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Structure of the Gene for Human Butyrylcholinesterase. Evidence for a Single Copy[†,‡]

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ABSTRACT: We have isolated five genomic clones for human butyrylcholinesterase (BChE), using cDNA probes encoding the catalytic subunit of the hydrophilic tetramer [McTiernan et al. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6682-6686]. The BChE gene is at least 73 kb long and contains four exons. Exon 1 contains untranslated sequences and two potential translation initiation sites at codons -69 and -47. Exon 2 (1525 bp) contains 83% of the coding sequence for the mature protein, including the N-terminal and the active-site serine, and a third possible translation initiation site (likely functional), at codon -28. Exon 3 is 167 nucleotides long. Exon 4 (604 bp) codes for the C-terminus of the protein and the 3′ untranslated region where two polyadenylation signals were identified. Intron 1 is 6.5 kb long, and the minimal sizes of introns 2 and 3 are estimated to be 32 kb each. Southern blot analysis of total human genomic DNA is in complete agreement with the gene structure established by restriction endonuclease mapping of the genomic clones: this strongly suggests that the BChE gene is present in a single copy.

Two types of cholinesterases exist in vertebrates: acetylcholinesterase (AChE,[1] EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). Although the two enzymes present parallel series of molecular forms including monomers, dimers, tetramers, and complex oligomers with collagen-like elements, they differ in their substrate specificity and inhibitor sensitivity [reviews in Massoulié and Bon (1982), Massoulié and Toutant (1988), and Chatonnet and Lockridge (1989)]. They are distinct enzymes encoded by two different but related genes.

The physiological function of AChE is to hydrolyze acetylcholine at cholinergic synapses, but the role of BChE remains unclear. The serum BChE, however, plays a key role in the hydrolysis of the muscle relaxant drug succinylcholine which is administered during anaesthesia (Kalow & Gunn, 1957). Patients with abnormal genetic variants of BChE can

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[1] Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; bp, base pair(s); nt, nucleotide(s); kb, kilobase(s).

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experience prolonged apnea when given standard doses of the drug [see Whittaker (1986) for a review]. A structural point mutation responsible for the reduced affinity in the atypical form of the enzyme has recently been identified in our laboratory (McGuire et al., 1989).

Plasma BChE is a hydrophilic tetramer of four identical subunits [Lockridge et al., 1979; see reviews in Whittaker (1986) and Lockridge (1988)]. The complete amino acid sequence (574 amino acids) of the catalytic subunit of human plasma BChE has been determined chemically (Lockridge et al., 1987). This allowed the construction of oligonucleotide probes and the isolation of cDNA clones from a human basal ganglia library (McTiernan et al., 1987). At the same time, another laboratory isolated overlapping cDNA clones from fetal brain and liver libraries (Prody et al., 1987). The deduced amino acid coding sequences of all these clones were a perfect match with the known amino acid sequence of adult plasma BChE, indicating that BChE in human liver, serum, and brain is identical.

We report here the isolation and characterization of several genomic clones covering the entire coding sequence of human serum BChE. Our results support the conclusion that a single gene exists for human BChE.

Materials and Methods

Materials. Restriction endonucleases were purchased from Bethesda Research Laboratories (Gaithersburg, MD) and Boehringer Mannheim (Indianapolis, IN). [α-^{32}P]dCTP (3000 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Packaging extracts were purchased from Stratagene (La Jolla, CA). Nylon membranes were from Dupont (Genescreen, NEF 972).

Genomic Blot Analysis. Genomic human DNA was isolated from white blood cells as described by Maniatis et al. (1982) and digested with restriction endonucleases. After electrophoresis on a 1% agarose gel, the DNA was transferred to a nylon membrane and hybridized with exon probes labeled by the random oligolabeling method (Feinberg & Vogelstein, 1984). The human genomic DNA was from a single male Caucasian donor having the usual BChE phenotype as determined by dibucaine and fluoride numbers.

Genomic Library Screening. A complete *Eco*RI digest of human white blood cell DNA was used to construct a genomic library in λgt11.

Two other human genomic libraries were gifts from Dr. P. J. Venta (Department of Human Genetics, University of Michigan, Ann Arbor). Each library was constructed from human white blood cell DNA from a single donor. A partial *Sau*3A1 digest was cloned into the *Bam*HI site of the polylinker region of the vector EMBL3. The plaque hybridization method of Benton and Davis (1977), as modified by Maniatis et al. (1982), was used for screening. cDNA probes (see Figure 1) were α-^{32}P-labeled according to Feinberg and Vogelstein (1984). Prehybridization and hybridization were in 0.25% instant nonfat dry milk (Johnson et al., 1984) in 6 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). Hybridization was for 15–20 h at 68 °C, except for the rabbit cDNA probe (58 °C). Posthybridization washes were in 0.1 × SSC/1% SDS at room temperature and then at 68 °C for 2 h with one fluid change (rabbit probe: 0.5 × SSC/0.5% SDS at room temperature and then 2 h at 58 °C).

DNA Sequencing. Exon-containing regions of genomic DNA were subcloned into M13mp18 and mp19 and sequenced by the dideoxynucleotide chain termination method of Sanger et al. (1977) using [α-^{32}P]dCTP. The 17 bp universal primer of M13 as well as oligonucleotide primers complementary to human BChE were used. Forced ligation of genomic clone fragments into M13mp18 and mp19, followed by sequencing with oligonucleotide primers whose orientation in the human BChE cDNA was known, allowed the 5′ to 3′ orientation of the genomic clones.

