cDNA cloning, characterization and stable expression of novel human brain carboxylesterase[1]

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Abstract The DNA sequence encoding a novel human brain carboxylesterase (CES) has been determined. The protein is predicted to have 567 amino acids, including conserved motifs, such as GESAGG, GXXXXEFG, and GDHGD which comprise a catalytic triad, and the endoplasmic reticulum retention motif (HXEL-COOH) observed in CES families. Their gene products exhibited hydrolase activity towards temocapril, *p*-nitrophenylacetate and long-chain acyl-CoA. Since the molecular masses of these gene products are similar to those that exist in capillary endothelial cells of the human brain [Yamamda et al. (1994) Brain Res. 658, 163–167], these CES isozymes may function as a blood-brain barrier to protect the central nervous system from ester or amide compounds.

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Key words: Carboxylesterase; Human brain; cDNA cloning; Expression

1. Introduction

Mammalian carboxylesterases (CESs, EC 3.1.1.1) comprise a multigene family whose gene products exist in many mammalian species and humans [1]. These enzymes are glycoproteins of the high mannose-containing type, and *N*-glycosylation may play an important role in the catalytic activity of CESs [2]. We recently described that a number of CES isozymes from mammals and humans are glycoproteins of high-mannose type [1]. These enzymes efficiently catalyze the hydrolysis of a variety of drugs or prodrugs containing ester and amide bonds [3]. Since ester derivatives of therapeutic agents have been used as prodrugs, CES is a major determinant of the pharmacokinetic behavior of most prodrugs, and its activity can be influenced by direct interactions with a variety of compounds either directly or at the level of enzyme regulation [3,4]. The enzyme also hydrolyzes endogenous long-chain fatty acid esters or thioesters [5–8]. Therefore, CES may play an important role in lipid metabolism as well as in drug metabolism.

Recently, we reported that human brain CES preparations were immunocross-reactive with human anti-liver CES antibodies [9]. The presence of CES in endothelial cells of the human brain has been established by immunohistochemistry using anti-human liver CES antibodies, suggesting the presence of similar isozymes in the central nervous system and the liver. Capillary endothelial cells of the brain function as a dynamic interface with regard to the transfer of nutrients and drugs from the circulation to brain interstitial fluid [10]. Therefore, the presence of CESs in capillary endothelial cells may be consistent with the enzyme acting to protect the central nervous system from toxic esters, a component of the so-called blood-brain barrier system. Since a significant number of drugs are metabolized by CES, altering the activity of endothelial CES may have important clinical implications. However, little is known about the differences in structure and hydrolytic capability of CES isozymes expressed in the brain of humans.

In the present study, we cloned and characterized novel CES cDNAs from the human brain, and we undertook the structural characterization of CES isozymes expressed in the human brain in order to learn more about the molecular basis responsible for their functional differences in drug and lipid metabolism.

2. Materials and methods

2.1. cDNA cloning of human brain CES

For isolation of cDNA encoding CES isozymes from the human brain, a λgt 11 cDNA library (Clontech Laboratories, CA, USA) was screened according to the general method using nitrocellulose filters with either antibodies to human liver CES HU1 [11] or hybridization with a coding region of CES HU1 cDNA [12] (PCR amplified using 5′-CTGAAAACACCTGAAGAGCTTCAA-3′ and 5′-CTCCCAAGGCCGGCTGGATCTTCA-3′) as a probe labeled with the ECL direct nucleic acid labeling and detection system (Amersham Pharmacia Biotech, Uppsala, Sweden). Inserts in phage DNAs of positive clones were isolated by *Eco*RI restriction and subcloned into plasmid pUC 118. To identify the 5′ end of the CES gene, 5′-RACE PCR was performed using total RNA from the human brain (Clontech) and a Marathon cDNA amplification kit (Clontech). The antisense primer was made according to the hBr0-13 clone, and the sequence was 5′-ATCCCCTGTGCTGAAGAATCC-3′. In order to obtain the full length of the CES cDNA, PCR amplification was performed, the sense primer was made according to the hBr5-3-20 clone (5′-CACAATGCGCCTCTACCCTC-3′) and the antisense primer was made according to the hBr0-13 clone (5′-GGAGCCGCCCATYCAAAGC-3′). The amplified DNA (hBr3) was subcloned into a pTARGET vector (Promega, OR, USA).

