Factors Which Affect the Activity of Glutaminase I in the Guinea Pig Kidney*

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(Received for publication, October 30, 1959)

Davies and Yudkin (1) have reported that the administration of dilute hydrochloric acid to rats for three months produced a 2-fold increase in renal glutaminase activity, and Rector et al. (2) found that administration of ammonium chloride to rats for four to six days caused a 4-fold rise in the activity of this enzyme. Enzyme activity was also increased 2-fold in the guinea pig by the administration of ammonium chloride for three weeks (3).

In the present study, renal and hepatic glutaminase I activity have been measured in guinea pigs treated with the urinary acidifying agents ammonium chloride and sulfur. dl-Ethionine has been used in an attempt to inhibit the increase in renal enzyme activity. The effects of adrenal steroid administration and variation of dietary protein intake have also been examined.

EXPERIMENTAL

Methods

Adult male guinea pigs (400 to 600 g) were maintained on Purina Guinea Pig Chow Checkers, water, and daily supplements of fresh cabbage.

The substances under investigation were administered twice daily at approximately 9:00 a.m. and 5:00 p.m. Ammonium chloride and sucrose were given in solution, and enzymatic casein hydrolysate and sulfur were suspended in water and administered by stomach tube. Hydrocortisone (Merck Sharp and Dohme) and deoxycorticosterone acetate (Ciba Pharmaceutical Products, Inc.) were suspended in 0.9% sodium chloride and injected intramuscularly. dl-Ethionine and dl-methionine (Nutritional Biochemicals Corporation) were dissolved in 0.9% sodium chloride solution and administered by intraperitoneal injection.

The animals were placed in rodent metabolism cages 2 hours before being killed, and the ammonia excreted in response to intragastric administration of 10 mmoles of NH₄Cl per kg of body weight was determined. Urinary ammonia concentration was measured by modified Seligson (4) technique.

Enzyme Assay—The animals were killed by a blow on the head. The kidneys were immediately removed, decapsulated and made into a 5% homogenate in cold 0.9% NaCl solution according to the technique of Potter and Elvehjem (5). The homogenate was then assayed for glutaminase I activity. This was measured by subtracting the activity of the inherent glutaminase from the total glutaminase activity, the former being measured in the absence of added phosphate and pyruvate ions (6). Each assay was performed (in duplicate in the absence of pyruvate) with and without added phosphate.

The following incubation mixture was found to be optimal for the assay of glutaminase I activity for both kidney and liver: 0.2 ml of 5% tissue homogenate, 0.1 ml of 0.1 M L-glutamine, 0.1 ml of 0.5 M Na₂HPO₄-NaH₂PO₄ (pH 7.5), 0.5 ml of 0.05 M Tris (pH 7.5), and 0.1 ml of 0.9% NaCl. Incubation was carried out in 25-ml bottles under air at 37 to 38° for 20 minutes.

At the end of the incubation period, the bottles were sealed with a sleeve-type rubber stopper holding a ground-glass cork moistened with 10 N H₂SO₄, and 1.0 ml of 20% Na₂O₂ solution was injected through the rubber stopper. The bottles were then rotated horizontally on a motor-driven wheel for 30 minutes. The glass rod was removed and rinsed with 3.0 ml of water into a separate vessel, and 2.0 ml of Koch-McMeekin Nessler's reagent were added and maximal color was allowed to develop. This was read in a Klett photometer at 420 μm wavelength.

Enzyme activity is expressed as mmoles of NH₃ liberated per 100 mg of renal deoxyribonucleic acid per minute of incubation. Deoxyribonucleic acid was measured in an aliquot of the same homogenate by the method of Schneider (7), and its concentration was determined by the color reaction of Dische (8) for deoxyribose.

