Action of Antidiuretic Hormone in Potassium-Depleted Rats; Relation to Aldosteronism. (23669)

HOWARD S. RICHTER (Introduced by K. E. Paschkis)
Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia

The syndrome of primary aldosteronism, usually caused by a functioning adrenocortical tumor ("aldosteroma"(1) or "Conn's tumor") is characterized by intermittent tetany, paresthesias, periodic muscle weakness, polyuria, polydipsia and hypertension, but *no edema*(2). The significant metabolic findings are hypokalemic alkalosis, hyperaldosteronuria and hyposthenuria which is unresponsive to antidiuretic hormone (ADH)(1,2,3).

Aldosterone causes sodium retention(4,5) and would therefore be expected to cause concomitant water retention, as do the other adrenal steroids. The fact that primary aldosteronism is associated with polyuria instead of edema calls for an explanation. The clinical observation that the hyposthenuria in primary aldosteronism is unresponsive to ADH and to sodium chloride suggests that the polyuria and water loss are due to ineffectiveness of endogenous ADH, just as in nephrogenic diabetes insipidus. In the later condition, the distal tubules are unresponsive to ADH due to a hereditary abnormality. One wonders, then, if aldosterone "blocks" the action of ADH on the otherwise normal tubule.

In secondary aldosteronism observed in cardiac failure, hepatic failure, nephrosis and eclampsia, high aldosterone levels are not associated with polyuria, but with edema. It therefore appears unlikely that aldosterone "blocks" or counteracts ADH action in the renal tubules.

Since severe hypokalemic alkalosis is associated with primary and not with secondary aldosteronism, it was thought of interest to determine whether potassium depletion would influence the action of ADH.

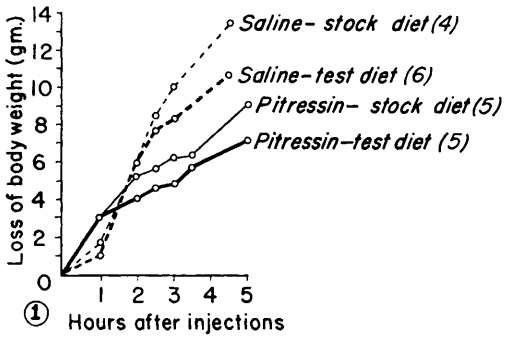
Two recent observations point in this direction. Hollander, *et al.*(5) have shown that ADH fails to produce urine concentration in potassium-depleted rats, the intensity of the hyposthenuria being related to the degree of potassium depletion. Five adult patients of Relman and Schwartz(6) with severe diarrhea producing severe potassium depletion demonstrated a vasopressin (Pitressin) resistant hyposthenuria. Urine volume and water retention were not mentioned in either paper.

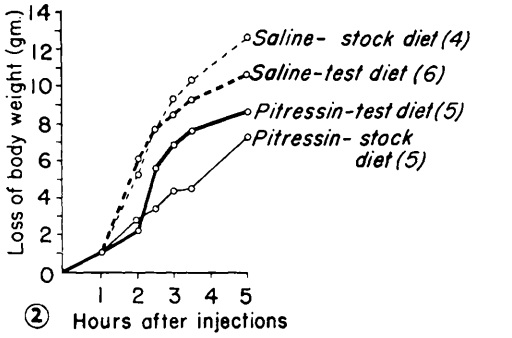
The present experiment is designed to measure the amount of water retention induced by ADH in rats depleted of potassium.

Materials and methods. Thirteen of 25 male rats of the Wistar strain weighing between 106 g and 170 g were fed a diet deficient in potassium for 100 days, while 12 controls received a stock diet (Purina laboratory chow) restricted only in quantity to keep their combined weights down to that of the experimental group. The potassium deficient diet(7,8) for the first 28 days was raw rice, 45%; dextrose, 28%; casein, 20%; cod liver oil, 3%; yeast, 2%; ammonium chloride, 1%; sodium chloride, 1%; thiamin, 75 mg/kg of food and distilled water *ad libitum.* When signs of severe hypokalemia (anorexia and weight loss) failed to appear, the diet was changed to dextrose, 35%; non-nutritive cellulose (Alphacel), 27%; casein, 25%; yeast, 4%; cod liver oil, 4%; sodium bicarbonate, 3%; sodium chloride, 1%; ammonium chloride, 1%; and distilled water *ad libitum.* No steroids or other drugs were used.

Potassium determinations were not performed, but the following presumptive evidence of potassium depletion in the test rats is strong: the diet was virtually potassium-free; only the test rats became lethargic; anorexia, as evidenced by flattening then dipping of the weight curve, has been the experience of other workers(5,7,8,9,10).

