J. Physiol. (1986), **381**, *pp.* 439–452
With 6 text-figures
Printed in Great Britain

THE INFLUENCE OF NEUROHYPOPHYSIAL HORMONES ON RENAL FUNCTION IN THE ACUTELY HYPOPHYSECTOMIZED RAT

BY R. J. BALMENT, M. J. BRIMBLE*, M. L. FORSLING† AND C. T. MUSABAYANE*

*From the * Department of Physiology, University of Zimbabwe, P.O. Box MP 167, Harare, Zimbabwe, † the Department of Physiology, Middlesex Hospital Medical School, London W1P 6DB and the Department of Zoology, University of Manchester, Manchester M13 9PL*

(*Received 5 March 1986*)

SUMMARY

1. Renal function and the effect of neurohypophysial hormone replacement was investigated in anaesthetized, acutely hypophysectomized, male rats.

2. Although urine production was only slightly lower over the 8 h post-operative study period in hypophysectomized rats, sodium excretion was greatly depressed reaching only $3{\cdot}5 \pm 1{\cdot}4$ μmol/min compared with a peak of $13{\cdot}2 \pm 1{\cdot}0$ μmol/min in intact animals.

3. In association with a decline in mean arterial blood pressure, glomerular filtration rate in hypophysectomized rats fell to $2{\cdot}1 \pm 0{\cdot}2$ ml/min 8 h after operation by comparison with a mean rate in intact rats of $3{\cdot}2 \pm 0{\cdot}2$ ml/min.

4. Plasma corticosterone levels were much lower in hypophysectomized (4 ± 2 ng/ml) than in intact (36 ± 4 ng/ml) rats, plasma aldosterone was reduced to a lesser extent ($0{\cdot}41 \pm 0{\cdot}08$ compared with $0{\cdot}76 \pm 0{\cdot}04$ ng/ml). While oxytocin was not detectable in hypophysectomized rat plasma, trace levels of vasopressin ($0{\cdot}16 \pm 0{\cdot}04$ μu./ml) were found. In intact unanaesthetized rats basal plasma levels of oxytocin were $0{\cdot}32 \pm 0{\cdot}13$ μu./ml and vasopressin were $0{\cdot}85 \pm 0{\cdot}19$ μu./ml.

5. Administration of oxytocin at 150 μu./min, which produced plasma hormone levels ($24{\cdot}0 \pm 2{\cdot}5$ μu./ml) greatly in excess of basal concentrations, increased renal sodium excretion but did not alter urine flow. Oxytocin administration at the lower rate of 15 μu./min producing plasma hormone levels of $2{\cdot}60 \pm 0{\cdot}1$ μu./ml, did not alter renal sodium excretion.

6. Arginine vasopressin administered at 12 μu./min induced plasma hormone levels of $1{\cdot}54 \pm 0{\cdot}09$ μu./ml and produced a large antidiuresis and small increase in the rate of sodium excretion.

7. The natriuretic response to vasopressin was potentiated by concurrent administration of oxytocin at 15 μu./min. The peak sodium excretion of $5{\cdot}8 \pm 1{\cdot}0$ μmol/min, however, remained well below that seen in intact rats.

8. It is concluded that, as restoration of posterior pituitary hormones at or above the physiological range only partially restored sodium excretion, the absence of anterior pituitary factors may also contribute directly or indirectly to the renal sodium retention of the hypophysectomized rat.

INTRODUCTION

A number of studies have examined the effects of chronic hypophysectomy on cardiovascular (Beznak, 1959, 1963; Simon, Honeyman & Fray, 1984) and renal function (Boss, Osborn & Renzi, 1952; Matthews, 1963; Bauman, 1967; Bauman & Phillips, 1970) in rats. Reduction of cardiac output, mean arterial pressure, renal blood flow and glomerular filtration rate (G.F.R.) has been described. It is generally assumed that these cardiovascular and renal changes reflect removal of the influence of the anterior rather than posterior pituitary hormones, secretion of neurohypophysial hormones being at least partially restored from the cut end of the pituitary stalk a few days after surgery (Billenstein & Levique, 1955).

There have been fewer studies of the acutely hypophysectomized animal where posterior pituitary function has not been restored nor the longer term changes associated with anterior pituitary hormone deficiency fully developed. Renal function does, however, appear to be compromised in the acutely hypophysectomized animal, these changes being most evident in animals presented with a saline load. By comparison with intact animals the hypophysectomized rat avidly retains sodium (Lichardus & Ponec, 1973; Ponec & Lichardus, 1977; Balment, Brimble, Forsling & Musabayane, 1984), resulting in elevated plasma sodium levels. Lichardus & Ponec (1973) concluded that the sodium retention was due to loss of posterior rather than anterior pituitary hormones and provided some evidence for the involvement of oxytocin deficiency. There is also evidence relating sodium retention to vasopressin deficiency, as a degree of renal sodium retention is seen in the saline-loaded Brattleboro rat, which congenitally lacks the ability to produce vasopressin but has normal or elevated plasma oxytocin levels (Balment, Brimble & Forsling, 1980) and a functional anterior pituitary (McCann, Antunes-Rodrigues, Nallar & Valtin, 1966; Arimura, Sawano, Redding & Schally, 1968). We have also noted marked sodium retention in ethanol anaesthetized, water-loaded rats (Musabayane, Brimble & Balment, 1985), which have suppressed vasopressin secretion (Forsling, Brimble & Balment, 1982). Indeed, our recent observation that vasopressin administration at doses within the physiological range has a natriuretic action in intact rats (Balment *et al.* 1984), would lend support to a role for the hormone in the regulation of sodium excretion.

