Effect of Pyridoxine Deficiency on Ornithine Aminotransferase in Rat Kidney and Liver

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Summary The hepatic and renal activities of ornithine aminotransferase (OAT) [L-ornithine 2-oxoacid aminotransferase, EC 2.6.1.13] were determined in male and female rats given diet with or without pyridoxine. OAT activities were measured in the presence of pyridoxal phosphate. Diet without pyridoxine caused a decrease in hepatic OAT activity in males and no change in females and an increase in renal OAT activity in males and a decrease in females. The increased renal OAT activity in males was associated with increased immunochemically recognizable OAT, and was due to increased synthesis of OAT as shown by measurement of [³H]leucine incorporation in vivo. Thus OAT is regulated by nutritional conditions in different ways in liver and kidney.

Key Words hepatic and renal OAT, synthesis of OAT, pyridoxine deficiency, pyridoxal phosphate, sexual difference

OAT activity is found in the mitochondrial matrix of many mammalian tissues, especially liver, kidney and small intestine (1–3). The enzyme from liver and that from kidney both consist of a single peptide species with a molecular weight of 43,000, as judged by dodecyl sulfate-polyacrylamide electrophoresis (4), and the two enzymes are indistinguishable immunologically (5); however, they differ in heat stability and cysteine content (6,7). They are also differentially regulated by various physiological conditions and environmental factors (8–13): the liver enzyme, but not the kidney enzyme, is induced by high-protein diet (5,14,15) or administration of glucagon (4,16) and the kidney enzyme is induced by administration of triiodothyronine (T₃) or estrogen as a result of change in its rate of synthesis (17–22).

Pyridoxine deficiency in animals results in various degrees of decrease in the activities of pyridoxal phosphate (PLP)-dependent enzymes (23–26). We found that
pyridoxine deprivation has different effects on the activity of the PLP-dependent enzyme aspartate aminotransferase [L-aspartate 2-oxoglutarate aminotransferase, EC 2.6.1.1] in different tissues and that the enzyme activity is regulated in different ways in different tissues (27–30).

In the present study we examined the changes in OAT activity in the liver and kidney of rats given diet with or without pyridoxine.

MATERIALS AND METHODS

Animals. Male and female Wistar strain rats weighing about 50 g each were fed ad libitum on 70% casein diet with or without pyridoxine for 4 weeks. The composition of the diet was as described previously (24).

Preparation of anti-OAT. Male Wistar strain rats, weighing about 200 g each, were fed on 70% casein diet for one week. They were then killed and the liver was promptly removed. The liver was homogenized in a Polytron (Kinematica) with 4 volumes (v/w) of 0.1 M potassium phosphate buffer (pH 7.4) containing 10^{-4} M PLP, sonicated at 200 W for 5 min (Kubota) and centrifuged. The supernatant obtained by centrifugation at 10,000 × g for 10 min was used for purification of OAT by a modification (4) of the method of Peraino et al. (31). The purified enzyme gave a single band on disc polyacrylamide gel and SDS-polyacrylamide gel electrophoresis. For preparation of antiserum to ornithine aminotransferase, 2 mg of purified enzyme was emulsified with an equal volume of complete Freund's adjuvant (Difco) and injected into rabbits intramuscularly. The injection was repeated 3 weeks later. The serum obtained 3 weeks after the second injection had a sufficiently high antigen titer for use. The antibody was partially purified and concentrated by ammonium sulfate fractionation (0–50%) and was stored at −70°C.

Incorporation of [3H]leucine into total protein and OAT in rat liver and kidney. Rats were fed on the experimental diet for 4 weeks, then starved for 16 h and given an injection of [3H]leucine (100 μCi/100 g body weight). They were killed 4 h later, and the liver and kidney were promptly removed. The liver and kidney were each homogenized in a Polytron with 0.1 M potassium phosphate buffer (pH 7.4) to prepare 20% and 10% homogenates, respectively. The homogenates were sonicated at 200 W for 5 min and centrifuged at 10,000 × g for 20 min. Portions of the supernatant were used for determinations of enzyme activity, its antigen activity and soluble protein synthesis; the remainder was centrifuged at 100,000 × g for 1 h and the supernatant was used for assay of OAT synthesis. The liver sample was heated in a water bath at 50°C for 2 min before centrifugation at 100,000 × g. For measurement of incorporation of radioactivity into total soluble protein, the preparation was treated with 10% trichloroacetic acid in the cold. The precipitate was suspended in 5% trichloroacetic acid–70% ethanol solution, heated at 85°C for 15 min and centrifuged at 3,000 × g for 20 min. The resulting precipitate was washed twice with the same solution and solubilized in 1 ml of formic acid and its radioactivity was determined in a scintillation counter (Aloka LSC-703) with xylene-
based scintillation fluid. For assay of OAT synthesis, an aliquot of the 100,000 × g supernatant was incubated with excess specific antibody for 30 min at 37°C and kept overnight at 4°C. The resulting immunoprecipitate was collected by centrifugation at 3,000 × g for 20 min and washed three times with cold 0.85% NaCl. It was then solubilized with 1 ml of formic acid and its radioactivity was determined as described above. For the blank, serum from non-immunized rabbits was used instead of antiserum.

