Influence of neurohypophysectomy on the renal actions of aldosterone
in the adrenalectomized rat

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Abstract. The influence of aldosterone administration on urine flow, Na⁺ and K⁺ excretion was examined in hypotonic saline infused, Inactin® anesthetised rats following removal of the adrenals or adrenals and posterior pituitary. Plasma adrenal steroid levels were considerably depressed but still detectable 10-14 days after adrenalectomy. Removal of the posterior pituitary markedly reduced Na⁺ excretion in adrenalectomized animals implying that Na⁺ retention following neurohypophysectomy is not dependent on adrenal gland function. In adrenalectomized rats aldosterone administration at 42 pmol/min reduced Na⁺ excretion and urine flow without significantly changing K⁺ excretion, though plasma K⁺ was reduced. In adrenalectomized/neurohypophysectomized rats aldosterone further reduced the already low rate of Na⁺ excretion and increased K⁺ excretion, though there was no observable effect on urine flow. The results obtained indicate that the Na⁺-retaining actions of aldosterone are largely independent of posterior pituitary influence. The K⁺-losing action of aldosterone was, however, only observed in animals in which the posterior pituitary was absent.

In recent years evidence has accumulated for the involvement of posterior pituitary hormones in the control of renal Na⁺ excretion in the rat and other species (1). This adds new interest to reports that have appeared over the years implying that the Na⁺ retaining action of aldosterone might be dependent on or modified by some factor of posterior pituitary origin (2-4). The potential interplay between neurohypophysial peptides and mineralocorticoids has been highlighted again recently by in vitro study of the isolated cortical collecting tubule. Both desoxycorticosterone acetate and vasopressin (AVP) stimulated sodium absorption across the perfused cortical collecting tubule (5). Combined hormone treatment however, produced a much larger response than observed when either were presented separately. This may reflect increased efficiency of intracellular cAMP production stimulated by AVP in the presence of desoxycorticosterone acetate, as adrenalectomy has been shown to impair the coupling between the vasopressin receptor and adenylate cyclase activity in the rat medulla (6). Indeed, the cortical collecting tubule from desoxycorticosterone acetate-treated rats exhibits a much larger than normal response in cAMP production to applied vasopressin (7).

The present study examines in the whole animal, the potential physiological consequence of this interplay between mineralocorticoid and posterior pituitary hormones. Accordingly, the influence of physiological levels of aldosterone on renal electrolyte excretion has been investigated in intact rats and in animals immediately following removal of the posterior lobe of the pituitary. The study has been undertaken in chronically adrenalectomized animals as adrenalectomy has been shown greatly to increase the sensitivity of rats to the Na⁺-retaining actions of aldosterone (8,9).
Materials and Methods

Animals
Male Sprague-Dawley rats were bred and housed in the Medical Faculty Animal House at the University of Zimbabwe, and allowed free access to food (Mouse com-}

proids, National Foods, Harare) and water.

Surgical preparation
Rats were anesthetised with Na+ pentobarbital (Sagatal®, May & Baker, Dagenham, UK, 55 mg/kg). The adrenal glands were exposed and removed via lateral incisions through the skin and body wall. The incisions were then closed with sutures and the skin surface sprayed with chloramphenicol/gentian violet mixture (Pedichlor®, CAPS, Zimbabwe). Following recovery from the anesthetic rats were caged individually and allowed free access to food, water and 0.077 mol/l NaCl. Body weight was well maintained in adrenalectomized animals on this regime (weight at adrenalectomy 357±11 g (mean ± SEM, N=20), weight 9 days later 361±9 g). Completeness of removal of adrenal glands was checked post mortem.

Renal excretion studies
Adrenalectomized animals were used 10 to 14 days after removal of the adrenals. Rats were anesthetised with Inactin® (sodium-5 ethyl-5-(1'methypropyl)-2-thiobarbiturate, Byk Gulden, Konstanz; 0.11 g/kg, ip) and a tracheotomy performed. The right jugular vein was cannulated with polythene tubing (Portex Hythe, Kent, UK; inner diameter 0.86 mm, outer diameter 1.27 mm) to allow iv infusion. The urinary bladder was also cannulated (Portex, inner diam-

eter 0.86 mm, outer diameter 1.27 mm) via an incision in the body wall, to allow collection of urine. Body temperature was maintained at 37±1°C by means of a heated table.

