Late-onset nonketotic hyperglycinemia and spinocerebellar degeneration

Investigation of a 15-year-old boy with progressive optic atrophy and spinocerebellar degeneration revealed elevated plasma, cerebrospinal fluid, and urine glycine concentrations. During an oral glycine loading test, the patient's plasma glycine concentration rose to a higher level than control values, although the initial rate of rise was slower; there was no concomitant rise in the plasma serine concentration. An oral serine loading test resulted in a prompt rise of both glycine and serine serum concentrations. The renal glycine clearance was elevated, and the renal tubular glycine reabsorption was diminished. These findings of decreased intestinal uptake and increased renal tubular glycine clearance suggest that a generalized derangement of glycine entry into cells may account for the phenotypic manifestations of the disorder.

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The hyperglycinemia syndromes, a clinically and biochemically heterogeneous group of metabolic disorders, are classified into ketotic and nonketotic categories. The hyperglycinemia associated with ketosis is a secondary phenomenon, reflecting an inhibition of glycine oxidation by the accumulation of organic acids. Nonketotic hyperglycinemia, in contrast, represents a primary disorder of glycine metabolism. Patients with either form of hyperglycinemia usually present early in infancy with a severe neurologic syndrome. A single sibship has been reported with nonketotic hyperglycinemia and a syndrome of progressive spastic paraparesis with onset between 2 and 23 years of age.

We describe the clinical and biochemical findings in an adolescent boy with nonketotic hyperglycinemia and spinocerebellar degeneration with optic atrophy. An unusual finding was diminished intestinal and renal tubular reabsorption of glycine.

CASE REPORT

Patient G.K. is a 15-year-old boy born in Kfarhghab, Lebanon, who came to the United States at the age of 2 years. He was the first born of dizygotic twins to nonconsanguineous parents. Birth history and early developmental milestones were within normal limits. A preschool examination at age 4 disclosed bilateral optic atrophy with nystagmus and mild spastic paraparesis. The spasticity was progressive; by the age of 9 he required lower limb braces to assist with ambulation. Presently, school performance is age appropriate although he attends classes for the visually handicapped. There is no bladder or bowel dysfunction. Results of examinations of both parents, an older brother, and a twin sister were within normal limits.

On physical examination, all abnormalities were restricted to the nervous system. Mental status examination was normal, with a mental age score of 71. There was bilateral optic atrophy with visual acuity in the left eye at finger counting, and in the right eye at 6/60. The extraocular movements were full, with rapid horizontal rotatory nystagmus noted in all positions of gaze. There was moderate dysmetria in the upper extremities. In the lower extremities, there was severe spastic paraparesis with bilateral footdrop. Reflexes were normal in the upper extremities, brisk at the knees, and absent at the ankles. Below the knees there was a mild vibratory and proprioceptive sensory loss.

Laboratory investigation revealed normal routine chemistry values and hematologic indices. Spinal fluid protein concentration was 19 mg/dl with a normal electrophoretic pattern. Results
Table I. Plasma amino acid levels (mg/dl ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Father</th>
<th>Mother</th>
<th>Brother</th>
<th>Sister</th>
<th>Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>4.87 ± 0.84</td>
<td>1.86</td>
<td>1.66</td>
<td>1.32</td>
<td>1.52</td>
<td>1.64 ± 0.12</td>
</tr>
<tr>
<td>Serine</td>
<td>0.98 ± 0.12</td>
<td>1.20</td>
<td>1.29</td>
<td>1.06</td>
<td>1.17</td>
<td>1.66 ± 0.04</td>
</tr>
</tbody>
</table>

*Based on 15 adult controls.

Table II. Urinary amino acid excretion (mg/24 hours ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Father</th>
<th>Mother</th>
<th>Brother</th>
<th>Sister</th>
<th>Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>1,317.2</td>
<td>101.9</td>
<td>290.6</td>
<td>175.0</td>
<td>112.5</td>
<td>112.8 ± 10.4</td>
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<tr>
<td>Serine</td>
<td>47.8</td>
<td>39.5</td>
<td>82.8</td>
<td>65.8</td>
<td>40.6</td>
<td>45.4 ± 4.0</td>
</tr>
<tr>
<td>Prol ine</td>
<td>Tr</td>
<td>2.07</td>
<td>Tr</td>
<td>0</td>
<td>0</td>
<td>Tr</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Based on 18 adult controls.
†Figures in parentheses represent range.

