Effect of Pyridoxine Administration on the Induction of Cytosolic Aspartate Aminotransferase in the Liver of Rats Treated with Hydrocortisone

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Summary The effects of pyridoxine and hydrocortisone administrations on the rate of synthesis of cytosolic aspartate aminotransferase in adrenalectomized pyridoxine-deficient rat livers were examined. Induction of cytosolic aspartate aminotransferase by hydrocortisone was observed 6 to 10 h after the injection. Treatment with pyridoxine daily for 6 days but not for the 2 days, resulted in suppression of the enzyme synthesis. Similar suppression of enzyme synthesis was observed in rats without hydrocortisone treatment. The activity of tryptophan oxygenase, which is known to be induced by glucocorticoid and does not contain pyridoxal phosphates, was higher in the livers of pyridoxine-deficient rats than in that of controls. The possible effects of pyridoxal phosphate on the action of glucocorticoid are discussed based on the results.

Key Words aspartate aminotransferase, pyridoxine deficiency, pyridoxine administration, effect of glucocorticoid

The cellular level of pyridoxal phosphate is known to influence both the activities and the contents of pyridoxal phosphate dependent enzymes (1-3). Various degrees of decrease in these enzyme activities in pyridoxine deficiency have been reported (4-6). Two isozymes of the pyridoxal phosphate-dependent enzyme aspartate aminotransferase [EC 2.6.1.1] are present in cells, one in the cytosol and the other in the mitochondria. These two isozymes are structurally and immunologically distinct, but do not show tissue specificity within a single species (7, 8). Recently, we found that in pyridoxine-deficient rats, the activities of both isozymes in the liver had decreased, but their antigenic activities remained normal (9). We also observed an increased rate of synthesis of the cytosolic isozyme in pyridoxine-deficient rat livers (10).

Pyridoxal phosphate is known to modulate the steroid hormonal system through its binding to the receptor. Disorbo et al. reported that pyridoxine...
deficiency resulted in an increased number of activatable hepatic glucocorticoid-receptor complexes in nuclei, in vitro (11). They also reported that in a cell culture system change in the intracellular level of pyridoxal phosphate affected the induction of hepatic tyrosine aminotransferase [EC 2.6.1.5] by glucocorticoid (12).

The cytosolic aspartate aminotransferase in rat liver is known to be induced by gluconeogenic conditions such as a high protein diet and administration of hydrocortisone (13). Thus, the increase in the rate of synthesis of the cytosolic aspartate aminotransferase in pyridoxine-deficient rat liver was thought to be related to an increase in the functional state of the glucocorticoid hormone.

In the present study, we examined the effects of the administration of pyridoxine and hydrocortisone on the synthesis of cytosolic aspartate aminotransferase in the liver of adrenalectomized rats.

**MATERIALS AND METHODS**

*Animals.* Male Wistar strain rats, weighing about 50 g, were administered a diet containing 70% casein (not vitamin-free) and B6-free vitamin mixture ad libitum. The composition of the diet has been described previously (9). After 2 weeks, the rats were adrenalectomized, and administration of the diet was continued for 5 days with 0.9% NaCl as drinking water to allow depletion of endogenous glucocorticoid. The rats were then divided into groups and were treated with drugs intraperitoneally as follows: Control, untreated but kept for the same period as experimental groups; group P, treated with pyridoxine (10 mg/100 g body weight) daily for 2 or 6 days; group H, treated with sodium hydrocortisone phosphate (12.5 mg/100 g body weight) 6 or 10 h before sacrifice; group PH, treated with both pyridoxine and hydrocortisone phosphate, as were groups P and H.

All rats after being starved for 16 h, were injected with [3H]leucine (80 µCi/100 g body weight) 4 h later the animals were sacrificed. In other experiments, male rats weighing about 50 g were given free access to a 70% casein (vitamin-free casein) diet, with or without added pyridoxine for 4 weeks.

*Antiserum.* Anti-aspartate aminotransferase of liver cytosol was prepared as described previously (9).

*Incorporation of [3H]leucine into total protein and cytosolic aspartate aminotransferase in rat liver.* Rat liver was homogenized in a Polytron (Kinematica), with 4 vol. of 1.19% KCl–10 mM potassium phosphate (pH 7.4)–0.1 mM pyridoxal phosphate. Thus, the 10,000 × g supernatant was obtained. Some of the supernatant was used for the determination of total protein synthesis. The rest was heated to 65°C, to inactivate mitochondrial aspartate aminotransferase, and then centrifuged at 100,000 × g for 1 h. The resulting supernatant was used for determination of the synthesis of cytosolic aspartate aminotransferase. For measurement of the incorporation of radioactivity into total protein, the protein was precipitated with cold 10% trichloroacetic acid, suspended in 5% trichloroacetic acid–70% ethanol solution, heated at 60°C for 15 min, and centrifuged at 1,500 × g for 20 min. The