In those animals where the posterior pituitary was to be removed, immediately prior to normal cannulation procedures the pituitary was exposed by the parapharyngeal approach, a dental drill being used to drill through the base of the skull. A small incision was made in the caudal tip of the exposed anterior lobe of the pituitary. This revealed the underlying posterior pituitary lobe which was then removed by gentle suction. Completeness of neurohypophysectomy was checked at operation by ex-

amination of the removed tissue, and at post mortem by macroscopic examination of the ventral brain surface. Mi-

croscopic examination of the anterior pituitary immediately after surgery confirmed an active surface blood circu-

lation. In an earlier study (10) we confirmed the func-

tional integrity of the remaining anterior pituitary, through measurement of plasma corticosterone levels comparable with those in intact animals, up to 8 h post-

surgery.

After the completion of all surgery, the rats were di-

rectly placed on a continuous iv infusion of 0.077 mol/l NaCl at 150 µl/min (Sage Syringe Pump model 351, Sage Instruments, Cambridge, MA). Following a 3-h equilibra-

tion period consecutive 20-min urine collections were made into preweighed plastic vials over the subsequent 5 h.

Determination of glomerular filtration rate and mean arterial blood pressure
Groups of adrenalectomized rats were surgically pre-
pared as described for the renal studies except that a heparinized cannula (inner diameter 0.86 mm, outer di-

ameter 1.27 mm) was also inserted into the femoral artery to permit recording of mean arterial blood pressure (Sta-

tham P23 a.c. pressure transducer and Grass model 7 polygraph, Quincy, MA) and withdrawal of blood sam-

dles. Animals were given a priming dose (0.3 µCi in 0.5 ml saline) of tritiated inulin (Amersham International plc, Bucks, UK) and then placed on a continuous iv infusion at 150 µl/min of 0.077 mol/l NaCl, containing inulin (0.14 µCi/ml). Urine collections were made every 20 min over the 8-h period and blood samples (200 µl) withdrawn at 2-h intervals. Blood was taken into heparinized hemato-

crit tubes prior to analysis of separated plasma. Aliquots of urine (100 µl) and plasma (50 µl) were counted on a Packard Tri Carb Liquid Scintillation Spectrometer using a Lumax scintillant (Lumac BV, Holland).

Aldosterone administration
In those animals in which the effect of exogenous aldoste-

rone was to be examined d-aldosterone (Sigma, Poole, UK) was administered via the infusate at 42 pmol/min (approx 2.5 µg · kg−1 · h−1), commencing 4 h after the start of the infusion. After 2 h of aldosterone adminis-
tration the rats were switched back to hormone-free in-

fusate for the final 2 h of the study. At the end of the experiment a 2-ml blood sample was taken by cardiac puncture, and the plasma was separated for electrolyte analysis. Urine volume was determined gravimetrically. Urine and plasma Na+ and K+ were determined by flame photometry (Corning model 435 flame photometer, Essex, UK).

Determination of plasma aldosterone and corticosterone levels
Parallel groups of adrenalectomized rats were prepared and infused as in the renal excretion studies, but at the end of the 2-h period of aldosterone administration or at the equivalent time in untreated animals receiving saline infusion only, the jugular cannula was clamped (to pre-

vent contamination of the blood sample with infusate), the animals decapitated, and trunk blood collected into cooled heparinized containers. Plasma was also collected from a parallel group of anesthetised rats with intact ad-

renal glands. Plasma was separated and aliquots of known volume (usually 2.5 ml) were freeze dried (VirTis Freeze Drier, New York, NY) and stored at -20°C until dis-

patched by air to the University of Manchester for mea-
measurement of aldosterone and corticosterone levels by radioimmunoassay.

Plasma aldosterone was measured by radioimmunoassay following initial separation from other steroids by LH20 chromatography by the method described by Milne et al. (11). Inter- and intra-assay coefficients of variation were 12.7% (N=42) and 14.4% (N=10), respectively, and the minimum detectable plasma aldosterone concentration was 0.06 pmol/l.

Plasma corticosterone was measured by radioimmunoassay of ethanol extracted samples as described by Kime (12). Inter- and intra-assay coefficients of variation were 10.1% (N=30) and 13.6% (N=10), respectively, and the minimum detectable plasma corticosterone concentration was 11 pmol/l.

**Data presentation**
All grouped data is presented as mean and SEM. Statistical comparisons are by paired or unpaired t-test as appropriate.

**Results**

**Plasma aldosterone levels**
Table 1 gives the levels of adrenal steroid hormones measured in the plasma of intact and adrenalectomized anesthetised rats after 6 h saline infusion. It is notable that both aldosterone and corticosterone were detectable in the plasma of all animals. The levels in adrenalectomized rats were, however, very much lower than those seen in intact, anesthetised rats for both aldosterone (p<0.01) and corticosterone (p<0.01), the reduction in aldosterone being proportionately greater than the reduction in corticosterone. Administration of exogenous aldosterone at 42 pmol/min caused a significant increase in plasma aldosterone in adrenalectomized animals (p<0.01).