Table III. Renal amino acid clearance and reabsorption

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Clearance* (ml/min/1.73 m²)</th>
<th>Control†</th>
<th>% Reabsorption</th>
<th>Control‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>90.5</td>
<td>2.7-5.8</td>
<td>29.8</td>
<td>96.7</td>
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<tr>
<td>Serine</td>
<td>13.5</td>
<td>1.9-3.0</td>
<td>90.2</td>
<td>97.0</td>
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<tr>
<td>Threonine</td>
<td>4.8</td>
<td>0.8-1.5</td>
<td>96.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Glutamine</td>
<td>4.7</td>
<td>0.7-1.8</td>
<td>96.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.1</td>
<td>0.3-0.9</td>
<td>97.8</td>
<td>99.2</td>
</tr>
<tr>
<td>Cystine</td>
<td>6.7</td>
<td>0.7-2.9</td>
<td>95.1</td>
<td>98.4</td>
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<tr>
<td>Tyrosine</td>
<td>5.9</td>
<td>1.0-1.7</td>
<td>95.7</td>
<td>98.4</td>
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<tr>
<td>Phenylal-amine</td>
<td>2.7</td>
<td>0.7-1.4</td>
<td>98.1</td>
<td>98.8</td>
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<tr>
<td>Lysine</td>
<td>4.5</td>
<td>0.2-1.9</td>
<td>96.7</td>
<td>99.5</td>
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<tr>
<td>Histidine</td>
<td>36.6</td>
<td>4.7-9.1</td>
<td>73.4</td>
<td>92.3</td>
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</table>

*Based on the average of two 20-minute clearance periods.
†From data of Cusworth and Dent, 1960.
‡From data of Scriver et al, 1964.

METHODS

Venous blood for amino acid analysis was drawn into a test tube containing heparin as an anticoagulant. The plasma was immediately separated by centrifugation and deproteinized with 3% sulfosalicyclic acid (1:1 v/v). Urine for amino acid analysis was collected on ice without the use of a preservative, and diluted with four volumes of 3% sulfosalicyclic acid prior to analysis. In most instances, the amino acid composition was determined on fresh samples. When this was not possible, the samples were kept frozen at −20°C until the assay could be performed. (Amino acid quantitation was performed on a Beckman Model 119 amino acid analyzer.)

Amino acid-loading tests were performed in the morning after an overnight fast. Baseline blood samples were obtained, and subjects then were given a single oral dose of either glycine (300 mg/kg) or serine (150 mg/kg) as a 10% solution in water. Subsequent blood samples for amino acid analysis were withdrawn at 30, 60, 120, 180, and 240 minutes.

The amino acid clearance study was performed in the morning after an overnight fast. An intravenous infusion of isotonic saline was administered at a rate of 100 ml/hour for two hours. In addition, water (500 ml/hour) was given by mouth during this two-hour preliminary period. Inulin was then administered as a 60 mg/kg bolus followed by an infusion of 10% inulin at 25 ml/hour. A separate saline infusion was continued at 150 ml/hour for the duration of the clearance study. After one hour, the first of two 20-minute collection periods was started after the patient had voided. At the midpoint of each 20-minute collection period, blood was withdrawn for determination of amino acids, creatinine, and inulin. Inulin was determined by the method of Waugh.¹
Nonketotic hyperglycinemia and spinocerebellar degeneration

RESULTS

The fasting plasma glycine levels were consistently elevated in the patient but not in his parents or his siblings (Table I). The plasma concentrations of all other amino acids, including serine, were within normal limits. The cerebrospinal fluid glycine concentration was five times the value reported for normal adults. The CSF amino-gram was otherwise unremarkable.

The patient's urinary glycine excretion was nearly seven times higher than were control values (Table II). Serine excretion was normal. Proline and hydroxyproline were excreted only in trace amounts, thus eliminating iminoglycinuria as a diagnostic possibility. Urinary amino acid excretion was normal in other family members with the exception of the patient's mother and brother, in whom glycine excretion was slightly higher than were control values (Table II).

Renal amino acid clearance data are given in Table III. Glomerular filtration rate, as determined by inulin clearance, was 140 ml/minute/1.73 m² and 130 ml/minute/1.73 m² during the two clearance periods. Renal clearance of all amino acids was somewhat elevated compared with published control values. The clearance of serine and histidine was more than four times normal values. The most striking abnormality was the elevated glycine clearance, which was approximately 16 times the upper limit for adult norms and nearly two-thirds the glomerular filtration rate. This exceptional elevation of glycine clearance was also reflected in a marked diminution of tubular fractional glycine reabsorption.

Oral glycine-loading tests in normal adults resulted in a sharp rise of plasma glycine concentration with a peak value occurring at one to two hours (Fig. 1). Levels declined steadily thereafter, approaching baseline values by the fourth hour. This fluctuation of plasma glycine concentration was accompanied by a parallel alteration of the plasma serine concentration, which also reached a peak value at one to two hours after the oral glycine load, and was only modestly elevated by the fourth hour. The results of the glycine-loading tests in the patient's family were essentially indistinguishable from control data with respect to both glycine (Fig. 1) and serine (Fig. 2) concentrations. In contrast, administration of an oral glycine load to the patient revealed a marked delay in the rise of blood glycine, with the peak value not occurring until the three-hour point. The slope of the curve describing the subsequent decline of blood glycine was comparable to that of the control group (Fig. 1), suggesting a defect of intestinal glycine uptake. A defect in some aspect of the conversion of glycine to serine is suggested by the minimal rise of the serine concentration after the glycine load (Fig. 2).

