mA anodal direct current (Lesion Maker, C.H. Stoehing Co., Chicago, Illinois) through a stainless-steel bipolar electrode (RMI, model SNE-200) for a duration of 15-20 sec. Penetrations of the electrode were made into the SFO at three different rostro-caudal levels. The stereotaxic coordinates used for SFO lesions are shown in Table 3. Figure 1 is a schematic diagram of the rat brain showing the SFO.

Table 3

Stereotaxic Coordinates' Used for SFO Lesions

Intracranial Site	Angle from Perpendicular (measured towards midline)	mm from Bregma: Anterior-posterior	Lateral	Vertical*
Subfornical Organ (SFO)	12^{o}	+0.2 - 0.2	1.0	4.4 - 4.6
Sham operation+	12^{o}	+0.2 - -0.2	1.0	3.4 - 3.6

*The vertical coordinate is the distance from the surface of the cortex to the most ventral position of the electrode.

'Modified from the atlas of Pelligrino and Cushman (1967).

+No current passed.

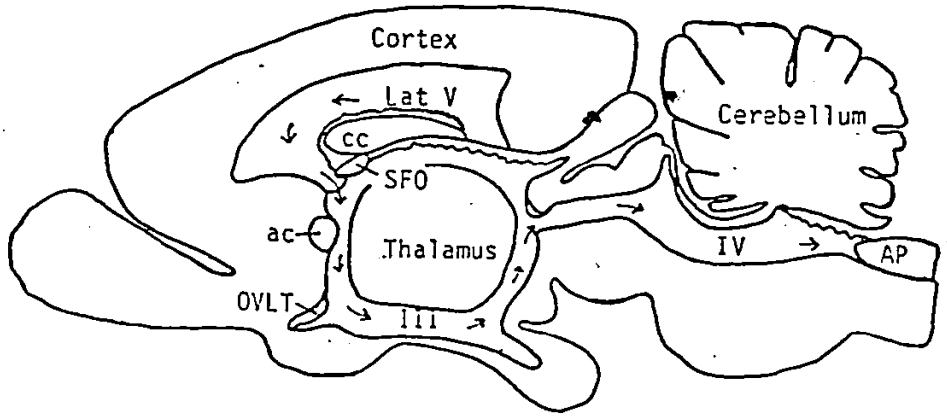
Figure 1a:

Location of the SFO in a mid-sagittal schematic of the rat brain, ac, anterior commissure; AP, area postrema; cc, corpus callosum, OVLT, organum vasculosum of the lamina terminalis; III, third ventricle; IV, fourth ventricle; Lat V, lateral ventricle; SFO, subfornical organ.

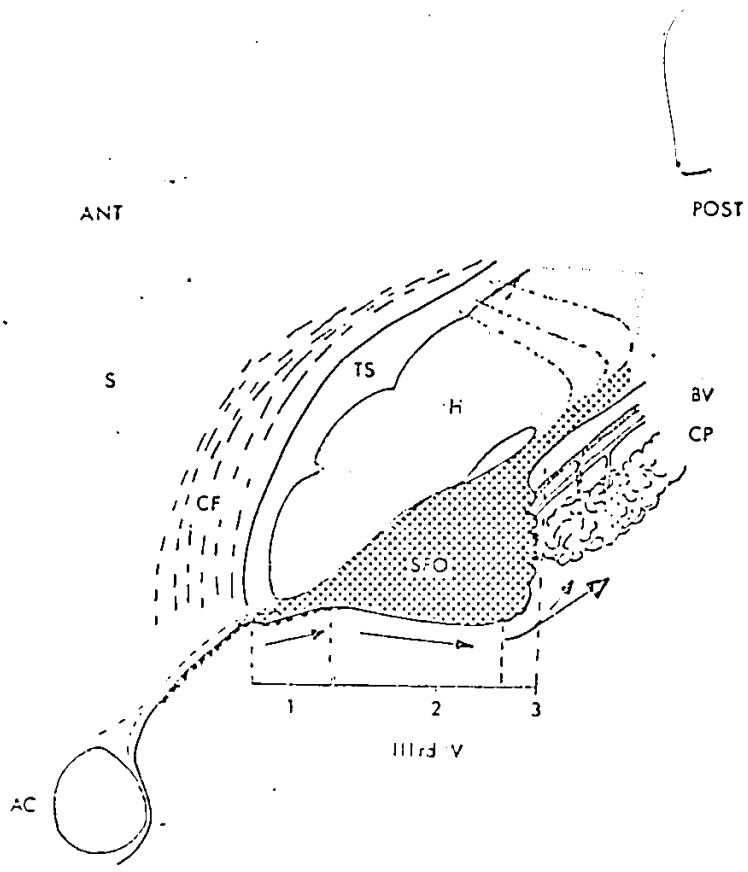
1b:

A schematic of a sagittal view showing 3 zones and relative position of the SFO to other structures. BV, blood vessels: subfornical artery dorsal and vein ventral; CP, choroid plexus; AC, anterior commissure; CF, column of fornix; H, hippocampus commissure; S, septum; TS, septalis triangalaris; IIIrd V, third ventricle. Arrows indicate possible flow patterns of the cerebrospinal fluid. (Phillips et al., 1974).

A.


Cortex
Lat V
cc
SFO
ac
Thalamus
OVLT
III
Cerebellum
IV
AP

B.


ANT
POST
S
TS
H
BV
CP
CF
SFO
1
2
3
IIIrd V
AC

2.4 Histology

All animals with stereotaxic lesions of the SFO or sham operations were perfused transcardially into the left ventricle of the heart with 60 ml of 10% buffered formalin. The brains were sectioned and set in paraffin. The placements of the lesions were determined in serial sections of the brain stained with cresyl violet with reference to a rat stereotaxic atlas (Pelligrino and Cushman, 1967). Analyses of each brain involved examination of serial coronal sections to determine the extent of damage to the subfonrical organ (SFO). Following examination of histological sections each rat was assigned to an experimental group (ie. sham-operation or lesions of the SFO sustaining damage to the body-caudal region or damage to the caudal region alone, otherwise the animal was discarded from the study if the lesions did not extend to include the SFO). All data was then statistically analysed according to the experimental grouping of these animals.

2.5 Statistical Analyses

All data were analyzed on a Wang 600 series computer. Quantitative results are expressed throughout as Mean ± Standard error of the mean (SEM). Paired and unpaired t-tests were used when only two related or unrelated groups were compared. For multiple comparisons a one-way ANOVA or two-way ANOVA was used depending on the number of independent variables being considered. A Scheffe test was used to determine where the statistical significance occurred in the ANOVA. A probability less than 5% ($p < 0.05$) was considered significant.

3.0 EXPERIMENTAL RESULTS AND DISCUSSION

The series of experiments which make up this study are described in the four parts of this section and the results obtained in each part are considered separately. A general discussion and summary of the results are given in Section 4 and Section 5, respectively.

3.1 Study 1 - Preliminary Study on Electrolyte Depletion as a Model for the Development of Polydipsia.

The current study depends on the use of a reliable model of polydipsia in order to test whether the observed drinking behaviour is mediated by the action of angiotensin II on central receptors located in the circumventricular organs of the brain. Rats fed a potassium deficient diet develop a polydipsia characterized by persistently elevated plasma renin activity (PRA) (Linas, 1981; Davis et al., 1976). Brokaw (1953) noted that water turnover was further augmented by removal of sodium from the potassium deficient diet. The purpose of the current study was to determine the possible role of the renin-angiotensin system in the development of polydipsia associated with dietary electrolyte depletion, more specifically K-depletion and salt depletion (low NaCl/ K-depletion).

METHODS

Study 1a involves a total of 30 male Sprague-Dawley rats housed in individual, wire mesh metabolic cages in a temperature controlled room on a 14 h:10 h light-dark cycle. They were allowed ad libitum access to either a dry synthetic control diet (n = 8), K-free

diet (n = 12) or low salt diet (low NaCl/K-free diet, n = 10), and ad libitum access to distilled water. Water intakes and urine outputs (± 1.0 g), as well as body weights (± 1.0 g), were measured at the same time each day for a total of 11 days. Blood samples were collected at the end via puncture of the abdominal aorta for analyses of plasma electrolytes, osmolality and plasma AI concentration and PRA. As shown in Figure 2, Study 1a, rats maintained on the low salt diet showed a significant and sustained increase in daily H_2O intake compared to controls. Therefore, the low salt diet provided a more suitable model for electrolyte depletion induced polydipsia from which further studies could be undertaken.

Another group (Study 1b) of 20 male Sprague-Dawley rats were housed and maintained under the same conditions as described above. They were allowed ad libitum access to either a control diet (n = 5), K-free diet (n = 8) and low salt diet (n = 7), and ad libitum access to distilled water. Water intake and body weights (± 1.0 g) were measured at the same time each day for a total of 5 days. Blood samples were collected via cardiac punctures at this time for analyses of plasma electrolytes and plasma osmolality.

A third group (Study 1c) of 20 male Sprague-Dawley rats were allowed ad libitum access to either a dry synthetic control diet (n = 5), K-free diet (n = 8) and low salt diet (n = 7), and ad libitum access to distilled water for a total of 11 days. Blood samples were collected via cardiac puncture on the 3rd and 11th day of the study for analyses of plasma electrolytes, plasma osmolality and plasma AI concentration and PRA.

RESULTS AND DISCUSSION

The main purpose of study 1 was to characterize the polydipsia associated with dietary electrolyte depletion (K-depletion and low NaCl/K-depletion) and the possible role of the renin-angiotensin system.

Table 4 summarizes results obtained from terminal blood samples taken via the abdominal aorta 11 days after introducing the three diets (control, K-free and low NaCl/K-free diets). Both dietary depletion groups showed decreased plasma K^+ and Cl^- concentrations whereas only the low salt diet groups showed a statistically significant decrease in plasma Na^+ concentration (control 141.75 ± 0.49 vs 138.92 ± 0.74 mEq/l plasma, $p < 0.05$). Hypokalemia was more pronounced in the K-free diet animals (control 3.77 ± 0.05 vs 1.94 ± 0.05 mEq/l plasma, $p < 0.005$). Both groups developed a hypoosmotic plasma compared to control diet fed animals.

Figures 2 and 3 show 24 h intakes of water and urine outputs, respectively, in the three groups studied. A statistically significant increase in water intake occurred as early as day 3 in the low salt diet group (control 12.77 ± 1.06 vs 30.44 ± 2.47 ml H_2O/24 h/100 g bwt, $p < 0.005$). The peak of polydipsia occurred by day 4 for both dietary depletion groups, whereas only the low salt diet group maintained a significantly increased water intake throughout the 11 day dietary electrolyte depletion study. The increased daily water intake of the low salt diet group was significantly higher than the K-free diet animals throughout this period. Both depletion groups developed polyuria concurrently with the polydipsia.

Figure 4 shows the mean daily body weights of rats in the control, K-free diet and low salt diet groups of this study. The low

Plasma electrolytes and plasma osmolality in control, K-free diet and low NaCl/K-free diet groups. Results are expressed as mean ± S.E.M. The number of animals are shown in parentheses. Blood samples were collected via abdominal aorta from rats 11 days after introducing the diets. *$p < 0.05$ and **$p < 0.005$ compared to control, +$p < 0.05$ and ‡$p < 0.025$ compared to K-free diet group using one-way ANOVA and Scheffé test.

TABLE 4

Study 1

	Control Diet (n = 8)	K-Free Diet (n = 12)	Low NaCl K-Free Diet (n = 10)
Plasma Electrolytes (mEq/l)			
Na^+	141.75 ± 0.49	140.48 ± 0.54	*138.92 ± 0.74
K^+	3.77 ± 0.05	**1.94 ± 0.05	‡** 2.47 ± 0.11
Cl^-	102.1 ± 0.81	**93.5 ± 0.89	+**90.85 ± 1.19
Plasma Osmolality ($mOsm/Kg\ H_2O$)	298.9 ± 0.85	*293.09 ± 1.46	*288.9 ± 2.47

Figure 2; Study 1a;

24 h water intakes by control, K-free diet and low NaCl/K-free diet groups. Results are expressed as means ± S.E.M. The number of animals are shown in parentheses. Data analysed using one-way ANOVA and Scheffé test.

$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$ compared to control.

$^{+}p < 0.25$, $^{\ddagger}p < 0.01$ and $^{\ddagger\ddagger}p < 0.005$ compared to K-free diet group.

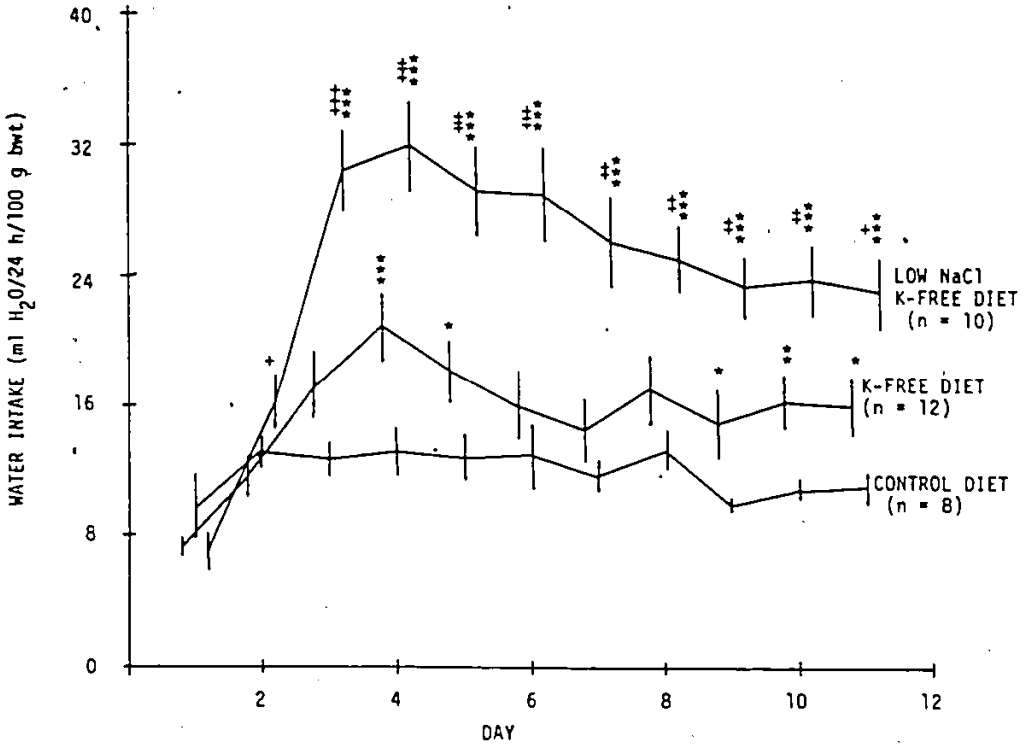

WATER INTAKE (ml H_2O/24 h/100 g bwt)
40
32
24
16
8
0
2
4
6
8
10
12
DAY
LOW NaCl
K-FREE DIET
(n = 10)
K-FREE DIET
(n = 12)
CONTROL DIET
(n = 8)

Figure 3; Study 1a:

24 h urine output by control, K-free diet and low NaCl/K-free diet groups. Results are expressed as means ± S.E.M. The number of animals are shown in parentheses. Data anlyzed using one-way ANOVA and Scheffé test.

$^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.005$ compared to control.

$^{+}p < 0.05$ and $^{+++}p < 0.005$ compared to K-free diet group.

