Tohoku J. exp. Med., 1969, 98, 289–296

Hyperglycinemia: A Defect in Glycine Cleavage Reaction

Keiya Tada, Kuniaki Narisawa, Toshio Yoshida, Tasuke Konno, Yoshimasa Yokoyama, Hiroshi Nakagawa, Kaneo Tanno, Keiko Mochizuki and **Tsuneo Arakawa**

Department of Pediatrics (Prof. Ts. Arakawa), Faculty of Medicine, Tohoku University, Sendai

Tadashi Yoshida and **Goro Kikuchi**

Department of Biochemistry (Prof. G. Kikuchi), Faculty of Medicine, Tohoku University, Sendai

A girl with hyperglycinemia of nonketotic type was presented. The liver biopsied from the patient was studied for glycine metabolism. It was found that the yield of $^{14}CO_2$ from glycine-1-^{14}C and the rate of ^{14}C incorporation into serine from glycine-1-^{14}C as well as glycine-2-^{14}C were extremely low in the patient's liver than in control livers, while the patient's liver showed normal activities of serine-hydroxymethylase and serine-dehydratase.

These findings indicate that the primary lesion of hyperglycinemia of nonketotic type is a defect in the glycine cleavage reaction which gives rise to the formation of CO_2, methylene-tetrahydrofolate and ammonia from glycine.

Hyperglycinemia is an inborn error of amino acid metabolism, in which elevated concentrations of glycine are found in the blood, urine and cerebrospinal fluid.[1,2] There seems to be at least two types of hyperglycinemia from the clinical aspects, one being ketotic and the other nonketotic type. The former is characterized by the neonatal onset of periodic ketosis, often leading to death in the early period of life, and subsequent developmental retardation. The latter type of hyperglycinemia lacks ketosis but has convulsions and mental retardation.

In 1968 Lindbald *et al.*[4] described an assumption in their paper of methylmalonic acidemia that the ketotic type of hyperglycinemia and methylmalonic acidemia were the same disorder because of the similarity of clinical features between both disorders and of the elevation of serum glycine found in cases of methylmalonic acidemia. On the other hand, Ando *et al.*[5] studied two patients with nonketotic hyperglycinemia and found a slower conversion of the first carbon of glycine to CO_2 and almost no conversion of the second carbon of glycine to the third carbon of serine by investigating the radioactivity of respiratory $^{14}CO_2$ and of serine in

Received for publication, April 19, 1969.

blood after the seperate intravenous injections of glycine-1-^{14}C and glycine-2-^{14}C. These findings suggest a defect in an enzyme system catalyzing the transformation of glycine to CO_2, NH_3 and methylene-tetrahydrofolate, the glycine cleavage reaction which had been shown to occur in pigeon liver and rat liver mitochondria.[7,8]

Recently we have had an opportunity of investigating glycine metabolism with use of liver biopsied from a patient with nonketotic hyperglycinemia. The results clearly indicated a defect of the glycine cleavage reaction in question.

Case Report

M. S., a girl, was born after a full term pregnancy and spontaneous delivery with a weight of 3,900 g. Her neonatal history was uneventful. The parents were healthy and not consanguineous. There was no sibling. Since the first attack at 7 months of age, the patient had occasional episodes of general convulsions. Because of retarded mental development, the patient was referred to our Clinic. On admission at the age of 1 year, physical examination revealed a moderately nourished girl with an apathetic countenance. She appeared to take no notice of her surroundings. She could not sit alone or turn over. The lungs and heart were clear to auscultation. Neither the liver nor the spleen was palpable. The extremities were hypotonic and the deep tendon reflexes were weak.

