Biochemical Evaluation of a Patient With a Familial Form of Leucine-Sensitive Hypoglycemia and Concomitant Hyperammonemia

Enrico Zammarchi, Luca Filippi, Elio Novembre, and Maria Alice Donati

A case of a child with recurrent episodes of severe hypoglycemia since the age of 6 months is reported. Biochemical evaluation extended to the first-degree relatives is consistent with a familial form of hypoglycemia due to a leucine-sensitive hyperinsulinism. In addition, this patient has a persistent elevation of serum ammonia levels of uncertain etiology that is more pronounced after meals. Urea cycle defects, organic acidurias, and β-oxidation defects have been ruled out, as well as a possible excessive deamination of glucogenetic amino acids. This unexpected hyperammonemia, which was also detected in the mother, might be related to leucine hypersensitivity.

LEUCINE-SENSITIVE hypoglycemia is presently classified among the hypoglycemias secondary to hyperinsulinism. In patients suffering from this metabolic disorder, there is an inappropriate insulin response to leucine ingestion with meals.

In 1956, Cochrane et al[1] speculated that some unexplained hypoglycemias could be due to protein-rich meals. This hypothesis was later confirmed by studies showing significant elevations of insulin levels in response to administration of L-leucine in patients suffering from otherwise unexplained hypoglycemic episodes.[2,3] Interestingly, such a response has also been identified in normal individuals[4]; leucine-sensitive hypoglycemia might then be due to the exaggeration of a physiologic phenomenon that could occur in situations such as pancreatic β-cell hyperplasia, adenoma, or nesidioblastosis.

We describe the case of a child with hypoglycemia secondary to a leucine-sensitive hyperinsulinism with a concomitant persistent hyperammonemia of uncertain nature. A similar, albeit less evident, picture is also present in the mother.

SUBJECTS AND METHODS

Assays

Measurements of plasma ammonia levels are based on the change in NADPH concentration due to enzymatic reductive amination of α-ketoglutarate to glutamate by glutamate dehydrogenase. This change is measured by UV spectrophotometry at 340 nm.[5] Venous blood collected without stasis was rapidly placed in a cold heparinized tube, and the plasma was separated in a refrigerated centrifuge. Measurements of plasma amino acid levels were performed by ion-exchange chromatography using a Carlo Erba (Milan, Italy) 3A30 automatic amino acid analyzer.[6] Gas chromatography/mass spectrometry (GC/MS) using a 3300 Varian Gas Chromatography (Walnut Creek, CA) and Ion Trap Detector Finnigan according to the method of Tanaka et al[7] was used to measure urinary organic acid levels. Urinary orotic acid and orotidine levels were measured with high-performance liquid chromatography method.[8] An allopurinol challenge test was performed according to a method previously described.[9,10] The leucine tolerance test was performed with oral administration of 150 mg/kg leucine; levels of glucose and insulin were measured before and 30, 60, and 90 minutes after leucine administration. Lactate, pyruvate, acetoacetate, and β-hydroxybutyrate were determined according to a semiautomatic[11] and miniaturized[12] method; readings were made by spectrophotometry (COBAS FARA; Roche, Basel, Switzerland).

Case Report

A 7-month-old boy was brought to us for evaluation of a seizure disorder. The parents are unrelated, and the family history is positive in the mother for nonfebrile seizures during infancy; moreover, she has always been reluctant to eat protein-rich meals. The baby was born at term by normal spontaneous vaginal delivery, with a birthweight of 2.680 g. Growth and development were normal. At the age of 6 months, he had his first generalized tonic-clonic seizure episode. He was found to have a normal electroencephalogram (EEG) and mild signs of cerebral damage on head computed tomographic scan, and was treated with phenobarbital.

