Characterization of Glutamate Dehydrogenase Isoproteins Purified from the Cerebellum of Normal Subjects and Patients with Degenerative Neurological Disorders, and from Human Neoplastic Cell Lines*

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Glutamate dehydrogenase (GDH) was purified to homogeneity from cerebellar tissue of three normal subjects and seven patients with four distinct types of degenerative neurological disorders. Nonequilibrium pH gradient gel electrophoresis showed that the purified enzyme consists of four major isoproteins designated GDH 1, 2, 3, and 4. With one exception, the relative abundance and isoelectric points of the GDH isoproteins decrease and the molecular weights increase progressively going from isoprotein 1 to isoprotein 4. The enzyme isolated from the brain of one patient with a variant form of multiple system atrophy and seven patients with four distinct types of neurologic control subjects displayed marked reduction of GDH isoprotein 1. The $K_m$ values of the patients' GDH for $\alpha$-ketoglutarate, glutamate, NADH, and NADPH were significantly increased as compared to GDH obtained from normal and neurologic control subjects. In addition, glutamate levels were markedly reduced in the patient's cerebellum. Pulse-chase studies have shown that both the human hepatoma HepG2 and the human glioma U373 cell lines synthesize exclusively GDH isoprotein 2. The different GDH isoproteins do not have a precursor-product relationship and may represent products of different GDH mRNA species.

Glutamate dehydrogenase (GDH)1 (EC 1.4.1.3) is known to catalyze the reversible deamination of $\alpha$-L-glutamate to $\alpha$-ketoglutarate using NAD and/or NADP as coenzyme thus providing a major pathway for the metabolic interconversion of $\alpha$-amino and $\alpha$-keto acids. GDH has been found in several mammalian tissues including liver, brain, kidney, heart, pancreas, ovaries, and lymph noder (1). The enzyme has been purified from the liver of several species and sequenced (1). The bovine liver enzyme is thought to be present in brain in concentrations that are 2-5-fold higher than those found in other organs. Furthermore, due to its neurotoxic potential, glutamate may be involved in the pathogenesis of human degenerative disorders (10-12).

Here we report characterization of GDH purified from human cerebellum of normal subjects and patients with degenerative neurological disorders and from human neoplastic cell lines. Two-dimensional polyacrylamide gel electrophoresis has shown that the purified enzyme is composed of four main isoproteins, whereas nascent GDH synthesized by HepG2 and U373 cells consists of a single major isoprotein. This analysis may be important for detection of enzyme mutations associated with human neurodegenerative disorders.

EXPERIMENTAL PROCEDURES

Materials

Bovine serum albumin, ovalbumin, lysozyme, trypsin inhibitor, neuraminidase Type X (Clostridium perfringens), bovine intestinal mucosa alkaline phosphatase type VII-S, deoxycholate, Triton X-100, 2-mercaptoethanol, TEMED, hydrogen peroxide (30%, w/w), Tris, agarose, phenylmethylene sulfonic fluoride (PMSF), and aprotinin were purchased from Sigma. Ampholines, pH 3.5-10, were purchased from Bio-Rad (Richmond, CA). PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; TEMED, N,N,N',N'-tetramethyleylenediamine.

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†An Established Investigator of the American Heart Association.