RESULTS

As shown in Table I, administration of 20 ml 1 M NH₄Cl per kg per day for 2 days caused renal glutaminase I activity to increase approximately 60% above control values. In 4 days, enzyme activity was approximately 80% above zero day control levels and remained at this level during 4 more days of treatment. Administration of a similar volume of water alone produced no significant rise in renal glutaminase I activity.

Intragastric administration of 10 mmoles of NH₄Cl per kg per day or less produced a temporary (at 2 days) rise in renal glutaminase I activity which was less than that produced by 20 mmoles. Doses of 30 and 40 mmoles per kg per day caused hemorrhagic pulmonary edema and could not be studied.

As shown in Table II, intragastric administration of 20 mmoles of NH₄Cl or sulfur per kg per day for 4 days produced a significant rise in renal glutaminase I activity. However, enzyme activity in the liver was not elevated; it was slightly, but not significantly, lowered. Intragastric administration of 20 mmoles
of sulfur per kg has been shown (unpublished observation) to produce a significant rise in the renal excretion of sulfate, hydrogen, and ammonium ions in the guinea pig.

The intramuscular administration of either hydrocortisone or deoxycorticosterone acetate (40 mg per kg per day) for 2 days had no significant effect on either basal renal glutaminase I activity or the response of the enzyme to the repeated administration of ammonium chloride (20 mmoles per kg per day). The mean and standard error (μmoles NH₃ per 100 mg of DNA per minute) of these groups were: control, 26 ± 2; hydrocortisone, 37 ± 4; and ammonium chloride plus deoxycorticosterone acetate, 37 ± 4.

As shown in Table III, intragastric administration of either 2.5 or 5 g of casein hydrolysate per day produced a significant rise in renal glutaminase I activity in 2 days. The decreased food consumption by the animals receiving 5 g of casein hydrolysate resulted in a daily protein intake by this group which was only slightly higher than that of the group which received 2.5 g of casein hydrolysate. The increase in enzyme activity in these animals was not accompanied by any significant change in urinary pH.

As shown in Table IV, intraperitoneal injection of 2 mmoles DL-ethionine per kg per day had no significant effect on basal renal glutaminase I activity. Injection of this same dose of DL-ethionine and DL-ethionine plus ammonium chloride resulted in a daily protein intake by this group which was only slightly higher than that of the group which received 2.5 g of casein hydrolysate. The increase in enzyme activity in these animals was not accompanied by any significant change in urinary pH.

### Table I

**Effect of repeated administration of ammonium chloride on renal glutaminase I activity**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutaminase I activity (μmoles NH₃ per 100 mg DNA per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control (20 ml H₂O per kg per day)</td>
<td>34±4*</td>
</tr>
<tr>
<td>NH₄Cl (20 mmoles per kg per day)</td>
<td>55±3†</td>
</tr>
</tbody>
</table>

Values are Mean ± S. E.

* Five animals in zero day control group, four animals in all other groups.
† Significantly different from zero day controls (P < 0.05).

### Table II

**Effect of repeated (4 days) ammonium chloride or sulfur administration on renal and hepatic glutaminase I activity**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Renal glutaminase I activity</th>
<th>Hepatic glutaminase I activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μmoles NH₃ per 100 mg DNA per min</td>
<td>μmoles NH₃ per 100 mg DNA per min</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>20 ± 1</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>NH₄Cl (20 mmoles per kg per day)</td>
<td>4</td>
<td>34 ± 2*</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>Sulfur (20 mmoles per kg per day)</td>
<td>4</td>
<td>32 ± 5†</td>
<td>62 ± 11</td>
</tr>
</tbody>
</table>

Values are Mean ± S. E.

* Significantly different from control group (P < 0.01).
† Significantly different from control group (P < 0.05).

