Clinica Chimica Acta, 129 (1983) 303–309
Elsevier Biomedical Press

CCA 2480

A new patient with 4-hydroxybutyric aciduria, a possible defect of 4-aminobutyrate metabolism

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(Received August 30th; revision December 31st, 1982)

Summary

A new patient with 4-hydroxybutyric aciduria is described, adding further evidence that 4-hydroxybutyric aciduria is a clinical entity. Main clinical symptoms were: motor and mental retardation, muscular hypotonia and ataxia. 4-Hydroxybutyric acid was increased in urine, plasma and cerebrospinal fluid.

Glutamic acid was shown to be a precursor and on valproate therapy concentrations in plasma and urine seemed to be enhanced.

Introduction

The excretion of 4-hydroxybutyrate (4HB) in a child with neurological abnormalities has been recently reported by Jakobs et al [1]: their patient was the 20-month-old son of related parents. He had a retarded motor and mental development, marked muscular hypotonia and ataxia. Examination of the urinary organic acids by gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC/MS) showed elevated amounts of 4HB in urine, serum and cerebrospinal fluid (CSF). The authors postulated a new inborn error of metabolism: a deficiency of succinic semialdehyde dehydrogenase (SSADH).

We wish to report metabolic studies in another patient with 4HB excretion and a similar clinical picture, adding further evidence that 4-hydroxybutyric aciduria is a clinical entity, presumably of an inherited character.

Case report

B.R., a boy, was born in 1975 as the second child of related Algerian parents. The delivery occurred at home and fetal distress with hypotonia was reported in the neonatal period.

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At 12 months he was hospitalized elsewhere for evaluation of his hypotonia and psychomotor retardation. During hospitalization an abnormal colour of the urine varying from green to dark green and reddish brown was observed. Screening tests for alkaptonuria, hemoglobinuria, and porphyria were all negative. In 1977 he was admitted to Hôpital Debrousse for investigation of his mental retardation, muscular hypotonia, cerebellar syndrome and ataxia. Screening of metabolite profiles revealed no major abnormalities. However, an unidentified small peak was noticed in the gas chromatogram of the volatile fatty acids. GC/MS was not available at that time.

In 1980 he was investigated again. The same peak was found in the volatile fatty acid profile of urine, plasma, and CSF. At that time the patient's epilepsy was treated with dipropylacetate (valproate) and the height of the excretion of the unkown product seemed to be somewhat related to this treatment. GC/MS analysis of urinary organic acids as methyl esters revealed no major abnormalities but the interpretation of the GC/MS profile was made difficult because of the excretion of the numerous valproate metabolites.

After withdrawal of the treatment a new urine sample was obtained in which the presence of 4HB was demonstrated.

Now this patient is hospitalized in an institute for mentally retarded children. His clinical status as well as the urinary excretion of 4HB have remained unchanged.