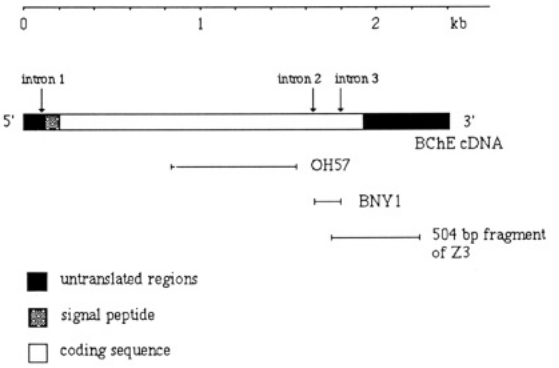


FIGURE 1: Location of cDNA probes used for the screening of human genomic libraries. BChE cDNA is schematically represented. The open bar represents the 1722 nucleotides coding for the mature protein (574 amino acids). The black boxes show 128 nucleotides of the 5′-untranslated region and 492 nucleotides of the 3′-untranslated region. The hatched bar represents 84 nucleotides of the signal peptide. The locations of the three introns are indicated by arrows. OH57 and Z3 are human BChE cDNA clones. OH57 spans nucleotides 625–1341 (716 bp). A 3′ fragment of Z3 (nucleotides 1521–2025, 504 bp) was used to screen for genomic clones containing the 3′ end of the gene. BNY1 is a rabbit BChE cDNA clone isolated from a rabbit cDNA liver library, and kindly provided by Dr. A. Chatonnet (INRA, Montpellier, France). This clone is 977 bp long; it contains 167 bp of coding sequence flanked by two intronic regions (487 bp on the 5′ side, 323 bp on the 3′ side).

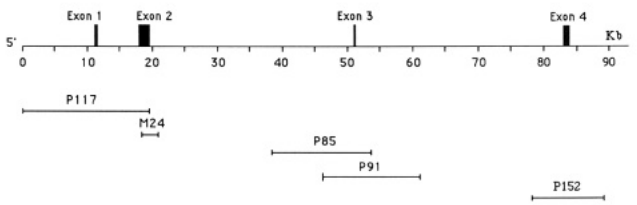


FIGURE 2: Location of the five human butyrylcholinesterase genomic clones. The top line represents the gene for human BChE. Exons are shown as black boxes. Clones P117 and M24 were isolated with OH57, P85 and P91 with BNY1, and P152 with the Z3 fragment. M24 is in λgt11. P117, P85, P91, and P152 are in phage EMBL3.

Results

All the nucleotide numbers used in the present study refer to the human BChE cDNA [original numbers in Figure 2 of McTiernan et al. (1987)].

Isolation and Characterization of Human Genomic Clones. Figure 1 shows the location of the cDNA probes. Screening of 2 × 10^6 plaques with OH57 gave 6 positive clones. One of them, P117, was further characterized. The insert released by *Sal*I cleavage was 19.4 kb long and contained the 5′ end of the cDNA. Sequence analysis revealed the existence of two introns: one between nucleotides (nt) −93/−92 (intron 1) and the second between nt 1432/1433 (intron 2, Figure 2). The 1525 bp exon (exon 2) present between these two introns thus contained 83% of the coding sequence, and included the active-site serine as well as the presumptive anionic site Asp-70 (McGuire et al., 1989). The size of intron 1 was 6.5 kb.

Only 44 bp of the 5′ end of intron 2 was present in P117. Genomic blot analysis had revealed the presence of a 2.4-kb *Eco*RI fragment hybridizing with exon 2 probe (Figure 3). We thus constructed an *Eco*RI genomic library in λgt11. Screening with OH57 gave one positive clone, M24, that has

