EFFECT OF HYDROCORTISONE ON AMMONIA INTOXICATION IN THE ADRENALECTOMIZED RAT

By Woon Ki Paik, Hyang Woo Lee and Sangduk Kim

ABSTRACT

Adrenalectomy induces a hypersensitivity in the rat to ammonia intoxication. Daily injection of hydrocortisone hydrochloride to adrenalectomized rats restored normal sensitivity to ammonia intoxication, with concomitant restoration of liver urea-synthesizing capacity to the normal value. When injected with a large dose of ammonium acetate, hydrocortisone-treated adrenalectomized rats were able to reduce the plasma ammonia concentration much more rapidly than the adrenalectomized control rats. However, neither the increase in liver urea synthesis nor the more rapid decrease in the plasma ammonia concentration were sufficient to explain the protective action of hydrocortisone against ammonia intoxication.

Gullino et al. (1955) first observed that the amino acids participating in the ornithine-urea cycle were highly effective in the detoxication of ammonia when injected into rats. Subsequently, γ-aminobutyric acid (Manning et al. 1964) and N-acetyl and N-carbamoyl derivatives of glutamic and aspartic acids (Chiosa et al. 1965; Kim 1960) were also found to be effective in the detoxication of ammonia in both rats and mice. Recently, we have reported that N-carbamoyl-L-glutamate in combination with L-arginine is far more effective in treating ammonia intoxication. In our previous report on urea synthesis in liver slices (Paik & Kim 1974), there is an unintentional error in Table 4. The values for urea synthesized should be reduced 20-fold and expressed in μmoles/h/g tissue.
effective in protecting rats from ammonia intoxication than when administered alone (Kim et al. 1972). On the other hand, the protective action of N-carbamoyl-L-glutamate plus L-arginine was significantly reduced in adrenalectomized rats (Paik & Kim 1974). Thus, with adrenalectomized rats, N-carbamoyl-L-glutamate plus L-arginine protected only 50% of the animals at a concentration three times that which afforded normal rats with 100% protection. In the present communication, we report that hydrocortisone alone can restore the effectiveness of N-carbamoyl-L-glutamate plus L-arginine in protecting adrenalectomized rats from ammonia intoxication.

MATERIALS AND METHODS

CFN rats (Wistar strain from Carworth Farms, Rockland, N. Y.), weighing 150-170 g, were fed ad libitum on a complete ration of Rockland Mouse diet, and were starved for a period of 24 h (with 1% saline solution ad libitum) prior to the injection experiments. N-Carbamoyl-L-glutamate and L-arginine were obtained from ICN Nutritional Biochemical Corp. (Cleveland, Ohio), hydrocortisone and aldosterone from Sigma Chemical Co. (St. Louis, Mo.) and ammonium acetate from Fisher Chemical Co. N-Carbamoyl-L-glutamate was recrystallized from water before use.

Adrenalectomy was performed at Carworth Farms, and the operated rats were shipped to us after a few days. Sodium [14C]bicarbonate (NaH14CO3; 4.7 mCi/mmole) was purchased from New England Nuclear Corp. (Boston, Mass.).

Detailed experimental procedures for ammonia intoxication and the protection by N-carbamoyl-L-glutamate plus L-arginine have been described previously (Kim et al. 1972; Paik & Kim 1974). Briefly, rats were starved for 24 h prior to experiments, and then weighed. All the solutions were prepared according to the body weight of individual rats so that a total of 2.5 ml of solution was administered to each rat. As the protective agent, a water solution of 2.5 ml containing 2 mmoles each of N-carbamoyl-L-glutamate and L-arginine per kg of body weight was injected intraperitoneally. One hour later, 10.8 mmoles of ammonium acetate per kg of body weight was injected ip. 5.4 mmoles were used to determine plasma ammonia concentration (Fig. 1). Unprotected rat dies generally within 30 min after the ammonia injection. If death did not occur within 30 min the animal survived.