‡The abbreviations used are: GDH, glutamate dehydrogenase; PMSF, phenylmethylene sulfonic acid; SDS, sodium dodecyl sulfate; TEMED, N,N,N',N'-tetramethyleylenediamine.
from LKB Instruments, Inc. Nonidet P-40 was purchased from Particle Data Laboratories, Ltd. Acrylamide was obtained from Serva Fine Chemicals. Glycine, methanol and glacial acetic acid were purchased from Fisher. Sodium dodecyl sulfate, bisacrylamide, ammonium persulfate, Coomassie Brilliant Blue, bromphenol blue, and Blue, which is a product of Biodyne. It was treated with 1 unit of neuraminidase type X for each 100,000 g. The supernatant was centrifuged for 1 h. The mixture was centrifuged for 30 s at 37 °C. The supernatant was then centrifuged at 400 × g for 10 min (at 0-5 °C). The resultant pellet was re-homogenized in buffer II and centrifuged for 30 min at 0-5 °C. The supernatant was centrifuged for 1 h. The mixture was centrifuged for 5 min in a microcentrifuge. Ten μl of antiserum was added to the supernatant and the mixture was incubated at 4 °C. The mixture was centrifuged for 30 s in a microcentrifuge. The pellet was washed once by resuspension into 100,000 g for 1 h. The final high speed supernatant contained 98% of the original GDH activity and was used as the starting material for the purification of GDH. The column was equilibrated in high concentration buffer (ESG) and Triton X-100. The enzyme was eluted from the column by incubation in a buffer containing 100 μM Tris-HCl, 1 mM EDTA, pH 7.15, buffer containing 50 mM KCl (to protect GDH activity) and loaded on a GTP-Sepharose affinity column prepared by the method described by Godinot et al. (18). The column was equilibrated with the same buffer and eluted at a flow rate of 1 ml of KCl (0.05-0.4 M). The enzyme was eluted at approximately 0.25-0.5 M KCl. Some purification experiments were performed in the presence of 5 mM EDTA and 0.1 mM PMSP or the combination of the above protease inhibitors plus 0.01% aprotinin in all the purification steps. Fractons containing GDH activity were pooled and used for the electrophoretic analysis and for immunization of rabbits.

Subjects

Cerebellar tissue obtained at autopsy from 10 subjects was used for these studies. Three of these subjects died of medical illnesses (myocardial infarction, etc.) and had no known neurological disorders while alive (non-neurologic subjects, ages 55, 60, and 70 years). Seven patients died of well characterized chronic degenerative neurological disorders (8-10). Of these, two had Parkinson's disease (ages 77, 82) and two had amyotrophic lateral sclerosis (ages 56, 44). The remaining three patients were afflicted with two distinct variants of multisystem atrophy. Two patients with multisystem atrophy (ages 31, 34) were members of Schut-Haymaker OPCA kindred (14). This disorder is HLA-linked and is characterized by adult onset and autosomal dominant transmission with complete penetrance of the mutant gene (15). Leukocytes from these patients have normal GDH activity (7).

The third patient was a 15-year-old male who was afflicted since age 7 by another form of multisystem atrophy characterized by cerebellar ataxia, mental degeneration, ophtalmoplegia, myoclonus, and dementia. His 42-year-old father and a 14-year-old brother have been similarly afflicted since ages 27 and 6 years, respectively. Thus, the disorder in this family appears to be inherited in an autosomal dominant manner with variable age of onset and frequent childhood occurrence. The disease appears to be similar to that described previously by Jampel et al. (16) and Colan et al. (17). HLA typing in members of this family failed to show a linkage between the disease and the HLA loci. Similar to previous studies (16), pathologic examination of the patient's brain at autopsy revealed changes consistent with a variant of olivopontocerebellar atrophy.

The patients died at various times after death ranging from 1 to 24 h and kept frozen at -80 °C, until the day of tissue preparation. (The post-mortem interval for each subject is shown in the legend of Fig. 2.)

Methods

GDH Purification

Tissue Preparation—About 5-10 g of brain tissue was dissected from the cerebellar hemispheres and subjected to 3-4 cycles of freeze-thaw on dry ice. A 20% (w/v) homogenate of minced tissue in 10 mM Tris-HCl, pH 7.4, buffer containing 0.1 mM PMSF, 0.5 mM EDTA, and 1% Triton X-100 (buffer I) was prepared by glass-to-glass shearing (0.004-0.006-inch clearance) for 5 min at 0-5 °C with an IKA-driven pestle (300 rpm). The homogenate thus obtained was diluted 5-fold with 10 mM Tris-HCl, pH 7.4, buffer containing 0.5 mM NaCl, and 1% Triton X-100 (buffer II) and allowed to stand at 0-5 °C for 1 h. It was then centrifuged at 480 x g for 10 min (at 0-5 °C). The resultant pellet was re-homogenized in buffer II and centrifuged as above. The two low speed supernatants were combined and subjected to a high speed centrifugation (100,000 x g for 1 h). The final high speed supernatant contained 98% of the original GDH activity and was used as the starting material for the purification of GDH. The column was equilibrated in high concentration buffer (ESG) and Triton X-100. The enzyme was eluted from the column by incubation in a buffer containing 100 μM Tris-HCl, 1 mM EDTA, pH 7.15, buffer containing 50 mM KCl (to protect GDH activity) and loaded on a GTP-Sepharose affinity column prepared by the method described by Godinot et al. (18). The column was equilibrated with the same buffer and eluted at a flow rate of 1 ml of KCl (0.05-0.4 M). The enzyme was eluted at approximately 0.25-0.5 M KCl. Some purification experiments were performed in the presence of 5 mM EDTA and 0.1 mM PMSP or the combination of the above protease inhibitors plus 0.01% aprotinin in all the purification steps. Fractons containing GDH activity were pooled and used for the electrophoretic analysis and for immunization of rabbits.