*J. Nutr Sci. Vitaminol.*
resulting precipitate was washed twice with the same solution, solubilized in 1 ml of formic acid, and then mixed with 10 ml of scintillation fluid (Aquasol: NEN). Its radioactivity was counted in an Aloka LSC-703 liquid scintillation counter. For determination of the synthesis of cytosolic aspartate aminotransferase, an aliquot of the 100,000 × g supernatant was incubated with excess specific antibodies (anti-cytosolic aspartate aminotransferase) for 30 min at 37°C, and kept overnight at 4°C. Immunoprecipitates were collected by centrifugation at 1,500 × g for 20 min, and washed three times with cold 0.9% NaCl. Then they were solubilized in 1 ml of formic acid, where radioactivity was determined as described above.

Analytical methods. Aspartate aminotransferase activity was determined by the method of Karmen (14). For determination of antigenic activity, a constant amount of liver supernatant was incubated with different amounts of anti-cytosolic aspartate aminotransferase and residual enzyme activity in the supernatant was determined. One unit of antigen activity was defined as the amount of immunoreactive substance, equivalent to the amount of antibody precipitating 1 U of enzyme activity of rat liver, which showed the highest enzyme activity of all groups in each experiment. Tryptophan oxygenase activity [EC 1.13.11.11] in the liver of rats on a diet, with or without pyridoxine, was determined by the method of Badawy and Evans (15). The pyridoxal and pyridoxal phosphate contents of rat liver were determined by the method of Chauhan and Dakshinamurti (16). For determination of the serum corticosterone level, blood obtained by decapitation of rats was used. Extracts of serum with dichloromethane were washed with a 0.1 N NaOH–10% Na2SO4 solution, and then treated with a mixture of H2SO4 and ethanol. The resulting fluorescence was measured in a fluorospectrophotometer (Hitachi, MPF-2A) by the method of Usui et al. (17). Protein was determined by the biuret reaction (18).

Chemicals. Dietary materials and malate dehydrogenase were obtained from Oriental Yeast Kogyo Co. [4,5-3H]l-Leucine(45 μCi/mmol) was from RCC Amersham. Pyridoxal phosphate, pyridoxine-hydrochloride, NADH, aspartate and α-ketoglutarate were obtained from Kyowa Hakko Kogyo. Corticosterone was from Sigma Chemicals Co. Sodium hydrocortisone phosphate (Medecort Injection) was from Kaken Seiyaku Co. and other chemicals were from Nakarai or Wako Pure Chemicals Co.

RESULTS

Animal conditions

The body, liver weights, and liver protein levels of rats in different groups were compared (Table 1). The average body weight of rats treated with pyridoxine daily for 6 days, was higher than those of rats treated with hydrocortisone alone and control rats, whereas the body weight of rats treated with pyridoxine for 2 days was very similar to that of untreated rats.
Table 1. Growth, liver weights and liver protein contents (10,000 × g supernatant) of rats treated with pyridoxine and hydrocortisone. Adrenalectomized rats were used. In experiment I rats received pyridoxine daily for 2 days, while in other experiments they received it daily for 6 days. Other experimental conditions were as described in the text.

| Experiment | Control | P | H(6 h) | PH(6 h) | P | H(10 h) | PH(10 h) |
|------------|---------|---|--------|---------|---|---------|----------|
| Body weight (g) | 132 ± 7 | 130 ± 0 | 119 ± 10 | 123 ± 9 | 114 ± 12 | 97 ± 8 | 132 ± 6 | 108 ± 16 | 152 ± 19 |
| Liver weight (g) | 4.8 ± 0.5 | 4.8 ± 0.1 | 4.8 ± 0.8 | 5.0 ± 0.3 | 5.0 ± 0.3 | 4.5 ± 0.1 | 6.0 ± 0.5 | 4.6 ± 1.2 | 6.5 ± 0.8 |
| % of body wt. | 3.6 | 3.6 | 4.0 | 4.1 | 4.4 | 4.6 | 4.5 | 4.3 | 4.3 |
| Protein content (mg/g liver) | 157 ± 7 | 154 ± 5 | 152 ± 8 | 152 ± 9 | 172 ± 10 | 165 ± 8 | 170 ± 12 | 160 ± 7 | 167 ± 9 |

Values are means ± SD for 3–4 rats.

**Enzyme and antigenic activities in rat liver**

The cytosolic levels of aspartate aminotransferase and its antigenic activity in the liver of rats in the different groups are shown in Table 2. The enzyme activities were higher in all groups treated with pyridoxine, whereas the antigenic activities in the livers of rats after the various treatments were similar with those in the control group (experiments II and III).

