Aldehyde dehydrogenase from adult human brain that dehydrogenates γ-aminobutyraldehyde: purification, characterization, cloning and distribution

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INTRODUCTION

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system. It is also present in significant amounts in mammalian peripheral tissues [1]. The major pathway for GABA synthesis is by the decarboxylation of t-glutamate by t-glutamate decarboxylase [2]. GABA can also be formed from putrescine by two pathways. The first involves the direct oxidative deamination of putrescine, by diamine oxidase, to give γ-aminobutyraldehyde [3]. The γ-aminobutyraldehyde is then converted into GABA by an aldehyde dehydrogenase. The second pathway involves the acetylation of putrescine to N-acetylputrescine, which is converted into N-acetyl-γ-aminobutyraldehyde by monoamine oxidase [4], and then to N-acetyl-GABA by an aldehyde dehydrogenase. N-Acetyl-GABA is deacetylated to form GABA. Levels of diamine oxidase in the mammalian brain are reportedly low [5], and so the main pathway of GABA formation from putrescine in the brain is believed to be through monoamine oxidase.

Aldehyde dehydrogenase (EC 1.2.1.3) catalyses the NAD⁺-linked oxidation of aldehydes to acids. It has a broad substrate specificity, with substrates ranging from straight-chain and branched aliphatic and aromatic aldehydes to the aldehyde metabolites of naturally occurring compounds, such as biogenic amines and corticosteroids [6]. Three isoenzymes of aldehyde dehydrogenase have been purified from the human liver [7,8]. They are the cytoplasmic E1 and E3 isoenzymes, and the mitochondrial E2 isoenzyme. The E3 isoenzyme was identified as E3 from the purified brain protein matched the amino acid sequence of the liver E3 isoenzyme. Employing liver E3 cDNA, a human cerebral cDNA library was screened and a 2.0 kb cDNA fragment was isolated. The cerebral cDNA yielded a derived primary structure which differed from the liver E3 amino acid sequence by a single serine-to-cysteine substitution at position 88 (position 84 in the liver sequence). Thus the γ-aminobutyraldehyde-metabolizing enzyme from human brain can be identified as E3, a variant of the E3 isoenzyme. The catalytic properties of the brain variant were indistinguishable from those of E3, and so the functional importance of this variant is at present unknown. The distribution of this enzyme in brain was investigated by Northern blot analysis, which demonstrated the presence of E3 mRNA in all regions of the human brain. mRNA levels were variable in the different brain areas, with the highest levels in the spinal cord and the lowest in the occipital pole.

More recently, aldehyde dehydrogenases which metabolize γ-aminobutyraldehyde were purified from rat and bovine brain [9,10]. The reported Kₘ values for γ-aminobutyraldehyde of the rat and bovine enzymes (151 and 154 μM respectively) were an order of magnitude higher than that of the E3 isoenzyme. Kₘ values of the brain enzymes for other substrates also differed from those of E3. These differences in kinetic properties, together with the reportedly low levels of brain diamine oxidase, suggested that the brain enzyme may be different from the liver E3 isoenzyme. Here we report the purification of an enzyme with γ-aminobutyraldehyde dehydrogenase activity from human brain. The kinetic, protein chemistry and molecular biology approaches used to characterize this enzyme are described.

