STUDY OF ADH SECRETION IN ADRENALECTOMIZED RATS AND EFFECTS OF DEXAMETHASONE

By

Jeannine Marchetti, Arlette Burlet and Michel Boulangé

ABSTRACT

Urinary ADH (antidiuretic hormone) excretion was determined in adrenalectomized rats which were given a 0.9% NaCl sodium as drinking water. The animals were adrenalectomized and studied after either 4 or 7 days. In both cases, urinary ADH excretion was twice as high as in sham-operated rats indicating that adrenalectomy enhances ADH release. The ADH content of neurohypophyses (LN) was also measured in these rats: 4 days after adrenalectomy no alteration in the hormonal content could be detected, but an increase of 40% occurred 7 days later. The accumulation of ADH in the neurohypophysis of these animals suggests that the hormonal synthesis is also increased after adrenalectomy and to a greater proportion than the amount of ADH released.

Urinary ADH excretion returned to normal after administration of pharmacological doses of dexamethasone. Three days of dexamethasone treatment prevented the increase of ADH in neurohypophyses of rats which had been adrenalectomized 4 days before the start of the treatment. The effects of a glucocorticoid lack and of dexamethasone treatment on the synthesis and release of ADH are discussed; moreover a control of the effects of glucocorticoids on ADH synthesis has to be taken into consideration.

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The increased plasma vasopressin associated with adrenocortical insufficiency has long been a problem and conflicting results have been reported (Kleeman et al. 1964; Ahmed et al. 1967). Opinions differ not only about the presence of hypervasopressinism but also about the pathophysiological factors involved. Thus, the increased plasma ADH concentration in adrenal insufficiency may be caused either by a reduction in extracellular fluid and in blood pressure as reported by Share & Travis (1970) or by the absence of glucocorticoid control on ADH secretion as has often been suggested (Dingman & Despointes 1960; Mialhe-Voloss et al. 1967; Ahmed et al. 1967; Travis & Share 1971).

These assumptions have not been definitely proved and in this field, the investigations progress slowly because of the difficulty of measuring ADH in plasma: the concentration of circulating hormone is very low. However, an easier approach to ADH physiology is possible by measuring urinary hormonal excretion which may, under some conditions, reflect its secretion (Miller & Moses 1971, 1972; Fressinaud et al. 1974; Khokhar et al. 1975). Such a process is valid only if ADH metabolism is not affected by the experimental conditions.

Many years ago, Birnie et al. (1953) and Ginsburg (1957) suggested that the lack of glucocorticoids reduced the ADH hepatic catabolism. More recently, however, Hankiss et al. (1965) showed that glucocorticoids did not influence the hepatic and renal metabolism of vasopressin in normal and adrenalectomized rats.

Thus, in this study, we measured urinary ADH excretion in order to investigate the eventual presence of hypervasopressinism in adrenalectomized rats which were given as sodium diet a 0.9 % NaCl solution in place of drinking water. We also investigated whether the increase in excreted ADH was associated with a modification of the neurohypophyseal hormonal content. Then to determine the part played by the lack of glucocorticoid in the modifications observed after adrenalectomy, measurements of urinary and neurohypophyseal ADH were performed in adrenalectomized rats treated with dexamethasone.

**MATERIAL AND METHODS**

Male albino Wistar rats (170–220 g body weight) were obtained from the Centre d’Elevage, Centre National de la Recherche Scientifique, Orléans, France. Surgical adrenalectomy was performed under 5 % pentobarbital anaesthesia (0.1 ml/100 g body weight, was injected intraperitoneally). Simultaneously, control rats were sham-operated. Adrenalectomized rats (Adx) were given a 0.9 % NaCl solution as drinking water and the control rats were kept on a normal diet.

For urine collection, there were 2 animals in each metabolic cage (Stigma, type MS inox). They were not given food but were allowed to drink freely. Urine was collected for 12 h, during the day in cooled graduated tubes. The urinary ADH excretion was measured in the 4th (group I) and 7th day (group II) after adrenalectomy. Two ex-
Experiments were performed on the 4th day after adrenalectomy and altogether 18 Adx rats as well as 16 control rats were studied. Other four experiments were performed on the 7th day after adrenalectomy; in each case, ADH excretion was measured in 8 Adx and 6 control rats (except for one experiment, where only 4 control rats were used). The day following the collection of urine, the animals were decapitated between 10 and 12 a.m. and the hypophyses were removed.