Table 1.
Plasma aldosterone and corticosterone levels.

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<thead>
<tr>
<th></th>
<th>Aldosterone nmol/l</th>
<th>Corticosterone nmol/l</th>
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<tbody>
<tr>
<td>Intact</td>
<td>4.37±0.71</td>
<td>263±40</td>
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<tr>
<td>N=7</td>
<td></td>
<td></td>
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<tr>
<td>Adrenalectomized</td>
<td>0.58±0.10</td>
<td>93±16</td>
</tr>
<tr>
<td>N=12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized + aldosterone, 42 pmol/min</td>
<td>2.76±0.44</td>
<td>74±13</td>
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<tr>
<td>N=7</td>
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**Renal excretion**
Urine flow, Na⁺ and K⁺ excretion rates in saline-infused adrenalectomized rats (N=6) is given in Fig. 1A. These animals exhibited considerable variability around a mean urine flow rate of approximately 100 μl/min and mean Na⁺ and K⁺ excretion rates of approximately 8 and 3 μmol/min, respectively.

**Effect of aldosterone administration**
Fig. 1B shows the effect of aldosterone administration at 42 pmol/min to a group of adrenalectomized animals (N=6). Two hours of hormone administration reduced Na⁺ excretion from the pre-aldosterone level of 8.0±0.9 to 4.2±1.6 μmol/min (p<0.01). The reduction in Na⁺ excretion persisted until the end of the experiment and was accompanied by a significant (p<0.05) reduction in urine flow from 93±17 to 54±16 μl/min.

A parallel group of adrenalectomized rats (N=6) exhibited a mean arterial blood pressure of 120±4 mmHg and a mean glomerular filtration rate (GFR) of 1.56±0.24 ml/min. Neither blood pressure nor GFR were significantly altered by aldosterone administration (Fig. 2), though there was an apparent, small, non-significant, decline with time in GFR.

The plasma Na⁺ levels at the end of the 8-h infusion in aldosterone-treated rats, 135±2 mmol/l (N=6) were not significantly different from those in untreated adrenalectomized animals, 132±1 mmol/l (N=6). Plasma K⁺ at 4.2±0.1 mmol/l (N=6) in the aldosterone-treated animals was, however, significantly lower (p<0.02) than the value of 6.0±0.6 mmol/l in untreated animals.

**Renal excretion in adrenalectomized/neurohypophysectomized rats**
Fig. 3A shows urine flow, Na⁺ and K⁺ excretion for adrenalectomized rats in which the posterior lobe of the pituitary had been acutely removed. The mean urine flow in these animals was comparable with that seen in adrenalectomized rats with intact pituitaries (Fig. 1A). Sodium excretion was, however, considerably lower (p<0.02) at 1.1±0.5 μmol/min in the 4th hour rising to 4.1±0.6 μmol/min in the 8th hour. In contrast to Na⁺ excretion, mean K⁺ excretion was similar in adrenalectomized rats with or without posterior pituitaries.

Fig. 3B shows the effect of aldosterone administration at 42 pmol/min in a group of adrenalectomized rats.
Renal excretion in adrenalectomized rats.
A. Urine flow, Na⁺ and K⁺ excretion rates in adrenalectomized (Adrenalect) rats (N=6) for the 4th to 8th hour of saline infusion.
B. The effect of aldosterone administration at 42 pmol/min (Aldo) on urine flow, Na⁺ and K⁺ excretion in adrenalectomized rats (N=6). Values are presented as means for each 20-min collection period; vertical bars indicate SEM.

tomized/neurohypophysectomized rats. The hormone produced a further significant reduction in the already low rate of Na⁺ excretion from 3.6±0.8 to 1.6±0.6 μmol/min (p<0.05). This was coupled with a significant increase in K⁺ excretion from 3.0±0.5 to 4.2±0.6 μmol/min (p<0.05), but in these animals there was no detectable effect of aldosterone on urine flow.

Discussion
The present study has confirmed the high plasma aldosterone levels reported previously in thiobutabarbital anesthetised intact rats (10,13,14) by comparison with unanesthetised rats (11,15-17). The high levels in anesthetised animals appear to be a

Fig. 2.
Glomerular filtration rate (GFR) (hatched column) and mean femoral arterial blood pressure (BP) (open column) in 6 adrenalectomized rats in the 2 h preceding aldosterone administration (Pre), the 2 h of aldosterone administration 42 pmol/min (Aldo), and the 2 h following cessation of aldosterone administration (Post). Values are presented as means ± SEM.
consequence of both barbiturate anesthesia and surgical intervention, which have been shown to cause a sustained ten-fold increase in aldosterone (18) to levels comparable with those seen in the present study. Such high circulating aldosterone levels may reduce receptor sensitivity (19) rendering the animal less responsive to exogenous aldosterone, which may explain the insensitivity of the anesthetised intact rat to aldosterone reported by Lockett & Roberts (8) and also noted by ourselves (Musabayane & Brimble, unpublished observations). However, Morris et al. (9) failed to observe a Na⁺-retaining action of aldosterone even in unaesthetised intact rats.