The administration of an oral serine load caused a comparable rise of the blood serine concentration in both the patient and adult controls, thus suggesting the absence in the patient of a defect of intestinal serine uptake. The plasma glycine concentration, however, rose much more
sharp in the patient (Fig. 3). This indicates that the process of glycine formation from serine in the patient is intact but that the subsequent disposal of the glycine so formed is impaired.

**DISCUSSION**

The clinical presentation of our patient clearly differs from that of patients with the “typical” forms of hyperglycinemia. He was well until age 4 years, when the first signs and symptoms of optic atrophy and spinocerebellar degeneration were appreciated. Convulsions and mental retardation were not present. The patient’s clinical course and biochemical findings are similar to those of the three brothers described by Bank and Morrow. These youngsters appear to have a discrete syndrome of nonketotic hyperglycinemia and spinocerebellar degeneration. The fact that all these children are of Lebanese extraction also suggests that they have a similar genotype.

The likely cause of most cases of nonketotic hyperglycinemia is a defect of the glycine cleavage system, which mediates the oxidation of the C-1 carbon of glycine to carbon dioxide and the transfer of the C-2 carbon to another glycine molecule to form serine. In our patient, the absence of a rise of blood serine following an oral glycine load and the sustained elevation of the blood glycine are consistent with such a defect. In both our patient and in infants with nonketotic hyperglycinemia, the administration of an oral serine load caused a prompt increase of blood glycine concentration. The obvious differences between the clinical presentation of our patient and that of infants with nonketotic hyperglycinemia indicate that the two groups do not share an identical metabolic defect. Since the glycine cleavage system is comprised of a network of distinct proteins, ample opportunity would exist for a broad heterogeneity of phenotypic expression, depending upon the nature of specific mutations.

The data obtained with the glycine loading test and renal amino acid clearance study are consistent also with a defect of glycine transport as the cause of the patient’s deranged glycine metabolism. Although plasma glycine values after an oral glycine load were much higher than control values, the initial rate of rise of plasma glycine was much lower than control values. Defective intestinal glycine transport could explain the diminished uptake, whereas impaired glycine transport into other tissues could explain the sustained hyperglycinemia. The absence of a rise of the blood serine concentration following the glycine load would then reflect the failure of glycine uptake into cells mediating the glycine-serine interconversion. The markedly increased renal glycine clearance suggests a defect of renal tubular glycine reabsorption. This defect is not referable to hyperglycinemia alone, since glycine clearance and reabsorption have been normal in other patients with nonketotic hyperglycinemia. Furthermore, studies in the dog indicate that a blood glycine concentration many times higher than the 6 to 7 mg/dl measured in our patient would have to exist before significant overflow glycinuria would be expected. Of interest is the fact that the patient’s glycine clearance is greater than that observed in familial iminoglycinuria. The coexistence of a glycine transport defect in both intestines and kidney has not been hitherto described.

The important role played by glycine as an inhibitory neurotransmitter in the spinal cord, medulla, and cerebellum suggests a physiologic basis for the relationship between the hyperglycinemia and the patient’s clinical presentation. The devastating symptoms of nonketotic hyperglycinemia, which commonly results in death during the first month of life, may be referable to profound impairment of neurotransmission by increased glycine levels in the brain and spinal cord. Patients with both ketotic and nonketotic hyperglycinemia also have dysmyelination in those areas of the brain which become myelinated after birth. Similar lesions have been observed in many other amino-acidopathies, including maple syrup urine disease and phenylketonuria. A likely cause of this abnormality is an inhibition of myelin-protein synthesis by intracytoplasmic amino acid imbalance. It is not clear in our patient why such demyelination, if indeed present, should be restricted to the cerebellum and spinal cord, although it may be
significant that these are the brain regions of highest glycine concentration in patients with nonketotic hyperglycinemia.

The genetics of this hyperglycinemic variant probably conform to an autosomal-recessive pattern. Oral glycine loading tests were performed in the patient's parents and siblings. The results did not differ from control values. All other family members are clinically well. The only abnormality detected in the family was increased 24-hour urine glycine excretion in the patient's mother. This finding, together with the fact that the patients reported by Bank and Morrow were all males, raises the possibility of an X-linked pattern of inheritance. The absence of any spinocerebellar degeneration in male ancestors of our patient's mother, however, is evidence against a mutation on the X chromosome.

REFERENCES