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Research Article

Cholinesterases are down-expressed in human colorectal carcinoma

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Abstract. The aberrations of cholinesterase (ChE) genes and the variation of ChE activity in cancerous tissues prompted us to investigate the expression of ChEs in colorectal carcinoma. The study of 55 paired specimens of healthy (HG) and cancerous gut (CG) showed that acetylcholinesterase (AChE) activity fell by 32% and butyrylcholinesterase (BuChE) activity by 58% in CG. Abundant AChE-H, fewer AChE-T, and even fewer AChE-R and BuChE mRNAs were observed in HG, and their content was greatly diminished in CG. The high level of the AChE-H mRNA explains the abundance of AChE-H subunits in HG, which as glycosylphosphatidylinositol (GPI)-anchored amphiphilic AChE dimers (G_2^A) and monomers (G_1^A) account for 69% of AChE activity. The identification of AChE-T and BuChE mRNAs justifies the occurrence in gut of A_{12}, G_4^H and PRiMA-containing G_4^A AChE forms, besides G_4^H, G_4^A and G_1^H BuChE. The down-regulation of ChEs might contribute to gut carcinogenesis by increasing acetylcholine availability and overstimulating muscarinic receptors.

Keywords. Cancer, cholinesterases, glycosylphosphatidylinositol, gut, real-time PCR.

Introduction

Worldwide, colorectal cancer is one of the most prevalent forms of cancer, accounting for 7–8% of all cancers in men and women. It still remains a substantial cause of death with poor 5-year survival rates. In Europe, more than 200 000 new cases (about 21 000 in Spain) and 112 000 deaths (approx. 11 000 in Spain) were reported in 1998, making it the second leading cause of cancer-related death in European countries [1].

Tumors arising from neural and non-neural tissues contain acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [2], and their possible involvement in malignancy is supported by the amplification of ChE genes in leukemias and ovarian carcinomas [3], their structural alteration or abnormal expression [4, 5], and the partial or complete deletion of the *ACHE* gene in myelodysplastic syndromes [6]. Moreover, the relationship between the overproduction of AChE/BuChE and the decrease of cell proliferation in human hematopoiesis [7], osteogenesis [8], myogenesis [9] and neurogenesis [10, 11] demonstrates that ChEs intervene in the control of cell division. Finally, the involvement of AChE in apoptosis [12, 13], and in astrocytic tumor development and aggressiveness [2] support a role for this enzyme in tumorigenesis.

Vertebrate tissues express three principal AChE mRNAs. The R, H and T transcripts encode the 'read through' AChE-R subunit, the 'hydrophobic' AChE-H subunit and

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the 'tailed' AChE-T subunit. An alternative exon 1 (E1d) has recently been identified and the mRNA produces an N-terminally extended AChE subunit (N-AChE) [14, 15]. Only one BuChE transcript has been found so far, which produces BuChE-T subunits [16]. The AChE-T (or BuChE-T) subunit may oligomerize giving globular forms (G_1, G_2 and G_4) with amphiphilic (G^A) or hydrophilic (G^H) properties. The T subunit can also form hetero-oligomers with structural subunits, such as the PRiMA in the G_4^A AChE and BuChE forms and the ColQ in the asymmetric species (A_4, A_8, A_{12}). The AChE-H subunit generates glycosylphosphatidylinositol (GPI)-linked G_1^A and G_2^A variants [17].

In spite of the early identification of AChE and BuChE in human gut, no information is available on the consequences of cancer on them. To gain insights into this issue, ChE activity and the molecular distribution of AChE and BuChE were determined in paired samples of healthy gut (HG) and cancerous gut (CG). This study may throw light on changes that intestinal ChEs undergo in cancerous tissues, keeping always in mind that the possible changes may be either a cause or an effect of neoplastic transformation. On the other hand, it is interesting to correlate the levels of the various AChE and BuChE transcripts with those of the corresponding subunits to evaluate their possible changes in colorectal carcinoma.

Materials and methods

Patients. Colorectal carcinoma specimens were taken from 29 male and 26 female patients, 41–92 years old and with a mean age of 68 years, during surgical resection at the Hospital Xeral de Vigo (Spain); 18 tumors were from colon, 19 from sigmoid colon, and 18 from rectum. Healthy gut (HG), at least 10 cm away from the cancerous gut (CG), was also taken. HG pieces weighing 0.05–4.0 g and CG specimens 0.03–3.0 g were frozen and stored at –80 °C until required. The presence in tumors of neoplastic cells was assessed by standard pathological techniques and tumors were staged according to Duke's and TNM classifications. The research was approved by Bioethics Commission of the University of Murcia. Patients were informed on the use of samples for research and gave their consent.

