WATER METABOLISM OF ADRENAL MEDULLECTOMIZED RATS

Yoshiyuki TOYOMASU AND Shinji ITOH*

Department of Physiology, Hokkaido University School of Medicine, Sapporo

On the water metabolism of adrenal medullectomized animals several reports have been appeared. STEIN and WERTHEIMER1) and DEXTER and STONER2) found that removal of the adrenal medulla resulted in a decrease in water excretion, while GAUNT, LILING and CORDSEN3) and HORRES, EVERSOLE and ROCK4) claimed that the water metabolism was not significantly altered by medullectomy. The present study was undertaken to clarify the part played by the adrenal medulla in the water metabolism, using medullectomized rats.

METHODS

Adult male rats of Wistar strain, weighing about 150 g, were used. Rats, which had been fasted and allowed access to water for about 18 hours, received a water load (5 ml per 100 g body weight) by a stomach tube and the rate of urine excretion was measured at 15 minutes intervals for a period of 180 minutes.

Extirpation of the adrenal medulla was performed as described in our previous paper5). The medullectomized rats were used for experiments 4 to 5 weeks after the operation.

Antidiuretic potency of blood serum was measured by the method of JEFFERS, LIVENZEY and AUSTIN6,7). One ml of fresh serum per 100 g body weight was injected into the femoral vein of hydrated test rats. Changes in the excretion rate were observed by comparing the volume of urine secreted in 20 minutes subsequent to injection with that for the same period just prior to injection. Inactivation of antidiuretic activity of vasopressin was determined by the method of BIRNIE8,9). Pitressin (Parke-Davis) was used as vasopressin preparation.

RESULTS

I. Water excretion rate of medullectomized rats; In medullectomized rats the rate of urine excretion after a standard dose of water (5 ml per 100 g) was considerably low, compared with that of normal rats (Fig. 1). Percentage water excreted at 90 minutes after water administration was 66±1.6 per cent in normal rats and 31±2.7 per cent in medullectomized ones.

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* 井上嘉幸，伊藤直次
Fig. 1. Diuretic response and effect of Pitressin in normal and medullectomized rats.

As reported in our previous paper, in normal rats administration of 0.01 mg adrenaline or noradrenaline was ineffective to enhance the water excretion rate. However, in medullectomized rats subcutaneous injection with this dose of adrenaline caused a significant increase in the excretion rate; in these rats percentage water excreted at 90 minutes was 45±4.0 per cent after 0.01 mg of adrenaline and 67±1.7 per cent after 0.04 mg of it. The percentage after 0.04 mg of noradrenaline was 72±1.3 per cent. These figures obtained after 0.04 mg of the hormones were the same as the normal rate (Figs. 2 & 3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of expt.</th>
<th>No. of test rats</th>
<th>Percentage water excreted at 90 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>42</td>
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<tr>
<td>Adrenaline 0.01 mg</td>
<td>4</td>
<td>12</td>
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<tr>
<td>Noradrenaline 0.01 mg</td>
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</table>

Fig. 2. Effect of adrenaline and noradrenaline on water excretion rate in normal rats.
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FIG. 3. Effect of adrenaline and noradrenaline on water excretion rate in medullectomized rats.

In the next series of experiments adrenaline (0.02 mg per 100 g, daily) was subcutaneously injected into medullectomized rats successively for a period of 3 to 4 weeks after the operation. In these chronically treated rats the water excretion rate was fairly high; the percentage was 54±5.3 per cent. Moreover, it was found that single injection of adrenaline or noradrenaline (0.04 mg per 100 g) into medullectomized rats 2 hours prior to the water load caused a similar increase in the rate of urine excretion; the percentage was 73±9.0 per cent in medullectomized rats treated with adrenaline and 67±3.4 per cent in those treated with noradrenaline. On the other hand, in normal rats the rate was not altered by the prior treatments with adrenaline and noradrenaline (Figs. 2 & 3).

II. Antidiuretic effect of Pitressin in medullectomized rats; If Pitressin (5 mu per 100 g) was injected intraperitoneally into normal rats simultaneously with water administration, urine excretion was completely inhibited for about 60 minutes and thereafter diuresis commenced at a considerably high rate. On the other hand, in medullectomized rats after the same dose of Pitressin the commencement of water elimination retarded and the rate of diuresis was low, as is shown in Fig. 1. From the results it may be inferred that the antidiuretic effect of Pitressin appears to be more efficient in medullectomized rats than in normal ones.

III. Inactivation of vasopressin by liver tissues of medullectomized rats; The above facts observed in medullectomized rats that the water diuretic response is poor and that the antidiuretic effect of Pitressin is prolonged may suggest that destruction of vasopressin in the body is reduced in the absence of adrenal medulla. In an attempt to ascertain this presumption, inactivation of antidiuretic activity
of Pitressin by liver tissue was examined. As illustrated in Fig. 4, Pitressin incubated with liver homogenates from normal rats markedly lost its antidiuretic activity, while the inactivation by liver tissues of medullectomized rats was considerably slight. The percentage water excreted at 90 minutes after injection of Pitressin (20 mu per 100 g, intraperitoneally) was 16±1.9 per cent, that after Pitressin incubated with normal liver tissues was 51±3.7 per cent, and after Pitressin incubated with liver tissues of medullectomized rats was 28±3.9 per cent. The results clearly indicate that the inactivation is markedly reduced after medullectomy, and may suggest that the decreased activity in inactivating vasopressin is possibly one of the causes for bringing about the deficient diuretic response in medullectomized rats.