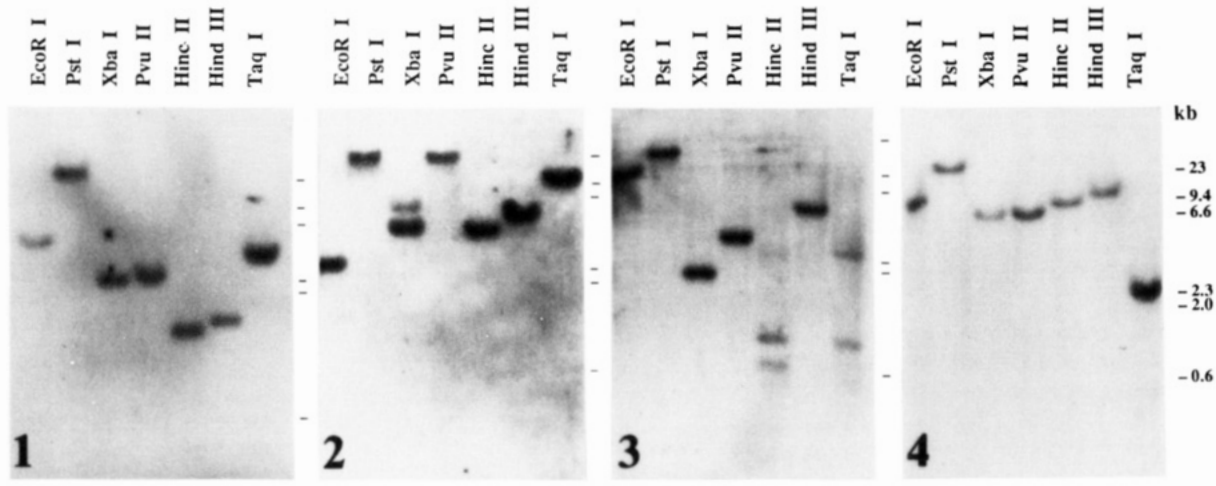


FIGURE 3: Genomic blot analysis of human white blood cell DNA with probes derived from the human butyrylcholinesterase gene. Genomic DNA was from a single individual. Each lane contained 10 μg of DNA. Blot 1 was hybridized with a 0.8-kb exon 1 probe; blot 2 with a 2.4-kb exon 2 probe; blot 3 with a 1.7-kb exon 3 probe; and blot 4 with a 0.6-kb exon 4 probe. The location of these probes is indicated in Figure 4. Hybridization was at 60 °C for 20 h in 6 × SSC/0.25% dry milk with probe (5 ng/mL). Blots were washed at 60 °C in 2 × SSC/0.1% SDS (3 times, 1 h each) and exposed for 14 h with an intensifying screen.

been entirely sequenced: it contains 1344 bp of exon 2 and 1022 bp of intron 2.

A 504 bp fragment of clone Z3 (see Figure 1) was used to screen for clones containing the 3′ end of the cDNA. One clone, P152, was found out of 10^6 plaques. It contained a 10.5-kb insert. Sequence analysis showed that this clone included the last 122 nucleotides of the coding sequence as well as the 3′-untranslated region. An intronic sequence was present between nt 1600/1601 as previously observed in an incompletely processed cDNA clone, Z35 (McTiernan et al., 1987). This intron is thus called intron 3, and the exon contained in P152 is exon 4.

There were two polyadenylation signals in the 3′-untranslated region: AATAAA (nt 2002–2007) and ATTAAA (nt 2182–2187). They were both followed by a CA polyadenylation site located downstream (17 and 15 bp, respectively) (see Figure 5). GT-rich regions were present in both cases after the CA site (GTG, TG, TTTT, and TGT for the first site and TTTGTTTTTT for the second site). cDNA clones have been previously characterized possessing poly(A) tracts at these positions (McTiernan et al., 1987), showing that both sites are likely functional.

To find additional genomic clones covering exon 3 (nt 1434–1600) and because no human probe was available that contained only exon 3, we used a probe of rabbit cDNA provided by Dr. A. Chatonnet (INRA, Montpellier, France). This probe (BNY1 in Figure 1) contained 167 bp of coding sequence at nt positions 1434–1600, sharing 85% homology with the nucleotide sequence of the human BChE cDNA. Screening the genomic library with the rabbit probe gave five positive clones, of which four were found identical by restriction mapping. Thus, only two clones were selected (P85 and P91, Figure 2) for further characterization. Both P85 and P91 contained exon 3 and flanking intronic sequences at positions 1433/1434 and 1600/1601. The inserts were 16.5 kb in P85 and 15 kb in P91; the two clones overlapped by 6.5 kb (Figure 2). Sequencing of 250 bp of intronic sequences on both sides of exon 3 in P85 and P91 gave identical results, showing that P85 and P91 belong to the same gene. P85 and P91 did not overlap with clones M24 or P152.

Genomic Blot Analysis. Blots from human white blood cell DNA were hybridized with probes containing exons 1, 2, 3, and 4 (Figure 3; probe locations are indicated in Figure 4). The sizes of the different fragments hybridizing with each probe are summarized in Table I. This allowed us to estimate the minimal sizes of introns 2 and 3 (each 32 kb) which were not entirely covered by the genomic clones. The minimal length of the total gene was estimated to be 73 kb.

Table I: Fragment Sizes on Genomic Blots Hybridized with Probes Derived from the Human Cholinesterase Gene[a]

	size (kb) of hybridizing fragments with probe				
enzyme	exon 1	exon 2	exon 3	exon 4	total[b]
*Eco*RI	4.3	2.4	12	9.5	28.2
*Pst*I	>25	>25	20	>25	>70
*Xba*I	2.3	5.2, 3.7	2	7	20.2
*Pvu*II	2.4	>25	3.2	7	>37.6
*Hinc*II	1.2	3.7	0.9, 0.7	9	15.5
*Kpn*I	>25	>25, 12	16	18	>71
*Hind*III	1.3	4.4	5	10.5	21.2
*Taq*I	3.0	12.5	2.7, 0.8	2.6	21.6

[a] Exon 1 probe is an 0.8-kb *Pst*I/*Hind*III fragment. Exon 2 probe is a 2.4-kb *Eco*RI/*Eco*RI fragment. Exon 3 probe is a 1.7-kb *Eco*RI/*Xba*I fragment. Exon 4 probe is a 0.6-kb *Rsa*I/*Rsa*I fragment. [b] Fragments that hybridized with more than one probe were counted only once.

A partial restriction map of the human BChE gene is shown in Figure 4. There was very good agreement between this restriction map, which had been essentially deduced from restriction endonuclease analysis of the five genomic clones, and the size of the hybridizing fragments observed in genomic blots. *This supports the existence of only one gene.*

Partial Nucleotide Sequence of the Human BChE Gene. The partial nucleotide sequence of the human BChE gene is shown in Figure 5. In the 5′ region, three potential translation initiation sites were found. The ATG corresponding to Met −28 lies in a consensus sequence for functional translation initiation sites and may, therefore, be the most frequently used initiator. However, two other ATG codons at Met −47 and −69 are in the same reading frame as Met −28 and could also be used for initiation. Thus, in Figure 5, we show the amino acid sequence of the signal peptide starting at Met −69.