Laboratory findings: Examination of blood on admission showed hemoglobin of 14.6 g/100 ml, R.B.C. 336×10^4/mm^3, W.B.C. 4,100/mm^3, with a differential count of 1% eosinophils, 76% lymphocytes, 13% monocytes and 10% neutrophils. Routine urinalysis showed no abnormal findings. Serum chloride was 111 mEq/L; calcium, 11.8 mg/100 ml; potassium, 4.4 mEq/L; phosphorus, 4.4 mg/100 ml; total protein, 5.6 g/100 ml; and total cholesterol, 220 mg/100 ml. The fasting blood sugar was 80 mg/100 ml. The liver function tests were within normal limits, Z.S.T. was 1.6 units; T.T.T. 0.6 units; GOT, 15 units; GPT, 9 units. Histological findings of the biopsied liver showed no abnormality. EEG shows HVS in both parietooccipital areas. Foundoscopic examination showed no abnormality. Laboratory data from subsequent hospitalization at the age of 14 months revealed the following: 418×10^4/mm^3 of R.B.C., 15.2 g/100 ml of hemoglobin, 39.5% hematocrit, 74,00/mm^3 of W.B.C. with 1% basophils, 1% eosinophils, 45% lymphocytes, 20% monocytes and 33% neutrophils. Using capillary blood, the following values were obtained by Astrup's apparatus; blood pH 7.35; Pco_2, 36.5 mmHg; base excess, -3.5 mEq/L; standard bicarbonate, 20.2 mEq/L; and buffer base, 45.5 mEq/L.

Metabolic Studies

Two dimensional thin-layer chromatogram of urinary amino acids revealed an excessive output of glycine, and the thin-layer chromatogram of serum also showed a striking elevation of glycine. These findings were confirmed by quantitative analysis with use of an automatic amino acid analyzer (cf. Tables 1 and 2), which

TABLE 1. *Serum amino acid levels in the patient with hyperglycinemia (mg/100 ml)*

Amino acids	Patient	Normal range
Aspartic acid	0.34	Trace −0.48
Threonine	0.91	0.37−1.05
Serine	1.51	0.85−2.40
Glutamine	3.07	1.96−3.44
Glutamic acid	0.65	0.66−2.19
Glycine	10.70	1.23−3.44
Alanine	1.95	1.30−3.91
Valine	1.43	0.94−2.87
Methionine	0.31	Trace −0.30
Isoleucine	0.38	0.28−0.94
Leucine	0.81	0.79−1.53
Tyrosine	0.63	0.36−1.25
Phenylalanine	0.52	0.50−1.14
Proline	1.27	0.66−2.19
Lysine	4.68	1.10−3.74
Histidine	0.59	0.33−1.05

TABLE 2. *Urinary amino acids in the patient with hyperglycinemia (γ/1 mg of creatinine)*

	Patient	Normal range[19]
Serine	17	56−209
Glycine	7,318	104−282
Alanine	102	45−164
Isoleucine	14	9−28
Leucine	20	12−49

showed levels of glycine in serum of 10.7 mg/100 ml and in urine of 7.318 mg per mg of creatinine (daily excretion being about 700 to 800 mg).

Basing upon these findings, a diagnosis of hyperglycinemia was established. Then the following examinations were made in respect of glycine metabolism. Serum folate (*L. casei*) was found to be subnormal (4 mγ/ml). Urinary output of formiminoglutamic acid after histidine loading was within normal range.
The activities of erythrocyte GOT, which were thought to be parallel with the amount of pyridoxal phosphate in blood, were determined by Karmen's method. The patient's erythrocytes showed normal activity. Blood ammonia was found to be 69 γ/100 ml (within normal ranges). Urinary excretion of oxalic acid was ranging from 1.8 to 2.3 mg/day (Controls: 2.0–10 mg/day). Methylmalonic aciduria was not detected by chromatography of urinary organic acids.

$^{14}CO_2$ in expired air following an intravenous dose of glycine-1-^{14}C: Glycine-1-^{14}C (specific activity of 10.6 mc/mM) was dissolved in physiologic saline, sterilized and injected intravenously to the hyperglycinemic patient in a dose of 1μc per kg of body weight in the fasting state. Expired carbon dioxide was collected for a three minute period each at 10, 30, 60 and 120 minutes after the radioactive glycine injection by the use of "Fukuda Metabolor" (an apparatus for the estima-

tion of basal metabolism equipped with a closed circulating system, in which expired $^{14}CO_2$ was captured in solid potassium hydroxide). Radioactivity in the expired CO_2 was determined by the method described previously[10]. As a control, a mentally retarded boy of the same age without abnormal aminoaciduria was subjected to the test.