Treatment of Purified GDH with Phosphatase and Neuraminidase

Purified human brain GDH (10 μg/5 μl) were dialyzed against water and adjusted to 0.1 M glycine, 1 mM ZnCl2, 1 mM MgCl2, pH 10.7, and treated with 10 units of alkaline phosphatase at 37 °C for 2 h. After incubation, the reaction mixture was diluted with water at 4 °C, lyophilized, suspended in 10 mM sodium phosphate, pH 7.2, 85 mM NaCl, 0.4 M). The enzyme was eluted at approximately 0.25-0.3 M KCl. Some purification experiments were performed in the presence of 5 mM EDTA and 0.1 mM PMSP or the combination of the above protease inhibitors plus 0.01% aprotinin in all the purification steps. Fractons containing GDH activity were pooled and used for the electrophoretic analysis and for immunization of rabbits.

Labeling of the Primary Cell Cultures and Immunoprecipitation of Nascent GDH

Human hepatoma (HepG2) (21) and human glioma (U373) (22) cells were plated in 35-mm diameter tissue culture dishes containing Eagle's minimum essential medium and Dulbecco's modified minimum essential medium supplemented with 10 and 20% fetal calf serum, respectively, and incubated at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. Ninety % confluent cell cultures were washed twice with methionine-free Dulbecco's modified Eagle's minimum essential medium supplemented with 2 mM glutamine and incubated in the same medium for the indicated time in the presence of 1 μCi of [35S]methionine (pulse). The [35S]methionine-containing medium was removed at the appropriate times. The cultures were washed twice with minimum essential medium and incubated with 1 ml of the same medium (chase) for various times. After labeling, the medium was discarded and the cells were lysed with addition of 10 mM sodium phosphate, pH 7.2, 55 mM NaCl, 5 mM KCl, 0.55% deoxycholate, and 1% Triton X-100. The mixture was centrifuged for 5 min in a microcentrifuge and the supernatant was diluted 1:1 with a solution of 10 mM sodium phosphate, pH 7.2, 85 mM NaCl, 5 mM KCl, 0.25% deoxycholate, 0.5% Triton X-100, and 0.5% sodium dodecyl sulfate was added to the cell lysate and incubated for 1 h at 4 °C. The mixture was centrifuged for 5 min in a microcentrifuge. Ten μl of antiserum was added to the supernatant and the mixture was incubated at 4 °C. The mixture was centrifuged for 30 s in a microcentrifuge. The pellet was washed once by resuspension into 100,000 g for 1 h. The final high speed supernatant contained 98% of the original GDH activity and was used as the starting material for the purification of GDH. The column was equilibrated in high concentration buffer (ESG) and Triton X-100. The enzyme was eluted from the column by incubation in a buffer containing 100 μM Tris-HCl, 1 mM EDTA, pH 7.15, buffer containing 50 mM KCl (to protect GDH activity) and loaded on a GTP-Sepharose affinity column prepared by the method described by Godinot et al. (18). The column was equilibrated with the same buffer and eluted at a flow rate of 1 ml of KCl (0.05-0.4 M). The enzyme was eluted at approximately 0.25-0.5 M KCl. Some purification experiments were performed in the presence of 5 mM EDTA and 0.1 mM PMSP or the combination of the above protease inhibitors plus 0.01% aprotinin in all the purification steps. Fractons containing GDH activity were pooled and used for the electrophoretic analysis and for immunization of rabbits.

