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ADH Levels during Salt Depletion in Dogs*

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Abstract. Two groups of trained dogs were subjected to sodium chloride depletion and plasma ADH concentration, renin activity, plasma sodium, plasma osmolality, blood volume, hematocrit and body weight were measured. In one group of animals, sodium depletion was created by restricting intake to 5 mEq/24 h. Despite a statistically significant decrease in body weight and blood volume and a corresponding increase in plasma renin activity, plasma ADH concentration was not seen to change significantly from control values. Similar findings were seen in a second group of dogs which were given a diuretic in addition to dietary sodium restriction. In these animals the decrease in blood volume and rise in plasma renin activity were proportionately greater. Plasma ADH concentration was not observed to change significantly in this group of animals either. Both groups of animals developed significant hyponatremia during the experiment. It is concluded that in these dogs, the secretion of ADH was not suppressed and consequently hyponatremia developed. It is suggested that endogenous angiotensin was responsible for the continued secretion of ADH at control levels.

Key words: ADH, renin-angiotensin, hyponatremia, salt depletion, blood volume, diuretic.

There is no information available regarding plasma concentrations of antidiuretic hormone during sodium chloride depletion. Despite this, many of the alterations in the body fluids attending sodium depletion have been explained on the basis of variations in ADH secretion. An animal in negative sodium balance might be expected to show alterations in ADH levels as volume is lost along with Na. This report deals with the results of our experiments in which plasma ADH was measured in trained dogs during sodium deprivation.

Methods

Eight adult mongrel dogs of both sexes weighing 14–29 kg served as the experimental animals. All dogs had chronic indwelling arterial catheters inserted at least two weeks before the experimental protocol was begun. The catheter was inserted into the aorta via the carotid artery in most dogs, but in some the femoral artery was used as previously described [1]. This procedure does not appear to interfere with ADH release as control values for these animals are similar to those reported by others as is their response to anesthesia, surgery and dehydration [1]. All animals underwent a period of training prior to the experiment in which they were taught to lie quietly on the examining table while arterial blood was sampled.

Two experimental protocols were used. In both protocols a pre-experimental control period and a post-experimental recovery period each of seven days duration, bracketed the experimental period. In protocol A the experimental period lasted 7 days, and in protocol B, it was of 10 days duration. In each protocol the animals ingested the same diet (h/d dog food, Hill Packing Co., Topeka, Kansas, U.S.A.) providing 5 mEq of Na per day. During the control and recovery periods additional sodium chloride was added to bring the sodium content of the diet up to 60 mEq/day. In protocol A, sodium depletion was accomplished solely by dietary means. In protocol B, a mercurial diuretic was used in addition to dietary sodium restriction. One ml of Mercuhydrin (Lakeside Laboratories, Milwaukee, Wisconsin, U.S.A.) supplying 39 mg of organic mercury was injected intramuscularly on days eight and nine of the experimental period.

In both protocols blood sampling was performed on days five and seven of the control period and on days three and seven of the recovery period. In protocol A, the animals were sampled on days three and seven in the experimental period. The animals participating in protocol B were sampled on the third, seventh and tenth day of the experimental period.

On the appropriate day, each dog was weighed, had an arterial microhematocrit performed and 50 ml of arterial blood was removed by means of an iced plastic syringe. The blood was centrifuged in the cold and the plasma separated for chemical analysis. The blood cells were then resuspended in an appropriate volume of sterile 6% dextran in isotonic saline (Gentran, Travenol Laboratories, Morton Grove, Illinois, U.S.A.) and returned to the animal. This procedure was shown not to effect ADH secretion rates [1]. The following determinations were performed on the plasma: sodium concentration, osmolality, ADH titer and plasma renin activity. In addition, plasma volume was determined in 10 experiments using Evans Blue Dye. In protocol A, plasma volume