**Syntheses of total protein and cytosolic aspartate aminotransferase in rat liver**

The incorporations of [3H]L-leucine into whole soluble protein and cytosolic aspartate aminotransferase in the liver were measured (Table 3). Very similar rates in the incorporation of leucine were observed in all groups in experiments I and III, but in experiment II the rates of leucine incorporation in groups treated with both pyridoxine and hydrocortisone were higher than those in other groups. This was probably due to a decrease in the leucine level in the liver, which resulted from improvement of amino acid metabolism by pyridoxine injection. We previously reported that amino acid contents in the liver from pyridoxine-deficient rats changed in various degrees, owing to impairment of amino acids metabolism, while the rate of protein synthesis in the deficient rat liver was maintained at normal
Table 2. Cytosolic aspartate aminotransferase and its antigenic activities in the livers of pyridoxine-deficient rats treated with pyridoxine and hydrocortisone. The same rats were used as for Table 1.

| Experiment  | Control | Enzyme activity (units/g liver) | Antigenic activity (units/g liver) |
|-------------|---------|---------------------------------|-----------------------------------|
| Experiment I | Control | 11.82 ± 3.24                    | ND                                |
|             | P       | 14.43 ± 1.39                    | ND                                |
|             | H(6 h)  | 10.61 ± 3.24                    | ND                                |
|             | PH(6 h) | 17.15 ± 1.36                    | ND                                |
| Experiment II | Control | 7.92 ± 1.16                     | 27.62                             |
|             | H(6 h)  | 14.11 ± 2.35                    | 38.52                             |
|             | PH(6 h) | 21.55 ± 1.98                    | 32.61                             |
|             | H(10 h) | 20.74 ± 4.39                    | 33.81                             |
|             | PH(10 h)| 28.12 ± 9.32                    | 28.12                             |
| Experiment III | Control | 7.54 ± 1.16                     | 17.14                             |
|              | P       | 13.89 ± 1.14                    | 15.27                             |

ND, not determined.

Table 3. Hepatic total protein and cytosolic aspartate aminotransferase in pyridoxine-deficient rats treated with pyridoxine and hydrocortisone. The same rats were used as for Table 1.

| Experiment | Total protein (A) (dpm/g liver × 10^-6) | AspATc (B) (dpm/g liver × 10^-3) | (B)/(A) (× 10^-3) |
|------------|----------------------------------------|---------------------------------|-------------------|
| Experiment I | Control 1.309 ± 0.206                   | 2.954 ± 1.188                   | 2.201 ± 0.632     |
|             | P 1.145 ± 0.012                        | 3.155 ± 0.622                   | 2.753 ± 0.516     |
|             | H(6 h) 1.162 ± 0.257                   | 6.189 ± 0.506                   | 5.471 ± 1.074     |
|             | PH(6 h) 0.937 ± 0.163                   | 5.559 ± 0.160                   | 6.042 ± 0.946     |
| Experiment II | Control 1.130 ± 0.395                   | 7.484 ± 0.937                   | 6.899 ± 1.583     |
|              | H(6 h) 0.913 ± 0.135                    | 8.082 ± 3.244                   | 8.628 ± 2.396     |
|              | PH(6 h) 1.400 ± 0.054                    | 4.477 ± 1.199                   | 3.197 ± 0.850     |
|              | H(10 h) 1.104 ± 0.451                    | 11.182 ± 3.241                  | 12.458 ± 6.147    |
|              | PH(10 h) 1.954 ± 0.091                   | 11.424 ± 2.220                  | 5.843 ± 1.058     |
| Experiment III | Control 1.618 ± 0.380                   | 5.669 ± 1.416                   | 3.496 ± 0.204     |
|              | P 1.529 ± 0.193                         | 2.248 ± 0.202                   | 1.483 ± 0.194     |

levels (3, 4). The rates of synthesis of aspartate aminotransferase, relative to total protein in the different groups were, therefore, compared. As shown in experiment I, the enzyme was induced 6 h after the injection of hydrocortisone, and the rate of
Table 4. Hepatic contents of pyridoxal and pyridoxal phosphate in pyridoxine-deficient rats treated with pyridoxine and hydrocortisone. Rats were treated in the same way as for Tables 1–3 (experiment II).

|                | Pyridoxal (mol/g liver $\times 10^{-9}$) | Pyridoxal phosphate (mol/g liver $\times 10^{-9}$) |
|----------------|------------------------------------------|---------------------------------------------------|
| Control        | 1.08 ± 0.57                              | 2.23 ± 0.52                                       |
| H              | 1.06 ± 0.16                              | 3.02 ± 0.46                                       |
| P              | 3.96 ± 1.14                              | 13.24 ± 2.55                                      |
| PH             | 4.67 ± 0.37                              | 23.77 ± 0.95                                      |

Values are means ± SD for 3–4 rats.