### Table III

**Effect of protein intake on renal glutaminase I activity**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Daily intake of chow pellets</th>
<th>Daily protein intake</th>
<th>Glutaminase I activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>μmoles NH₃ per 100 mg DNA per min</td>
</tr>
<tr>
<td>Controls</td>
<td>11</td>
<td>28 ± 3</td>
<td>6.7</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Sucrose (2.5 g per animal per day for 2 days)</td>
<td>4</td>
<td>32 ± 2</td>
<td>7.7</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Sucrose (5 g per animal per day for 2 days)</td>
<td>4</td>
<td>22 ± 0</td>
<td>5.3</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Casein hydrolysate (2.5 g per animal per day for 2 days)</td>
<td>4</td>
<td>28 ± 2</td>
<td>9.2</td>
<td>39 ± 2*</td>
</tr>
<tr>
<td>Casein hydrolysate (5 g per animal per day for 2 days)</td>
<td>4</td>
<td>20 ± 2</td>
<td>9.8</td>
<td>41 ± 5*</td>
</tr>
</tbody>
</table>

Values are Mean ± S. E.

* Significantly different from controls (P < 0.05).

### Table IV

**Effect of injection of DL-ethionine and DL-ethionine plus DL-Methionine on the response of renal glutaminase I activity to repeated (2 days) ammonium chloride administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>Glutaminase I activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (20 ml 0.9% NaCl per kg per day intraperitoneally)</td>
<td>23</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>DL-Ethionine (2 mmoles per kg per day intraperitoneally)</td>
<td>4</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>NH₄Cl (20 mmoles per kg per day) + 0.9% NaCl (20 ml per kg per day intraperitoneally)</td>
<td>10</td>
<td>51 ± 2*</td>
</tr>
<tr>
<td>NH₄Cl (20 mmoles per kg per day) + DL-ethionine (2 mmoles per kg per day intraperitoneally)</td>
<td>18</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>NH₄Cl (20 mmoles per kg per day) + DL-ethionine (2 mmoles per kg per day intraperitoneally) + DL-methionine (2 mmoles per kg per day intraperitoneally)</td>
<td>6</td>
<td>52 ± 4*</td>
</tr>
</tbody>
</table>

Values are Mean ± S. E.

* Significantly different from control group (P < 0.001).

The intraperitoneal administration of either 2.5 or 5 g of casein hydrolysate per day produced a significant rise in renal glutaminase I activity. Injection of this same dose of DL-ethionine completely inhibited the rise in enzyme activity which follows repeated (2 days) ammonium chloride administration. The urinary acidity of animals receiving ethionine or ethionine plus ammonium chloride was slightly greater than that of the animals receiving ammonium chloride alone. The average urinary pH of the three groups was 6.3, 5.8, and 6.6 respectively. Administration of DL-methionine (2 mmoles per kg per day) completely prevented the inhibition by DL-ethionine of the renal glutaminase I response to repeated ammonium chloride administration (Table IV). The average urinary pH of the last group was 5.5.

Since a significant relation has been found between renal glutaminase I activity and ammonia excretion in the rat (2),...
Our data for the guinea pig have been plotted as a function of urinary ammonia excretion. As shown in Fig. 1, a positive correlation ($r = +0.67$) was found between mean renal glutaminase I activity and mean ammonia excretion.

**Discussion**

Renal glutaminase I activity was significantly elevated after 2 days of ammonium chloride administration, and maximal enzyme activity was observed by the fourth day of treatment. Thus, the “adaptation” period of this enzyme is between that of enzymes which are increased within a period of hours (9), and those which require more than a week (10). A similar time course has been observed for the “adaptation” of this enzyme in the rat (2).

Although repeated administration of either ammonium chloride or sulfur produced an increase in renal enzyme activity, enzyme activity in the liver was not elevated. The data thus support the positive correlation found between renal glutaminase I activity and urinary ammonia excretion. The reverse situation has been reported for the “adaptation” of xanthine oxidase activity in the mouse. Dietrich (10) found that repeated administration of xanthine to mice produced a rise in hepatic xanthine oxidase activity, but not in renal xanthine oxidase activity.