In the present study we have re-investigated renal function in the acutely hypophysectomized rat and have attempted to establish whether the associated renal retention of sodium is due to lack of either or both of the neurohypophysial hormones, or whether other factors consequent on anterior pituitary deficiency contribute. To this end we have examined renal excretion in acutely hypophysectomized rats during oxytocin and/or vasopressin administration. The induced plasma levels of neurohypophysial hormones and circulating levels of adrenal steroid hormones have been measured. A preliminary report of part of this work has already been published (Balment, Brimble, Forsling & Musabayane, 1985).

METHODS

Animals

Experiments were performed on male Sprague–Dawley rats (300–450 g body weight) bred and housed in the Medical Faculty animal house at the University of Zimbabwe. Animals were maintained on a 12 h light–12 h dark regime and allowed free access to food (Mouse Comproids, National Foods, Harare) and water.

TABLE 1. Experimental groups of intact and hypophysectomized rats

Group	Oxytocin administration rate (μu./min)	Vasopressin administration rate (μu./min)
Intact control ($n = 9$)	—	—
Hypophysectomized control ($n = 8$)	—	—
Hypophysectomized ($n = 8$)	150	—
Hypophysectomized ($n = 10$)	15	—
Hypophysectomized ($n = 10$)	—	12
Hypophysectomized ($n = 10$)	15	12

1 μu. oxytocin equals approximately 2 pg.
1 μu. vasopressin equals approximately 2·2 pg.

Renal Excretion Studies

Rats were anaesthetized by intraperitoneal injection of Inactin (5-ethyl-5-(1′-methylpropyl)-2-thiobarbiturate; Byk Gulden) at 0·11 g/kg body weight. The pituitary was exposed by the parapharyngeal approach, a dental drill (Shick HS 1000/18) being used to drill through the base of the skull. The whole pituitary (anterior and posterior lobes) was then removed by suction, completeness of removal being checked at operation and post-mortem.

Immediately after hypophysectomy the urinary bladder was cannulated with polythene tubing (Portex i.d. 0·86 mm, o.d. 1·27 mm) via an incision in the abdominal wall (dead space in the bladder cannula was approximately 150 μl). The right jugular vein was also cannulated (Portex i.d. 0·86 mm, o.d. 1·27 mm) to allow intravenous infusion. Additional groups of sham-hypophysectomized rats (in which the pituitary was exposed but not removed) and of non-hypophysectomized (intact) rats were similarly prepared. Body temperature was maintained at 37 ± 1 °C by means of a heated table.

Animals were placed on a continuous intravenous infusion of 0·077 M-NaCl at 150 μl/min (Sage Syringe Pump model 351) and consecutive 20 min urine collections made into pre-weighed plastic vials over the subsequent 8 h. Neurohypophysial hormone administration was achieved by switching the infusate after 3 h to one of identical ionic composition but containing synthetic oxytocin (Sigma) and/or synthetic arginine vasopressin (Sigma Grade V). The experimental groups and rates of hormone infusion employed are listed in Table 1. Hormone administration was continued for a period of 3 h, after which animals were returned to hormone-free infusate for the final 2 h of the experiment. At the end of the experiment a 2 ml blood sample was taken by cardiac puncture, plasma separated and assayed for sodium and potassium.

Urine volume was determined gravimetrically. Urinary and plasma concentrations of sodium and potassium were determined by flame photometry (Corning model 435 flame photometer).

Determination of plasma oxytocin and vasopressin concentrations

Parallel groups of hypophysectomized rats were prepared and infused as for the renal studies, however, at the end of the 3 h period of hormone administration (or at the equivalent time for animals receiving no hormone) the jugular cannula was clamped (to prevent contamination of the blood sample with infusate), the animals decapitated and trunk blood collected into cooled heparinized containers. All blood collections were completed within 30 s of stopping the infusion. Plasma was separated and an aliquot of known volume (usually 2 ml) from each plasma sample was freeze dried (Virtis Freeze Drier) and stored at −20 °C until dispatched by air to the Middlesex Hospital Medical School for determination of oxytocin or arginine vasopressin content by radioimmunoassay. The validity of this procedure for plasma storage and transport was verified by assay of hormone in plasma from hypophysectomized rats to which known amounts of oxytocin or vasopressin had been added. Additional groups of unanaesthetized intact rats were similarly bled by decapitation to determine normal basal hormone levels.

Vasopressin was determined in plasma extracted with bentonite as described by Stromberg, Forsling & Akerlund (1981). The results are expressed in terms of the First International Standard for Vasopressin (77/501). Plasma oxytocin was determined by radioimmunoassay of samples extracted using Spherosil glass beads (Forsling, 1974). The labelled oxytocin was prepared using a solid-phase lactoperoxidase method (Karonen, Morsky, Siren & Seuderling, 1975). The standard used was the Fourth International Standard for Oxytocin and the limit of detection was 0·1 μu./ml).

Determination of plasma aldosterone and corticosterone levels

Further groups of intact and hypophysectomized rats were prepared as described for the renal studies. After 6 h of hypotonic saline infusion rats were decapitated and trunk blood collected into cooled heparinized containers. Separated plasma was freeze dried and stored at −20 °C until dispatched by air to Manchester University for measurement of aldosterone and corticosterone levels by radioimmunoassay. Steroid hormone levels were also determined in a group of unanaesthetized intact rats bled by decapitation.

Plasma corticosterone was measured by radioimmunoassay of ethanol extracted samples as described by Kime (1977). Plasma aldosterone levels were also measured by radioimmunoassay following initial separation from other steroids by LH20 chromatography (Milne, Balment, Henderson, Mosley & Chester Jones, 1982).

Determination of G.F.R., mean arterial blood pressure and haematocrit

Groups of intact and hypophysectomized rats were surgically prepared as described for the renal studies except that a heparinized cannula (i.d. 0·86 mm, o.d. 1·27 mm) was also inserted into the femoral artery to permit recording of mean arterial blood pressure (Statham P23 a.c. pressure transducer and Grass model 7 Polygraph) and withdrawal of blood samples. Animals were given a priming dose (0·3 μCi in 0·3 ml saline) of tritiated inulin (Amersham International) and then placed on a continuous intravenous infusion at 150 μl/min, of 0·077 M-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 haematocrit tubes for measurement of haematocrit 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).