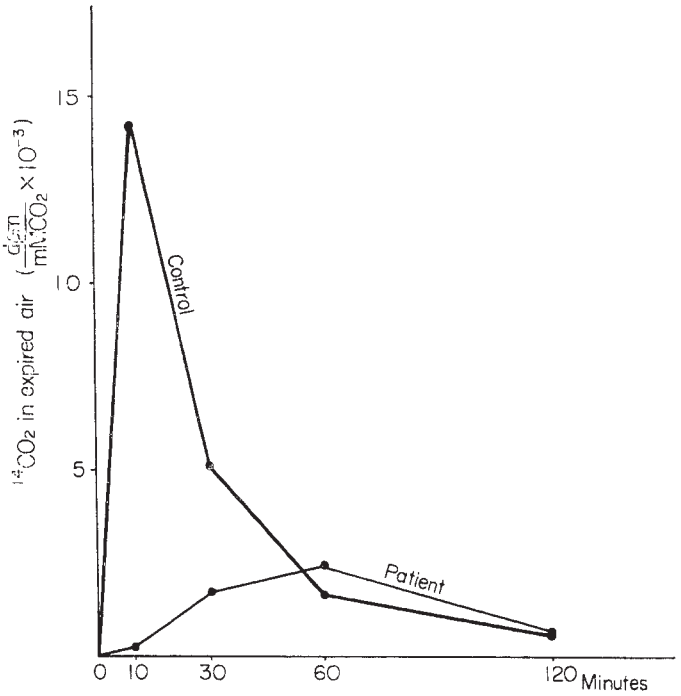

Fig. 1. $^{14}CO_2$ in the expired air after an intravenous dose of glycine-1-^{14}C in the patient with hyperglycinemia and a control individual.

Results obtained were illustrated in Fig. 1. In the control, the specific activity of $^{14}CO_2$ in the expired air showed a high peak at 10 minutes after the injection of radioactive glycine and declined with time. In contrast, the curve obtained form the patient was rather flat, rising slowly to the hightest value at 60 minutes after injection. These findings are in agreement with the results obtained by Ando *et al.*[5] in two patients with hyperglycinemia and strongly suggest a defect in the formation of CO_2 from the carboxyl carbon of glycine in this particular disorder.

Enzyme analyses: In order to confirm the above-mentioned suggestion, enzymatic analysis was made using the liver specimen biopsied surgically from the hyperglycinemic patient. As controls, the liver specimens were obtained at laparotomy of five surgical patients without liver involvement.

Procedure: The biopsied liver specimens were immediately homogenized with 0.1M KCl-0.05M patassium phosphate buffer (pH 7.4). For the assay of the activity of glycine cleavage reaction, the liver homogenate was aerobically incubated with glycine-1-^{14}C at 37°C for 60 minutes and the yield of $^{14}CO_2$ and the

radioactivity of ^{14}C incorporated into serine were determined as described in the preceding papers.[8,9] In the same way another set of tubes was incubated using glycine-2-^{14}C as the substrate instead of glycine-1^{14}C.

Results showed that the $^{14}CO_2$ formation from glycine-1-^{14}C was extremely low in the patient's liver as compared with those in control livers while there was no significant difference in $^{14}CO_2$ liberated from glycine-2-^{14}C between the patient's liver and control livers (cf. Table 3). Addition of pyridoxal phosphate (PALP) to the incubation system failed to increase the $^{14}CO_2$ formation from glycine-1-^{14}C in the patient's liver. With homogenates of control livers, the amounts of ^{14}C-serine formed from glycine-2-^{14}C were nearly two times those from glycine-1-^{14}C, indicating that the glycine cleavage reaction was operating also in human liver. In the patient system, however, the ^{14}C incorporation into serine from glycine-1-^{14}C or glycine-2-^{14}C was found to be both markedly diminished than control liver systems (cf. Table 4). These findings indicate a defect in process of conversion of glycine to CO_2, NH_3 and methylene-tetrahydrofolate.