EXPERIMENTAL

Materials

Acetaldehyde and γ-aminobutyraldehyde diethyl acetal were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Propionaldehyde, from J. T. Baker Chemical Co. (Phillipsburg, NJ, U.S.A.), was redistilled before use. Ammonium sulphate was from Schwarz/Mann Co. (Orangeburg, NY, U.S.A.). CM-Sephadex C-50, DEAE-Sephadex A-50, 5'-AMP–Sepharose 4B, Blue Sepharose CL-6B, FPLC Mono-P column, Pharmalyte and the Isoelectric Focusing Calibration Kit (pH range 3-10) were from Pharmacia Biotech Co. (Piscataway, NJ, U.S.A.). Formamide and tetramethylammonium chloride were from Fisher Scientific (Pittsburgh, PA, U.S.A.). NAD⁺ was from Boeringer-Mannheim Co. (Indianapolis, IN, U.S.A.). The Sequenase Version 2.0 DNA Sequencing Kit was from United States Biochemical (Cleveland, OH, U.S.A.). The Multiprim
DNA Labeling System, Hybond-N nylon membranes and all radioactive nucleotides were from Amersham Co. (Arlington Heights, IL, U.S.A.). Immobilon poly(vinylidene difluoride) (PVDF) membranes were from Millipore Corp. (San Francisco, CA, U.S.A.). Nitrocellulose filters were from Schleicher and Schuell (Keene, NH, U.S.A.). Betaine aldehyde chloride, glycolaldehyde, N-acetylputrescine, catalase, monoamine oxidase, β-mercaptoethanol, p-Nitro Blue Tetrazolium chloride, phenazine methosulphate, salmon testes DNA, SDS and Fuji RX film were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The human cerebellar cDNA library was from Stratagene (La Jolla, CA, U.S.A.). The human fetal brain cDNA library was from Clontech Laboratories (Palo Alto, CA, U.S.A.). E1, E2 and E3 aldehyde dehydrogenase isoenzymes from human liver were purified using previously described procedures [7,8].

Preparation of aldehyde substrates

γ-Aminobutyraldehyde was prepared by the acid hydrolysis of fractionally redistilled diethyl acetal, as described by Ambroziak and Pietruszko [11]. N-Acetyl-γ-aminobutyraldehyde was generated from N-acetylputrescine by the action of monoamine oxidase, as described in [11].

Enzyme activity assay

γ-Aminobutyraldehyde-metabolizing activity was assayed in 0.1 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, 500 µM NAD⁺ and 500 µM γ-aminobutyraldehyde. Activity towards propionaldehyde was assayed in 0.1 M sodium pyrophosphate buffer, pH 9.0, containing 1 mM EDTA, 500 µM NAD⁺ and 1 mM propionaldehyde. Reactions were initiated by the addition of enzyme. All assays were carried out at 25 °C. NADH formation was monitored at 340 nm using a Gilford spectrophotometer. A molar absorption coefficient of 6.22 mM⁻¹·cm⁻¹ for NADH was used. Kᵦ values were determined by the single reaction method of Yun and Sueltzer [12], and also by the Lineweaver–Burk method [13].

Protein determination

Protein concentration was determined as described by Goa [14], using BSA as a standard. Protein was also assayed spectrophotometrically at 280 nm, using a molar absorption coefficient of 1.0 (mg/ml)⁻¹·cm⁻¹ [8].

Isoelectric focusing

Agarose plates composed of 1 % (w/v) agarose, 12 % (w/v) sorbitol and 0.063 % (v/v) Pharmalyte (pH 3–10) were used for isoelectric focusing. Gels were run for 16–18 h at 120 V and stained for activity with γ-aminobutyraldehyde (500 µM) using 0.1 M sodium phosphate buffer, pH 7.4, and for activity with propionaldehyde (13 mM) using 0.1 M Tris/HCl buffer, pH 8.5. The staining solution also contained NAD⁺ (10 mg/100 ml), p-Nitro Blue Tetrazolium (10 mg/100 ml), phenazine methosulphate (1 mg/100 ml) and 1 mM EDTA. The Pharmacia Isoelectric Focusing Calibration Kit (range pH 3–10) was used, according to the manufacturer’s instructions, to determine isoelectric points.

Subunit molecular mass determination and Western blotting

Subunit molecular mass was determined by SDS/PAGE according to the procedure of Laemmli [15]. The standards were from Sigma Chemical Co. Protein bands were transferred from the gel to an Immobilon PVDF membrane using a Milliput Graphite Electroblotter System (Millipore Corp.). Western blot analysis was then carried out using the polyclonal anti-E3 antibody described by Kurys et al. [16].

