European Journal of Neuroscience, Vol. 24, pp. 381–386, 2006 doi:10.1111/j.1460-9568.2006.04898.x

Astroglia up-regulate transcription and secretion of 'readthrough' acetylcholinesterase following oxidative stress

C. E. Bond,[1] P. Patel,[2] L. Crouch,[1] N. Tetlow,[2] T. Day,[1] S. Abu-Hayyeh,[2] C. Williamson[2] and S. A. Greenfield[1]
[1]University Department of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK
[2]Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK

Keywords: astrocyte, degeneration, hypoxia, neurotoxicity, rat, RNA

Abstract

Novel and diverse functions of glial cells are currently the focus of much attention [A. Volterra and J. Meldolesi (2005) *Nature Rev.* 6, 626–640]. Here we present evidence that rat astroglia release acetylcholinesterase (AChE) as part of their response to hypoxic damage. Exposure of astroglia to tert-butyl hydroperoxide, and hence oxidative stress, subsequently leads to a switching in mRNA from the classical membrane-bound T-AChE to a preferential increase in the splice variant for a soluble form, R-AChE, This change in expression is reflected in increased perinuclear and reduced cytoplasmic AChE staining of the insulted glial cells, with a concomitant and marked increase in extracellular secretion that peaks at 1 h post-treatment. An analogous increase in R-AChE, over a similar time scale, occurs in response to psychological stress [D. Kaufer *et al.* (1998) *Nature* 93, 373–377], as well as to head injury and stroke [E. Shohami *et al.* (1999) *J. Neurotrauma* 6, 365–76]. The data presented here suggest that glial cells may be key chemical intermediaries in such situations and, perhaps more generally in pathological conditions involving oxidative stress, such as neurodegeneration.

Introduction

It is now well established that acetylcholinesterase (AChE, EC 3.1.1.7) has multiple biological functions other than hydrolysing acetylcholine (Soreq & Seidman, 2001; Greenfield & Vaux, 2002). Studies showing the trophic effect of AChE have been performed in the postnatal developing brain (Keymer *et al*., 1999; Day & Greenfield, 2002), as well as in embryonic development (Whyte & Greenfield, 2003; Dori *et al*., 2005). As yet, little is known of the mechanisms underlying these trophic actions of AChE. However, answers to this question are of particular urgency in the light of the parallels between mechanisms of development and those of neurodegeneration, where released AChE could be a critical signalling molecule (Greenfield & Vaux, 2002; Toiber & Soreq, 2005). The role of glial cells in the degenerative process has been generally overlooked, despite increasing awareness of their active and diverse functions (Volterra & Meldolesi, 2005). This issue is particularly significant because reactive astrogliosis has been recognized as the distinguishing feature characterizing both acute and chronic central nervous system damage in most degenerative pathologies (Marchetti *et al*., 2005).

Alternative splicing of AChE yields multiple variants with different respective functions (Meshorer *et al*., 2004). The most likely isoform to be involved in any extracellular signalling by glia is the 'readthrough' form of AChE (R-AChE), as R-AChE remains monomeric and soluble, and therefore secretable, unlike T-AChE, which predominantly exists in tetrameric membrane-bound form (Grisaru *et al*., 1999). R-AChE, specifically, appears transiently during critical periods of neurogenesis (Coleman & Taylor, 1996), displays haemopoietic growth-promoting activities, and increases during psychological stress (Pick *et al*., 2004) and head injury (Shohami *et al*., 1999). We have therefore investigated whether cultures of rat brain astroglia could secrete R-AChE when exposed to the kind of insult that would be common in both head injury and neurodegeneration, i.e. oxidative stress.

Correspondence: Dr Cherie Bond, as above
Email: cherie.bond@pharm.ox.ac.uk

Received 10 November 2005, revised 18 April 2006, accepted 20 April 2006

Materials and methods

All reagents were purchased from Sigma-Aldrich Co. Ltd, Poole, UK, unless otherwise noted.

Preparation of astroglia

Astroglia were prepared as described previously (Whyte & Greenfield, 2003). Briefly, P1 rats were killed by anaesthetic overdose of isoflurane (Schedule 1, Animal Scientific Procedures Act, 1986). Then the cerebrum was removed, cut into $\sim$1-mm^3 pieces, and dissociated with gentle trituration in Dulbecco's modified Eagle medium (DMEM; Life Technologies Ltd, Paisley, UK) containing 10% fetal calf serum, 1% penicillin/streptomycin and 2.5 μg/mL amphotericin B, then incubated at 37 °C in a humidified atmosphere (95% air, 5% CO_2) for 7 days. Astroglia were then passaged, placed into clean 75-cm^2 flasks and allowed to reach confluency. After a second passage, glia were plated onto six-well plates or 13-mm coverslips in 12-well plates and allowed to recover for 3 days. Plates and coverslips were precoated with poly-D-lysine; coverslips were also precoated with collagen (Farenheit Laboratory Supplies, Ltd, Milton Keynes, UK).

