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# THE IMPAIRED EXPRESSION OF GLYCINE DECARBOXYLASE IN PATIENTS WITH HYPERGLYCINEMIAS

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SUMMARY: Glycine decarboxylase, a constituent of the glycine cleavage system, in patients with either nonketotic or ketotic hyperglycinemia (NKH and KH) was examined using an anti-chicken glycine decarboxylase antibody. Patients with NKH who have lesion in glycine decarboxylase are differentiated by its expressed level in the liver. One group is cases of the neonatal onset type who have neither activity of the enzyme nor protein reactive to the antibody. The other is a case of the late onset type who shows low but detectable activity of the enzyme and the desirable amount of the immunoreactive material. In the liver of a patient with KH not showing the appreciable activity of H-protein, ubiquitous amount of protein reactive to anti-H-protein IgG is detected and amount of glycine decarboxylase has also been lowered. It is suggested that several mechanisms may be involved in determining the expressed level of glycine decarboxylase in patients with hyperglycinemias. 

It has been accepted that hyperglycinemia, an overwhelming disease in man, results from deficit of activity of the glycine cleavage system (1-3) which consists of four constituents such as glycine decarboxylase (4), H-protein (5) (also verified as aminomethyl carrier protein (4)), tetrahydrofolate requiring protein (T-protein)(6), and lipoamide dehydrogenase (6) as an enzyme complex (7). As would be expected from the observation that the mode of existence of glycine decarboxylase and H-protein in mitochondria closely links to expression of those function (8), an anomaly in at least one of the constituents may cause total loss of the glycine cleavage activity forming $CO_2$, $NH_3$, $CH_2$-$H_4$folate and $NADH+H^+$. NKH in which glycine solely accumulates in body fluids has been differentiated from ketotic one which accompanies with organic acidemias (9-11), and thus far several types of NKH (typical and the neonatal onset type, and atypical and the infantile or late onset type) have been classified from the clinical aspects (3). Various abnormalities of the

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enzyme system resulting in the symptom have also been reported. Among them, most cases with NKH have a lesion in glycine decarboxylase (3), and the H-protein activity is undetectable in patients with KH during metabolic acidosis (12). In this regard, it is of interest to classify immunochemical properties of the enzyme system in the patients with hyperglycinemias and to compare the features with each other. For this purpose, we examined the occurrence of immunoreactive materials in patients with either NKH or KH using anti-chicken glycine decarboxylase and anti-rat H-protein antibodies. In this report we show that a novel case, who is classified as the late onset type of NKH, has low-abundance glycine decarboxylase, and that an inactive H-protein accumulates, accompanying with simultaneous reduction of glycine decarboxylase, in a case with KH. Possible mechanisms for the altered level of expression of glycine decarboxylase are discussed.

## EXPERIMENTAL PROCEDURES

CASES Under the clinical diagnosis of either NKH or KH, all the autopsied and biopsied specimens of livers were transported through Prof. Tada (Tohoku University) to Toyama Medical and Pharmaceutical University in dry ice, and stored at -80°C until examination. Clinical diagnosis for NKH was given by doctors as follows: patient V. M. (died on the 10th day of life) by Dr. P. Ferreira, Department of Pediatrics and Laboratory Medicine, Health Science Center, University of Alberta, Edmonton, Canada; patient F. S. (died on the 6th day of life) by Dr. S. Schelley, Genetics Center, Stanford University School of Medicine, Stanford, CA., U.S.A.; as the neonatal onset type, and patient D. T. ( alive, 20 years old) by Dr. M. Batshaw, Department of Pediatrics, Johns Hopkins Hospital, Baltimore, MD., U.S.A. ; as the late onset type. Patient T. I. (died on the 30th day of life) was diagnosed as KH (propionic acidemia) by Dr. M. Bamba, Yokosuka Kyosai Hospital, Yokosuka, Kanagawa, Japan. Control human livers were obtained from autopsy at Toyama Medical and Pharmaceutical University School of Medicine from patients without hyperglycinemia.

