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Original article

Characterization of an unstable variant (BChE115D) of human butyrylcholinesterase

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An unstable variant of human butyrylcholinesterase (BChE) is described in four apparently unrelated individuals sensitive to succinylcholine. Sequencing of genomic DNA revealed a single nucleotide substitution which results in the replacement of amino acid residue Gly115 by Asp. This variant can be recognized by its increased instability under extremes of temperature such as heating and also freezing and thawing, both in homozygous and heterozygous states. When in heterozygous combination with the Atypical variant, it produces dibucaine and fluoride numbers which are intermediary between those of Atypical homozygotes and heterozygotes. After repeated freezing and thawing, however, these values approach those of homozygous Atypical plasma. Measurement of activity and immunoreactive BChE protein in plasma of individuals representing different combinations of this allele indicated that the presence of the Usual or Atypical enzymes seems to partially protect this variant from denaturation *in vivo*. Phenotyping fresh serum or plasma samples, before they are frozen, is critical for the identification of this, and possibly some other, unstable variants.

Keywords: human BChE variant, butyrylcholinesterase, cholinesterase, unstable BChE.

Introduction

Human butyrylcholinesterase (BChE; EC 3.1.1.8) has a broad substrate specificity and is the enzyme responsible for the hydrolysis of the muscle relaxants succinylcholine and mivacurium. Patients with low levels of BChE activity or genetically determined BChE variants can develop prolonged paralysis, sometimes lasting for several hours, after receiving standard doses of these drugs. The best known BChE variants (Atypical and Fluoride-resistant variants) can be distinguished from the Usual enzyme by their increased resistance to several inhibitors, the most commonly used being dibucaine (Kalow & Genest, 1957) and sodium fluoride (Harris & Whittaker, 1961). The percentage inhibition produced by dibucaine and fluoride, the dibucaine (DN) and fluoride (FN) numbers, allow the identification of most variants in several genotypic combinations. The Silent BChE variants are characterized by complete or very reduced BChE activity levels. They can be identified by the absence of or very low levels of BChE activity (in homozygotes or compound heterozygotes for two Silent alleles) or by unexpected segregation of BChE phenotypes. A number of comprehensive reviews are available on the variants and genetic aspects of BChE (Whittaker, 1986; Lockridge, 1992; Massoulié, 1993; Pantuck, 1993). The determination of the amino acid sequence of human BChE (Lockridge *et al.*, 1987) and the respective cDNA and genomic sequences (McTiernan *et al.*, 1987; Prody *et al.*, 1987; Arpagaus *et al.*, 1990) have allowed the characterization of these variants at the DNA level. All variants are characterized by nucleotide substitutions, deletions and/or insertions which result in unique amino acid substitutions and other alterations in the BChE protein (Table 1).

The usual BChE and all the previously described variants are highly stable. Neither the activity nor the inhibition characteristics are affected by storage at room temperature for several days or at low temperatures for several years (Lanks & Sklar, 1976; Turner *et al.*, 1985). This degree of stability has been

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Table 1. Human *BCHE* alleles characterized at the DNA level and their effects on the BChE molecule