The whole coding region of the gene as well as the 3′-untranslated region of exon 4 was sequenced in the genomic clones. Four nucleotide variations were found compared to the previously published cDNA sequences, at nt −116, 1139, 1615, and 1914 (Table II). It has recently been shown that the variation at nt 1615 corresponded to the mutation responsible for the K variant (Bartels et al., 1989). It is possible

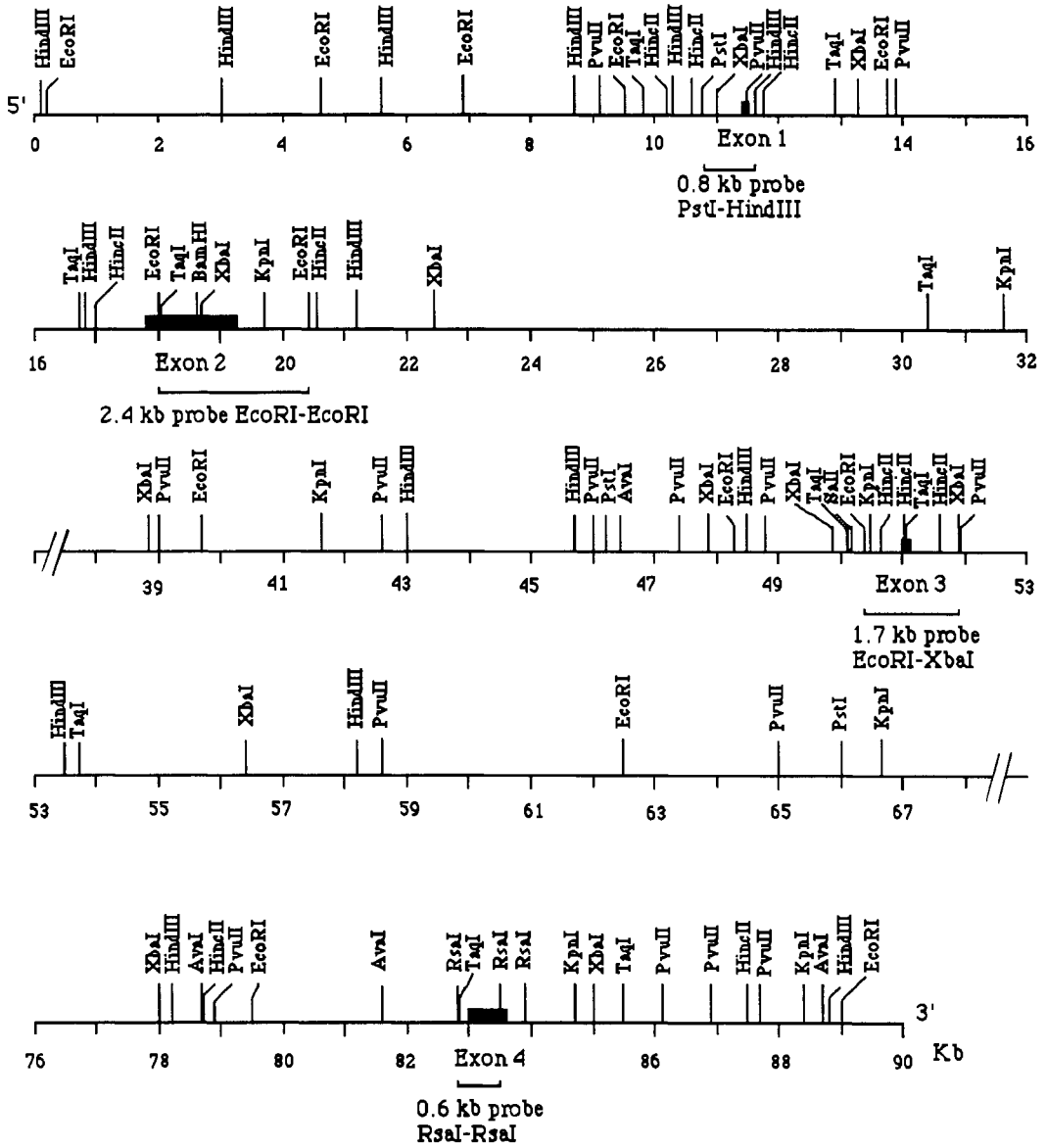


FIGURE 4: Partial restriction map of the human BChE gene. The restriction sites were determined either by restriction mapping of the five genomic clones or by genomic blot analysis of total human genomic DNA in the regions not covered by the genomic clones. The location of four exon probes used for the genomic blots in Figure 3 is shown.

