Inherited 3-methylglutaconic aciduria in two brothers—Another defect of leucine metabolism

Two brothers, aged 7 and 5 years, who excreted large amounts of the leucine metabolites 3-methylglutaconic acid, 3-methylglutaric acid, and 3-hydroxyisovaleric acid, are described. The excretion of these metabolites could be enhanced by increasing the leucine intake. Restriction of the protein intake resulted in a marked reduction of the metabolite excretion. However, the excretion of the ultimate leucine metabolite, 3-hydroxy-3-methylglutaric acid, remained unchanged at a low level. The only clinical abnormality was speech retardation. A (partial) deficiency of 3-methylglutaconyl coenzyme A hydratase is proposed to be the most likely underlying defect.

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THE PRESENT KNOWLEDGE of the inherited disorders of leucine metabolism has been collected by the careful study of patients who were suspected to have a so-called organic acidemia.[1] The most prominent symptoms have included metabolic acidosis with or without ketosis or hypoglycemia, feeding difficulties, neurologic abnormalities, and an abnormal body odor. Branched-chain ketoaciduria, isovaleric acidemia, 3-methylcrotonyl-CoA carboxylase deficiency, and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (Figure) have been well documented. All defects can be observed as a severe (and often lethal) neonatal form or as a more benign, late-onset variant. There appears to be a positive correlation between the rate of metabolite production and the administration of the parent amino acid, leucine. One disorder of leucine metabolism, presumed 3-methylglutaconyl-CoA hydratase deficiency, has not yet been studied in detail because of the limited number of patients.[2,3] The excretion of 3-methylglutaconate by the first patient was only transient (B. H. Robinson, personal communication), whereas the other two patients excreted very small amounts. The latter finding probably represents only a minor biochemical lesion, which is difficult to correlate with the severe clinical findings in those patients.

We describe two brothers with excessive excretion of 3-methylglutaconic acid, 3-methylglutaric acid, and 3-hydroxyisovaleric acid. The only clinical abnormality in these patients was retarded speech development.

CASE HISTORIES

Patient 1, a 7-year-old boy, was the second child of healthy, nonconsanguineous Moroccan parents. The neonatal period was unremarkable, but at the age of one year, when still in Morocco, he had an attack of unconsciousness which lasted for almost a day. His psychomotor development was reported to be delayed: he started to walk at the age of 2. In that period the family emigrated to the Netherlands. The patient was admitted at the age of 7 years for an evaluation of speech retardation. His speech consisted of single words only. On psychologic testing he had a short attention span. He had nocturnal enuresis. On physical examination no abnormalities were found. Routine laboratory investigations gave normal results. Results of the electroencephalogram was normal. Screening for inherited metabolic disorders revealed an abnormal urinary organic acid profile with excessive amounts of 3-methylglutaconic acid, 3-hydroxyisovaleric acid, and less 3-methylglutaric acid. The excretion of 3-hydroxy-3-methylglutaric acid appeared to be normal. The blood glucose concentration decreased to 3 mmol/L upon fasting for 18 hours, with a simultaneously

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Table. Urinary excretion of leucine metabolites in two brothers with 3-methylglutaconic aciduria

	3-Methyl-glutaconic acid	*3-Methyl-glutaric acid*	*3-Hydroxy-isovaleric acid*	*3-Hydroxy-3-methyl-glutaric acid*
Patient 1				
Basal excretion	4,587-7,429	40-80	1,269-1,819	44-71
High-protein intake	13,305	101	2,704	64
Fasting experiment	13,543	116	2,727	78
0-12 hr after leucine	20,581	125	4,281	58
12-24 hr after leucine	17,991	140	2,169	50
Patient 2				
Basal excretion	6,736-8,221	42-63	1,523-2,334	95-114
High-protein intake	19,221	128	3,367	78
Protein restriction	3,339	48	691	34
0-12 hr after leucine	26,361	173	4,125	72
12-24 hr after leucine	23,936	237	3,114	78
Controls (2-13 yr)	50	nd	72 ± 35	85 ± 65

Concentrations are given in μmol/gm creatinine.

occurring compensated metabolic acidosis (base excess of −7.7 mmol/L). At that time the patient had the clinical symptoms of hypoglycemia, such as profuse sweating.