Adrenalectomized rats were maintained on 1% saline solution and a complete ration of Rockland Mouse diet ad libitum and were injected daily for 7 days with various hormones dissolved in 0.2 ml of corn oil. Detailed experimental procedures for studying urea synthesis in liver slices have been described previously (Paik & Kim 1974): The main feature of the experimental procedures involve incorporation of NaH14CO3 into urea in the liver slices, and subsequent determination of 14CO2 liberated by the action of urease on the urea synthesized. Liver slices approximately 0.44 mm in thickness and weighing about 30 mg were prepared in the cold room using a Brinkman tissue slicer. The slices were placed in a 25 ml Erlenmeyer flask containing 4.0 ml of Eagle's medium, 0.1 ml of 1.0 M phosphate buffer at pH 7.2, 0.20 ml of 0.1 M L-ornithine (pH of water solution was adjusted to 7.0) 0.15 ml of 0.05 M NaH14CO3 (4.5 x 10^6 cpm) and 0.1 ml of NH4Cl. The reaction was carried out at 37°C for 1 h in a Dubnoff shaking incubator. The reaction was stopped by the addition of 1.0 ml of 10% trichloracetic acid and centrifuged at 39000 g for
10 min. The supernatant was then aerated with CO₂ for 15 min to remove unreacted \(^{14}\)CO₂ and the samples were adjusted to a pH 7.0. A 1.0 ml aliquot of each sample was placed in a 25 ml Erlenmeyer flask with a tightly fitting rubber stopper and center well. To the center well was added 0.25 ml of 1 N NaOH and a small strip of filter paper for the absorption of \(^{14}\)CO₂ liberated from urea-\(^{14}\)C after treatment with urease. To each flask 3–5 units of urease (product of Sigma Chemical Co.) was added to convert urea to ammonia and the reaction was allowed to proceed for 1 h at 37°C in the shaking incubator. The reaction was stopped by the addition of 0.25 ml of 1 N HCl to each flask. The incubation was allowed to continue for another 30 min at 37°C to release any \(^{14}\)CO₂ dissolved in the medium. The contents of the center well were then transferred to scintillation vials containing 10 ml of scintillation fluid.

Plasma ammonia concentration after injection of ammonium acetate (Fig. 1) was determined as follows (Natelson 1961); after injection of 5.4 mmoles of ammonium acetate per kg of body weight the rat was anaesthetized at various time periods and blood was drawn directly from the exposed heart with a heparinized syringe. The blood was centrifuged in a table-top clinical centrifuge and the ammonia concentration in the plasma was determined by the colorimetric method.

RESULTS AND DISCUSSION

Three groups of adrenalectomized rats were treated with one daily injection of either aldosterone, hydrocortisone or corn oil for 7 days prior to the administration of N-carbamoyl-L-glutamate plus L-arginine followed by an in-

<table>
<thead>
<tr>
<th>Hormone treated</th>
<th>No. of rats used</th>
<th>No. of rats survived</th>
<th>Per cent survival</th>
<th>P value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>6</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>19</td>
<td>5</td>
<td>26.3</td>
<td>0.94</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>20</td>
<td>15</td>
<td>75.0</td>
<td>0.003</td>
</tr>
</tbody>
</table>

All rats, weighing 150–170 g, were adrenalectomized and treated with daily injection of aldosterone (1.0 mg in 0.2 ml of corn oil; 6.3 mg/kg body weight/day) or hydrocortisone (5.0 mg in 0.2 ml of corn oil; 31.5 mg/kg body weight/day) ip for 7 days before ammonia intoxication experiment. The control animals were injected daily with 0.2 ml of corn oil. Two mmoles each of N-carbamoyl-L-glutamate and L-arginine per kg of body weight of rat were injected ip 1 h prior to the injection of 10.8 mmoles of ammonium acetate per kg of body weight.

\(^a\) Calculated relative to the control. Chi square values were calculated by two way classification as described by Sachs (1971) and corresponding P values were obtained from a Chi square table for one degree of freedom.
jection of a lethal dose of ammonium acetate. As seen in Table 1, aldosterone treatment had no effect on the protective action. Hydrocortisone, on the other hand, increased the protective action of N-carbamoyl-L-glutamate plus L-arginine. It should be pointed out that the concentration of N-carbamoyl-L-glutamate plus L-arginine used in Table 1 (2 mmoles each per kg of body weight afforded complete protection of normal rats from ammonia intoxication (Kim et al. 1972).

