Gyrate Atrophy of the Choroid and Retina with Hyperornithinemia

CHARACTERIZATION OF MUTANT LIVER
L-ORNITHINE:2-OXOACID AMINOTRANSFERASE KINETICS

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A B S T R A C T  Deficient activity of L-ornithine:2-oxoacid aminotransferase is associated with gyrate atrophy of the choroid and retina with hyperornithinemia, an autosomal recessive disease leading to blindness. Liver tissue from two patients contained trace activity of the enzyme. The Michaelis ($K_m$) value of the mutant enzyme for ornithine was 200 mM, 50-fold higher than normal, but increasing the concentrations of $\alpha$-oxoglutarate and pyridoxal 5'-phosphate to 10 times those giving maximal activity of the normal enzyme had no effect on the mutant enzyme. Substrate inhibition of the mutant could not be demonstrated at 1,000 mM ornithine concentration, whereas ornithine concentrations above 70 mM inhibited the normal enzyme. The data suggest that the abnormal L-ornithine:2-oxoacid aminotransferase in the two patients studied has an altered binding site for ornithine.

INTRODUCTION

Gyrate atrophy of the choroid and retina with hyperornithinemia (GA) is an autosomal recessive disease characterized by night blindness and myopia by age 5–9 yr, posterior subcapsular cataracts, and progressive constriction of the visual fields often leading to blindness after 20–40 yr (1–3). The circular patchy atrophic lesions begin in the peripheral retina, enlarge, and coalesce, leaving only small central area of the posterior retina unaffected. In skeletal muscle, type II fibers are atrophic and partly filled with tubular aggregates, and the mitochondria may be altered (4). Plasma ornithine is 10–20 times higher than normal (5). In stimulated lymphocytes and cultured fibroblasts of the patients, the activity of L-ornithine:2-oxoacid aminotransferase (OAT) is usually unmeasurably low (6–11). In patients with GA, elevation of plasma ornithine after an intravenous arginine load is followed by a more rapid decrease in extracellular fluid ornithine than could be expected from urinary excretion and protein synthesis from arginine (12). This suggests that, in patients with GA, ornithine is degraded by some other mechanism, possibly by residual activity of the major catabolic enzyme, OAT. We report here that residual OAT activity with altered kinetics for ornithine is present in liver biopsies from two patients with GA.

METHODS

Preparation of liver homogenates. Percutaneous needle biopsies of the liver were performed in two patients with typical GA, a sister and brother, 32 and 28 yr old. These tissues and normal control biopsies initially obtained for other reasons under laparotomy (patients aged 4–82 yr), were frozen immediately in liquid nitrogen, transported in dry ice, and stored at -20°C until used for enzyme measurements. The liver samples were homogenized in 40 parts of 0.1 M phosphate buffer, pH 7.4, freeze-thawed three times, and, after addition of Triton X-100 to a final concentration of 0.1% and standing for 10 min at room temperature, centrifuged at 3,000 rpm for 10 min. The supernate was used for enzyme assays.

Enzyme assays. A micromodification of a method previously described (13) was used. For measurement of the enzyme kinetics the assay system contained 0.5–1,000 mM L-ornithine, 3.75 mM $\alpha$-oxoglutarate, and 4 $\mu$g/ml pyridoxal 5'-phosphate in a total volume of 25 $\mu$L. 40 $\mu$g of GA and 1–10 $\mu$g of control liver homogenate protein was used. The reaction was stopped after incubation for 60 min by adding 5 $\mu$L of a mixture of 6 mg ortho-amino-benzaldehyde in

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500 μl methanol and 500 μl concentrated HCl. The generation of dihydroquinazolinium from the product of the enzyme reaction, Δ²-pyrroline-5-carboxylic acid, was complete in 30 min at room temperature, after which precipitated protein was removed by centrifugation at 3,000 rpm for 10 min, and 10 μl of the clear supernate was used for measurement of dihydroquinazolinium by high-pressure liquid chromatography with detection at 254 nm. The integrated peak values were compared with an external standard of dihydroquinazolinium. The results represent means of duplicate determinations. Under the conditions used, the assays were linear with time and with added homogenate. In simultaneously run blanks, the stopping mixture was added before incubation, and they never showed any Δ²-pyrroline-5-carboxylic acid formation.

**Calculations.** The Michaelis constant (K_m) and maximum velocity of the enzyme reaction (V_max) were computed after nonlinear curve fitting to V = V_max (S)/K_m + (S).