Nucleotide sequences were determined by the dideoxy method using a Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech) and an automated DNA sequencer (Shimadzu DSQ-2000 system, Shimazu, Kyoto, Japan).

2.2. Transfection and G418 selection

The parental V79 cells (V79-4, American Type Culture Collection CL93, Rockville, MD, USA) were cultured in Dulbecco's modified

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[1] The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession numbers AB025026 and AB025028.

Abbreviations: CES, carboxylesterase; D-MEM, Dulbecco's modified Eagle's medium; HPLC, high-performance liquid chromatography; 5′-RACE, 5′-rapid amplification of cDNA end

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Table 1
Catalytic activity of xenobiotic or endogenous substrates in V79 cells expressing CES isozymes

Substrate	Hydrolase activity (nmol/mg/min)	
	hBr3/pTARGET	HU1/pTARGET
p-Nitrophenylacetate	200.8	43.8
Temocapril	0.260	0.070
Palmitoyl-CoA	6.14	0.05
Oleoyl-CoA	20.9	1.35

hBr3/pTARGET: hBr3/pTARGET-transfected V79 cells; HU1/pTARGET: HU1/pTARGET- transfected V79 cells.

Eagle's medium (D-MEM; Gibco, Grand Island, NY, USA) supplement with 10% heat inactivated fetal bovine serum (Gibco), penicillin/streptomycin (Gibco) and L-glutamine (Gibco) in an atmosphere of 5% CO_2 and 95% air at 37°C. The V79 cells were plated at 1×10^6 cells/100 mm plate. The next day, 10 μg of vector DNA (hBr3/pTARGET, HU1/pTARGET or pTARGET) was transfected into the cells using SuperFect Transfection Reagent (Qiagen, Hilden, Germany) with OPTI-MEM medium (Gibco). On day 3, the cells were split into 100 mm plates with media containing the selective agent, G418 (Gibco), at 400–800 μg/ml. The medium was changed every 2–3 days, and the cells were maintained for at least 3 weeks to obtain stable expression colonies. Ten colonies were identified and homogenized by SET buffer (0.25 M sucrose, 1 mM EDTA, 100 mM Tris-HCl buffer, pH 7.4), and the expression of hBr3 or HU1 was determined by examining *p*-nitrophenylacetate, long-chain acyl-CoA and temocapril hydrolase activities or by immunoblot analysis. These G418-resistant colonies could be stored in liquid N_2 for several months without loss of expression level.

2.3. Enzyme assays

Temocapril hydrolase activity was determined as follows. An assay mixture consisting of 50 μl of 200 mM HEPES buffer (pH 7.4), 50 μl of cell homogenate at a suitable concentration, 100 μl of temocapril solution (100 μM or 500 μM in water; final concentration of 50 μM or 250 μM, respectively) was incubated at 37°C for 20–40 min. The reaction was performed in a linear range with respect to protein concentration and incubation time. After the reaction was stopped by addition of 200 μl of cold ethanol, the mixture was centrifuged at $10\,000\times g$ for 15 min, and 200 μl of the supernatant was analyzed by HPLC as described below.

The hydrolytic metabolite of temocapril, temocaprilat, was determined using the following HPLC method. The HPLC system consisted of a model L-6000 pump (Hitachi, Tokyo, Japan), a model L4000H UV detector (Hitachi), a model AS-2000 autosampler (Hitachi), a model D-2500 integrator (Hitachi), and a 4.6×150 mm YMC-Pack Ph A-402 column (YMC, Tokyo, Japan). For the determination of temocaprilat, the mobile phase consisted of 0.2% phosphoric acid-acetonitrile (60:40, v/v) and was delivered at a flow rate of 0.8 ml/min. The eluate was monitored at a wavelength of 233 nm. A calibration curve was generated from 5 to 250 μM by processing the authentic standard substance through the entire procedure. Temocapril and temocaprilat were kindly obtained from Dr. Toshihiko Ikeda, Analytical and Metabolic Research Laboratories, Sankyo Co., Hiromachi, Shinagawa-ku, Tokyo, Japan. Michaelis-Menten kinetic parameters (K_m and V_{max}) for *p*-nitrophenylacetate hydrolase activity were estimated by non-linear least-squares regression analysis. *p*-Nitrophenylacetate and long-chain acyl-CoA hydrolase activities were determined as described previously [5].