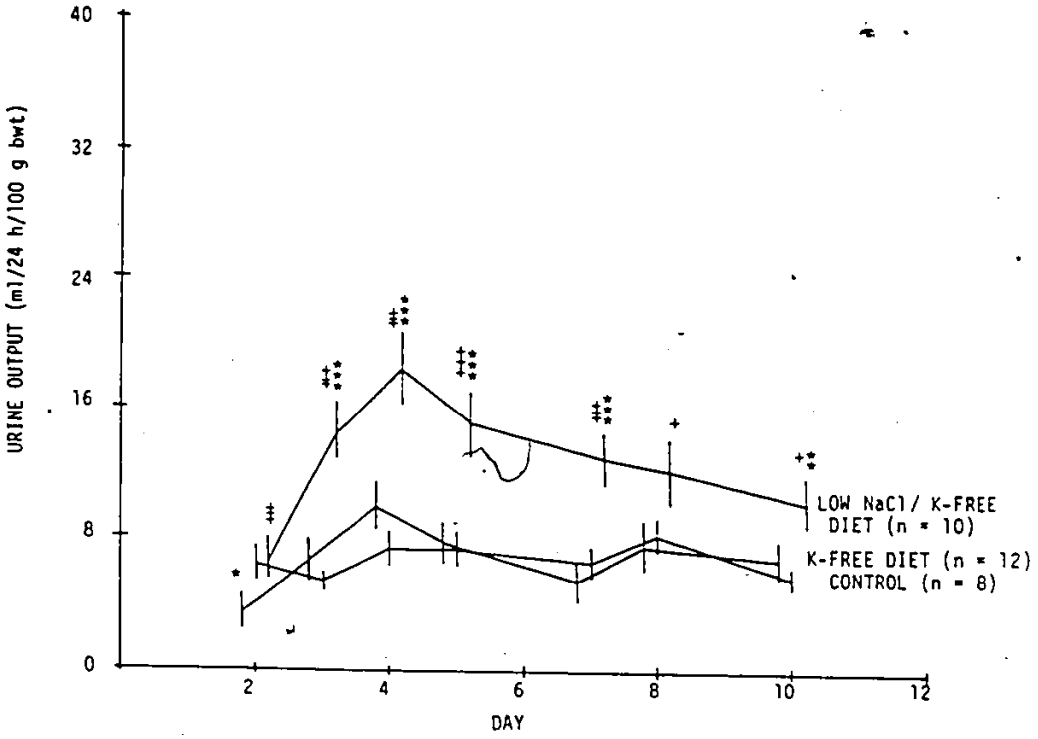
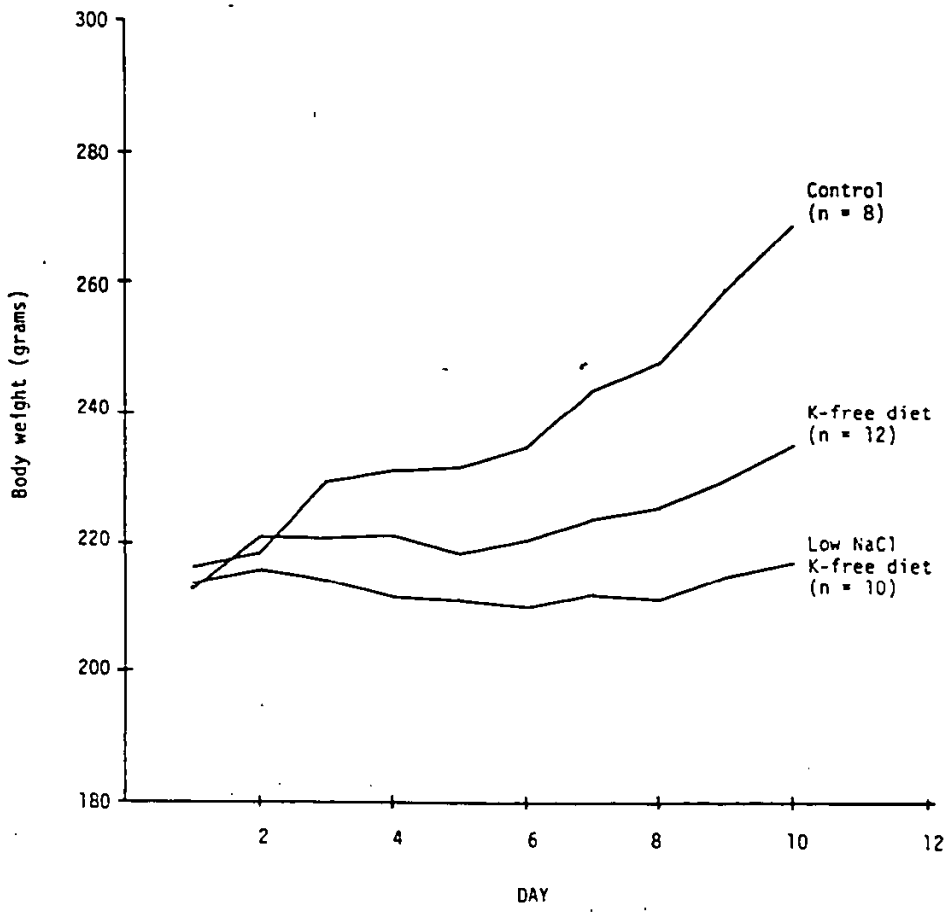

URINE OUTPUT (ml/24 h/100 g bwt)
40
32
24
16
8
0
2
4
6
8
10
12
DAY
LOW NaCl/ K-FREE DIET (n = 10)
K-FREE DIET (n = 12)
CONTROL (n = 8)

Figure 4; Study 1a:

Mean daily body weight of control, K-free diet and Low NaCl/K-free diet groups. Results are expressed as means. The number of animals are shown in parentheses.


300
280
260
240
220
200
180
Body weight (grams)
2
4
6
8
10
12
DAY
Control
(n = 8)
K-free diet
(n = 12)
Low NaCl
K-free diet
(n = 10)

salt diet animals remained at a relatively constant body weight throughout the 11 day study. On the other hand, the K-free diet group showed a slow but steady increase in body weight. Animals maintained on a control diet showed the normal rate of growth (body weight) from day to day. The greatest retardation of growth occurred in the low salt diet animals.

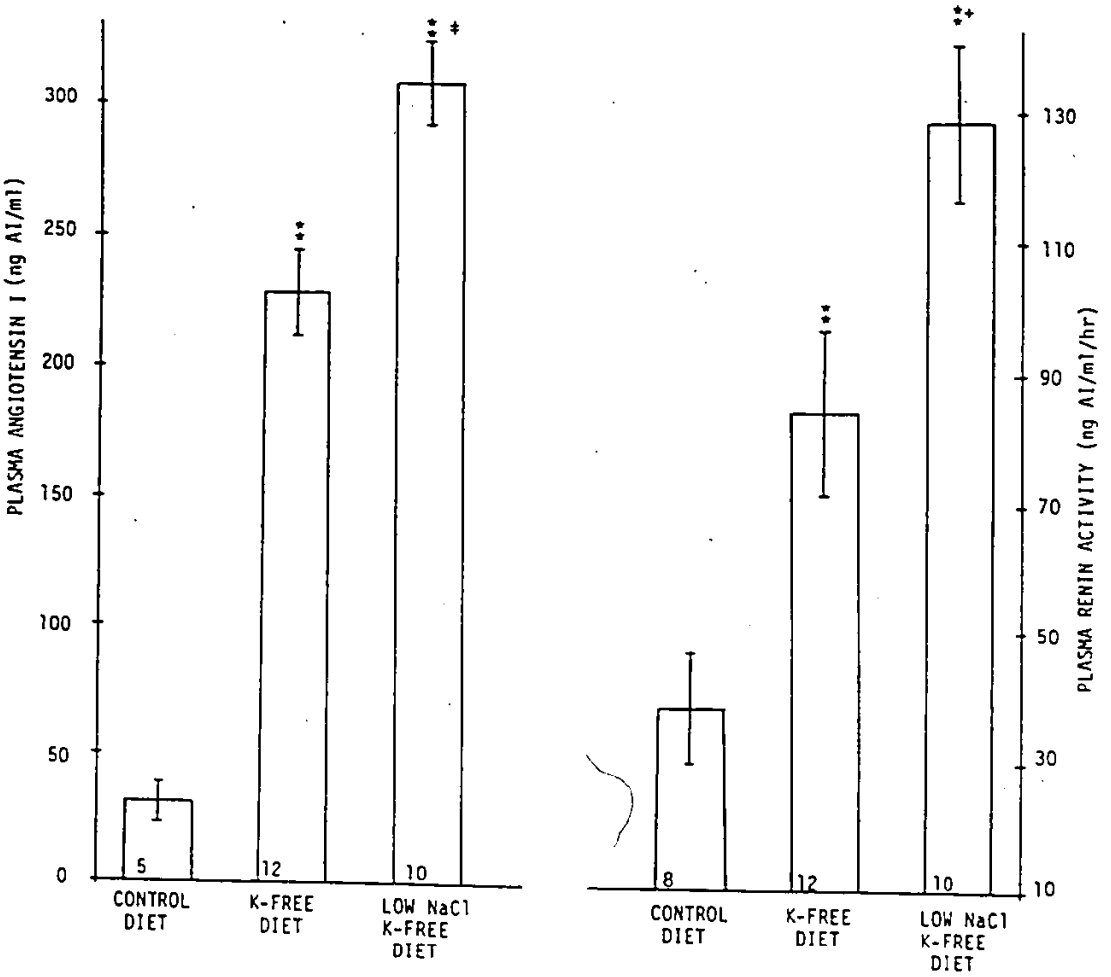
Figure 5 is a histogram of plasma renin activity (PRA) and angiotensin I(AI) concentrations in control, K-free diet and low salt diet groups, sampled on day 11 of the study. Both groups show significantly elevated AI and PRA plasma levels compared to control animals. The low salt diet group had significantly higher AI concentrations (308.69 ± 16.61 vs 227.64 ± 16.47 ng AI/ml) and PRA (128.21 ± 12.05 vs 83.96 ± 12.45 ng AI/ml/hr) levels than the K-free diet animals ($p < 0.005$, respectively). In both cases the levels were significantly elevated from control AI concentration (31.03 ± 7.67 ng AI/ml) and PRA (37.97 ± 8.78 ng AI/ml/hr) levels ($p < 0.005$).

Table 5, Study 1b summarizes results obtained from blood samples taken via cardiac puncture 5 days after introducing the diets show decreased plasma K^+ and Cl^- concentrations in both experimental groups. Again hypokalemia was more pronounced in the K-free diet animals (control 4.31 ± 0.04 vs 2.52 ± 0.07 mEq/l plasma, $p < 0.005$). Both the K-free diet group (275.86 ± 1.58 mOsm/kg H_2O) and the low salt diet group (273.86 ± 1.97 mOsm/kg H_2O) developed a hypoosmotic plasma by day 5 of the study when compared to control (280.4 ± 1.12 mOsm/Kg H_2O) animals ($p < 0.05$ and $p < 0.025$, respectively).

Figure 6 shows 24 h intakes of water for of the three diet groups in study 1b. A statistically significant increase in water intake

Figure 5; Study 1a:

Plasma renin activity (PRA) and angiotensin I (AI) concentrations in control, K-free diet and low NaCl/K-free diet groups. The blood samples were collected via abdominal aorta on the 11th day of the study. The results are expressed as means ± S.E.M. The number of animals is shown in the lower left hand corner of the histogram. $^{*}p < 0.05$ and $^{\ddagger}p < 0.005$ compared to control. $^{+}p < 0.01$ and $\ddagger p < 0.005$ compared to K-free diet group using a one-way ANOVA and Scheffé test.


PLASMA ANGIOTENSIN I (ng AI/ml)
300
250
200
150
100
50
0
5
12
10
CONTROL DIET
K-FREE DIET
LOW NaCl K-FREE DIET
PLASMA RENIN ACTIVITY (ng AI/ml/hr)
130
110
90
70
50
30
10
8
12
10
CONTROL DIET
K-FREE DIET
LOW NaCl K-FREE DIET

Plasma electrolytes and plasma osmolality in control, K-free diet and low NaCl/K-free diet groups. Results are expressed as mean ± S.E.M. The number of animals are shown in parentheses. Blood samples were collected via cardiac punctures from rats 5 days after introducing the diets. *$p < 0.05$, **$p < 0.025$ and ***$p < 0.005$ compared to control, +$p < 0.025$ and ‡$p < 0.01$ compared to K-free diet group using a one-way ANOVA and Scheffé test.

TABLE 5*
Study 1b

	Control Diet (n = 5)	K-free Diet (n = 8)	Low NaCl K-free Diet (n = 7)
Plasma Electrolyte (mEq/l plasma)			
Na^+	139.8 ± 0.51	140.58 ± 0.46	+138.75 ± 0.62
K^+	4.31 ± 0.04	***2.52 ± 0.07	‡***3.08 ± 0.14
Cl^-	103.8 ± 0.8	***97.5 ± 0.57	**99 ± 1.40
Plasma Osmolality (mOsm/kg H_2O)	280.4 ± 1.12	*275.86 ± 1.58	**273.86 ± 1.97

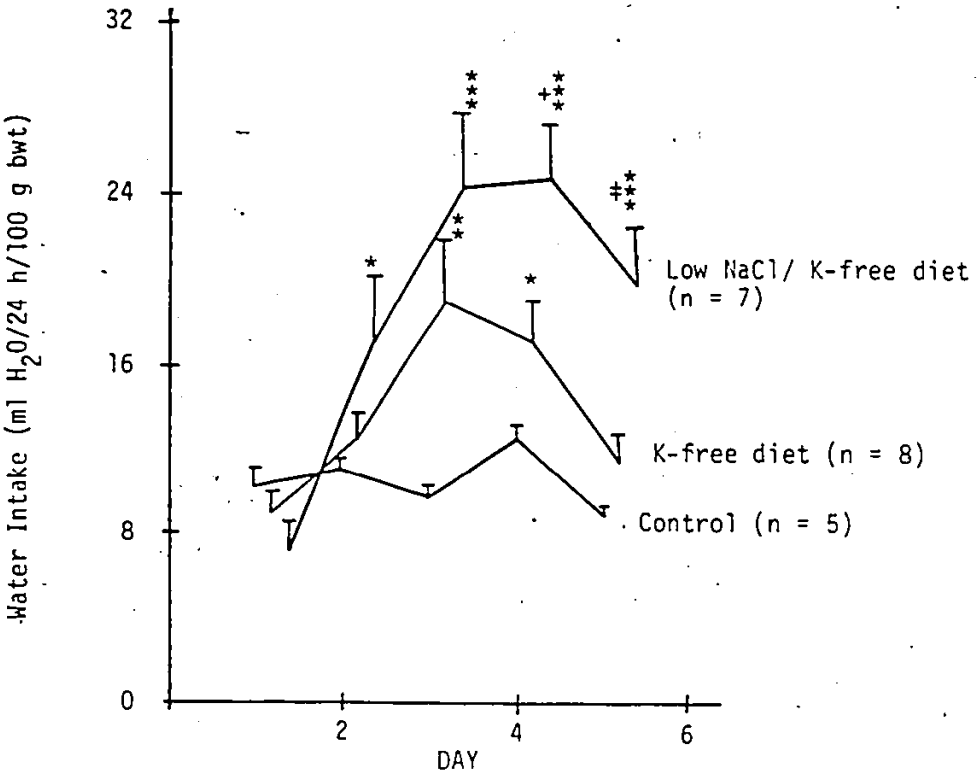
*In reference to control diet fed rats of study 1 a range of normal plasma osmolality levels in each series of animals (Study 1a, b and c [to follow p. 48]) is observed. We attribute some of these discrepancies in pOsm to animal variability. It is important to note that the supply of experimental animals was from two different sources (Charles River and a colony from the University of Ottawa when available). Furthermore, in each case we deal with a small sample size ($n < 8$). One approach would have been to pool all control data for comparison to experimental rats and in this way reduce sample variation due to a small sample size. However, we found it appropriate to have a control with each experimental group or have animals serve as their own controls. In this way we may minimize variability due to the source of rats and different times during which the studies were conduced. Also it is important to be aware that it is Study 1b which shows the lower extreme of control pOsm data. I may be that in these rats the water conserving ability is not to the same capacity as rats of the other studies and may account for the lower normal pOsm. Finally, it is important to note that in every case, rats maintained on the depletion diets develop a plasma osmolality that is isotonic or hypotonic to control diet fed animals, further supporting the fact that hyperosmolality does not mediate electrolyte depletion induced polydipsia.

Figure 6; Study 1b:

24 h water intakes by control, K-free diet and low NaCl/K-free groups. Results are expressed as means ± S.E.M. The number of animals are shown in parentheses. Data analyzed using one-way ANOVA and Scheffé test.

$^{*}p < 0.05$, $^{\ddagger}p < 0.025$ and $^{\ddagger\ddagger}p < 0.005$ compared to control.

$+p < 0.05$ and $\ddagger p < 0.005$ compared to K-free diet group.


32
24
16
8
0
2
4
6
DAY
Water Intake (ml H_2O/24 h/100 g bwt)
Low NaCl/ K-free diet
(n = 7)
K-free diet (n = 8)
Control (n = 5)

occurred as early as day 2 in the low salt diet group (control 11.07 ± 0.52 vs 17.34 ± 2.82 ml H_2O/24 h/100 g bwt, $p < 0.05$) and day 3 in the K-free diet group (control 9.70 ± 0.58 vs 19.08 ± 2.79 ml H_2O/24 h/100 g bwt, $p < 0.025$). The increased daily water intake of the low salt diet group was greater than the K-free diet animals throughout the 5 day study.