Data presentation and statistical analysis

All values are presented as mean ± S.E. of mean. The renal excretion data is presented graphically showing 20 min collections over the 8 h of study. Statistical comparisons are by paired or unpaired *t* test as appropriate.

RESULTS

Renal function in intact and hypophysectomized rats

Urine flow rates in intact and hypophysectomized rats through the 8 h period of study are compared in Fig. 1. Both groups showed a similar pattern, urine flow rising progressively to come close to the infusion rate of 150 μl/min 2 h after the start of infusion. The mean urine flow rate remained at or above the infusion rate until the

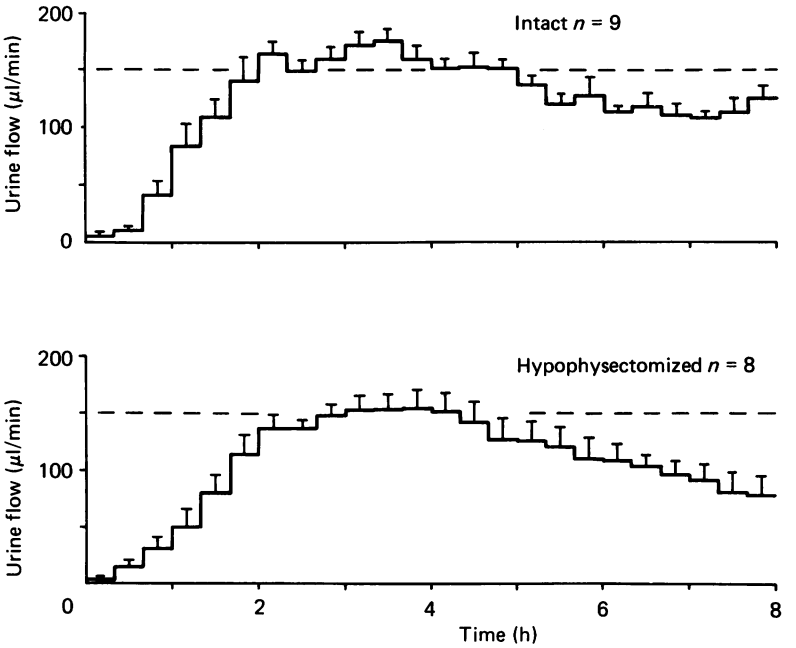

Fig. 1. Urine production in intact and hypophysectomized rats. Values are means for each 20 min collection during the 8 h period of post-operative infusion; vertical bars indicate S.E. of means. Dashed line indicates rate of infusion.

fourth or fifth hour and then declined. The reduction in urine production was more marked in hypophysectomized rats, flow rate falling to 79 ± 17 μl/min in the last collection by comparison with 126 ± 10 μl/min for intact animals ($P < 0{\cdot}05$). Urine flow in sham-hypophysectomized rats ($n = 7$) closely paralleled that in intact rats reaching a peak of 177 ± 31 μl/min after 4 h and falling to 119 ± 14 μl/min by the last collection.

The pattern of sodium excretion (Fig. 2) differed much more between the two groups than did urine flow. In intact rats sodium excretion, like urine flow, rose progressively to equal the rate of infusion (11·6 μmol/min) after 3 h 20 min. After 5 h sodium excretion began to decline, falling to $6{\cdot}6 \pm 0{\cdot}6$ μmol/min by the last collection. By contrast, sodium excretion in hypophysectomized rats remained well below the infusion rate throughout the 8 h period. After 4 h 20 min, when sodium excretion had reached its peak in intact rats ($13{\cdot}2 \pm 1{\cdot}0$ μmol/min), the rate of sodium excretion in hypophysectomized animals was $2{\cdot}2 \pm 0{\cdot}8$ μmol/min and had only risen

to $3{\cdot}5 \pm 1{\cdot}4$ μmol/min by the last collection. This marked difference in renal sodium handling is also evident when total sodium excreted between the third and sixth hour in intact (1946 ± 124 μmol) and hypophysectomized (339 ± 121 μmol) rats is compared ($P < 0{\cdot}01$). Sodium excretion in the sham-hypophysectomized (1744 ± 176 μmol)

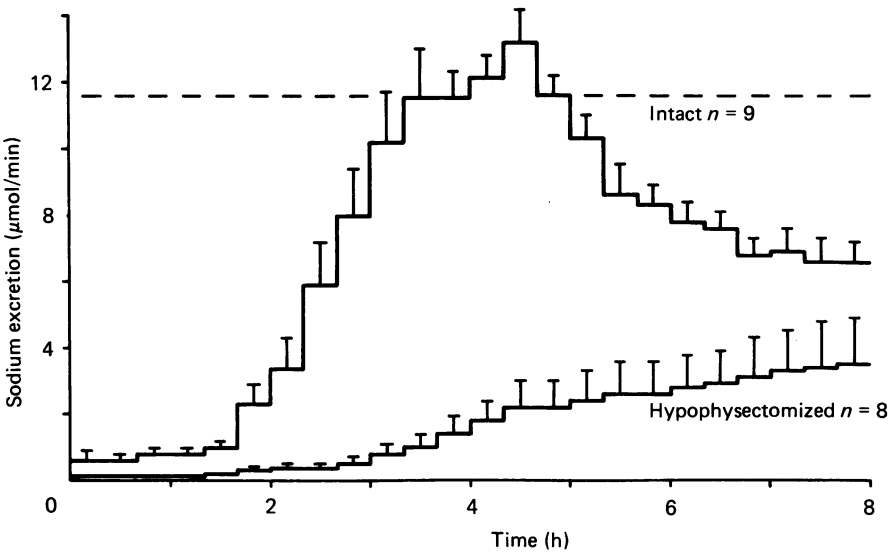

Fig. 2. Sodium excretion in intact and hypophysectomized rats. Values are means for each 20 min collection during the 8 h period of post-operative infusion; vertical bars indicate S.E. of means. Dashed line indicates rate of infusion.

animals did not differ significantly from that in intact rats. During this 3 h period all rats had received 2080 μmol sodium via the infusate. The sodium retention of hypophysectomized rats was associated with significantly elevated ($P < 0{\cdot}01$) terminal plasma sodium levels (151 ± 2 mmol/l, $n = 8$) as compared with intact animals (142 ± 1 mmol/l, $n = 9$).

