SALURETIC ACTION OF ALDOSTERONE IN THE PRESENCE OF INCREASED SALT INTAKE AND RESTORATION OF NORMAL ACTION BY PROLACTIN OR BY OXYTOCIN

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SUMMARY

The effects of aldosterone on sodium, potassium and water excretion during various treatment regimes were studied in six Merino ewes. Urine was collected from 12.00 to 14.00 h and from 14.00 to 16.00 h each day. Intravenous injections of either 1 ml isotonic saline or 500 \( \mu \text{g} \) aldosterone were given at 13.30 h and excretion during the second collection period compared with that in the first. When each animal was given a salt (\( \text{NaCl} \)) supplement of 80 mequiv./day by i.v. injection, aldosterone caused marked sodium retention with no effect on potassium. When salt supplements of 400 mequiv./day were given, aldosterone lost its sodium-retaining action in all animals and caused a marked saluresis with a small increase in potassium excretion in five sheep out of six. Injections of sheep pituitary prolactin or of oxytocin restored the sodium-retaining action of aldosterone in spite of a continued high salt intake. The animals gained very little weight when treated with 400 mequiv. salt alone but did gain significantly when treated with salt plus prolactin. The weight gain was rapidly lost when the prolactin and high salt intake were discontinued.

INTRODUCTION

In normal subjects, continued administration of mineralocorticoid hormones causes an initial retention of sodium followed by 'escape' from the renal sodium-retaining effects of the steroid (Perera, 1948; August, Nelson & Thorn, 1958). The mechanism of the escape is unknown but it does not occur in sodium-depleted states (Strauss & Earley, 1959), in dogs with thoracic vena caval constriction, arterio-venous fistulae or heart failure (Davis, 1964), in patients with Addison's disease or in bilaterally adrenalectomized animals (Soffer, Lesnick, Sorkin, Sobotka & Jacobs, 1944). The present paper demonstrates that in normal animals a phenomenon akin to 'escape' can be induced simply by a very high level of sodium intake and that the continued presence of abnormally large amounts of mineralocorticoids is not necessary for its development.

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Prolactin is a powerful sodium, potassium and water retainer in man (Horrobin, Lloyd, Lipton, Burstyn, Durkin & Muiruri, 1971). In perfused kidneys, Lockett and co-workers have demonstrated that when the kidney is supplied with blood from a headless animal aldosterone is saluretic: the usual salt-retaining action can be restored by adding growth hormone, prolactin or oxytocin to the perfusate (Davey & Lockett, 1960; Lockett & Roberts, 1963; Lockett, 1965). The present paper demonstrates in intact animals that aldosterone can be saluretic and that its normal effect can be restored by treatment with prolactin or with oxytocin.

METHODS

In six dioestrous Merino ewes, the effects of aldosterone on sodium, potassium and water excretion were studied. The sheep were examined by a veterinarian and appeared clinically healthy. However, when jugular venous cannulae were inserted one was found to have a high venous pressure of 15–20 cm H₂O which persisted for the duration of the experiments. There was no obvious reason for this and the animal was not killed at the end of the experiment as it was required for further study.

Each animal was prepared by inserting a plastic cannula into one jugular vein and a self-retaining balloon catheter into the bladder. The cannulae and catheters were left in place for the duration of the experiment. No signs of infection were noted around the venous cannulae. The bladder was irrigated with 1:200 Savlon solution immediately after insertion of the catheter. Once a week, 50 ml of the dilute Savlon was left in the bladder between 16.30 and 17.00 h. The urine remained clear and free from blood on this regime without any antibiotic treatment.

The sheep were housed in individual pens. They were given tap water (sodium and potassium concentration less than 1 mequiv./litre) in a bucket and chopped lucerne ad libitum. Water and lucerne were both given at 08.30 h, the water being replenished at 16.30 h. Although no precise measurements of water and food intake were made, food intake appeared to remain steady throughout while water intake increased during the period of supplementation with 400 mequiv. salt/day.