Table 6 lists the plasma electrolytes and osmolalities of blood samples taken via cardiac puncture on day 3 and day 11 after introducing the diets (control, K-free and low NaCl/K-free diets) of rats in study 1c. Both dietary depletion groups showed decreased plasma K^+ concentration by day 3, whereas only the low salt diet animals showed a decreased plasma Cl^- level (control 101.7 ± 0.51 vs 98.21 ± 0.82 mEq/l plasma, $p < 0.005$). The low salt diet group show a statistically significant decrease in plasma osmolality as early as the 3rd day of the study (control 294 ± 0.45 vs 290.07 ± 1.32 mOsm/kgH_2O, $p < 0.05$). By day 11 after introducing the diets, both dietary depletion groups developed hypochloremia and hypokalemia. The hypokalemia was most significant in the K-free diet animals (control 4.47 ± 0.08 vs 2.70 ± 0.13 mEq/l plasma, $p < 0.005$). By day 11, the low salt diet animals showed a statistically significant decrease in plasma Na^+ concentration. Both dietary depletion groups developed a hypoosmotic plasma at this time.

Figures 7 and 8 are histograms of angiotensin I (AI) concentrations and plasma renin activity (PRA), respectively, from blood samples on day 3 and again on day 11 after introducing the diets (control, K-free and low NaCl/ K-free diet) of the study. In both experimental groups, PRA and AI concentrations were significantly

Plasma electrolytes and plasma osmolality in control, K-free diet and low NaCl/K-free diet groups. Results are expressed as mean ± S.E.M. The number of animals are shown in parentheses. Blood samples were collected via cardiac punctures from rats on day 3 and day 11 of the study. Data was analysed using two-way ANOVA and Scheffé test.

TABLE 6

Study 1c

	Control diet (n = 5)	K-free diet (n = 8)	Low NaCl K-free diet (n = 7)
Day 3 Blood Samples			
Plasma Electrolytes (mEq/l plasma)			
Na^+	140.51 ± 0.52	141.23 ± 0.44	140.86 ± 0.62
K^+	4.76 ± 0.25	***3.69 ± 0.09	***3.62 ± 0.09
Cl^-	101.7 ± 0.51	100.71 ± 0.5	‡***98.21 ± 0.82
Plasma Osmolality (mOsm/kg H_2O)	294 ± 0.45	290.86 ± 1.71	*290.07 ± 1.32
Day 11 Blood Samples			
Plasma Electrolytes (mEq/l plasma)			
Na^+	140.56 ± 0.32	140.58 ± 0.46	†**138.75 ± 0.62
K^+	4.47 ± 0.08	***+++2.70 ± 0.13	‡***++3.23 ± 0.09
Cl^-	102.7 ± 0.41	***+++92.79 ± 1.13	‡***+++84.93 ± 1.06
Plasma Osmolality (mOsm/kg H_2O)	295.1 ± 0.51	***289.43 ± 1.19	†***++286.14 ± 1.39

*p < 0.05, **p < 0.01, ***p < 0.005 compared to control.
++p < 0.01, +++p < 0.005 compared to Day 3.
†p < 0.05, ‡p < 0.025, ‡p < 0.005 compared to K-free diet group.

Figure 7; Study 1c:

Angiotensin I (AI) concentrations in control, K-free diet and low NaCl/K-free diet groups. The blood samples were collected via cardiac puncture on day 3 (blank bars) and on day 11 (hatched bars) of the study. The results are expressed as means ± S.E.M. The number of animals is shown in parentheses. $^{*}p < 0.005$ compared to control, and $\ddagger p < 0.005$ compared to K-free diet group and $+p < 0.005$ compared to day 3 data using a two-way ANOVA and Scheffé test.

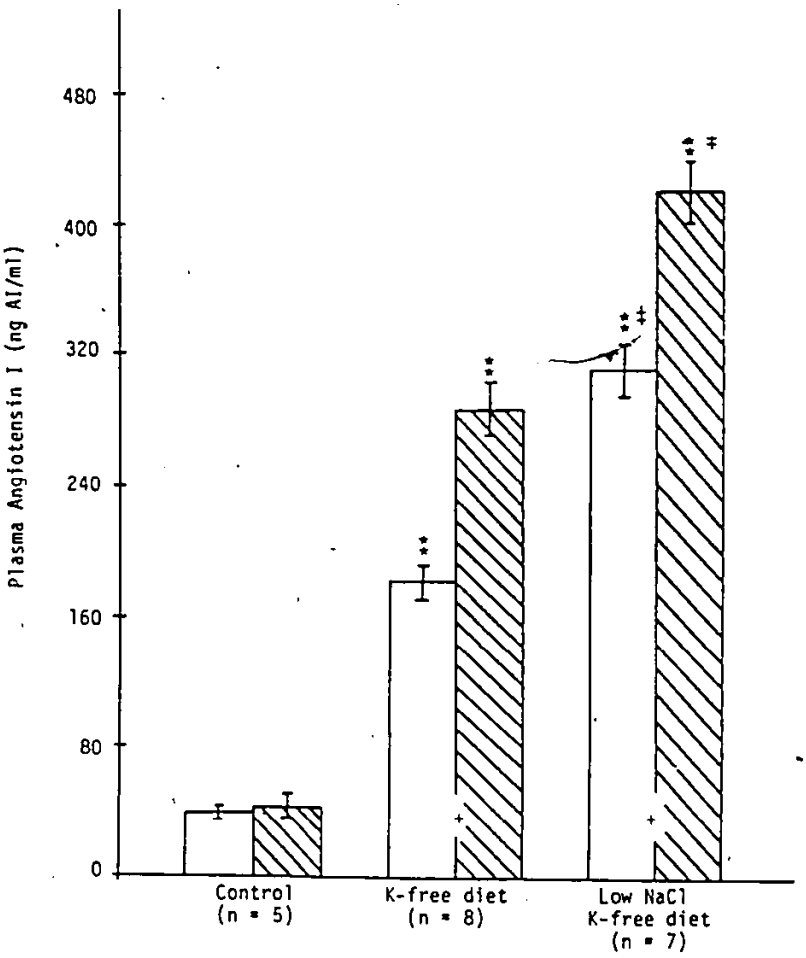
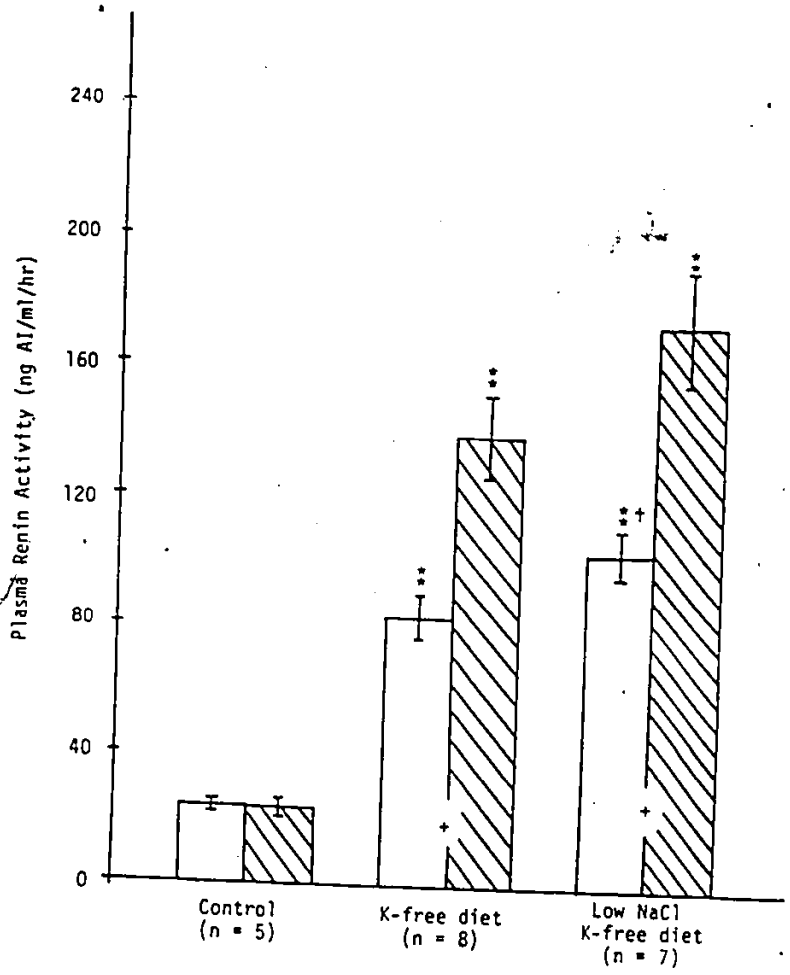

Plasma Angiotensin I (ng AI/ml)
480
400
320
240
160
80
0
Control
(n = 5)
K-free diet
(n = 8)
Low NaCl
K-free diet
(n = 7)

Figure 8; Study 1c:

Plasma renin activity (PRA) in control, K-free diet and low NaCl/K-free diet groups. The blood samples were collected via cardiac puncture on day 3 (blank bars) and on day 11 (hatched bars) of the study. The results are expressed as means ± S.E.M. The number of animals is shown in parentheses. $^{*}p < 0.005$ compared to control, $+p < 0.05$ compared to K-free diet group and $+p < 0.005$ compared to day 3 using a two-way ANOVA and Scheffé test.


Plasma Renin Activity (ng AI/ml/hr)
240
200
160
120
80
40
0
Control
(n = 5)
K-free diet
(n = 8)
Low NaCl
K-free diet
(n = 7)

elevated as early as day 3 of the study. The low salt diet group had significantly higher AI concentrations (311.41 ± 16.35 vs 183.2 ± 10.23 ng AI/ml, p 0.005) and PRA (104 ± 7.28 vs 83.25 ± 7.30 ng AI/ml/hr, p 0.05) levels as compared to K-free diet animals, respectively. In both cases these levels are significantly increased from control AI concentrations (38.86 ± 3.56 ng AI/ml) and PRA (23.66 ± 1.14 ng AI/ml/hr) levels at this time (p 0.005). Both dietary electrolyte depletion groups had significantly higher levels of AI concentrations and PRA on day 11 compared to day 3, whereas control animals showed no significant changes between day 3 and day 11 of the study.

Brokaw (1953) has reported that water turnover was further augmented by removal of sodium from the potassium deficient diet while the water turnover of potassium-deficient rats drinking saline was only slightly greater than that of the saline drinking controls. In the present study, 3 groups of animals were used in order to determine the most reliable model for the development of polydipsia (control, K-free and low NaCl/K-free diets). Both of the dietary depletion groups showed significantly elevated intakes of water by days 3-5 on the diets, whereas only the low salt diet group remained at the elevated level (Figs. 2 and 6). With the development of polydipsia there was a concurrent increase in urine output (Fig. 3). Dietary electrolyte depletion suppresses normal growth in animals. A retardation of growth was observed, measured as the relative rate of increase in daily body weight, was suppressed in the dietary depletion groups (Fig. 4). Plasma analyses of electrolytes (Tables 4 and 6) showed that these dietary depleted rats are hypochloremic and hypokalemic on day 11 of the

diets. The decrease of plasma potassium concentrations was less in rats receiving the low salt diet because continuing sodium deprivation reduces renal potassium excretion by increasing the rate of potassium reabsorption by the collecting duct (Peterson et al., 1977). Plasma sodium concentration shows a statistically significant decrease in the low salt diet group by day 11 of the study. Plasma osmolality for both groups of dietary electrolyte depleted rats have a hypoosmotic plasma as compared to controls on day 11 of the diets (Tables 4 and 6). The data shows that dietary electrolyte depletion results in a decreased plasma osmolality as early as day 5 compared to control (Table 5). This corresponds temporally with the initial attenuation of water intake (Fig. 6).

In order to correlate the peak of polydipsia with circulating AII levels, blood was sampled on the 3rd and 11th days of the depletion diets. The data shows that both plasma AI concentrations and PRA are significantly increased whereas statistically significant changes in plasma osmolality are not yet seen as early as day 3 (Table 6 and Figs. 7 and 8). The depleted animals show significant decreases of plasma K levels by day 3 and the low salt diet group shows a statistically significant decrease in plasma Cl at this time (Table 6). Finally, both plasma renin activity (PRA) and AI concentration is significantly elevated in the dietary depleted rats as compared to control (Figs. 5, 7 and 8).

The low salt diet is a more potent stimulus for the development of polydipsia as well as for increased plasma renin activity (PRA) and AI concentrations (Figs. 2, 5, 6, 7 and 8) than dietary potassium depletion alone. This data suggests that the development of dietary

electrolyte depletion induced polydipsia may involve the renin-angiotensin system, mediated by the action of elevated circulating AII acting on central receptors. With continued dietary depletion the developing hypotonicity of plasma may act as an inhibitory osmotic influence on thirst, thus antagonizing the action of elevated levels of circulating AII on the drinking behaviour of these animals. The hypotonicity of plasma may attenuate any further development of polydipsia or may even overide the dipsogenic action of circulating AII.

Paller et al. (1983) has reported that rats maintained on a K-deficient diet for 2 weeks develop a sustained polydipsia and hypertonic plasma. This contradicts the present findings concerning the development of a hypotonic plasma. A hypertonic plasma would provide an osmotic stimulus for drinking, therefore potentiating any action of elevated circulating AII in the development and maintenance of polydipsia throughout the dietary electrolyte depletion period.

It is important to note the geographical differences between this laboratory and that of Berl, Linas and Paller. Their work was done in Denver, Colorado (altitude $\sim$ 5280 ft, Bar. pres. $\sim$ 630 mmHg, O_2 tension $\sim$ 130 mmHg) where the humidity is less and acclimatization to altitude may be an important factor. Under the conditions of high altitudes the continuous water loss from the skin and through respiration (insensible water loss) and a variable amount of sweat (sensible water loss) in the regulation of body temperature may be increased relative to studies at sea level conditions. Also, when using the rat as the experimental model, an animal with a high relative rate of metabolic activity and respiration rate (resp. rate $\sim$80-150 per min.), the effects of altitude changes on water metabolism become more

significant. These considerations will be discussed further in section 4, General Discussion.

3.2 Study 2 - Preliminary Study of Urine Concentrating Ability in Relation to Dietary Electrolyte Depletion.

An impairment of urine concentrating ability associated with hypokalemia has been observed in man (Rubini, 1961) and in animals (Mannitus et al., 1960; Bennett, 1970). In potassium depletion, the water-losing disorder is characterized by polydipsia, polyuria and an impaired maximal urine concentrating ability (Berl, 1980). There is evidence that polydipsia and the urine concentrating defect occur entirely independent of each other (Berl et al., 1977). Studies concerned with the effects of anions on urine concentrating mechanisms show that hypochloremia influences renal function (Abboud et al., 1979; Galla et al., 1981; and Luke et al., 1977).

This study was undertaken to determine if the development of increased water intake was a primary event or secondary to the development of a urine concentrating defect during dietary electrolyte depletion.

METHODS

All rats were housed and maintained under the conditions described in section 2.1, General Methods. Maximal urinary concentrating ability (Max Uosm) was evaluated in all animals by water depriving for 24 hrs and food depriving for the last 4-6 hrs of the 24 hr period. Urine was collected from clean metabolic cages into urine bottles under oil to prevent any evaporative water loss during the last 4-6 hrs of the 24 hr test period.

A total of 34 rats were maintained on synthetic control diet during which time maximum urine concentrating ability (Max Uosm) was tested (Figs. 9, 10 and 11). After a minimum 3 day recovery period following dehydration, a group of 12 rats was fed a K-free diet and evaluated for Max Uosm 11 days post commencement (Fig. 9). The other group of 22 rats were maintained on the low salt diet of which 10 animals were evaluated for Max Uosm on day 6 and day 11 of the depletion period (Fig. 11). The remaining group of 12 rats were evaluated for Max Uosm on the 11th day of the low salt diet followed by control chow and evaluated for Max Uosm on day 5 of the dietary electrolyte repletion period (control diet) (Fig. 10).

RESULTS AND DISCUSSION

The main purpose of this study was to determine how soon following dietary electrolyte depletion does an impairment of urine concentrating ability manifest itself. Figures 9, 10 and 11 are histograms of maximum urine osmolality (Max Uosm) measured prior to dietary electrolyte depletion and at various times during depletion on the K-free and low salt diets. Figure 9 shows a significant impairment of maximum urine concentrating ability on the 11th day of dietary electrolyte depletion of animals maintained on the K-free diet (control 3120 ± 73.63 vs 2458.62 ± 74.73 mOsm/kg H_2O, $p < 0.001$). Similarly, animals maintained on the low salt diet show a significant decrease in Max Uosm by the 11th day of dietary depletion (Figs. 10 and 11). Figure 10 shows that the impairment of urine concentrating ability seen in rats following 11 days on the low salt diet is corrected with dietary

electrolyte repletion within 5 days (repletion 3075 ± 138 vs 2587.8 ± 146 mOsm/kg H_2O, p 0.005). Figure 11 shows no significant impairment of urine concentrating ability on day 6 of dietary electrolyte depletion on the low salt diet (control 2918 ± 148 vs 2751.4 ± 170 mOsm/kg H_2O), whereas an impairment was confirmed by day 11 of the diet (control 2918 ± 148 vs 2363 ± 166 mOsm/kg H_2O, p 0.005).