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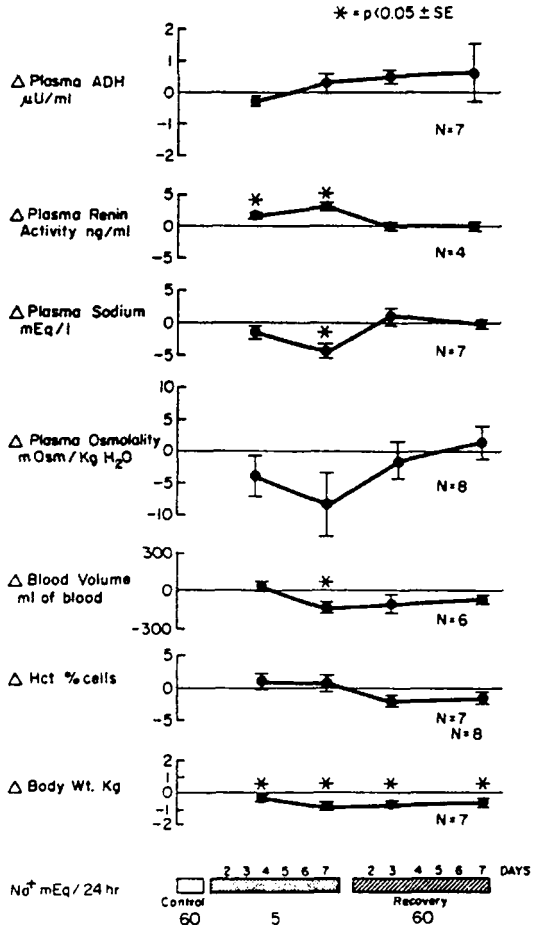

Fig. 1. Effect of dietary sodium deprivation on plasma ADH and renin, blood volume, body weight and plasma composition. Each point represents the mean ± SEM. Statistics done by paired sample analysis, by comparing each value with the mean of the two control values

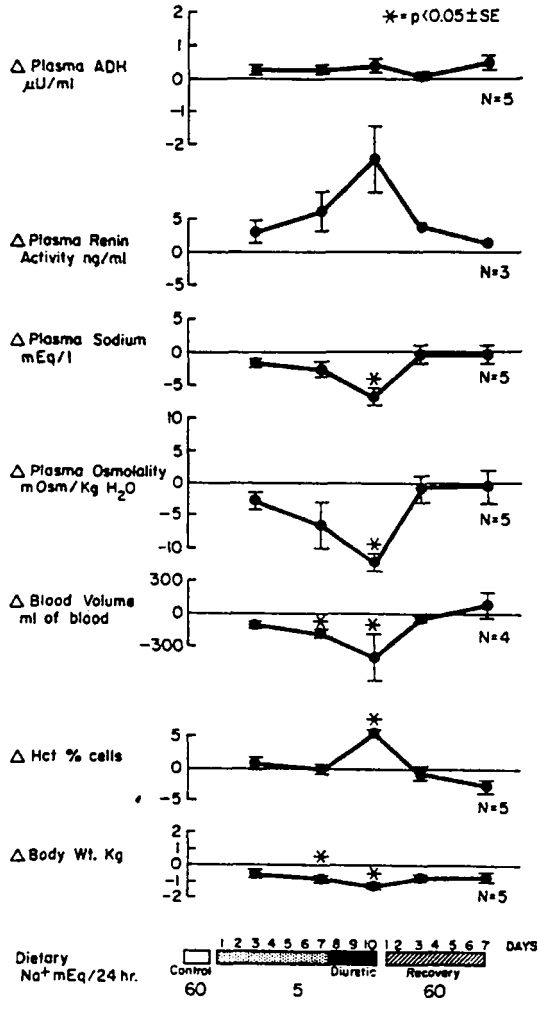

Fig. 2. Effect of dietary sodium deprivation and mercurial diuretic on plasma ADH and renin, blood volume, body weight and plasma composition. Each point represents the mean ± SEM. Statistics done by paired sample analysis

was determined in 6 experiments, and in 4 experiments using protocol B. Blood volume was calculated from the plasma volume and large vessel hematocrit.

Plasma ADH was estimated by a modification of the method of Share and Levy [1]. In general this method depends upon the extraction and concentration of ADH from plasma. The extract is then injected into a water loaded, alcohol anesthetized rat and the inhibitory effect on the diuresis compared to standards. Each sample was run in quadruplicate. The modification introduced here was to omit use of diethyl ether in removal of tricloracetic acid from the extract and to omit adjusting the tricloracetic acid to pH 4.5. Both steps were shown to be unnecessary. The standard deviation of replicate analysis on the same sample was 10%, and the sensitivity of the method such that no sample in this work had concentrations below detectable levels. Plasma renin activity was estimated from a modification of the method of Boucher [2]. Renin activity is expressed as angiotensin equivalents in nanograms per milliliter of plasma. Sodium concentrations were measured by flame photometry, osmolality by freezing point depression using an Advanced Instruments Osmometer. Plasma volume was estimated by measuring the volume of distribution of Evans Blue determined 10 minutes following intravenous injection.

Results

The results of both protocols are summarized in Figs. 1 and 2. The experimental values have been compared to the mean of the two control values and examined statistically by paired sample analysis.