Extraction and assay of ChEs. Extraction of HG and CG in two steps, the first with a detergent-free saline buffer (SB; 1 M NaCl, 50 mM $MgCl_2$, 10 mM Tris, pH 7.0) and anti-proteinases [18], and the second with 1% Brij 96 in SB and anti-proteinases, allowed us to separately obtain loosely bound ChEs in the S1 supernatant and tightly bound ChEs in S2. ChE activity in homogenates and S1 and S2 extracts was assayed by the Ellman method, using suitable substrate and inhibitors to distinguish AChE and BuChE activities [18]. ChE activity is given in nmol of substrate split per min at 37 °C (mU). Acetylthiocholine (ATCh) degradation due to unspecific esterases in HG or CG amounted to 15–20% and that of butyrylthiocholine (BuTCh) to 25–30%. Substrate hydrolysis by unspecific esterases was always measured and subtracted for calculating true ChE activity. In sedimentation profiles, ChE activity is given in arbitrary units (AU), one unit of activity referring to an increase of 0.001 absorbance units per microliter of sample and per min, but normalized for the volume of sample added to the gradient. Inhibition of BuChE activity by Triton X-100 was counteracted by adding 0.5% Brij 96 to the assay mixture. Protein was determined by a Lowry method.

Characterization of ChE components. AChE and BuChE molecules were separated by centrifugation analyses in sucrose gradient and characterized by their sedimentation coefficients [18]. Overlapping peaks in the sedimentation profiles were resolved by using the PEAK-FIT program from SPSS Inc. The percentage of each AChE form was estimated by comparing the AChE activity under each peak area and under the entire profile.

The distinct migration of ChE components depending on the detergent (Brij 96 or Triton X-100) added to sucrose gradients showed their amphiphilic properties, which were further confirmed using hydrophobic chromatography in phenyl-agarose [18, 19]. The asymmetric structure of 16.2S AChE was assessed by cleaving its ColQ tail with collagenase [20]. The dimeric state of the 4.4S species (in gradients with Brij 96) and the monomeric state of the 3.2S components were assessed by the conversion of the former into the latter variants by reducing the disulfide bond which tethers the subunits in dimers [21]. The linkage of GPI to G_2^A AChE was tested by its exposure to phosphatidylinositol-specific phospholipase C (PIPLC) from *Bacillus thuringiensis*. A sample of bovine erythrocyte G_2^A AChE, which fully loses its hydrophobic domain with PIPLC [22], was used as a positive control. PIPLC alone failed to convert gut G_2^A into G_2^H AChE, which indicated the presence of acyl chains linked to the inositol ring. Taking advantage of the capacity of alkaline hydroxylamine for removing the acyl groups, the conversion of G_2^A into G_2^H forms was attempted by subjecting the sample to hydroxylaminolysis before the PIPLC treatment [21].

Identification of ChE mRNAs. RNA was extracted from colorectal pieces with TRIzol (Life Technologies, UK). For reverse transcription (RT), RNA (5 µg) was heated at 70 °C, 10 min. After cooling, a mixture of dithiothreitol, dNTPs, random decamers, ribonuclease inhibitor and buffer was added, and samples were heated 2 min at 42 °C. Then, Moloney murine leukemia virus (MMLV) reverse transcriptase (200 U, Invitrogen) was added, and

synthesis of cDNA was carried out for 50 min at 42 °C, in a volume of 20 µl. Finally, samples were heated 15 min at 72 °C and kept frozen. ChE mRNAs were amplified by PCR with primer pairs specific for each AChE mRNA, R, H or T, and the BuChE mRNA.

Since the three AChE mRNAs share the 5′ region (exons E1-E4) followed by either I4,E5,E6 (R mRNA), E5,E6 (H mRNA) or only E6 (T mRNA), primers (Invitrogen) were made to cover exon-exon splicing sites of the AChE sequence [23]. The forward primers were as follows: for R mRNA, primer p61, which is targeted to the exon 3/exon 4 (E3/E4) junction, for amplifying only processed transcripts (mature mRNAs); for H mRNA, p63 (located in E3); for T mRNA, p65 (in E3). The reverse primers were: for R mRNA, primer p62 (in intron 4); for H mRNA, p64 (in E4/E5); for T mRNA, p66 (in E4/E6). The cDNA corresponding to the BuChE mRNA was amplified using as the forward primer p71, targeted to E2, and as the reverse primer p72, located in exon 4. The primer sequences were: p61, AACTTTGCCCGCACAGGGGA; p62, ACCTGGCGGGCTCCCACTC; p63, CCCCTGGACCCCTCTCGAAAC; p64, GGGAGCCTCCGAGGCGGT; p65, GGATCCCCCTGGACCCCTCT; p66, GCCTCGTCGAGCGTGTCGGT; p71, TGTCTTTGGTTTACCTCTGGAA; p72, CACTCCCATTCTGCTTCATC. The expected PCR products for the R, H and T AChE mRNAs and the BuChE mRNA have 243, 273, 280 and 297 bp, respectively.