The results of the study confirm the development of a urine concentrating impairment of the kidney in rats maintained on the electrolyte free diets (Figs. 9, 10 and 11). The impairment of urine concentrating ability becomes significant at least by the 11th day of dietary depletion, whether the rats are maintained on a low NaCl/K-free diet or the K-free diet alone. Berl et al. (1977) demonstrated that the increase in water intake precedes the development of a concentrating defect in potassium depletion, and that the polyuria is largely a consequence of the polydipsia. To determine whether the primary effect of the electrolyte deplete diets is to induce polydipsia, rather than being secondary to the development of a urine concentrating impairment and polyuria, rats maintained on the low salt diet were tested for Max Uosm on day 6, a time by which the elevated daily water intake was well established in rats maintained on the depletion diets (Figs. 2 and 11).

The data are in agreement with the findings of other investigators (Berl, 1980). The fact that increases in the intake of water precedes an impairment of water conserving ability and the development of a hypoosmotic plasma in this study suggests that the stimulus for thirst is not hyperosmolality but rather of a nonosmolar nature. It seems that the polydipsia induced by dietary electrolyte depletion is concomittant with elevated circulating AII levels, whereas

Figure 9:

Maximum urine osmolality (Max Uosm) made prior to dietary electrolyte depletion (blank bar) and on day 11 of the K-free diet (hatched bar). Results are expressed as means ± S.E.M. The number of animals is shown in the lower left hand corner of the histogram. $^{**}p < 0.001$ compared to control using an unpaired t-test.

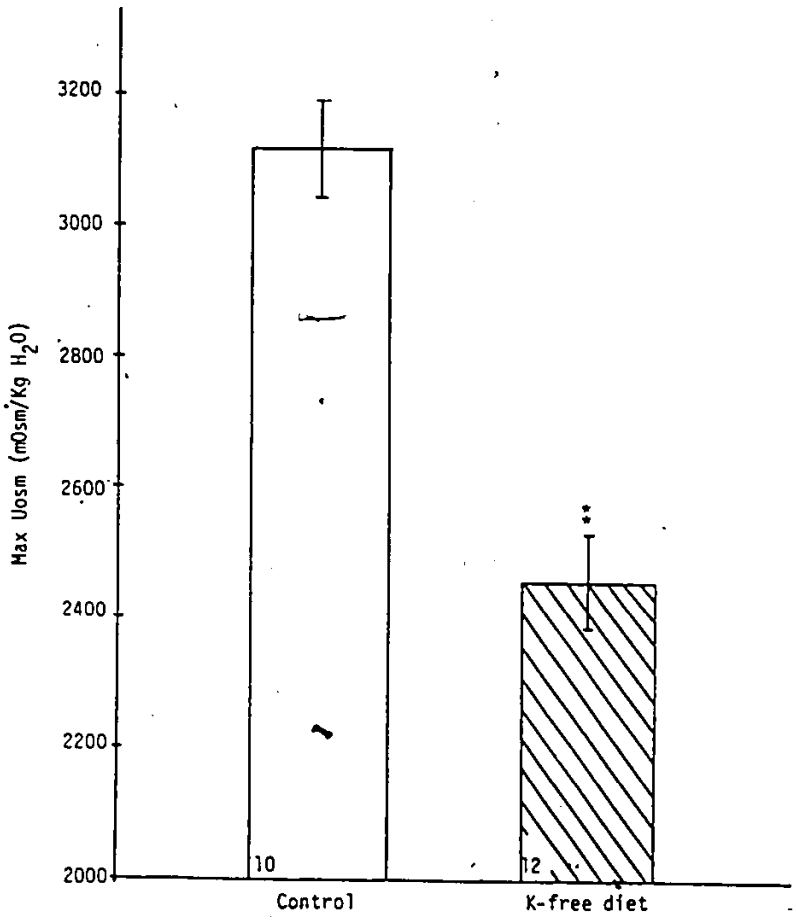

Max Uosm (mOsm/Kg H_2O)
3200
3000
2800
2600
2400
2200
2000
10
12
**
Control
K-free diet

Figure 10:

Maximum urine osmolality (Max Uosm) made prior to dietary electrolyte depletion (blank bar), on day 11 (hatched bar) of the low NaCl/K-free diet and on day 5 (blank bar) of dietary electrolyte repletion (control diet). Results are expressed as means ± S.E.M. The number of animals is shown in the lower left hand corner of the histogram. *$p < 0.005$ compared to control and +$p < 0.005$ compared to repletion using a one-way ANOVA and Scheffé test.

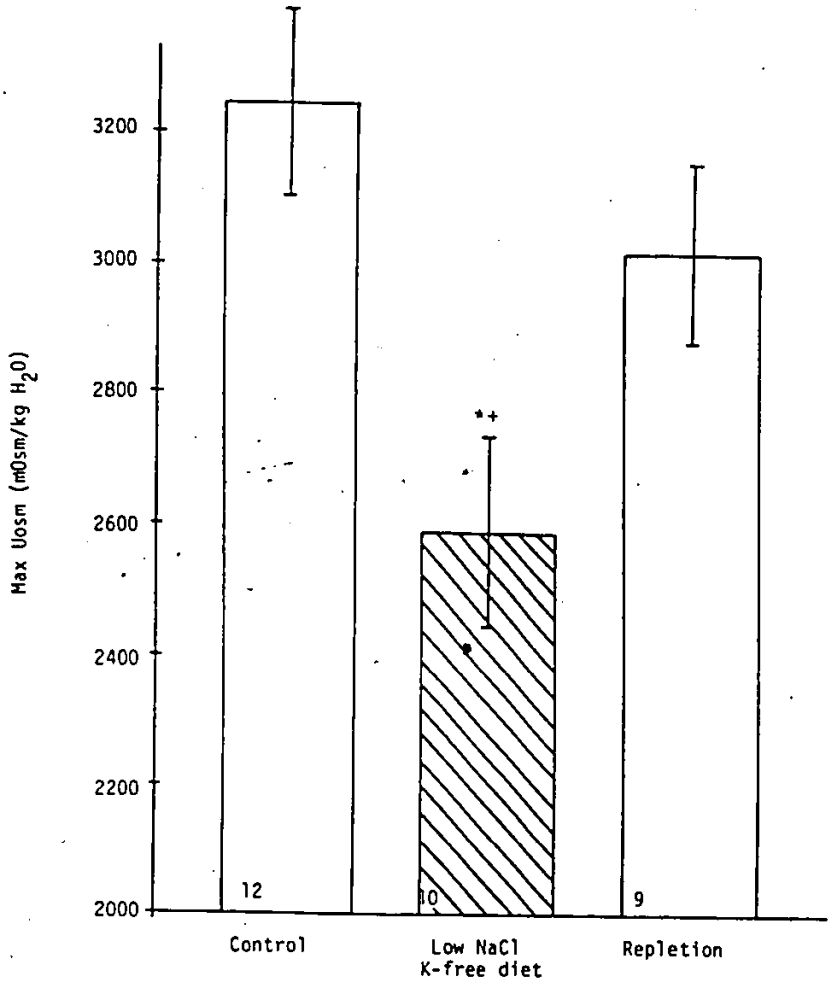
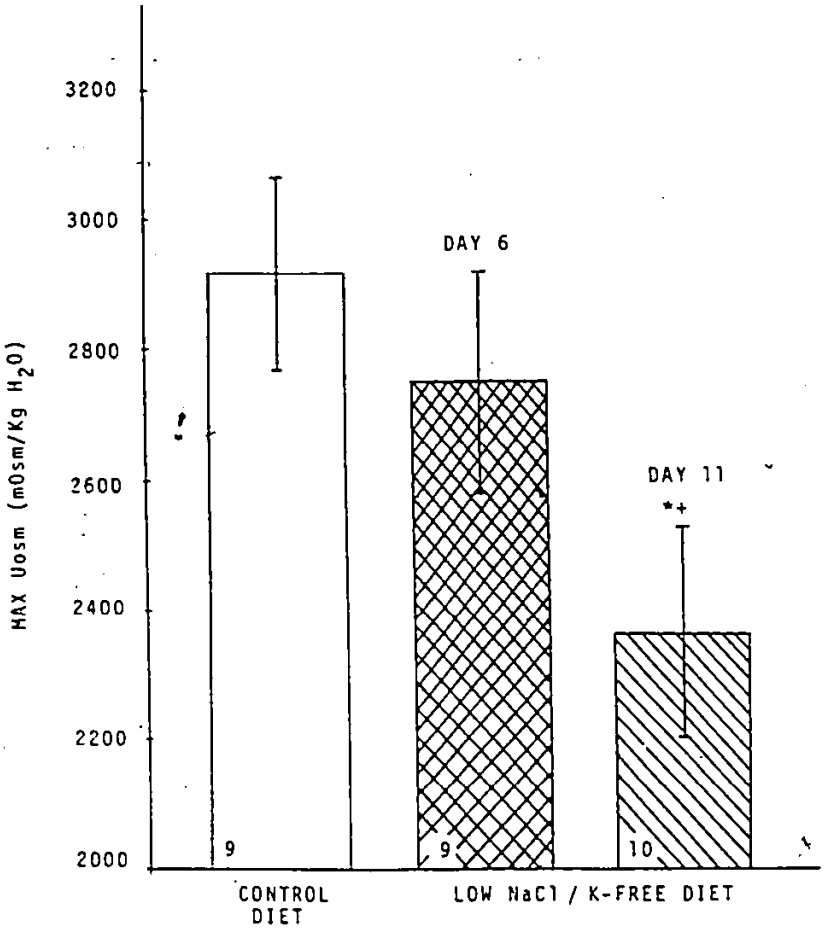

Max Uosm (mOsm/kg H_2O)
3200
3000
2800
2600
2400
2200
2000
*+
12
10
9
Control
Low NaCl
K-free diet
Repletion

Figure 11:

Maximum urine osmolality (Max Uosm) made prior to dietary electrolyte depletion (blank bar), on day 6 (cross-hatched bar) and day 11 (hatched bar) of the low NaCl/K-free diet. Results are expressed as means ± S.E.M. The number of animals is shown in the lower left corner of the histogram. *$p < 0.01$ compared to control and +$p < 0.01$ compared to day 6 using a one-way ANOVA and Scheffé test.


MAX Uosm (mOsm/Kg H_2O)
3200
3000
2800
2600
2400
2200
2000
DAY 6
DAY 11
*+
9
9
10
CONTROL DIET
LOW NaCl / K-FREE DIET

the urine concentrating defect may result from an impaired NaCl transport by the thick ascending limb (TAL) coupled with reduced solute delivery to the loop of Henle in dietary electrolyte depletion (Gutsche et al., 1984; and reviewed in Peterson, 1984).

3.3 Study 3 - Preliminary Study on the Effect of Subfornical Organ Lesions on the Development of Polydipsia Induced by Dietary Electrolyte Depletion

Since rats maintained on a low salt diet are characterized by elevated PRA and plasma AI concentrations as demonstrated in this lab, and angiotensin II is a potent dipsogen (Fitzsimons, 1976), it is possible that this is the mechanism responsible for the polydipsia induced by dietary electrolyte depletion. The purpose of the third study was to determine whether ablation of the SFO, a small midline circumventricular structure in the brain believed to mediate angiotensin-induced thirst in the rat would prevent the development of polydipsia in rats fed a low salt diet.

METHODS

A total of 27 animals with stereotaxic lesions of the SFO, 9 sham-operated rats and 6 non-lesioned rats were housed and maintained under the conditions described earlier. All rats were allowed a minimum of 7 days post-surgical recovery while being maintained on control diet.

The group of 27 SFO-lesioned rats were fed a low salt diet for 11 consecutive days followed by 5 days of repletion on the control diet.

Water intakes, urine outputs and body weights (± 1.0 g) were measured daily. Blood samples (1.0 ml whole blood) were taken via cardiac puncture (n = 14) during the post-surgical recovery period for plasma electrolytes and osmolality analyses. Four days later this group of 14 rats were fed the low salt diet during which time blood samples (2.0 ml whole blood) were taken via cardiac puncture on day 1 (n = 5), day 2 (n = 5), day 4 (n = 4) and again on day 11 (n = 14) for analyses of plasma electrolytes, osmolalities, plasma AI concentrations and PRA. On the 5th day of repletion (control diet), blood samples (n = 14) were taken via cardiac puncture for analyses of plasma electrolytes, osmolalities, plasma AI concentrations and PRA. The remaining group of 13 SFO-lesioned rats were tested for Max Uosm on the 11th day of the low salt diet period and again on day 5 of the repletion period (control diet). The sham-operated rats were tested in the same manner for urine concentrating ability on the low salt diet and repletion diet (control diet). Blood samples were taken via cardiac puncture from six non-lesioned rats while on the control diet, day 2, and day 11 of the low salt diet, and again on day 5 of the repletion period, in the same manner as the lesioned rats.

At the end of the study all rats with stereotaxic lesions or sham operations were perfused transcardially with 10% buffered formalin solution for histological analyses for localization of the lesions.

RESULTS AND DISCUSSION

The purpose of the current study was to determine whether ablation of the SFO would prevent the development of polydipsia associated with dietary electrolyte depletion (low NaCl/K-free diet).

On the basis of histological analyses of brain tissue, the rats were divided into three experimental groups. "Group 1" rats (n = 9) had sham operations; "Group 2" rats (n = 17) had lesions of both the medial and posterior regions of the SFO; and "Group 3" rats (n = 10) had lesions of the posterior part of the SFO. All lesioned animals in the study sustained varying degrees of SFO damage, as well as non-specific damage extending into the adjacent hippocampal and thalamic structures. Behavioural data were assessed with reference to the sham operation group. Photomicrographs showing the location of SFO lesions are given in Fig. 12.

Table 7 lists the plasma electrolytes and osmolalities of blood samples taken via cardiac puncture from rats with electrolytic lesions of the SFO and without lesions. Samples were collected from rats fed control diet, at different times during the 11 consecutive days on the low salt diet and again on day 5 of dietary electrolyte repletion (control diet). Both SFO-lesioned animals and controls had decreased plasma K^+ concentrations by day 1 and 2 after introducing the low salt diet as compared to their pre-dietary depletion values, respectively. The SFO-lesioned animals showed a decreased plasma Cl^- level (control 100.64 ± 0.69 vs 97.75 ± 0.75 mEq/l plasma, $p < 0.025$) by day 4 of the low salt diet. At this time a significant decrease in plasma osmolality was seen (control 287 ± 1.11 vs 283.7 ± 1.76 mOsm/Kg H_2O, $p < 0.025$). By day 11 of the low salt diet both SFO-lesioned animals and controls

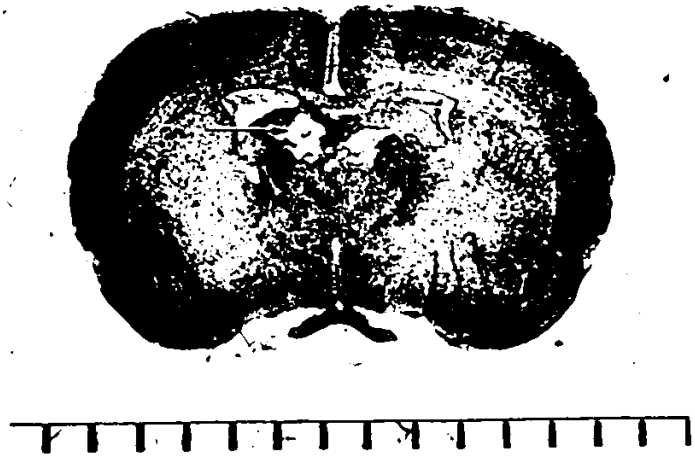
Figure 12:

Coronal photomicrographs of 40-60 thick brain sections from two animals. Thick black arrow shows section with an intact SFO. The sites of lesions are designated by Thin black arrows. Adjacent vertical bars are 1 mm apart.

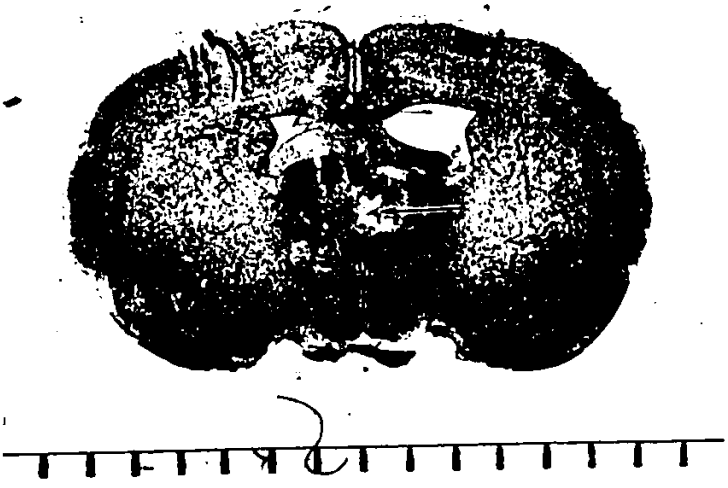
A. lesion centered in ventrolateral hippocampus in which there is partial damage to the body and caudal part of the SFO (Group 2).