Table 5. Hepatic activities of tryptophan oxygenase and serum contents of corticosterone in rats given diets with or without pyridoxine.

|                | Tryptophan oxygenase (units/g liver) | Protein contents (mg/g liver) | Corticosterone contents (μg/dl) |
|----------------|-------------------------------------|------------------------------|---------------------------------|
| Control        | 5.860 ± 1.575                       | 127.97 ± 4.59                | 12.36 ± 3.96                    |
| Deficient      | 12.552 ± 8.027                      | 118.55 ± 8.11                | 15.34 ± 12.38                   |

Values are means ± SD for 10 rats.

enzyme synthesis was not affected by daily pyridoxine injections administered for 2 days. On the other hand, when rats were treated with pyridoxine daily for 6 days, the induction of the enzyme by hydrocortisone administration was suppressed. In experiment III, treatment of adrenalectomized rats with pyridoxine only for 6 days resulted in a lower rate of enzyme synthesis than that in the controls.

Liver pyridoxal and pyridoxal phosphate contents
The pyridoxal and pyridoxal phosphate contents of the liver of rats in the various groups were determined (Table 4). The livers of rats treated with pyridoxine daily for 6 days had normal levels of pyridoxal and pyridoxal phosphate.

Hepatic tryptophan oxygenase activity and serum corticosterone content
The activity of tryptophan oxygenase, which is known to be induced by glucocorticoid, was determined in the liver of rats fed a diet with or without pyridoxine (Table 5). The enzyme activity in the liver of pyridoxine-deficient rats was higher than that in the controls. The contents of serum corticosterone were also determined in these two groups, but no significant difference was observed between the average values.

J Nutr. Sci. Vitaminol.
We studied the effects of the administrations of pyridoxine and hydrocortisone on synthesis of cytosolic aspartate aminotransferase in the liver of adrenalectomized rats receiving a pyridoxine-deficient diet. As shown in Table 2, the antigenic activities induced by hydrocortisone were not sufficient to permit their quantitation. This may be explained as follows: aspartate aminotransferase is present at high levels in the liver cytosol, and its turnover rate is very slow. Thus antigenic activity induced in a short time, such as 6 or 10 h after a single injection of hydrocortisone, will be negligible compared with the total amount. In Table 3-experiment II, the incorporation of [³H]leucine into total protein in the liver was higher in groups treated with both pyridoxine and hydrocortisone than in other groups. This seemed to be due to differences in the pool sizes of free leucine. So we compared the rate of synthesis of cytosolic aspartate aminotransferase with that of total protein. In experiment I, two injections of pyridoxine had no effect on the relative rate of synthesis of cytosolic aspartate aminotransferase in either hydrocortisone-treated or untreated rats. On the other hand, treatment with pyridoxine daily for 6 days resulted in a decrease in the relative rate of enzyme synthesis, in both hydrocortisone-treated and untreated rats. The failure of two daily injections of pyridoxine to suppress enzyme synthesis suggests that when pyridoxine is injected into pyridoxine-depleted rats, it is preferentially incorporated into the apoprotein of pyridoxal phosphate-dependent enzymes, because of their high affinity for pyridoxal phosphate.

Glucocorticoid is known to be involved in several metabolic regulatory systems by acting on target organs (19). In these systems glucocorticoid first binds to a specific receptor in the cytosol, resulting in an activated hormone-receptor complex, and then the activated complex binds to chromatin inducing gene expression. Cak et al. reported that pyridoxal phosphate inhibits the binding of the activated receptor complex to DNA-cellulose in vitro (20). Sekula et al. found that pyridoxal phosphate exerts dual effects, stimulating the rate of activation, but inhibiting the subsequent binding of the activated complex to DNA-cellulose or nuclei (21). According to Disorbo et al., pyridoxine deficiency results in an increase in the number of activatable hepatic glucocorticoid-receptor complexes, and an accelerated rate of translocation of the complexes to the nuclei in vivo (11). They also reported that incubation of rat hepatomas in a pyridoxine-free medium resulted in a decrease in the intracellular level of pyridoxal phosphate and significant enhancement of the induction of tyrosine aminotransferase (12).

In the present work we have examined the effect of pyridoxal phosphate on the glucocorticoid action, using studies on the regulation of cytosolic aspartate aminotransferase in rat liver. We found that pyridoxine when administered to adrenalectomized pyridoxine-deficient rats, caused repression of the synthesis of aspartate aminotransferase induced by hydrocortisone. The same phenomenon was observed in adrenalectomized rats without treatment, with hydrocortisone as was
the case in experiment III. It is possible that these rats might have an ability to compensate for the absence of adrenal function to some extent, but their corticosterone level was too low to measure.

In any case our results suggest that pyridoxal phosphate may be a modulator of cytosolic aspartate aminotransferase in rat livers.

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*J. Nutr. Sci. Vitaminol.*
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