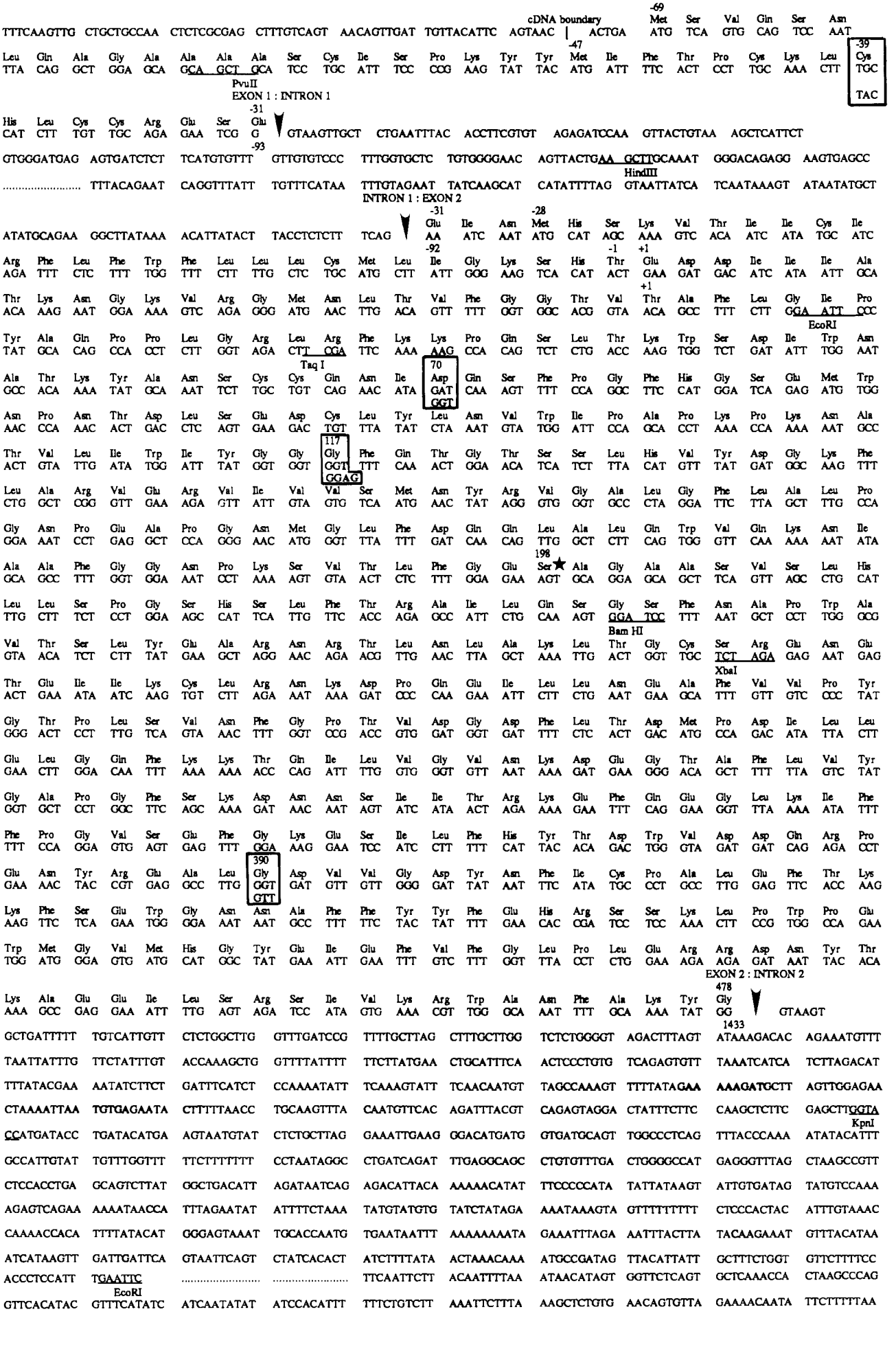
Table II: Nucleotide Variations in the Human Butyrylcholinesterase Exons[a]

nucleotide no.	variation	amino acid alteration	clones
−116	A/G	Tyr −39 (TAC) to Cys (TGC)	A in Z13 cDNA; G in P117; G in FL 39 cDNA[b]
209[c]	A/G	Asp 70 (GAT) to Gly (GGT)	A in all cDNA and genomic clones; G in atypical BChE
351[d]	T/AG	Gly 117 (GGT) to GGAG	T in all cDNA and genomic clones; AG in silent BChE
1169	G/T	Gly 390 (GGT) to Val (GTT)	G in P117; T in Z3 and Z19 cDNA; G in FL 39 cDNA
1615[e]	G/A	Ala 539 (GCA) to Thr (ACA)	G in Z3 and Z19 cDNA; A in Z35 cDNA and P152; A in K variant of BChE; G in FL 39 cDNA
1914	G/A	3'-untranslated region	A in Z3 cDNA; G in Z19, Z35 cDNA, and P152; A in FL 39 cDNA

[a] The nucleotide numbers are the same as in Figure 5. Nucleotide −116 is in a translated sequence only if Met −69 or Met −47 is used as a translation initiation site. cDNA clones Z3, Z13, Z19, and Z35 had been previously isolated in our laboratory from a fetal brain cDNA library made from a single donor (McTiernan et al., 1987). Clones P117 and P152 were isolated from two different genomic libraries, each one constructed from a single donor. The nucleotides found by Prody et al. (1987) in their liver BChE cDNA clone FL39 (*b*) and structural mutations responsible for the atypical (*c*), the silent (*d*) (McGuire et al., 1989), and the K (*e*) (Bartels et al., 1989) variants of the enzyme are indicated.

that the mutations at positions −116, 1139, and 1914 could also correspond to genetic polymorphisms, and account for some other genetic variants. We have indicated in Table II the two structural mutations responsible for the atypical (nt 209) and one of the silent (nt 351) genetic variants of BChE (McGuire et al., 1989). All these variations also appear in Figure 5 as boxed codons.