Statistically significant depression of the plasma sodium concentration was observed in both groups of dogs during the experimental period. Plasma osmolality also decreased during the period of salt depletion, but statistical significance was only achieved when diuretic injections were superimposed on the low

sodium diet. Blood volume was depressed significantly in both groups and became more pronounced after the diuretic was administered, and was accompanied by a statistically significant rise in the arterial hematocrit. Animals of both groups lost weight during the experiment after three and seven days on the low sodium diet in protocols A and B respectively. Plasma renin concentrations were significantly elevated after three days of sodium restriction in the animals participating in protocol A and increased an even greater amount after the seventh experimental day. A similar pattern was observed in the animals of protocol B, but due to the small number of animals in this group statistical significance was not achieved.

Plasma ADH concentrations did not change significantly during the experiment in either group. In Fig. 1 the mean decrease in ADH concentration on day 3 was 0.24 μU/ml from the average control value of 1.0 μU/ml; a decrease which was not statistically significant.

Discussion

The lack of variation in the concentration of plasma sodium despite large fluctuations in sodium intake is a well known phenomenon. The production of negative sodium balance by moderately reducing dietary intake is generally not associated with a decrease in the plasma sodium concentration of man [3] or dog [2]. Severe sodium depletion, on the other hand, which results in significant changes in the intravascular volume is commonly associated with hyponatremia [4]. This usually requires a greater sodium loss than that obtained by dietary restriction alone. At any given level of sodium deficit, it is the regulation of the extracellular water content which determines the plasma concentration of the ion. Thus with mild reductions in total body sodium, the extracellular water content is reduced and the concentration of sodium in plasma does not change significantly. It is felt that water leaves the extracellular space and enters the intracellular compartment as well as being excreted as solute free water by the kidney [5]. It has classically been taught that the renal water excretion in this case was secondary to inhibition of antidiuretic hormone release from the pituitary under the control of osmoreceptors located in the brain. The movement of water into cells was best explained by osmotic shifts.

Presumably, when the degree of sodium loss is severe and the volume of intravascular compartment is compromised by a further loss of salt and water, the kidney no longer excretes solute free water and the volume of the intravascular space is preserved. The mechanism by which the body defends the blood volume at the expense of the tonicity of the body fluids has not been elucidated but is felt to involve increased secretion of ADH mediated through thoracic volume receptors [6].

Our data do not support the classical theory regarding ADH secretion during salt depletion. Despite progressive decrements in both plasma osmolality and sodium concentration, plasma ADH levels did not change significantly. In keeping with past thought, one would have expected a significant decrease in ADH concentration to have occurred initially as negative sodium balance developed.

It is likely that the continued secretion of ADH at or near the control level during the experimental period was responsible for the trend toward hyponatremia and hypoosmolality observed in the two series. It is, of course, possible that the kidney is sensitive to extremely small variations in plasma ADH concentrations which fall outside the limit of sensitivity of our bioassay.

Another possible explanation for failure of ADH to be supressed during the early period of salt deprivation is that the rising level of plasma renin resulted in continued ADH secretion. It has been demonstrated that intravenous infusions of angiotensin will increase plasma levels of ADH [8], presumably by increasing secretion rates. The available evidence suggests that an angiotensin receptor is present in the brain, but this has not been proven [9]. This is not to imply that a reduction in blood volume plays no role in regulating secretion rates of ADH. Rather we believe that a combination of factors, reduced volume and increased circulating levels of renin and presumably angiotensin serve to maintain ADH secretory rates at or near control levels.

It is surprising that the administration of the diuretic which resulted in a reduction in blood volume of 12–15% did not result in a significant increase in ADH concentration. It has been demonstrated that a decrease in blood volume of this magnitude if produced by hemorrhage is a reliable stimulus for ADH release [10]. Recently Johnson, Zehr and Moore have demonstrated that hypoosmolality is a stronger inhibitor of ADH secretion than previously thought. They found that a 10% reduction in blood volume was not sufficient to elevate ADH levels in the face of moderate hypotonicity in the conscious sheep [7]. The failure of ADH levels to increase after salt restriction and administration of the diuretic in our animals may be due to the inhibitory influence of the hypotonicity.

The remainder of the data from these experiments dealing with renin are what one would expect from past work in this field [2, 11]. It should be mentioned, however, that relatively few investigators have induced sodium deficiency in dogs by dietary means alone. It would appear that additional work should be done in this area particularly in view of our unexpected results regarding the secretion of ADH during sodium deficiency.

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