B. lesion centered in dorsal midline thalamus in which there is partial damage to the caudal part of the SFO (Group 3).

A.



B.



Plasma electrolytes and plasma osmolality in rats with lesions of the SFO and without lesions at various times during control, low NaCl/K-free and repletion (control) diet periods of the study. Results are expressed as means of ± S.E.M. The number of animals shown in parentheses. Data anlyzed using one and two-way ANOVA and Scheffé test. $^{*}p < 0.025$, $^{**}p < 0.01$, $^{***}p < 0.005$ compared to pair controls.

TABLE 7

Low NaCl/ K-Free Diet

	Control Diet	DAY 1	DAY 2	DAY 4	DAY 11	Repletion (control Diet)
CONTROL (n = 6)						
Plasma Electrolytes (mEq/l)						
Na^+	141.4 ± 0.3		139.7 ± 0.4		*138.1 ± 0.6	*145.3 ± 2.0
K^+	4.37 ± 0.10		* 3.52 ± 0.1		* 3.12 ± 0.04	* 4.79 ± 0.10
Cl^-	99.7 ± 1.3		97.7 ± 1.2		93.5 ± 0.6	98.5 ± 0.7
Plasma Osmolality ($mOsm/kgH_2O$)	293.2 ± 1.3		294 ± 0.4		*286 ± 1.2	*297 ± 1.1
SFO LESIONS (n = 14)		(n = 5)	(n = 5)	(n = 4)		
Plasma Electrolytes (mEq/l)						
Na^+	143.6 ± 1.0	141.8 ± 0.8	141.2 ± 0.2	143.9 ± 1.2	*141.1 ± 1.2	144.3 ± 0.8
K^+	4.45 ± 0.01	* 3.73 ± 0.03	* 3.39 ± 0.06	* 3.19 ± 0.31	* 2.81 ± 0.13	4.46 ± 0.07
Cl^-	100.6 ± 0.6	* 98.2 ± 0.5	100.2 ± 0.7	* 97.8 ± 0.8	* 90.1 ± 1.3	99.8 ± 0.04
Plasma Osmolality ($mOsm/kg\ H_2O$)	287 ± 1.1	290 ± 1.3	290 ± 0.5	*283.7 ± 1.8	*282.2 ± 1.6	*290.8 ± 1.3

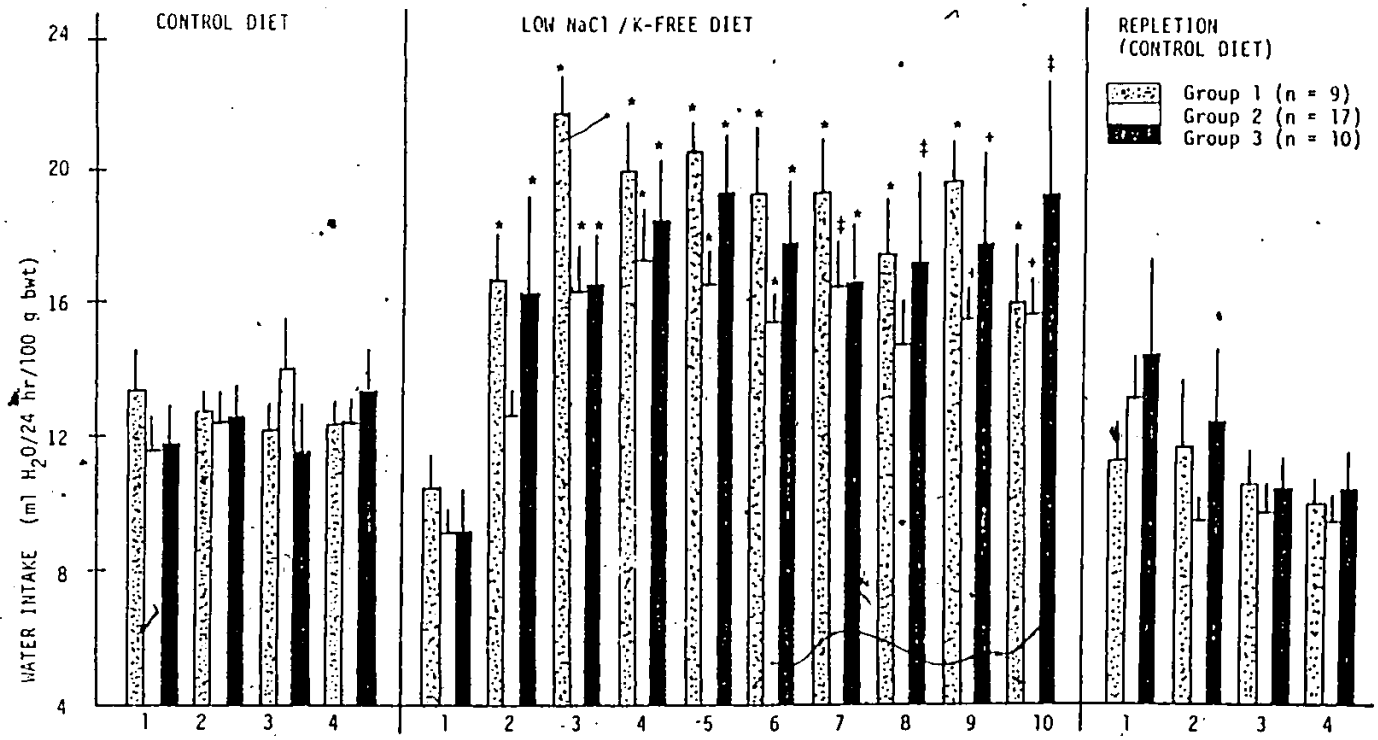
developed a hypochloremic, hypokalemic and hypotonic plasma. All animals showed a statistically significant increase in plasma osmolality by day 5 of dietary repletion (control diet), returned to their pre-dietary treatment levels, of both plasma electrolytes, and osmolality.

Figures 13 and 14 show 24 h intakes of water and urine outputs, respectively. A statistically significant increase in water intake occurred as early as day 2-3 of introducing the low salt diet in group 1 (sham-operated), group 2 and group 3 animals. In these rats the peak of polydipsia occurred by day 3-5 and they maintained significantly increased intakes of water compared to their pre-dietary electrolyte depletion levels. Only group 2 shows statistically significant lower intakes of water than the sham-operation animals (group 1) on days 2, 3, 5, 6 and 9 of the dietary electrolyte depletion period of this study. Group 3 does not show any statistically significant difference from the sham operation animals for the same period. All three groups show a return to control levels for intakes of water following dietary repletion (control diet). Figure 14 shows that during the same period of dietary electrolyte depletion, sham operation and group 2 animals developed polyuria concurrently with the polydipsia.

Figures 15 and 16 are histograms of plasma renin activity (PRA) and angiotensin I (AI) concentration, respectively, of rats with lesions of the SFO (groups 2 and 3) and without lesions. Data was obtained from blood samples taken via cardiac puncture while on the control diet, at different times during the 11 consecutive days on the low salt diet and again on day 5 of dietary electrolyte repletion (control diet). Both groups show significantly elevated levels of AI and PRA as compared to

Figure 13:

A histogram of 24 hr intakes of water by rats with sham operation (Group 1) and lesions of the SFO (Group 2 = medial-caudal damage; and Group 3 = caudal damage). Results are expressed as means ± S.E.M. The number of animals are shown in parentheses. Data analyzed using paired t-test for comparison between dietary conditions within groups, and one-way ANOVA and Scheffé test for between group comparisons for a given condition. +$p < 0.05$, ‡$p < 0.025$ and *$p < 0.005$ compared to pair control.

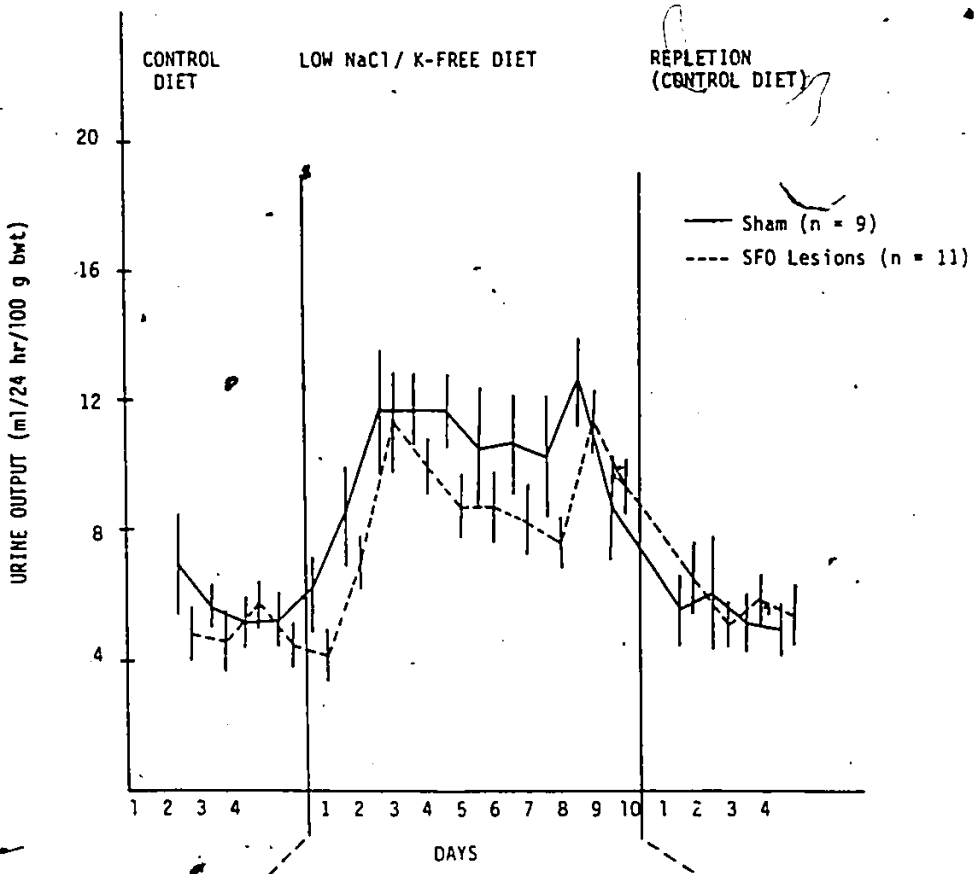


P Values

Group 1 vs Group 2	NS	0.05	0.025	NS	0.05	0.05	NS	NS	0.05	NS
Group 1 vs Group 3	NS	NS	0.025	NS	NS	NS	NS	NS	NS	NS
Group 2 vs Group 3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figure 14:

Mean daily urine output of rats with sham operation and lesions of the SFO. Results are expressed as means ± S.E.M. Data analyzed using paired t-test and one-way ANOVA.



P Values

Sham vs Lesion	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sham vs Sham Control Diet	NS	.025	.005	.005	.005	.005	.005	.005	.005	.025
Lesion vs Lesion Control Diet	NS	.025	.005	.005	.005	.005	.005	.005	.005	.025

Figure 15:

Plasma renin activity (PRA) in rats with electrolytic lesions of the SFO and without lesions at various times during the control diet, low NaCl/K-free diet and repletion (control diet) periods of the study. Results are expressed as means ± S.E.M. The number of animals shown in the lower left hand corner of the histogram. Data analyzed using one-way and two-way ANOVA and Scheffé test. *$p < 0.005$ compared to control diet.

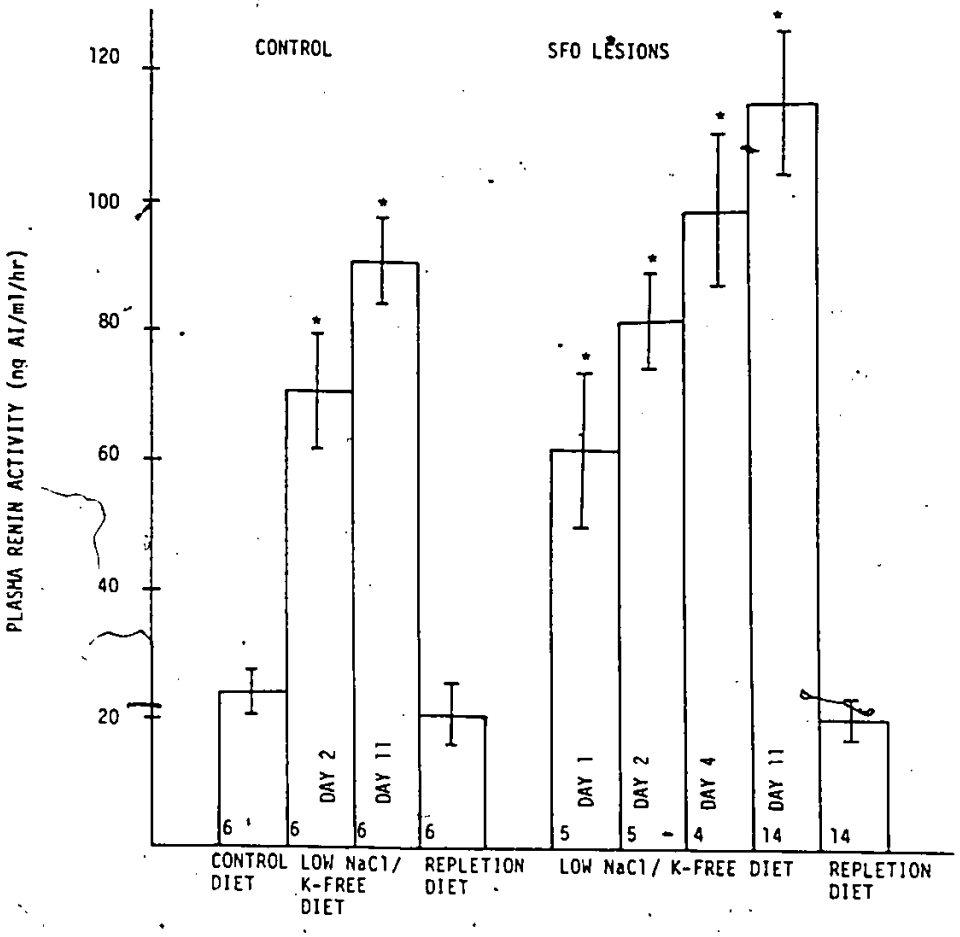
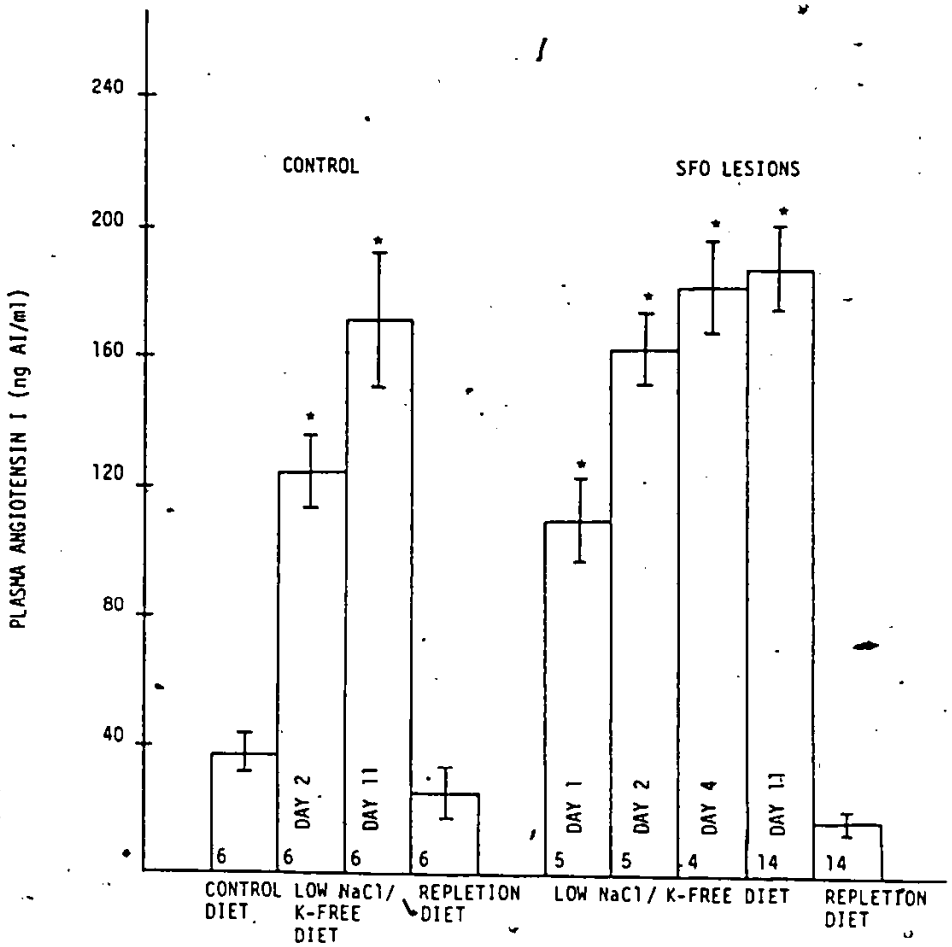

CONTROL
SFO LESIONS
PLASMA RENIN ACTIVITY (ng AI/ml/hr)
120
100
80
60
40
20
DAY 2
DAY 11
DAY 1
DAY 2
DAY 4
DAY 11
6
6
6
6
5
5
4
14
14
CONTROL DIET
LOW NaCl/ K-FREE DIET
REPLETION DIET
LOW NaCl/ K-FREE DIET
REPLETION DIET

Figure 16:

Plasma angiotensin I concentrations (AI) in rats with electrolytic lesions of the SFO and without lesions at various times during the control diet, low NaCl/K-free diet and repletion (control diet) periods of the study. Results are expressed as means ± S.E.M. The number of animals shown in the lower left hand corner of the histogram. Data analyzed using one-way and two-way ANOVA and Scheffé test.
*$p < 0.005$ compared to control diet.