Allele	*Effect on BChE molecule*[a]
*BCHE*70G* (Atypical)[b]	Aspartate 70 → glycine
*BCHE*243M* (fluoride-resistant)[c]	Threonine 243 → methionine
*BCHE*390V* (fluoride-resistant)[c]	Glycine 390 → valine
*BCHE*539T* (K-variant)[d]	Alanine 539 → threonine
*BCHE*497V* (J-variant)[e]	Glutamate 497 → valine
*BCHE*142M* (H-variant)[f]	Valine 142 → methionine
*BCHE*FS117* (Silent)[g]	Frameshift at codon 117
*BCHE*ALU355* (Silent)[h]	Insertion of *Alu* element at codon 359
*BCHE*365R* (Silent)[i,j]	Glycine 365 → arginine
*BCHE*FS315* (Silent)[j]	frameshift at codon 315
*BCHE*418S* (Silent)[k]	Phenylalanine 418 → serine
*BCHE*515C* (Silent)[k]	Arginine 515 → cysteine
*BCHE*465STOP* (Silent)[k]	Stop codon at codon 465
*BCHE*FS6* (Silent)[l]	Frameshift at codon 6
*BCHE*33C* (Silent)[l]	Tyrosine 33 → cysteine
*BCHE*37S* (Silent)[l]	Proline 37 → serine
*BCHE*125F* (Silent)[l]	Leucine 125 → phenylalanine
*BCHE*170E* (Silent)[l]	Aspartate 170 → glutamate
*BCHE*198G* (Silent)[l]	Serine 198 → glycine
*BCHE*201T* (Silent)[l]	Alanine 201 → threonine
*BCHE*271STOP* (Silent)[l]	Stop codon at codon 271
*BCHE*471R* (Silent)[l]	Tryptophan 471 → arginine
*BCHE*500STOP* (Silent)[l]	Stop codon at codon 500
*BCHE*518L* (Silent)[l]	Glutamine 518 → leucine
*BCHE*I2E3-8G* (Silent)[l]	Altered splicing of intron 2

[a] Amino acid residues are numbered according to Lockridge *et al.* (1987); [b] McGuire *et al.* (1989); [c] Nogueira *et al.* (1992); [d] Bartels *et al.* (1992b); [e] Bartels *et al.* (1992a); [f] Jensen *et al.* (1992); [g] Nogueira *et al.* (1990); [h] Muratani *et al.* (1991); [i] Hada *et al.* (1992); [j] Hidaka *et al.* (1992); [k] Maekawa *et al.* (1995); [l] Primo-Parmo *et al.* (1996).

extremely important in the study of BChE variants. It has enabled the creation of reference collections of plasma samples, by our and other laboratories over the years, which can be used as reference samples, with reproducible results. It was, therefore, surprising to find that the plasma samples of some individuals produced dibucaine and fluoride numbers which changed after the samples had been frozen and thawed. The aim of the present study was to characterize the new BChE variant responsible for this abnormal finding.

Materials and methods

BChE phenotyping

BChE activity was determined according to Kalow & Lindsay (1955) using 50 μM benzoylcholine as substrate in 67 mM phosphate buffer, pH 7.4, at 25 °C. Phenotypes were determined by the inhibition of BChE activity by 10 μM dibucaine (Kalow & Genest, 1957) and by 50 μM sodium fluoride (Harris & Whittaker, 1961).

DNA amplification and sequencing

Genomic DNA isolated from white blood cells (Muellenbach *et al.*, 1989) was amplified by PCR (Saiki *et al.*, 1988) using pairs of primers designed to amplify the four *BCHE* exons and intron-exon boundaries (Arpagaus *et al.*, 1990). It was not possible to obtain fresh blood samples from individuals 1-I-1, MP and JoW, and DNA was amplified directly from the original plasma samples. In these cases, two rounds of amplification using internal primers were usually necessary to obtain enough PCR product for sequencing. The sequences of the primers used are described in Bartels *et al.* (1992b). The additional primers used were: AP38 (5′-TGTCTTTATACTAAAGTCTACCCCAGAGACC) which anneals from 63 to 94 nucleotides downstream of the splice site between exon 2 and intron 3, and AP39 (5′-GGTTTCTCATCATGTATGACTGCCTGCAGGGTC) which anneals from −804 to −771 nucleotides upstream from the splice site between exon 1 and intron 1. The PCR products were directly sequenced by the dideoxynucleotide chain-termination procedure (Sanger *et al.*, 1977) with internal oligonucleotide primers (Bartels *et al.*, 1992b).