Patient 2 was the younger brother of the first patient. He had no physical abnormalities. His speech development, tested at a chronologic age of 5.4 years, appeared to be retarded: receptive language 3.9 years, expressive language 2.4 years, number of words 2.8 years, structure of the language 2.2 years. Prolonged fasting did not lead to hypoglycemia or metabolic acidosis. The organic acid excretion was similar to that of his older brother. Both children attend a school for children with speech and hearing disabilities.

Two more children in this family have completely normal development, including their speech. They excreted normal amounts of leucine metabolites.

METHODS

Urinary and plasma organic acids were analyzed by gas-liquid chromatography of the trimethylsilyl derivatives.[4] A quantitative conversion of 3-hydroxyisovalerate to its di-TMS derivative was achieved only with *N,O*-bis (trimethylsilyl) acetamide (BSA)/ pyridine/trimethylchlorosilane (5:1:0.5 v/v). The samples have to be analyzed immediately after derivatization, because a rapid decrease of the peaks representing 3-methylglutaconic acid takes place. All other clinical chemical determinations were done by routine methods.

RESULTS

The profile of urinary organic acids in Patient 1 showed large peaks of 3-methylglutaconic acid (8.8 mmol/L or 6.9 mmol/gm creatinine), 3-hydroxyisovaleric acid (2.2 mmol/L or 1.8 mmol/gm creatinine), and a moderate amount of 3-methylglutaric acid (102 μmol/L or 80 μmol/gm creatinine). The excretion of 3-hydroxy-3-methylglutaric acid was not increased: 89 μmol/L or 70 μmol/gm creatinine. Comparable values were observed in the younger brother (Table). Other compounds of leucine degradation, such as 3-methylcrotonylglycine, could not be detected. An amino acid chromatogram of the plasma and urine gave normal profiles, especially with regard to the leucine concentrations.

An investigation of the urine from both parents and from the two healthy siblings while they ingested a normal diet revealed normal excretion levels of all leucine metabolites.

A determination of the activity of 3-hydroxy-3-methylglutaryl coenzyme A lyase in the patients' leukocytes failed to reveal a deficiency of this enzyme: the activities were 7.0 and 3.6 nmol/minute/mg protein, respectively (control value 8.6 nmol/minute/mg protein). An attempt was made to assay the 3-methylglutaconyl coenzyme A hydratase activity in leukocytes or cultured fibroblasts by following the backward reaction starting with 3-hydroxy-3-methylglutaryl coenzyme A. However, even normal cells did not show sufficient activity. A liver biopsy was not done for ethical reasons.

The following in vivo experiments were carried out in order to explore the nature of the disorder and the possible implications for treatment: (1) An increased protein intake in the form of natural foods (4 gm/kg) was given for two days. The excretion of 3-methylglutaconic acid in Patient 1 increased from 3 to more than 6 mmol/24 hours. A similar increase of excretion was observed in Patient 2. (2) Protein restriction (daily intake 1.5 gm/kg) day was attempted in Patient 2. On this regimen he excreted only 0.8 to 1.0 mmol/24 hours of 3-methylglutaconic acid. (3) Prolonged fasting in Patient 1 did not lead to hypoglycemia. The excretion of 3-methylglutaconic acid increased to 5.6

mmol/24 hours during the fast, possibly as a result of the release of muscle leucine. (4) An oral loading test with 100 mg/kg of L-leucine was performed. Peak levels of 3-methylglutaconic acid excretion were recorded at this stage: Patient 1 excreted 8.6 mmol of 3-methylglutaconic acid in the first 24 hours after loading, and Patient 2 excreted 7.9 mmol in the same period of time. Approximately 10% of the load was excreted in the form of leucine metabolites. There were no clinical side-effects of this loading test. The plasma leucine concentrations, which were normal under basal conditions in both patients (112 and 146 μmol/L, respectively), reached peak values of 819 and 875 μmol/L, respectively, in Patients 1 and 2 at 60 minutes after loading. Although these values were somewhat higher than those in controls, the leucine concentrations returned to baseline values at a normal rate in both patients.