In another experimental series, 14 Adx rats were treated with dexamethasone (Sigma) and compared with 16 control rats. Each Adx rats received 7 injections of dexamethasone administered twice daily from the 5th to the 8th day after the adrenalectomy. The 7th and last injection was given 2 h before decapitation which had been performed as previously between 10 and 12 a.m. Dexamethasone was dissolved in pure ethanol and then diluted in a sterile 0.9 % NaCl solution just before injection. The volume administered to the experimental rats was 0.1 ml per 100 g body weight. Each injection was equal to a dose of 100 mg dexamethasone/100 g body weight. Control rats were given an equivalent volume of 0.9 % NaCl solution.

During preliminary experiments, urinary and neurohypophysis ADH levels were measured, by radioimmunoassay, in rats which weighed between 250 and 300 g and were kept under normal conditions.

**Chemical procedures**

a) *Preparation of urinary samples for ADH determination*

For preparation of ADH urine extracts, a modified version of the Moran technique (Moran et al. 1964) was used (Miller & Moses 1971). The urine was first adjusted to pH 4.5 and then passed through a column (diameter: 1.6 cm) containing 4 cm³ of cation exchange resin (Amberlite CG 50, type II). The resin was obtained in H⁺ form according to Hirsch et al. (1953). After the column had been washed with 25 ml distilled water to pH 4.5 and with 25 ml of 50 % ethanol/water at pH 4.5, AVP was eluted by using 50 ml of 75 % ethanol/water at pH 2. The eluates were evaporated to dryness in a rotatory evaporator; the dry residue was redissolved in 2 ml water and evaporated so as to eliminate traces of acid. The flasks containing the ADH to be measured were kept at −30°C until the following day when immunoad assay was performed. Just before assay, the dry residues were dissolved in 2 ml of 0.01 M phosphate buffer containing 0.15 M NaCl and 300 mg % lysosyme. The pH of these samples was checked and when necessary, adjusted to pH 7.5 by addition of very small amounts of 0.1 N NaOH solution. The efficiency of the extraction procedure was studied either by adding [¹²⁵I]IVP to 10 ml of 0.9 % NaCl solution, adjusted to pH 4.5 or by adding 1–10 mU to 10 ml of urine from diabetes insipidus patients.

b) *ADH extraction from the neurohypophysis*

Extracts were prepared according to Amatruda et al. (1963). Immediately after decapitation, the pituitaries were completely removed and placed in 2 or 3 ml cold acetone. After about 5 h, the acetone was renewed and the pituitaries were kept at 4°C for 20 h. The glands were placed in a dessicator in the presence of phosphoric anhydride and maintained under vacuum for 30 min. The neurohypophyses were then dissected, weighed on a Cahn-Gram electro-scale and homogenized in 0.25 % acetic acid. After centrifugation, the supernatants were frozen at −30°C until the time of
 assay. Under these conditions, it was possible to keep the extracts for several months without noticeable losses of ADH. To perform ADH measurements, the extracts were diluted to 100, 200 or 400 times their volume in 0.01 M phosphate buffer, pH 7.5, containing 0.15 M NaCl and 300 mg % lysozyme for immunoassay and in 0.9 % NaCl solution for bioassay.

c) Radioimmunoassay

ADH was determined using the type of radioimmunoassay previously described for LVP (Marchetti 1973). According to this system, anti-LVP antibodies cross-reacted completely with AVP, but did not recognize oxytocin. The titer of the serum used in the present studies was 1/3000. In these experiments, LVP (Sandoz – solution to 10 U/ml) was used as the standard and the lowest sensitivity of assay was 2.5 μU.