2.4. Immunoblotting

Immunoblot analysis was performed as reported previously [5]. Briefly, purified CES isozymes (0.2 μg/well) and V79 cell homogenates (5 μg/well) were electrophoresed on SDS-polyacrylamide gels, transferred onto a nitrocellulose sheet, and stained immunochemically.

3. Results and discussion

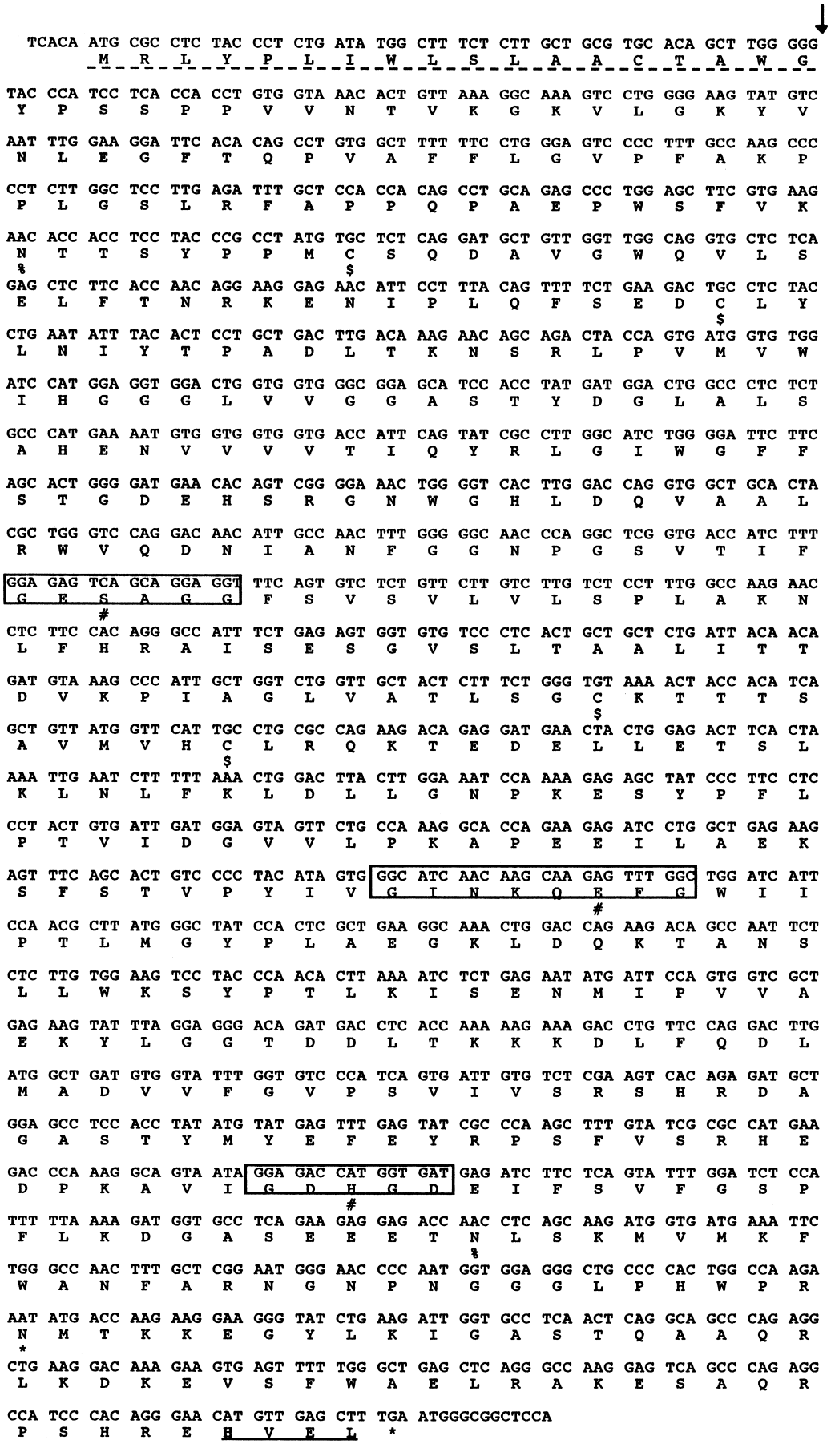
3.1. Screening of a cDNA library

A λgt11 cDNA library, constructed from the human brain, was screened by plaque hybridization using a 1093 bp PCR fragment of CES cDNA prepared from human liver [2,12]. From 1.8×10^5 plaques tested, 12 positive clones were isolated, and the cDNA insert was subcloned into pUC118 and sequenced with Thermo Sequenase DNA polymerase and fluorescent labeled primer using an automated DNA sequencer. These clones contained cDNA inserts of 0.5–1.3 kb. The largest clone, designated hBr0-13, spanned 1285 bp, with an open reading frame encoding a 345 amino acid polypeptide, followed by a termination codon (TAG), and followed in turn by 251 nucleotides of a 3′-non-coding sequence which contained a consensus polyadenylation signal (AATAAA) and a 69 bp long poly(A) tail (data not shown). The authenticity of the coding region of hBr0-13 cDNA was supported by matching its deduced amino acid sequence with conserved motifs in mammalian CESs. The clone showed 71.5% homology to the human liver CES isozyme HU1 [12] or hCE [2].

