PHYSIOLOGICAL SIGNIFICANCE OF GLYCINE CLEAVAGE SYSTEM IN HUMAN LIVER AS REVEALED BY THE STUDY OF A CASE OF HYPERGLYCINEMIA*

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Summary: Deficiency of the glycine cleavage activity was shown to compose the primary lesion in hyperglycinemia of non-ketotic type in human infant. This led to the conclusion that the direct cleavage of glycine is quantitatively the most significant pathway of glycine catabolism in normal human individuals. Further, evidence was obtained that in human liver considerable portions of serine may be catabolized by way of the preliminary cleavage to methylene-THF and glycine followed by their further oxidation to yield CO_2 as the final product.

Introduction: Glycine can be metabolized in animals by several routes; such as, (1) conversion to serine followed by its deamination to yield pyruvate, (2) oxidative cleavage of glycine into methylene-THF, CO_2 and NH_3, (3) deamination to glyoxylate and its subsequent oxidation to CO_2, formic acid or oxalate, (4) the succinate-glycine cycle or aminoacetone cycle, and others (cf. ref. 1). The routes (3) and (4) may represent only minor

Abbreviation: THF, tetrahydrofolic acid.

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pathways of glycine catabolism (cf. ref. 1 and 2). The route (2) is usually accompanied by the concomitant synthesis of serine from methylene-THF and another molecule of glycine and the whole process of route (2) has been demonstrated to proceed reversibly[3,4]. However, it still remains unclear which of the routes (1) and (2) is really the major pathway of glycine catabolism under the physiological condition.

On the other hand, recently we have experienced a case of congenital hyperglycinemia of nonketotic type in human infant. Hyperglycinemia is characterized by extremely high levels of glycine in blood and urine, suggesting that hyperglycinemia is associated with a decreased catabolism of glycine resulted by some inborn anomaly. If the site(s) of the primary lesion in hyperglycinemia could be identified, this would, in turn, provide an excellent opportunity to answer the question as to which of the known routes of glycine metabolism is most significant in the physiological catabolism of glycine. This communication is concerned with a study on these aspects.

Materials: Hyperglycinemia patient was a 15 month old girl at the time of examination, showing several sings of retarded mental and physical development. Laboratory investigations revealed that the glycine content in the blood was about 11 mg/100 ml while the values for the normal control humans were in the range of 1.2 to 3.4 mg/100 ml. Urinary glycine was also very high (7.3 mg of glycine/1 mg of creatinine). The level of serine in blood (1.5 mg/100 ml) of the patient was in the normal range; no other amino acid was increased beyond the respective control values in either blood or urine. Levels of urinary oxalic acid and blood ammonia were also in the normal ranges, but serum folate (L. casei)

being subnormal. About 1 g wet weight of liver was taken from the patient by surgical biopsy and homogenized in nine times the volume of 0.1 M KCl-0.05 M potassium phosphate buffer (pH 7.4) with a tefron homogenizer. The homogenate was passed once through two layers of cheese cloth and used as such. For the preparation of soluble liver fraction which were used for the assay of serine hydroxymethyltransferase and serine dehydratase, an aliquote of the homogenate was centrifuged at 30,000 xg for 120 min. Livers used as control specimens were taken from five adult humans without hepatic involvement on the occasions of laparatomy in the University Hospital and processed in the same way as above.

Results and Discussion: As shown in Table I, the homogenates

TABLE I Catabolism of ^{14}C-glycine and ^{14}C-serine by liver homogenates from hyperglycinemia patient and control humans

Source liver	$^{14}CO_2$ formed from ^{14}C-labeled substrate listed below (cpm/10 mg protein/hr)				^{14}C-Serine formed from ^{14}C-glycine listed below (cpm/10 mg protein/hr)	
	G-1-^{14}C	G-2-^{14}C	S-1-^{14}C	S-3-^{14}C	G-1-^{14}C	G-2-^{14}C
Patient	430	458	1295	3180	855	1060
Control A	3785	358	3127	861	3838	8500
Control B	5683	358	3330	1006	5660	9410
Control C	8080	520	2050	1430	7240	12010
Control D	5480	530	1220	1849	5008	8190
Control E	5050	879	2100	2745	4170	7155

G and S stand for glycine and serine, respectively.
Reaction mixtures contained in a final volume of 2 ml: 10 μmoles of ^{14}C-glycine (0.05 mc/mmole) or 20 μmoles of ^{14}C-DL-serine (0.05 mc/mmole), 190 μmoles of KCl, 95 μmoles of potassium phosphate buffer (pH 7.4) and 1 ml of homogenates containing 12 to 13 mg of protein, respectively. Reactions were carried out in Warburg manometric flasks for 1 hr at 37° in air. Amounts of $^{14}CO_2$ and ^{14}C-serine formed were assayed as described previously[4].

