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Glycine Cleavage System in Ketotic Hyperglycinemia: a Reduction of H-Protein Activity

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Summary

Glycine cleavage activity was compared in the livers from three cases of ketotic hyperglycinemia (two cases of propionic acidemia and one case of methylmalonic acidemia) and three controls. In one case of propionic acidemia, glycine cleavage activity (5.2 nmole/mg protein/hr) was normal in the liver obtained at biopsy when the patient was well controlled by the treatment with low protein diet (0.8 g/kg/day) and the level of serum glycine was lowered to normal. In the two other cases of ketotic hyperglycinemia, glycine cleavage activity was significantly reduced in the liver obtained at autopsy when the patients died in the state of metabolic acidosis. Its activity in the liver of one case of propionic acidemia (0.7 nmole/mg protein/hr) was 6–26% of that in controls (2.7–10.8 nmole/mg protein/hr), and 2–7% in a case of methylmalonic acidemia (0.2 nmole/mg protein/hr).

Analysing of the individual components of the glycine cleavage system, a marked decrease in the activity of H-protein was revealed in the livers of the both patients; it (0.2 nmole/mg protein/hr) was only 3–4% of that in controls (4.9–6.3 nmole/mg protein/hr). These findings suggest that the reduction of the glycine cleavage system in the liver of ketotic hyperglycinemia occurs secondarily as speculated previously and is caused mainly by a decrease of H-protein activity.

Speculation

The reduction of glycine cleavage activity in ketotic hyperglycinemia may be caused at first by an inactivation of H-protein. Subsequently the activities of other components of the glycine cleavage system may be decreased.

The glycine cleavage system is a major pathway of catabolism of glycine in vertebrates (7, 22). The enzyme system is composed of four protein components: P-protein (a pyridoxal phosphate enzyme), H-protein (a lipoic acid-containing enzyme), T-protein (a tetrahydrofolate-requiring enzyme), and L-protein (a lipoamide dehydrogenase) (8, 12).

Hyperglycinemia, a group of metabolic disorders characterized by elevated concentration of glycine in body fluids, is associated with decreased catabolism of glycine caused by reduction of the activity of glycine cleavage system (19–21).

These disorders are generally classified into two types, ketotic hyperglycinemia and nonketotic hyperglycinemia. The ketotic hyperglycinemia accompanies a deficiency of some specific enzyme involved in the metabolism of branched-chain amino acids (1, 3, 6, 14, 16, 19–21), whereas nonketotic hyperglycinemia does not show any concomitant disorder of organic acid metabolism (20). Perry *et al.* (15) had suggested that there was a defect in H-protein in the brain of the patient with nonketotic hyperglycinemia. Recently, we (5) reported that there was an abnormal H-protein appeared to be devoid of lipoic acid in the liver of the patient with nonketotic hyperglycinemia. In a case of ketotic hyperglycinemia (methylmalonic acidemia), Motokawa *et al.* (13) reported that the contents of P-protein, H-protein, and T-protein were reduced, suggesting that some metabolites of branched-chain amino acids accumulating in the liver may act to reduce the level of the glycine cleavage system. Now we had an opportunity of analysing the glycine cleavage system in the livers of three cases of ketotic hyperglycinemia (two cases of propionic acidemia and one case of methylmalonic acidemia) and found that the activity of glycine cleavage system was normal in one case and in the other two cases it was decreased mainly due to a reduction of H-protein activity.

MATERIALS AND METHODS

CHEMICALS

[1-^{14}C] glycine and [^{14}C] bicarbonate were obtained from New England Nuclear, Boston, MA. NAD^+, NADH, dithiothreitol, and lipoamide, from Sigma Chemical Company, St. Louis, MO; pyridoxal phosphate, from Kyowa Hakko Kogyo, Tokyo; leupeptin (a microbial protease inhibitor), from Protein Research Foundation, Osaka.

DL-Tetrahydrofolate was prepared by catalytic hydrogenation of folic acid in glacial acetic acid (11).

PATIENTS

Patient K.H. (propionic acidemia) was born at term after an uneventful pregnancy and normal delivery, but she started vomiting at 12 days of age. Thereafter the patient became progressively ill, showing muscular hypotomia and frequent convulsions. Amino acid analysis of serum revealed a marked increase in glycine (1787 μmole/liter). She died in the state of hyperglycinemia and severe metabolic acidosis at the age of 53 days. After her death, a diagnosis of propionic acidemia was made by a marked excretion of propionic acid in urine (5.6 μmole/mg of creatinine). The activity of propionyl CoA carboxylase in her liver obtained at autopsy was found to be 0.01 nmol/min/mg protein (control 2.89 ± 0.15 nmol/min/mg protein).