Renal potassium excretion in intact rats reached a peak of $4{\cdot}7 \pm 0{\cdot}4$ μmol/min in the third hour of the experiment and thereafter declined slowly falling to $1{\cdot}8 \pm 0{\cdot}1$ μmol/min by 8 h. In hypophysectomized rats potassium excretion was initially lower and rose slowly to reach only $2{\cdot}2 \pm 0{\cdot}3$ μmol/min at 8 h. The generally lower rates of potassium excretion were associated with raised ($P < 0{\cdot}01$) terminal plasma potassium concentration ($5{\cdot}1 \pm 0{\cdot}3$ mmol/l, $n = 8$) in hypophysectomized rats by comparison with intact ($3{\cdot}9 \pm 0{\cdot}1$ mmol/l, $n = 9$) animals.

Mean arterial blood pressure (Fig. 3) declined progressively and to a similar extent in both intact and hypophysectomized rats over the first 4 h of study. However, in the latter half of the experiment blood pressure stabilized in intact animals around 106 mmHg but continued to decline in hypophysectomized rats reaching 87 ± 4 mmHg by 8 h. Against these changes in systemic blood pressure glomerular filtration rate remained relatively stable in intact rats but declined progressively from the second hour in the hypophysectomized group. In the last hour of the experiment, mean glomerular filtration rate had fallen to $2{\cdot}1 \pm 0{\cdot}2$ ml/min in hypo-

physectomized rats by comparison with the mean rate of $3{\cdot}1 \pm 0{\cdot}2$ ml/min in intact animals ($P < 0{\cdot}01$). The measurements of haematocrit were again relatively stable in intact rats at around 45% (Fig. 3) but in hypophysectomized rats there was a significant ($P < 0{\cdot}01$) fall in haematocrit to $42{\cdot}3 \pm 1{\cdot}3\%$ ($n = 10$) by 7 h 30 min, presumably indicative of an expansion of blood volume.

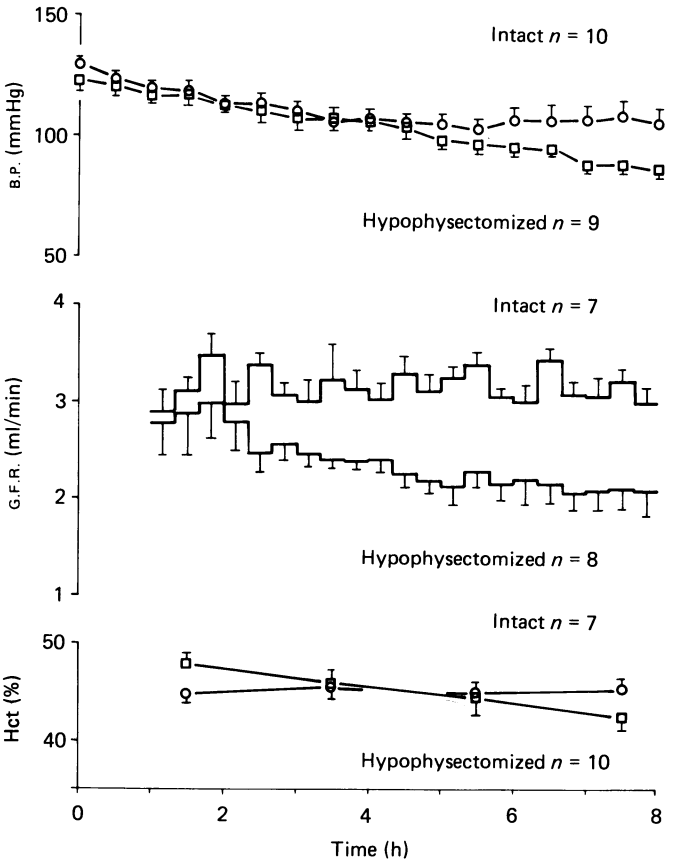

Fig. 3. Mean arterial blood pressure (B.P.), G.F.R. and haematocrit (Hct) in intact and hypophysectomized rats. Values are means for the times indicated during the 8 h post-operative study period; vertical bars indicate S.E. of means.

Plasma hormone levels

Plasma corticosterone concentration was considerably lower in anaesthetized (36 ± 4 ng/ml, $n = 7$) than in unanaesthetized (134 ± 22 ng/ml, $n = 5$) intact rats ($P < 0{\cdot}01$) and was further reduced ($P < 0{\cdot}01$) in hypophysectomized (4 ± 2 ng/ml, $n = 8$) animals.

Plasma aldosterone levels were similar in anaesthetized ($0{\cdot}76 \pm 0{\cdot}04$ ng/ml, $n = 7$) and unanaesthetized ($0{\cdot}57 \pm 0{\cdot}07$ ng/ml, $n = 5$) intact rats. Aldosterone concentration was, however, somewhat reduced ($P < 0{\cdot}01$) in hypophysectomized

(0·41 ± 0·08 ng/ml) by comparison with intact anaesthetized rats, though to a much lesser extent than corticosterone.