Treatment with t-BHP

Astroglia were conditioned overnight in serum-free DMEM, then *tert*-butyl hydroperoxide (t-BHP) was added to the cells for 1 h. Cells were washed twice with 2 mL DMEM to remove residual t-BHP and placed back into serum-free media for various recovery periods.

Neu-N and GFAP immunostaining

Cultures were washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde, followed by methanol and 33% hydrogen peroxide 9 : 1, then treated with 0.4% sodium borohydride. Non-specific antibody binding was blocked with 3% normal goat serum, 0.3% Triton X-100 and 0.2% acetylated bovine serum albumin (BSA). Primary mouse monoclonal antibodies (Chemicon International, Ltd, Eastleigh, UK) were applied to astroglia (Neu-N 1 : 100, GFAP 1 : 100) and organotypic cultures [neuron-specific nuclear protein (Neu-N) 1 : 200, glial fibrillary acidic protein (GFAP) 1 : 300] in PBS containing 1% goat serum, 0.1% Triton X-100 and 0.2% BSA, and incubated at 4 °C for 18 h. After washing, cultures were incubated with biotinylated goat anti-mouse (Vector Laboratories Ltd, Peterborough, UK) 1 : 500 in PBS with 1% goat serum and 0.2% BSA for 2 h at 23 °C, then in ABC Elite (Vector Laboratories Ltd) as per the manufacturer's recommendations. Peroxidase was revealed using 0.025% diaminobenzidine and 0.03% hydrogen peroxide in Tris-HCl buffer, pH 7.6, for 8 min at 23 °C. The reaction was quenched with excess Tris-buffered saline, pH 7.6, before dehydrating and mounting on slides.

AChE histochemistry

Staining for AChE was performed as described (Hedreen *et al*., 1985). Briefly, cultures were fixed in 1.5% glutaraldehyde and 0.5% paraformaldehyde for 1 h at 23 °C. Cultures and sections were incubated in 0.3 M sodium acetate buffer, pH 6.0, containing 0.5 mg/mL acetylthiocholine iodide, 4 mM sodium citrate, 3 mM $CuSO_4.5H_2O$ and 0.1 mM $K_3Fe(CN)_6$ for 15 min (brain sections) or 25 min (astroglia cultures). After successive washes with 1% $(NH_4)_2S$ and 0.1 M $NaNO_3$, cultures were stained with 0.1% silver nitrate for 1 min, washed, dehydrated and mounted onto slides.

Measurement of cholinesterase activity

Media removed from the cells was centrifuged and 25-μL aliquots were taken from the supernatant for analysis. The method of Ellman *et al*. (1961) was used to determine the levels of cholinesterase. Absorbance was read at 405 nm over 20 min using a Molecular Devices plate reader (Alpha Laboratories Ltd, Hampshire, UK). Activity was also measured in the presence of 100 μM tetra-isopropyl pyrophosphoramide (Iso-OMPA) or 100 μM 1,5-bis(4-allydimethylammoniumphenyl)-pentan-3-one dibromide (BW284c51). For intracellular AChE activity measurements, cells were washed twice with PBS, then scraped from the plate into 1 mL PBS. Cell homogenates were assayed for AChE activity as above. AChE activity was calculated (V_{max} × enzyme efficiency factor × dilution factor = mU/mL) and statistical analyses were performed by use of Student's *t*-test.

LDH assay

Lactate dehydrogenase (LDH) activity was measured in media from control and treated astroglial cultures by the method of Allen *et al*. (1994) using an LDH diagnostic assay kit (Sigma). Briefly, samples for each treatment group were incubated with the kit A/B reagent mixture at 25 °C for 30 s, and the change in absorbance was then measured at 340 nm over a period of 3 min using a UV-160A spectrophotometer (Shimadzu Europe Ltd, Milton Keynes, UK). LDH activity (U/L) was calculated for each sample and statistical analysis was performed by using Student's *t*-test.