On the 12th, 19th, 27th, 31st, 35th, 49th, 61st and 100th days, food and water were withdrawn and 2 hours later all rats were hydrated by an injection of 10 ml of distilled water intraperitoneally. Both the rats on the stock diet, and those on the potassium deficient diet were divided into 2 equal groups so that each of 8 experiments were carried out with 4 groups. One stock diet group and one





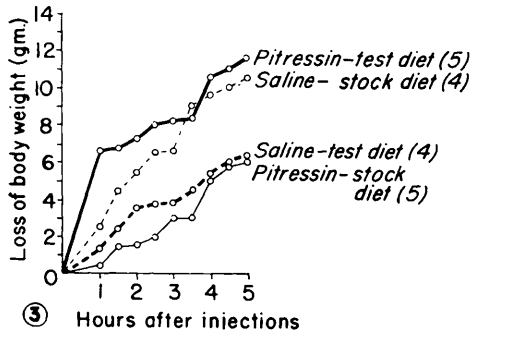


FIG. 1. Weight loss of hydrated rats over a 5-hr period on the 31st day of diet. One potassium deficient and 1 control group injected with 2 IU Pitressin, 1 potassium deficient and 1 control group injected with vehicle (saline) only. Numbers in brackets indicate the number of rats in each group. The variation in weight losses within each group of 4-6 rats at 3 hr, the point at which the curves were analyzed for significance, is given by a standard deviation of ± 1.75 g/group.

FIG. 2. Weight loss of hydrated rats over a 5-hr period on the 35th day of diet. One potassium deficient and 1 control group injected with 2 IU Pitressin, 1 potassium deficient and 1 control group injected with vehicle (saline) only. Numbers in brackets indicate number of rats in each group. Variation in weight losses within each group of 4-6 rats at 3 hr is given by a standard deviation of ± 1.33 g/group.

FIG. 3. Weight loss of hydrated rats over a 5-hr period on the 100th day of diet. One potassium-deficient and 1 control group injected with 2 IU Pitressin, 1 potassium-deficient and 1 control group injected with vehicle (saline) only. Numbers in brackets indicate number of rats in each group. Variation in weight losses within each group of 4-5 rats at 3 hr is given by a standard deviation of ± 2.46 g/group.

test diet group were injected subcutaneously with 2 IU of aqueous vasopressin (Pitressin, Parke Davis) in 0.5 ml of isotonic saline solution. The rats of the other 2 groups, one on stock diet and one on potassium deficient diet, served as controls and received 0.5 ml of isotonic saline subcutaneously.

All rats were weighed to the nearest gram immediately after being injected, and then weighed again at appropriate intervals. All rats were squeezed abdominally before each weighing to stimulate micturition. Feces were not weighed since the weight of even relatively large amounts is insignificant. In consecutive experiments control groups (saline only) and experimental groups (Pitressin) were rotated, the control groups of one experiment serving as experimental groups in the following.

Results. On about day 33, the weight curves began to flatten and on the 90th day they began to descend. The test rats had become progressively more lethargic beginning about the 50th day. The change in the rates of excretion of the 10 g water load became progressively more significant as the length of time on the test diet increased. Since the results of the first 3 injections were not significantly different from those of the 4th, and the 6th and 7th produced the same results as the 5th, only the results of the 4th, 5th and 8th experiments will be reported in detail. The control groups automatically cancel variations of body weight due to humidity, temperature, etc.

Fig. 1 shows average water loss over a period of 5 hours on the 31st day of the diet (3rd day of the improved diet), just before the test animals began to manifest signs of potassium depletion. The mildly potassium-depleted animals seemed to excrete their water load slower than the controls, but the

difference was not statistically significant. Pitressin slowed the water excretion in both groups to a highly significant degree ($P < .001$)* after a small initial diuresis.

Fig. 2 represents results of the injections given on the 35th day, just after the test rats began to show signs of potassium depletion. There was significant interaction ($P < .05$); *i.e.*, the injection effect was not independent of the diet effect. The effect of Pitressin on water retention in the normal animals was again highly significant ($P < .001$); in the moderately potassium-depleted rats Pitressin failed to induce a significant amount of water retention ($P > .05$). In the 2 groups given Pitressin, failure of the moderately depleted rats to retain as much water as the controls was probably significant ($P < .05$).

Fig. 3 shows how severely potassium-depleted rats (showing increasing lethargy and progressive weight loss) handle a water load with and without Pitressin. Again, there was significant interaction ($P < .01$). The severely depleted rats given a dose of isotonic saline seemed unable to excrete the water as fast as those on the control diet, but the difference was not statistically significant. Whereas Pitressin had previously produced water retention, now it not only failed to produce water retention in the depleted animals ($P < .05$), but actually enhanced its excretion in the first hour after injection. Furthermore, of the 2 groups given Pitressin, the depleted rats excreted their water significantly faster ($P < .01$).