Plasma oxytocin and vasopressin concentrations in unanaesthetized intact rats and anaesthetized hypophysectomized groups are presented in Table 2. In the hypo-

TABLE 2. Plasma oxytocin and vasopressin levels in unanaesthetized intact rats and in anaesthetized hypophysectomized rats

Group	Oxytocin (μu./ml)	Vasopressin (μu./ml)
Unanaesthetized intact	0·32 ± 0·13 (6)	0·85 ± 0·19 (7)
Hypophysectomized control (no hormone administration)	Undetectable	0·16 ± 0·04 (8)
Hypophysectomized OXY 150 μu./min	24·0 ± 2·5 (7)	—
Hypophysectomized OXY 15 μu./min	2·59 ± 0·39 (10)	—
Hypophysectomized AVP 12 μu./min	—	1·54 ± 0·09 (8)

Values are mean ± S.E. of mean, *n* values are shown in parentheses.
AVP, arginine vasopressin; OXY, oxytocin.

physectomized rat plasma oxytocin was undetectable though trace levels of vasopressin were present. The plasma hormone levels induced in hypophysectomized animals by the administration of oxytocin and vasopressin are also shown in Table 2.

Effect of neurohypophysial hormones on renal excretion in hypophysectomized rats

Oxytocin. Administration of oxytocin at 150 μu./min had no significant effect on urine flow but was associated with increased sodium excretion which reached a peak of 5·8 ± 0·3 μmol/min (Fig. 4). Following cessation of hormone administration sodium excretion fell rapidly to 2·8 ± 0·5 μmol/min by the end of the experiment. Total sodium excreted during the 3 h period of hormone administration (782 ± 120 μmol) exceeded that previously seen in animals receiving saline alone ($P < 0·01$). Administration of oxytocin at 15 μu./min had no discernible effect on the rate of sodium excretion (Fig. 4) but slightly raised urine production (32·4 ± 2·4 ml) during the 3 h period of hormone infusion by comparison with animals receiving no hormone (24·8 ± 2·7 ml, $P < 0·05$).

Vasopressin. Arginine vasopressin administered at 12 μu./min induced the expected large reduction in urine production, which fell to 23 ± 4 μl/min (Fig. 5). This was accompanied by a small increase in sodium excretion to a peak of 3·4 ± 0·6 μmol/min, which was significantly ($P < 0·02$) higher than the mean rate of excretion during the last hour of the experiment. The total sodium excreted during the 3 h of hormone administration (461 ± 80 μmol) was, however, not significantly different from that in hypophysectomized animals receiving no hormone.

Combined oxytocin and vasopressin. Administration of oxytocin at 15 μu./min (a dose which administered alone had no detectable natriuretic action, see above) together with vasopressin (12 μu./min) produced a larger increase ($P < 0·05$) in

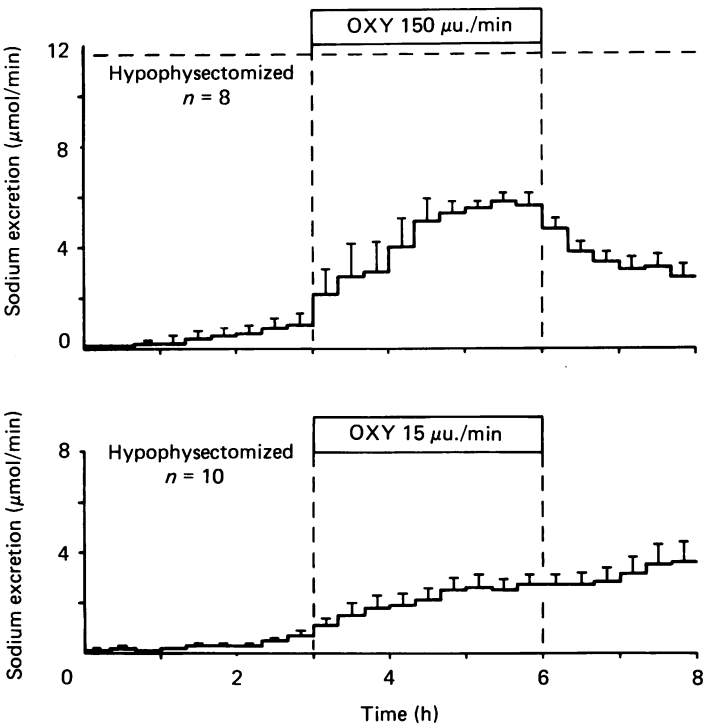

Fig. 4. The effect of oxytocin (OXY) administration at 150 and 15 μu./min on sodium excretion in hypophysectomized rats. Values are means for each 20 min collection period, vertical bars indicate S.E. of means. Dashed line indicates rate of infusion.

sodium excretion than that seen when vasopressin was administered alone. Sodium excretion rate reached a peak of $5{\cdot}8 \pm 1{\cdot}0$ μmol/min after 1 h but had fallen back to $3{\cdot}7 \pm 0{\cdot}4$ μmol/min by the end of the 3 h period of hormone administration (Fig. 6). The total sodium excreted between the third and sixth hour was 803 ± 115 μmol. Although this was greater ($P < 0{\cdot}02$) than that seen in hypophysectomized animals receiving no hormone it remains considerably lower ($P < 0{\cdot}01$) than that excreted by intact rats in the same time period.

The antidiuretic action of vasopressin was slightly but significantly reduced ($P < 0{\cdot}01$) when administered together with oxytocin (Fig. 6). Urine production during the 3 h of combined hormone administration was $8{\cdot}7 \pm 0{\cdot}7$ ml by comparison with $6{\cdot}0 \pm 0{\cdot}6$ ml when vasopressin was given alone.

Potassium excretion was apparently unaffected by oxytocin administration at either 150 or 15 μu./min. Vasopressin infusion produced a small initial transient increase in potassium excretion to $2{\cdot}9 \pm 0{\cdot}3$ μmol/min after 40 min of vasopressin administration. This was significantly higher ($P < 0{\cdot}05$) than the corresponding rate of potassium excretion ($1{\cdot}9 \pm 0{\cdot}4$ μmol/min) in hypophysectomized rats receiving no hormone. A similar marginal increase in potassium excretion was also observed in animals receiving combined oxytocin and vasopressin administration.