However, hBr0-13 did not contain an in-frame initiation codon (ATG), suggesting that this cDNA lacked part of the 5′ end of the coding region. To obtain a cDNA corresponding to this missing region, 5′-RACE-Marathon-Ready cDNA, prepared from human brain total RNA, was amplified by 5′-RACE according to the instructions of the manufacturer (Clontech). As a result, five cDNAs of different lengths between 465 bp and 1359 bp were obtained and were subcloned into a pGEM-T vector (Promega) by TA cloning methods and then sequenced. A 1298 bp 5′-RACE PCR product (hBr5-3-20) had a start codon and open reading frame consisting of 431 amino acids, which overlapped with the cDNA of hBr0-13 by 620 bp. To obtain the full-length cDNA sequence of human brain CES, further PCR was performed using the sense primer from hBr5-3-20 and the antisense primer from hBr0-13, and a final PCR product designated hBr3 was isolated. hBr3 was subcloned into a pTARGET vector and sequenced. We also obtained another 1359 bp 5′-RACE PCR product designated hBr1, which had a start codon and open reading frame consisting of 429 amino acids. However, the hBr1 did not completely overlap the 5′ end of hBr0-13. The homology of this nucleotide sequence with hBr3 is 71%, and the clone did not include a stop codon. Although we tried to

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Fig. 1. The nucleotide and the deduced amino acid sequence of human brain CES hBr3. A putative signal peptide is indicated by dotted underlining. The arrow shows the putative restriction site of the signal peptidase. Ser (S), Glu (E) and His (H), which are presumed to be the active sites (#), potential glycosylation sites (*) and cysteine ($), are labeled. The box shows the conserved catalytic triad. The C-terminal retention signal is indicated by single underlining.