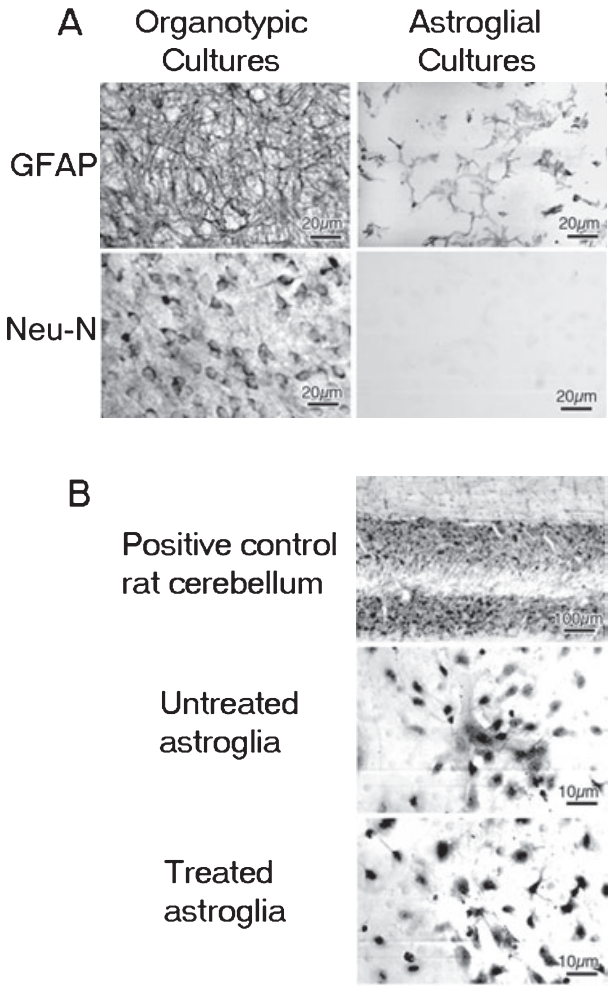


FIG. 1. Immunohistochemical analysis of cultures. (A) Organotypic (~300 μm thick) and monolayer astroglial cultures stained for glial fibrillary acidic protein (GFAP) and neuron-specific nuclear protein (Neu-N). (B) Acetylcholinesterase (AChE) staining in brain tissue and astroglial cultures. Positive control shows high level of AChE present in a 50-μm tissue slice through the granular layer of the anterior lobe of the cerebellum. Prominent perinuclear staining is visible in both treated and control astroglial cultures. Punctate AChE staining is visible in the cytoplasm of untreated astroglia, but not in those treated for 1 h with 0.5 mM t-BHP.

Total RNA Isolation, cDNA preparation and real-time PCR

Total RNA was isolated from astroglial cells using the Sigma GenElute™ Mammalian Total RNA kit, and Taqman® analysis performed as previously described (Patel *et al*., 2003). Gene-specific primers and probes for Taqman analysis of T-AChE and R-AChE mRNA were designed using the PrimerExpress software program (PE Applied Biosystems, UK). Primers and probe for the Tata binding protein (Tbp) have been previously described (Eriksson *et al*., 2000). All probes were labelled at the 5′ end with the reporter dye FAM