As shown in Table 2, the adrenalectomized control (not treated with hormone subsequent to adrenalectomy) and aldosterone-treated rats have a significantly lowered capability of urea synthesis in the liver slices. On the other hand, hydrocortisone-treated adrenalectomized rat liver is shown to have a normal level of urea synthesizing capacity.

In order to further study a possible relationship existing between the increased sensitivity of the adrenalectomized rats to ammonia intoxication and urea synthesizing capacity of the liver, plasma ammonia concentration was determined at various time periods after injection of half the amount of lethal dose of ammonium acetate (5.4 mmoles per kg of body weight). This concentration rather than the lethal dose was chosen because it was impossible to accurately assess the ammonia concentration with the lethal dose which caused the adrenalectomized control rats to die within a few minutes. As shown in Fig. 1, the ammonia level in the normal corn-oil-treated animals showed an only slight increase. On the other hand, the plasma ammonia concentration

\[ \text{Table 2.} \]

Effect of various hormone-treatment on the urea synthesis of adrenalectomized rat liver.

<table>
<thead>
<tr>
<th>Hormone injected to the adrenalectomized rat</th>
<th>Urea synthesis*</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μMoles/h/g tissue</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.6 ± 1.7</td>
<td>0.54</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>21.3 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>48.5 ± 3.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Normal***</td>
<td>39.1 ± 7.3</td>
<td></td>
</tr>
</tbody>
</table>

* Average of three determinations with duplicate runs.

** Calculated relative to the control by using Student's t-test.

*** Non-adrenalectomized and not treated with hormone.

All the conditions for hormone-treatment are the same as in Table 1.
Effect of hydrocortisone-treatment on the plasma ammonia concentration of adrenalectomized rats. Each point is the average values from 3 animals with standard deviation expressed by vertical lines. Ad. x represents adrenalectomized rats, and the value on the ordinate represents the ammonia concentration in animals which had not previously been injected with ammonium acetate. More detailed experimental procedures for hormone-treatment and plasma ammonia determination are described under Methods.

in the adrenalectomized control rats increased to a high level of 4.8 mmol/l followed by a gradual decrease. A similar high ammonia level was reached, although at a faster rate, in the plasma of hydrocortisone-treated adrenalectomized rats. Subsequently, the ammonia concentration decreased rapidly to low levels within approximately 20 min in contrast to the gradual decrease seen in the adrenalectomized controls.

It has been previously reported from this laboratory that N-carbamoyl-L-glutamate in combination with L-arginine is much more effective in protecting rats from ammonia intoxication than when administered alone (Kim et al. 1972; Paik & Kim 1974). The rationale behind this effect was based on the fact that N-carbamoyl-L-glutamate, which is an analogue of naturally occurring activator N-acetyl-L-glutamate, stimulates carbamoyl phosphate synthetase [EC 2.7.2.5 (NH₄)] thereby enhancing the removal of free ammonia, and that L-arginine accelerates the synthesis of endogenous N-acetyl-L-glutamate
as well as supplying more L-ornithine to accelerate the urea cycle (Kim et al. 1972).

The results presented in the present communication however, do not seem to support the contention that the protective of N-carbamoyl-L-glutamate plus L-arginine from ammonia intoxication in rats is due to the accelerated removal of injected ammonium acetate, or that restoration of the protective action of this amino acid mixture from ammonia intoxication by hydrocortisone in adrenalectomized rats is brought about by the restoration of urea cycle activity: As shown in Table 2, liver slices of hydrocortisone-treated adrenalectomized rats can synthesize urea at the rate of 48.5 μmoles/h/g liver. This corresponds to 4.85 μmoles of urea synthesized per min per animal (6 g liver per 150 g rat). This rate of removal of ammonium acetate constitutes less than 0.6 %/h per min per rat of the injected amount of ammonium acetate. Since the lethal effect occurs within a few minutes after the injection of ammonium acetate, the protective action of N-carbamoyl-L-glutamate plus L-arginine from ammonia intoxication can not be explained on the basis of urea synthesis. Furthermore, the plasma ammonia concentration of the hydrocortisone-treated rat is as high as that of the corn-oil treated one (Fig. 1), although the urea-synthesizing capacity of the corn-oil treated rat is very much impaired (Table 2). Thus, the changes in the urea cycle activity after hydrocortisone-treatment might have been a secondary accompanying effect of some other primary action of the hormone.

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REFERENCES


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