TABLE 3. *$^{14}CO_2$ formation from glycine-1-^{14}C or glycine-2-^{14}C with use of liver homogenates*

		$^{14}CO_2$ from glycine-1-^{14}C (cpm/10 mg of protein)	$^{14}CO_2$ from glycine-2-^{14}C (cpm/10 mg of protein)
Control	A*	3,785	358
		3,685	365
〃	B*	5,683	358
		6,080	367
〃	C	8,080	520
〃	D	5,840	530
〃	E	5,050	879
Patient*		430	458
		412	445
+PALP 3μmoles		387	370

Note: Reaction mixtures contained in a final volume of 2 ml: 10μmoles of ^{14}C-glycine (0.05 mc/mmole), 190 μmoles of KCl, 95 μmoles of potassium phosphate buffer (pH 7.4) and 12 to 13 mg as protein of respective homogenates. Reactions were carried out in Warburg manometric flasks for 1 hr. at 37°C in air.

* The experiment was made duplicated.

TABLE 4. *Incorporation of ^{14}C from glycine-1-^{14}C or glycine-2-^{14}C into serine in the liver from the patient with hyperglycinemia*

		Incorpation of glycine-1-^{14}C to serine (cpm/10 mg of protein)	Incorporation of glycine-2-^{14}C to serine (cpm/10 mg of protein)
Control	A	3,838	8,500
〃	B	5,660	9,410
〃	C	7,240	12,010
〃	D	5,008	8,190
〃	E	4,170	7,155
Patient		855	1,060

Note : The assay system is described in Table 3.

TABLE 5. *Activity of serine dehydratase and serine hydroxymethylase in the liver from the patient*

		Serine dehydratase (mμmoles/mg of protein/hr)	Serine hydroxymethylase (μmoles/mg of protein/hr)
Control	A	25.4	1.48
〃	B	19.6	1.68
〃	C	23.8	1.76
〃	D	31.5	2.23
〃	E	33.5	1.67
Patient		27.9	2.37

Note : The assay system of serine dehydratase consists of 20 μmoles of DL-serine, 0.3 ml of Krebs-Ringer phosphate buffer (0.5M, pH7.1), 3.0 μmoles of PALP, 0.25 μmoles of NADH, lactic dehydrogenase (amount of 50γ protein) and 0.2 ml of the supernatant of liver homogenate and water to a final volume of 3.0 ml. Incubation was crraied out at 37°C for 20 minutes. The assay system of serine hydroxymethylase consists of 1.0 μmole of DL-tetrahydrofolate, 5μmoles of L-serine, 100 μmoles of phosphate buffer (pH 7.5), 10 μmoles of mercaptoethanol, 0.6 μmoles of NADP, and 0.25 ml of the 20 times diluted supernatant of liver homogenate and water to a final volume of 1.0 ml. Incubation was carried out at 37°C for 30 minutes. The supernatant of liver homogenates contained approximately 5 to 6 mg of protein per ml.

Table 5 showed the activities of serine hydroxymethylase and of serine dehydratase. The activities of serine hydroxymethylase and of serine dehydratase were estimated by Bertino *et al.*'s method[11] and Freedland and Avery's method,[12] respectively, using the supernatant of the liver homogenate obtained by the centrifugation at 30,000 g for 120 minutes.

Therapeutic trial: At the age of 16 months, the patient with hyperglycinemia was given intramuscular injections of 3 mg of Leucovorin (N^5-formyltetrahydrofolate) every day for a week. Before and after the treatment, the fasting level of glycine in serum was determined. There was no significant change in serum glycine by the treatment (7.04 and 8.16 mg/100 ml, respectively). In the next place, 30 mg of Leucovorin were intravenously given by drip-infusion mixed with physiologic saline, in 30 minutes. Serum level of glycine was determined before and at the end of the drip-infusion and thereafter 30 and 60 minutes following the infusion. Significant, but temporary, decrease in serum glycine was observed at the end of the drip-infusion (cf. Fig. 2).

