Glycolysis in the Brain and Liver of Rats with Experimentally Induced Phenylketonuria

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The genetically-linked deficiency in the activity of hepatic phenylalanine 4-hydroxylase (EC 1.14.16.1) leads to a marked accumulation of L-phenylalanine (L-Phe) and some of its metabolic products, namely phenylpyruvate, phenyl-lactate and o-hydroxyphenylacetate, in the blood and tissues of patients with uncontrolled phenylketonuria (PKU). In order to explain the biochemical mechanisms by which the accumulation of L-Phe and/or its metabolites causes mental retardation in PKU, several hypotheses have been suggested. Weber et al. (1) demonstrated that either L-Phe or phenylpyruvate inhibited competitively in vitro the brain pyruvate kinase (PK) (EC 2.7.1.40) and hexokinase (HK) (EC 2.7.1.1.).

In this paper we report the in vivo effects of high concentrations of L-Phe on the levels of glycolytic intermediates in brain and liver of rats with experimentally induced phenylketonuria. The effect of L-Phe on the PK activity in rat liver has been also investigated.

METHODS

Adult female rats of the Wistar strain weighing 150–200 g were used. Animals were treated with DL-p-chlorophenylalanine (p-CPA), inhibitor of phenylalanine hydroxylase in vivo (2), and esculin, pteridine antagonist (3), to reinforce the inhibition produced by p-CPA (4). p-CPA (360 mg/kg) and esculin hydrate (680 mg/kg) were injected intraperitoneally 36 hr and 1 hr, respectively, before killing. All the animals had free access to food and water during the experiments.

Treated rats were injected intraperitoneally with L-Phe (1 g/kg). Litter-mate controls received doses of 0.9% NaCl. One hour after injection the animals were sacrificed by decapitation, the heads being completely

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frozen by immersion into liquid N$_2$. One lobe of the liver was cut off very rapidly and freeze-clamped at the temperature of liquid N$_2$.

Tissue extracts were prepared as described by Hems et al. (5). Glucose was determined by the glucose-oxidase method (6, 7). Glucose 6-phosphate and fructose 6-phosphate were determined as described by Hohorst (8), and fructose 1,6-diphosphate, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate by the method of Bucher and Hohorst (9). 3-Phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate and pyruvate were determined as described by Czok and Eckert (10). PK activity was assayed spectrophotometrically as described by Weber (1). Phe and tyrosine (Tyr) were determined by ion-exchange chromatography on the short column of a Carlo Erba 3A27 amino acid analyzer.

RESULTS

Phe and Tyr concentrations in liver and brain of treated and control rats are reported in Table 1. As seen, a 50 fold and 17 fold increase of the values of L-Phe were found, respectively, in liver and brain. However, Tyr concentration remained near the control values in both tissues.

The patterns of glycolytic metabolites in brain and liver of rats with induced PKU are shown in Fig. 1. Increased levels of the intermediates leading from fructose 1,6-diphosphate to phosphoenolpyruvate were found. On the contrary, the concentrations of glucose 6-phosphate, fructose 6-phosphate, and pyruvate were significantly decreased.

Table 2 shows the effect of L-Phe and its metabolites on the PK activity in rat liver. Any effect was observed when phenylpyruvate, phenylacetate, phenyl-lactate, phenyl-ethylamine, o-hydroxyphenylacetate and p-CPA were used. Nevertheless, L-Phe strongly inhibited the enzyme (about 68%). A Dixon plot demonstrate that L-Phe is a competitive inhibitor of the PK activity, with a $K_i$ approximately 3 mM (Fig. 2).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>PHENYLALANINE AND TYROSINE LEVELS IN LIVER AND BRAIN OF RATS WITH EXPERIMENTAL PHENYLKETONURIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Phe</td>
<td>0.066 ± 0.011</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.067 ± 0.010</td>
</tr>
</tbody>
</table>

The results expressed in $\mu$mole/g wet tissue are the means ± SEM of six experiments.
GLYCOLYSIS IN PHENYLKETONURIA

FIG. 1. Changes in the glycolytic intermediates in the brain and liver of rats with experimentally induced phenylketonuria. Each point represents mean ± SEM of 8–12 determinations expressed as a percentage of control values. The symbols +, ++, and +++ indicate values significantly different from control values below 5, 1 and 0.1% levels, respectively. The abbreviations are: Glu, glucose; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FDP, fructose 1,6-diphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde phosphate; 3PGA and 2PGA, 3- and 2-phosphoglycerate; PEP, phosphoenolpyruvate and Pyr, pyruvate. Glycolytic intermediate concentrations (mean ± SEM) in the brain and liver from control groups of 10–14 rats, expressed as μmole/g of wet tissue, were respectively: Glu, 0.772 ± 0.04 and 22.16 ± 1.74; G6P, 0.063 ± 0.005 and 0.553 ± 0.044; F6P, 0.033 ± 0.003 and 0.144 ± 0.012; FDP, 0.105 ± 0.013 and 0.086 ± 0.008; DHAP, 0.038 ± 0.011 and 0.043 ± 0.003; GAP, 0.0086 ± 0.002 and 0.020 ± 0.008; 3PGA, 0.060 ± 0.003 and 0.213 ± 0.033; 2PGA, 0.030 ± 0.002 and 0.056 ± 0.005; PEP, 0.031 ± 0.003 and 0.113 ± 0.010; Pyr, 0.129 ± 0.005 and 0.212 ± 0.016.
Addition