PCRs were carried out in 50 µl buffered medium with 1.5 µl cDNA, AChE and BuChE primers (0.3 µM), dNTPs, dimethyl sulfoxide (5%) and Hotmaster Taq DNA polymerase (50 U/ml, Eppendorf). The reactions included an initial denaturing step of 2 min at 94 °C, followed by 40 cycles with 20 s at 94 °C, 20 s at 63 °C (for AChE) or 57 °C (for BuChE), and 40 s at 65 °C. PCR products were separated in 1.5% agarose gels and revealed with ethidium bromide. Their length was calculated with DNA size markers and the GelPro Analyzer program, version 3.1 (Media Cybernetics).

Determination of the relative content of ChE mRNAs. The possible variation between CG and HG in the content of AChE and/or BuChE mRNAs was tested using real-time fluorescent PCR. Since the TRIzol extract did not yield satisfactory results when used for real-time PCR, total RNA was isolated using an RNA extraction kit of Invitrogen, according to the manufacturer's instructions. RT was performed as indicated above.

Amplifications were carried out in 20 µl buffered medium with various volumes (0.05–2 µl) of cDNA, 0.3 µM AChE or BuChE primers and the PCR reaction kit (Takara) with SYBR Green I, dNTPs, and Taq DNA polymerase (50 U/ml). Reactions included an initial denaturing step of 30 s at 95 °C, followed by 40–50 cycles with 5 s at 95 °C, 10 s at 60 °C, and 15 s at 72 °C, and were performed in a LightCycler instrument (Roche). The β-actin cDNA product was used as the internal standard using AGAAAATCTGGCACCACACC and GGGGTGTTGAAGGTCTCAAA as forward and reverse primers. The relative content of AChE and BuChE cDNAs, with respect to β-actin cDNA, was determined with the LightCycler software. In addition, the specificity of the primers was assessed by analyzing the PCR products in agarose gels as above.

Statistics. The results are expressed as the mean ± SD. The statistical difference in ChE activity between normal and malignant gut samples was assessed by the Student's *t*-test. The results were analyzed by considering total (the full set of control and cancerous pieces) and paired samples (control and neoplastic samples of the same patient).

Results

AChE and BuChE activities in healthy and neoplastic gut. ChE activity in healthy and cancerous colon, sigmoid colon and rectum is given in Table 1. The lack of statistically significant differences between the three anatomical regions of the human bowels in AChE and BuChE activities allowed us to group them for comparing their mean values. The comparison showed that HG contains twice as much BuChE (4.16 ± 2.41 mU/mg) than AChE activity (2.17 ± 1.07 mU/mg), and that AChE activity decreases by 32% and BuChE activity by 58% in CG.

AChE components in healthy and cancerous colorectal pieces. Most of the AChE (85 ± 10%) and BuChE (96 ± 4%) activities in HG and CG were released using the two-step extraction protocol. Nearly one-third (27 ± 10%) of AChE activity was recovered in the S1 supernatant and the rest (58 ± 11%) in S2. The opposite applied to BuChE (68 ± 12% in S1 and 28 ± 10% in S2). This result indicates that most AChE molecules are tightly bound to membranes, whereas most BuChE molecules are not. No major differences between HG and CG in the extent of AChE or BuChE extraction were observed.

Centrifugation of the S1 supernatant in Brij 96-containing sucrose gradients revealed that HG contains abundant AChE molecules with sedimentation coefficients of 4.4 ± 0.3S and 3.2 ± 0.2S, and fewer of 16.2 ± 0.3S, 10.2 ± 0.3S, 9.2 ± 0.3S and 6.1 ± 0.3S (Fig. 1, upper panels). The 10.2S, 9.2S, 4.4S and 3.2S forms were also identified in S2 (Fig. 1, lower panels). The change in migration of the 9.2S, 4.4S and 3.2S AChE in gradients with Brij 96 to 10.4 ± 0.2S, 5.5 ± 0.2S and 4.0 ± 0.2S in gradients with Triton X-100 (profiles not shown) and the adsorption of AChE activity in phenyl-agarose (80% in a mixture of S1+S2) proved the amphiphilic behavior of

Table 1. Acetyl- (AChE) and butyrylcholinesterase (BuChE) activities in paired samples of healthy gut and colorectal carcinoma.

