Inhibition of beta-ureidopropionase by propionate may contribute to the neurological complications in patients with propionic acidaemia

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Inhibition of $\beta$-ureidopropionase by propionate may contribute to the neurological complications in patients with propionic acidaemia


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Propionic acidaemia is due to a primary deficiency of propionyl-CoA carboxylase (EC 6.4.1.3) activity. The clinical picture is characterized by repeated relapses and neurological sequelae are common. Among the neurological complications, focal and general seizures as well as EEG abnormalities are often observed. During relapse substantial accumulation of propionate occurs in all body fluids.

$\beta$-Ureidopropionase (UP, EC 3.5.1.6) is the third enzyme in the degradation pathway of uracil and thymine. It catalyses the degradation of both $\beta$-ureidopropionic acid and $\beta$-ureidoisobutyric acid to $\beta$-alanine and $\beta$-aminoisobutyric acid, respectively. A deficiency of UP or one of the other enzymes of pyrimidine degradation leads to a diminished production of $\beta$-alanine, a neurotransmitter amino acid.

Diminished production of $\beta$-alanine also occurs in other pyrimidine degradation defects and is presumed to be a contributing factor in the neurological abnormalities seen in the patients with those defects (Van Gennip et al 1997). Propionate has been reported to inhibit UP in Euglena gracilis (Wasternack et al 1979). We wondered whether inhibition of UP by propionate or $\beta$-hydroxypropionate could be demonstrated in vitro in human liver and in vivo in patients with propionic acidaemia.

MATERIALS

Patients: Patient J.R., a boy, was diagnosed as having propionic acidaemia at the age of 10 days. Despite adequate treatment the patient had frequent relapses with severe ketoacidosis, vomiting, lethargy and other neurological manifestations. During these relapses generally large amounts of characteristic metabolites were excreted (Table 1).

Patient J.V., a 24-year-old mentally retarded woman, was diagnosed as having a mild
form of propionic acidaemia. The patient’s history revealed severe problems in the perinatal and neonatal period, but since then relapses had not recurred. The organic aciduria was repeatedly mild without exacerbations (Table 1). Both the classical and the phenotype of the patient had very low activities of propionyl-CoA carboxylase in leukocytes (<1% residual activity).

Four urine samples of each patient were analysed for β-ureidopropionate. To investigate a possible storage effect, four control urines stored under the same conditions for a comparable period of time were also analysed.

Liver tissue: Frozen human liver from a transplant bank was available as control material for diagnostic investigations.

Chemicals: [2-14C]Dihydouracil was obtained from Moravek. The compound was purified before use by reversed-phase HPLC.

METHODS

Preparation of liver homogenate: A homogenate (20%, w/v) of frozen human liver was prepared in a buffer containing 10mmol/L MOPS–NaOH (pH 7.4), 1mmol/L EDTA, 10mmol/L dithiothreitol, 5mmol/L 4-(2-aminoethyl)benzenesulphonylfluoride hydrochloride and 10µg/ml leupeptin with the aid of a Teflon–glass homogenizer. After centrifugation (11000g at 4°C for 20min), the supernatant was removed and stored in liquid nitrogen until further analysis.

Determination of the activity of β-ureidopropionase: The activity of UP was determined in a reaction mixture containing 0.1mol/L Tris-HCl (pH 8.0), 1mmol/L dithiothreitol and 500µmol/L [2-14C]dihydouracil at 37°C. The reaction was started by injection of supernatant corresponding to 0.1–0.2mg protein into the mixture and after 1h of incubation was terminated by addition of 25µl of 10% (v/v) perchloric acid. 14CO2 was trapped in 2mol/L NaOH during incubation, and after termination 14CO2 trapping was continued at 4°C for 2h. Radioactivity of the trapped carbon dioxide was measured by liquid scintillation counting. The reaction mixture was centrifuged (11000g for 5min) to remove the protein and the supernatant was stored at −20°C until HPLC analysis.

The analysis of radiolabelled dihydouracil and radiolabelled β-ureidopropionate was accomplished by HPLC on a Supelcosil LC-18-S Column (250×4.6mm, 5µm particle size, Supelco Inc., Bellefonte, PA, USA) with 50mmol/L NaH2PO4 (pH 4.5) at a flow rate of 1ml/min. Radioactivity was detected on-line with a Radiomatic 525 TR detector (Packard Instrument Company, Meriden, CT, USA) equipped with a 500µl liquid flow cell. Full details of the method will be published elsewhere. To test the effect of propionate and β-hydroxypropionate, respectively, on the activity of UP in human liver, increasing amounts of propionate and β-hydroxypropionate were added to the homogenate before the incubation.

Organic acids: The index metabolites for propionic acidaemia were measured as TMS derivatives, after their extraction from urine with ethyl acetate (Wadman et al 1984).

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Pyrimidine catabolites: Dihydropyrimidines (dihydouracil, dihydrothymine) and N-carbamyl-β-amino acids (β-ureidopropionate, β-ureidoisobutyrate) were determined by amino acid analysis after their isolation and conversion into the corresponding β-amino acids as previously described (Van Gennip et al 1993).

RESULTS AND DISCUSSION

As shown in Figure 1 increasing amounts of propionate resulted in an increased production of radiolabelled β-ureidopropionate versus a diminished production of radiolabelled CO₂. In contrast, β-hydroxypropionate added in comparable amounts had no effect. The concentrations of propionate and β-hydroxypropionate used in these experiments are of the same magnitude as are assumed to occur in the liver of patients with propionic acidaemia. These results therefore indicate that UP may be inhibited in these patients. The findings are in accordance with the reported inhibition of UP by propionate in Euglena gracilis (Wasternack et al 1979). The results of the measurements of β-ureidopropionate in the urine samples of the patients with propionic acidaemia and controls are presented in Table 1. The excretion of β-ureidopropionate appeared to be elevated in three out of the four samples of patient J.R. with severe propionic acidaemia, but normal in the samples of patient J.V. with mild propionic acidaemia. The elevated excretion of β-ureidopropionate in patient J.R. suggests that, at least during relapse, inhibition of UP can occur.
Although patients with UP deficiency have not yet been described, because of the frequent occurrence of neurological symptoms in patients with other pyrimidine degradation defects (Van Gennip et al. 1994) it is reasonable to assume that UP-deficient patients are likely to present neurological symptoms as well. A shortage of $\beta$-alanine caused by these defects could be an aetiological factor in the neurological symptomatology.

**REFERENCES**


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**Table 1. Urinary $\beta$-ureidopropionate vs $\beta$-hydroxypropionate and methylcitrate in a child (J.R.) with the classic, severe and an adult (J.V.) with mild presentation of propionic acidemia**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\beta$-Hydroxypropionate</th>
<th>Methylcitrate</th>
<th>$\beta$-Ureidopropionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.R.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3000</td>
<td>1857</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>4389</td>
<td>6111</td>
<td>163</td>
</tr>
<tr>
<td>3</td>
<td>203</td>
<td>448</td>
<td>122</td>
</tr>
<tr>
<td>4</td>
<td>234</td>
<td>516</td>
<td>136</td>
</tr>
<tr>
<td>J.V.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>170</td>
<td>163</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>568</td>
<td>135</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>780</td>
<td>101</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>723</td>
<td>131</td>
<td>23</td>
</tr>
<tr>
<td>Storage controls* ($n=4$)</td>
<td></td>
<td></td>
<td>11–60</td>
</tr>
</tbody>
</table>

*Urines of controls stored under the same conditions for a comparable period of time*