WATER METABOLISM IN THE HYPOPHYSECTOMIZED BRATTLEBORO (DI) RAT

By

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ABSTRACT

To re-examine the relative roles of the anterior and posterior pituitary hormones in diabetes insipidus, the effects of hypophysectomy and hormone replacement therapy in hereditary diabetes insipidus rats (Brattleboro strain) was compared with effect of similar treatment of rats without diabetes insipidus.

In agreement with experiments on surgically induced diabetes insipidus, hypophysectomy sharply reduced water intake in rats with hereditary diabetes insipidus (DI rats). Water intake remained considerably higher, however, in these rats than in the non-DI rats following hypophysectomy.

ADH treatment reduced water intake nearly to levels seen in non-DI rats. In water deprivation studies it was found that urine concentrating ability of the hypophysectomized non-DI rats was almost twice that of the hypophysectomized DI rats.

None of the hormones tested restored water turnover of the hypophysectomized DI rats to that seen in sham operated controls. ACTH and T₃ increased water intake, but not to levels seen in DI rats with intact pituitary glands.

These experiments lead us to concur with those who hold that maximal flow in diabetes insipidus requires the participation of the anterior pituitary hormones. In addition these experiments indicate that ADH or ADH like material is physiologically functional in hypophysectomized rats (other than the Brattleboro strain), and that its presence accounts for the absence of diabetes insipidus following pituitary removal.

The relative roles of the anterior and posterior pituitary hormones in the development of diabetes insipidus (DI) have been disputed. One group (Keller 1942; Heinbecker et al. 1947; Lipsett & Pearson 1957) held that the anterior pituitary gland was not essential for maximal flow in diabetes insipidus if all
neurohypophysial material was removed. Another group (von Hann 1918; Richter 1934) contended that the anterior pituitary hormones were essential for maximal flow. The argument appeared to be resolved by the work of Gale et al. (1961) who found that hypophysectomy ameliorated water turnover in rats with electrolytically induced diabetes insipidus, and that median eminence lesions produced sharply increased water turnover in hypophysectomized rats. It thus seemed that while the absence of ADH is the primary cause of diabetes insipidus, anterior pituitary hormones are required for maximal water turnover.

These excellently designed studies were, however, open to criticism on the grounds that no definition of the extent of the median eminence lesion was established. In addition, the conclusions would have been strengthened even more had the authors been able to show that replacement of anterior pituitary hormones reversed more completely the effect of hypophysectomy in lesioned rats, and more importantly, that replacement of ADH reversed the effects of lesioning in hypophysectomized rats.

In this report we describe studies on water turnover in hypophysectomized and sham-operated rats with hereditary diabetes insipidus (Brattleboro strain). These rats presumably have a discrete lesion in the ADH synthesizing apparatus, other endocrine systems remaining intact (McCann et al. 1964; Galton et al. 1966; Valtin et al. 1966). We have also investigated the effect of ADH administration to the hypophysectomized DI rat. In an attempt to further characterize the effect of ADH in these rats we also examined the effects of various anterior pituitary hormones and ADH on urine concentrating ability of hypophysectomized Sprague-Dawley and Brattleboro rats following water deprivation.

The results of these studies confirm the conclusions of Gale et al. (1961) with respect to the roles of anterior pituitary and posterior pituitary hormones in the development of diabetes insipidus. We have also found that triiodothyronine (T₃) as well as ACTH augments water turnover in these rats. Additionally, we have studied the effect of exogenous ADH in the hypophysectomized Brattleboro (DI) rat, and interpreted the role of ADH and anterior pituitary hormones in preventing permanent diabetes insipidus following hypophysectomy in non-DI rats.

**METHODS**

Female rats weighing 158–210 g of the Brattleboro strain were obtained through the courtesy of Drs. H. Green and H. Valtin of Dartmouth College Medical School. Hypophysectomized Sprague-Dawley strain rats weighing about 170 g were obtained from a commercial supplier (Charles River Breeding Laboratories, North Wilmington, Mass.), and had been hypophysectomized for more than two weeks at the time of experiment. The Brattleboro rats were maintained in our laboratory for more than a week before
hypophysectomy during which time daily water intake and body weight records were kept. Two groups of animals were studied. The first group was subjected to water deprivation studies, while the second group was used only in the study on water turnover. Because of the variability in water turnover in DI rats each group was again subdivided, making a total of four groups, so that a comparable distribution with respect to water consumption was present in each subdivision.