	Number of pairs	AChE activity				BuChE activity			
		mU/mg tissue	*p* value	mU/mg protein	*p* value	mU/mg tissue	*p* value	mU/mg protein	*p* value
Colon	18		0.018		0.012		0.001		< 0.001
Normal (a)		0.23 ± 0.18		1.75 ± 1.24		0.39 ± 0.28		3.65 ± 2.52	
Tumoral (b)		0.13 ± 0.10		1.09 ± 0.68		0.17 ± 0.11		1.34 ± 0.85	
Sigmoid colon	19		0.043		0.018		< 0.001		< 0.001
Normal (c)		0.30 ± 0.22		2.18 ± 1.15		0.65 ± 0.29		4.50 ± 2.95	
Tumoral (d)		0.16 ± 0.06		1.45 ± 1.19		0.25 ± 0.15		1.81 ± 0.98	
Rectum	18		0.049		< 0.001		0.012		< 0.001
Normal (e)		0.24 ± 0.11		2.30 ± 1.07		0.45 ± 0.25		4.74 ± 2.45	
Tumoral (f)		0.16 ± 0.10		1.46 ± 0.65		0.19 ± 0.11		1.86 ± 1.03	
All regions	55		< 0.001		< 0.001		< 0.001		< 0.001
Normal (a + c + e)		0.26 ± 0.17		2.17 ± 1.07		0.50 ± 0.29		4.16 ± 2.41	
Tumoral (b + d + f)		0.16 ± 0.08		1.40 ± 0.89		0.21 ± 0.13		1.65 ± 0.87	

AChE and BuChE activities are given as mean ± SD; *p* values were calculated using the Student's *t*-test. One milliunit (mU) of ChE activity represents one nmol of substrate (ATCh or BuTCh, respectively) hydrolyzed per minute. When BuChE activity is assayed with ATCh as the substrate, the activity is ~50% of that measured with BuTCh. Mean values of activity ratios in paired samples of tumoral/normal gut were 0.68 ± 0.31 for AChE and 0.42 ± 0.23 for BuChE.

the principal AChE molecules. According to previous data ([5] and references therein), the AChE variants were tentatively assigned to asymmetric AChE (A_{12}, 16.2S), hydrophilic and amphiphilic tetramers (G_4^H, 10.2S, and G_4^A, 9.2S), dimers (G_2^H, 6.1S, and G_2^A, 4.4S), and monomers (G_1^A, 3.2S).

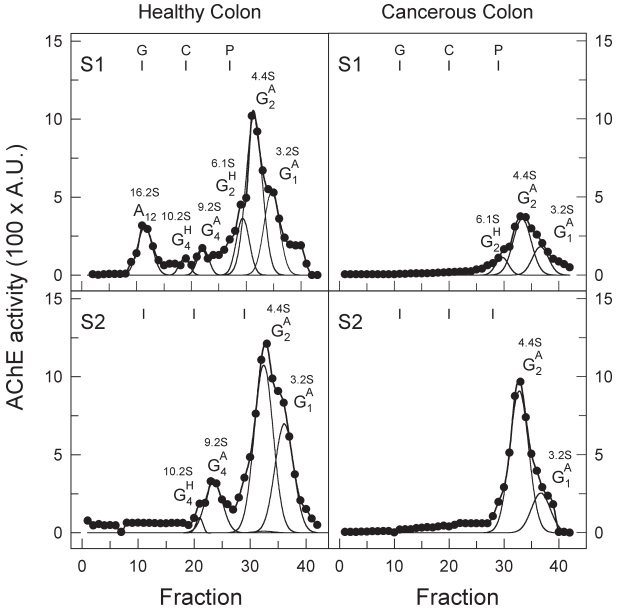

Figure 1. Representative sedimentation profiles with AChE forms in healthy and in cancerous colon. Enzyme components in the S1 supernatant, with soluble and weakly membrane-bound AChE, and the S2 extract, with tightly bound AChE, were resolved by centrifugation in 5–20% sucrose gradients containing Brij 96. AChE components were identified by their sedimentation coefficients. The internal sedimentation markers are: G, 16.0S; C, 11.4S; P, 6.1S. Note the abundance of G_2^A AChE forms in control and cancerous colon, and the loss of A_{12} and G_4 species in the latter. Similar sedimentation profiles were obtained when testing pieces of sigmoid colon or rectum.