The patients with GA gave their informed consent, and the control liver samples were obtained with approval of the Children's Hospital's ethical committee.

**RESULTS**

When the samples from the patients with GA were assayed under conditions optimal for normal OAT, at 35 mM L-ornithine and 3.75 mM α-oxoglutarate concentrations, only trace amounts of Δ²-pyrroline-5-carboxylic acid were generated, but they were clearly detectable by high-performance liquid chromatography. This activity did not increase with increasing concentrations of α-oxoglutarate and/or pyridoxal 5'-phosphate. 100-fold concentration of the latter inhibited the reaction. In the patients, Lineweaver-Burk plots of the OAT assays gave K_m values about 50 times that of the mean of controls. Apparent V_max values were in the mean four times higher in control homogenates and showed a large deviation (Fig. 1, Table I). Adding the mutant liver homogenate to the control sample did not decrease the activity of the normal enzyme. In the control homogenates, OAT activity showed substrate inhibition at ornithine concentrations 70 mM (20 times K_m) (Fig. 1B), but in the patients with GA, no inhibition was seen at 1,000 mM ornithine (5 times K_m).

**Table I**

**Kinetic Constants for Liver OAT**

<table>
<thead>
<tr>
<th></th>
<th>K_m</th>
<th>V_max</th>
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<tbody>
<tr>
<td></td>
<td>mM</td>
<td>nmol/hmg protein</td>
</tr>
<tr>
<td>Gyrate atrophy patients</td>
<td>206</td>
<td>79.9</td>
</tr>
<tr>
<td></td>
<td>213</td>
<td>86.4</td>
</tr>
<tr>
<td>Controls</td>
<td>3.91</td>
<td>662</td>
</tr>
<tr>
<td></td>
<td>4.98</td>
<td>312</td>
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<td></td>
<td>3.68</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>4.01</td>
<td>219</td>
</tr>
<tr>
<td>Mean±SD in controls</td>
<td>4.15±0.57</td>
<td>333±231</td>
</tr>
</tbody>
</table>

The solubility of ornithine precluded assays at higher substrate concentrations, and kinetic studies with α-oxoglutarate or pyridoxal 5'-phosphate could not be performed because of the small amounts of sample available.

**DISCUSSION**

The residual OAT activity in liver homogenates of patients with GA had a K_m value for ornithine far above that in normal liver and far above the ornithine concentrations seen in physiological fluids. It was ~250-fold the mean plasma ornithine concentration of these two patients. The mean K_m in the control samples was ~50-fold the normal mean fasting plasma ornithine concentration. It is unlikely that any other enzyme but OAT could have been functioning in the assay, because the specific product of delta transamination
of ornithine, Δ1-pyrroline-5-carboxylic acid, was measured (13). This unique delta transamination is not known to be catalyzed by other transaminating enzymes in mammals. However, final proof of the presence of OAT enzyme protein remains to be demonstrated with antiserum against human OAT.

Previous studies of OAT in patients with GA report only trace activities, not clearly differing from zero (6–10). The negligible OAT activity in most patients with GA may be due to the low ornithine concentrations (0.7–35 mM) used in these assays which were performed on fibroblasts or stimulated lymphocytes. Such results may lead to the misinterpretation that patients with GA totally lack the OAT enzyme. An assay based on specific chemical measurement of the products and performed with metabolically active liver tissue is better suited for use in measurements of the kinetic constants. The fibroblasts of several patients (11, 14, 15) show OAT activity at high pyridoxal 5'-phosphate concentrations, indicating a kinetic mutation for pyridoxal 5'-phosphate. In our liver homogenates, elevation of pyridoxal 5'-phosphate only inhibited the reaction. Increasing the α-oxoglutarate concentration up to 37.5 mM did not result in higher OAT activity. In our patients with GA, the mutation in OAT is probably in its site for ornithine binding.

These results are well in accordance with the observations in vivo. After an intravenous arginine load, plasma ornithine t½ is 360 min in patients with GA compared with 97 min in controls (4). The fasting plasma concentrations in different Finnish patients with GA vary considerably (5). On the other hand, fasting ornithine concentrations taken at different times from the same individual are far more constant. These individual plasma ornithine levels could be determined by the individual Kₘ for OAT in the subject. This could be the reason for variable ornithine concentrations in GA, but the pathophysiologic importance of the severity of hyperornithinemia remains to be clarified.

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REFERENCES