Gradient SDS-Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gradient gel electrophoresis was performed on vertical slab gels of 17% to 5% acrylamide separating gel consisted of linear 5-15% acrylamide (acrylamide to bisacrylamide ratio 2.92 to 0.08%) gradient in 0.375 M Tris-HCl.
buffer, pH 8.8, containing 0.1% SDS, 0.05% ammonium persulfate, and 0.03% TEMED. The stacking gel comprised 4.5% acrylamide and 0.1% bisacrylamide in 75 mM Tris-HCl, pH 6.8, containing 0.1% SDS, 0.05% ammonium persulfate, and 0.1% TEMED. Electrophoresis was performed under cooling at a constant current of 50 mA until the marker dye reached the bottom of the separating gel.

**Two-dimensional Nonequilibrium pH Gradient Gel Electrophoresis and Isoelectric Point Determination**

Two-dimensional nonequilibrium pH gradient polyacrylamide gel electrophoresis was performed according to the method of O'Farrell et al. (20). The pH gradient electrophoresis was performed in cylindrical tubes of 12.0 cm length with a diameter of 3.0 mm. The pH gradient gel consisted of 4.4% acrylamide (Bio-Rad), 0.12% bisacrylamide, 10 mM urea, 2.2% Nonidet P-40, 0.25% ampholines (pH 3.5–10 ampholines or pH 3–10 Biolytes), 0.25% ammonium persulfate, and 0.017% TEMED. The electrophoresis was performed in cylindrical apparatus (Hoefcr Scientific) at room temperature. The bottom and top chambers contained 2.5 liters of 0.01 M NaOH and 500 ml of 0.01 M phosphoric acid, respectively. For electrophoresis, polarity of the current was reversed and electrophoresis was performed at 150 constant volts for 1 h and at 500 constant volts for 5 h. The cylindrical gels were quickly eluted and frozen in 0.0625 M Tris-HCl buffer, pH 6.8, containing 2% SDS, 1% glycerol, and 0.05% 2-mercaptoethanol.

For the second dimension, the cylindrical gels were thawed quickly and electrophoresis was performed on vertical slab gels of 19.5 X 20.5 cm with a thickness of 0.5–0.75 mm. The slab gels consisted of the separating gel of 9% acrylamide to isoelectric point marker. The pH gradient gel consisted of 2.92 to 0.8% in acrylamide from isoelectric point marker to pH 8.8. The gradient gel comprised of 4.4% acrylamide, 0.12% bisacrylamide in 0.25% Tris-HCl pH 6.8, containing 0.1% SDS, 0.05% ammonium persulfate and 0.2% TEMED. Electrophoresis was performed at a constant voltage of 50 volts for 1 h and then at 85 volts until the marker dye reached the bottom of the separating gel. After electrophoresis, the one- or two-dimensional gels were fixed in 50% methanol, 10% acetic acid for 30 min to an hour.

**Enzyme Assays and Kinetic Studies**

GDH activity was measured spectrophotometrically or fluorometrically in the direction of reductive amination of α-keto-glutarate in a medium containing 50 mM triethanolamine buffer, pH 8.0, 100 mM ammonium acetate, 90 μM NADH, 2.6 mM EDTA, and 1 mM ADP and the reaction started with the addition of α-keto-glutarate to 8 mM (7).

GDH activity was also measured fluorometrically in the direction of glutamate oxidation in the same buffer containing 1.4 mM NAD+, 2.6 mM EDTA, and 1 mM ADP. The reaction started by addition of glutamate to 25 mM final concentration.

Kinetic studies were carried out by varying the substrate under investigation while keeping the other substrates and reagents at the optimal concentration indicated above. α-keto-glutarate was varied between 0.5 and 8 mM, NADH and NADPH between 15 and 120 μM, and glutamate between 2.5 and 50 mM. The post-mortem intervals of tissues utilized as enzyme source for kinetic studies were between 0.5 and 24 h after death.