All intron–exon junctions were sequenced at least 100 bp into the intron. Some of the intronic sequences were used for the designing of oligonucleotide primers used in polymerase chain reaction experiments to amplify human genomic DNA and identify the structural mutations in the exons mentioned above. The 20 bp of each boundary are shown in Table III. They all possess the starting GT or terminal AG consensus


cDNA boundary
-69
-47
-39
PvuII
EXON 1 : INTRON 1
-31
-93
HindIII
INTRON 1 : EXON 2
-31
-28
-92
-1
+1
+1
EcoRI
Taq I
70
117
198
BamHI
XbaI
390
EXON 2 : INTRON 2
478
1433
KpnI
EcoRI

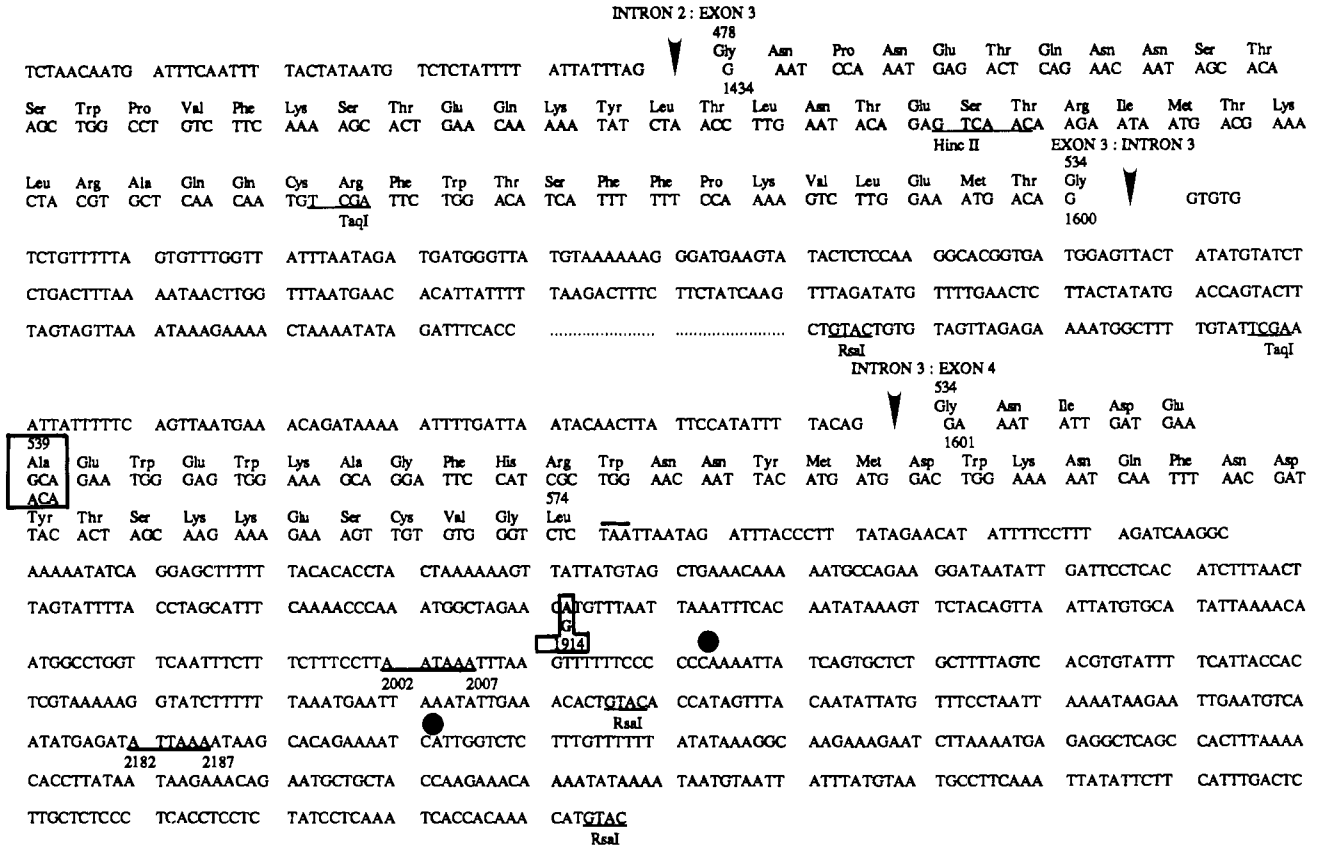


FIGURE 5: Partial nucleotide sequence of the human BChE gene. Nucleotide +1 is the first nucleotide of Glu, the NH_2 terminus of the mature protein (amino acid +1). The active-site serine is Ser +198 (★). Nucleotide numbers (below sequence) and amino acid numbers (above sequence) are negative in the signal peptide and upstream. We have indicated translation starting at Met −69 (one of the three possible translation initiation sites, see text). The two other sites (Met −47 and −28) are in the same reading frame. Arrowheads indicate intron–exon splice junctions. Introns have been only partially sequenced; the unsequenced regions are indicated by dots. The mature protein ends at Leu +574; the stop codon is overlined. Two polyadenylation sites (CA, solid circles) and two polyadenylation signals (AATAAA and ATTAAA, underlined) are indicated. Three nucleotide variations identified by sequencing the genomic clones (at nt −116, Cys−39; nt +1169, Gly +390; and nt +1914, 3′-untranslated) and three structural mutations responsible for the atypical (nt +209, Asp +70), one of the silent (nt +351, Gly +117), and the K (nt +1615, Ala +539) variants are boxed (see also Table II).

