STUDIES ON THE MECHANISM OF NITROGEN STORAGE

II. EFFECTS OF ANTERIOR PITUITARY GROWTH HORMONE PREPARATIONS ON KIDNEY GLUTAMINASE

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Glutamine has been suggested as an immediate source of nitrogen for the synthesis of essential nitrogenous components of protoplasm (1-3). Utilizing anterior pituitary growth hormone preparations for the production of nitrogen storage in dogs, we were unable to obtain any evidence that glutamine functions as an interim storage form of amino nitrogen (4). Changes produced in plasma glutamine and the excretion of urinary ammonia, however, emphasized the importance of the glutamine-glutaminase system in the transport and storage of ammonia (5) and suggested that kidney glutaminase might be directly or indirectly affected by anterior pituitary growth hormone.

Studies were therefore undertaken to determine whether or not growth hormone alters the activity of kidney glutaminase in vitro, or its concentration in vivo, in normal or hypophysectomized rats.

EXPERIMENTAL

Animals—Female rats of the Sprague-Dawley strain, both normal and hypophysectomized, were used in all experiments. The hypophysectomized animals were supplied by the Hormone Assay Laboratories of Chicago and were 26 to 28 days old at the time of operation. The animals usually required from 10 to 14 days to reach a plateau in body weight after the operation. In some experiments, normal female rats 5 to 6 months old were used.

Diets—The rats were maintained on a diet consisting of 24 per cent casein, 55.5 per cent white flour, 10 per cent whole milk powder, 1.5 per cent calcium carbonate, 1 per cent sodium chloride, 8 per cent corn oil supplemented with a vitamin mixture (6), and fresh lettuce.

Growth Hormone Preparations1—Preparations assayed by means of gain in weight of normal or hypophysectomized rats were used in all experiments. In some of the studies, preparations were used which had also been shown

1 We are especially indebted to Dr. D. A. McGinty and Mr. L. W. Donaldson of Parke, Davis and Company for supplying assayed preparations.
to produce both nitrogen storage and gain in weight in adult female dogs (4, 7).

Methods—Lyophilized growth hormone preparations were taken up in 0.8 per cent saline and prepared fresh at 2 day intervals. The solutions were stored frozen, and were thawed and warmed to 37° before administration. Injections were made once a day by the intraperitoneal route.

**Table I**

Kidney Glutaminase of Normal and Hypophysectomized Rats and of Hypophysectomized Rats Treated with Anterior Pituitary Growth Hormone Preparations

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Female rats, 25-28 days old</th>
<th>Feeding method and treatment</th>
<th>Body weight</th>
<th>Glutaminase* per gm. kidney</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial gm.</td>
<td>gm.</td>
<td>Terminal gm.</td>
</tr>
<tr>
<td>6</td>
<td>Hypophysectomized</td>
<td>*Ad libitum; no treatment</td>
<td>72.0</td>
<td>73.5</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>*Ad libitum; 10 mg. Preparation Rx-099816‡</td>
<td>74.0</td>
<td>91.5</td>
<td>17.5</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>Stomach tube;§ no treatment</td>
<td>99.0</td>
<td>92.5</td>
<td>6.5</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>*Ad libitum; no treatment</td>
<td>124.0</td>
<td>150.5</td>
<td>26.5</td>
</tr>
</tbody>
</table>

* The values shown are the average of two assays. Each assay was conducted on the pooled kidneys of two to three rats. The values in parentheses are the standard deviations of the mean.
† From Fisher's (14) table of t. Values of 0.01 or less are considered highly significant.
‡ Growth hormone, Preparation Rx099816, assayed at 1361 units per gm. in the normal adult female rat. The 10 mg. dose was administered in daily 1 mg. injections over a 10 day period. A 200 mg. dose of this preparation produced nitrogen storage of 0.7 gm. per kilo of body weight in an adult female dog (4).
§ Based on the average food consumption of the hypophysectomized rats treated with growth hormone.

In general, a 5 day control period followed by a 10 to 15 day period of treatment with the growth hormone preparation constituted an experiment. At the end of the experiment the rat was fasted for 24 hours and then sacrificed by a sharp blow on the head and decapitated. The kidneys were quickly removed, decapsulated, frozen, and stored in dry ice until assayed. Glutaminase assays were conducted according to the method of Archibald (8) by micro ammonia distillation and nesslerization (9).
Results

In Table I are recorded the results of studies on kidney glutaminase of normal and hypophysectomized rats and of hypophysectomized rats treated with a growth hormone preparation. Treatment of hypophysectomized rats with growth hormone resulted in a marked increase in the concentration of kidney glutaminase, which approached the value obtained in normal rats.