Plasmid constructs

The nucleotide substitution characterizing the new variant described in the present study was introduced in the pRcCMV vector containing Usual BCHE by site-directed mutagenesis. The 5′-phosphorylated mutagenic oligonucleotide (5′-GGATTTATGATGGTGGTTTTC) was annealed to single-stranded M13mp19/Usual*BCHE* together with the upstream PUC/M13 forward primer (Promega). Extension and ligation were done with T7 DNA polymerase (Promega) and T4 DNA (Promega) ligase. The product of these reactions was subjected to PCR using oligonucleotides AP3 and AP23 (Bartels *et al.* 1992b). After purification, the PCR product was digested with *Sph* I and *Xba* I, and ligated into the pGEM7f(-)/Usual BCHE cut with the same restriction enzymes. Competent *E. coli* HB101 were transformed with the constructs and grown in LB medium plates containing 100 μg ml^{-1} of ampicillin. Resistant colonies were selected with toothpicks and placed into a 100 μl PCR reaction mixture. The amplified product was sequenced to verify the desired mutation. The BCHE insert was, then, cloned from pGEM7f(-) into pRcCMV using *Apa* I

and *Hind* III restriction enzymes. The pRcCMV/BCHE115D was used to transform competent HB101 *E. coli*, and the plasmid was isolated with the QIAGEN plasmid DNA purification kit. Both M13mp19 and pRcCMV vectors containing the Usual BCHE were kindly provided by Dr Oksana Lockridge.

Expression of BChE in 293 cells

293 cells were grown in MEM supplemented with 20% of fetal bovine serum, in 25 cm^2 flasks. This fetal human kidney cell line does not express endogenous BChE. When the cell lawns were 50% confluent, cells were transformed with 1 μg of the purified pRcCMV/BCHE vector using the calcium-phosphate method according to Ausubel *et al.* (1993). Cells were incubated with the DNA for 3 h, and were then exposed to 15% glycerol for 2 min. Cells were rinsed with PBS and the same, used medium, containing the calcium phosphate-vector, was put back on the cells. Twenty-four hours later the medium was replaced by fresh medium. The next day (48 h after tranformation) cells were exposed to Geneticin (Gibco) at a concentration of 0.5 mg ml^{-1} of medium. This antibiotic was present in the culture medium in all subsequent steps. Quadruplicate experiments were performed. The resistant cells were pooled in order to minimize differences between experiments. Non-transformed cells were used as a negative control.

Immunoquantitation of BChE

The amount of immunoreactive BChE protein in the plasma of patient 1-I-1, in cell culture medium and in 293 cells lysates was determined by ELISA. A monoclonal anti-human BChE, kindly provided by Dr S. Brimijoin, was used as the capture antibody and a rabbit polyclonal anti-human BChE (Accurate) as the secondary antibody. The BChE-antibody complexes were quantitated with a goat anti-rabbit IgG, human-adsorbed (Promega), conjugated to alkaline phosphatase. The development of color, after addition of the alkaline phosphatase substrate solution (Promega), was measured in a microtiter plate reader at 405 nm.

Enzymatic analyses of expressed BChE

Secreted expressed BChEs were assayed in serum-free culture medium. When resistant cells reached 100% confluency, the former medium was replaced by SFRE 199-2 medium (Sigma) supplemented with 150 U ml^{-1} of insulin. After 2 days, both the medium and the cells were harvested. The intracellular BChE was assayed in 10 mg of washed cells lysed with 150 μl of lysis buffer (50 mM Tris/Cl, 1% Triton X-100, 0.15 M NaCl, 1 μg ml^{-1} aprotinin, pH 8.0). The lysate was centrifuged at 16 000 rpm for 15 min and only the supernate was used. Enzymatic analyses were done as above, and the effect of temperature on the expressed BChEs was determined by maintaining the enzymes at −20 °C, 4 °C, and 25 °C overnight, or at 56 °C from 5 to 50 min, after which the samples were placed on ice for about 2 min before being analysed.

Table 2. Phenotypic characteristics of all individuals analysed in the present study. The results refer to analyses performed in our laboratory. Individuals are numbered according to Fig. 1