DISCUSSION

It has been considered that the major pathway of glycine catabolism is a route *via* its conversion to serine possibly through the function of serine hydroxymethylase.[13] It has, therefore, been presumed that primary lesion of hyperglycinemia may lie in the conversion of glycine to serine.[14,15] The present study

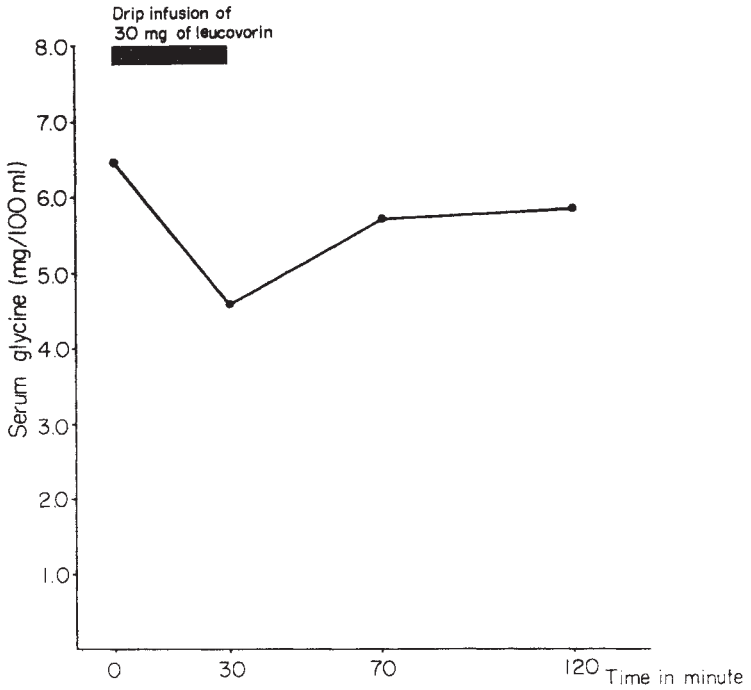

Fig. 2. Effect of leucovorin on the levels of serum glycine of the patients with hyperglycinemia.

revealed that the $^{14}CO_2$ formation from glycine-1-^{14}C and the C^{14} incorporation to serine from either of glycine-1-^{14}C and glycine-2-^{14}C were extremely low in the patient liver and that there was no great difference between the yields of ^{14}C-serine formed from glycine-1-^{14}C and glycine-2-^{14}C in the patient liver. These findings are consistent with a defect in the glycine cleavage reaction. In *in vitro* systems, the glycine cleavage reaction is usually accompanied by the concomitant synthesis of serine as can be predicted from the following sequences of the reaction.

(1) Glycine + THF $\rightleftharpoons$ methylene-THF + CO_2 + NH_3 + 2H

(2) Methylene-THF + glycine $\rightleftharpoons$ serine + THF

Reaction (2) is known to be catalyzed by serine hydroxymethylase and this activity was shown to be normal in this patient. The enzyme catalyzing reaction (1) is a complex one, requiring pyridoxal phosphate and NAD as cofactors,[16] and the whole process of the glycine cleavage reaction was demonstrated to proceed reversibly.[8,9,16,17] There was evidence[20] that methylene-tetrabydrofolate (THF) formed in reaction (1) could also be oxidized to CO_2, independently of reaction (2), by mitochondrial enzyme system. It is, therefore, reasonable to assume that the primary lesion of hyperglycinemia lies in a defect in reaction (1). A minute amount of radioactivity found in serine fraction from glycine-^{14}C in patient's liver may be due to the function of serine hydroxymethylase. It is quite possible that methylenetetrahydrofolate other than that derived from glycine will participate in the reaction of serine hydroxymethylase, although it may be minor. This supposition may be supported by the fact that a temporary but significant decrease in serum level of glycine was observed with the treatment of massive dose of Leucovorin.