Table II

Kidney Glutaminase of Hypophysectomized Rats Treated with Anterior Pituitary Growth Hormone Preparations

Each group consisted of ten hypophysectomized female rats, 26 to 28 days old.

<table>
<thead>
<tr>
<th>Feeding method and treatment</th>
<th>Body weight</th>
<th>Glutaminase* per gm. kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial gm.</td>
<td>Terminal gm.</td>
</tr>
<tr>
<td>Ad libitum; no treatment</td>
<td>76.9</td>
<td>77.0</td>
</tr>
<tr>
<td>Pair-fed with untreated animals; 0.8 mg. Preparation Rx099816†</td>
<td>76.1</td>
<td>90.0</td>
</tr>
</tbody>
</table>

* The values shown are the average of two glutaminase assays. Each assay was conducted on the pooled kidneys of five rats.
† Growth hormone, Preparation Rx099816, is the same preparation used and described in Table I.

Table III

Kidney Glutaminase of Normal Rats Treated with Anterior Pituitary Growth Hormone Preparations

Each group consisted of three normal female rats, 5 to 6 months old.

<table>
<thead>
<tr>
<th>Feeding method and treatment</th>
<th>Body weight</th>
<th>Glutaminase* per gm. kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial gm.</td>
<td>Terminal gm.</td>
</tr>
<tr>
<td>Controls fed ad libitum; no treatment</td>
<td>278.8</td>
<td>274.2</td>
</tr>
<tr>
<td>Pair-fed with controls fed ad libitum; 3.5 mg. Preparation Rx099916†</td>
<td>274.2</td>
<td>284.0</td>
</tr>
<tr>
<td>Pair-fed with controls fed ad libitum; 7 mg. Preparation Rx099916</td>
<td>265.5</td>
<td>288.5</td>
</tr>
</tbody>
</table>

* The values shown are for assays on pooled kidney preparations prepared from the number of rats shown in each group.
† Growth hormone, Preparation Rx099916, assayed at 3502 units per gm. in the normal adult female rat. In hypophysectomized rats this preparation assayed at an activity equivalent to about 91 per cent of that obtained at the 10 γ level for a 10 day injection period with pure crystalline growth hormone described by Fishman, Wilhelmi, and Russell (15). The injections in this experiment were spread over a 15 day period.
mal rats fed the same amount of food by stomach tube. Normal rats of the same age group, fed ad libitum, gave a still higher assay of kidney glutaminase. Growth hormone-treated rats had an average daily food intake during the injection period of 5.9 gm., while the untreated hypophysectomized animals consumed 6 gm. per day.

**Table IV**

**Kidney Glutaminase of Normal Rats Treated with Anterior Pituitary Growth Hormone**

Each group consisted of three normal female rats, 5 to 6 months old.

<table>
<thead>
<tr>
<th>Feeding method and treatment</th>
<th>Body weight</th>
<th>Glutaminase* per gm. kidney</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial gm.</td>
<td>Terminal gm.</td>
<td>Change gm.</td>
</tr>
<tr>
<td>Ad libitum; no treatment</td>
<td>258.3</td>
<td>256.3</td>
<td>-2.0</td>
</tr>
<tr>
<td>Pair-fed with group fed ad libitum; 20 mg. Preparation Rx099916†</td>
<td>259.8</td>
<td>297.5</td>
<td>37.7</td>
</tr>
</tbody>
</table>

* The values shown are the average of two assays. One assay was conducted on the pooled kidneys from two rats, while the other assay was conducted on the kidneys from one rat.

† Growth hormone, Preparation Rx099916, is the same as that described in Table III. The total dosage of 20 mg. was administered over a 15 day period.

**Table V**

**Effects In Vitro of Anterior Pituitary Growth Hormone Preparations on Kidney Glutaminase**

<table>
<thead>
<tr>
<th>Growth hormone* added to assay tube</th>
<th>Amide nitrogen hydrolyzed in 15 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14.3</td>
</tr>
<tr>
<td>0.1</td>
<td>14.7</td>
</tr>
<tr>
<td>1.0</td>
<td>14.7</td>
</tr>
<tr>
<td>10.0</td>
<td>14.4</td>
</tr>
<tr>
<td>100.0</td>
<td>14.0</td>
</tr>
<tr>
<td>1000.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

* Growth hormone, Preparation Rx099816, described in Table I, was used in this experiment.