Individuals	*BChE activity*[a]	*DN*[b]	*FN*[c]	BCHE *genotype*
1-I-1	absent	–	–	*BCHE*115D/*115D*
1-II-1	0.709 0.620	81 81	61 60	*BCHE*U/*115D*
1-II-2	1.299 0.930	81 80	57 59	*BCHE*U/*115D*
1-III-1	1.550 1.510	83 81	61 60	*BCHE*U/*U*
1-III-2	1.000 0.831	82 80	59 59	*BCHE*U/*115D*
1-III-3	0.881 0.910	82 80	61 58	*BCHE*U/*U*
1-IV-1	0.388 0.295	37 25	40 29	*BCHE*70G/*115D*
1-IV-2	0.567 0.465	35 25	51 28	*BCHE*70G/*115D*
2-I-1	0.500	81	67	DNA not sequenced
2-I-2	0.300	58	53	DNA not sequenced
2-II-1	0.190	17	–	*BCHE*70G/*115D*
2-II-2	0.220	14	25	*BCHE*70G/*115D*
MP	0.095 0.072	36 24	51 31	*BCHE*70G/*115D*
JoW	0.412 0.370	28 22	29 25	*BCHE*70G/*115D*

[a] Micromoles of benzoylcholine hydrolysed per min per ml of plasma; in each column, the numbers to the left represent activity when the samples were first assayed in our laboratory, and the ones to the right after two cycles of freezing and thawing.
[b,c] Dibucaine and fluoride numbers, respectively.

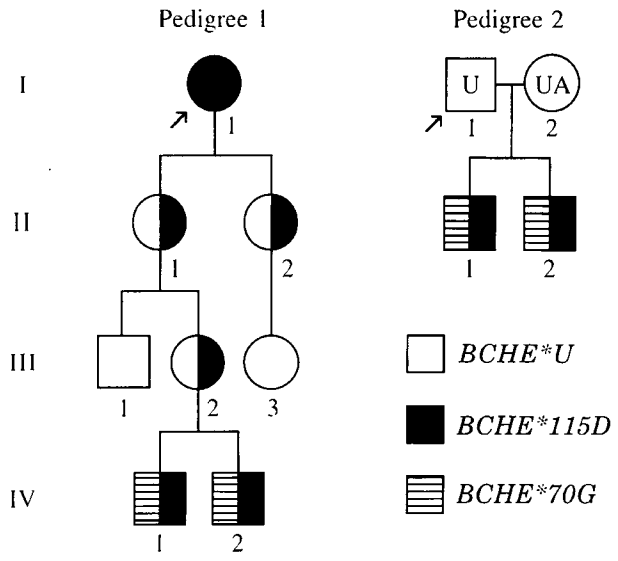

Fig. 1. Pedigrees of patients 1-I-1 and 2-I-1. It was not possible to analyse DNA from individuals 2-I-1 and 2-I-2, and the letters inside their symbols represent their BChE phenotype: U, Usual phenotype; UA, phenotype consistent with the genotype *BCHE*U/*70G*.

Results

Table 2 shows the values of BChE activity and the percentage of inhibition by dibucaine (DN) and sodium fluoride (FN) for all individuals analysed in the present study. Patient 1-I-1 was first believed to have a Silent BChE phenotype, since no BChE activity was detected in her plasma. This sample was received by our laboratory in 1981; however, the sample had been assayed earlier by another laboratory and it was reported to have very low BChE activity. Patient 1-I-1 is now deceased, but we were able to collect fresh blood samples from several relatives (Fig. 1). Most of these had the Usual BChE phenotype; the exception being 1-IV-1 and 1-IV-2, whose results were close to those reported for the BCHE 70G,497E (AJ) phenotype (Table 3). The phenotyping of individuals 1-IV-1 and 1-IV-2 was repeated a few days later, after the samples had been frozen at −20 °C and thawed twice. Surprisingly, the new DN and FN were now similar to those of the Atypical phenotype. The changes in inhibition characteristics were associated with a decrease in total BChE activity of 24% for individual 1-IV-1 and 18% for 1-IV-2. This treatment also lowered the activity of individuals 1-II-1, 1-II-2, and 1-III-2 (13%, 28%, and 17%, respectively), but not of individuals 1-III-1 and 1-III-3 (Table 2). Those unexpected results were interpreted as indicating a new, unstable, BChE variant that loses activity upon freezing and thawing. This interpretation accounts for the discrepancies referred above for the sample from patient 1-I-1. We assume that this sample may have been frozen before being sent to our laboratory. Unfortunately, we do not know how the samples were handled before being sent to us.