Primary structure analysis

Approx. 2 µg of the main protein band was transferred from an SDS/PAGE gel to a nitrocellulose filter using the Milliput Graphite Electroblotter System. The filter was then stained with 0.1 % Ponceau S in order to visualize the protein bands, and the sample band was cut out. In situ tryptic digestion, MS and microsequencing of the band were carried out by the Microchemistry Core Facility, Memorial Sloan-Kettering Cancer Center, New York, NY, U.S.A., as described by Erdjument-Bromage et al. [17].

Screening of a human brain cDNA library

A human cerebellar cDNA library constructed in λ ZAP was screened (1 x 10⁶ plaque-forming units) using a 1.6 kb human liver E3 cDNA [16] and a 24-mer oligonucleotide (5’ GCTAT-CACCTGGCGGTTGCTGGC 3’) complementary to the 5’-end region of the E3 cDNA (nt 3–26). Filters were prehybridized for 4 h at 65 °C in a solution containing 6 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 10 x Denhardt’s solution, 0.5 % (w/v) SDS, 0.05 % (w/v) sodium pyrophosphate and 50 µg/ml denatured salmon testes DNA [18]. The cDNA probe was ³²P-labelled by the random priming method using [α-³²P]dCTP, and the oligonucleotide probe was labelled at the 5’ end with [γ-³²P]ATP. The specific radioactivities of the cDNA and oligonucleotide probes were 3 x 10⁶ and 2 x 10⁵ d.p.m./µg respectively. Hybridization with the cDNA probe was carried out in the same solution as above, containing 50 % formamide, at 37 °C for 12 h, whereas hybridization with the oligonucleotide probe was carried out in 6 x SSC, 10 x Denhardt’s solution, 0.05 % sodium pyrophosphate and 20 µg/ml yeast tRNA at 45 °C for 12 h [18]. cDNA-probed filters were washed four times for 15 min in 2 x SSC/0.1 % SDS at room temperature. Oligonucleotide-probed filters were washed twice for 30 min in 6 x SSC/0.05 % sodium pyrophosphate and twice for 20 min in 3 M tetramethylammonium chloride, 50 mM Tris, pH 8.0, 2 mM EDTA and 0.1 % SDS at 50 °C. Three rounds of screening yielded five plaque-purified positive clones which, after in vitro excision of the λ vector, formed phagemids containing the cloned inserts. The clones were then characterized by restriction enzyme analysis and DNA sequencing [19].

Northern blot analysis

Two Multiple Tissue Northern blots (Clontech Laboratories) containing 2 µg aliquots of poly(A)+ mRNA obtained from various regions of the human brain were probed using the 1.6 kb human liver E3 cDNA [16], and also with a 2.0 kb β-actin control cDNA provided with the blots by Clontech. Each blot was prehybridized for 3 h at 65 °C in 10 ml of a solution containing 5 x SSPE (1 x SSPE = 0.15 M NaCl, 0.001 M EDTA and 0.01 M sodium phosphate, pH 7.4), 10 x Denhardt’s solution, 2 % (w/v) SDS and 100 µg/ml denatured salmon testes DNA. The cDNA probes were labelled as described for the cDNA library screen, and hybridization was carried out in 10 ml of the above solution at 65 °C for 24 h. The blots were washed three times for 15 min in 2 x SSC/0.05 % SDS at room temperature, and twice for 20 min in 0.1 x SSC/0.1 % SDS at 50 °C. They were then exposed to Fuji RX film at −70 °C for various
periods of time. The specific radioactivity of all the probes used in these hybridizations was $3 \times 10^6$ d.p.m./pg.