In Table II are summarized the data obtained in similar experiments conducted at lower growth hormone dosage levels, with pair-fed hypophysectomized rats. It is quite apparent that, although the dosage levels employed were effective in producing body weight gains of from 17 to 19 per cent, no significant changes in kidney glutaminase were produced.

Table III shows the results of a study conducted on 5 to 6 month-old
normal female rats. Animals treated with doses of 3.5 and 7.0 mg. of growth hormone and pair-fed with controls receiving food *ad libitum* showed increases in weight but no significant change in glutaminase concentration.

In Table IV are shown data obtained at a higher level of growth hormone treatment. 5 to 6 month-old normal females, treated with a total dosage of 20 mg. of growth hormone and pair-fed with controls fed *ad libitum*, showed slightly diminished kidney glutaminase concentrations.

Table V summarizes the results of a typical study in which the effects of anterior pituitary growth hormone preparations were tested *in vitro* on kidney glutaminase preparations obtained from hypophysectomized rats. A series of assay tubes was prepared as in the usual assay procedure, and to each tube was added 1 ml. of an 0.8 per cent saline solution of the growth hormone preparation containing the desired concentration of the preparation to be tested. The tubes were incubated for 15 minutes and the amide nitrogen hydrolyzed was determined by the usual micro ammonia distillation procedure. Added concentrations of growth hormone ranging from 0.1 γ to 1 mg. produced no significant effect.

**DISCUSSION**

From the results shown in Table I, it is apparent that hypophysectomy results in a decrease in the concentration of kidney glutaminase. Treatment of the hypophysectomized animals with growth hormone preparations at the total dosage level of 10 mg. produced a highly significant increase in kidney glutaminase, yet treatment with doses approximately one-tenth as great, as shown in Table II, failed to produce any change. Since treatment of the rats at the low dosage level produced a marked gain in weight, this response appears to be more sensitive than the increase in kidney glutaminase concentration.

Of considerable interest is the apparent increase in the utilization of food consumed by hypophysectomized rats treated with growth hormone. The food intake of both treated and untreated animals was nearly identical, yet the treated animals showed an increase in body weight of approximately 24 per cent above their control level. This finding is in agreement with results reported by Lee and Schaffer (10) and Nilson et al. (11) on normal pair-fed rats and on normal rats genetically equivalent with respect to the utilization of food.

No significant changes in kidney glutaminase concentrations were produced in 5 to 6 month-old normal female rats treated at three different dosage levels with a highly active growth hormone preparation. That this was not due to rapid excretion or inactivation of the hormone by normal animals is indicated by their gain in weight.
Failure to obtain significant effects from growth hormone preparations at physiological levels in experiments both *in vivo* and *in vitro* seems to indicate that anterior pituitary growth hormone has no direct effect on kidney glutaminase. The production of a significant increase in the concentration of kidney glutaminase in hypophysectomized animals at higher dosage levels, however, suggests an indirect effect. Explanation of such an effect might be found in an increased stimulation of renal glutaminase through ketosis (12), produced in rats treated with growth hormone (13).

**SUMMARY**

1. Effects of anterior pituitary growth hormone preparations on kidney glutaminase were studied in hypophysectomized animals, in normal animals, and in experiments *in vitro*.
2. Growth hormone preparations, at dosage levels well above those required to produce the physiological response of body weight gain, produce a significant increase in the concentration of kidney glutaminase in 26 to 28 day-old hypophysectomized rats. At dosage levels just sufficient to produce body weight gain, no change occurred in kidney glutaminase.
3. Anterior pituitary growth hormone had no effect on the kidney glutaminase of normal 5 to 6 month-old female rats which had reached a weight plateau.
4. Anterior pituitary growth hormone preparations produced no effect *in vitro* on the hydrolysis of amide nitrogen from glutamine by kidney glutaminase.

**BIBLIOGRAPHY**

STUDIES ON THE MECHANISM OF NITROGEN STORAGE: II. EFFECTS OF ANTERIOR PITUITARY GROWTH HORMONE PREPARATIONS ON KIDNEY GLUTAMINASE
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