He was brought to our institution for the first time at age 7 months for another seizure episode that occurred after a meal. On admission, his weight was 7.770 g (25th percentile) and head circumference was 42 cm (third percentile). Blood chemistry analyses showed a glucose level of 1.94 mmol/L (normal, 3.9 to 6.1) and an ammonia level of 205 μg/dL (normal, 10 to 80). On EEG, a diffuse slowing rhythm together with occasional paroxysmal activity was detected. During the first 24 hours after admission, he suffered from numerous tonic-clonic convulsive episodes, always after meals, with facial myoclonic movements, upper-limb jerking, occasional apneic episodes, cyanosis, and postictal hypotonia. During one of these episodes, a blood glucose level of 0.67 mmol/L was documented together with an insulin level of 60 mU/L (normal, 6 to 21), absent ketonuria, normal serum lactate and pyruvate levels, and a serum ammonia level of 200 μg/dL. On the basis of these findings, a diagnosis of hypoglycemia with hyperinsulinism was made. The serum amino acid profile was typical of hyperinsulinism,[13] with a low level of branched-chain amino acids (valine 86.2 μmol/L, normal 145 to 315; leucine 44.2 μmol/L, normal 76 to 176; isoleucine 25.7 μmol/L, normal 40 to 100). On abdominal ultrasound and computed tomographic scan, no pancreatic abnormality was detected.

A leucine tolerance test had to be interrupted due to symptomatic hypoglycemia (Fig 1). Phenobarbital administration was discontinued, and a diet with natural proteins containing less than 115 mg/kg/d leucine together with a synthetic leucine-free amino acid supplement was initiated. Within a few days, serum glucose levels stabilized in the range of 3.3 mmol/L, without the need for diazoxide administration. No further seizure episodes were noted,

From the Neurometabolic Diseases Unit, Department of Pediatrics, University of Florence, Florence, Italy.

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Address reprint requests to Enrico Zammarchi, MD, Dipartimento di Pediatria, Ospedale Anna Meyer, via Luca Giordano 13, 50132 Firenze, Italy.

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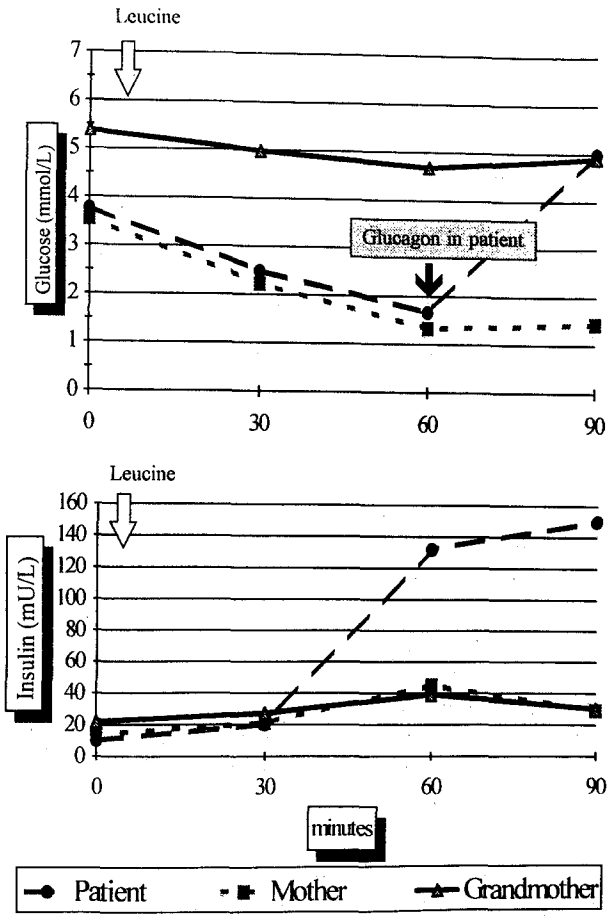

Fig 1. Glucose and insulin levels after oral leucine challenge test (150 mg/kg) in affected family members.

even though the infant had a mild motor delay and was somewhat hypotonic. Developmental milestones were otherwise appropriate for age, and the EEG picture was improved.

However, serum ammonia levels remained elevated, ranging between 130 and 250 μg/dL. Urinary excretion of orotic acid during the fasting state (0.074 mmol/mol creatinine), postprandially (0.79 mmol/mol creatinine), and after a protein challenge test (0.61 mmol/mol creatinine) was consistently normal. Urinary organic acids determined by GC/MS were normal, whereas plasma amino acid quantitative analysis showed occasional increased levels of glutamine (up to 1,097 μmol/L; normal, 420 to 906).

To test the possibility that the leucine-free amino acid supplement might be contributing to the hyperammonemia, it was suspended, with no improvement.