Discussion. Experiments here presented show that rats fed a potassium-deficient diet become progressively less responsive to the antidiuretic action of Pitressin; of the severely potassium-depleted animals, the Pitressin-treated animals even lost water more rapidly than their controls. These results, as well as the work of Hollander, *et al.*(5), suggest that severe potassium depletion "blocks" the action of ADH. Welt(12) had previously suggested this hypothesis. The mechanism by which this "block" is brought about is, at present, unknown. It is of interest that the effects of potassium depletion on ADH action seem to be biphasic inasmuch as mild potassium depletion appears to enhance the antidiuretic effect of ADH, whereas in severe potassium depletion ADH seems to induce diuresis.

These results may have a bearing on the differences in water balance in primary and secondary aldosteronism. The fact that ADH is ineffective in the presence of potassium depletion may explain the absence of edema in primary aldosteronism. In secondary aldosteronism, severe potassium depletion is not observed and endogenous ADH would be expected to be fully effective; if mild potassium loss would occasionally occur, the effect of ADH might even be potentiated. The polyuria associated with high levels of aldosterone seen in primary aldosteronism would then be analogous to nephrogenic diabetes insipidus, since endogenous ADH is ineffective in both; in the case of nephrogenic diabetes insipidus, polyuria is due to a primary defect in the kidney tubules, while in primary aldosteronism it is probably due to the ineffectiveness of endogenous ADH resulting from severe potassium depletion.

The results reported here may possibly have therapeutic implications. It is conceivable that in cases of secondary aldosteronism, especially nephrosis and cirrhosis, the production of hypokalemia with adrenocortical steroids, with or without added ADH, might induce a prompt diuresis, as seen in the test rats.

Summary and conclusions. Rats were depleted of potassium and half the group was injected with ADH when mildly depleted, moderately depleted and severely depleted; simultaneously, half the rats on stock diet were given ADH. The antidiuretic action of ADH was enhanced in the presence of mild potassium depletion; in moderate potassium depletion the antidiuretic effect of ADH was diminished; in severe potassium depletion ADH induced prompt polyuria. These results suggest that the lack of edema, and the presence of polyuria in primary aldosteronism are due to ineffectiveness or to blocking of endogenous ADH, not as a result of a di-

* All data were tested at the 3 hour point after injection. Results were analyzed statistically by the method of factorial arrangements(11).

rect antagonistic action of aldosterone, but as a result of the potassium depletion.

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NaCl Appetite of Adrenalectomized Rats.* (23670)

Melvin J. Fregly (Introduced by A. B. Otis)
Department of Physiology, J. Hillis Miller Health Center, University of Florida, Gainesville

One of the striking behavioral characteristics of adrenalectomized rats is their spontaneous NaCl appetite when given choice between water and .15 M NaCl solution as drinking fluids(1,2). This characteristic has been used by some investigators to assess completeness of adrenalectomy(3,4). It was therefore deemed worthwhile to determine whether the NaCl appetite of adrenalectomized rats was concentration dependent; that is, whether the rats preferred NaCl solution to water at some but not all concentrations of the former. It was also the object of this study to determine the NaCl concentration at which adrenalectomized rats ingested most salt solution. Knowledge of this particular concentration would be especially useful in experiments in which completeness of adrenalectomy is to be assessed, since it would assure that maximal differences could be obtained between intakes of normal and adrenalectomized rats.

Methods. Two separate experiments were performed. The materials and methods, similar for both experiments, are described below. Male rats of the Holtzman strain were used. All rats were kept in individual cages in a room maintained at 26 ± 1°C and illuminated from 8 a.m. to 6 p.m. The animals were allowed Purina Laboratory Chow *ad libitum.* Intakes of food were not measured. Each rat was given choice of 2 drinking fluids, distilled water and NaCl solution. The type of fluid containers used has been described (5). The positions of the 2 bottles were interchanged daily to avoid habit formation in the selection of the fluids. Fluid intakes were measured daily by weight difference and are expressed as ml/100 g body weight/day. Body weights of the rats were also measured daily. *Exp. 1.* Five adrenalectomized and 5 control rats ranging in initial weight from 180 to 200 g were used. Adrenalectomy was performed one week prior to beginning any measurements, and all animals were maintained on .15 M NaCl solution and water until the experiment began. Each of 4 different concentrations of NaCl solution was offered, with distilled water as the second fluid choice, for 4 days. Chronologically the concentrations given were: .25, .35, .50 and .15 M. *Exp. 2.* Five adrenalectomized and 5 control rats ranging in initial weight from 220 to 240 g

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