Patient C.A. (propionic acidemia) was born after an uneventful pregnancy and full term delivery, but she suffered from frequent episodes of vomiting and lethargy associated with acidosis soon after birth. The gain in weight was poor. Laboratory findings revealed hyperglycinemia (1440 μmole/liter) and hyperammone-

mia (176 μmole/liter). The glycine level of the cerebrospinal fluid was found to be 31 μmole/liter. Since 65th day of age, the patient was put on a low protein diet (0.8 g/kg/day). Her clinical symptoms improved with the decrease of blood ammonia (43 μmole/liter), of plasma glycine (287 μmole/liter) and of cerebrospinal fluid glycine (8 μmole/liter). On 118th day of age when the patient had normal levels of plasma glycine and blood ammonia and no acidosis under the low protein treatment, the liver specimen was biopsied for enzymatic diagnosis. Then the defect of propionyl CoA carboxylase was demonstrated (0.02 nmole/min/mg protein, control 2.89 ± 0.15 nmole/min/mg protein). Retrospectively her serum obtained at the episode of metabolic acidosis was investigated by gaschromatography which showed a marked increase of propionic acid (1986 μmole/liter).

Patient S.H. (methylmalonic acidemia) had methylmalonic aciduria and deficiency of methylmalonyl CoA mutase activity in leukocytes as described in our previous report (18). Until 10 months of age, she was well controlled by dietary therapy, normally developed in our hospital and transferred to other ward. Since 20 months of age, she could not be well controlled and was dead at 21 months in the state of metabolic acidosis. The level of serum glycine in this state was not analysed.

PREPARATION OF HOMOGENATES AND SONIC EXTRACTS OF HUMAN TISSUES

The liver specimens of patient K.H. and patient S.H. were obtained at the time of autopsy, frozen, and stored at −80°C within 4 hr after death.

The liver specimen of patient C.A. was frozen immediately after obtained by biopsy and stored.

For control studies, samples of livers were obtained at autopsy from 5-month-old female who died of congenital heart disease (control A.B.), from 8-year-old female who died of sudden cardiac arrest of unknown cause (control K.Y.), and from 53-year-old male who died of lung tumor (control Y.A.). All specimens of controls were frozen within 2–6 hr after death. Frozen tissues were thawed and homogenized with 4 volumes of 20 mM potassium phosphate buffer (pH 7.0) containing 1 mM dithiothreitol, 1 mM pyridoxal phosphate, and 10 μg/ml of Leupeptin. The homogenates were passed through four layers of gauze and used for the assay of glycine cleavage activity. For the assays of individual components of glycine cleavage system and the assay of lipoamide dehydrogenase, the homogenates were subjected to sonic treatment at 140 W for 5 min and the supernatants were used after centrifugation at 105,000 × *g* for 50 min.

P-PROTEIN AND H-PROTEIN FROM CHICKEN LIVER

These were purified from chicken liver mitochondria by the method of Hiraga and Kikuchi (4).

ASSAY OF ENZYME ACTIVITIES

The activity of the glycine cleavage reaction was assayed essentially as described in previous paper (17). The reaction mixture contained, in a final volume of 1 ml, 100 μmole of potassium phosphate buffer (pH 7.4), 0.4 μmole of pyridoxal phosphate, 1 μmole of NAD^+, 1 μmole of DL-tetrahydrofolate, 10 μmole of [1-^{14}C] glycine (specific radioactivity, 0.05 Ci/mole) and homogenate. The reaction was conducted at 37°C for 20 min in a Warburg flask with shaking and terminated by the addition of 0.1 ml of 50% trichloroacetic acid. $^{14}CO_2$ formed was measured by the procedure described previously (9).

Activities of P-protein and H-protein were assayed by the glycine-$^{14}CO_2$ exchange reaction essentially according to the method of Motokawa and Kikuchi (11). For the assay of P-protein activity the assay system was supplemented with 50 μg of H-protein of purified from chicken liver mitochondria and for the assay of H-protein activity 8 μg of P-protein purified from chicken liver mitochondria was added.