**Purification procedure**

Human brains obtained at autopsy (6–24 h after death) were stored at $-70$ °C until use. Four purifications were attempted, and the sources of the brains used were: (1) a 47-year-old Caucasian male (death by hanging); (2) a 20-year-old Caucasian male (motor vehicle accident); (3) a 72-year-old Caucasian female (myocardial infarction); and (4) a 44-year-old Caucasian male (cause of death unknown). The purification procedure was based on those described for rat brain [9] and bovine brain [10] γ-aminobutyraldehyde dehydrogenases.

During purification the following buffers were used: buffer A, 5 mM sodium phosphate buffer, pH 6.0, 1 mM EDTA, 0.1 % (v/v) 2-mercaptoethanol; buffer B, 5 mM sodium phosphate buffer, pH 7.0, 1 mM EDTA, 0.1 % (v/v) 2-mercaptoethanol. These buffers were the same as those described by Abe et al. [9] and Lee and Cho [10]. Buffer C consisted of 100 mM sodium phosphate buffer, pH 8.0, 1 mM EDTA and 0.1 % (v/v) 2-mercaptoethanol. All buffers were de-aerated and nitrogenated exhaustively. All operations were carried out at 4 °C.

**Extraction**

About 500 g of brain tissue was homogenized in 4 vol. of buffer A. The homogenate was left to extract at 4 °C for 90 min and then centrifuged at 15000 g for 70 min. The supernatant was collected.

(NH$_4$)$_2$SO$_4$ fractionation

The supernatant was fractionated with (NH$_4$)$_2$SO$_4$. γ-Aminobutyraldehyde dehydrogenase activity was in the 40–70 % satd. fraction. The suspensions were centrifuged at 15000 g for 50 min. The pellet was resuspended in a minimal volume of buffer A, and the sample was dialysed extensively against buffer A.

**CM-Sephadex**

The dialysed sample was applied on to a CM-Sephadex column (8 cm $\times$ 14 cm) equilibrated with buffer A [10]. The column was washed with buffer A. The enzyme did not bind to the column. Active fractions were pooled, and the sample pH was adjusted to 7.0 using a saturated solution of Tris base.

**DEAE-Sephadex**

The CM-Sephadex flow-through sample was then applied on to a DEAE-Sephadex column (6.5 cm $\times$ 20 cm) equilibrated with buffer B [10]. The column was washed with buffer B and then eluted with a linear gradient of 0–0.4 M NaCl. Fractions containing the enzyme activity were pooled and the pH was adjusted to 6.0 using a saturated solution of monobasic sodium phosphate.

**5′-AMP–Sepharose**

The active sample from the DEAE-Sephadex column was applied on to a 5′-AMP–Sepharose column (1.5 cm $\times$ 7 cm) equilibrated with buffer A [10]. The enzyme did not bind to the column, so the column was washed with buffer A until no more enzyme came off. The active fractions were collected and the pH was adjusted to 7.0.

**Blue Sepharose**

The active sample from the 5′-AMP–Sepharose column was applied on to a Blue Sepharose column (3.5 cm $\times$ 11 cm) equilibrated with buffer B [9]. The column was washed with buffer B and then with buffer C. The enzyme was eluted with buffer C containing NAD$^+$ (1 mg/ml).

**FPLC Mono-P HR 5/20**

Active fractions from the Blue Sepharose column were pooled and concentrated. The sample was then applied on to an FPLC Mono-P column equilibrated with 10 mM sodium phosphate buffer, pH 6.8, 1 mM EDTA [8]. The column was washed with the same buffer and the enzyme was eluted with a linear 0–1 M NaCl gradient. Active fractions were pooled.

**RESULTS**

**Purification of the γ-aminobutyraldehyde-metabolizing enzyme**

The purification from brain 1 is summarized in Table 1. The procedure attempted to follow those already published for the rat and bovine enzymes [9,10]. However, this was not always possible. For example, the bovine enzyme became attached to the 5′-AMP–Sepharose 4B column whereas the rat and human enzymes did not. Thus the 5′-AMP column could only be used for the removal of other nucleotide-binding enzymes. The procedure represents one of four purifications attempted, using four different brains, none of which yielded homogeneous enzyme in the final Blue Sepharose step. The material obtained from this step was, however, analysed by both isoelectric focusing and SDS/PAGE (see below). It was also electroblotted on to a nitrocellulose filter in order to obtain pure material for protein sequence analysis.