The conversion of the 16.2S form into the 10.2S (lytic G_4^H) variant with collagenase (Fig. 2a) confirmed the asymmetric structure of the former and the tetrameric one of the latter. Moreover, the 70% conversion of the 4.4S into the 3.2S AChE species by reducing the disulfide bond, which tethers the subunits in dimers (Fig. 2b), proved their correct assignment to G_2^A and G_1^A AChE. Although the GPI-linked G_2^A AChE from bovine erythrocytes was fully converted into its hydrophilic variant after exposure to PIPLC alone (profile not shown), the digestion failed to do so with the isoforms of gut. Nevertheless, the conversion to an important extent of G_2^A AChE into G_2^H with alkaline hydroxylamine plus PIPLC (Fig. 2c) demonstrated that a fraction at least of AChE dimers (and monomers) in the human bowels bear GPI residues.

Taking the percentages of AChE activity in S1 and S2 extracts and the relative content of the enzyme forms in them, the mean proportions of AChE variants in healthy gut are: A_{12}, 3%; G_4^H, 3%; G_4^A, 21%; G_2^H, 4% and G_2^A+G_1^A, 69%. The percentage of G_2^A+G_1^A AChE rose to 82% in CG at the expense of other variants, especially of G_4^A AChE, which dropped to 5% (Fig. 1). The similar AChE activity in colon, sigmoid colon and rectum, the 32% decrease of AChE activity in CG and the mean proportion of each AChE form in HG and CG allowed us to conclude that the absolute content of all enzyme species drops in colorectal carcinoma.

Distribution of BuChE components in unaffected and neoplastic gut. Velocity sedimentation analysis of the S1 and S2 supernatants in Brij 96-containing sucrose gradients revealed that the human bowels contain abundant BuChE molecules of 11.9 ± 0.1S, 9.9 ± 0.2S and 4.5 ± 0.2S (Fig. 3). The 9.9S forms shifted to 10.5S in gradients with

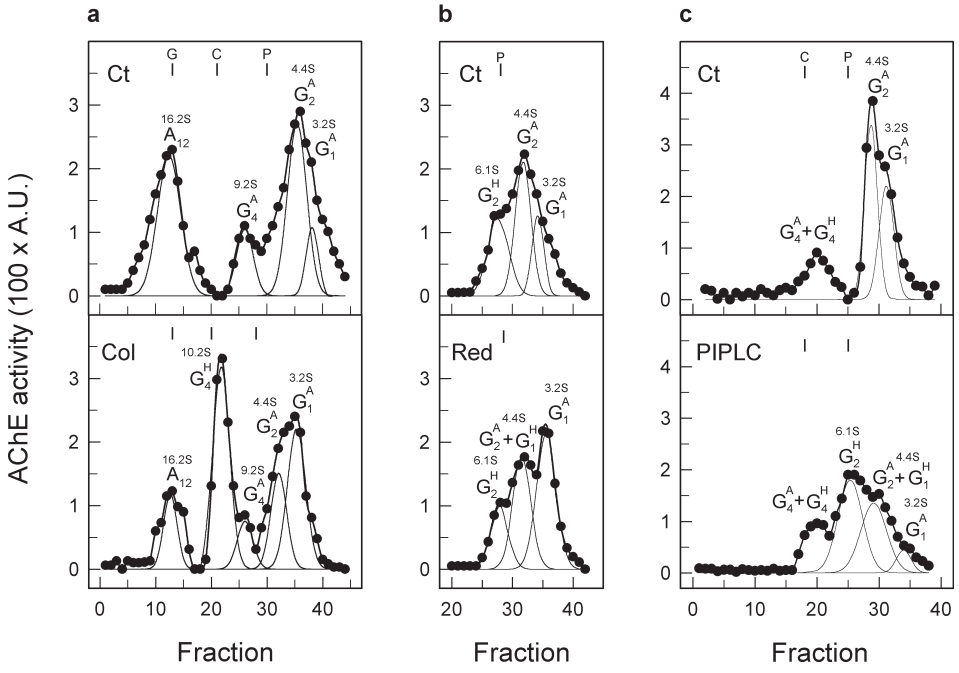

Figure 2. Asymmetric nature of the 16.2S AChE, dimeric structure of the 4.4S forms and presence of GPI in them. (*a*) Cleavage of the collagen-like tail in 16.2S AChE. The S1 supernatant of HG was incubated without (control, Ct) and with collagenase (Col), and then analyzed in sucrose gradients with Brij 96. The conversion of the 16.2S into the 10.2S species demonstrates the asymmetric nature of the former and the tetrameric state of the latter. (*b*) Dissociation of AChE subunits in dimers. After sedimentation analyses, the peak fractions rich in dimers were pooled, dialyzed, reduced and alkylated. AChE forms in non-reduced (Ct) and reduced samples (Red) were identified by sedimentation analysis. The conversion of the 4.4S into the 3.2S species confirmed the presence in gut of G_2^A and G_1^A AChE. (*c*) Removal of GPI residues in G_2^A and G_1^A with hydroxylamine plus PIPLC. The formation of 6.1S AChE (G_2^H) at the expense of the 4.4S forms (G_2^A), and the drop of the 3.2S species (G_1^A) indicate that the light AChE forms contain GPI residues.