**Analytical methods.** OAT activity was determined by the method of Katunuma et al. (14) or of Lyons and Pitot (4), and enzyme activity was expressed in units in terms of micromoles of pyrroline-5-carboxylate formed per hour at 37°C. A molar extinction coefficient of 2.71 × 10³ was used to calculate the amount of pyrroline-5-carboxylate formed (32). For determination of antigenic activity, a constant amount of liver or kidney supernatant was incubated with different amounts of anti-OAT in 0.1 M potassium phosphate buffer (pH 7.4) containing 10⁻⁴ M pyridoxal phosphate at 37°C for 30 min. The reaction mixture was then kept at 4°C overnight to allow complete precipitation and centrifuged at 3,000 × g for 20 min, and residual enzyme activity in the supernatant was measured. One unit of antigen activity was defined as the amount of immunoreactive substance equivalent to the amount of antibody precipitating 1 unit of enzyme activity of control rat tissue. Protein was determined by either the biuret reaction (33) or Lowry’s method (34).

**Chemicals.** Dietary materials were purchased from Oriental Yeast Kogyo Co. [4,5-³H] L-Leucine was obtained from RCC Amersham and o-amino-benzaldehyde from Maruwaka Kogyo Co. Other reagents were purchased from Nakarai or Wako Pure Chemical Co.

**RESULTS**

**Enzyme activity in the liver and kidney of male and female rats**

The OAT activities in the liver and kidney of male and female rats fed on diet with or without pyridoxine were determined (Table 1). The enzyme activity in the liver of control rats was twice as high in females as in males in both the presence and absence of PLP added to the assay system. In preparations from controls of both sexes, the liver OAT activity was the same when measured in either the presence and absence of PLP, but kidney OAT activity was overwhelmingly higher when measured in the presence of PLP. This means that most of the OAT exists as the holoenzyme in the liver and as the apoenzyme in the kidneys in both male and female rats. Pyridoxine deprivation caused a marked decrease in holoenzyme activity in the liver and kidney of rats of both sexes. By the addition of PLP to the assay system, the hepatic enzyme activity was completely restored to the control level in females and partially restored in males, and the renal activity was partially restored in females and moreover increased above the control level in males. Thus pyridoxine deprivation had differential effects on OAT activity in liver and kidney
Table 1. Ornithine aminotransferase in liver and kidney of male and female rats on diet with or without pyridoxine.
Weanling rats were fed on experimental diet for 4 weeks and then killed. Enzyme activity was measured in both the presence and absence of $4 \times 10^{-4}$ M pyridoxal phosphate. Values are the absorbancy at 440 nm of quinazolium compound produced per hour at 37°C per g tissue. Other conditions are as described in the text. Values are means for 6 rats ± SD.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Pyridoxine in diet</th>
<th>Male OAT (OD$_{440}$/g tissue)</th>
<th>Female OAT (OD$_{440}$/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>−PLP</td>
<td>+PLP</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>123.5 ± 9.8</td>
<td>128.2 ± 14.3</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>59.0 ± 10.4$^b$</td>
<td>92.9 ± 16.2$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(47.7)</td>
<td>(72.4)</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
<td>20.4 ± 3.3</td>
<td>154.5 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>10.9 ± 2.4$^b$</td>
<td>229.7 ± 15.3$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(53.4)</td>
<td>(148.6)</td>
</tr>
</tbody>
</table>

$^a$ and $^b$ are significantly different from control values at $p<0.01$ and $p<0.001$, respectively. Figures in parentheses show values as percentages of control values.

Table 2. Ornithine aminotransferase and its antigen activities in the liver and kidney of male rats on diet with and without pyridoxine.
Enzyme activity was measured in the presence of $4 \times 10^{-4}$M PLP. Values are for pooled samples from four rats. Details of conditions are described in the text.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Pyridoxine in diet</th>
<th>E Enzyme activity (units/g tissue)</th>
<th>A Antigen activity (units/g tissue)</th>
<th>E/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>+</td>
<td>135.6</td>
<td>135.6</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>80.6</td>
<td>131.9</td>
<td>0.61</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
<td>121.8</td>
<td>121.8</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>273.0</td>
<td>256.1</td>
<td>1.03</td>
</tr>
</tbody>
</table>

in males and females.