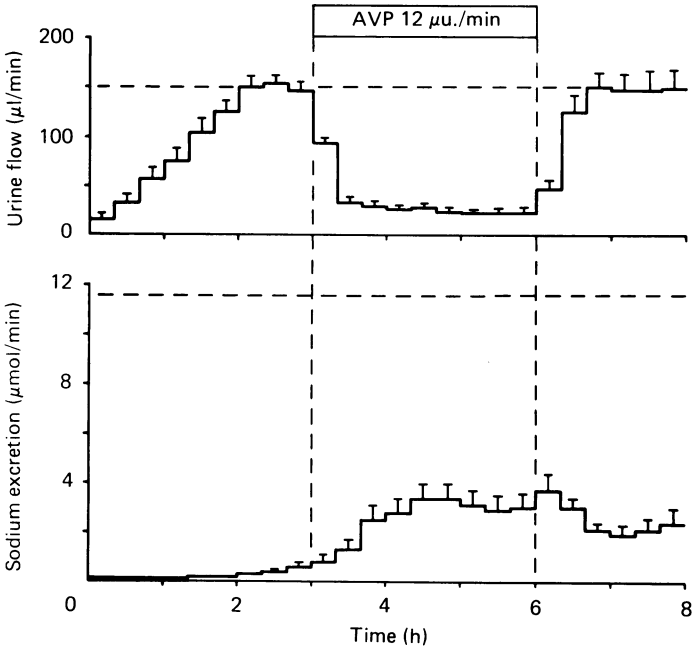

Fig. 5. The effect of arginine vasopressin administration (AVP 12 μu./min) on urine production and sodium excretion in hypophysectomized rats. Values are means for each 20 min collection period; vertical bars indicate S.E. of means. Dashed lines indicate rates of infusion.

DISCUSSION

The present study shows that the marked reductions in arterial blood pressure and G.F.R., well documented in chronically hypophysectomized rats, can also be seen in the acutely hypophysectomized animal within a few hours of removal of the pituitary. It would seem unlikely that the fall in G.F.R. resulted solely from the reduced arterial pressure, as glomerular filtration declined progressively from the second post-operative hour whereas femoral arterial pressure only fell significantly below that in intact animals some 4 h after surgery. Increased sympatho-adrenal activity may have been responsible for the initial maintenance of arterial blood pressure and depression in G.F.R. However, as the decline in blood pressure and glomerular filtration was not apparent in animals in which only the posterior lobe of the pituitary had been removed (Balment, Brimble, Forsling, Kelly & Musabayane, 1986), it would appear to result from the loss of one or more of the anterior pituitary factors, or of the hormones whose secretion they regulate. There is indeed evidence that glomerular filtration is impaired if glucocorticoid secretion is reduced to low levels as a result of loss of pituitary corticotrophin (Cutler, Kleeman, Koplowitz, Maxwell & Dowling, 1962; Kleeman, Levi & Better, 1975), and it has been shown that administration of exogenous corticotrophin can partially restore arterial blood pressure and glomerular filtration in chronically hypophysectomized rats (Bauman, 1967; Bauman & Phillips, 1970).

The present investigation has confirmed previous reports (Lichardus & Ponec,

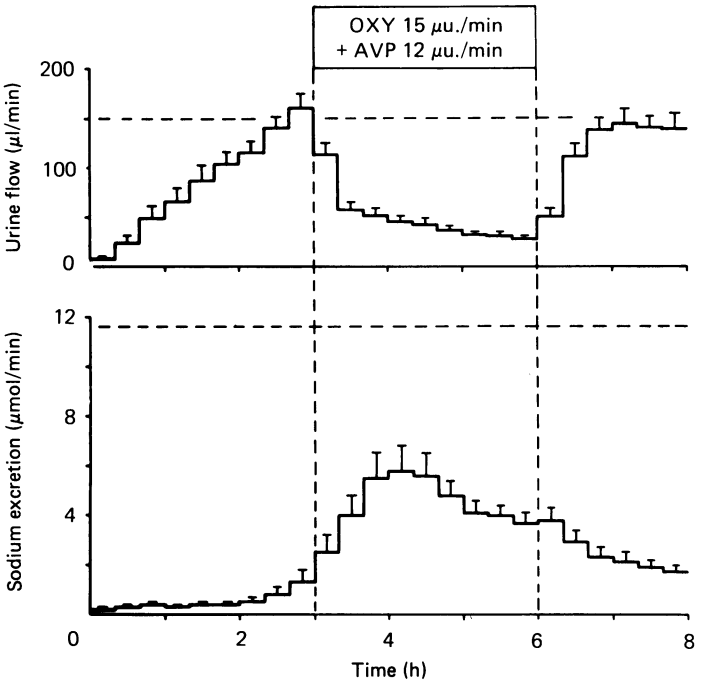

Fig. 6. The effect of combined oxytocin and arginine vasopressin administration (OXY 15 μu./min + AVP 12 μu./min) on urine production and sodium excretion in hypophysectomized rats. Values are means for each 20 min collection period; vertical bars indicate S.E. of means. Dashed lines indicate rates of infusion.

1973; Balment *et al.* 1984) that hypophysectomized rats receiving saline avidly retain sodium. This sodium retention occurred despite lowered plasma aldosterone and excretion of much of the accompanying water load, resulting in hypernatraemia. Hypophysectomized animals also exhibited reduced rates of potassium excretion and had somewhat elevated plasma potassium levels. The fluid retention indicated by the decline in urine flow towards the end of the 8 h period of study in hypophysectomized rats, together with the observed reduction in haematocrit, would suggest that the marked retention of sodium was associated with an expansion of blood volume. It is well established that, in intact animals, expansion of blood volume, particularly if coupled with hypernatraemia, leads to enhanced urinary sodium excretion which appears to be partly due to the release of a natriuretic factor (De Wardener & Clarkson, 1985). The natriuretic factor has been claimed to be of atrial (Lang, Thölken, Ganten, Luft, Ruskoako & Unger, 1985), renal (Louis & Favre, 1980) or intracranial origin (Sedláková, Lichardus & Cort, 1969). It would seem necessary to postulate that hypophysectomy inhibits either the release or the action of any such factor.