PLASMA ANGIOTENSIN I (ng AI/ml)
240
200
160
120
80
40
0
CONTROL
SFO LESIONS
DAY 2
DAY 11
DAY 1
DAY 2
DAY 4
DAY 11
6
6
6
6
5
5
4
14
14
CONTROL DIET
LOW NaCl/ K-FREE DIET
REPLETION DIET
LOW NaCl/ K-FREE DIET
REPLETION DIET

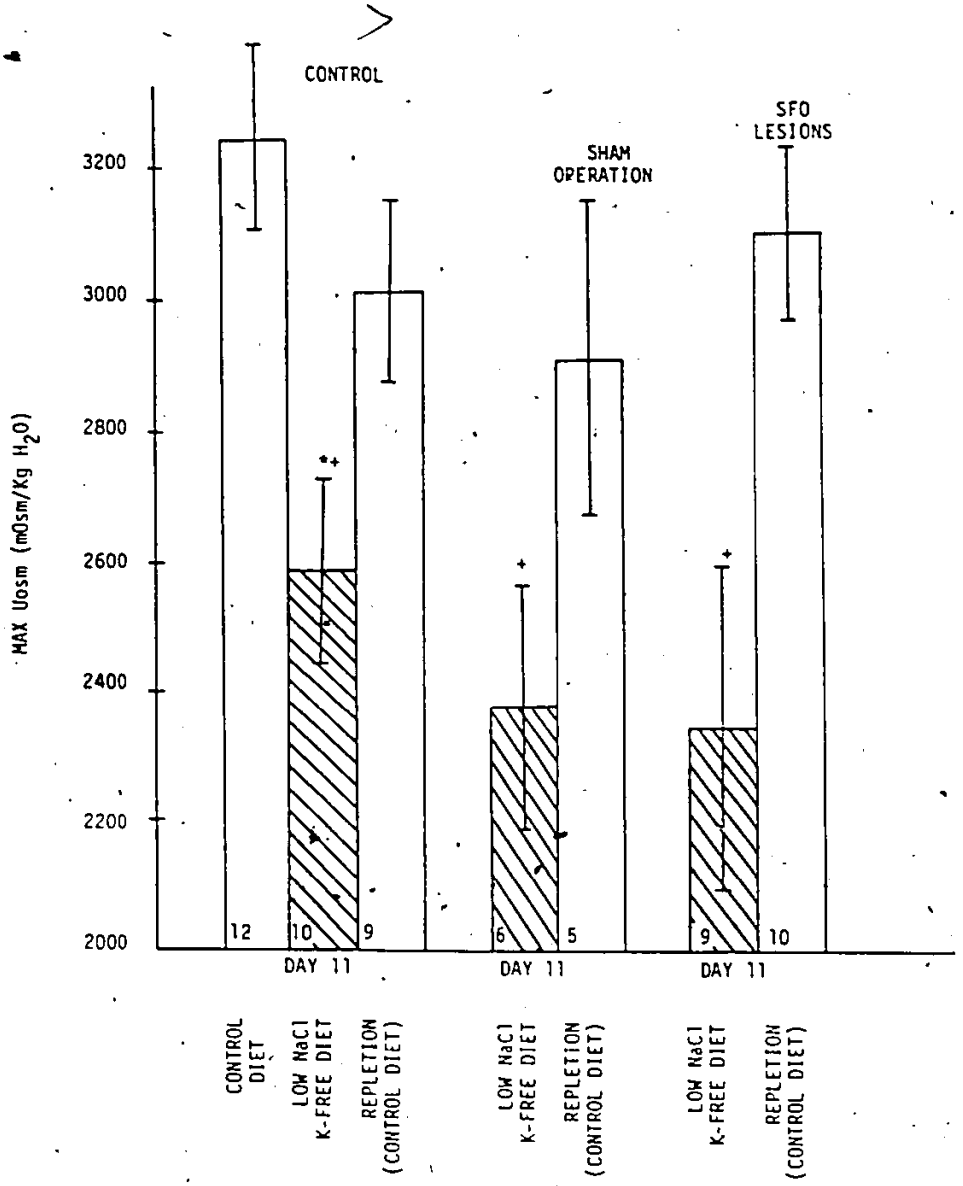
pre and post-dietary electrolyte depletion levels while on the control diets. The SFO-lesioned animals show significantly higher AI concentrations (control 37.25 ± 5.90 vs 110.28 ± 13.52 ng AI/ml, $p < 0.005$) and PRA (control 24.27 ± 3.27 vs 61.44 ± 12.33 ng AI/ml/hr, $p < 0.005$) levels compared to the pre-dietary electrolyte depletion levels of control animals. Throughout the depletion period both groups of animals maintained elevated AI concentrations and PRA levels. No significant differences in PRA or AI levels were observed between SFO-lesioned and control animals for corresponding days of the study.

Figure 17 is a histogram of Max Uosm of control animals (no lesions) from study 3.2 (Figure 10) and rats with SFO lesions and sham-operation on day 11 of the low salt diet and day 5 of dietary electrolyte repletion (control diet). In all three cases a urine concentrating impairment was confirmed by day 11 of the dietary electrolyte depletion period. The impairment of urine concentrating ability is corrected with dietary repletion in both the sham-operation group (repletion 2914 ± 242.6 vs 2381.7 ± 193.7 mOsm/Kg H_2O, $p < 0.005$) and SFO-lesioned animals (repletion 3105 ± 132 vs 2348 ± 254 mOsm/Kg H_2O, $p < 0.005$) as compared to the depletion Max Uosm data..

Based on histological analyses (Fig. 12) of rat brain, animals were assigned into three groups depending on the extent of damage to the subfornical organ. Behavioural data of the daily water intake and urine volume were analyzed according to this grouping. All these animals showed significantly elevated intakes of water by day 3-5 on the low salt diet which remained elevated thereafter (Fig. 13). With the development of polydipsia there was a concurrent increase in urine volume (Fig. 14). Plasma analyses of electrolyte concentrations

Figure 17:

Maximum urine osmolality (Max Uosm) of controls, sham-operation and SFO lesioned animals made prior to dietary electrolyte depletion (control diet) (blank bar), on day 11 of the low NaCl/K-free diet (hatched bar) and on day 5 (blank bar) of dietary electrolyte repletion (control diet). Results are expressed as means ± S.E.M. The number of animals is shown in lower left hand corner of histogram. Data analyzed using one-way ANOVA with Scheffé test and unpaired t-test. *$p < 0.005$ compared to control and +$p < 0.005$ compared to repletion.


CONTROL
SHAM OPERATION
SFO LESIONS
MAX Uosm (mOsm/Kg H_2O)
3200
3000
2800
2600
2400
2200
2000
12
10
9
6
5
9
10
DAY 11
DAY 11
DAY 11
CONTROL DIET
LOW NaCl K-FREE DIET
REPLETION (CONTROL DIET)
LOW NaCl K-FREE DIET
REPLETION (CONTROL DIET)
LOW NaCl K-FREE DIET
REPLETION (CONTROL DIET)

(Table 7) showed that rats with or without SFO lesions maintained on the diet became hypochloremic, and hypokalemic, and developed a hypoosmotic plasma which was corrected within 5 days of dietary electrolyte repletion (control diet). Plasma sodium concentration showed a statistically significant decrease by the 11th day on the low salt diet. Both plasma renin activity (PRA) and plasma AI concentrations are significantly elevated as early as the 1st day of introducing the low salt diet (Figs. 15 and 16). An impairment of urine concentrating ability is confirmed by the 11th day of introducing the low salt diet in control, sham-operation and SFO-lesioned animals (Fig. 17). This impairment was corrected within days by allowing the rats access to control diet.

The general aim of this study was to prevent the development of polydipsia that is associated with dietary electrolyte depletion (low NaCl/K free diet). Histological analyses show that animals with lesions experience varying degrees of damage to the SFO involving nonspecific damage into adjacent areas without complete ablation of the SFO. The data from this study would seem to suggest that although these animals become polydipsic when maintained on the low salt diet, the magnitude of increase in water turnover of these rats maybe influenced by the extent and specificity of damage to the SFO and surrounding structures and therefore available AII receptor sites.

The physiological basis for the observed differences in water intakes between sham-operated (Group 1), Group 2 and Group 3 (SFO lesioned) rats can only be speculated on. The SFO is a small midline circumventricular structure attached to the hippocampal commissure and protrudes into the third ventricle above the intraventricular foramen.

The SFO contains neurons, glial cells, and fenestrated capillaries (Dellman and Simpson, 1979). The arterial supply of the SFO include the subfornical artery which immediately before reaching the SFO anastomoses with the arterior and posterior choroidal arteries. Dellman et al. (1976) in their transmission and scanning electron microscopic studies of the subfornical organ have demonstrated regional differences within the structure of the SFO. Depending upon which regions are damaged, the lesions may have different effects on function. For example, lesions confined to the rostral region would affect afferent/efferent nerve fibres in passage. Lesions of the central region would damage the body of the SFO and lesions of the caudal region may interfere with the blood supply to the entire structure. Therefore, an understanding of the role of the SFO in electrolyte depletion induced polydipsia requires an evaluation of the experimental results with respect to the anatomical regions affected by any experimental treatment.

It is well established that circulating AII activates receptors in the subfornical organ (SFO), resulting in drinking, vasopressin release and by a pressor effect an increase in mean arterial pressure (Simpson, 1981). Circulating AII of peripheral origin binds the central part of the SFO (Van Houten et al., 1980), a region which is highly vascularized and contains most of the neuronal population of the SFO. This region also contains AII-immunoreactive terminal fields which appear to arise from cells in the lateral hypothalamus, zona incerta and nucleus reuniens (Lind et al., 1984 and 1985). The zona incerta and lateral hypothalamic area are involved in the behavioural control of fluid balance, particularly to osmotic stimuli. This may be achieved in part by AII-containing projections to the SFO, where neural and humoral

inputs of AII from two different sources may be integrated (Lind et al., 1985).

Neuroanatomical studies have shown that the neural outputs of the SFO also contain AII (Lind et al., 1984). These efferent pathways from the SFO project to several areas of the forebrain and hypothalamus involved in fluid-electrolyte metabolism (Lind et al., 1982; Miselis, 1981). Many efferents to the anteroventral third ventricular area of the preoptic region emerge from the rostal SFO. The second pathway is out of the body a[illegible] anterior stalk of the SFO to the hypothalamus, particularly to the neurosecretory magnocellular nuclei. This has led to proposals that information from blood-borne and synaptically released AII in the SFO may be relayed to the hypothalamic structures controlling behavioural and endocrine responses by way of a neural pathway that employs the same peptide, AII, as a neurotransmitter (Lind et al., 1982 and 1985). Although the subfornical organ (SFO) is one central site of angiotensin II dipsogenic receptivity, this does not preclude a dipsogenic action of AII at other loci. This is discussed further in section 4, General Discussion.

3.4 Study 4 - Effect of SFO Lesions on the Development of Polydipsia Induced by Dietary Electrolyte Depletion and Urine Concentrating Ability Following Electrolyte Depletion

The previous study suggested that animals maintained on the low salt diet become polydipsic, and that the magnitude of increase in water turnover may be influenced by the extent and specificity of damage to the SFO and therefore available AII receptor sites. The impairment of

urine concentrating ability of rats maintained on the diet is independent of these lesions. As already mentioned, the central sites for angiotensin II dipsogenic efficacy are likely not confined to the SFO. The SFO has ventrally directed efferents which mediate drinking response, carrying information to structures in the region of the anteroventral third ventricle (AV3V) which plays a crucial role in the central neural control of fluid balance (Buggy et al., 1977; Lind et al., 1982; Miselis, 1981). This area seems necessary for the normal dipsogenic, antidiuretic and pressor effects of intraventricular and circulating AII. The AV3V includes the medial portions of the medial preoptic area, arterior hypothalamus, median and supraoptic portions of the preoptic region and the organum vasculosum of the laminae terminalis (OVLT), a circumventricular structure lacking the blood-brain barrier (Buggy et al., 1977). Lesions of the AV3V, which include the OVLT ventrally and extend to the arterior commissure dorsally as well as many neural efferent/afferent terminals, cause disturbances in both osmotic and nonosmotic regulation of water balance (Buggy et al., 1977 and Brady et al., 1978). Anatomical data describing the efferent pathways of the SFO suggest that deficits in AII mediated drinking produced by lesions in the AV3V region may be due in part to destruction of efferents of the SFO (Miselis et al., 1977 and 1981; Shapiro et al., 1978).

The purpose of the current study was to determine whether ablation of multiple CNS structures believed to have AII receptors, the SFO and OVLT, will prevent the development of polydipsia induced by the low salt diet. The second objection was to assess whether the UCD is a consequence of inadequate release of ADH from the magnocellular neurosecretory neurons of the supraoptic nucleus (SON). The difficulty

with interpreting the present experiments in rats is that the lesions impinge on many structures with consequent destruction of many neural pathways.

METHODS

A total of 18 animals with stereotaxic lesions of the OVLT + SFO (n = 12) and SFO alone (n = 6), as well as 6 sham-operated and 6 non-lesioned rats were housed and maintained under the conditions described earlier. All rats were allowed a minimum of 7 days for post-surgical recovery while being fed a control diet. The stereotaxic coordinates used are shown in Table 8.

Table 8

Stereotaxic Coordinates Used for Lesioning Electrode in Study 4

Intracranial Site	Angle from Perpendicular (Measured towards midline)	mm from Bregma Anterior-Posterior	Lateral	Vertical*
Subfornical Organ	refer to Table 3			
Organum vasculosum° of the laminae terminalis (OVLT)	12°	-0.3	1.0	7.3-7.6
Sham operation +	12°	-0.3	1.0	5.5

*The vertical coordinate is the distance from the surface of the cortex to the most ventral position of the electrode.

°current passed 1.5 mA/15-20 sec.

+no current passed.

All rats were fed a low salt diet for 10 consecutive days. Water intakes and body weights (± 1.0 g) were measured daily. At the end of the study one-half of the lesioned rats (n = 9) and the non-lesioned rats (n = 6) were tested for Max Uosm on day 11 of the depletion period. Another group of non-lesioned rats (n = 9) were tested for Max Uosm while being maintained on the control diet. Following tests of Max Uosm, all animals were killed by decapitation and blood was collected into chilled EDTA tubes for analysis of plasma ADH. RIA for plasma ADH concentration was carried out in the lab of Dr. Daniel Bichet, Sacred Heart Hospital, Montreal, Quebec, Canada.

At the end of the study all rats with stereotaxic lesions and sham operations that were not sacrificed by decapitation were perfused transcardially with 10% buffered formalin solution for histological analyses for localization of the lesions.

RESULTS AND DISCUSSION

The main focus of the current study was to determine whether lesions of circumventricular structures, the SFO and OVLT, sites of angiotensin-mediated thirst would prevent the development of polydipsia induced by dietary electrolyte depletion (low NaCl/K-free diet).

Based on histological analyses of brain tissue, the rats were divided in two experimental groups: Sham-operated rats (n = 6), and lesioned rats (n = 18) with varying degrees of SFO damage extending from the body to the caudal region of the SFO. Animals with surgical lesions involving the SFO and OVLT showed varying degrees of SFO damage with no

ablation of the OVLT. Lesions directed at the OVLT were either not evident in the serial sections of rat brain or were confined to an area dorsolateral to the optic recess away from the AV3V region. When the fluid intake from rats with lesions of the SFO alone and those with multi-lesions of the SFO and AV3V region missing the OVLT were compared, no statistically significant differences were observed. For this reason, the SFO and OVLT lesioned rats were combined into the one experimental group described above. The water intake was compared to the sham-operated group in this study. Photomicrographs showing the location of SFO and OVLT lesions are given in Fig. 18.