It was notable that both aldosterone and cortisol could always be detected in experimental animals 10-14 days after adrenalectomy, though aldosterone levels were only one seventh and cortisol levels one third of those in intact animals. These results would be consistent with the persistence of patches of extra-adrenal steroidogenic tissue, under strong stimulation by corticotropin following complete removal of the adrenal glands (20).

In adrenalectomized rats GFR was lower than in intact animals (21) and comparable with that reported previously in chronically adrenalectomized animals (22). Administration of aldosterone at 42 pmol/min to adrenalectomized animals resulted in plasma hormone levels comparable with those induced in intact rats by dietary Na⁺ depletion (15), which would thus appear to be within the physio-
logical range. This rate of aldosterone administration produced a marked, long lasting reduction in Na⁺ excretion. This confirms a number of previous reports of a marked Na⁺-retaining action of aldosterone in the adrenalectomized rat (8,9,23-25).

In agreement with Field et al. (22), Na⁺ retention was found to be associated with a significant reduction in urine flow. Consistent with earlier observations (22,25) aldosterone administration did not alter either blood pressure or GFR.

In the present study there was no kaliuretic response to aldosterone administration in adrenalectomized rats. This contrasts with the findings of Morris et al. (9), Campen et al. (24), and Horisberger & Diezi (25), but is in agreement with those of other groups (8,22,23). This discrepancy may reflect the reported sensitivity of the kaliuretic action of aldosterone to the state of potassium balance (23), the rate of Na⁺ excretion (25), and/or urine flow rate (22). In a micropuncture study on the distal tubule, Field et al. (22) found that aldosterone did increase K⁺ secretion, but in the whole animal this was masked by the antikaliuretic effect of reduced urine flow. Perhaps it is significant, therefore, that in the present study a clear aldosterone-induced kaliuresis was observed only in the adrenalectomized/neurohypophysectomized rat in which aldosterone did not have an antidiuretic action.

The adrenalectomized rats in the present study exhibited a characteristic hyponatremia and hyperkalemia. The hyperkalemia was corrected by aldosterone administration. In the absence of a measurable kaliuretic action of the hormone this may reflect an extrarenal action of aldosterone to promote cellular uptake of K⁺, as has already been suggested both in the rat (22,26) and in the dog (27).

We have previously reported that removal of the posterior pituitary results in a marked reduction in Na⁺ excretion in rats with intact adrenals (10). This reduced Na⁺ excretion occurs in the absence of any significant fall in GFR (10). In these earlier studies restoration of Na⁺ excretion rates comparable with those in intact animals, required replacement of both oxytocin and vasopressin, suggesting that both neurohypophysial hormones may play a role in the regulation of renal Na⁺ excretion. The observation in the current work that neurohypophysectomy is also associated with a marked reduction in Na⁺ excretion in adrenalectomized animals, makes it unlikely that such inappropriate Na⁺ retention could be secondary to increased aldosterone secretion. Furthermore, as aldosterone further reduced the already low Na⁺ excretion rates in the adrenalectomized/neurohypophysectomized rat, the Na⁺-retaining action of aldosterone would not appear to require the presence of a circulating factor of posterior pituitary origin as might be suggested by the work of Lockett & Roberts, (8) and Burstyn et al. (3). However, although the antina-triuretic activity of aldosterone may be evident in the neurohypophysectomized animal, this clearly does not preclude a more subtle effect of oxytocin and vasopressin to modulate the steroid's renal action as suggested by the isolated renal tubule studies of Reif et al. (5).

In summary, the present work shows that it cannot be assumed that the circulating levels of adrenal steroids in chronically adrenalectomized animals are negligible. Indeed, the hyperaldosteronemic effect of barbiturate anesthesia and surgery results in plasma aldosterone levels within the range reported in unanesthetised intact rats in normal Na⁺ balance, (11,15-17). It is clear that the Na⁺ retention seen following removal of the posterior pituitary is not dependent on intact adrenal glands, nor is the Na⁺-retaining action of aldosterone dependent on an intact posterior pituitary. Removal of the posterior pituitary did, however, abolish the antidiuretic effect of aldosterone and perhaps in consequence, did allow a kaliuretic action of aldosterone to be observed.

Acknowledgments

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