**Isoelectric focusing**

The determination of the isoelectric point is shown in Figure 1. It can be seen that the major band, visualized either by protein staining (Figure 1A, lane 2) or by activity towards γ-aminobutyraldehyde (lane 1), has a pI of about 5.3. Although the enzyme appears homogeneous (lane 2), there were several minor protein bands visualized on the gel which are not visible on the photograph.

**Subunit molecular mass and Western blot analysis**

The main protein component had a subunit molecular mass of 55000 Da (Figure 2, lanes 1 and 3). A PVDF membrane blotted

| Table 1 Purification of the γ-aminobutyraldehyde-metabolizing enzyme from human brain |
|---------------------------------|------------------|--|------------------|------------------|
| Purification step               | Total protein (mg) | Total activity (nmol/min) | Specific activity (nmol/min per mg) | Yield (%) |
| (NH$_4$)$_2$SO$_4$ pptn. and dialysis | 4600 | 6400 | 1.4 | 100 |
| CM-Sephadex                     | 1100 | 2660 | 2.4 | 40  |
| DEAE-Sephadex                   | 330  | 1590 | 4.8 | 24  |
| 5′-AMP–Sepharose                | 190  | 1000 | 5.3 | 15  |
| Blue Sepharose                  | 3.5  | 480  | 137 | 7.4 |
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Figure 1  Isoelectric focusing of the human brain enzyme

(A) Isoelectric focusing gel: development for activity towards \( \gamma \)-aminobutyraldehyde (lane 1), and staining with Coomassie Brilliant Blue (lanes 2 and 3). Lanes 1 and 2, brain enzyme; lane 3, standards. (B) Isoelectric point determination. The Pharmacia standards used were: amyloglucosidase (pI 3.5), soybean trypsin inhibitor (pI 4.55), \( \beta \)-lactoglobulin A (pI 5.2), bovine carbonic anhydrase B (pI 5.85), human carbonic anhydrase B (pI 6.55), horse myoglobin (pI 7.35), lentil lectins (pI 8.15, 8.45 and 8.65). The brain enzyme (+) is indicated.

with samples of the brain enzyme, liver E3 isoenzyme, a mixture of liver E1 and E2 isoenzymes and standards was probed with anti-E3 polyclonal antibody. The antibody interacted with only the major 55000 Da component of the brain sample and the liver E3 isoenzyme.

A PVDF membrane blotted with human cerebellar, putamen and hippocampal extracts, following SDS/PAGE, was also probed with anti-E3 antibody. The antibody interacted with a 54000 Da component in all three extracts, the most intense interaction being with the hippocampal extract.

**Specific activity determination**

In order to determine the specific activity of the brain enzyme, the purification was repeated and an additional chromatographic step, using an FPLC Mono-P column, was performed. The specific activity of the eluted enzyme was found to be 1.4 \( \mu \text{mol/min} \) per mg. The E3 isoenzyme is extremely unstable and sensitive to atmospheric oxygen. The large increase in specific activity in the FPLC step is due to the removal of inactive enzyme.

**Kinetic properties**

All the \( K_m \) values determined for the human brain enzyme were comparable with those of the liver E3 isoenzyme (Table 2). \( K_m \) values for the human brain enzyme were also determined under the conditions used by Abe et al. [9] and Lee and Cho [10] for the determination of rat and bovine brain enzyme \( K_m \) values. Comparison of these \( K_m \) values (Table 3) showed that only the rat enzyme \( K_m \) value for acetaldelyde was comparable with that of the human brain enzyme.