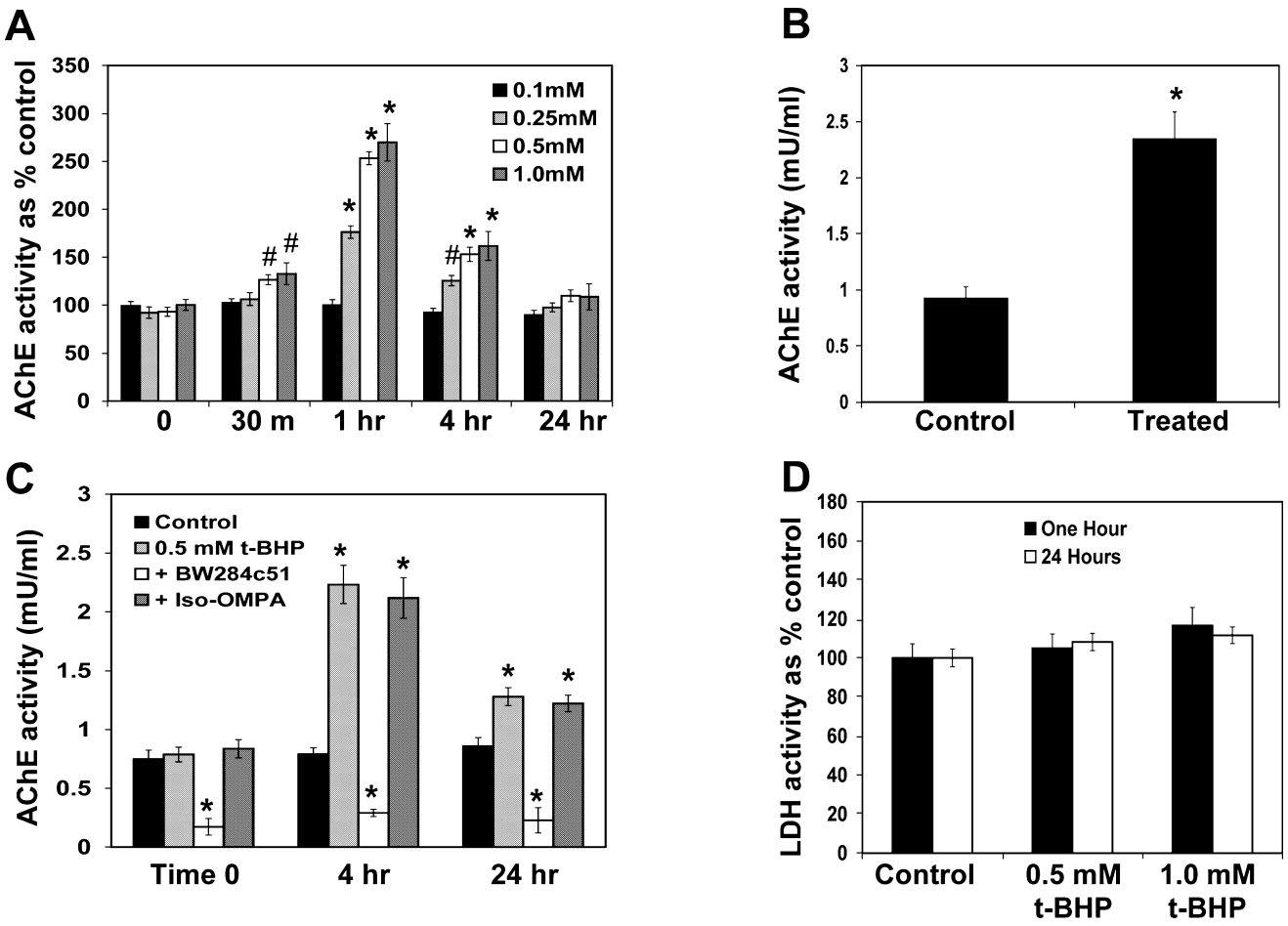


FIG. 2. AChE release from astroglia in response to oxidative stress. (A) Effect of treatment concentration and post-treatment incubation time on AChE release. Each experiment was performed on a pool of astroglia harvested from 20 to 27 P1 rat pups from two litters. The results shown represent the combined data from three independent experiments with $n = 3$, $n = 6$ and $n = 6$ astroglia, respectively. Values shown (mean ± SEM) were determined by comparison with untreated control glia from the same time point. Asterisks indicate values significantly different from controls (*$P < 0.001$; #$P < 0.01$). (B) Intracellular AChE activity in astroglia exposed to 0.5 mM t-BHP for 1 h, then allowed to recover for 1 h in serum-free medium. Results shown are from a single experiment ($n = 6$). The experiment was repeated twice. Results (mean ± SEM): control = 0.93 ± 0.099; treated = 2.35 ± 0.236 mU/mL cell lysate. (C) Effect of specific inhibitors on AChE activity. Glia were exposed to 0.5 mM t-BHP for 1 h and media were sampled for AChE activity in the absence and presence of 100 μM BW284c51 or 100 μM Iso-OMPA at time 0, 1 h and 4 h post-treatment. Data were compiled from two independent experiments, each with $n = 6$ glia. Asterisks indicate results significantly different from control values at the same time point ($P < 0.001$). (D) LDH assay results. Cell media were sampled for LDH activity after 1 h treatment with 0.5 or 1.0 mM t-BHP, followed by 1 h recovery (105 ± 7.3 and 116 ± 9.3% of control values, respectively) or 24 h recovery (108 ± 7.7 and 111 ± 7.4%) in serum-free medium ($n = 6$). There was no significant difference in LDH release into the medium from treated astroglia as compared with controls at either time point.

(6-carboxyfluorescein) and at the 3′ end with the quencher dye TAMRA (6-carboxytetramethylrhodamine). Single tube Taqman analysis was performed using 300 nM primers and 200 nM probes. Concentrations for each gene were obtained as described in *PE User Bulletin #2* (Perkin Elmer Applied Biosystems).