TCACA ATG CGC CTC TAC CCT CTG ATA TGG CTT TCT CTT GCT GCG TGC ACA GCT TGG GGG 54
M R L Y P L I W L S L A A C T A W G -1
TAC CCA TCC TCA CCA CCT GTG GTA AAC ACT GTT AAA GGC AAA GTC CTG GGG AAG TAT GTC 114
Y P S S P P V V N T V K G K V L G K Y V 20
AAT TTG GAA GGA TTC ACA CAG CCT GTG GCT TTT TTC CTG GGA GTC CCC TTT GCC AAG CCC 174
N L E G F T Q P V A F F L G V P F A K P 40
CCT CTT GGC TCC TTG AGA TTT GCT CCA CCA CAG CCT GCA GAG CCC TGG AGC TTC GTG AAG 234
P L G S L R F A P P Q P A E P W S F V K 60
AAC ACC ACC TCC TAC CCG CCT ATG TGC TCT CAG GAT GCT GTT GGT TGG CAG GTG CTC TCA 294
N T T S Y P P M C S Q D A V G W Q V L S 80
GAG CTC TTC ACC AAC AGG AAG GAG AAC ATT CCT TTA CAG TTT TCT GAA GAC TGC CTC TAC 354
E L F T N R K E N I P L Q F S E D C L Y 100
CTG AAT ATT TAC ACT CCT GCT GAC TTG ACA AAG AAC AGC AGA CTA CCA GTG ATG GTG TGG 414
L N I Y T P A D L T K N S R L P V M V W 120
ATC CAT GGA GGT GGA CTG GTG GTG GGC GGA GCA TCC ACC TAT GAT GGA CTG GCC CTC TCT 474
I H G G G L V V G G A S T Y D G L A L S 140
GCC CAT GAA AAT GTG GTG GTG GTG ACC ATT CAG TAT CGC CTT GGC ATC TGG GGA TTC TTC 534
A H E N V V V V T I Q Y R L G I W G F F 160
AGC ACT GGG GAT GAA CAC AGT CGG GGA AAC TGG GGT CAC TTG GAC CAG GTG GCT GCA CTA 594
S T G D E H S R G N W G H L D Q V A A L 180
CGC TGG GTC CAG GAC AAC ATT GCC AAC TTT GGG GGC AAC CCA GGC TCG GTG ACC ATC TTT 654
R W V Q D N I A N F G G N P G S V T I F 200
GGA GAG TCA GCA GGA GGT TTC AGT GTC TCT GTT CTT GTC TTG TCT CCT TTG GCC AAG AAC 714
G E S A G G F S V S V L V L S P L A K N 220
CTC TTC CAC AGG GCC ATT TCT GAG AGT GGT GTG TCC CTC ACT GCT GCT CTG ATT ACA ACA 774
L F H R A I S E S G V S L T A A L I T T 240
GAT GTA AAG CCC ATT GCT GGT CTG GTT GCT ACT CTT TCT GGG TGT AAA ACT ACC ACA TCA 834
D V K P I A G L V A T L S G C K T T T S 260
GCT GTT ATG GTT CAT TGC CTG CGC CAG AAG ACA GAG GAT GAA CTA CTG GAG ACT TCA CTA 894
A V M V H C L R Q K T E D E L L E T S L 280
AAA TTG AAT CTT TTT AAA CTG GAC TTA CTT GGA AAT CCA AAA GAG AGC TAT CCC TTC CTC 954
K L N L F K L D L L G N P K E S Y P F L 300
CCT ACT GTG ATT GAT GGA GTA GTT CTG CCA AAG GCA CCA GAA GAG ATC CTG GCT GAG AAG 1014
P T V I D G V V L P K A P E E I L A E K 320
AGT TTC AGC ACT GTC CCC TAC ATA GTG GGC ATC AAC AAG CAA GAG TTT GGC TGG ATC ATT 1074
S F S T V P Y I V G I N K Q E F G W I I 340
CCA ACG CTT ATG GGC TAT CCA CTC GCT GAA GGC AAA CTG GAC CAG AAG ACA GCC AAT TCT 1134
P T L M G Y P L A E G K L D Q K T A N S 360
CTC TTG TGG AAG TCC TAC CCA ACA CTT AAA ATC TCT GAG AAT ATG ATT CCA GTG GTC GCT 1194
L L W K S Y P T L K I S E N M I P V V A 380
GAG AAG TAT TTA GGA GGG ACA GAT GAC CTC ACC AAA AAG AAA GAC CTG TTC CAG GAC TTG 1254
E K Y L G G T D D L T K K K D L F Q D L 400
ATG GCT GAT GTG GTA TTT GGT GTC CCA TCA GTG ATT GTG TCT CGA AGT CAC AGA GAT GCT 1314
M A D V V F G V P S V I V S R S H R D A 420
GGA GCC TCC ACC TAT ATG TAT GAG TTT GAG TAT CGC CCA AGC TTT GTA TCG CGC CAT GAA 1374
G A S T Y M Y E F E Y R P S F V S R H E 440
GAC CCA AAG GCA GTA ATA GGA GAC CAT GGT GAT GAG ATC TTC TCA GTA TTT GGA TCT CCA 1434
D P K A V I G D H G D E I F S V F G S P 460
TTT TTA AAA GAT GGT GCC TCA GAA GAG GAG ACC AAC CTC AGC AAG ATG GTG ATG AAA TTC 1494
F L K D G A S E E E T N L S K M V M K F 480
TGG GCC AAC TTT GCT CGG AAT GGG AAC CCC AAT GGT GGA GGG CTG CCC CAC TGG CCA AGA 1554
W A N F A R N G N P N G G G L P H W P R 500
AAT ATG ACC AAG AAG GAA GGG TAT CTG AAG ATT GGT GCC TCA ACT CAG GCA GCC CAG AGG 1614
N M T K K E G Y L K I G A S T Q A A Q R 520
CTG AAG GAC AAA GAA GTG AGT TTT TGG GCT GAG CTC AGG GCC AAG GAG TCA GCC CAG AGG 1674
L K D K E V S F W A E L R A K E S A Q R 540
CCA TCC CAC AGG GAA CAT GTT GAG CTT TGA ATGGGCGGCTCCA 1717
P S H R E H V E L * 549

