Tyrosinemia Induced by a Pyridoxine Antagonist, Desoxypyridoxine

E. Joanne Easton, Ian Simpson, J. Kenneth Martin, and Donald J. Campbell

Tyrosinemia was produced in Wistar rats by feeding a diet deficient in vitamin B₆ and containing 1 mg of 4-desoxypyridoxine (DOP) per 100 g of diet. Concentrations of serum tyrosine were greatest in lactating rats, with smaller increases in nonpregnant, nonlactating females and adult males. Paper chromatography of urine indicated that DOP feeding also probably inhibits formation of most normal urinary metabolites of tyrosine. Livers of several rats showed no evidence of cirrhosis after 18 days on the diet containing DOP, indicating that this experimentally produced tyrosinemia is not a true model of tyrosinosis.

Additional Keyphrases rat • tyrosinosis • diagnosis of B₆ deficiency

During studies on Wistar rats fed nor, an antimetabolite of pyridoxine, we detected abnormally high concentrations of tyrosine in their serum. We sought to determine if an experimental model for tyrosinosis had been produced.

Materials and Methods

Wistar rats were studied and three diets were used: (a) a commercial rat diet, (b) a vitamin B₆-deficient diet, and (c) a vitamin B₆-deficient rat diet, containing in addition 1 mg of DOP per 100 g of diet.

After parturition, four lactating females were placed on diet a and four on diet b.

A pair of 159-176 g rats, one adult male and one adult nonpregnant female, was placed on diet a, another such pair on diet b, and another on diet c.

Serum tyrosine (1) and phenylalanine (2, 3) concentrations were measured for lactating animals and some of the offspring after 18 days, and for the nonlactating adults on the 3rd, 7th, 10th, 14th, and 18th days.

Urine (24-h) from nonlactating adults were tested by two-dimensional paper chromatography (4, 5) for tyrosine, pHPPA, pHPAA, PHPA, VMA, HVA, HGA, and cystathionine.

When the animals were killed after 18 days on the diet, some livers were examined histologically (hematoxylin and eosin stain).

Results

Serum tyrosine and phenylalanine. As seen in Table 1, serum tyrosine was most enhanced in the lactating rats, with smaller increases in the nonpregnant, nonlactating females, and the smallest increases in the adult males. The offspring of nursing females also had increased serum tyrosine values, but lower than the dams.

Serum phenylalanine was slightly, but significantly (P < 0.05) increased in lactating rats on nor (mean, 4.4 mg/100 ml ± 0.8 sp). We measured phenylalanine because vitamin B₆ is involved in the metabolism of both tyrosine and phenylalanine.

Urinary metabolites of tyrosine by two-dimensional paper chromatography. To interpret the results of the paper chromatography of urine, it is helpful to recall the

Table 1. Effect of DOP on Concentration of Tyrosine in Rat Serum

<table>
<thead>
<tr>
<th>Tyrosine, mg/100 ml serum</th>
<th>Control*</th>
<th>B₆-def. diet†</th>
<th>DOP-contg. diet‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating rats</td>
<td>1.7 ± 0.5</td>
<td>...</td>
<td>52.0 ± 13.1</td>
</tr>
<tr>
<td>Offspring</td>
<td>3.7 ± 1.2</td>
<td>...</td>
<td>8.7 ± 3.1</td>
</tr>
<tr>
<td>Adult males</td>
<td>2.5 ± 0.1</td>
<td>3.2 ± 0.5</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td>Adult females</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>21.4 ± 8.8</td>
</tr>
</tbody>
</table>

* 1 mg pyridoxine/100 g B₆-deficient diet.
† pyridoxine-deficient diet.
‡ 1 mg desoxypyridoxine/100 g pyridoxine-deficient diet.

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1 Nonstandard abbreviations used: DOP, 4-desoxypyridoxine; pHPPA, p-hydroxyphenylpyruvic acid; pHPAA, p-hydroxyphenylactic acid; PHPA, p-hydroxyphenylalactic acid; VMA, vanillylmandelic acid (4-hydroxy-3-methoxymandelic acid); HVA, homovanillic acid; HGA, homogentisic acid; CVSTA, cystathionine.