Bioassay

The technique used for bioassay was that of Jeffers et al. (1942) modified by Gharib (1967). Test animals were female Sprague-Dawley rats (150–200 g) obtained from the Centre d’Elevage des Oncins, Saint-Germain sur l’Arbresle. Rats were anaesthetized and overloaded with 6 % alcohol solution. The quantity of solution administered in two times, by mouth was equal to 8 % of the body weight. Then, the jugular vein was catheterized, and after cystotomy, a thin rubber tube was passed into the bladder. The overload was maintained by perfusing (0.1 ml/min) the rat with a solution which contained 0.3 % NaCl, 1.67 % glucose and 1.2 % ethanol. Assay started when diuresis was constant, and greater than 8 drops per min. The extract was injected into the jugular vein and the anti-diuresis observed was compared to that obtained with a LVP solution. A “4 point assay” was performed: each rat received alternatively two LVP injections and two injections of the unknown extract; the quantities administered were in a 1/2 ratio. The sensitivity of the test was 10 μU LVP.

The specificity of dosage was checked by adding Na+ thioglycolate (final concentration: 0.01 M) to the LVP solution and the extract containing AVP. The mixture was kept for 2 h at room temperature. Under these conditions, thioglycolate inhibited the anti-diuretic response due to ADH.

Creatinine was determined by a Technicon Auto Analyser. Student's t-test was used for statistical analysis and P values below 0.05 were considered significant.

RESULTS

Antidiuretic hormone determinations

When measured with [225I]LVP, the efficiency of the procedure used to extract ADH from urine was 69.9 ± 2.4 % (n = 36). When non-radioactive LVP was added to 10 ml of urine from patients with diabetes insipidus, the percentage of hormone recovered after extraction measured by radioimmunoassay was 63.3 ± 5.2 % (n = 12). It was also checked that no ADH could be detected in the extracts obtained from the urine (10 ml) of “diabetes insipidus” patients.

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Urinary ADH excretion determined by radioimmunoassay, in normal (non-operated) rats weighing from 250 to 300 g, was found to be 0.245 ± 0.016 mU/12 h/100 g (n = 30).

The neurohypophyses of these rats contained a quantity of ADH equal to 429 ± 30 mU/LN corresponding to a hormonal concentration of 1431 ± 153 mU/mg dry weight of the neurohypophysis.

Fig. 1 shows a good correlation between the ADH measurements performed by radioimmunoassay and bioassay. The linear regression line equation was

\[ Y = 1.41x \pm 0.01 \quad (r = 0.94) \]

for ADH measurements of urinary extracts and

\[ Y = 1.47x \pm 29.97 \quad (r = 0.91) \]

for measurements of neurohypophysial extracts. The slope of the linear regression line is not equal to one, the bioassay values being somewhat higher than the immunoassay values.

**Increase of urinary excretion in adrenalectomized rats**

Table 1 shows that urinary ADH excretion was significantly twice as high in adrenalectomized rats than in the control, sham-operated rats. This increase occurred during both types of experiments: *i.e.* the 4th (group I) and 7th (group II) day after adrenalectomy. The increase of excreted ADH was not due to a modified glomerular filtration since creatinine urinary excretion showed no alteration after adrenalectomy: thus it was 995 ± 54 μg/12 h/100 g.
Table 1.
Effect of adrenalectomy on urinary ADH excretion (mU/12 h/100 g).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th day after adrenalectomy</td>
</tr>
<tr>
<td>Control rats</td>
<td>0.234 ± 0.022 n = 8</td>
</tr>
<tr>
<td>Adrenalectomized rats</td>
<td>0.540 ± 0.113 n = 9</td>
</tr>
<tr>
<td></td>
<td>( P &lt; 0.05 )</td>
</tr>
</tbody>
</table>

Control rats were sham-operated and fed a normal diet. Adrenalectomized rats were given a 0.9% NaCl solution as drinking water. There were 2 rats per cage: \( n \) = number of measurements (number of animals: 2 \( n \)). Values are means ± SEM.

![Graph showing ADH content](image)

Fig. 2.
ADH content of the neurohypophysis after adrenalectomy. Sham-operated rats were used as controls and kept on a normal diet. Soon after the operation adrenalectomized rats were given a 0.9% NaCl solution as drinking water. The number of animals in each group is shown in the lower part of the bars. Means are accompanied by their standard errors. Adrenalectomized rats are compared to the control rats included in the same experiments.
in control rats, $1190 \pm 67 \, \mu g/12 \, h/100 \, g$ in group I and $1153 \pm 64 \, \mu g/12 \, h/100 \, g$ in group II.