During all experiments the excretion of 3-hydroxyisovaleric acid and of 3-methylglutaric acid followed the same course as that of 3-methylglutaconic acid. In contrast, the excretion of 3-hydroxy-3-methylglutaric acid remained stable (Table).

DISCUSSION

With this report the number of patients excreting abnormal amounts of 3-methylglutaconic acid without concomitant 3-hydroxy-3-methylglutaric aciduria has increased to five. Our patients excreted 10 to 50 times higher amounts of leucine metabolites than those of Greter et al.[3] There also has been a considerable variation in clinical presentation within this small group of patients. The first patients[2,3] developed normally during the first months of life, after which developmental regression started, leading to severe mental retardation and neurologic abnormalities. The only abnormality in our two patients—the delay in speech development—appeared to coincide with the metabolic abnormality, at least in this family.

Although the amount of leucine metabolites excreted was considerable, a balance study revealed that the presumed metabolic defect was only partial: approximately 10% of the ingested leucine was excreted in the form of metabolites.

The difference between our patients and 3-hydroxy-3-methylglutaryl-CoA lyase–deficient patients can be expressed in the ratio of 3-methylglutaconic acid to 3-hydroxy-3-methylglutaric acid excretion. In five lyase-deficient patients a mean ratio of 1.90 (range 0.44 to 3.73) was observed. The ratio in the present patients ranged from 59 to 366 (mean 181). However, the potential hazard of these calculations is exemplified by Truscott et al's patient,[5] who had a ratio of 3-methylglutaconic acid to

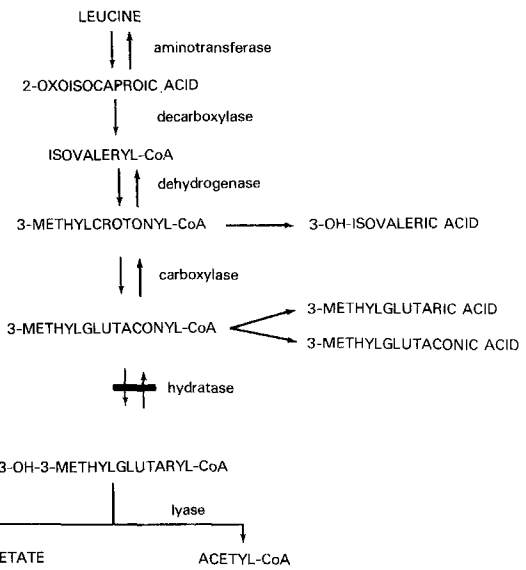

Figure. Metabolic pathway of leucine metabolism. The proposed defect is indicated.

3-hydroxy-3-methylglutaric acid of 2.56, but normal lyase activity. The latter patient did not excrete enhanced amounts of 3-hydroxyisovaleric acid, in contrast to our patients with 3-methylglutaconic aciduria and also the patients with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency.[6]

Our patients did not have consistent signs of hypoglycemia and had normal ketone body production. There was an increase of metabolite excretion during the fasting experiment, as was to be expected. Excessive muscle protein catabolism has been identified under fasting conditions. Metabolites which are characteristic of muscle protein breakdown, such as N^{τ}-methylhistidine, have not been measured in our patients.

Variation of the protein intake was promptly reflected in the amount of urinary leucine metabolites. Hence it is our opinion that the chemical abnormality in this condition can be partially reversed by reduction of the daily protein intake. The effect of protein restriction upon the clinical condition of the patients remains to be assessed in spite of our attempts. It was very difficult to motivate these Northern African people to adhere to the diet, perhaps in part because of the moderate clinical symptoms.

Delayed speech development has been associated with other inborn errors of metabolism, e.g., histidinemia[7] and formiminoglutamic aciduria.[8] Thus this clinical symptom is a valid reason to screen for inherited metabolic disease in the affected patients. Such a screening program should always include a chromatographic analysis of urinary organic acids, whether or not these patients have a metabolic acidosis.

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