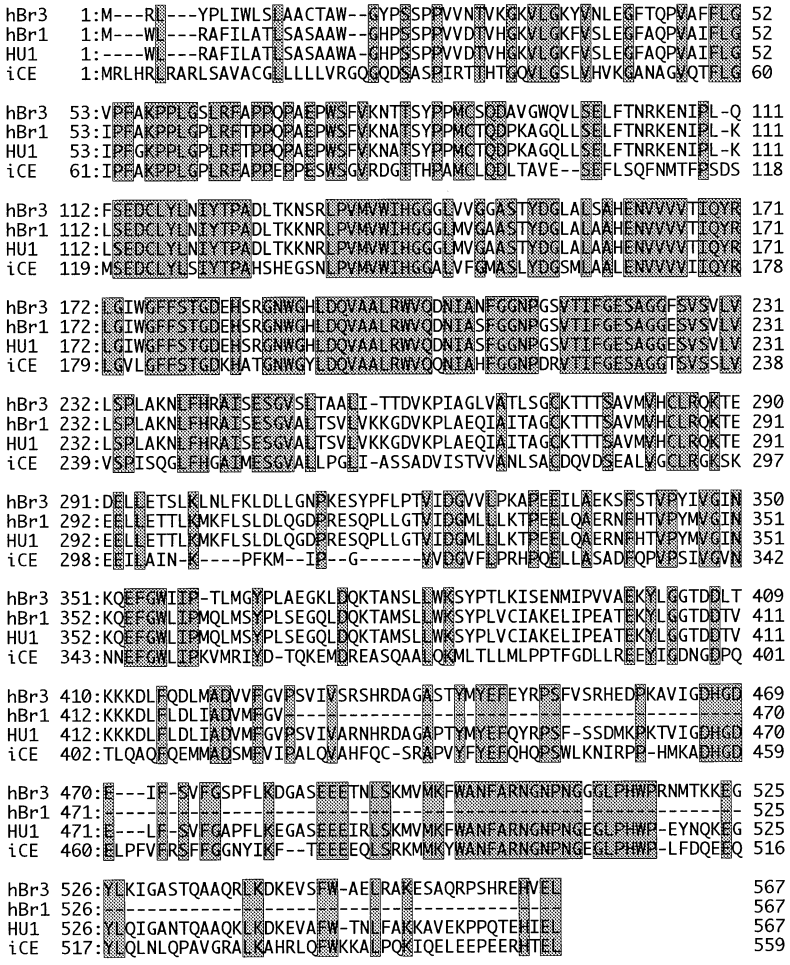


Fig. 2. Alignment of the predicted amino acid sequences of human brain CES hBr3, with human brain CES hBr1 (present study), human liver CES HU1 [12] and human intestine CES iCE [16]. Identical amino acid residues are shown in gray boxes.

obtain full-length cDNA of hBr1 by 3′-RACE methods, we were not successful.