References

1) Childs, B., Nyhan, W.L., Borden, M., Bard, L. & Cooke, R.E. Idiopathic hyperglycinemia and hyperglycinuria: A new disorder of amino acid metabolism. *Pediatrics*, 1961, **27**, 522–538.
2) Tada, K., Yoshida, T., Morikawa, T., Minagawa, A., Wada, Y., Ando, T. & Shimura, K. Idiopathic hyperglycinemia. *Tohoku J. exp. Med.*, 1963, **80**, 218–226.
3) Ziter, F.A., Bray, P.R., Madsen, J.A. & Nyhan, W.L. The clinical findings in a patient with nonketotic hyperglycinemia. *Pediat. Res.*, 1968, **2**, 250–253.
4) Lindbald, B., Lindbald, B.S., Olin, P., Svanberg, B. & Zetterstrom, R. Methylmalonic acidemia. *Acta Pediat. scand.*, 1968, **57**, 417–424.
5) Ando, T., Nyhan, W.L., Gerritsen, T., Gong, L., Heiner, D.C. & Bray, P.F. Metabolism of glycine in the nonketotic form of hyperglycinemia. *Pediat. Res.*, 1968, **2**, 254–263.
6) Richert, D.A., Amberg, R. & Wilson, M. Metabolism of glycine by avian liver. *J. biol. Chem.*, 1962, **237**, 99–103.
7) Sato, T., Motokawa, Y., Kochi, H. & Kikuchi, G. Glycine synthesis by extracts of acetone powder of rat-liver mitochondria. *Biochem. biophys. Res. Commun.*, 1967, **28**, 495–501.
8) Sato, T., Kochi, H., Sato, N. & Kikuchi, G. Glycine metabolism by rat liver mitochondria. III. The glycine cleavage and the exchange of carboxyl carbon of glycine with bicarbonate. *J. Biochem.*, 1969, **65**, 77–83.
9) Kawasaki, H., Sato, T. & Kikuchi, G. A new reaction for glycine biosynthesis. *Biochem. biophys. Res. Commun.*, 1966, **23**, 227–233.
10) Arakawa, Ts., Tada, K., Narisawa, K., Tamura, T., Mochizuki, K., Wada, Y. & Yoshida, T. $^{14}CO_2$ in expired air after radioactive histidine injection in formiminotransferase deficiency syndrome. *Tohoku J. exp. Med.*, 1968, **96**, 341–344.
11) Bertino, J.R., Simons, B. & Donohue, D.M. Purification and properties of the formate activating enzyme from erythrocytes. *J. biol. Chem.*, 1962, **237**, 1314–1318.
12) Freedland, R.A. & Avery, E.H. Studies on threonine and serine dehydrase. *J. biol. Chem.*, 1964, **239**, 3357–3360.
13) McElory, W.D. & Glass, H.B. A symposium on amino acid metabolism. The Johns Hopkins Press, Baltimore, 1955, pp. 637–657.
14) Tada, K. & Ando, T. Idiopathic hyperglycinemia: A possibility of heterozygosity in the parents. *Tohoku J. exp. Med.*, 1964, **82**, 164–167.
15) Tada, K. & Ando, T. Congenital heperglycinemia: Demonstration of a minor metabolic defect in the parents. *Tohoku J. exp. Med.*, 1965, **85**, 105–107.
16) Motokawa, Y. & Kikuchi, G. Glycine metabolism by rat liver mitochondria. II.Methylene-tetraphydrofolate as the direct one carbon donor in the reaction of glycine synthesis. *J. Biochem.*, 1965, **65**, 71–75.
17) Sato, T., Kochi, H. & Kikuchi, G. Glycine metabolism by rat liver mitochondria. I. Synthesis of two molecules of glycine from one molecule each of serine, bicarbonate and ammonia. *J. Biochem.*, 1969, **65**, 63–70.
18) Tada, K. Serum amino acid pattern in normal children, assayed by automatic amino acid analyzer. *Rinshoshoniigaku* (Jap.), 1964, **12**, 129–132.
19) Tada, K., Morikawa, T. & Arakawa, Ts. Tryptophan load and uptake of tryptophan by leukocytes in Hartnup disease. *Tohoku J. exp. Med.*, 1966, **90**, 337–346.
20) Yoshida, T., Motokawa, Y. & Kikuchi, G. Metabolism of glycine and serine by rat liver. *Seikagaku* (Jap.), 1968, **40**, 547.