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Biotin-dependent organic aciduria

Excretion of Organic Acids Associated with Biotin Deficiency in Chronic Anticonvulsant Therapy*

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Summary: Urinary organic acids, known to be elevated in children with biotin deficiency, were determined in 7 epileptics under long-term therapy with anticonvulsants and in three controls. Four patients administered phenytoin, primidone, phenobarbital, or carbamazepine, alone or in combination, had reduced plasma biotin levels (<250 ng/l) and an elevated excretion of certain organic acids indicating a possibly decreased activity of propionyl CoA carboxylase (3-OH-propionate, methylcitrate) and 3-methylcrotonyl CoA carboxylase (3-methylcrotonate and the glycine conjugate, 3-OH-isovalerate). Two epileptics receiving sodium valproate alone had normal circulating biotin levels and no changes in level of the investigated urinary acids were found. These findings indicate that the reduced biotin levels seen in epileptics receiving other anticonvulsants than sodium valproate lead to an elevated excretion of certain organic acids in urine.

Introduction

In the urine of patients with an inborn error of metabolism of biotin-dependent carboxylases elevated concentrations of organic acids (e.g. methylcitrate, 3-hydroxypropionate, propionylglycine, tiglic acid, 3-methylcrotonic acid, 3-hydroxyisovaleric acid) are found [1]. These metabolites are excreted due to a blockage in the catabolism of branched chain amino acids and are caused by a reduced activity of the biotin-dependent enzymes propionyl CoA carboxylase and 3-methylcrotonyl CoA carboxylase (Figure 1).

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Pyruvate carboxylase deficiency has also been described as being accompanied by a typical urinary pattern, especially by an elevation of lactic acid [13] (Fig. 1). The regulation of fatty acid synthesis at the acetyl CoA carboxylase step is well known but its clinical relevance has not yet been elucidated. A deficient holocarboxylase synthetase, which attaches biotin to the apocarboxylase, or reduced circulating biotin levels due to an altered absorption, transport, and/or metabolism of biotin can be the reasons for the reduced carboxylase activities in patients with an inborn error of metabolism [1]. Some of the above mentioned organic acids have also been reported in the urine of a boy with a dietary biotin deficiency [9] and in a girl with short-gut syndrome during parenteral alimentation without biotin [8]. In both patients low circulating biotin levels and a reduced activity of biotin-dependent enzymes were found.

A marked reduction of plasma biotin concentrations has been noted in epileptics on long-term therapy with anticonvulsants [6, 7]. The purpose of this study was to evaluate whether epileptics with low plasma biotin levels also excrete abnormal organic acids in their urine.

Patients and methods

24-h-samples of urine were collected from seven long-term treated epileptics and from three controls (two males, aged 30 and 34 years, one female, aged 27 years). The clinical data of the patients are summarized in Table I. In the morning after urine collection blood was taken for the microbiological determination of plasma biotin levels, using Lactobacillus plantarum as test organism [4], and for measuring serum concentrations of anticonvulsants.

Tab. 1: Clinical data of the patients

Patient	age (years)	sex	type of seizures	anticonvulsants	duration of treatment (years)	daily intake at time of examination (mg/kg body weight)
A	25	male	partial seizures with complex symptomatology and tonic-clonic seizures	phenytoin phenobarbital	24 24	4.2 1.0
B	37	male	partial seizures with complex symptomatology	primidone carbamazepine	12 2	10.1 13.5
C	28	male	tonic-clonic seizures	phenytoin	1	4.8
D	30	male	partial seizures with complex symptomatology and tonic-clonic seizures	phenytoin carbamazepine	0.1 5	5.4 none
E	24	female	complex absences, partial seizures with complex symptomatology and tonic-clonic seizures	phenytoin sodium valproate	7 10	5.8 34.6
F	25	male	tonic-clonic seizures	sodium valproate	3	15.6
G	22	male	tonic-clonic seizures	sodium valproate	3	9.8

Determination of organic acids, which are known to be involved in inborn errors of biotin-dependent enzymes, or nutritional biotin deficiency, was carried out by combined gaschromatography and mass spectroscopy: Aliquots of the urines containing 5 mg of creatinine were lyophilized, then acidified to pH 2,5 with H_2SO_4 and separated on a column of silicic acid into 4 fractions using 2-methyl-2-butanol/chloroform mixtures as eluents [5].