These results were therefore consistent with the presence of hypervasopressinism in the adrenalectomized rats.

*Modified ADH content in the neurohypophysis after adrenalectomy*

The ADH content in the neurohypophysis was also measured 4 or 7 days after adrenalectomy. Fig. 2 shows that there was no change 4 days later, but unexpectedly the hormonal content of post-pituitary increased significantly by 40 % 7 days after operation. In addition, the ADH content of the neurohypophysis remained high 15 days after adrenalectomy.

*Effects of dexamethasone on urinary ADH excretion and on the ADH content of neurohypophysis*

Determinations of ADH excretion were made after the first and fifth dexamethasone injection. Since glucocorticoids are known to increase glomerular filtration (*Finkenstaedt et al. 1954; Garrod et al. 1955; Liddle et al. 1954*), creatinine excretion was measured at the same time and the urinary ADH/urinary creatinine ratio was established.

Table 2 shows a higher percentage (40 %) of urinary creatinine in adrenalectomized rats treated with dexamethasone; but the ratio ADH/creatinine determined in these animals did not significantly differ from that found in the untreated sham-operated control rats. Dexamethasone therefore prevented ADH excretion from rising above the normal level in adrenalectomized rats.

With regard to the ADH content of the neurohypophysis, there was no significant difference between experimental and control rats ($186 \pm 15 \, mU/NL$ in control rats versus $216 \pm 9 \, mU/NL$ in treated adrenalectomized rats). It was the same for the hormonal concentration ($1408 \, mU/mg$ of dry weight in control rats and $1318 \, mU/mg$ of dry weight in experimental rats). Thus, the increase in post-pituitary vasopressin observed 7 days after adrenalectomy did not occur when adrenalectomized rats were given dexamethasone.

**DISCUSSION**

The present experiments show a good correlation between bioassay and radioimmunoassay both for the ADH neurohypophyseal and urinary extracts. The slope of the linear regression line is not equal to one. This is because LVP was used to plot standard curve, whereas the hormonal extracts measured
Table 2.
ADH urinary excretion in adrenalectomized rats after one and five injections of dexamethasone.

<table>
<thead>
<tr>
<th>Urinary parameters</th>
<th>Animals</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control rats</td>
<td>4 days after adrenalectomy</td>
<td>7 days after adrenalectomy</td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td>+1 injection n = 7</td>
<td>+5 injections n = 7</td>
</tr>
<tr>
<td>Creatinine μg/12 h/100 g</td>
<td>1126 ± 90</td>
<td>1650 ± 169 P &lt; 0.01</td>
<td>1491 ± 224 P &lt; 0.01</td>
</tr>
<tr>
<td>ADH mU/12 h/100 g</td>
<td>0.200 ± 0.034</td>
<td>0.200 ± 0.033 n. s.</td>
<td>0.324 ± 0.066 n. s.</td>
</tr>
<tr>
<td>ADH/creatinine × 1000</td>
<td>0.176 ± 0.021</td>
<td>0.199 ± 0.032 n. s.</td>
<td>0.195 ± 0.041 n. s.</td>
</tr>
</tbody>
</table>

Four days after adrenalectomy, the rats were given twice daily im injections of dexamethasone (100 μg/100 g body weight). During the same experiment, sham-operated rats were given a placebo solution. There were 2 rats per cage (n = number of measurements, number of animals = 2 n). ADH and creatinine were measured in urine collected during the day following injection No. 1 (the first day of treatment) and after the fifth injection (the 3rd day of treatment).

contained AVP. This fact did not affect immunoassay since both AVP and LVP have the same affinity in reactivity to antibodies (Marchetti 1973). However, it was not the same for the determination of biological activity of the extracts, since AVP was more antidiuretic than LVP, i.e. 1 mole AVP had the same antidiuretic effect as 1.67 moles LVP (Berde & Boissonnas 1968).