Sample 24-h urine collections showed that daily sodium excretion on this diet varied from 15 to 50 mequiv./day. In order to reduce the variation between the animals each was given a daily supplementation of 80 mequiv. sodium chloride via the jugular venous cannula; 40 mequiv. in 20 ml sterile water was given by slow injection over 5 min at about 08.00 h and a similar injection was given at 17.00 h. On days 6–27 inclusive (see below) 200 mequiv. of sodium chloride in 50 ml water was given at 08.00 h and another 200 mequiv. at 17.00 h.

The animals were accustomed to the various handling procedures for 10 days before the formal experiment began and after increasing the salt intake by 80 mequiv./day. Each day urine was collected for two successive 2-h periods from 12.00 to 14.00 h and from 14.00 to 16.00 h. The volume of each sample was noted and its sodium and potassium concentrations estimated using an EEL clinical flame photometer. The mean quantities of water, sodium and potassium excreted each day in the period 12.00 to 14.00 h are shown in Fig. 3.

Each day at 13.30 h an injection of either 500 μg aldosterone (Aldocorten, Ciba) or of 1 ml isotonic saline was given to each animal via the venous cannula. Control
and aldosterone injections were usually given on alternate days except on two occasions when the treatment pattern was changing and it seemed desirable to follow the aldosterone response on two successive days. The excretion of water, sodium and potassium during the second collection period (14.00 to 16.00 h) was compared with that in the first period by the following method. For each animal and for each parameter the percentage change in excretion during the second period was calculated by taking the excretion during the first period on that day as 100%. Mean percentage changes for the whole group were then estimated and are shown in Fig. 1.

The experiment was divided into the following periods: Days 1–5: supplementation by 80 mequiv. sodium chloride. Days 6–13: supplementation by 400 mequiv. sodium chloride. Days 14–18: 400 mequiv. salt plus 5 mg ovine pituitary prolactin (Ferring, Malmo, guaranteed free of vasopressin and oxytocin) given intramuscularly at 08.00 h each day. Days 19–22: 400 mequiv. salt only as on days 6–13. Days 23–26: 400 mequiv. salt plus two units of oxytocin (Pitocin, Parke Davis) given intramuscularly at 12.00, 13.00, 14.00 and 15.00 h. Day 27: 400 mequiv. salt only. Days 28–31: salt supplementation reduced to 80 mequiv./day.

The animals were weighed at the start of the experiment and on days 5, 13, 18, 22, 26 and 31 between 10.00 and 11.00 h.

Fig. 1. Mean urinary excretion of sodium, potassium and water (%) in the period 14.00 to 16.00 h each day. For each parameter in each animal excretion during the 12.00 to 14.00 h period on the same day was taken as 100%. All six sheep are included in the means. C indicates a control day when saline was injected at 13.30 h while A indicates a day when 500 µg aldosterone was injected. The treatments given are indicated at the bottom: 80 indicates salt supplementation of 80 mequiv./day; 400, supplementation of 400 mequiv./day. P indicates the period during which prolactin was administered and O indicates the period when oxytocin was given. On day 27 400 mequiv. salt was given alone. The bars indicate standard errors of the mean.
RESULTS

Figure 1 shows the mean percentage changes in urinary sodium, potassium and water excretion, comparing the periods 12.00 to 14.00 h and 14.00 to 16.00 h and taking the excretion in the first of these periods as 100%. Sodium excretion shows the most striking changes. Throughout the experiment there was a tendency for sodium excretion on control days to be slightly greater during the second period of collection, presumably because of normal diurnal variation. The effect of aldosterone changed sharply as the treatment was altered. During supplementation of intake by 80 mequiv. salt/day, aldosterone had its expected sodium-retaining action, excretion during the second collection period falling to about 25% of that in the first. On increasing salt supplementation to 400 mequiv./day, the sodium-retaining action of aldosterone was completely abolished. It had a suggestive saluretic action in the first two trials and an unequivocal one in the second two. Injections of prolactin given at 08.00 h restored the sodium-retaining action of aldosterone given at 13.30 h. The effect seemed to increase progressively so that by the third trial the aldosterone had a sodium-retaining action equivalent to that seen when the animals were receiving 80 mequiv. salt daily. There then followed a 4-day period during which the prolactin was discontinued while the 400 mequiv. supplementation was maintained. Two trials of the effect of aldosterone again showed unequivocal saluretic effects. Oxytocin treatment again restored the normal sodium-retaining action of aldosterone. When oxytocin was
discontinued, on day 27 aldosterone again caused a saluresis. During the last 4 days of the experiment a drop in salt supplementation to 80 mequiv./day produced a return to the usual sodium-retaining action of aldosterone.