from control humans actively catalyzed the decarboxylation of glycine and the decarboxylation was accompanied by the concomitant synthesis of serine. When glycine-1-^{14}C was the substrate, the amounts of ^{14}C-serine formed were close to the amounts of $^{14}CO_2$ formed in the respective reaction systems. When glycine-2-^{14}C was employed, the amounts of ^{14}C-serine formed were almost doubled, while the amounts of $^{14}CO_2$ obtained were very small. These results are quite consistent with the view that the glycine cleavage reaction as has been shown to occur in pigeon liver[5] and rat liver mitochondria[3,4] also occurs in human liver. With liver homogenate from the hyperglycinemia patient, however, the amount of $^{14}CO_2$ formed from glycine-1-^{14}C was only one-tenth to twentieth of those obtained with liver homogenates from controls. The yields of ^{14}C-serine were also very small with either glycine-1-^{14}C or glycine-2-^{14}C as the substrates. Moreover, the amounts of $^{14}CO_2$ as well as ^{14}C-serine formed from either glycine-1-^{14}C or -2-^{14}C were nearly equal, respectively. These data strongly suggest that the patient is deficient, if not nil, of the activity of glycine cleavage. Indirect evidence suggesting the same conclusion has also been obtained by Ando *et al.*[8] through a *in vivo* study of two cases of hyperglycinemia. Small amounts of $^{14}CO_2$ liberated from either glycine-1-^{14}C or -2-^{14}C in the patient system may be due mainly to the function of alternate pathways of glycine catabolism such as the route *via* serine which could be formed from glycine and endogenous one carbon donor and the behaviour of the ^{14}C-serine formation could be explained in the same way. In support of this view the activities of both serine hydroxymethyltransferase and serine dehydratase in liver of the patient were similar to those found for control livers, respectively (Table II).

TABLE II Activities of serine hydroxymethyltransferase and serine dehydratase in livers from hyperglycinemia patient and control humans

Source of liver	Serine hydroxymethyltransferase (μmoles of serine cleaved/1 ml of soluble fraction/hr)	Serine dehydratase (μmoles of serine deaminated/1 ml of soluble fraction/hr)
Patient	12.3	0.145
Control A	7.70	0.132
Control B	8.74	0.102
Control C	9.15	0.124
Control D	11.6	0.164
Control E	8.70	0.176

Activities of serine hydroxymethyltransferase were assayed spectrophotometrically by the method of Bertino, Simmons and Donohue[6]. Reaction mixtures contained, in a final volume of 1 ml: 5 μmoles of L-serine, 0.6 μmole of THF, 0.6 μmole of NADP, 100 μmoles of potassium phosphate buffer (pH 7.5), and 0.25 ml of 20 times diluted soluble fractions, respectively. Reactions were carried out for 30 min at 37°. Serine dehydratase activities were assayed by the method of Freeland and Avery[7]. Reaction mixtures contained in a final volume of 3 ml: 20 μmoles of DL-serine, 0.25 μmole of NADH, 3 μmoles of pyridoxal phosphate, 150 μmoles of potassium phosphate buffer (pH 7.4), 50 μg as protein of lactate dehydrogenase, and 0.2 ml of soluble fraction, respectively. Reactions were carried out for 20 min at 37°. Control experiments omitting serine were also carried out. Soluble fractions contained approximately 5 to 6 mg of protein per ml.

It should be emphasized that the hyperglycinemia patient who was shown to be normal with respect to serine hydroxymethyltransferase and serine dehydratase exhibited a greatly elevated glycine level in blood while the serine level was in the normal range. These facts would indicate that the glycine cleavage system is quantitatively the most significant pathway of glycine catabolism under the physiological condition in man; the route *via* serine and pyruvate, though functioning, may be only of secondary importance.

In Table I are also shown the amounts of $^{14}CO_2$ formed from

serine-1-^{14}C or serine-3-^{14}C. It is worth noting that the liver homogenate from the patient significantly catalyzed the decarboxylation of either of serine-1-^{14}C and serine-3-^{14}C. Furthermore, the amount of $^{14}CO_2$ formed from serine-3-^{14}C was much larger than that from serine-1-^{14}C, while in most of the control systems the amounts of $^{14}CO_2$ formed from serine-1-^{14}C were larger than or close to those obtained from serine-3-^{14}C. The observed $^{14}CO_2$ formation from serine-1-^{14}C in the patient system may reflect the decarboxylation of pyruvate-1-^{14}C which was derived from serine-1-^{14}C, but the fact that the $^{14}CO_2$ formation from serine-3-^{14}C was higher than that from serine-1-^{14}C could only be accounted for by assuming that considerable portions of serine were cleaved preliminary into glycine and methylene-THF and the latter was oxidized to CO_2, while the glycine formed remained practically unaffected. The oxidation of methylene-THF to CO_2 is probably catalyzed by a mitochondrial enzyme system, for we have observed that intact rat liver mitochondria which were free from the serine dehydratase activity catalyzed the $^{14}CO_2$ formation from either of serine-1-^{14}C and serine-3-^{14}C, while the soluble liver fraction hardly catalyzed the $^{14}CO_2$ formation from either of serine-1-^{14}C and serine-3-^{14}C (to be published). Kutzbach and Stokstad[9] isolated an enzyme system from pig liver which catalyzed the NADP-dependent oxidation of formyl-THF to CO_2. In livers of control humans the glycine thus formed from serine may be catabolyzed to CO_2 through the function of the glycine cleavage system. The results obtained with ^{14}C-labeled serine point to the possibility that although serine can be catabolized via pyruvate, the glycine cleavage system would also play an important role in the serine catabolism in normal human liver. Studies are in progress to determine quantitatively the relative

contributions of these two pathways in the physiological serine catabolism in various animals.

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