Sequencing of DNA isolated from the original plasma sample of individual 1-I-1 disclosed a single nucleotide substitution in homozygous state, which results in the replacement of glycine 115 by aspartate. This new *BCHE* allele, *BCHE*115D*, was present in heterozygous combination with the Atypical allele in individuals 1-IV-1 and 1-IV-2, and with the Usual allele in the other relatives (Fig. 1).

Two other patients, MP and JoW, had the genotype *BCHE*70G/*115D*. Their DN, FN, and BChE activity

Table 3. Reference values of BChE activity with benzoylcholine and inhibition by dibucaine and sodium fluoride for different BChE phenotypes

Phenotype[a]	*Activity*[b] *mean ± SD*	*DN*[c] *mean (range)*	*FN*[d] *mean (range)*
BCHE U	0.81 ± 0.25	80 (77–83)	57 (51–66)
BCHE U, 70G	0.77 ± 0.20	63 (58–68)	49 (44–57)
BCHE 70G	0.50 ± 0.17	20 (13–27)	23 (14–37)
BCHE 70G,243M	0.50 ± 0.25	48 (42–53)	30 (27–33)
BCHE 70,390V			
BCHE U,243M	0.80 ± 0.36	75 (73–77)	48 (45–50)
BCHE U,390V			
BCHE 70G,539T[e]	0.53 ± 0.08	54 (50–61)	47 (42–50)
BCHE 70G,497E[f]	0.33 ± 0.18	39 (35–44)	38 (36–41)
BCHE 70G,142M[g]	0.20 ± 0.05	32 (30–36)	27 (24–31)

[a] U, Usual; 70G, Atypical; 243M and 390V, fluoride resistant; 539T, K-variant; 497E, J-variant; 142M, H-variant. [b] Micromoles of benzoylcholine hydrolysed per min per ml of plasma. [c,d] Dibucaine and fluoride numbers, respectively. [e] Results based on Bartels *et al.* (1992b). [f] Results based on Bartels *et al.* (1992a). [g] Results based on Jensen *et al.* (1992).

Table 4. Comparison between expressed, secreted, Usual BChE and BChE115D

Enzyme	*BChE activity*[a]	*BChE activity after overnight incubation at*[b]			*Immunoreactive BChE protein*[c]
		−20 °C	4 °C	25 °C	
Expressed Usual BChE	0.155 ± 0.012	0.164 ± 0.009 ($p > 0.27$)	0.151 ± 0.009 ($p > 0.20$)	0.160 ± 0.089 ($p > 0.12$)	100
Expressed BChE115D	0.002 ± 0.001	Not detected	0.0014 ± 0.0006 ($p > 0.17$)	0.0013 ± 0.0004 ($p > 0.05$)	30 ± 4

[a] Micromoles of benzoylcholine hydrolysed per min, per ml of culture medium. The values represent mean ± SD of five measurements.
[b] Statistical comparisons represent one-tailed *t*-tests.
[c] The results refer to ELISA experiments with one uncharacterized monoclonal anti-BChE antibody, and they represent percentages relative to the expressed Usual BChE.

levels also decreased after freezing and thawing, but these findings were not evaluated further at the time when their plasma samples were analysed (1981 and 1979, respectively). The *BCHE*115D* allele was also found in pedigree 2 (Fig. 1), with no evidence of BChE instability. The plasma samples of the individuals in this pedigree were analysed in 1973, and it was thought that a Silent *BCHE* allele was segregating, since the proband with the Usual BChE phenotype had two sons with DN and FN characteristic of the Atypical phenotype. These results did not change when these plasma samples were reanalysed recently. As mentioned above, these conflicting results regarding stability of BChE activity, DN and FN may be due to the different conditions under which the plasma samples were preserved before being analysed.

A significant amount of immunoreactive BChE protein, about 40% of the average levels, was detected in the plasma sample of patient 1-I-1. The unavailability of fresh samples from homozygous individuals led us to express this new variant in cell culture, in order to evaluate its activity characteristics in the homozygous state. The activity of the expressed BChE115D represented only about 1% of that obtained for the Usual enzyme expressed under identical conditions (Table 4). The immunoreactive BChE protein, however, was present in about 30% of the amount obtained for the Usual enzyme expressed under identical conditions. This agrees with the results obtained for the sample of patient 1-I-1, which showed only trace activity when first tested at another laboratory (the actual numbers are not available).