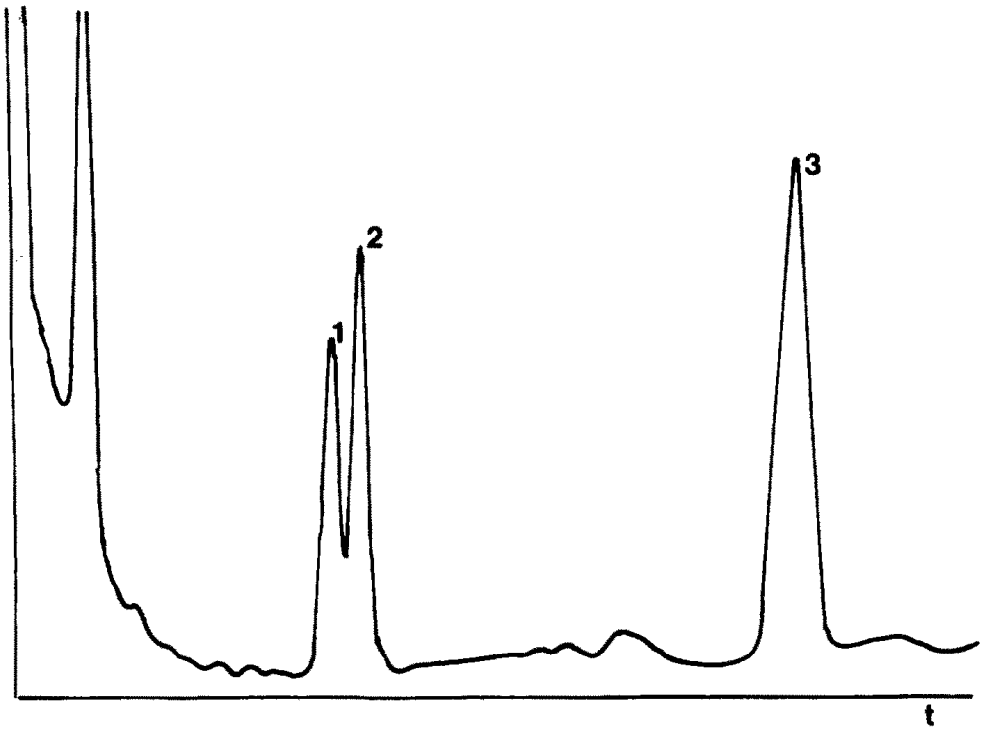

Fig. 1. Gas chromatogram of volatile fatty acids in plasma of R.B. on treatment with valproic acid; 1, butyrolactone (t_R 7.5 min); 2, valeric acid (internal standard, t_R 8.4 min); 3, valproic acid (t_R 18.5 min).

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Methods

Amino acids were analysed in plasma by ion-exchange chromatography using an automated amino acid analyser, Biotronik LC 6000.

Volatile fatty acids (VFA) were analysed in urine, plasma and cerebrospinal fluid using steam distillation and GC on neopentyl glycol adipate/H_3PO_4 as described previously [2].

Analyses of 4HB in urine, plasma, and CSF were done by GC after transformation of 4HB in butyrolactone as described by Lettieri et al [3].

To 200 μl of the sample were added: 0.4 ml of concentrated H_2SO_4; after cooling 0.4 ml of 1.2 mol/l KOH followed by 0.05 ml of internal standard (valerolactone 0.2 mg/ml). Extraction was performed by $CHCl_3$ (2 × 1 ml). The pooled organic phases were evaporated to approximately 0.25 ml. A volume of 2 μl was injected into the gas chromatograph. GC conditions: 2-m glass column filled with 10% diethylene glycol succinate, oven temperature 140°C isothermal, injection port and detector temperature 220°C.

GC/MS of the trimethylsilyl-derivatives of the urinary organic acids was performed as described previously [4,5].

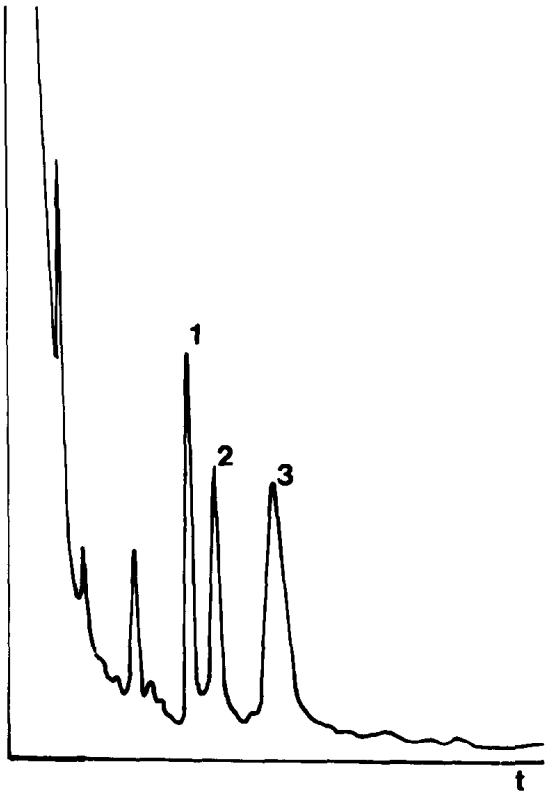

Fig. 2. Gas chromatogram of lactones in plasma of patient R.B. on treatment with valproic acid; 1, valerolactone (internal standard, t_R 4.7 min), 2, butyrolactone (t_R 5.4 min); 3, 4-hydroxyvalproatelactone (t_R 6.7 min).