PREPARATION OF LIVER EXTRACTS AND ENZYME ASSAYS About 1 g each of the frozen livers was thawed and homogenized with 9 volumes of 20 mM potassium phosphate buffer (pH 7.0) containing 1 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 10 ug/ml of leupeptin, and 2 ug/ml of pepstatin A. Aliquots were taken to assay for the glycine decarboxylation. The remnants were sonicated at an intensity of 200 watts for 2 min in ice. Spinning cellular debris down at 105,000 g for 60 min, the supernatants were subjected to enzyme assays which were essentially performed as reported previously (1), and immunoblot analyses. Protein amount was determined by the method of Lowry et al. (13).

IMMUNOBLOT ANALYSIS An antibody raised against glycine decarboxylase obtained from chicken liver by the method of Hiraga and Kikuchi (4) was purified on a glycine decarboxylase-Sepharose column. The immunopurified anti-rat H-protein antibody (14) was also used. Immunoblot of glycine decarboxylase and H-protein was carried out by the method of Towbin et al. (15) using [$^{125}$I]anti-rabbit IgG antibody (Amersham).

## RESULTS AND DISCUSSION

First, we examined the activity of glycine decarboxylation in the liver specimens by measuring the amount of $^{14}CO_2$ formed from [1-$^{14}$C]glycine. As shown in Table I, the liver homogenates from patients with NKH of the neonatal

Table 1. Identification of the impaired constituent in livers of patients with NKH. Assays were performed as previously reported (1) and briefly as follows. The glycine cleavage activity was expressed as amount of $^{14}CO_2$ formed by the incubation with liver homogenate from [1-$^{14}$C]glycine. Glycine decarboxylase and H-protein were assayed by measuring amount of fixed $^{14}CO_2$ at the carboxyl carbon of glycine in the glycine-$^{14}CO_2$ exchange reaction which was catalyzed with those enzymes in the presence of excess amount of H-protein for glycine decarboxylase or glycine decarboxylase for H-protein. T-Protein was assayed by [1-$^{14}$C]glycine synthesis from $CH_2$-$H_4$folate, NADH+$H^+$, $NH_3$, and $^{14}CO_2$ in the reaction mixture supplemented with glycine decarboxylase, H-protein, and lipoamide dehydrogenase. The lipoamide dehydrogenase activity in the liver specimens was not measured.

| EXPERIMENT | LIVER SPECIMEN | ENZYME ACTIVITY (nmol of products formed/mg of protein X h) | | | |
|---|---|---|---|---|---|
| | | GLYCINE CLEAVAGE | GLYCINE DECARBOXYLASE | H-PROTEIN | T-PROTEIN |
| 1. | control 1 | 2.83 | 165.0 | 90.5 | 5.5 |
| | control 2 | 6.04 | 106.0 | 88.8 | 5.5 |
| | V.M. | 0.01 | 0.4 | 83.1 | 7.1 |
| | F.S. | 0.00 | 0.6 | 104.0 | 17.4 |
| 2. | control 1 | 3.80 | 163.0 | 93.3 | 9.8 |
| | control 2 | 6.65 | 89.0 | 84.5 | 11.8 |
| | D.T. | 0.14 | 4.4 | 106.0 | 15.1 |

onset type cannot show the significant activity. The homogenate from patient D.T. (the late onset type), however, can form small amount of $CO_2$, suggesting that the putative abnormality in the constituents is not complete in this case. We further tested activities of the individual constituents of the system. Glycine decarboxylase of patients V.M. and F.S. seems to be defective because of extremely low activity (less than 0.5 % of the control values), while the enzyme of patient D.T. shows about 3 % of the activity in control livers. As the other constituents express activities higher than those of the controls, we judged that the patients listed in Table 1 would have the primary lesion in glycine decarboxylase, although nature of the anomaly in patient D.T. might differ from those of others.