Since we have found that the lowered diuretic response of medullectomized rats was restored to normal level after successive treatments with adrenaline, the inactivating capacity of liver tissues of rats which had been medullectomized and treated with adrenaline (0.02 mg per 100 g, daily) successively for a period of 4 weeks after the operation was examined. As shown in Fig. 4, the value obtained was the same as the normal one, indicating the inactivating capacity had been completely restored to normal by this treatments.

![Table 1](image)

<table>
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<tr>
<td>Normal liver</td>
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<tr>
<td>Medullect. liver</td>
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<td>33</td>
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<tr>
<td>+ Adrenaline 0.02 mg 4 weeks</td>
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<tr>
<td>+ Adrenaline (in vitro)</td>
<td>3</td>
<td>9</td>
<td></td>
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</tbody>
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![Fig. 4](image)

Next, to see whether adrenaline affects directly on the vasopressin inactivating system of the liver, 0.05 mg of adrenaline was added into 5 ml of the incubation medium. Such in vitro addition of adrenaline was, however, ineffective to promote the inactivating activity. It may be assumed that adrenaline does not affect
directly on the 'vasopressinase'.

IV. Serum antidiuretic potency of medullectomized rats; From the above results it is considered that the destruction of vasopressin may be retarded in medullectomized rats. If so, serum antidiuretic potency of these animals is expected to be increased. Experiments were done, determining the potency by the method of Jeffers et al. As shown in FIG. 5, water excretion rate after injection of normal control serum was 60±6.5 per cent, while after that of medullectomized rats was only 20±2.9 per cent, indicating a marked increase in the potency in serum of medullectomized rats. If medullectomized rats were treated with adrenaline (0.02 mg per 100 g, daily for a period of 4 weeks), serum antidiuretic activity decreased significantly; the excretion rate after serum injection was 53±5.1 per cent. These results may indicate that the reduction of water diuretic response in medullectomized rats is plausibly associated with the increased antidiuretic potency of serum.

DISCUSSION

As to the effect of adrenaline and noradrenaline on the water metabolism, a considerable number of studies have been reported. At present it is mostly accepted that these hormones affect urine formation in two ways: relatively small amounts of the hormones increase urine output indirectly by elevating the arterial pressure, while large amounts of them reduce urine excretion by acting directly on the kidney. Anyhow different results may be obtained according to the dose and route of administration. In the present experiments subcutaneous injection of adrenaline and noradrenaline in a dose of 0.04 mg per 100 g body weight into hydrated rats was effective to increase urine output; in this case the effect of noradrenaline was more intense than that of adrenaline. This effect may be caused by the elevation of the arterial pressure. In normal rats 0.01 mg of adrenaline had no effect to increase water elimination, while in medullectomized rats this amount of adrenaline significantly increased urine excretion.
rate. Moreover, in medullectomized rats, but not in normal ones, prior treatments with adrenaline resulted in an increase in the water excretion rate. Lowered diuretic response of these rats was also restored to normal level by daily treatments with adrenaline. This effect of prior or successive treatments is difficult to be explained by changes in the renal hemodynamics. It may be inferred that derangement of the water metabolism caused by the absence of adrenal medulla is improved by such treatments with adrenaline.

Posterior pituitary antidiuretic hormone may participate in the deficient diuretic response of medullectomized rats. The fact that the antidiuretic effect of Pitressin is prolonged in medullectomized rats suggests that the destruction of antidiuretic hormone in the body delays in the absence of adrenal medulla. Actually it was found that the vasopressin-inactivating ability of liver tissue is markedly decreased after medullectomy. In the medullectomized rats high antidiuretic potency of blood serum was also proved. From the results the following conclusion may be given: (a) Poor diuretic response to administered water in medullectomized rats is ascribed, at least in part, to an accumulation of the antidiuretic hormone in the circulation, and (b) reduced ability of tissues to inactivate vasopressin may be responsible for the accumulation of the hormone.

It was reported by PICKFORD that injection of adrenaline, intravenously or intracarotid, prevented the antidiuretic hormone-releasing action of acetylcholine\textsuperscript{12,13}. As a cause of the diminished diuretic response in medullectomized rats, removal of the inhibitory effect of adrenaline on the discharge of antidiuretic hormone should be considered. However, in the present study this possibility is open to question, since the part played by adrenaline in the central control on the release of antidiuretic hormone is complex and its physiological function uncertain.

SUMMARY

1. Medullectomized rats showed a diminished diuretic response to ingested water.
2. Subcutaneous injection of adrenaline in a small dose, which failed to increase urine output in normal rats, caused an increase in the water excretion rate of medullectomized rats. Treatments with adrenaline, 2 hours prior to water load or successively for 3 to 4 weeks, restored the lowered diuretic response of medullectomized rats to normal level.
3. Pitressin was more effective to elicit antidiuresis in medullectomized rats than in normal ones.
4. Vasopressin inactivating capacity of liver tissue was reduced after medullectomy. The reduced inactivation restored to normal level after treatments with adrenaline. \textit{In vitro} addition of adrenaline into incubation medium of liver homogenate did not affect the inactivating system.
5. Serum from medullectomized rats contained more antidiuretic activity than
that from normal rats. The accumulation of antidiuretic material in serum in the absence of adrenal medulla could be prevented by chronic treatments with adrenaline.

REFERENCES