Figure 19 shows 24 h intakes of water of sham-operated and SFO lesioned animals maintained on control diet followed by 10 consecutive days of being fed the low salt diet. The sham-operated rats showed a statistically significant increase in their daily water intake, which remained elevated throughout the duration of the diet, as in our previous studies. The lesioned rats did not show any significant increases of daily fluid intake throughout the dietary treatment. Rather, they maintained pre-dietary depletion levels, but with greater variability.

Figure 20 is a histogram of Max Uosm of control rats (no lesions) maintained on control diet, control rats (no lesions) and lesioned rats following 11 consecutive days on the low salt diet. In both control and lesioned rats a UCD was confirmed by day 11 of the diet, as in the previous studies. Following the test for Max Uosm these animals were decapitated for analysis of plasma ADH concentration after maximal stimulation by 24 hour water deprivation. Figure 21 is a histogram of plasma ADH concentrations in these control and lesioned water-deprived

Figure 18:

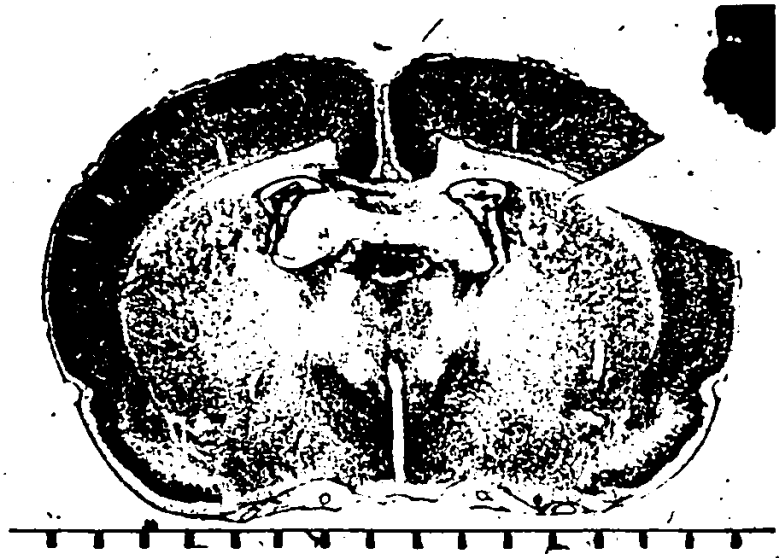
Coronal photomicrographs of rat brain sections. Thick black arrow shows section with an intact SFO. Thin black arrows point to sections where the SFO has been ablated.

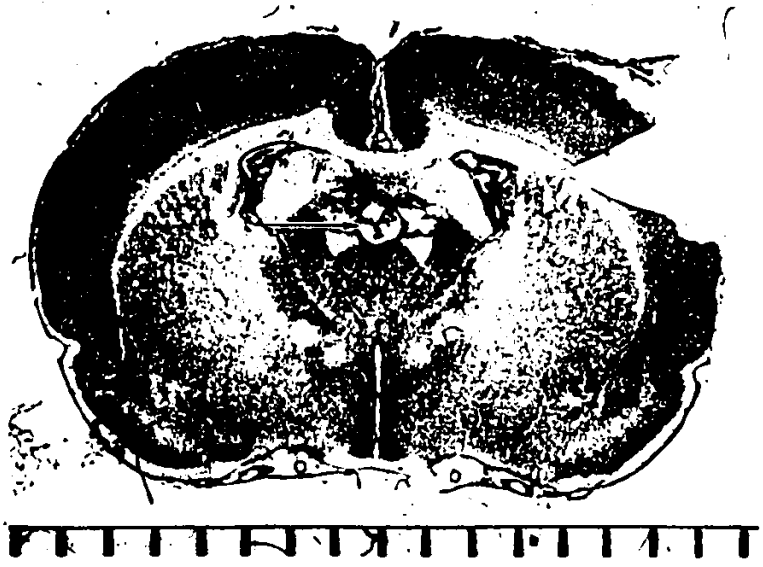
A. shows a locus of a lesion in which the body and caudal part of the SFO was destroyed.

B. Serial sections of a rat brain showing a lesion in which the caudal part of the SFO was destroyed.

A.

B.





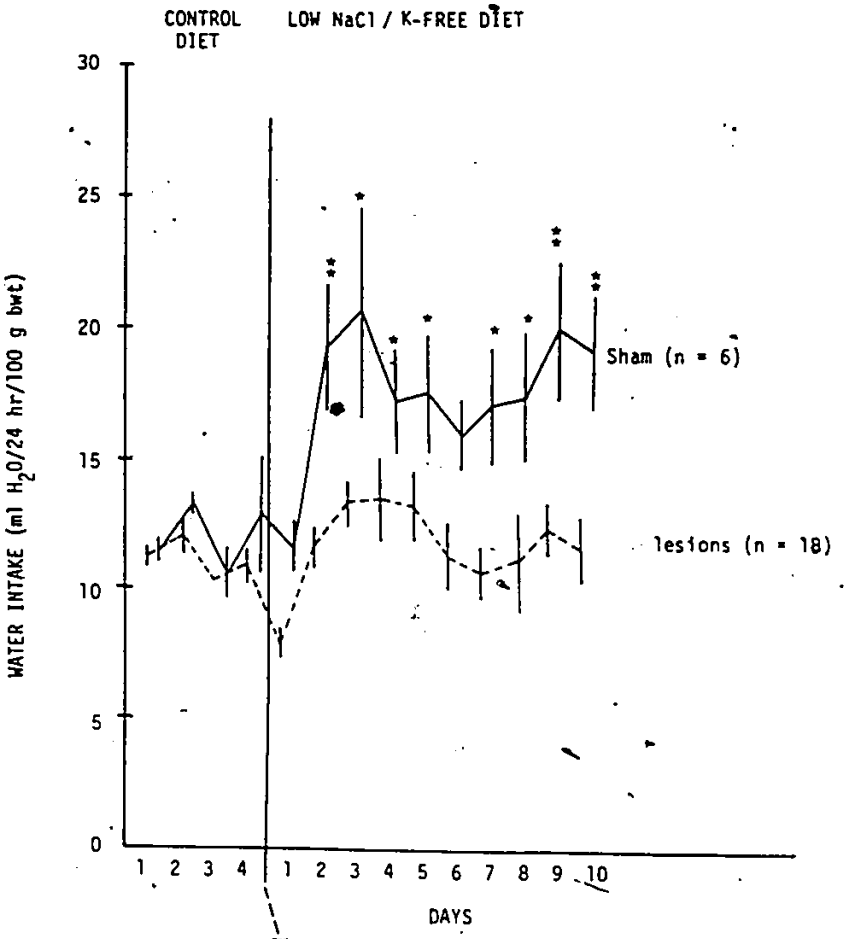
animals. ADH levels measured after water deprivation were similar in lesioned and non-lesioned animals fed either the low salt diet (29.15 ± 3.56 vs 40.03 ± 9.39 pg ADH/ml, respectively) or the control food (non-lesioned animals 25.63 ± 2.63 pg ADH/ml).

Based on histological analyses (Figs. 18) of rat brains, animals were assigned into two experimental groups, sham-operated and lesioned rats according to the rational described earlier. Daily water intakes were compared in these 2 groups. Sham-operated rats showed significantly elevated intakes of water by day 2 on the low salt diet which remained elevated, whereas the lesioned animals never achieved a statistically significant change in their daily fluid intakes throughout the duration of the diet (Fig. 19). An impairment of urine concentrating ability was confirmed by the 11th day after introducing the low salt diet in control and lesioned animals (Fig. 20). The ADH release in response to water deprivation was not abolished or limited following dietary electrolyte depletion with or without lesions of the SFO (Fig. 21).

The general aim of this study was to prevent the development of the polydipsia associated with dietary electrolyte depletion (low NaCl/K-free diet). This would require specific lesions of CNS structures involved in angiotensin-mediated thirst, assuming that the polydipsia is mediated by stimulation of AII receptors located in circumventricular structures. Histological data has revealed that animals with lesions experience partial to complete damage to the SFO with minimal encroachment and non-specific damage into adjacent areas which was a common occurrence in study 3.3. The data from this study would seem to suggest that lesions of the SFO, although not completely

Figure 19:

Mean daily water intakes of rats with sham operations and lesions of the SFO. Results expressed as means ± S.E.M. The number of animals shown in parentheses. Data analyzed using paired t-test and one-way ANOVA. $^{*}p < 0.05$ and $^{\ddagger}p < 0.025$ compared to pair control.



P Values

Sham vs lesion	0.025	0.025	NS	NS	0.05	0.05	0.05	NS	0.05	0.025

Figure 20:

Maximum urine osmolality (Max Uosm) of controls and SFO lesioned animals made prior to dietary electrolyte depletion and on day 11 of the low NaCl/K-free diet. Results are expressed as means ± S.E.M. The number of animals is shown in the lower left hand corner of histogram. Data analyzed using two-way ANOVA and Scheffe test. *$p < 0.025$ and ‡$p < 0.005$ compared to control diet.

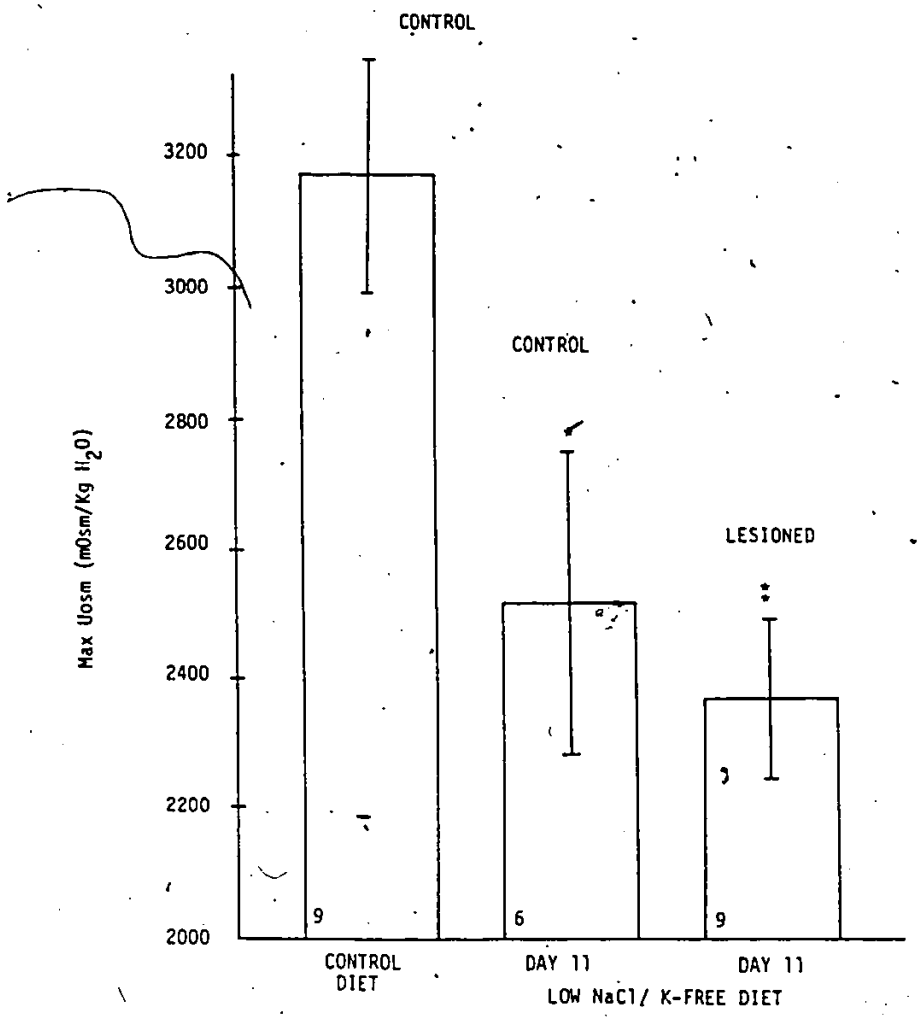
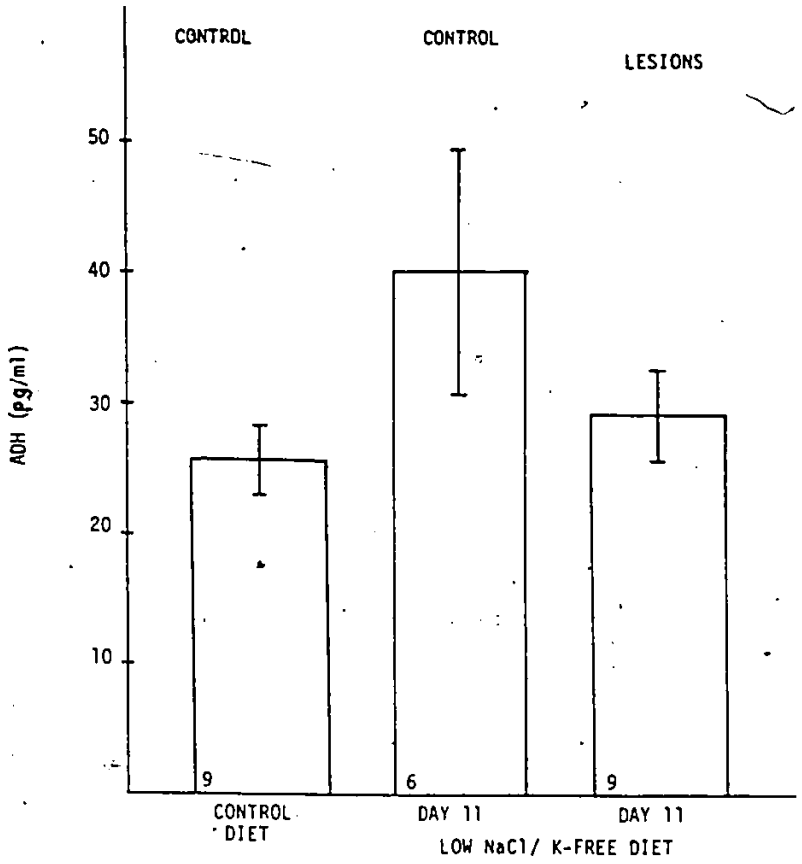

CONTROL
CONTROL
LESIONED
3200
3000
2800
2600
2400
2200
2000
Max Uosm (mOsm/Kg H_2O)
9
6
9
CONTROL DIET
DAY 11
DAY 11
LOW NaCl/ K-FREE DIET

Figure 21:

Plasma antidiuretic hormone (ADH) concentrations in control and SFO lesioned animals following maximum stimulation by water depriving the animals for 24 hours. Results are expressed as means ± S.E.M. The number of animals is shown in the lower left hand corner of histogram. Data analyzed using two-way ANOVA and Scheffé test.


CONTROL
CONTROL
LESIONS
ADH (pg/ml)
50
40
30
20
10
9
6
9
CONTROL DIET
DAY 11
DAY 11
LOW NaCl/ K-FREE DIET

ablating the structure, were effective in preventing the development of polydipsia, a primary effect of dietary electrolyte depletion. This would support the possibility that elevated circulating angiotensin is the mechanism responsible for the polydipsia.

Lesions of the SFO would interfere with angiotensin-mediated thirst mechanisms by disrupting the pathways described in study 3.3, Results and Discussion. This involves the interruption of blood-borne AII which has been demonstrated by RIA to be elevated in diet-depleted rats in the present study. Depletion of plasma K and Cl levels have been demonstrated to influence renin release from the juxtaglomerular cells of the kidney (Linas, 1981; Opara-Stitzer et al., 1976). The activation of the peripheral RAS system is responsible for high levels of circulating AI, and assuming normal converting enzyme activity, elevated levels of circulating AII. Mediation of angiotensin II drinking behaviour appears to involve interaction of the hormone with brain receptors at the angiotensin-receptive neurons of the circumventricular organs bordering the cerebral ventricles. These structures, particularly the SFO, possess vascular specializations that would allow circulating AII or other blood-borne peptide hormones to equilibrate with the interstitial fluid in the brain.

In the present study animals on the low salt diet had a primary renal concentrating defect, independent of the polydipsia. Since plasma becomes hypoosmotic as a consequence of NaCl and K deficiency, the second focus of this study was to assess whether ADH secretion from the SON was impaired in the animals which might contribute to the presence of the UCD. The primary polydipsia characteristic of electrolyte depletion does not appear to be responsible for the impairment of urine

concentrating ability, which instead results from changes in intrarenal factors (Berl et al., 1977 and 1980). RIA to establish ADH levels following 24 hour dehydration revealed that plasma ADH is not significantly different in hypokalemic rats compared to normokalemic rats (Berl et al., 1977). In potassium depletion studies, the mechanism whereby hypokalemia may impair the response to vasopressin has led to proposals of a role for increased prostaglandin levels on ADH action (Rutechi et al., 1982; Berl et al., 1977).