**Levels of Amino Acid Putative Transmitters and Glutamine in Cerebellar Cortex**

The levels of amino acid putative transmitters and glutamine in cerebellar cortex of normal controls and the patient with abnormal GDH electrophoretic pattern were determined as follows: approximately 200 mg of frozen cerebellar cortex were homogenized (10%, w/v) at 4°C in 0.4 N ice-cold perchloric acid. The homogenate was allowed to stay at 0–4°C for about 10–20 min and then centrifuged at 19,000 X g for 10 min (4°C). The supernatant was analyzed for its amino acid profile by high performance liquid chromatography (Perkin-Elmer) with an ion exchange column and a lithium citrate buffer elution system as described (24).

**RESULTS**

**Purification of GDH to Homogeneity from Human Cerebellum—**GDH was purified 547-fold to apparent homogeneity from whole homogenates of cerebellar tissue of three neurologically normal subjects and seven patients with four distinct types of degenerative neurological disorders. Table I shows the results of the various purification steps employed. The overall recovery of enzymatic activity was 56.6%. The purified enzyme shows a single band of approximately 60,000 daltons on denaturing SDS-polyacrylamide gels (Fig. 1).

**Isoform Protein Composition of GDH Purified from the Cerebellum of Neurologically Normal Subjects and Patients with Degenerative Neurological Diseases—**Nonequilibrium pH gradient gel electrophoretic analysis of the purified enzyme showed that it consists of four major isoproteins (designated 1, 2, 3, and 4) which differ in charge and size (Fig. 2, A–C). All isoproteins are more basic than human apolipoprotein E3 (25) which was used as an internal isoelectric point marker.

**TABLE I**

<table>
<thead>
<tr>
<th>Activity/recovery</th>
<th>Specific activities</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmol/h (%)</td>
<td>amol/mg protein/h</td>
<td>fold</td>
</tr>
<tr>
<td><strong>Tissue homogenate</strong></td>
<td>14,099 (2,177) (100)</td>
<td>17.5 ± 3.5</td>
</tr>
<tr>
<td><strong>Tissue extract</strong></td>
<td>13,877 ± 1,231 (98.4) (n = 9)</td>
<td>24.8 ± 4.3</td>
</tr>
<tr>
<td>30–65% (NH₄)₂SO₄</td>
<td>10,403 ± 1,899 (73.8) (n = 10)</td>
<td>61.1 ± 12.8</td>
</tr>
<tr>
<td>Phenyl-Sepharose</td>
<td>9,536 ± 1,018 (67.6) (n = 10)</td>
<td>672.4 ± 204.8</td>
</tr>
<tr>
<td>eluate</td>
<td>7,986 ± 1,467 (56.6) (n = 10)</td>
<td>9,580 ± 4,608</td>
</tr>
</tbody>
</table>

**FIG. 1. Gradient polyacrylamide gel electrophoresis of purified GDH.** GDH purified from human cerebellum was analyzed by polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. Lanes A and C contain 1 and 5 μg, respectively, of GDH purified from normal cerebellum; Lane B contains GDH purified from the cerebellum of a patient with HLA-linked multisystem atrophy (Sohx-Haymaker type); BL indicates bovine liver GDH (Boehringer Mannheim) that was used as molecular weight marker.
This indicates that the apparent isoelectric point of these proteins is higher than 6.2. The relative abundance and the molecular weights of these isoproteins follows the order 1 > 2 > 3 > 4. The GDH isoprotein profile was not affected when the purification was performed in the presence of protease inhibitors PMSF, aprotinin, and EDTA as described under “Methods.” These GDH isoproteins were also unaffected by treatment with alkaline phosphatase or neuraminidase, indicating that the acidic forms do not arise by post-translational sialation or phosphorylation.

GDH also purified from the cerebellum of seven patients with various forms of neurological disorders with primary neuronal degeneration and similarly characterized by nonequilibrium pH gradient gel electrophoresis. This analysis showed that the two-dimensional pattern of GDH isolated from patients with Parkinson’s disease (Fig. 2, D and E), amyotrophic lateral sclerosis (Fig. 2, G and H), and the Schut-Haymaker type of multiple system atrophy (14) (Fig. 2, I and J) was similar to that of controls (Fig. 2, A–C). However, the patient with the variant of the multiple system atrophy associated with blindness, ophthalmoplegia, and myoclonus showed marked reduction of GDH isoprotein 1 (Fig. 2F).