This idea was confirmed by immunoblot using the anti-glycine decarboxylase antibody. The antibody reacts with a human mitochondrial protein having about 100 kDa of molecular weight (Fig. 1A, lane 2) similar to that of glycine decarboxylase purified from chicken liver (lane 1). Since we have confirmed that the antibody could neutralize the human glycine decarboxylase activity (1), it is acceptable to consider the band in lane 2 as the signal for location of the human enzyme. Although the extracts from the control also contain the protein (lanes 3 and 4, and also in Fig. 1B, lanes 1 and 2), those from the patients of the neonatal onset type do not reveal any signal (lanes 5 and 6), indicating that glycine decarboxylase is absent from the livers. The result is consistent with the finding that those extracts hardly show activity of the enzyme. In contrast, liver specimen from patient D.T. gives clear sig-

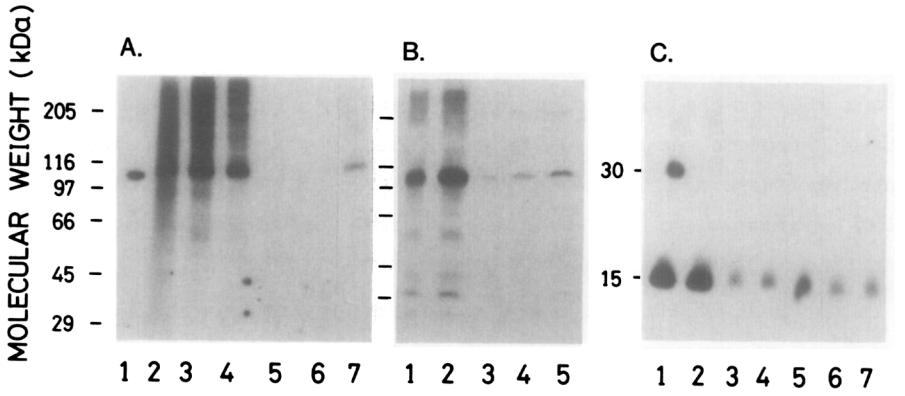


Fig. 1. Immunoblot of glycine decarboxylase and H-protein in the liver specimens from patients with either nonketotic or ketotic hyperglycinemia. A; the liver extracts (100 ug each of protein) of patients with the neonatal onset type (V.M. and F.S., lanes 5 and 6) or with KH (T.I., lane 7), and the controls (lanes 3 and 4) were boiled with 3 % sodium dodecyl sulfate (SDS) for 3 min and subjected to polyacrylamide gel electrophoresis using 7.5 % gel in the presence of 0.1 % SDS (17). Purified chicken glycine decarboxylase (100 ng, lane 1) and the human mitochondrial fraction (300 ug, lane 2) were processed in parallel. The proteins were transferred to a nitrocellulose filter and treated with anti-glycine decarboxylase IgG followed by [$^{125}$I] anti-rabbit IgG antibody. Bands were located by autoradiography. B; The liver extracts from patient D.T. (50, 100, and 200 ug as protein, lanes 3, 4, and 5) and control 2 (100 and 200 ug as protein, lanes 1 and 2) were treated by the same way as described in A. C; Purified human H-protein (lane 1, 40 ng), human mitochondrial fraction (lane 2, 100 ug), and the liver extract (6 ug each of protein) from control 1 and 2, (lanes 3 and 4), patient T.I. (lane 5), and patient V.M. and F.S. (lanes 6, and 7) were processed as described in A, except that 15 % of gel concentration and the immunopurified anti-rat H-protein antibody were used.

nals increasing in intensity with increasing amounts of protein loaded (Fig. 1B, lanes 3, 4, and 5). The bands, however, seem to be less intense than those in controls in lanes 1 and 2 (in Fig. 1B) to which protein amounts equal to those to lanes 4 and 5 have been loaded. In fact, the bands in lanes 2 and 5 were densitometrically expressed as 406 and 12, respectively, in arbitrary units. By comparison, content of glycine decarboxylase in the liver of patient D.T. is calculated to be about 3 % of the control and the value is very close to that determined on the basis of specific enzyme activity shown in Table 1, suggesting that the protein molecule is apparently normal.