The present data (Fig. 21) shows that ADH responses to 24 hour water deprivation (osmotic and nonosmotic stimuli) far exceed 5 pg/ml plasma, a level that normally produces maximum urine concentration (Robertson, 1983). The data demonstrate that the UCD in salt depleted rats persists in control and lesioned animals in the presence of normal ADH plasma levels. This is discussed further in section 4, General Discussion.

4.0 GENERAL DISCUSSION

Electrolyte depletion in animals is associated with some very pronounced behavioural, endocrinological and renal changes. For this reason, we have aimed to achieve some understanding of the causal relationship between these electrolyte depletion induced changes. This has involved an assessment of the neuroendocrine factors which influence fluid balance following dietary electrolyte depletion.

Electrolyte depletion in rats, produced by dietary restriction of K alone or K, Na and Cl is known to cause a polydipsia characterized by persistently elevated plasma angiotensin II (AII) levels, and polyuria with significant impairment of urine concentrating ability (Linas, 1981). In order to determine whether the behavioural changes are mediated by the renin-angiotensin system (RAS), young adult male rats were fed electrolyte restricted diets during which measurements were made of daily water intakes, urine outputs, plasma osmolalities and electrolytes, and PRA and angiotensin I (AI) concentrations. The major findings may be summarized as follows:

1. The primary effect of electrolyte depletion is to induce polydipsia and concurrently polyuria. Subsequently this is followed by the development of a UCD.

2. Ablation of the SFO, a structure which has been shown to mediate angiotensin-induced thirst, attenuates the polydipsia but does not prevent the UCD. The polydipsia therefore appears to result from stimulation of AII receptors at the SFO by high levels of circulating AII.

Although there are many studies of fluid electrolyte metabolism and renal function following selective electrolyte depletion, especially potassium depletion, none have been aimed at precisely defining the role of the RAS in electrolyte depletion induced polydipsia. The results of study 3.1 and study 3.2 underscore the suitability of the electrolyte depletion model for polydipsia used in this lab. Rats maintained on the low salt diet were hypokalemic, hypochloremic, and had a hypoosmotic plasma with persistently elevated PRA and AI concentrations. The latter two diet induced plasma changes suggest that an activation of the RAS may be the mechanism responsible for the increased daily fluid intakes of the animals following introduction of the electrolyte deplete diets.

Studies on water metabolism in potassium deplete animals have focused on the possible mechanisms that come into play at the central hypothalamic-pituitary and renal level. Berl et al. (1977 and 1980) have shown that rats on a potassium deficient diet drink larger volumes of water than their pair-fed controls by the seventh day. At which time polydipsia and polyuria are well established, no significant impairment of Max Uosm was noted. In a subsequent study, the water intake of animals on a potassium-restricted diet, was matched to that of normokalemic rats. Urine outputs were correspondingly reduced, suggesting that the polyuria is largely a consequence of the polydipsia. These results clearly demonstrate that in potassium depletion, the increase in water intake precedes the development of a concentrating defect. This further demonstrates that the substantial increase in

water intake, which has been shown to washout medullary solutes (Levitin et al., 1962) is not responsible for the renal concentrating defect in these polydipsic, polyuric and hypokalemic rats. Our results of study 3.1 and study 3.2 establish a similar argument for rats maintained on the low NaCl/K-free diet. This diet was found to be more effective for the development of polydipsia compared to dietary potassium depletion alone (Brokaw, 1953).

The fact that increased water intake precedes a defect in water conservation implies that the stimulus to thirst is not hyperosmolality but rather of a nonosmolar nature. In order to test further the hypothesis that the primary increase in water intake and the renal concentrating impairment occur independently of one another we attempted to prevent the development of electrolyte depletion induced polydipsia. The results of studies 3.3 and 3.4 demonstrate that ablation of central receptor sites of angiotensin-mediated thirst in the SFO attenuate the development of increased fluid intake seen within a few days of introducing the electrolyte deplete diet. The normodipsic-lesioned rats did however, develop an impairment of urine concentrating ability to the same degree as polydipsic control animals when maintained on the low salt diet. These studies indicate that activation of the RAS is the probable mechanism responsible for mediation of the primary polydipsia characteristic of electrolyte depletion. This may involve elevated circulating levels of angiotensin II action onto receptor sites located in regions of the brain lacking a complete blood-brain barrier such as the subfornical organ, a circumventricular structure in the brain.

A pharmacological approach to understanding the mechanism of electrolyte depletion induced polydipsia has been inconclusive (Berl,

1980). Studies employing captopril (SQ 14,225), an inhibitor of angiotensin I-converting enzyme (ACE) and other ACE inhibitors have been inconclusive since at many doses these drugs cause an increase in water turnover and plasma renin activity (PRA) (Lehr et al., 1973; Berl, 1980; and Schiffrin et al., 1981 and 1982). The increased fluid intake following captopril treatment may be caused by the effects of elevated angiotensin I concentrations achieved after blockade of ACE and stimulation of renin secretion by captopril (Schiffrin et al., 1982). Furthermore, Schiffrin et al. (1982) demonstrated that systemic administration of an AII antagonist abolishes the dipsogenic action of oral captopril. If the mechanism for electrolyte depletion induced polydipsia involves stimulation of central AII receptors by high levels of the circulating hormone, as suggested by the results of studies 3.3 and 3.4, an appropriate experiment may be to use the AII competitive antagonist, saralasin, to attenuate the development of the polydipsia. This would involve chronic infusion of [Sar'Ile^{8}] ANG II to attempt to block receptors for blood-borne AII (Van Houtten et al., 1980).

Although the effects of dietary electrolyte depletion on hemodynamic parameters were not assessed, there have been extensive studies of the hemodynamic changes associated with potassium depletion and chloride depletion. The various factors known to increase renin release have been discussed earlier. Increased PRA, inturn, will result in activation of the renin-angiotensin system. In conditions of chronic electrolyte depletion, particularly chronic potassium deficiency, it has been demonstrated in the conscious rat that there is a decrease in systemic vascular resistance (SVR) which results in a decrease in mean arterial pressure (MAP) (Linas et al., 1982). Despite peripheral

vasodilation, there is an increase in renal vascular resistance (RVR) which results in a decreased renal blood flow (RBF). The increased RVR is mediated by both angiotensin II and products of prostaglandin endoperoxide metabolism because angiotensin antagonism or prostaglandin inhibition was effective in restoration of RBF to normal values in these potassium depleted animals (Linas et al., 1982). The decrease in peripheral vascular resistance occurs in the presence of increased PRA (Linas et al., 1981) which suggests that the pressor effect of angiotensin II is blunted in potassium deficiency. Paller et al. (1983) investigated the possibility of other pressor hormones which might contribute to the maintenance of peripheral vascular resistance and mean arterial pressure in potassium deficient animals. They were able to demonstrate that ADH is important in the support of blood pressure. In the potassium deficient conscious rat by decreasing either the concentration or efficacy of ADH resulted in a decrease in blood pressure. Following the decrease of endogenous plasma ADH they were able to restore mean arterial pressure in potassium deficient rats by infusing vasopressin.

In the present studies, similar plasma-induced changes were observed following a period of dietary electrolyte depletion on both the K-free and low NaCl/K-free diets. Although hemodynamic parameters were not assessed directly it seems likely that the changes would parallel those seen in studies involving dietary potassium depletion alone. Paller et al. (1983), found that potassium deficient animals show increased PRA, AII and ADH levels. The stimulus of a hyperosmotic plasma additionally favours ADH release. These increases in ADH and AII resulting from nonosmotic and osmotic stimuli may activate thirst

mechanisms resulting in a significant and sustained polydipsia in the potassium deficient animal. In the present studies involving dietary K-depletion alone and low NaCl/K-depletion the animals showed similar changes in plasma hormones except for the development of a hypoosmotic plasma, which may inhibit the development of polydipsia and ADH release.

The mechanism by which dietary electrolyte depletion may bring about activation of the RAS to influence fluid metabolism was not the focus of this study. However, the activation of the RAS leading to elevated circulating AII appears to be due to increased plasma renin activity (PRA) mediated by renal vascular receptors and renal tubular receptors in the macula densa (Linas, 1981). Furthermore, I would like to emphasize the present study does not attempt to elucidate the pathogenesis of the water conserving impairment characteristic of electrolyte depletion. Rather, the aim of the present study was to confirm that the observed polydipsia is not secondary to the development of a urine concentrating impairment. Changes in renal hemodynamics, medullary solute concentration and antidiuretic hormone release are several of the possibilities to have been postulated to mediate the urine concentrating defect. It is well known that potassium depletion alone results in a renal concentrating defect (Berl, 1980; Berl et al., 1977; and Eknoyan et al., 1970). This defect can be corrected by dietary potassium repletion. We found this to be the case with rats maintained on the low salt diet in the present study. Following dietary repletion all plasma parameters measured (K^+, Cl^-, Na^+, osmolality, PRA and AI) returned to control levels.

One possible explanation for the failure to normally concentrate urine during electrolyte depletion is a defect in vasopressin release. ADH release is governed by the state of excitability and impulse frequency in neurosecretory neurons in the supraoptic and paraventricular nuclei (Douglas, 1974) and this is influenced by several factors such as plasma osmolality, baroreceptor input and CSF AII levels. It has been demonstrated by radioimmunoassay that ADH levels in K-depleted rats following 24 hour dehydration do not differ significantly from normokalemic rats (Linas, 1983). This would suggest that the UCD may be due to a decreased renal unresponsiveness to ADH rather than to deficient ADH release. This interpretation is supported by the results of study 3.4. There were no significant differences in ADH levels following 24 hour water deprivation between control animals and depletion animals with or without lesions of the SFO. Although the SFO stimulates the release of vasopressin following activation of AII receptors in the SFO (Iovine et al., 1984), water deprivation leads to dehydration of both cellular fluid compartments involving osmotic and nonosmotic control of ADH release (Fitzsimons, 1972 and 1979; and Schrier, 1979).

A possible explanation for the concentrating defect in the present study may involve an impaired NaCl transport by the thick ascending limb (TAL) coupled with reduced solute delivery to the loop of Henle in dietary electrolyte depletion (Gutsche et al., 1984; Peterson, 1984). Micropuncture studies have shown that Cl-depletion leading to hypochloremia decreases net reabsorption of NaCl by the TAL and K-depletion impairs NaCl reabsorption by the TAL (Galla et al., 1981; Gutsche et al., 1984).

The extent of chloride depletion following these dietary manipulations may be an important factor. Study 3.1 shows that the low NaCl/K-free diet fed animals are more hypochloremic than the K-free diet fed animals in comparison to controls. Many studies have shown that Cl-depletion leads to renin release (Abboud et al., 1979; Kirchner et al., 1978; Kotchen et al., 1976 and 1978). Since Cl transport occurs as a secondary active process in co-transport with Na^+ (Greger, 1981; Knepper et al., 1983), occurring in the thick ascending limb, the Cl effect on renin release may be mediated by a macula densa mechanism. This may account for a greater stimulus for activation of the renin-angiotensin system leading to increased thirst behaviour overriding any osmotic inhibition to the development of polydipsia in rats maintained on the low salt diet. Studies of chronic metabolic alkalosis which can be maintained by feeding a Cl-free diet are characterized by hypochloremia, hyponatremia, hypokalemia, plasma hypo-osmolality, polydipsia, polyuria and a renal concentrating defect. All or some of these changes are common to conditions of potassium depletion and chloride depletion and may have a common etiology of renal and extrarenal function.

SUMMARY

Electrolyte depletion in rats induced by dietary restriction of K alone or K, Na and Cl as studied in this lab is characterized by a hypokalemic, hypochloremic, and hypoosmotic plasma with persistently elevated plasma renin activity (PRA) and plasma angiotensin I (AI) concentrations. Measurements of daily water intake, Max Uosm, and ADH

response of control rats and rats with electrolytic lesions of the subfornical organ (SFO) suggest the following:

1) The primary effect of electrolyte depletion is to induce polydipsia and concurrently polyuria, followed later by the development of a urine concentrating defect, therefore the polydipsia is not secondary to an impaired water conserving ability of these animals.

2) Ablation of central structures (circumventricular organs) which mediate angiotensin-induced thirst attenuates the polydipsia, but does not prevent the development of the urine concentrating defect.

3) The polydipsia therefore results from stimulation of AII receptors at the circumventricular structures (the SFO investigated in the present study) by high levels of circulating AII.

4) The urine concentrating defect (UCD) is independent of the polydipsia, involving both renal and extrarenal factors. The UCD is not a consequence of inappropriate ADH release. The salt depleted animals were presented with chronic plasma hypoosmolality that may have an effect on normal ADH release and the magnitude or attenuation of the observed polydipsia. Furthermore, the accompanying electrolyte changes may be in part responsible for the concentrating defect and influence on overall fluid metabolism.

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APPENDIX 1

Angiotensin 1 Radioimmunoassay

Sample Collection:

Blood was drawn into a syringe containing sufficient EDTA to yield a final concentration of 1 mg/ml. Samples were maintained in an ice bath before separating the plasma in a refrigerated centrifuge at 4°C for 15 minutes at 1200 x g. The clear plasma was placed in an ice bath for immediate assay or stored frozen at -20°C.

Angiotensin 1 Generation - Specific Protocol

1. Known high and low control samples were assayed with each run.
2. The frozen plasma samples were thawed in a refrigerator at 4°C.
3. 1 ml of each plasma sample was pipetted into a clear polystyrene tube in an ice bath. 10 µl of the Dimercaprol solution and 10 µl of the 8-Hydroxyquinoline solution were added to each tube.
4. 2.0 ml of the pH 6.0 Maleate Buffer was added to each tube and mixed thoroughly.
5. 1 ml of the contents of each tube was transferred to a similarly labelled polystyrene tube. The 1 ml aliquots were incubated for 1 hour, at 37°C, while maintaining the remainder of each solution in an ice bath for the same period of time.
6. At the end of the incubation period the samples incubated at 37°C were matched against their 4°C control in the ice bath and the angiotensin 1 measured by radioimmunoassay. (All samples may be stored frozen at this stage, if desired. Precautions must be taken to thaw later at temperatures that do not exceed 4°C). Incubated samples were kept in an ice bath while pipetting all reagents.

Radioimmunoassay Protocol

All reagents and tubes were kept in an ice bath while pipetting.

1. A series of 16 tubes for the standard curve and 4 tubes for each sample to be run were numbered.
2. 500 µl of Assay Buffer was pipetted into tubes 1 and 2 (Blank tubes).
3. 100 µl of 5% BSA was pipetted into tubes 1 and 2 (Blank tubes) and 3 and 4 ("0" Standard).
4. 100 µl of each Standard was pipetted into the appropriate tubes, i.e., 0.1 ng/ml into tubes 5 and 6, 0.25 ng/ml into tubes 7 and 8, 0.5 ng/ml into tubes 9 and 10, 1.0 ng/ml into tubes 11 and 12, 2.5 ng/ml into tubes 13 and 14, and 5.0 ng/ml into tubes 15 and 16.
5. 100 µl or less (Assay Buffer added to maitnain uniform incubation volumes) of the sample was pipetted as follows: 37°C generated sample into tubes 17 and 18 and the matched 4°C control into tubes 19 and 20. Continue in this manner for all other samples. The value obtained is then multiplied by the appropriate dilution factor.
6. 100 µl of Tracer solution was pipetted into all tubes.
7. 500 µl of Antiserum was pipetted into all tubes except tubes 1 and 2.
8. All tubes were stirred using a Vortex mixer for 2-5 seconds.
9. All tubes were incubated at 4°C for 18-24 hours.
10. Prior to the completion of the incubation, the diluted Charcoal Suspension was actively stirred using a magnetic stir bar.
11. At the end of the 4°C incubation all samples were returned to an ice bath and 1.0 ml of the Charcoal Suspension was pipetted into each tube. Active stirring of the Charcoal Suspension was maintained during the transfer.

12. All tubes were vortexed and centrifuged at 1200 x g for 15-20 minutes. All tubes in the assay group were processed as a unit to minimize variations in handling during the separation procedure.

13. The supernatant fraction of each tube was decanted to appropriately numbered polystyrene tubes for gamma counting. Great care was taken to transfer the supernatant free of charcoal particles. Transer was maximized by rimming the tubes. Antibody-bound angiotensin 1 is contained in the supernatant fraction and unbound angiotensin 1 is absorbed by the charcoal.

14. The supernatant tubes were counted in sequence using a well-type, solid-crystal scintillation counter. Each tube was counted for 5 minutes.

15. All values were multipled by the appropriate dilution factor.

Taken from Instruction Manual, Rianen, Angiotensin 1 (I^{125}) Radioimmunoassay Kit, New England Nuclear, North Billerica, MA., 1983. Cat. No. NEA-026,022

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