The fractions were evaporated until dry, following which the TMS derivatives formed by addition of MSTFA (Machery & Nagel) were analyzed by GC/MS on a 50 m glass-capillary SE-54 (Jaegi, Trogen, Switzerland) with a Du Pont 21-492 B mass-spectrometer (Programm 75° (10 min), 4°/min up to 220° C). Identification and quantification were performed by multiple specific ion detection (MSID) of the characteristic mass-ions of the acids to be measured. Calibrations of the available reference compounds were performed as for samples. The internal standards (dibutylacetic acid and C_{19} alkane) were added after silicic acid chromatography and before evaporation of the chromatographic fraction. Reference compounds were not available for 3-methylglutaconic acid and propionylglycine. Identification of both compounds was confirmed by mass-spectrometric analyses of enriched samples. For quantification of propionylglycine (m/z 159) isobutyrylglycine (m/z 158) was used as reference due to the almost equal intensities of the mentioned mass-ions. In the case of 3-methylglutaconic acid (cis + trans) 3-methylglutaric acid was applied using the M^+-15 mass-ions for quantification; probable error was assumed to be less than ±30%.

Results and Discussion

The results of the determination of organic acids in urine are summarized in table II, together with the serum concentrations of anticonvulsants and the plasma biotin levels. As expected from our previous study [7], plasma biotin levels in the five epileptics (patients A–E) treated with phenytoin, primidone, phenobarbital or carbamazepine were clearly lower than in the two patients receiving sodium valproate alone (F + G) or in the three controls. Four of the five epileptics (A–D) had a reduced plasma biotin concentration of less than 250 ng/l.

A deficient activity of the propionyl CoA carboxylase might be deduced from elevated amounts of methylcitric acid and 3-OH-propionic acid in 3 cases (A, B, C). In contrast to our controls, propionylglycine could be detected in 2 patients (A, C). The excretion rates of tiglic acid and its glycine conjugate are too small to result in a clear indication, although the highest values of tiglic acid have been found in patients A and B. 3-OH-2-methylbutyric acid was distinctly elevated only in patients B and C. A possibly influenced activity of the 3-methylcrotonyl CoA carboxylase was indicated by elevation of 3-OH-isovaleric acid in 3 cases (A, B, C), of 3-methylcrotonic acid in 4 cases (A, B, C, D) and of its glycine conjugate in patients B and D. The determinations of the metabolites following the 3-methylcrotonyl CoA carboxylase reaction (see Fig. 1) revealed, that in one patient (C) both metabolites were absent; in 2 other (B, D) 3-OH-3-methylglutaric acid was detectable in very little amounts, whereas the precursor was shown to be absent. High values of lactic acid were found in patients A–D; that seems to be consistent with decreased activity of the pyruvate carboxylase.

Little is known about the connection between reduced biotin levels and changes in excretion of metabolites in consequence of decreased activity of carboxylases [1, 8,

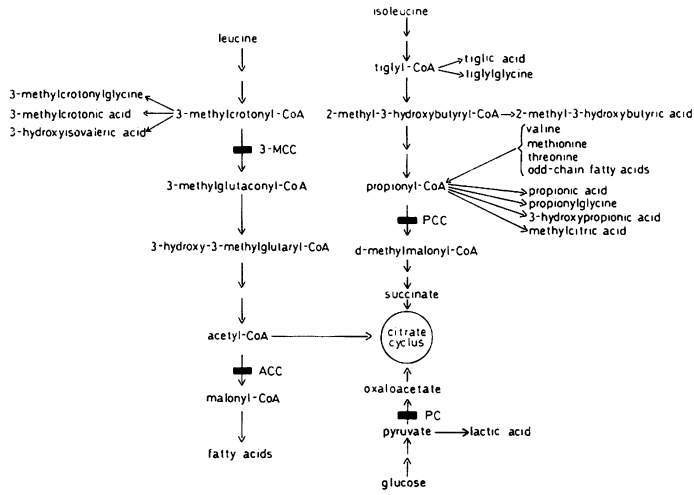

Fig. 1: Metabolic pathways involving biotin-dependent carboxylases (3-MCC = 3-methylcrotonyl CoA carboxylase, PCC = propionyl CoA carboxylase, PC = pyruvate carboxylase, ACC = acetyl CoA carboxylase)