**Kinetic Parameters of Normal and Variant GDH Forms**

The $K_m$ values of GDH substrates were determined for the soluble brain enzyme isolated from non-neurologic and neurologic control subjects and the patient who displayed reduction in the relative concentration of isoprotein 1 (Fig. 2F). To avoid proteolytic alterations due to aging of the tissue specimen, which could affect the kinetic analysis of GDH (26) the kinetic analysis was performed on enzymes isolated from brain with similar post-mortem intervals. The kinetic analysis shows that the $K_m$ values of the patients’ GDH for $\alpha$-ketoglutarate, glutamate, NADH, and NADPH are increased significantly as compared to control GDH (Table II).

**Amino Acid Content of Cerebellar Cortex of Neurologically Normal Subjects and the Patient with the Abnormal GDH Profile**

Determination of the amino acid putative transmitter content showed that the glutamate and aspartate contents of the patient’s cerebellar cortex were 25 and 40%, respectively, of the control values, whereas the patient’s values of taurine, glutamine, and $\gamma$-aminobutyric acid were within the normal range (Table III).

**Synthesis of GDH by HepG2 and U373 Cells**

To study the sequence of events involved in the generation of the GDH isoproteins, the human hepatoma (HepG2) cell line was pulsed with $^{35}$S-methionine for 12 min and chased for various periods. Similarly, the human glioma cell line (U373) was pulsed continuously from 10 min to 1 h. The enzyme was immunoprecipitated with anti-bovine GDH and analyzed by two-dimensional nonequilibrium pH gradient gel electrophoresis and autoradiography. This analysis showed that the GDH synthesized by both cell lines after short pulses (10–15 min) consisted of a single major isoprotein which co-migrated with isoprotein 2 of the human cerebellar GDH (Fig. 3). No other isoproteins were generated during the course of pulse-chase experiments. Immunoblotting analysis of the purified brain enzyme using rabbit anti-bovine GDH (13) gave the same isoprotein pattern as that of Fig. 2 which was obtained by protein staining (data not shown). This suggests that the antibodies used recognize all four GDH isoproteins.

**DISCUSSION**

In this study, we purified and characterized human brain GDH isolated from the cerebellum of neurologically normal individuals and patients with degenerative disorders. Two-dimensional nonequilibrium pH gradient isoelectric focusing polyacrylamide gel electrophoresis of purified GDH showed that the enzyme consists of four major isoproteins. With one exception the relative abundance of these isoproteins is $1 > 2 > 3 > 4$. These isoproteins could be the products of different genes or could arise by intracellular post-translational modification involving the endoplasmic reticulum and the Golgi complex. Two GDH isoproteins with different molecular weight electrophoretic mobilities, antigenic properties, and peptide maps have been described in bovine heart (27). To study the possibility of intracellular post-translational modification of GDH in the endoplasmic reticulum-Golgi system we performed pulse-chase labeling studies of HepG2 and U373 cells. These studies showed that the enzyme synthesized by both cell lines consisted of a single isoprotein which co-migrated with isoprotein 2 of the human cerebellum. The failure of HepG2 isoprotein 2 to be converted to other isopro-
Glutamate Dehydrogenase Isoproteins

**TABLE II**

Kinetic parameters of glutamate dehydrogenase obtained from different human subjects

The $K_m$ values of non-neurologic and neurologic controls were not significantly different. Numbers in parentheses indicate number of experimental determinations.