**Protein sequence analysis**

Tryptic digestion and amino acid sequencing of the main SDS/PAGE gel protein band (see Figure 2A) resulted in the determination of the primary structure of one peptide, T44. Its sequence matched that of the liver E3 isoenzyme at residues

**Table 2**  Comparison of the Michaelis constants for the \( \gamma \)-aminobutyraldehyde-metabolizing enzymes from human brain and liver

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Human brain</th>
<th>Human liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \gamma )-Aminobutyraldehyde</td>
<td>7</td>
<td>5*</td>
</tr>
<tr>
<td>NAD(^+)</td>
<td>4</td>
<td>14†</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>40</td>
<td>50†</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>200</td>
<td>240</td>
</tr>
<tr>
<td>Betaine aldehyde</td>
<td>140</td>
<td>260†</td>
</tr>
<tr>
<td>N-Acetyl-( \gamma )-aminobutyraldehyde</td>
<td>110</td>
<td>100</td>
</tr>
</tbody>
</table>

\( * \): Kurys et al. [8]; \( † \): Abe et al. [9]; \( ‡ \): Lee and Cho [10].
Table 3 Comparison of the Michaelis constants for the γ-aminobutyraldehyde-metabolizing enzymes purified from human, rat and bovine brain

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( K_m (\mu M) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human brain</td>
<td>Rat brain</td>
</tr>
<tr>
<td>γ-Aminobutyraldehyde</td>
<td>70</td>
</tr>
<tr>
<td>NAD(^+)</td>
<td>4</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>22</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 4 Identification of tryptic peptides obtained from the purified brain enzyme that metabolizes γ-aminobutyraldehyde

Amino acid residues are numbered according to the human liver E3 isoenzyme sequence reported by Kurys et al. [16].

<table>
<thead>
<tr>
<th>Peptide Method of identification</th>
<th>Sequence</th>
<th>Amino acid positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>T35 MS</td>
<td>SPBIFSDDMNNAVP</td>
<td>227–242</td>
</tr>
<tr>
<td>T20 MS</td>
<td>VCLGGDIVPDPFK</td>
<td>321–334</td>
</tr>
<tr>
<td>T36 MS</td>
<td>ANDTGFIALGTVTR</td>
<td>380–394</td>
</tr>
<tr>
<td>T44 Amino acid sequencing</td>
<td>VVAELQGICTFRNYNVXPV</td>
<td>402–421</td>
</tr>
</tbody>
</table>

DISCUSSION

Enzyme purification, cDNA cloning and Northern blot analysis were three major approaches utilized during this investigation in an attempt to determine the identity and occurrence of the enzyme that metabolizes γ-aminobutyraldehyde in the adult human brain. The purification (Table 1; Figures 1 and 2) did not result in complete homogeneity, but the γ-aminobutyraldehyde-metabolizing enzyme was the major component. In three human brains, only one protein band with activity towards γ-aminobutyraldehyde was observed on the isoelectric focusing gel (Figure 1, lanes 1 and 2). Its \( pI \) value of 5.3 (Figure 1) was identical with that previously determined for the major component of the human liver E3 isoenzyme [8]. The minor component of the liver enzyme (\( pI \) 5.45) was observed in only one human brain (brain 4). One major protein band was also visualized by SDS/PAGE (Figure 2, lanes 1 and 3). Its subunit molecular mass of 55000 Da was comparable with that of the liver E3 isoenzyme (54000 Da) [8]. The major SDS/PAGE band also interacted with a polyclonal anti-E3 antibody on a Western blot. Tryptic digestion of this band yielded a 20-amino-acid peptide whose sequence completely matched part of that of the E3 isoenzyme, and three other peptides which were matched to the E3 sequence by MS (Table 4).