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Results

A chromatogram of volatile fatty acids in plasma is given in Fig. 1. The 'unknown' peak emerging before the internal standard, valeric acid, has been found in all the patient's samples. Its concentration seemed to be higher when the patient was on valproate therapy.

Injection of an authentic standard of 4HB produced a peak in the same position. Under the conditions of acidic pH and the high temperature of the injector, 4HB is converted to gamma-butyrolactone. The absolute identification of the butyrolactone was performed by GC/MS. Urinary 4HB was measured by the method described above. A linear response was obtained for concentrations from 0.1 mmol/l to 2 mmol/l. A gas chromatogram is shown in Fig. 2. The results of 4HB determinations in plasma, CSF and urine are summarised in Table I. In normal plasma and urine 4HB is absent. This has been checked in 10 normal children and in 20 children on valproate therapy. No 4HB was detected in the CSF of two patients with neurological disease.

The patient was loaded with L-glutamic acid (100 mg/kg per os). Glutamic acid and 4HB were monitored in plasma. The results are summarised in Table II. The

TABLE I

CONCENTRATIONS OF 4HB IN BODY FLUIDS

The samples from 1979 and 1980 had been stored at −20°C, those collected in 1981 were analyzed immediately.

Sample data	Valproate therapy (mg/day)	4-Hydroxybutyrate		
		plasma (μmol/l)	urine (mmol/mol creat.)	CSF (μmol/l)
13-12-79	600	301	180	
14-12-79	0			
20-12-79	0	240		
15-1-80	600	282		
16-1-80	0	374		
17-1-80	0	172	192	
21-1-80	0	194	90	245
8-6-81	400	172	153	
12-6-81	400	220	287	
13-6-81	0		239	
14-6-81	0		94	
15-6-81	0		52	
17-6-81	0		62	
18-6-81	0		69	350
19-6-81	0	128	116	
20-6-81	0		57	
23-6-81	0	154		
24-6-81	0	149	45	
25-6-81	0		107	

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TABLE II

RISE OF 4HB AFTER A LOAD OF L-GLUTAMIC ACID (100 mg/kg per os)

Plasma	4-OH-Butyric acid (μmol/l)		Glutamic acid (μmol/l)		Glutamine (μmol/l)	
	patient	control	patient	control	patient	control
T: 0	149	ND	56	80	474	250
+1 h	166	ND	70	89	505	280
+2 h	171	ND	82	92	485	259
+3 h	179	ND	62	112	490	319
+4 h	247	ND	162	102	400	318
+48 h	170	ND	–	–	–	–

concentration of 4HB increased significantly while in the plasma of the control no 4HB could be detected.

Discussion

Until now, 4HB has been found in measurable amounts in human biological fluids in three subjects [1]. Two of them were only briefly mentioned. The clinical picture of the first patient described was similar to that of the present patient. Both exhibited mental retardation, muscular hypotonia, a cerebellar syndrome and ataxia in spite of the fact that our patient had lower 4HB values that the first one of Jakobs et al. However, the order of magnitude of CSF 4HB is the same (Table III).

In the patient of Jakobs et al, urinary excretion of 4HB was nearly 35 times higher than succinic semialdehyde (SSA). In our patient urinary SSA was below the detection limit of 30 μmol/l.

4HB has been found in mammalian brain and is known to be a metabolite of 4-aminobutyric acid. The difference in 4HB concentrations between cerebrospinal fluid and plasma can be considered to be indicative of the cerebral origin of this substance. Its biosynthesis via the transamination product SSA has recently been reviewed [6]. Under normal conditions the SSA concentration in brain is very low, 10^{-10} mol/g according to Matsuda and Hoshino [7]. Its metabolism is under the control of three enzymes: SSADH and two SSA reductases.

TABLE III

4-OH-BUTYRIC ACID (μmol/l)

	Patient of Jakobs et al	Our patient B.R.
Plasma	943	130
CSF	596	350
Urine	2702	780

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