<table>
<thead>
<tr>
<th>Phosphoenolpyruvate (μM)</th>
<th>PHE (μM)</th>
<th>Phenylacetaate</th>
<th>Phenyl-ethylamine</th>
<th>Phenyl-lactate</th>
<th>o-Hydroxyphenylacetate</th>
<th>p-CPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>40.6 ± 0.7</td>
<td>13.0 ± 0.4</td>
<td>40.2 ± 0.7</td>
<td>38.7 ± 2.1</td>
<td>42.5 ± 1.8</td>
<td>40.7 ± 1.7</td>
</tr>
<tr>
<td>Phc</td>
<td>13.0 ± 0.4</td>
<td>13.0 ± 0.4</td>
<td>40.2 ± 0.7</td>
<td>38.7 ± 2.1</td>
<td>42.5 ± 1.8</td>
<td>40.7 ± 1.7</td>
</tr>
<tr>
<td>Phenylacetaate</td>
<td>40.2 ± 0.7</td>
<td>13.0 ± 0.4</td>
<td>40.2 ± 0.7</td>
<td>38.7 ± 2.1</td>
<td>42.5 ± 1.8</td>
<td>40.7 ± 1.7</td>
</tr>
<tr>
<td>Phenyl-ethylamine</td>
<td>38.7 ± 2.1</td>
<td>13.0 ± 0.4</td>
<td>40.2 ± 0.7</td>
<td>38.7 ± 2.1</td>
<td>42.5 ± 1.8</td>
<td>40.7 ± 1.7</td>
</tr>
<tr>
<td>Phenyl-lactate</td>
<td>42.5 ± 1.8</td>
<td>13.0 ± 0.4</td>
<td>40.2 ± 0.7</td>
<td>38.7 ± 2.1</td>
<td>42.5 ± 1.8</td>
<td>40.7 ± 1.7</td>
</tr>
<tr>
<td>o-Hydroxyphenylacetate</td>
<td>40.5 ± 1.4</td>
<td>13.0 ± 0.4</td>
<td>40.2 ± 0.7</td>
<td>38.7 ± 2.1</td>
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<td>p-CPA</td>
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</tr>
</tbody>
</table>

DISCUSSION

The Phe concentration in the brain of treated animals has been found to be very close to that reported in the brain of phenylketonuric patients (11). The tyrosine levels remained unchanged.
The patterns of the glycolytic metabolites in brain and liver of rats with experimental PKU suggest that in vivo the elevated concentration of L-Phe leads to an enhancement of the intermediates of glycolysis from fructose 1,6-diphosphate to phosphoenolpyruvate and to a decrease of the pyruvate levels. The inhibition of the PK activity by L-Phe (1) can account for these findings. On the other hand, the decrease of the concentrations of glucose 6-phosphate and fructose-6-phosphate can be explained either by the inhibition of the HK activity by L-Phe (or some of its metabolites) (1) or by altering the permeability of glucose (12).

Alterations in the pattern of the glycolytic metabolites in the brain of the rats with induced PKU could be expected because of the inhibition of brain PK activity as observed in vitro by Weber (1), and the results recently reported in vivo by Miller et al. (12) after L-Phe administration. However, the behavior in such a condition of the liver, which have a different pattern of PK isoenzymes, remained unexplained.

Essays in vitro on the liver PK activity demonstrated that the enzyme was inhibited by L-Phe with a Ki 3 mM. These results are in good agreement with those reported by Carbonell et al. (13) showing that the class L of PK, the major component of the enzyme in liver, was inhibited by L-Phe as it is the class M, the major component of the brain enzyme.

When the effect of p-CPA and Phe metabolites on liver PK activities was investigated no inhibition was observed. These results suggest that only L-Phe may inhibit the PK activity in vivo being responsible for the decreased levels of pyruvate found in both tissues.

The inhibition of brain glycolysis by Phe can influence the rate of the synthesis of fatty acids and cholesterol, precursors of myelin. It is worth note that impaired myelination (14) and decreased cholesterol concentrations (15) have been found in PKU brains. The inhibition of brain glycolysis in PKU can play an important role in the etiology of mental retardation, specially during the early stages of brain development, when the processes of the functional differentiation of the central nervous system are more rapid.

SUMMARY

The effect of Phe on the glycolytic pathway in brain and liver of rats with experimentally induced phenylketonuria-like characteristics have been investigated. The Phe concentrations in the rat tissues were similar to those found in phenylketonuria patients. Important changes in the levels of glycolytic metabolites in brain and liver have been observed: The marked decrease of pyruvate concentrations and the increase of the intermediates from fructose 1,6-diphosphate to phosphoenolpyruvate, are especially significant. Essays in vitro on the liver pyruvate kinase activity
have shown that phenylalanine is also a competitive inhibitor of the liver enzyme.

ACKNOWLEDGMENT

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REFERENCES