Triton X-100 (profiles not shown), which demonstrated their amphiphilic properties. Thus, the 11.9S, 9.9S, and 4.5S BuChE forms were assigned to G_4^H, G_4^A and G_1^H molecules ([5] and papers referred therein), their mean proportions being 51%, 19% and 30% in S1; 35%, 45% and 20% in S2; and 48%, 22% and 30% in S1+S2. The fact that 80% of BuChE activity in a mixture of S1+S2 passed freely through phenyl-agarose (elution profile not given) confirmed the abundance of hydrophilic BuChE species in human gut. As seen for AChE, the percentages of BuChE forms were not statistically different between colon, sigmoid colon and rectum.

A comparison of the sedimentation profiles obtained with extracts of HG and CG revealed that the proportion of G_4^A BuChE dropped from 22% to 12%, that of G_4^H forms rose from 48% to 58% and that of G_1^H remained unmodified in colorectal carcinoma (Fig. 3, right panels). On the basis of the 58% reduction of BuChE activity in CG (Table 1) and the mean percentage of BuChE forms in HG and CG, we conclude that malignancy produces an important decrease in the absolute content of the whole set of BuChE forms in the human bowels.

Levels of AChE and BuChE mRNAs in HG and CG. Application of RT-PCR allowed us to identify the three principal AChE mRNAs (T, H and R) and the BuChE

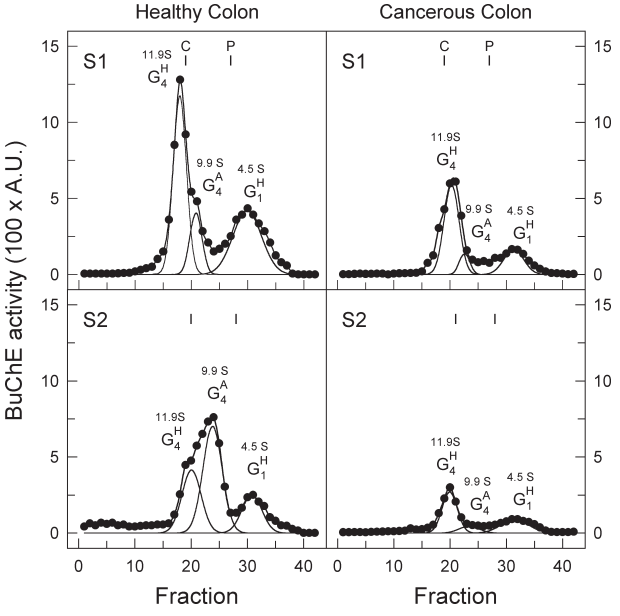

Figure 3. Sedimentation profiles with BuChE molecules in healthy and cancerous colon. BuChE forms in S1 and S2 extracts were resolved by centrifugation in Brij 96-containing sucrose gradients and identified by their sedimentation coefficients. While the hydrophilic tetramers (G_4^H) predominate in S1 of healthy colon, the amphiphilic variants (G_4^A) prevail in S2. Note the lowered content of BuChE species in cancerous colon.

transcript in HG (Fig. 4). Although our experimental approach only gives an approximate notion of the relative levels of the mRNAs, the results of real-time PCR indicated that the AChE-H transcript predominates in the human bowels, followed by the AChE-T and the AChE-R mRNAs (Fig. 4). Regarding the changes in the content of AChE mRNAs in cancerous gut, the levels of the AChE-H and AChE-R mRNAs were about 2.7-fold lower in CG than in HG, whereas the content of the AChE-T transcript decreased more than 10-fold. It was more difficult to estimate the content of the BuChE mRNA, due to its very minor level in human gut. It was roughly estimated that there are less than 10 copies of the BuChE mRNA per million of copies of β-actin mRNA in HG, and about 1000-fold less in CG.

