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Short Communication

Inhibition of β-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.

β-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 β-ureidopropionic acid and β-ureidoisobutyric acid to β-alanine and β-aminoisobutyric acid, respectively. A deficiency of UP or one of the other enzymes of pyrimidine degradation leads to a diminished production of β-alanine, a neurotransmitter amino acid.

Diminished production of β-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 β-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 phenotypically mild 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-^{14}C]Dihydrouracil 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, 10 mmol/L dithiothreitol, 5mmol/L 4-(2-aminoethyl)benzenesulphonylfluoride hydrochloride and 10 μg/ml leupeptin with the aid of a Teflon–glass homogenizer. After centrifugation (11 000*g* 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 500 μmol/L [2-^{14}C]dihydrouracil 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 25 μl of 10% (v/v) perchloric acid. $^{14}CO_2$ was trapped in 2mol/L NaOH during incubation, and after termination $^{14}CO_2$ trapping was continued at 4°C for 2 h. Radioactivity of the trapped carbon dioxide was measured by liquid scintillation counting. The reaction mixture was centrifuged (11 000*g* for 5min) to remove the protein and the supernatant was stored at −20°C until HPLC analysis.

The analysis of radiolabelled dihydrouracil and radiolabelled β-ureidopropionate was accomplished by HPLC on a Supelcosil LC-18-S Column (250×4.6mm, 5 μm particle size, Supelco Inc., Bellefonte, PA, USA) with 50mmol/L NaH_2PO_4 (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 500 μl 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).

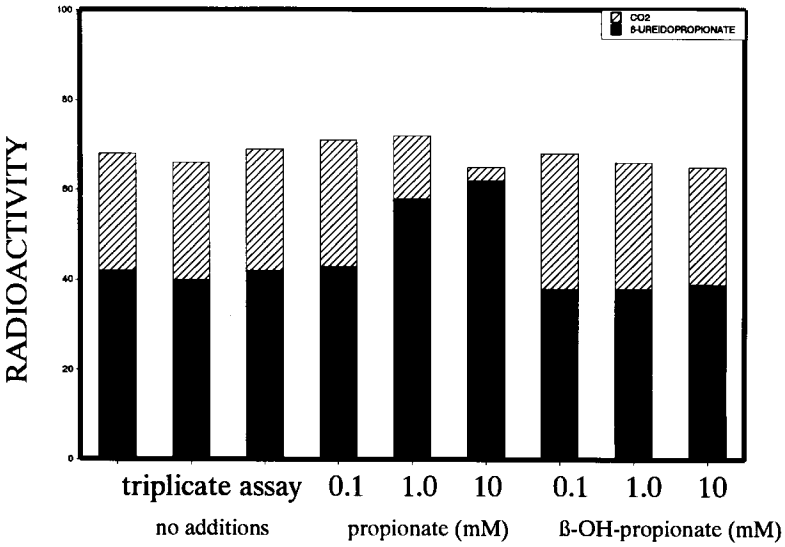

Figure 1 Effect of propionate or β-hydroxypropionate on UP activity in a homogenate of human liver. The height of each bar represents the activity of dihydropyrimidinase (nmol/h per mg protein), which was calculated on the radioactivity incorporated into CO_2 (hatched bars) and β-ureidopropionate (solid bars). The ratio of the radioactivity incorporated into CO_2 vs β-ureidopropionate is a measure of the activity of β-ureidopropionase. The scale on the ordinate is chosen arbitrarily

Pyrimidine catabolites: Dihydropyrimidines (dihydrouracil, 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 CO_2. 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.

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

	Concentration (μmol/mmol creatinine)		
Sample	β-*Hydroxypropionate*	*Methylcitrate*	β-*Ureidopropionate*
J.R.			
1	3000	1857	60
2	4389	6111	163
3	203	448	122
4	234	516	136
J.V.			
1	170	163	21
2	568	135	24
3	780	101	30
4	723	131	23
Storage controls[a] ($n=4$)			11–60

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

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 β-alanine caused by these defects could be an aetiological factor in the neurological symptomatology.

REFERENCES

Van Gennip AH, Busch S, Elzinga L, et al (1993) Application of simple chromatographic methods for the diagnosis of defects of pyrimidine degradation. *Clin Chem* **39**: 380–385.

Van Gennip AH, Abeling NGGM, Stroomer AEM, Van Lenthe H, Bakker HD (1994) Clinical and biochemical findings in six patients with pyrimidine degradation defects. *J Inher Metab Dis* **17**: 130–132.

Van Gennip AH, Abeling NGGM, Vreken P, Van Kuilenburg ABP (1997) Genetic metabolic disease of pyrimidine metabolism: implications for diagnosis and treatment. *Int Pediatr* ***12***: 35–40.

Wadman SK, Beemer FA, De Bree PK, et al (1984) New defects of pyrimidine metabolism. *Adv Exp Med Biol* **165**: 109–114.

Wasternack C, Lippmann G, Reinbotte H (1979) Pyrimidine degrading enzymes: purification and properties of β-ureidopropionase of *Euglena gracilis. Biochim Biophys Acta* **570**: 341–351.