3.2. Nucleotide and amino acid sequence of hBr3

The nucleotide and deduced amino acid sequence of hBr3 are shown in Fig. 1. The deduced amino acid sequence of hBr3 was not identical to any reported sequence in the database. The cDNA is 1722 bp long with an open reading frame of 1701 bp encoding a polypeptide of 567 amino acid residues (i.e. 18 amino acid residues for a signal peptide and 549 amino acid residues for a mature subunit). The nucleotide sequence surrounding the first ATG codon was a putative start site for translation, being located in the consensus sequence for eukaryotic initiation codes [13].

The deduced amino acid sequence of hBr3 contained several motifs characterizing CES isozymes and other serine hydrolases [3,14]. For example, it contained four Cys residues, including Cys-98, in the structure of the polypeptide responsible for the mature subunit, and one residue in the presumed signal peptide. These Cys residues, except the one in the signal peptide, are highly conserved in most CES isozymes and may function as specific disulfide bonds. Among them, Cys-98 is the most highly conserved in CES and other serine hydrolases and has been proposed to be one of the structurally important amino acid residues in esterase, lipase and related enzymes, on the basis of X-ray structures of *Torpedo californica* acetylcholinesterase and *Geotrichum candidum* lipase [14]. Another motif characterizing CES is the two Asn residues at positions 61 and 472 in the N-X-S/T motif, which is known to be a putative *N*-glycosylation site. The amino acid sequence of hBr3 also contains common structures of serine hydrolase superfamilies, Ser-203 (GE<u>S</u>AGG), Glu-335 (GXXXX<u>E</u>XG) and His-449 (GD<u>H</u>XD), which comprise a catalytic triad and Gly-123–Gly-124, which may be a part of the oxyanion hole [3]. This cDNA clone also encoded the same structure as the endoplasmic reticulum retention motif (HXEL-COOH) observed in CES families [3]. Since several proteins of the endoplasmic reticulum lumen have a similar carboxy-terminal sequence (KDEL-COOH) which binds the KDEL receptor to be retained in the endoplasmic reticulum, the polypeptide en-

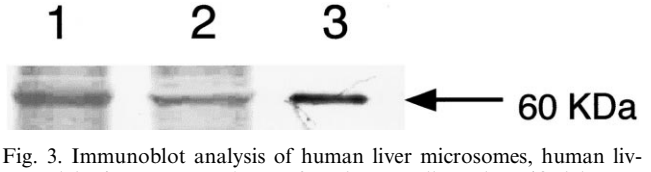


Fig. 3. Immunoblot analysis of human liver microsomes, human liver and brain CES cDNA-transfected V79 cells and purified human liver CES HU1 with antibodies to human liver CES HU1. 1, HU1/pTARGET-transfected V79 cells; 2, hBr3/pTARGET-transfected V79 cells; 3, purified human liver CES HU1.

Table 2
Kinetic parameters for the hydrolysis of *p*-nitrophenylacetate in V79 cells expressing CES isozymes

Isozyme	Range of substrate concentrations (μM)	K_m (μM)	V_{max} (nmol/mg/min)	V_{max}/K_m
hBr3/pTARGET	10–250	22.9	201.3	8.79
HU1/pTARGET	10–300	365.1	107.9	0.295

hBr3/pTARGET: hBr3/pTARGET-transfected V79 cells; HU1/pTARGET: HU1/pTARGET- transfected V79 cells.

coded by hBr3 is considered to be localized on the luminal side of the endoplasmic reticulum [15].

3.3. Comparison of the deduced amino acid sequences of hBr3 and other carboxylesterases

Fig. 2 shows the alignment of the deduced amino acid sequences of the human brain hBr3 and hBr1, human liver CES HU1 [12], and human intestine iCE [16]. Ser-203, Glu-335, and His-447, which constitute a catalytic triad, are highly conserved in these isozymes. In addition, four Cys involved in disulfide bonds and the endoplasmic reticulum retention motif HXEL-COOH are also conserved. The sequence of hBr3 showed 76%, 76%, and 50% amino acid sequence identity with hBr1, HU1, and iCE, respectively.

In contrast, the deduced amino acid sequence of hBr1 was almost identical to that of human liver CES HU1 [12] and hCE [2], having a sequence identity of more than 99% (Fig. 2). Therefore, hBr3, HU1 and hCE are considered to be the same gene product.