A leucine load test performed in both parents and grandparents showed a significant increase in serum insulin levels in the mother and the maternal grandmother (normal control, <8 mU/L[14]), but only in the former was this accompanied by hypoglycemia (Fig 1). A protein load test (1 g/kg) performed in the mother was followed by hypoglycemia (glucose, 2.33 mmol/L; insulin, 39.9 mU/L) and hyperammonemia (ammonia, 128 μg/dL); hyperammonemia frequently occurred after meals. The mother had normal urinary excretion of orotic acid and orotidine at baseline and after allopurinol challenge (300 mg).

When the subject was 7 years old, several studies to determine if the hyperammonemia could be a consequence of an increased deamination rate of gluconeogenetic amino acids secondary to hypoglycemia-induced gluconeogenesis were performed. Ammonia, glucose, insulin, C-peptide, lactate, pyruvate, β-hydroxybutyrate, and acetoacetate levels were measured during continuous infusion of glucose 5 mg/kg/min and then 10 mg/kg/min, with no significant changes (Table 1). Fasting studies did not show an increase in ammonia after 20 hours (12 hours after the meal, 186 μg/dL; 15 hours, 165 μg/dL; 20 hours, 170 μg/dL).

We suspected a defect in *N*-acetyl-glutamate synthetase (NAGS), an enzyme that catalyzes the synthesis of *N*-acetyl-glutamate, an activator of the first step of the urea cycle, and therefore an *N*-carbamyl-glutamate load test was performed. After oral administration of 25 mg/kg, the ammonia level decreased from 145 μg/dL to 81 μg/dL within 4 hours, alanine progressively decreased (the child had fasted for many hours), glutamine decreased slightly in the first 3 hours, citrulline showed a slight increase, and urinary orotic acid remained unchanged (Table 2). Even if *N*-carbamyl-glutamate doses were increased up to 200 mg/kg, no further modifications were noted. Enzymatic activities of carbamyl phosphate synthetase (CPS-I), first step of the urea cycle, and of its activator, NAGS, on liver biopsy (Professor Jean Pierre Colombo, Inselspital, Bern, Switzerland) were within normal limits (CPS-I, 1.40 μmol/60 min/mg protein, normal, 0.7 to 2.1; NAGS, 96.6 nmol/min/g protein, normal, 34 to 203).

Presently, the 9-year-old child is developmentally normal with a head circumference of 52 cm (50th percentile) and an IQ of 97; cerebral magnetic resonance imaging is normal. Serum glucose levels are within normal limits, insulin levels are at the upper limit of normal, and ammonia levels are still elevated.

DISCUSSION

Leucine-sensitive hypoglycemia is a well-described clinical picture belonging to the hypoglycemias with hyperinsulinism. The diagnosis can be difficult, since baseline glycemia and insulin levels are generally normal and hypoglycemic crises episodic. In our patient, the condition was suspected on the basis of a documented severe hypoketotic hypoglycemia with hyperinsulinemia during a seizure episode. A leucine challenge test resulting in a decrease of serum

Table 1. Patient's Response to the Infusion of 10 mg/kg/min of Glucose

	Ammonium (μg/dL)	Glucose (mmol/L)	Insulin (mU/L)	C-Peptide (ng/mL)	Lactate (mmol/L)	Pyruvate (μmol/L)	β-OHB (μmol/L)	AA (μmol/L)
T_0	154	3.27	8.8	0.9	2.1	92.9	44.5	63.8
T_{30}	148	7.44	28.3	3.2	2.1	82.9	63.2	70.0
T_{60}	160	7.27	28.1	4.8	2.9	57.1	24.1	55.6
T_{90}	130	5.72	26.0	4.2	2.6	71.8	18.0	58.5
T_{120}	130	6.61	34.1	4.4	2.3	67.2	10.7	57.4
T_{150}	138	6.77	35.6	4.1	1.9	31.8	14.3	54.9
T_{180}	146	6.55	27.3	4.1	1.8	55.0	2.6	54.9
Normal values	10-80	3.9-6.1	6-21	1-3	0.63-2.44	45-190	20-90	10-40

Abbreviations: β-OHB, β-hydroxybutyrate; AA, acetoacetate.