<table>
<thead>
<tr>
<th>$K_m$ values</th>
<th>NADH</th>
<th>NADPH</th>
<th>Glutamate</th>
</tr>
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<tbody>
<tr>
<td>mM</td>
<td>$\mu$M</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td>Non-neurologic control</td>
<td>1.3 ± 0.1 (9)</td>
<td>77.8 ± 39.6 (9)</td>
<td>51.2 ± 22.8 (6)</td>
</tr>
<tr>
<td>Neurologic control</td>
<td>53.1 ± 10.8 (6)</td>
<td>32.9 ± 5.3 (7)</td>
<td>17.6 ± 2.0 (7)</td>
</tr>
<tr>
<td>Patient</td>
<td>4.8 ± 0.5 (7)</td>
<td>368.0 ± 70.1 (7)</td>
<td>172.8 ± 22.1 (9)</td>
</tr>
<tr>
<td>Significance (p)</td>
<td>0.001$^{a,b}$</td>
<td>0.001$^{a,b}$</td>
<td>0.001$^{a,b}$</td>
</tr>
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</table>

$^a$ Significant $K_m$ differences, non-neurologic control versus patient.

$^b$ Significant $K_m$ differences, neurologic control versus patient.

**TABLE III**

Levels of amino acid putative transmitters and glutamine for cerebellar cortex

<table>
<thead>
<tr>
<th>Normal controls$^a$</th>
<th>Control range</th>
<th>Patient$^b$</th>
</tr>
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<tbody>
<tr>
<td>$\mu$mol/g wet weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>3.16 ± 1.03</td>
<td>1.00–3.98</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.24 ± 0.60</td>
<td>0.52–2.03</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8.75 ± 1.72</td>
<td>6.75–11.20</td>
</tr>
<tr>
<td>Glutamine</td>
<td>5.38 ± 0.95</td>
<td>3.90–5.90</td>
</tr>
<tr>
<td>$\gamma$-Aminobutyric acid</td>
<td>1.18 ± 0.48</td>
<td>0.68–2.08</td>
</tr>
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</table>

$^a$ The control values were determined from specimens obtained from seven neurologically normal subjects (mean ± S.D.).

$^b$ The patient's values represent the mean ± S.D. of two independent determinations done on separate specimens of cerebellar cortex.

mitochondria matrix with concomitant loss of a $M, 6000$ fragment, presumed to be a signal peptide (31). The existence of a 53-amino acid signal for hepatic GDH peptide has been verified recently by sequencing of a full length GDH cDNA clone (29). It is interesting that the amino-terminal residues of the putative signal peptide sequence are Ala-Arg-Arg-His-Tyr (29). It is thus possible that an initial cleavage at the Ala residue and subsequent slow removal of amino-terminal Arg residues may generate acidic GDH isoprotein species. Multiple mRNA forms generated by alternative splicing are responsible for the mRNA and protein diversity in the amino-terminal regions of human tyrosine hydroxylase (28). A similar process could also generate GDH isoproteins with few amino acid differences in the mature protein, but very similar antigenic properties. Further studies are required to elucidate the GDH polymorphism.

Analysis of cerebellar GDH from four neurologically normal individuals and comparison with the enzyme of four patients affected by Parkinson's disease and amyotrophic lateral sclerosis revealed an almost identical electrophoretic pattern in all these individuals. This was also true for the cerebellar GDH from two patients, members of an HLA-linked dominant kindred afflicted by a form of multisystem atrophy that is not associated with GDH deficiency. However, this analysis revealed that a patient from a non-HLA linked pedigree with another variant of multisystem atrophy (16, 17) showed a major reduction in isoprotein 1 which is most abundant in the normal individuals. This could result from defective synthesis or post-translational formation of isoprotein 1.

Kinetic analysis of the soluble brain GDH obtained from non-neurologic and neurologic control subjects has shown that the $K_m$ values of the patient's enzyme are significantly higher than those of the controls. The increase in $K_m$ values is also associated with significant decreases in glutamate and aspartate levels in the cerebellum, apparently due to ineffi-
cient conversion of the α-ketoglutarate to glutamate. Since aspartate in brain is primarily derived from glutamate and oxaloacetate by transamination, the decrease in the aspartate levels could be secondary to the reduction of the glutamate pool (24). These findings suggest that the altered electrophoretic pattern of this patient’s enzyme is associated with altered catalytic activity and function. Further studies are needed to substantiate this hypothesis and to test whether the marked reduction in isoprotein 1 is the result of a mutant gene or defective processing of the enzyme.

This report provides the first description of human brain GDH polymorphism and suggests that the study of this polymorphism could provide clues toward the understanding of human nondegenerative disorders.

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