3.4. Stable expression of hBr3 and HU1 in V79 cells

The cDNAs for hBr3 and human liver CES HU1 were inserted separately into the pTARGET mammalian expression vector (hBr3/pTARGET and HU1/pTARGET, respectively) and transfected into V79 cells. As shown in Fig. 3, immunoblot analysis of the cell extracts from pTARGET-, hBr3/pTARGET- and HU1/pTARGET-transfected V79 cells with anti-human liver CES HU1 antibodies gave protein bands that were approximately 60 kDa. These results suggest that both the cDNA of hBr3 and that of HU1 were stably expressed in V79 cells and that these two gene products (CES hBr3 and CES HU1) were immunocross-reactive with anti-human liver CES HU1 antibodies. Previously, we reported that human brain preparations were immunocross-reactive with human anti-liver CES antibodies and that the position of migration is the same as that of human liver preparations in immunoblotting analysis [9]. We also showed by immunohistochemistry using anti-human liver CES antibodies that CES was expressed in capillary endothelial cells of the human brain [9]. These findings coupled with the present results suggest that gene products of hBr3 and/or hBr1 are immunocross-reactive with anti-HU1 antibodies, which have been reported to be distributed in capillary endothelial cells of the human brain.

Table 1 shows the hydrolysis activities of *p*-nitrophenylacetate and temocapril, a prodrug of an angiotensin-converting enzyme inhibitor, in cell extracts of V79 cells expressing CES hBr3 or CES HU1. Both activities were much higher in cells expressing CES hBr3 than those expressing CES HU1, although the expression level of CES hBr3 was commensurate with that of CES HU1 (data not shown). Table 2 shows the kinetic parameters of the hydrolysis of *p*-nitrophenylacetate by V79 cells expressing CES hBr3 and CES HU1. The V79 cells expressing CES hBr3 showed a lower Michaelis constant (K_m) and higher V_{max}, so that the V_{max}/K_m values for *p*-nitrophenylacetate hydrolysis were much higher than those of CES HU1. Similarly, V79 cells expressing CES hBr3 showed much higher temocapril hydrolase activity than CES HU1 in 5–500 μM substrate concentration (data not shown). The results suggest that CES hBr3 possesses a different catalytic property from CES HU1, and this may be caused by the structural difference in the amino acid sequences (Fig. 2).

Table 1 also shows the hydrolysis of endogenous substrates by CES hBr3 and CES HU1. We previously reported that the most effectively metabolized endogenous substrate of CES was the long-chain acyl-CoAs [5]. Therefore, hydrolysis of acyl-CoAs of different acyl chain lengths was tested in the present study. The V79 cells expressing CES hBr3 showed high hydrolytic activity toward oleoyl-CoA (Table 1); however, acyl-CoAs of medium chain length were poorly, or not, hydrolyzed by CES hBr3 (data not shown). These results suggest that the human brain CES isozyme possesses similar substrate specificity toward acyl-CoAs reported previously and may play an important role in lipid metabolism in the brain.

Although the physiological function of brain CES isozymes remains unclear, Ishizuka et al. [17] reported that temocaprilat, which is a hydrolytic metabolite of temocapril, is transported by a canalicular multispecific organic anion transporter (cMOAT). In addition, Kusuhara et al. [18] reported that an organic anion transporter exists in brain capillary endothelial cells and suggested that the transporter is responsible for the unidirectional, energy-dependent efflux of organic anions from the brain into the circulating blood across the blood-brain barrier. Therefore, the brain CESs in capillary endothelial cells may cooperate with these transporters for effluxing organic anions that are produced in the brain to the circulating blood through the metabolism via CES.

In conclusion, we isolated and sequenced two cDNA clones encoding CES, referred to as hBr1 and hBr3, from a human brain cDNA library. The latter clone was a novel one but the former was considered to be the same as human liver CES HU1. Although the physiological function of these brain CES isozymes remains unclear, they may exist in capillary endothelial cells of the human brain and may function as a blood-brain barrier by co-operating with a multidrug resistance family of transporters that efflux organic anions from the brain into the circulating blood.

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