The 1.6 kb E3 cDNA probe hybridized to mRNA in all regions of the brain represented on the Multiple Tissue Northern blots (Figure 4). Only one 2.9 kb band was seen in all lanes, demonstrating the presence of only one species of E3 mRNA in the human brain. On re-probing the blots with the control \( \beta \)-actin cDNA, the expected 2.0 kb band was observed in each lane (not shown). As can be seen in Figure 4, the E3 mRNA levels were not uniform. Autoradiographs of the two blots were scanned using a Mustek MFS 6000 scanner and the SigmaGel gel analysis software (Jandel Scientific), in order to compare band intensities and thus determine the distribution of E3 mRNA in the brain. The order of distribution, starting with the highest levels of mRNA, was as follows: spinal cord > corpus callosum > subthalamic nucleus > medulla > thalamus > amygdala > cerebral cortex > substantia nigra > hippocampus > caudate nucleus > temporal lobe > cerebellum > putamen > frontal lobe > whole brain > occipital pole.

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Figure 3  cDNA isolated from a human cerebellar library and derived amino acid sequence

The cerebellar cDNA is longer than the published liver E3 cDNA [16] at the 5’ and 3’ ends (underlined regions). Nucleotide differences from the liver E3 cDNA are marked by *, and the nucleotide insertion is doubly underlined.

were also observed in the fetal brain cDNA, they were most probably not due to reverse transcription errors during the making of the cerebellar cDNA library. Of the five nucleotide substitutions found in the coding region of the brain cDNA, only one resulted in an amino acid substitution. As a result, the brain enzyme amino acid sequence differs from the liver E3 sequence by one amino acid, a cysteine at position 88 (position 84 in liver E3) instead of a serine. The presence of serine at position 84 in liver E3 was corroborated by both cDNA and protein sequencing [16]. Thus the human brain contains a variant of the E3 isoenzyme, designated E3'.

Like the liver E3 isoenzyme, the brain enzyme catalysed the dehydrogenation of betaine aldehyde, acetaldehyde, propionaldehyde and other aldehydes as well as that of \( \gamma \)-aminobutyraldehyde. Its \( K_m \) values for these substrates were similar to those determined for the liver E3 isoenzyme (Table 2). Interestingly, at 37 °C the \( K_m \) values for acetaldehyde of both the brain and liver enzymes decreased to about 50% of their values at 25 °C (compare Tables 2 and 3), which suggests that E3 may be important in alcohol metabolism. Following gradient elution from the FPLC Mono-P column, the specific activity of the brain enzyme (1.4 \( \mu \)mol/min per mg) and its \( \gamma \)-aminobutyraldehyde/propionaldehyde activity ratio (3.3) were also similar to those of the E3 isoenzyme (1.7 \( \mu \)mol/min per mg and 3.2 respectively [8]). All of the above results suggest that the catalytic properties of the brain enzyme and the liver E3 isoenzyme are similar.

Comparison of the properties of the human brain \( \gamma \)-aminobutyraldehyde-metabolizing enzyme with those of the
similar enzymes from rat and bovine brain [9,10] revealed some similarities and some differences. All three enzymes have a subunit molecular mass of 50000–58000 Da, and a similar and broad substrate specificity. However, the \( K_m \) value for \( \gamma \)-aminobutyaldehyde appeared to be higher for the rat and bovine enzymes than for the human brain enzyme. These differences could have been due to the assay conditions used. Therefore \( K_m \) value determinations for the human brain enzyme were repeated at pH 8.0 and 37°C, the conditions used to determine the rat and bovine enzyme \( K_m \) values (Table 3). The human brain enzyme \( K_m \) value for \( \gamma \)-aminobutyaldehyde increased 10-fold to 70 \( \mu \)M, but it was still half that of the rat and bovine enzymes. At high pH and high temperature, \( \gamma \)-aminobutyaldehyde is likely to cyclize more rapidly into \( \Delta^2 \)-pyrroline, which could result in a higher apparent \( K_m \) value for \( \gamma \)-aminobutyaldehyde. \( K_m \) values for the brain enzyme for \( \gamma \)-aminobutyaldehyde and \( \gamma \)-aminobutyraldehyde remained the same (compare Tables 2 and 3); only the \( K_m \) value for acetaldehyde agreed with that of the rat enzyme under these conditions. Thus it appears that, while the \( \gamma \)-aminobutyaldehyde-metabolizing enzyme from human brain is similar to the liver E3 isoenzyme [8], it may be different from the \( \gamma \)-aminobutyaldehyde-metabolizing enzymes from rat and bovine brain [9,10].