It would have made this study all the more interesting if one had been able to perform ADH measurements in plasma. But unfortunately, our radio-immunoassay was not sensitive enough to allow the measurements in the rat; only a few laboratories are in a position to do it (Dunn et al. 1973; Möhring & Möhring 1975). However, determination of urinary AVP has the advantage of presenting a cumulative index of the hormonal secretion over a given period, whereas determination of plasma AVP only indicates the concentration at the time of sampling.

Thus, in adrenalectomized rats, the increase in ADH excretion indicated an enhanced vasopressin release. In addition, 7 days after adrenalectomy, the ADH content in the neurohypophysis was also increased in these animals. As the neurohypophyseal content is the result of a balance between the rate of
hormonal production and release, the ADH accumulation in the neurohypophysis of these animals which excrete large amounts of hormone suggests that the synthesis of ADH is increased and is proportionally higher than its release.

The neurohypophyseal content varied differently, when adrenalectomized rats were given water containing no salt. In this case, the hormonal content of the pituitary dropped by 40% compared to control rats (110±8 mU/neurohypophysis versus 197±14 mU/neurohypophysis). In these animals, ADH secretion was very high because sodium losses were not at all compensated; consequently, the plasma volume was considerably reduced. Thus, ADH secretion from the neurohypophysis was higher than hormonal synthesis, and ADH pituitary reserves were released.

In the present study, one can wonder what part is played by the lack of glucocorticoid in release and/or in synthesis of ADH? As regards hormonal release, its increase may also result from a reduction in extracellular fluid caused by sodium losses insufficiently compensated by salt contained in drinking water. Indeed PRA (plasma renin activity) which is considered as a satisfactory index for variations in plasma volume, was enhanced in adrenalectomized rats, even though they were given a 0.9% NaCl solution as drinking water (unpublished observation). Under these conditions, it is difficult to know exactly how the lack of glucocorticoids affects ADH release.

Pharmacological doses of dexamethasone prevented the increase of ADH release in adrenalectomized rats. This drug did not act by correcting the reduction of extracellular fluids, since doses of the drug are known to be diuretic, natriuretic and kaliuretic (Chabria & Gaitonde 1966; Marchetti 1975). Under these conditions, dexamethasone would prevent ADH secretion caused by the reduction in extracellular volume, probably, by affecting the mechanism of hormonal release.

A glucocorticoid effect preventing ADH secretion caused by different kinds of stimuli has already been reported:

- Dingman & Despointes (1960), Mialhe-Voloss et al. (1967) and Share & Travis (1971) observed that a single injection of glucocorticoid reduced the ADH release caused by nicotine, as well as neurogenic and emotional stress.
- Aubry et al. (1965) showed that cortisol raised the osmotic threshold for AVP release in response to the osmotic stimulus.
- Vilhardt (1970) demonstrated that prednisolone prevented ADH release from the neural lobes of rats isolated and stimulated by adding potassium ions to the incubation medium. However, prednisolone did not affect normal vasopressin release from the non-stimulated neurohypophysis.

As for the increase of ADH synthesis observed in adrenalectomized rats, it could result from lack of glucocorticoid, since dexamethasone (the main effect of which is glucocorticoid) prevents the accumulation in the neurohypophysis of treated animals.

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In conclusion, our results demonstrate an increase in ADH release in adrenalectomized rats despite the 0.9% NaCl solution given as drinking water. This increase may not only be due to the absence of glucocorticoids but also to the reduction in extracellular fluids since 0.9% NaCl solution is not sufficient to correct this reduction. However, the lack of glucocorticoids could be responsible for the increase of hormonal synthesis. The ability of dexamethasone to correct hypervasopressinism and prevent the rise in neurohypophyseal ADH suggests that glucocorticoids prevent ADH release from the stimulated neurohypophysis and reduces the hypothalamic synthesis increased after adrenalectomy.

A negative feedback effect of glucocorticoids on ADH synthesis may therefore be considered, but to demonstrate it, definitively, other more relevant studies than those so far undertaken would be necessary, for example: measurements of incorporation of labelled amino acids into isolated hypothalamus (cf. Takabatake & Sachs 1964).

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REFERENCES

Marchetti J.: Experientia (Basel) 29 (1973) 351.

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