9, 11]. Both the pattern and the concentration of organic acids excreted in urine were found to vary greatly in children with an inborn error of biotin-dependent enzymes, being dependent on protein intake or clinical status [1]. Therefore, not so much the individual concentrations or the pattern of organic acids excreted seem to be important, but the fact that in the urine of the epileptics under phenytoin, primidone, phenobarbital, or carbamazepine treatment, indications are given of higher levels of some organic acids possibly demonstrating a deficient activity of the carboxylases, especially of the propionyl CoA carboxylase, in which several increased metabolites support the assumption. In contrast, the 2 patients (F and G) on sodium valproate monotherapy exhibited no excretion rates indicating a possibly reduced activity of the carboxylases. As their plasma biotin levels were found to be normal a reduced enzymatic activity would not be expected. The reduced activity of the 3-methylcrotonyl CoA carboxylase in patients B, C and D seems furthermore to be indicated by the lower excretion of 3-methylglutaconic acid and 3-hydroxy-3-methylglutaric acid, which appear after the carboxylation step in the catabolism of leucine, when compared to controls.

The results of patient E, treated with sodium valproate and additional phenytoin, were intermediate between those of controls and epileptics given sodium valproate alone and those of the remaining epileptics: 3-hydroxyisovaleric acid and tiglic acidwere found to be higher in the urine of patient E. The excretion of 3-methylglutaconic acid and of 3-hydroxy-3-methylglutaric acid was comparable to that of patients F and G. These findings indicate that the activity of the biotin-

Tab. II: Concentrations of anticonvulsants (PHT = phenytoin, PB = phenobarbital, PRM = primidone, CBZ = carbamazepine, VPA = sodium valproate) in serum, biotin in plasma and organic acids in urine (μmol/mg creatinine) (n.d. = not detectable)

	patients A	B	C	D	E	F	G	controls 1	2	3	normal values from the literature
anticonvulsants in serum (μg/ml)	PHT 6.1 PB 9.9	PRM 8.1 PB 30.0 CBZ 7.6	PHT 12.6	PHT 46.2	VPA 53.6 PHT 15.6	VPA 75.4	VPA 50.7	—	—	—	
biotin in plasma (ng/L)	<150	240	197	198	280	343	325	327	305	413	>250 [7]
propionylglycine	0.004	n.d	0.002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.01 [10]
methylcitric acid	0.07	0.03	0.05	trace	n.d.	trace	n.d.	n.d.	n.d.	n.d.	<0.01 [10]
3-hydroxypropionic acid	0.025	0.03	0.09	trace	trace	n.d.	n.d.	n.d.	0.001	n.d.	<0.01 [3]
3-hydroxy-2-methylbutyric acid	0.004	0.04	0.03	0.004	0.004	0.005	0.008	n.d.	0.003	0.002	
tiglic acid	0.003	0.003	n.d.	n.d.	0.001	n.d.	n.d.	n.d.	trace	n.d.	<0.001 [10]
tiglylglycine	n.d.	trace	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.01 [10]
3-methylcrotonic acid	0.01	0.05	0.02	0.04	n.d.	0.004	n.d.	n.d.	trace	n.d.	
3-methylcrotonylglycine	n.d.	0.09	trace	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.01 [11]
3-hydroxyisovaleric acid	0.37	0.42	0.22	0.15	0.17	0.06	0.06	0.03	0.01	0.1	<0.09[2]
3-methylglutaconic acid	trace	n.d.	n.d.	n.d.	0.005	0.01	0.006	trace	trace	trace	
3-hydroxy-3-methylglutaric acid	0.01	trace	n.d.	0.002	0.03	0.03	0.02	0.01	0.003	0.02	<0.01 [10]
lactic acid	0.74	0.61	0.65	0.84	0.3	0.4	0.35	0.19	0.15	0.3	<0.22 [12]

dependent enzymes is less reduced in patient E than in the epileptics treated with anticonvulsants other than sodium valproate; this is in accordance with the intermediate plasma biotin level of 280 ng/l detected in patient E.

For the first time an elevated excretion of some organic acids, known to occur in the urine of children with an inborn error of metabolism of biotin-dependent enzymes or of children with a dietary biotin deficiency, has been detected in epileptics treated with phenytoin, primidone, phenobarbital or carbamazepine. This elevated excretion seems to be caused by the reduced circulating biotin levels found in these epileptics. Whether a connection exists between the reduced plasma biotin levels and the anticonvulsant efficacy of these drugs remains to be studied.

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