Table 2. N-Carbamyl-Glutamate Load (25 mg/kg)

	Plasma				Urine
	Ammonium (10-80 μg/dL)	Alanine (269-572 μmol/L)	Glutamine (420-906 μmol/L)	Citrulline (3-42 μmol/L)	Orotic Acid (0.5-3.3 mmol/mol creatinine)
T_0	145	178.22	692.34	37.83	0.66
T_{60}	114	186.73	686.52	42.56	
T_{120}	93	132.76	623.39	42.80	0.79
T_{180}	84	134.80	675.40	43.67	
T_{240}	81	162.58	787.67	32.48	0.01

NOTE. Normal values in parenthesis.

glucose to 1.66 mmol/L and an increase of serum insulin to 132.3 mU/L at 60 minutes established the diagnosis of leucine-sensitive hypoglycemia. Several features of the clinical history were also consistent with this diagnosis: the first hypoglycemic episodes manifested at 6 months of age, coincidentally with the introduction of foods such as meat or cow's milk–based formulas, which have a higher leucine content compared with breast milk; these episodes never occurred during the fasting period, but instead after a meal and always with no accompanying ketonuria; and low levels of plasma branched-chain amino acids (valine, leucine, and isoleucine) were also typical of hyperinsulinism.[13] The results of GC/MS urinary organic acid studies and the normal tolerance to prolonged fasting ruled out other causes of hypoketotic hypoglycemia such as β-oxidation defects.

The family history was also consistent with a familial form of leucine-sensitive hypoglycemia.[1,15,16] This autosomal dominant disorder can affect multiple family members. In this family, the mother suffered from seizure episodes, and both she and the maternal grandmother had abnormal leucine tolerance curves. The increase in insulin levels in the mother and the maternal grandmother was significantly higher compared with the modest increases observed in other patients both by us and by others.[14]

The mechanism underlying leucine-sensitive hypoglycemia has not been fully elucidated; it is known that leucine can normally induce insulin secretion from pancreatic islet cells, and leucine-sensitive hypoglycemia could be an exaggeration of this normal phenomenon. Recently,[15] gastric inhibitory polypeptide has been proposed to act synergistically with leucine to enhance this effect.[17]

Treatment consists of simply limiting leucine intake; administration of diazoxide[18] or surgical intervention are generally avoidable. In our case, we were able to control the disease with a diet containing 115 mg/kg/d leucine without the need for diazoxide. The patient had only one seizure episode at 3 years of age, which was attributable to poor compliance with the diet.

We found very intriguing the persistent hyperammonemia, which was relatively independent of the meals and was occasionally accompanied by increased plasma glutamine levels (up to 1,097 μmol/L).

A defect in the last four steps of the urea cycle was ruled out by the plasma amino acid profile and a normal urinary excretion of orotic acid both at baseline and after a protein load. A defect in the activation of the first step of the urea cycle[19] might have been suggested by a 45% reduction in serum ammonia levels induced by a *N*-carbamyl-glutamate load; however, this defect and a defect in the first step were ruled out by a normal NAGS and CPS-I activity on liver biopsy. The significant reduction of plasma ammonia levels induced by *N*-carbamyl glutamate might be attributable to an overstimulation of the first step of the urea cycle. Organic acidurias were ruled out by normal urinary organic acids.

Hyperammonemia could also be a consequence of an increased deamination rate of gluconeogenetic amino acids secondary to a hypoglycemia-induced gluconeogenesis. This mechanism was excluded because ammonia levels did not decrease after glucose infusion at a rate of 5 and then 10 mg/kg/min, which should adequately fulfill caloric requirements and hence inhibit a possible ongoing gluconeogenesis, and because ammonia levels were not increased with fasting.

In our patient and his mother hyperammonemia accompanied leucine hypersensitivity. The family distribution of leucine hypersensitivity is consistent with an autosomal dominant inheritance. This unexpected association could be explained by two linked genetic defects; however, since we ruled out both a urea cycle defect and organic acidurias and since there are no known autosomal dominant hyperammonemias, it is possible that this metabolic finding is secondary to leucine sensitivity via a mechanism that remains to be elucidated.

To our knowledge, the association of hyperammonemia and leucine-sensitive hypoglycemia has never been previously reported; we are aware of another patient with the same metabolic findings (Galli V., personal communication).

In conclusion, we believe that the routine determination of ammonemia in all patients with leucine hypersensitivity is very important: this determination could help us to understand if hyperammonemia is a consistent feature of this disease and if there is a biochemical link or simply two genes in linkage-disequilibrium. In fact, if this association is frequent, a true underlying biochemical mechanism should be likely; on the other hand, a sporadic association should lead in the direction of a genetic linkage or a genetic variant of leucine hypersensitivity.

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