Lichardus & Ponec (1973) claimed that the change in the renal handling of sodium in hypophysectomized rats was a result of the loss of posterior rather than anterior pituitary hormones. They were able to restore sodium excretion by the administration of homogenates of posterior but not anterior pituitary lobes. Furthermore they were

able to partially restore sodium excretion with an injection of oxytocin but felt it unlikely that oxytocin deficiency was the sole factor leading to sodium retention in hypophysectomized animals. We have been able to confirm that oxytocin administration at 150 μu./min does partially restore sodium excretion in hypophysectomized male rats. This rate of oxytocin administration, however, produces plasma hormone levels well above the basal concentration. In a previous study (Balment *et al.* 1980) we reported that plasma oxytocin levels in unanaesthetized rats were less than 2 μu./ml and in this work for the first time we have been able to present measurements of basal levels in normally hydrated male rats of approximately 0·3 μu./ml. Oxytocin administration at the lower rate of 15 μu./min, which produced a plasma hormone level closer to the physiological range, had no detectable effect on urinary sodium excretion. Thus in hypophysectomized rats, as in intact animals (Balment *et al.* 1980), oxytocin only exerts a significant natriuretic action when present at supra-physiological levels. It is, therefore, unlikely that lack of oxytocin alone is responsible for the sodium retention of hypophysectomized rats.

We have recently shown the other neurohypophysial hormone, arginine vasopressin, to be natriuretic at physiological levels in intact rats (Balment *et al.* 1984) and that the natriuretic effect was blunted in hypophysectomized animals. Failure to observe a natriuretic action may have reflected the short (30 min) period of hormone administration in this earlier study. However, in the current work, in which the longer 3 h period of vasopressin administration was employed, the results were similar. Vasopressin administered at 12 μu./min induced plasma hormone levels within the physiological range (Balment *et al.* 1984) but produced only a marginal increase in sodium excretion despite a profound antidiuretic action. A larger increase in sodium excretion was produced, however, when this dose of vasopressin was combined with oxytocin administration at 15 μu./min, a dose rate which had no natriuretic action on its own. This suggests that oxytocin may potentiate the natriuretic action of vasopressin.

The rate of vasopressin administration and the lower of the two rates of oxytocin administration employed in this study were chosen from experience in previous studies (Balment *et al.* 1980, 1984; Forsling *et al.* 1982) to induce plasma hormone levels within the physiological range. Measurement of induced plasma hormone concentrations has confirmed them to be within or, in the case of oxytocin, possibly slightly above the physiological range. Thus we believe that we have adequately compensated for loss of posterior pituitary function in hypophysectomized rats but this has failed to fully restore sodium excretion to the level seen in intact animals. It seems unlikely that loss of neurohypophysial hormones alone can fully account for the sodium retention of hypophysectomized rats, unless there is, as suggested by Sedláková, Prusik, Skopkova, Barth, Kluh & Cort (1974), an additional posterior lobe natriuretic factor. Quamme, Hwang, Friesen & Dirks (1976) could, however, detect no significant natriuretic activity other than that attributable to oxytocin or vasopressin in the posterior pituitaries of either the cow or pig and it seems unlikely that the rat would differ in this respect. Thus we conclude that loss of anterior pituitary factors probably contributes to the sodium retention of the hypophysectomized animals either directly, or indirectly via altered trophic influence on the endocrines they control. The large fall in plasma glucocorticoid levels,

consequent on pituitary corticotrophin deficiency, may be of particular importance. Depressed corticosteroid levels are associated with reduced renal blood flow and G.F.R.s and altered renal handling of water and electrolytes (Cutler *et al.* 1962; Kleeman *et al.* 1975).

In summary the present study has shown that the failure of acutely hypophysectomized rats to excrete a salt load cannot be explained by elevated plasma aldosterone levels or simple deficiency of posterior pituitary hormones. Although ineffective alone, physiological levels of oxytocin apparently potentiate the natriuretic action of vasopressin but even in combination the two neurohypophysial hormones failed to fully restore sodium excretion in the hypophysectomized animal. This suggests that loss of anterior pituitary factors also contributes, possibly via the observed reduction in G.F.R. To investigate the effects of posterior pituitary hormone deficiency without the complication of concurrent removal of anterior pituitary influence, a technique for neurohypophysectomy in the rat was developed and is described in the accompanying paper.

The authors thank Byk Gulden, Konstanz, for the gift of Inactin.

REFERENCES

ARIMURA, A., SAWANO, S., REDDING, T. W. & SCHALLY, A. V. (1968). Studies on the retarded growth in rats with hypothalamic diabetes insipidus. *Neuroendocrinology* **3**, 187–192.

BALMENT, R. J., BRIMBLE, M. J. & FORSLING, M. L. (1980). Release of oxytocin induced by salt loading and its influence on renal excretion in the male rat. *Journal of Physiology* **308**, 439–449.

BALMENT, R. J., BRIMBLE, M. J., FORSLING, M. L., KELLY, L. P. & MUSABAYANE, C. T. (1986). A synergistic effect of oxytocin and vasopressin on sodium excretion in the neurohypophysectomized rat. *Journal of Physiology* **381**, 453–464.

BALMENT, R. J., BRIMBLE, M. J., FORSLING, M. L. & MUSABAYANE, C. T. (1984). Natriuretic response of the rat to plasma concentrations of arginine vasopressin within the physiological range. *Journal of Physiology* **352**, 517–526.