Table III: Sequences of Exon–Intron Boundaries of Human BChE Gene[a]

5′ boundary		3′ boundary
exon 1 A TCG G -93	intron 1 (6.5 kb) **gtaag**ttgctctgaatttac ... tatacttacctctctttcag	exon 2 AA ATC -92
exon 2 TAT GG 1433	intron 2 (>32 kb) **gtaag**tgctgatttttgtc ... gtctctattttattatttag	exon 3 G AAT C 1434 GLY 478
exon 3 G ACA G 1600	intron 3 (>32 kb) gtgtgtctgtttttagtgtt ... acttattccatattttacag	exon 4 GA AAT 1601 GLY 534

Consensus sequence of intron-exon boundary :

C a ttttttttt c
AG gta gt n ag G
A g ccccccccccc t

[a] Exon sequences are in capital letters, and intron sequences are in lower-case letters. Numbers below the DNA sequence are the nucleotide numbers. Amino acid residues of the protein are shown below the nucleotide number. Sequences in bold letters are in agreement with the consensus sequence (Mount, 1982).

sequences (Mount, 1982). All the introns interrupt an exon codon. Sikorav et al. (1988) found a cDNA clone of *Torpedo* AChE containing at its 3′ end a sequence encoding a 66 amino acid peptide which could account for a minor hydrophobic subunit. This sequence would be an intronic sequence corresponding to the 5′ end of our intron 3. We checked for this possibility in the human BChE gene: reading the homologous sequence leads to a stop codon after only 12 amino acids. It is thus unlikely that this intronic region could be used as an alternative coding sequence in the human BChE gene.

Discussion

The entire cDNA encoding the catalytic subunit of the G4 hydrophilic BChE is contained in four exons. It is of interest to note that the sum of all exon sizes is 2416 bp, a value in good agreement with the 2.5-kb band detected on Northern blots from human liver and brain poly(A+)RNA by Prody et al. (1987). We cannot, however, rule out the possibility of the existence of an additional exon upstream of exon 1, especially since no TATA- or CAAT-like sequences have been identified in the sequence upstream of the cDNA boundary. An interesting feature is that 83% of the coding sequence (28 amino acids of the signal peptide and 478 amino acids out of 574 of the mature protein) is encoded by exon 2, including the esteratic (serine 198) subsite. The minimal size of the BChE gene is estimated to be 73 kb, due to the presence of two unusually long introns (introns 2 and 3).

Six nucleotide variations have been identified in exons 1, 2, and 4. A nucleotide insertion at glycine 117 (GGT to GGAG) has been found in one silent family (McGuire et al., 1989). It results in the appearance of a stop codon at amino acid 121, leading to a truncated protein lacking the active-site serine, and thus devoid of any catalytic activity. The point

mutation at nt 209, changing aspartate 70 (GAT) to glycine (GGT), is the only difference in the coding sequence identified in individuals presenting the atypical phenotype of the enzyme. Since the atypical enzyme is characterized by a reduced affinity for positively charged substrates and inhibitors, this unique mutation identifies aspartate 70 as a component of the anionic site. The mutation at nt 1615, which changes alanine 539 (GCA) to threonine (ACA), is responsible for the quantitative K variant (Bartels et al., 1989), characterized by a 33% decrease in the enzyme activity. Interestingly, the mutations at nt 209 and 1615 appear to be in close linkage. The physiological significance of the three other variations at positions −116, 1169, and 1914 is still unclear. They might correspond to other genetic variants or have no influence on the amount or level of activity of the protein.

One Gene for Human BChE. Soreq et al. (1987) found two sites of in situ hybridization of human chromosomes with a full-length BChE cDNA. One was on chromosome 3, corresponding to the position where the BChE structural gene (locus E1) had been previously mapped by genetic linkage studies (Sparkes et al., 1984; Yang et al., 1984). The second site, on chromosome 16, was tentatively suggested by Soreq et al. to be the E2 locus, which is implicated in the production of the C5 variant (Harris et al. 1963a,b). The C5 isozyme, which appears as an additional slow-migrating band in electrophoresis, is present in only 8% of the Caucasian population (Whittaker, 1986). Soreq et al. (1987) thus suggested that the E2 locus might have become a pseudogene expressed only in C5+ individuals. Recent genetic linkage studies, however, show that the E2 locus is linked to the γ-crystallin gene cluster and is therefore assigned to chromosome 2 (Eiberg et al., 1989). In fact, we have never obtained any evidence for more than one BChE gene. Genomic blots of total white blood cell DNA show very few bands, and these are in agreement with the gene structure deduced from restriction endonuclease mapping of the genomic clones (Figure 4). We have never observed additional bands which would indicate the presence of a second gene, or a pseudogene. Several screenings of three genomic libraries from different individuals and the isolation of genomic clones have never supported the existence of a second gene differing in the coding sequence, or in its structural organization. Another argument comes from polymerase chain reaction experiments used in the identification of the atypical mutation (McGuire et al., 1989). Total genomic DNA from homozygous atypical individuals was amplified with oligonucleotide primers located in the coding sequence and flanking the mutated site, and the double-stranded amplified piece was sequenced directly. The sequencing gels never showed any band heterogeneity at the place of the atypical mutation. This would have been expected if a second gene were present, since it is highly improbable that this other gene would have the same rare mutation. If one accepts the hypothesis of the existence of a single BChE gene, the genomic basis for the C5 variant remains puzzling. It has been suggested either that the C5 variant could be a hybrid molecule formed by the association of butyrylcholinesterase (product of E1) with a different protein (product of E2) (Scott & Powers, 1974) or that it could originate from the modification of BChE by a neuraminidase-like enzyme expressed in only a small percentage of the population (Ogita, 1975). The first hypothesis is supported by the recent finding that the C2 isozyme is a hybrid molecule composed of a BChE subunit covalently linked to albumin via a disulfide bridge (Masson, 1989).