Antigen activity in liver and kidney of male rats
In pyridoxine-deficient male rats, although the enzyme activity in the liver was decreased, the antigen activity was maintained at the control level (Table 2), while the enzyme activity in the kidney increased in parallel with the antigen activity, suggesting that this increase in the renal activity in the deficient rats was associated with an increase in the amount of enzyme.

Syntheses of protein and OAT in liver and kidney of male rats
The incorporations of $[^{3}H]$leucine into total soluble protein and OAT in the

Table 3. Incorporations of $[^3H]$L-leucine into ornithine aminotransferase and soluble proteins in liver and kidney of male rats on diet with and without pyridoxine. Enzyme activity was measured in the presence of $4 \times 10^{-4}$M PLP. Values are means for 3 rats ± SD. Other experimental conditions are as described in the text. Figures in parentheses show values as percentages of control values.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Pyridoxine in diet</th>
<th>Enzyme activity (units/g tissue)</th>
<th>$[^3H]$L-Leucine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A (dpm/g tissue)</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>155.2 ± 21.7</td>
<td>8,958 ± 1,138</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72.0 ± 18.7b</td>
<td>6,232 ± 242⁴(70)</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
<td>134.0 ± 4.9(169)</td>
<td>4,181 ± 570</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>226.0 ± 25.6b(169)</td>
<td>7,979 ± 1,562⁴(190)</td>
</tr>
</tbody>
</table>

$^{a}$ and $^{b}$ are significantly different from control values at $p < 0.05$ and $p < 0.01$, respectively.

The OAT activities in the liver and kidney of male and female rats were determined. As shown in Table 1, most OAT exists as the holoenzyme in the liver and as the apoenzyme in the kidney in rats. This might be explained by a difference in coenzyme contents between kidney and liver, because we obtained a higher value of PL plus PLP in the liver than in the kidney, i.e., 2.9 µg/g liver and 1.08 µg/g kidney (48). On the other hand, a similar affinity of PLP for both liver and kidney apoenzymes has been reported (5). When the OAT activity was assayed in the presence of PLP, pyridoxine deprivation was found to have different effects on renal OAT activities depending on the sex of rat: an increase in male and a decrease in female. This increase in male kidney was shown to be due to an increase in the
quantity of enzyme resulting from increased synthesis of OAT as determined by incorporation of \(^{3}H\)leucine (Tables 2 and 3). Although the decrease of renal enzyme activity in female rats being due to a decrease in antigenic activity or not is unknown, the existence of estrogen in large quantities might have resulted in a different effect from that in males. Inactive or less active enzyme molecules seemed to be present in the liver of pyridoxine-deficient rats because the antigenic activity of OAT remained unchanged, although the enzyme activity decreased (Table 2). On the other hand, as shown in Table 3, OAT synthesis in the liver also decreased slightly in pyridoxine deficiency. Thus further studies are necessary to elucidate the regulation of OAT in the liver of pyridoxine-deficient rats.

The elevation of renal OAT activity by estrogen was first reported by Herzfeld and Knox (3) and, according to them, this hormone is responsible for the high levels of OAT in female rat kidneys. Recently, the induction of renal OAT activity by estrogen and thyroid hormone has been clarified as resulting from increased synthesis of the enzyme, which is accompanied by an increase in the level of its mRNA due to an increased rate of transcription of the OAT gene (35). For OAT action, estrogen is bound to a specific receptor in target organs, the hormone-receptor complex is activated and then the activated complex becomes bound to chromatin (36–38). Recently, some inhibitory effects of pyridoxal phosphate on the action of steroid hormones such as estrogen and glucocorticoid have been reported (39–47). Details of the mechanism of the inhibitory effect of pyridoxal phosphate on the action of estrogen are unknown; it is believed to affect the processes of both activation of the hormone-receptor complex and binding of the activated complex to chromatin.

At present, it is unknown whether the increased synthesis of OAT in the kidney of pyridoxine-deficient rats is due to such an effect of estrogen, but it is conceivable that the action of estrogen is suppressed under usual conditions and desuppressed in pyridoxine deficiency. We are now studying the regulation of OAT by estrogen in rat kidney.

Degradation of OAT is reported to increase in the small intestine of pyridoxine-deficient rats (49). Thus OAT is regulated by many nutritional and hormonal factors, and in different ways in liver, kidney and small intestine of rat.

REFERENCES


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