The two groups of hypophysectomized DI numbering 11 in the first group and 13 in the second, were operated under hexobarbital anaesthesia, the pituitary glands being removed via the parapharyngeal route. Control DI rats were sham operated at the same time. Sham operation consisted of opening the neck region, inserting a tracheal cannula and preparing the floor of the skull for drilling, but did not include drilling through the skull. After surgery each rat was weighed and placed in an individual cage. Since the surgical preparation of these rats was performed in the late afternoon, 24 hour water intake was measured and recorded at 4 p.m. each day throughout the experimental period. Throughout the course of these experiments body weight was measured at least 5 times per week. Completeness of pituitary removal was based on adrenal weights measured at termination of the experiment.

In the water deprivation experiments water was removed at 3-4 p.m. At 9 a.m. the following day micturition was induced and plastic covered boards placed directly under the individual cages. Urine accumulating on this board during the two hour collection period was picked up at frequent intervals in a syringe and pooled with that obtained by again inducing micturition at the end of the period. Urine osmolalities were determined on the Fiske osmometer.

For hormone replacement 50 μg growth hormone (NIH-GH-B12 Bovine) in a 0.1 ml aqueous solution, 2 μg triiodothyronine (Smith, Kline and French, lot No. DRG-2892-69B) in divided doses of 1 μg each and 4 IU ACTH (Acthar® HP, Armour Pharmaceutical Co., Chicago, Illinois) were given daily by subcutaneous injection. Corticosterone (Mann Research Laboratories, New York, New York) and aldosterone (Ciba Pharmaceutical Co., Summit, New Jersey, lot No. M-1081) were given in the doses described as an oil suspension by the same route as was ADH (Pitressin® tannate, Parke, Davis & Co., Ann Arbor, Michigan).

RESULTS

Water intake: The effects of hypophysectomy and of hormone replacement on water intake are summarized in Figs. 1 and 2. In the first 24 hour period following hypophysectomy, water intake fell precipitously to an average of 60 ml/100 g body weight for that group. Water intake by the sham operated group also fell markedly, but only to 81 ml/100 g in this initial 24 hour period. On the second day, water intake, on a body weight basis, was actually greater in the hypophysectomized than in the sham operated group. Actual water consumption for the two groups was identical. After the third day water consumption, both absolute and per unit body weight, steadily declined until, by the eighth day it was about half that of the control group. This level was constantly found throughout the 92 day observation period, except during hormone replacement periods. Water intake by the hypophysectomized DI rats remained
Mean daily water intake of 9 sham operated (solid line) and 7 hypophysectomized (dotted line) DI rats. Rats were operated on day 0. Also shown is mean daily intake of 10 hypophysectomized Sprague-Dawley rats (dashed line) measured two weeks after hypophysectomy. ACTH, 4 IU per day, was injected daily to the hypophysectomized group as indicated by the arrows.

about twice that seen in hypophysectomized rats possessing an intact source of neurohypophysial substance (see Fig. 1).

On day 56–57 ADH was administered in two doses of 100 mU each, the first dose being given at 4 p.m. and the second at 9 a.m. the following day. This treatment reduced water intake of six hypophysectomized DI rats from an average of 61 ml/100 g to 39 ml/100 g. A marked reduction of water intake was observed in four hypophysectomized DI rats, although in one the reduction was only from 88 ml to 75 ml and in another from 50 ml to 39 ml.