The activity of the expressed, secreted, Usual BChE was not affected by being kept overnight at −20 °C, 4 °C, and 25 °C. Freezing and thawing of the expressed enzyme completely inactivated the BChE115D variant; however, incubation at 4 °C and 25 °C decreased its activity slightly, although the differences were not statistically significant (Table 4). The BChE115D variant is also much more unstable with incubation at 56 °C than the Usual enzyme, as can be seen in Fig. 2. This shows the residual activity of both enzymes at different times after incubation at this elevated temperature.

Attempts to determine the degree of inhibition of the expressed BChE115D by dibucaine and fluoride were not successful, since the activity of the inhibited reactions were too low to be accurately determined. This variant BChE protein is present in plasma of homozygotes at about 40% of the levels of the Usual enzyme, although its activity is less than 1% of the Usual levels. The range of BChE activity in healthy

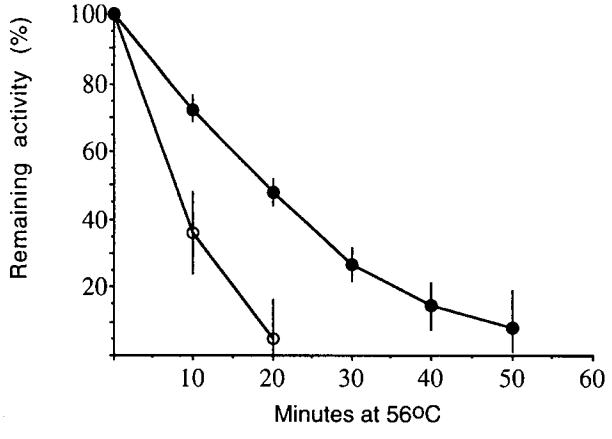

Fig. 2. Heat inactivation of expressed BChE U (●) and BChE115D (○). The expressed, secreted, enzymes were incubated at 56 °C, and aliquots were taken at the times shown. BChE activity was assayed with benzoylcholine as substrate. After 20 min of incubation the activity of BChE115D was too low to be measured with confidence. Numbers represent the means of three measurements.

individuals is rather broad (0.81–1.25 U ml^{-1}), and this makes it difficult to estimate directly the amount of BChE protein encoded by the *BCHE*115D* allele in individuals with other genotypes. However, the DNs of individuals 1-IV-1 and 1-IV-2 provide a means of estimating it. Individuals 1-II-1, 1-II-2 and 1-III-2 (genotype *BCHE*U/*115D*) had DN values which fell within the range characteristic of the Usual phenotype (Table 1). This indicates that the BChE produced by BChE115D has a dibucaine number similar to that of the Usual enzyme. The departure from the average DN of Atypical homozygotes (DN = 20) in individuals 1-IV-1 and 1-IV-2 (*BCHE*70G/*115D*) must be due to the contribution of the active BChE115D. The proportion of activity due to BChE115D in plasma of these individuals can be estimated by the expression $36 = 80X + 20(1-X)$, where X represents the percentage of BChE115D molecules, and 80 and 20 are the average DN for the Usual and Atypical phenotypes, respectively. The results indicate that about 27% of the total activity could be attributed to BChE115D, when the plasma samples were received by our laboratory 1 day after being collected and sent to us at room temperature. After freezing and thawing the value of DN fell to 25, so only about 8% of the remaining activity could still be attributed to BChE115D.