BALMENT, R. J., BRIMBLE, M. J., FORSLING, M. L. & MUSABAYANE, C. T. (1985). Renal sodium retention in acutely hypophysectomized rats. *Journal of Physiology* **361**, 56*P*.

BAUMAN, J. W. (1967). The effect of hypophysectomy and corticotrophin on the renal sensitivity to antidiuretic hormone. *Journal of Endocrinology* **38**, 107–114.

BAUMAN, J. W. & PHILLIPS, E. S. (1970). Failure of blood pressure restoration to repair glomerular filtration rate in hypophysectomized rats. *American Journal of Physiology* **218**, 1605–1608.

BEZNAK, M. (1959). Hemodynamic changes in hypophysectomized rats. *Circulation Research* **7**, 907–916.

BEZNAK, M. (1963). Cardiovascular effects of thyroxine treatment in hypophysectomized rats. *Circulation Research* **12**, 333–340.

BILLENSTEIN, D. C. & LEVIQUE, T. F. (1955). The reorganisation of the neurohypophysial stalk following hypophysectomy in the rat. *Endocrinology* **56**, 704–717.

BOSS, W. R., OSBORN, C. M. & RENZI, A. A. (1952). Effects of adrenal cortical extracts on renal function in hypophysectomized rats. *Endocrinology* **51**, 66–74.

CUTLER, R. E., KLEEMAN, C. R., KOPLOWITZ, J., MAXWELL, M. H. & DOWLING, J. T. (1962). Mechanisms of impaired water excretion in adrenal and pituitary insufficiency. III. The effect of extracellular or plasma volume expansion, or both, on the impaired diuresis. *Journal of Clinical Investigation* **41**, 1524–1530.

DE WARDENER, H. E. & CLARKSON, E. M. (1985). Concept of natriuretic hormone. *Physiological Reviews* **65**, 658–759.

FORSLING, M. L. (1974). Extraction of neurohypophysial hormones for bioassay. *Journal of Physiology* **241**, 35–36*P*.

FORSLING, M. L., BRIMBLE, M. J. & BALMENT, R. J. (1982). The influence of vasopressin on oxytocin-induced changes in urine flow in the male rat. *Acta endocrinologica* **100**, 216–220.

KARONEN, S. L., MORSKY, P., SIREN, M. & SEUDERLING, U. (1975). An enzymatic solid phase method for the trace iodination of proteins and peptides with ^{125}iodine. *Analytical Biochemistry* **67**, 1–10.

KIME, D. E. (1977). Meausrement of 1α hydroxycorticosterone and other corticosteroids in elasmobranch plasma by radioimmunoassay. *General and Comparative Endocrinology* **33**, 344–351.

KLEEMAN, C. R., LEVI, J. & BETTER, O. (1975). Kidney and adrenocortical hormones. *Nephron* **15**, 261–278.

LANG, R. E., THÖLKEN, H., GANTEN, D., LUFT, F. C., RUSKOAKO, H. & UNGER, TH. (1985). Atrial natriuretic factor – a circulating hormone stimulated by volume loading. *Nature* **314**, 264–266.

LICHARDUS, B. & PONEC, J. (1973). On the role of the hypophysis in the renal mechanism of body fluid volume regulation. *Endokrinologie* **61**, 403–412.

LOUIS, F. & FAVRE, H. (1980). Natriuretic factor in rats acutely expanded by Ringer's versus albumen solution. *Kidney International* **18**, 20–28.

MCCANN, S. M., ANTUNES-RODRIGUES, J., NALLAR, R. & VALTIN, H. (1966). Pituitary–adrenal function in the absence of vasopressin. *Endocrinology* **79**, 1058–1064.

MATTHEWS, B. F. (1963). Effects of hormones, placental extracts and hypophysectomy on inulin and para-aminohippurate clearances in the anaesthetized rat. *Journal of Physiology* **165**, 1–9.

MILNE, C. M., BALMENT, R. J., HENDERSON, I. W., MOSLEY, W. & CHESTER JONES, I. (1982). Adrenocortical function in the Brattleboro rat. *Annals of the New York Academy of Sciences* **394**, 230–240.

MUSABAYANE, C. T., BRIMBLE, M. J. & BALMENT, R. J. (1985). Renal sodium retention and vasopressin induced kaliuresis in ethanol anaesthetised rats. *Acta endocrinologica* **110**, 214–220.

PONEC, J. & LICHARDUS, B. (1977). The effect of small doses of antidiuretic hormone on renal excretion of sodium and water in saline loaded acutely hypophysectomized rats. *Experientia* **33**, 685–686.

QUAMME, G. A., HWANG, P., FRIESEN, H. G. & DIRKS, J. H. (1976). Characterization of natriuretic activity from posterior pituitary lobes. *Canadian Journal of Physiology and Pharmacology* **54**, 561–576.

SEDLÁKOVÁ, E., LICHARDUS, B. & CORT, J. H. (1969). Plasma saluretic activity: its nature and relation to oxytocin analogs. *Science* **164**, 580–582.

SEDLÁKOVÁ, E., PRUSIK, Z., SKOPKOVA, J., BARTH, T., KLUH, I. & CORT, J. H. (1974). Isolation of a tridecapeptide from natriuretic fractions of bovine posterior pituitary. *European Journal of Clinical Investigation* **4**, 285–292.

SIMON, C. D., HONEYMAN, T. W. & FRAY, J. C. S. (1984). Renin–angiotensin system in hypophysectomized rats. I. Control of blood pressure. *American Journal of Physiology* **246**, E84–88.

STROMBERG, P., FORSLING, M. L. & AKERLUND, M. (1981). Effects of prostaglandin inhibition on vasopressin levels in women with primary dysmenorrhea. *Obstetrics and Gynecology* **58**, 206–208.