Eight days after hypophysectomy hormone replacement treatment was begun. Treatment with 4 IU ACTH per rat per day for 10 days resulted in an increased water intake by the hypophysectomized DI rats to near intact DI levels after 8–9 days. This dose of ACTH, however, failed to maintain this increased intake on the 10th day of treatment. Administration of various doses or combinations of doses of corticosterone or corticosterone and aldosterone, resulted in negligible increases in water intake. Fig. 2 shows the effect of 20 mg corticosterone given daily for 6 days, with 20 mg aldosterone as well as corticosterone injected on days 5 and 6. Other doses and combinations of adrenal hormones tested without effect on water intake were: 40 mg corticosterone plus 2 mg aldosterone; 80 mg
Mean daily water intake of 9 sham operated (solid line) and 6 hypophysectomized (dotted line) DI rats. Arrows indicate days of hormone administration. B + aldosterone: first four days, corticosterone (B) only, fifth and sixth days corticosterone plus aldosterone. Triiodothyronine: T₃ administered in two daily doses of 1 µg each.

corticosterone plus 4 mg aldosterone and 160 mg corticosterone plus 8 mg aldosterone.

Also in Fig. 2 is shown the effect of triiodothyronine administration. While T₃ failed to increase water intake to control levels, this hormone unquestionably increased water intake. Administration of T₃ plus corticosterone on days 77–80 produced no significant increase in water intake. Indeed, the administration of T₃ alone on days 71–77 produced a smaller increase than did the earlier T₃ treatment.

The daily variation of water intake by both groups of rats is also indicated in Fig. 1. Although the range of intake was large, the daily variation was about the same for each individual animal in the group as for the group as a whole. In other words, the daily intake of any individual rat paralleled rather closely the mean intake of the group.

Water deprivation: The results of water deprivation for a period of 17–19 hours in treated and untreated hypophysectomized DI rats and hypophysectomized Sprague-Dawley controls are presented in Table 1. Hypophysectomized DI rats were able to concentrate urine to a surprising degree. The mean value for this group after this period of water deprivation was 648 mOsm/kg. Growth hormone, ACTH and triiodothyronine all failed to increase urine concentrating
Table 1.

Urine concentrating ability of hypophysectomized Brattleboro rats with and without hormone treatment.

<table>
<thead>
<tr>
<th>Strain of hypophysectomized rat</th>
<th>Hormone treatment</th>
<th>n</th>
<th>Uosm (mOsm/kg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean range</td>
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<tr>
<td>A. Brattleboro</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>ACTH</td>
<td>3</td>
<td>840 (765–1034)</td>
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<tr>
<td>ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>STH</td>
<td>4</td>
<td>870 (735–1050)</td>
</tr>
<tr>
<td>STH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ADH</td>
<td>4</td>
<td>790 (717–912)</td>
</tr>
<tr>
<td>ADH</td>
<td></td>
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<tr>
<td>T₃</td>
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</tr>
<tr>
<td>T₃</td>
<td></td>
<td>6</td>
<td>1490 (1120–1740)</td>
</tr>
<tr>
<td>B. Sprague-Dawley</td>
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</tbody>
</table>

Hormone treatment for 6–8 days was as follows: ACTH, 4 IU/day; STH, 50 µg/day; T₃, 2 µg/day. ADH was given in two 100 mU doses, at the start of water deprivation and one half hour before urine collection. Water deprivation period = 19 hours.

ability. ADH, given in the rather large dose of 200 mU in the 17 hour period, also failed to increase urine concentrating ability as indicated by urine osmolality in these rats. In no case, treated or untreated, was the urine concentrating ability of the Brattleboro rats seen to be as high as that of the hypophysectomized Sprague-Dawley group.

Hypophysectomy renders the Brattleboro rats extremely labile. Of the 11 hypophysectomized rats in the first group we studied only 4 survived more than two weeks. In the second group only 5 of the original thirteen survived the entire 90 day study. Death in these operated rats appears to be caused by some respiratory infection (lungs are quite haemorrhagic at autopsy), and the onset of the disease is indicated by a sudden decrease in water intake as well as obvious respiratory difficulty. The present data, of course, do not include those from animals showing signs of disease.

DISCUSSION

It was our purpose in these experiments to re-examine the relative roles of anterior pituitary hormones and ADH in diabetes insipidus using rats with a physiologically more discrete lesion than those with electrolytic median eminence lesions. If such is the case with the Brattleboro strain, then these studies have clearly corroborated the view of those who propose that anterior pituitary hormones are required for maximal water turnover in diabetes insipidus. Hypo-
physicectomy sharply reduced water intake in the Brattleboro strain (DI) rats. And while the hormones tested did not re-establish water intake at levels found in the sham operated DI rats, both ACTH and T₃, in the doses used here, did increase water intake.