Activity of T-protein was assayed in the reaction mixture for glycine cleavage with 32 μg of P-protein and 50 μg of H-protein which were purified from chicken liver mitochondria. Other assay conditions were same as for the assay of the glycine cleavage activity.

The activity of lipoamide dehydrogenase was determined as previously reported (10).

PROTEIN DETERMINATION

This was done by the biuret method with bovine serum albumin as standard (2).

RESULTS

The glycine cleavage activity in the livers of patient K.H. (propionic acidemia) and patient S.H. (methylmalonic acidemia) was significantly reduced: it was 2–26% of the activity in control human livers as shown in Table 1. However, the glycine cleavage activity in patient C.A. (propionic acidemia) was not reduced in the liver, which was obtained at biopsy when the serum level of glycine of the patient had been kept within normal range (311 μmole/liter) under the treatment with a low-protein diet (0.8 g/kg/day).

For further analysis of the reduction of glycine cleavage system in patient K.H. (propionic acidemia) and patient S.H. (methylmalonic acidemia), comparison of the activities of the individual protein components of the glycine cleavage system in patients with controls was made with the extracts of liver. As can be seen from Table 2, a marked decrease in the activity of H-protein was found in the livers of patients K.H. and patient S.H.; it was only 3–4% of that in controls. The activity of lipoamide dehydrogenase in the patients' livers was not decreased.

Table 1. *Glycine cleavage activity in livers of patients and controls*[1]

Source of tissue	$^{14}CO_2$ formed (nmole/mg protein/hr)
Patient K.H. (propionic acidemia)	0.7
Patient C.A. (propionic acidemia)	5.2
Patient S.H. (methylmalonic acidemia)	0.2
Control A.B.	10.8
Control K.Y.	5.2
Control Y.A.	2.7

[1] Nine to 12 mg of liver homogenate was used as protein.

Table 2. *Activities of individual components of the glycine cleavage system in livers of patient K.H., patient S.H. and controls*[1]

Source of tissue	P-protein[2]	H-protein[2]	T-protein[2]	Lipoamide dehydrogenase[3]
Patient K.H.	27.4	0.2	55.2	49.3
Patient S.H.	5.1	0.2	38.6	39.3
Control A.B.	10.3	4.9	39.6	35.2
Control K.Y.	9.3	5.1	21.9	35.1
Control Y.A.	10.9	6.3	30.2	42.8

[1] Activities of P-protein, H-protein, T-protein, and lipoamide dehydrogenase were assayed with sonicated homogenates (1–4 mg as protein). P-protein was assayed in the presence of 50 μg of purified chicken liver H-protein, and H-protein was asssayed in the presence of 8 μg of purified chicken liver P-protein. The activity of T-protein was determined by glycine cleavage activity in the reaction system supplemented with 32 μg of chicken liver P-protein and 50 μg of chicken liver H-protein. Other reaction conditions were as described in "Materials and Methods."

[2] nmole of product /mg protein/hr.

[3] nmole/mg protein/min.

DISCUSSION

It has been reported the glycine cleavage system in the liver was defective in all cases of ketotic hyperglycinemia so far investigated (1, 14, 19–21). But liver specimens in these cases seem to have been obtained by either biopsy or autopsy at the presence of hyperglycinemia and organic acidemia.

The present findings showed that the glycine cleavage system in the liver was normal when the patient with propionic acidemia (patient C.A.) had been in no metabolic acidosis and in normal level of serum glycine under low protein diet. On the other hand, the glycine cleavage system was found to be disturbed in the liver when patient K.H. and patient S.H. had been in metabolic acidosis suggesting high levels of organic acidemia. These findings support the hypothesis that the defect of the glycine cleavage system seen in ketotic hyperglycinemia occurs secondarily.

Furthermore, it was found that the disturbance of glycine cleavage system in ketotic hyperglycinemia is caused mainly by specific reduction of H-protein activity. Motokawa *et al.* (13) previously reported the reduced content of P-protein, H-protein and T-protein in the liver from a patient with methylmalonic acidemia, although the extent of reduction was greatest in H-protein. A speculation may be possible that metabolites of branched-chain amino acids accumulating in the tissues cause first an inactivation of H-protein which subsequently lead to decrease of the other components P-protein and T-protein.

Further study is needed to confirm the reduction of H-protein and to clarify the mechanism of its reduction in ketotic hyperglycinemia.

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