	115
Human BChE[a]	NATVLIWIY**G**GGFQTGTSS
Rabbit BChE[b]	NATVMIWIY**G**GGFQTGTSS
Mouse BChE[c]	NATVMVWIY**G**GGFQTGTSS
Bovine BChE[d]	NATVMIWIY**G**GGFQTGTSS
Dog BChE[d]	NATVMIWIY**G**GGFQTGTSS
Pig BChE[d]	NATVMIWIY**G**GGFQTGTSS
Sheep BChE[d]	NATVMIWIY**G**GGFQTGTSS
Rhesus BChE[d]	NATVLIWIY**G**GGFQTGTSS
Rabbit AChE[e]	PTPVLVWIY**G**GGFYSGASS
Human AChE[f]	PTPVLVWIY**G**GGFYSGASS
Mouse AChE[c]	PTPVLIWIY**G**GGFYSGAAS
Rat AChE[g]	PTPVLIWIY**G**GGFYSGASS
Chicken AChE[h]	PPVLVVWIY**G**GGFTGGSVS
Bovine AChE[i]	PTPVLVWIY**G**GGFYSGASS
T. marmorata AChE[j]	SATVMLWIY**G**GGFYSGSST
T. californica AChE[k]	STTVMVWIY**G**GGFYSGSST
C. elegans AChE[l]	KLAVMVWIY**G**GGFWSGTAT
D. melanogaster AChE[m]	GLPILIWIY**G**GGFQTGSAT

Fig. 3. Amino acid sequences neighboring glycine 115, shown in bold type, in BChEs and AChEs. [a] Lockridge *et al.* (1987); [b] Jbilo & Chatonnet (1990); [c] Rachinsky *et al.* (1990); [d] Arpagaus *et al.* (1991); [e] Jbilo *et al.* (1994); [f] Soreq *et al.* (1993); [g] Legay *et al.* (1993); [h] Randall *et al.* (1994); [j] Krejci *et al.* (1991); [k] Schumacher *et al.* (1986); [l] Arpagaus *et al.* (1994); [m] Hall & Spierer (1986).

Discussion

This first example of an unstable variant of human BChE is characterized by the substitution of amino acid residue glycine 115 by aspartate. This amino acid substitution occurs in a highly conserved region in all BChEs and AChEs sequenced to date (Fig. 3), which reflects its importance for the maintenance of the proper conformation of the enzyme.

The discrepancies found between the level of activity of the BChE115D variant in homozygotes and in heterozygotes indicate that BChE115D is protected to some degree against denaturation when it is present in combination with another form of BChE. BChE is present in blood mostly in tetramers which are formed by random association of monomers (La Du & Choi, 1975). Individuals of heterozygous genotypes, for example BCHE*70G/*115D, can produce five different types of tetramers in the proportions $1A_4 : 4A_3115D_1 : 6A_2115D_2 : 4A_1115D_3 : 1\ 115D_4$. The tetramer $115D_4$ is highly unstable as shown by the low activity of homozygotes. If the tetramer A_1115D_3 were also very unstable, most of the activity represented by BChE115D should be present in the other tetramer combinations of mixed composition (A_3115D_1 and A_2115D_2). The percentage of BChE115D molecules among $A_4 + A_3115D_1 + A_2115D_2$ would be about 33%, which is very similar to the former estimate (27%) obtained considering the values of DN. It is possible to suggest, therefore, that BChE115D is protected against denaturation in the two latter tetramers.

This new variant of human BChE adds to the increasing variety of variants described to date. Its unstable character points to the necessity of assaying samples as soon as possible after being collected, and specially before being frozen. We recommend that plasma or serum be separated from the blood cells and both be sent in wet ice or at room temperature. If the time in transit is to be longer than a day the blood cells or the blood clot should be sent refrigerated in order to provide adequate DNA for analyses. This can be done easily by placing the tubes with the blood cells inside a small Styrofoam container with blue ice, separated from the plasma or serum.

It is possible that other examples of unstable BChE variants exist and that they have been overlooked because of the assumption that BChE is a very stable enzyme, but they may be identified by testing samples exposed to different variations of temperature. Their recognition will contribute to our understanding of the structural characteristics of the BChE molecule.

Most variants of BChE have been recognized by their unusual behavior with the muscle relaxant succinylcholine. The use of other drugs, substrates of

this enzyme (e.g. mivacurium) is expected to identify other BChE variants which show increased (or decreased) hydrolytic characteristics, leading to unexpected clinical responses.

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