The effect of ACTH, which we observed and which Gale et al. (1961) also observed, does not appear to be due solely via its effect on the adrenals. Neither corticosterone, nor aldosterone, nor any of several combinations of these two hormones had any significant effect on water intake in these experiments. In view of the finding of Chester-Jones (1957) that adrenalectomy reduces water turnover in neurohypophysectomized rats, it may be that the adrenals exert a permissive effect on water metabolism, and that other hormones, in the presence of intact adrenals, are required to augment water intake. Which hormones these may be, or how ACTH may activate them is not presently evident.

The second question to which we addressed these studies was the question of the reason for the failure of permanent diabetes insipidus to develop following hypophysectomy (in rats other than the Brattleboro strain). Two hypotheses are usually advanced, one, that anterior pituitary hormones, which augment water turnover, are absent, and the other, that ADH-like material is present in the median eminence and stalk. The considerable evidence that hormones under anterior pituitary control can augment diuresis (and water turnover) has been reviewed by Smith (1951). On the other hand, evidence that significant amounts of ADH-like material remain in the hypothalamus following hypophysectomy has been convincingly presented by van Dyke (1957). To our knowledge, however, no evidence that anterior pituitary hormones increase water turnover in hypophysectomized rats exists. Nor is there any convincing evidence that physiologically active ADH exists in the hypophysectomized rat. Although Lloyd & Peirog (1955) have reported its presence in plasma following hypophysectomy, this finding has not been verified by other bioassay methods.

While direct evidence in support of either argument could not be produced from our experimental animals, indirect evidence in support of the view that ADH-like material prevented permanent diabetes insipidus following hypophysectomy could. First, if exogenous ADH reduced water intake of the hypophysectomized Brattleboro rats to levels observed in the hypophysectomized Sprague-Dawley rats, it would indicate that this hormone was the only endocrine difference between these two strains with respect to water turnover. Our finding that two doses of ADH, as the tannate complex, reduced water intake of the hypophysectomized DI rats from a mean of 61 ml/100 g/day to 39 ml/100 g/day supports that argument. In view of the finding by Harrington & Valtin (1963) that DI rats do not initially respond normally to Pitressin® tannate, it is expected that continued treatment of our experimental animals would produce even greater reduction in daily water turnover.
While this observation suggests that the ADH presumed present in the hypophysectomized Sprague-Dawley rat is active physiologically and can therefore account for the absence of diabetes insipidus in these rats, further evidence is needed. It could be argued, for example, that in these rats ADH may not be active and that certain anterior pituitary hormones could elevate water turnover to levels greater than those observed in the hypophysectomized Brattleboro rats. The further evidence that ADH or ADH-like material is active in the hypophysectomized Sprague-Dawley rats is, we believe, provided by the water deprivation experiments. Following water deprivation hypophysectomized Sprague-Dawley rats excreted a urine nearly twice the osmolality of that excreted by hypophysectomized Brattleboro rats subjected to the same period of water deprivation. Since ADH is the only known hormone with such antidiuretic activity, it follows that there is a high probability that ADH is physiologically functional in hypophysectomized animals (other than DI).

As we have previously shown for hypophysectomized Sprague-Dawley rats (Bauman 1965), growth hormone and ACTH have no significant effect on urine concentrating ability of hypophysectomized DI rats. Triiodothyronine, which exerts an antidiuretic effect probably via ADH (Bauman et al. 1969) also has no effect in the DI rats, as would be expected. In view of its effect on water intake, and the response of unoperated DI rats to ADH (Sawyer & Valtin 1967; Vierling et al. 1967) it was expected that ADH, as Pitressin® tannate, also failed to increase the urine osmolality significantly following water deprivation in the DI rats. ADH does increase somewhat the urine concentrating ability of hypophysectomized Sprague-Dawley rats, although its effect is limited, and at present we can offer no explanation for its apparent failure to do so in the DI rats.

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