Although the precise mechanism is quite unknown, a similarly reduced level of the enzyme was also found in the liver of patient T.I. with KH. This patient appeared to have no activity of the glycine decarboxylation and the primary lesion in H-protein (less than 3 % of the activity in control livers). Moreover, the liver extract only retained about 10 to 25 % of the glycine decarboxylase activity relative to the controls. Content of the enzyme in patient T.I. is also estimated as about 10 to 18 % of control livers by the same way as above from the density in lane 7 of Fig. 1A. Conversely, in spite of the low activity of H-protein, the patient has ubiquitous amount of a

which recognizes the carboxyl-terminal region of dynorphin A. Synthetic κ opiate agonists affect dihydropyridine binding to L-type channels in rat brain (28), and dynorphin A [1-13] inhibits PN 200-110 binding in brain synaptic vesicles ($K_i$ = 10 μM). However, dynorphin A [3-13] is without effect (50 μM). Therefore, dynorphin A-like peptides have multiple effects on neuronal $Ca^{2+}$ channels, but interaction between dynorphin A and ω-CgTX receptors most likely does not involve L-type channels.

Venom of the spider Plectreurys tristes contains peptides which interfere with synaptic transmission in drosophila by apparently blocking presynaptic $Ca^{2+}$ channels (15). This venom caused potent concentration-dependent inhibition of ω-CgTX binding with an $IC_{50}$ of 30 ng venom protein/ml (Fig. 3A). Saturation experiments indicate that inhibition is noncompetitive (Fig. 3A-inset). In separate experiments, venom was found to affect kinetics of ω-CgTX binding: $k_{-1}$ is increased (Fig. 3B), as is $k_1$. Thus, it is probable that a peptidyl-venom component binds to a site which is allosterically coupled to the ω-CgTX receptor. Importantly, this venom (5 μg protein/ml) has no effect on PN 200-110, D-600 or diltiazem binding in brain, indicating that interaction between venom and L-type channels is unlikely. Moreover, crude venom (50 μg protein/ml) has no effect on L- or T-type channel activity in $GH_3$ cells as determined by voltage clamp protocols (G. Suarez-Kurtz, and G. Kaczorowski, unpublished). These data

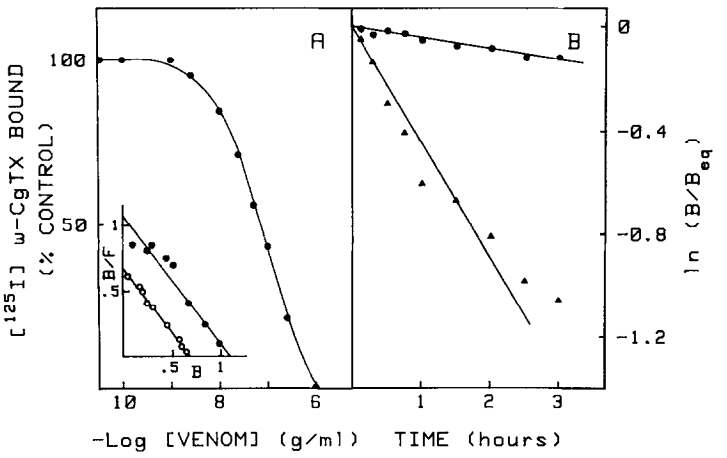


Fig. 3. **Effect of venom of Plectreurys tristes on ω-CgTX binding** (A). Membrane vesicles were incubated with 1 pM ω-[$^{125}$I]CgTX in the absence or presence of increasing concentrations of venom of Plectreurys tristes. Specific binding data is presented relative to an untreated control. Insert: Scatchard representation of ω-[$^{125}$I]CgTX binding in the absence (●) or presence (○) of 25 ng/ml venom of Plectreurys tristes. Units of the y- and x-axes are pmol/mg protein/pM and pmol/mg protein, respectively. (B). Dissociation kinetics of ω-[$^{125}$I]CgTX from its receptor were measured by addition of 10 nM ω-CgTX alone (●) or with 0.5 μg/ml of venom of Plectreurys tristes present (▲) and incubating at 25°C for different periods of time. A semilogarithmic plot of the first-order dissociation reaction is presented.

rearrangement of the gene encoding glycine decarboxylase, including replacement of a single base in one exon by which the enzyme becomes labile but still active in catalytic function. Similarly, a structural change at the regulatory site of the gene may cause the altered level of expression. Further study would shed the light on the problem in more detail concerning structure of the glycine decarboxylase and H-protein genes in patients with hyperglycinemias.

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