The E3 isoenzyme has been postulated to play a role in the formation of GABA from putrescine [8]. The reportedly low levels of diamine oxidase in mammalian brain [5] point to the formation of GABA from acetylated putrescine via monoamine oxidase. It was, therefore, expected that the brain enzyme would reflect this in its \( K_m \) value for N-acetyl-\( \gamma \)-aminobutyaldehyde. However, there is no difference in the \( K_m \) values for N-acetyl-\( \gamma \)-aminobutyaldehyde between the human brain E3' and liver E3 enzymes. Thus the brain enzyme may be involved in the formation of GABA from putrescine either directly via diamine oxidase, or indirectly through acetylated putrescine via monoamine oxidase. Since GABA is synthesized mainly by glutamate decarboxylase in the central nervous system, the physiological role of the E3' isoenzyme in the brain is still uncertain. Recently the E3 isoenzyme has been identified as a human betaine aldehyde dehydrogenase [20], which suggests a role for it in choline metabolism. Choline is converted into betaine aldehyde by choline dehydrogenase, and then into betaine by betaine aldehyde dehydrogenase. Betaine appears to be necessary for osmoregulation in all living organisms [21,22]. In mammals it has been implicated in the alleviation or even prevention of fatty and cirrhotic liver [23]. It also functions as a methyl donor and a nitrogen source [24,25].

The occurrence of different isoenzymic forms in different tissues has been known for a long time. A prime example is lactate dehydrogenase, a tetramer made up of two types of subunits (H and M); the H type predominates in the heart and the M type predominates in liver and skeletal muscle [26]. Both forms of the enzyme have become subtly adapted to the different metabolic requirements of these tissues. For the brain E3' enzyme, the serine-to-cysteine substitution does not appear to have any effect on those catalytic constants that have been determined so far. During purification, however, it was noted that the brain enzyme appeared to be more stable than the liver E3 isoenzyme. It is, therefore, possible that the serine-84-to-cysteine substitution leads to some structural rearrangement, perhaps the introduction of a disulfide bond, which renders the cysteine-substituted E3' enzyme more stable. However, the functional significance of the serine-to-cysteine replacement in the brain enzyme is as yet unknown.

During the purification of the brain enzyme it was observed that the amount of \( \gamma \)-aminobutyaldehyde-metabolizing activity was not proportional to the weight of brain used for enzyme purification. This suggested that different brain regions may vary in enzyme content. Western blot analysis of cerebellar, putamen and hippocampal extracts demonstrated the presence of anti-E3-antibody-interacting protein in all three brain areas, with the strongest interaction in the hippocampus. Northern blot analysis (Figure 4) showed that a single 2.9 kb band of E3' mRNA was present in all regions of the brain that were tested. This suggested that E3' mRNA is localized throughout the whole brain. Comparison of E3' mRNA levels in the cerebellum, putamen and hippocampus by autoradiograph scanning showed that the hippocampus had the highest levels, confirming the results from Western blotting, and also suggesting that E3' mRNA levels reflect the amount of expressed E3' protein in the brain. When all brain areas were compared, E3' mRNA levels were highest in the spinal cord. Interestingly, it has been reported that betaine levels are higher in the spinal cord than in the brain of guinea pigs [27]. This could result from the presence of higher levels of an enzyme metabolizing betaine aldehyde. Thus the E3' enzyme may also be involved in the synthesis of betaine in the central nervous system.

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