Figure 2 shows the percentage changes in sodium excretion in animal 6 which showed the most marked and consistent saluretic effect and in animal 5 which did not once show a clear saluresis. Number 5 was the animal which had a sustained high central venous pressure.

Aldosterone produced no significant changes in potassium excretion. However, during the two periods when 400 mequiv. salt supplementation was given without any other treatment, aldosterone consistently produced a small rise in potassium excretion which did not reach statistical significance. Water excretion showed no clear pattern: there may have been a very slight tendency for aldosterone to promote water retention during treatment with oxytocin.

The absolute values for the mean excretion of sodium, potassium and water during the 12.00 to 14.00 h period (i.e. without the influence of exogenous aldosterone) are shown in Fig. 3. As expected the rise in sodium supplementation produced a rise in sodium excretion which, however, did not appear to have reached a peak even after 8 days. Prolactin caused a drop in sodium excretion and oxytocin had a similar but much less marked effect. Excretion dropped sharply when the supplement was reduced again to 80 mequiv./day. The rise in sodium supplementation to 400 mequiv./day produced a sharp rise in potassium excretion followed by a steady decline to below
control levels. The outstanding feature of the potassium pattern was the very sharp fall in excretion during the period of prolactin treatment. Oxytocin had no apparent effect on potassium.

Changes in water excretion were erratic.

The weight of the animals showed no change while the animals were on an 80 mequiv. salt supplement, a small but not significant rise while they were on a 400 mequiv. supplement and a sharp rise during the period of prolactin treatment (Table 1). About half the increase was lost when the prolactin was discontinued and the other half when sodium supplementation was reduced to 80 mequiv./day.

Table 1. Mean changes in body weight of six sheep at various points during the experiment. The weight on the first day of the experiments was taken as 100% and the animals were then weighed on the last day of each treatment period.

<table>
<thead>
<tr>
<th>Treatment periods</th>
<th>Treatment*</th>
<th>Weight (%)</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>None</td>
<td>100-0</td>
<td>—</td>
</tr>
<tr>
<td>Days 1-5</td>
<td>80 mequiv. salt/day</td>
<td>100-5</td>
<td>2-4</td>
</tr>
<tr>
<td>Days 6-13</td>
<td>400 mequiv. salt/day</td>
<td>102-0</td>
<td>2-2</td>
</tr>
<tr>
<td>Days 14-18</td>
<td>400 mequiv. salt + 5 mg prolactin</td>
<td>104-6</td>
<td>5-5</td>
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<tr>
<td>Days 19-22</td>
<td>400 mequiv. salt/day</td>
<td>104-8</td>
<td>4-4</td>
</tr>
<tr>
<td>Days 23-26</td>
<td>400 mequiv. salt + oxytocin</td>
<td>104-7</td>
<td>4-3</td>
</tr>
<tr>
<td>Days 28-31</td>
<td>80 mequiv. salt/day</td>
<td>100-5</td>
<td>3-7</td>
</tr>
</tbody>
</table>

* See text p. 371 for further details.

DISCUSSION

The experiments demonstrated that a rise in sodium intake can abolish the normal sodium-retaining action of aldosterone, and can convert aldosterone into a saluretic hormone. Only one animal failed to show a clear saluresis and that was the sheep which had an increased central venous pressure of unknown origin. During the periods when the sheep were receiving supplements of 400 mequiv. salt/day and no other treatment, the aldosterone-induced increase in sodium excretion was associated with a small but consistent increase in potassium excretion.