Received July 2, 1971; accepted Sept. 20, 1971.
metabolism of tyrosine (Figure 1). Basically, there are three metabolic pathways for dealing with increased amounts of tyrosine: (a) DOP to HVA or VMA (catecholamine synthesis), (b) pHPAA to HGA, and (c) tyramine to tyrosine. All of these steps require vitamin B₆ as a co-factor. Urinary tyrosine was increased, in keeping with the enhanced serum concentrations. Lesser amounts of VMA and HVA were found in DOP urines than in normal controls; pHPAA showed only a slight increase (1+ to 2+) with DOP feeding. Tyrosine transaminase was also probably inhibited, because pHPFA and pHPAA concentrations were unchanged in the face of increased concentrations of serum tyrosine.

Slightly higher concentrations of cystathionine were detected in the urines of the DOP-fed animals than in controls.

Liver. Livers of several animals showed no histologic evidence of cirrhosis after 18 days on DOP.

Discussion

Tyrosinemia has been defined as a blood tyrosine concentration greater than 5 mg/100 ml (6). By this criterion, pyridoxine deficiency alone does not increase tyrosine concentrations. However, addition of DOP to the diet causes a marked increase (Table 1), particularly in lactating rats. This may be due to a relative deficiency of B₆ owing to increased estrogen levels during lactation (7). The lower concentrations of tyrosine in the serum of males may reflect the stimulatory effect of testosterone on tyrosine transaminase activity. However, the major reason for tyrosine increase is probably DOP inhibition of tyrosine transaminase (8). This enzyme, metabolically labile, is thought to be the rate-limiting enzyme in the metabolism of tyrosine (9). Pyridoxine is a co-enzyme for tyrosine transaminase and can increase the amount of both the holoenzyme and the apoenzyme (10-18). Pyridoxal phosphate, by combining with the apo-enzyme, may remove transaminase from equilibrium with its precursors and degradation products, thereby causing increased transaminase synthesis (18).

Changes in amounts of tyrosine metabolite excreted in urine were interpreted from two-dimensional urine chromatograms as follows: Chromatograms of urine from normal and DOP-treated rats were run and developed at the same time. A 0 to +3 grading system was used by three independent observers. The results are given in Table 2.

With increased serum tyrosine, one might expect increased utilization of one or more of the metabolic routes, with concomitant increase in the tyrosine metabolites involved. The slight increase in pHPAA in DOP urine indicates that some excess serum tyrosine was decarboxylated to tyramine, despite DOP being a known inhibitor of tyrosine decarboxylase (14). However, considering the serum tyrosine concentrations, the increase over normal was very slight.

Apparent decreased amounts of VMA and HVA in DOP urines imply DOP inhibition of B₆-dependent dopa decarboxylase. Urinary pHPAA was not increased, but its metabolite, HGA, disappeared from urine. This may be the result of reversible inactivation of p-hydroxyphenylpyruvate hydroxylase by the increased tyrosine concentrations (16). Expected pigment changes could not be noted because we used albino rats in these experiments.

Thus, DOP feeding increases serum tyrosine con-

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**Table 2. Urine Metabolites of Tyrosine in DOP-Fed Rats**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Diet</th>
<th>TYR</th>
<th>VMA</th>
<th>HVA</th>
<th>pHPAA</th>
<th>pHPFA</th>
<th>HGA</th>
<th>pHPAA</th>
<th>CYSTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult male</td>
<td>Normal diet</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>Adult female</td>
<td>Normal diet</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>Adult male</td>
<td>B₆-def.</td>
<td>3+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Adult female</td>
<td>B₆-def.</td>
<td>3+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Adult male</td>
<td>B₆-def. and 1 mg DOP</td>
<td>3+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Adult female</td>
<td>B₆-def. and 1 mg DOP</td>
<td>3+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
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</tr>
</tbody>
</table>

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centration by also blocking several pathways of metabolism, particularly those involving hGA and catecholamine formation.

Cirrhosis of the liver develops in tyrosinosis. After 18 days on a low protein diet, the livers of our rats showed no evidence of cirrhosis. Thus, although we produced a tyrosinemia, we did not produce a true model of tyrosinosis. A diet of L-tyrosine, fed for six months, is uniformly fatal to rats (16), but if rats are made B6 deficient the diet is less toxic.

Urine cystathionine was checked to see if it might be a useful index of the degree of vitamin B6 deficiency, as suggested by Serter and Hutchison (17). Slightly more cystathionine was detected in the urines of the nor-fed animals. However, as cystathionine is also present in control urines, diagnosis of B6 deficiency on this basis does not seem possible.

References