The administration of either deoxycorticosterone acetate or hydrocortisone had no effect on basal renal glutaminase I activity or the response of this enzyme to repeated ammonium chloride administration. These observations are in agreement with the results obtained by Iacobellis et al. (11) in the rat. Furthermore, Wilson and Seldin (12) showed that adrenalectomy in rats had no effect on the capacity of kidney to respond to repeated ammonium chloride administration with respect to the elevation of glutaminase I activity. Thus, the adrenal steroids do not seem to play a major role in the increase of activity of this enzyme.

It has been shown that enzyme activity may be increased by chemical compounds which are not substrates of the enzyme (13). The rise in renal glutaminase I activity after repeated administration of ammonium chloride does not seem to be substrate-induced, but is rather a nonspecific effect of such substances as urinary acidifying agents.

The increase in enzyme activity which follows the administration of a chemical compound may be due to activation of preexisting, inactive enzyme or to the synthesis of new enzyme. The amino acid antagonist L-ethionine has been used in differentiating between these two phenomena. Ethionine inhibits normal protein synthesis by preventing the incorporation of methionine (14) and other amino acids (15) into protein. Ethionine is itself incorporated into proteins (16). In previous studies with other enzyme systems (10, 13, 17, 18), ethionine was found to inhibit the increase in enzyme activity which resulted from the administration of the inducer and, in each case, injection of an equimolar amount of methionine eliminated the effect of ethionine. DL-Ethionine was used in all these experiments. It is assumed that the D isomer is as effective as the L isomer, since Wachstein and Meisel (19) have shown that both isomers are equally potent in producing tissue damage. In the present study, it was found that intraperitoneal injection of ethionine completely inhibited the rise in renal glutaminase I activity produced by the repeated administration of ammonium chloride. Furthermore, injection of an equimolar quantity of methionine eliminated the effect of ethionine. These results are consistent with the hypothesis that the increase in renal glutaminase I activity after repeated ammonium chloride administration is due to the formation of new enzyme. However, it is possible that ethionine had an effect on some system which regulates glutaminase I activity. For example, the synthesis of a renal glutaminase I activator may have been inhibited. Thus, although the data obtained with the use of ethionine and methionine suggest that new enzyme synthesis is responsible for the increase in renal glutaminase I activity, this point is not conclusively established.

Rector et al. (2) have shown that repeated administration of ammonium chloride to rats produces a parallel rise in both renal glutaminase I activity and ammonia excretion. These results suggest that the production of urinary ammonia is, at least in part, controlled by renal glutaminase activity. The positive correlation found between renal glutaminase I activity and ammonia excretion in the guinea pig supports this hypothesis.

**Summary**

1. Intragastric administration of 20 mmoles of NH$_4$Cl or sulfur per kg per day to guinea pigs increased renal glutaminase I activity 80% above control values in 4 days. Hepatic glutaminase I activity was not elevated by either treatment.
2. Intramuscular administration of neither deoxycorticosterone acetate nor hydrocortisone (40 mg per kg per day for 2 days) had any effect on renal glutaminase I activity. Furthermore, deoxycorticosterone acetate administered simultaneously with NH$_4$Cl did not affect the rise in enzyme activity produced by NH$_4$Cl administration alone. Casein hydrolysate (2.5 g or 5 g per day), orally, for 2 days increased enzyme activity 50% above control values.
3. The rise in renal glutaminase I activity produced by intragastric administration of 20 mmole NH$_4$Cl per kg per day for 2 days was completely inhibited by the simultaneous intraperitoneal administration of D-ethionine (2 mmole per kg per day). Administration of D-methionine with D-ethionine (3 mmole per kg per day of each) and 20 mmole NH$_4$Cl per kg per day resulted in an increase in enzyme activity that was different from that produced by NH$_4$Cl alone.
4. A positive correlation was found between renal glutaminase I activity and ammonia excretion.
REFERENCES

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