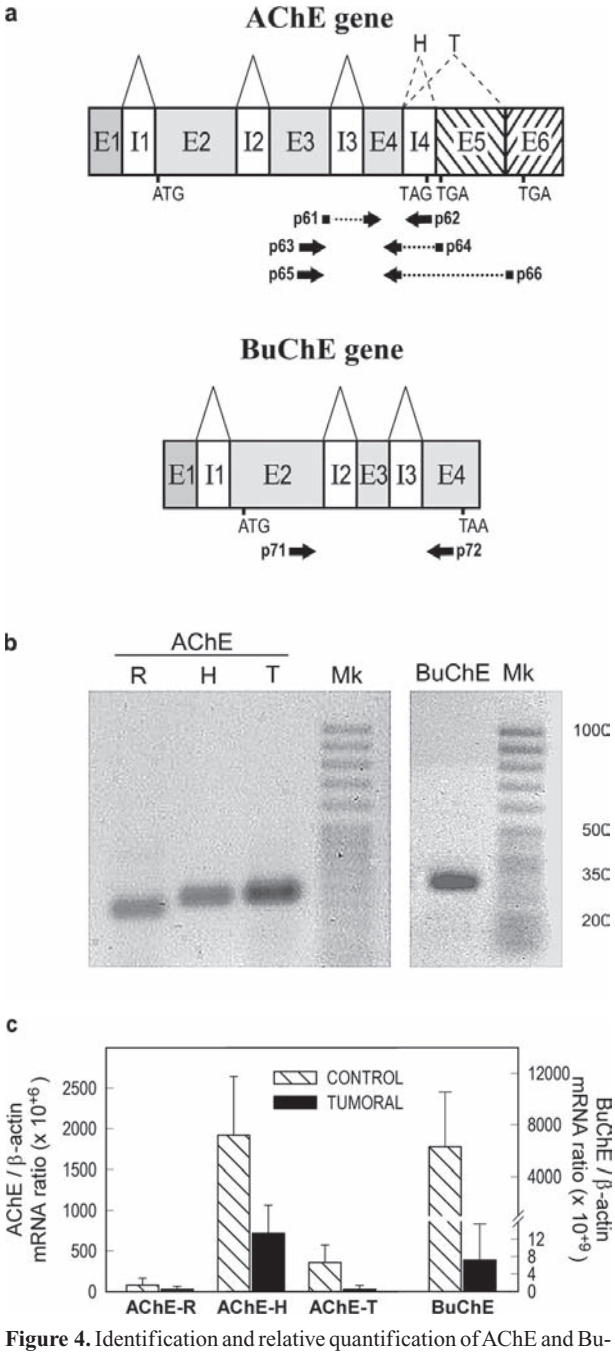

Figure 4. Identification and relative quantification of AChE and BuChE mRNAs in gut. (*a*) Scheme showing the position of the primers used for RT-PCR assays (gene regions are not drawn to scale). (*b*) Agarose gel of the RT-PCR products for AChE mRNAs (R, H and T) and the BuChE mRNA, with the expected sizes: 243, 273, 280 and 297 bp, respectively. (*c*) Histogram comparing the estimated relative levels of ChE mRNAs in HG and CG ($n = 4$ for AChE, $n = 6$ for BuChE). Please note the different scale used for AChE and BuChE mRNAs, and the discontinuity in the BuChE scale.

Discussion

Although it has long been known that the human bowels display AChE and BuChE activities [24], no information exists regarding the effects of cancer on them. The prevalence of BuChE over AChE activity in colorectal pieces, regardless of their anatomical localization (Table 1), contrasts with Sine's results. Moreover, the quantification of the alternatively spliced AChE mRNAs reveals for the first time that human gut possesses principally AChE-H mRNA (80% of total AChE mRNA) and less AChE-T mRNA (15%). The scant amount of the AChE-R mRNA (3%) in gut agrees with its very minor quantity (2%) in neural cell lines [25]. Our results concerning sedimentation analysis, phenyl-agarose chromatography (elution profiles not shown), the splitting of AChE dimers, and the treatments with collagenase and with PIPLC (Figs. 1, 2) demonstrate that human colon, sigmoid colon and rectum contain major GPI-linked G_2^A and G_1^A AChE forms (made of AChE-H subunits, and accounting for 69% of intestinal AChE activity), and minor G_2^H, G_4^A, G_4^H and A_{12} species (all made of AChE-T subunits). The prevalence of the GPI-anchored AChE species over the other variants agrees with the higher content of AChE-H than AChE-T mRNAs in colorectal pieces.