The presence of a single gene also implies that the genetic variants identified in serum should be expressed in all tissues that normally have BChE, as previously shown for the atypical enzyme (Liddell et al., 1963).

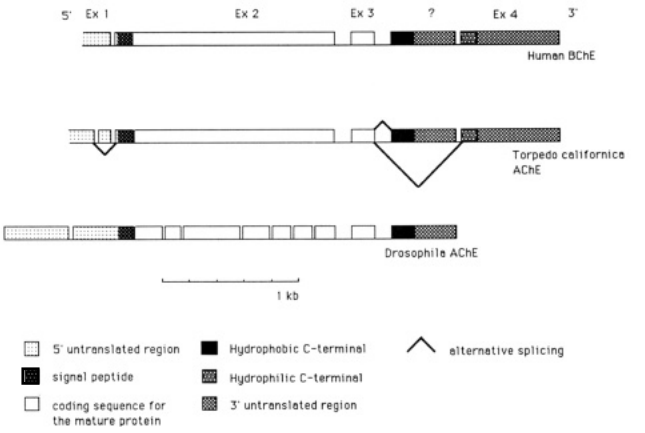


FIGURE 6: Comparison of the human butyrylcholinesterase gene with the *Torpedo californica* and *Drosophila melanogaster* AChE genes. Exons are represented as boxes. Introns are not on scale. A hypothetical alternative exon at the 3′ end of the human BChE gene has been indicated with a question mark (see Discussion). Data for the *Torpedo californica* AChE gene are from Schumacher et al. (1988), Gibney et al. (1988), and a personal communication from Dr. P. Taylor (University of California, San Diego). Data for the *Drosophila* AChE gene are from Fournier et al. (1989).

Comparison of the Human BChE Gene with Other Cholinesterase Genes. The amino acid sequence of the plasma human BChE displays 54% identity with *Torpedo californica* and *Torpedo marmorata* AChE and 38% identity with *Drosophila melanogaster* AChE (Mc Tiernan et al., 1987). Figure 6 presents a comparison of these gene structures based on the currently available results. Introns 2 and 3 of human BChE, which flank the small 167 bp exon (exon 3), are present at the same location in the *Torpedo* (Dr. P. Taylor, personal communication) and *Drosophila* (Fournier et al., 1989) AChE genes. The *Torpedo* gene also possesses a large exon containing most of the coding sequence, equivalent to our exon 2. This great similarity between the human BChE and the *Torpedo* AChE gene structures suggests that vertebrate AChE and BChE derive from a common ancestral gene. The *Drosophila* gene, however, contains 7 exons in the region where vertebrate AChE and BChE have a unique exon (Figure 6). This indicates that the divergence between insect and vertebrate cholinesterases occurred before the vertebrate cholinesterase gene was split into AChE and BChE.

Existence of Amphiphilic Forms of BChE and Their Possible Origin. The structure of the human BChE gene, as described in this report, explains the generation of a hydrophilic catalytic subunit which self-associates into disulfide-linked dimers in the hydrophilic G4 form present in plasma (Lockridge et al., 1979). This hydrophilic subunit is also produced in the brain (McTiernan et al., 1987; Prody et al., 1987). Atack et al. (1986) suggested that part of the BChE G4 form was membrane-bound in the human brain. One likely hypothesis is that the hydrophobic tetramer is made of the association of catalytic subunits to a noncatalytic hydrophobic element (not coded by the BChE gene), that mediates the membrane attachment, in a way similar to the G4 form of AChE in the bovine brain (Inestrosa et al., 1987). Atack et al. (1986) also suggested the existence of a membrane-bound monomeric form of human BChE, and hydrophobic monomers and dimers of BChE were also demonstrated in *Torpedo* heart (Toutant et al., 1985; Bon et al., 1988). These observations raise the possibility of the existence of a *hydrophobic* type of catalytic subunit in BChE. The *Torpedo* AChE gene produces

the two types of catalytic subunits (hydrophilic, hydrophobic) by alternative splicing of two exons in the 3′ region (Sikorav et al., 1988; Schumacher et al., 1988). Due to the structural similarity of the BChE and AChE genes, one may wonder whether such a mechanism also exists in the BChE gene. In this case, another exon should be present at the 3′ end of the gene, and it should be alternatively spliced with exon 4 (see Figure 6).

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Registry No. DNA (human butyrylcholinesterase gene coding region), 123962-87-8; butyrylcholinesterase (human precursor reduced), 123962-88-9; butyrylcholinesterase (human reduced), 123962-89-0; butyrylcholinesterase, 9001-08-5.

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