THE SOURCE OF AROMATIC KETOACIDS IN TYROSINAEMIA AND PHENYLKETONURIA*

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SUMMARY

The studies reported here support the observation that elevated excretion of β-hydroxyphenylpyruvic acid could occur in the presence of deficient hepatic tyrosine aminotransferase activity. The ketoacid need not have come either from the liver or from the kidney. It appears possible that the urinary ketoacid, both in tyrosine aminotransferase deficiency and in phenylketonuria, originates not in the liver but in other tissues which possess transaminase but which lack hydroxylase activity. What emerges from these studies is the view that the consequence of a single enzyme deficiency in one tissue may be modified by the distribution of isozymes or related enzymes in that tissue as well as in other tissues.

Tyrosinaemia of prematurity and hereditary tyrosinaemia are two disorders of aromatic amino acid metabolism. They are characterised by the presence of hyper-tyrosinaemia and the excretion of β-hydroxyphenylpyruvic acid (pHPPA) and its metabolites in the urine. These findings can be readily explained by deficient activity of β-hydroxyphenylpyruvate (pHPP) hydroxylase activity (E.C. 1.14.2.2) in liver and kidney. Such a deficiency has indeed been demonstrated in some patients with hereditary tyrosinaemia1-2. An alternative hypothesis has been advanced by La Du2 to explain the excretion of phenolic acids in the original patient with tyrosinosis described by Medes3. He suggested that this patient may have suffered from deficient hepatic tyrosine aminotransferase activity and that the observed elevation of urinary pHPPA was due to deamination of tyrosine by the kidney2. The same mechanism was recently suggested by Rosenberg and Scriverv to account for the urinary excretion of phenolic acids in a patient with a demonstrated deficiency of hepatic tyrosine aminotransferase (E.C. 2.6.1.5) described by us4,5. Further work in our laboratories has enabled us to propose an alternative hypothesis for the production of the aromatic ketoacids in tyrosinaemia and also in phenylketonuria.

Our patient exhibited hypertyrosinaemia and markedly elevated urinary levels

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of pHPPA and related metabolites while possessing normal hepatic pHPP hydroxylase activity. Further studies showed that the hepatic cytosol tyrosine aminotransferase activity was deficient, but that the enzyme activity was present in normal amounts in mitochondria. These findings explained the hypertyrosinaemia but failed to explain the massive amounts of the ketoacid that were present in the patient's urine. In view of the fact that pHPP hydroxylase activity in the patient's liver was normal, how could we account for the large amounts of ketoacid in the urine?

We advance the following hypothesis which can account for these observations. pHPPA, formed by mitochondrial transamination of tyrosine in liver or kidney cells, would be oxidised further by the intact hydroxylase system of these tissues. However, the elevation of plasma tyrosine, resulting from the absence of hepatic cytosol tyrosine aminotransferase, would increase the amount of tyrosine which could be transaminated in other tissues, some of which are richly endowed with the mitochondrial form of the enzyme. If these latter tissues lacked pHPP hydroxylase activity, then pHPPA produced there, by the mitochondrial enzyme, would accumulate and eventually appear in the blood. Some of the ketoacid would then be filtered through the renal glomeruli and be excreted in the urine unless it was reabsorbed efficiently by the renal tubules. This hypothesis is illustrated diagrammatically in Fig. 1A.

To test this hypothesis, we measured the activity of pHPP hydroxylase and mitochondrial tyrosine aminotransferase in fresh rat and monkey and autopsied human liver, kidney, brain, heart and skeletal muscle tissue homogenates. Mitochondrial tyrosine aminotransferase activity was present in all tissues examined but pHPP hydroxylase activity was present only in the liver and kidney extracts (Table I).
TABLE I

DISTRIBUTION OF TYROSINE AMINOTRANSFERASE AND p-HYDROXYPHENYLPYRUVIC HYDROXYLASE ACTIVITIES

<table>
<thead>
<tr>
<th>Specie</th>
<th>n</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Muscle</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Mitochondrial tyrosine aminotransferase. Specific activity = μmoles product/30 min/mg protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>3</td>
<td>0.042</td>
<td>0.037</td>
<td>0.025</td>
<td>0.040</td>
<td>0.027</td>
</tr>
<tr>
<td>Monkey</td>
<td>6</td>
<td>0.019</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Human*</td>
<td>2*</td>
<td>0.028</td>
<td>0.011</td>
<td>0.027</td>
<td>0.002</td>
<td>0.014</td>
</tr>
<tr>
<td>B. p-Hydroxyphenylpyruvate hydroxylase. Specific activity = μmoles product/h/mg protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>2</td>
<td>1.16</td>
<td>0.34</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Monkey</td>
<td>7</td>
<td>2.24</td>
<td>1.23</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Human*</td>
<td>5*</td>
<td>0.82</td>
<td>0.08</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

n = Number of animals or human subjects tested. The values represent average specific activities observed.

* Autopsy tissue.

With a specific activity of 0.002 for human muscle mitochondrial tyrosine aminotransferase a muscle protein mass of 0.4 kg in a 10-kg child could theoretically account for the production of 7 g of pHPPA per day in muscle alone. Since this tissue contains no detectable hydroxylase activity, we could expect a correspondingly large excretion of pHPPA in the urine provided it is efficiently cleared by the renal tubules.

Gentz et al.⁶ have recently published data on a plasma disappearance curve and urinary excretion of pHPPA in a normal subject who received a single intravenous injection of this material. From their data, we have calculated that the renal clearance of pHPPA in this subject was 290 ml/min using the method of Dominguez et al.⁷. This value is more than twice the normal glomerular filtration rate and hence we conclude that pHPPA is not conserved well and may be secreted by the renal tubule.

The hypothesis can be extended to explain the excretion of phenylpyruvic acid in phenylketonuria. This disorder of phenylalanine metabolism is caused by a defect in hepatic phenylalanine hydroxylase activity. The disease is characterised by marked elevation of plasma phenylalanine and the excretion of phenylpyruvic acid and its metabolites in the urine. The commonly held belief is that the failure of hepatic phenylalanine hydroxylation directs the hepatic metabolism of the amino acid through transamination to phenylpyruvic acid, some of which is released into the circulation and excreted by the kidney. The liver, however, possesses phenylpyruvate hydroxylase (which is identical to pHPP hydroxylase)⁸ in sufficient quantity that all hepatic phenylpyruvic acid should be metabolised to o-hydroxyphenylacetic acid, leaving none of the ketoacid to appear in the urine. This explains the urinary output of o-hydroxyphenylacetic acid but raises some speculation regarding the origin of the urinary ketoacid.

Phenylpyruvic acid produced by hepatic or kidney transaminase should be oxidised to o-hydroxyphenylacetic acid in these tissues. However, the elevated plasma phenylalanine levels in this disease would lead to increased transamination of phenylalanine in other tissues⁹, such as muscle, brain and heart, which lack hydroxylase activity (see Table I). The phenylpyruvic acid so formed would then be released into the circulation and excreted in the urine, assuming adequate clearance by the kidney. This mechanism is illustrated in Fig. 1B. Further studies to investigate the renal handling of aromatic ketoacids are in progress.
REFERENCES