Both prolactin and oxytocin were able to restore the sodium-retaining action of aldosterone even in the face of a continued high salt intake. In addition prolactin had a moderate sodium-retaining and powerful potassium-retaining action in its own right. Oxytocin had no effect on potassium but appeared to have a slight sodium-retaining action.

It is one of the paradoxes of electrolyte physiology that although plasma potassium levels normally remain remarkably constant, no text even attempts to explain the mechanism whereby this constancy is attained. The only known factor which alters potassium excretion is the action of aldosterone on the distal tubule. This forces potassium excretion to fluctuate secondarily as a consequence of primary changes in sodium excretion. It seems very likely that there must be some other potassium-regulating system. In view of the powerful action of prolactin on potassium excretion it seems possible that it may play a physiological role.

No direct measurements were made of body fluid compartments. However, the mean weight of the six animals was the same at the end of the experiment as at the
beginning and it seems probable that the changes in weight observed during the experiment reflected changes in the total body water content. Eight days of treatment with 400 mequiv. salt daily produced a mean 1.5% rise in body weight which was not significant. Five days of treatment with 400 mequiv. salt plus prolactin produced a further rise of 7.6% which was significant at the $P < 0.02$ level. Four days after stopping the prolactin approximately half the additional weight had been lost. Weight then remained steady during the oxytocin treatment but returned to its original level after 4 days treatment with 80 mequiv. salt/day. These results suggest that prolactin-like material may be necessary for significant retention of water to occur.

The results described here indicate that sodium excretion may be regulated partly by variations in the level of aldosterone and partly by variations in the levels of other hormones whose presence or absence can vary the action of aldosterone from being markedly salt-retaining to being markedly saluretic. They do not exclude the existence of another saluretic hormone although some of the experimental findings in this area may need re-evaluation. Several other workers have suggested that the action of aldosterone may be modulated by another hormone of extra-adrenal origin (De Wardener, Mills, Clapham & Hayter, 1961; Davis, Holman, Carpenter, Urquhart & Higgins, 1964; Rovner, Conn, Knopf, Cohen & Hsueh, 1965). Our findings suggest that prolactin and oxytocin may be involved in this modulation while Lockett’s findings indicate a possible role of growth hormone. Which, if any, of these three plays an actual physiological role can be determined only by further experiment. For the sake of brevity in the remainder of this discussion the expression ‘prolactin-like’ will be used to describe hormones which have this modulating action: this description refers strictly to the interaction with aldosterone and is not meant to have any other chemical or physiological implications. In particular it is not intended to imply that prolactin is the physiological modulator although it is clearly one of the candidates.

These experiments provided no direct evidence of the site of interaction between aldosterone and the prolactin-like hormones. The work of Lockett on perfused kidneys suggests that the interaction is a direct one at the renal level and does not involve an indirect endocrine link (Davey & Lockett, 1960; Lockett, 1965). There is some evidence from micropuncture studies that the escape phenomenon depends on proximal tubular changes and that the distal tubule may not be affected (Dirks, Cirksena & Berliner, 1965; Brenner & Berliner, 1969). Indirect evidence pointing in the same direction is the small rise in potassium excretion which occurred at the same time as the aldosterone-induced saluresis. This could be explained by a reduction in proximal sodium reabsorption with increased delivery of sodium to the distal tubule. If aldosterone continued to stimulate sodium reabsorption distally, the rise in potassium excretion in exchange would be expected. Actions of aldosterone at other sites may not be modulated by prolactin-like hormones since rat colon does not appear to show the escape phenomenon (Thompson & Edmonds, 1971).

The experiments give some clues as to the factors which may govern the level of secretion of the prolactin-like material. Its output seems to be suppressed by a rise in salt intake and increased by a rise in venous pressure since the animal with a high venous pressure failed to show any saluresis. It is possible that its secretion may be
suppressed by high levels of glucocorticoids since patients with Cushing's syndrome show sodium loss rather than the expected sodium retention when treated with deoxycorticosterone acetate (Soffer et al. 1944). The study was partly supported by the Human Reproduction Unit of the World Health Organization. The flame photometer used for electrolyte determinations was purchased with the aid of a grant from the Wellcome Trust.

REFERENCES