Albeit fluorescence 'in situ' hybridization (FISH) assays are needed for ascribing AChE mRNAs to particular intestinal cells, the observation of asymmetric AChE in smooth muscle [26], besides its absence from epithelial cells scraped from intestine [24] and from Caco-2 cells [27] support the muscular origin of the A_{12} AChE species identified in gut ([28], and this work). Moreover, the great increase of G_4^A AChE in Hirschsprung's disease, which arises from hypertrophy of submucosal nerve trunks [28] points to the neuronal origin of intestinal G_4^A AChE. The abundance of GPI-linked G_2^A and G_1^A AChE in human gut (Figs. 1, 2), breast and meningioma [5] suggests that they are the principal, if not the only, components produced by intestinal mucosa and other epithelial tissues.

The cytochemical observation of BuChE in human intestine [24], besides the presence of BuChE activity in Caco-2 cells and its increase by insulin [29] demonstrate the capacity of intestinal epithelial cells for producing BuChE. The very minor amount of the BuChE mRNA

in colorectal pieces compared with the total content of AChE mRNAs (Fig. 4) contrasts with the higher BuChE activity in gut. Although the data regarding the number of copies of the BuChE mRNA can be affected by some bias on the RT step, a lack of relationship between the content of ChE transcripts and the level of activity has also been observed in rat spinal cord neurons, which show a greater amount of AChE than BuChE transcripts, despite the prevalence of BuChE activity in them [30]. The scant amount of BuChE mRNA in gut does not necessarily mean that the same occurs with the mature protein, considering the different steps at which the protein content can be regulated [31].

As regards the molecular distribution of BuChE, the results of sedimentation analyses (Fig. 3) and phenyl-agarose chromatography (not shown) reveal that HG contains G_4^H (48%), G_4^A (22%), and G_1^H (30%) BuChE forms. The overwhelming amount of G_4^H BuChE in human blood plasma could lead to the belief that the gut isoforms come from it. However, the incomplete binding of intestinal G_4^H BuChE with the lectin LCA (authors' data) compared with the full binding of the plasma variants with it [32] shows that a part at least of the gut G_4^H BuChE arises from the tissue itself. Moreover, the lack of G_4^A and G_1^H BuChE in plasma demonstrates their intestinal origin. The abundance of G_4^A AChE and BuChE in brain [33] and their scant amount (when any) in epithelial tissues, such as meningioma and breast [5], support the neuronal origin of G_4^A BuChE in gut. The lower proportions of muscular A_{12} AChE and neuronal G_4^A AChE and G_4^A BuChE in colorectal carcinoma than in healthy colorectal pieces (Figs. 1–3) can reflect changes in the histological composition of samples or the damage caused by cancer in the gut layers. The degradation of the muscular basement membrane, where asymmetric AChE resides [9], by myofibroblasts infiltrating colon carcinoma [34] supports the last possibility.

The decrease in AChE and BuChE activities (Table 1) and the lower level of AChE and BuChE mRNAs in neoplastic gut indicate that both enzymes are down-regulated in colorectal carcinoma. The up-regulation of AChE is associated with differentiation of glial [35] and Caco-2 cells [27]. If the overexpression of AChE in Caco-2 cells is associated with differentiation, the under-expression may reflect the shifting of gut mucosal cells to a less differentiated state. The down-regulation of AChE and BuChE in colorectal carcinoma, the increase in AChE besides the drop in BuChE activity in breast cancer [18], the fall of AChE but not of BuChE activity in metastasized lymph nodes [20], and the variation of both activities in lung tumors depending on their histological features [15] indicate that the change in ChE activity depends on the kind of cell from which the tumor originates. This view agrees with the variable expression of AChE in proliferating cell systems, so that cell division is associated with up-regulation of AChE in mouse hematopoiesis (after transient blockade of its expression) [36], but with down-regulation in human hematopoiesis [37], megakaryocytopoiesis [38], and osteogenesis [8]. The variation of AChE and BuChE activities depending on the cellular origin of tumors and the mutually exclusive expression of AChE and BuChE in developing neural systems [10] highlight the differences between tumorigenesis and neurogenesis with regards to the expression of ChEs.

Although it remains to be established whether AChE can itself be tumorigenic and even whether the variation in ChE activity is cause or effect of neoplastic transformation, the observed down-expression of AChE and BuChE in colorectal carcinoma may collaborate with tumor development. Thus, the presence in many classes of cells of the proteins involved in ACh metabolism and signal transduction [39] suggests a role for the neurotransmitter in the control of cell division. An increase in the availability of ACh probably enhances cell proliferation, a process associated with the over-stimulation of cholinergic receptors [40]. The involvement of cholinergic responses in lung [41] and colon cancers [42, 43] supports a tumorigenic significance for the lowered AChE and BuChE activities observed in colorectal carcinoma.

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