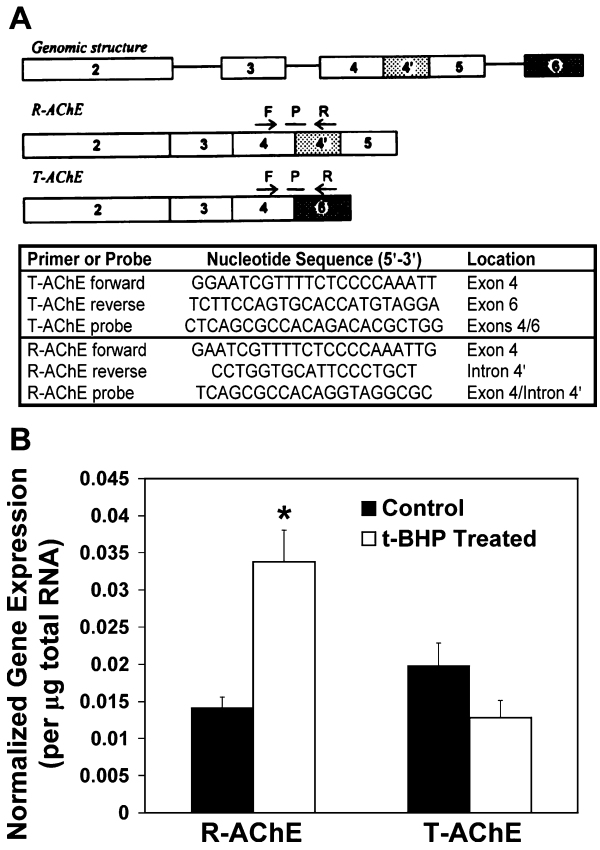
Results

AChE staining in pure astroglial cultures

To ensure that the astroglial cultures were uncontaminated by neuronal cell types, we stained for the presence of Neu-N and glia-specific GFAP. Astroglial cultures were negative for Neu-N but positive for GFAP, whereas control organotypic brain cultures were positive for both (Fig. 1A). AChE staining was readily discerned in brain slice preparations, as well as in astroglial cultures (Fig. 1B). Distinct perinuclear AChE staining was visible in control glial cultures and in those exposed to oxidative stress, whereas cytoplasmic AChE staining was detected only in control cells.

Astroglia secretion of AChE in response to t-BHP-induced oxidative stress

To ascertain the extent and time course of AChE release in response to oxidative stress, we exposed astroglia to various concentrations of t-BHP then measured subsequent release of AChE into the media over the course of 24 h. In preliminary experiments both t-BHP and hydrogen peroxide were tested as stress-inducing agents. Although both induced similar levels of AChE release, results were more variable with hydrogen peroxide, and thus t-BHP was chosen for further experimentation. Serum-free medium was used to eliminate exogenous AChE activity in the assay. For each time point, media were sampled from replicate six-well plates containing one well of each t-BHP treatment concentration, one well of no treatment control, and one well of media control. A distinct concentration-dependent AChE release was observed that reached a maximum at 1 h post-treatment (Fig. 2A). The induced release diminished by approximately 50% at 4 h and returned to control levels by 24 h post-treatment. No response was detected at the lowest concentration of t-BHP tested



Primer or Probe	Nucleotide Sequence (5'-3')	Location
T-AChE forward	GGAATCGTTTTCTCCCCAAATT	Exon 4
T-AChE reverse	TCTTCCAGTGCACCATGTAGGA	Exon 6
T-AChE probe	CTCAGCGCCACAGACACGCTGG	Exons 4/6
R-AChE forward	GAATCGTTTTCTCCCCAAATTG	Exon 4
R-AChE reverse	CCTGGTGCATTCCCTGCT	Intron 4'
R-AChE probe	TCAGCGCCACAGGTAGGCGC	Exon 4/Intron 4'

FIG. 3. Real-time PCR analysis of AChE isoform expression in control and t-BHP treated astroglia. (A) Schematic representation of the readthrough (R-AChE) and synaptic (T-AChE) alternative splicing products arising from the AChE gene. The forward (F) and reverse (R) primers and the probe (P) used for real-time RT-PCR studies are indicated for the R-AChE and T-AChE transcripts. The table lists sequence and location of primers and probes used. (B) R-AChE and T-AChE expression 1 h post-treatment in control glial cultures and those treated for 1 h with 0.5 mM t-BHP: 1 µg total RNA was reverse transcribed into cDNA; 200 ng cDNA was amplified by PCR with isoform-specific primers. Average R-AChE expression is increased 240% ($P < 0.001$, by both Mann–Whitney test and Student's t-test), while T-AChE expression is decreased by 65% in treated cells as compared with controls. The decrease in T-AChE was found not to be significant ($P = 0.054$). Results were obtained from ten experiments each performed in triplicate. Values were normalized to internal TATA-binding protein (Tbp) controls, which showed no variability between control and treated samples.

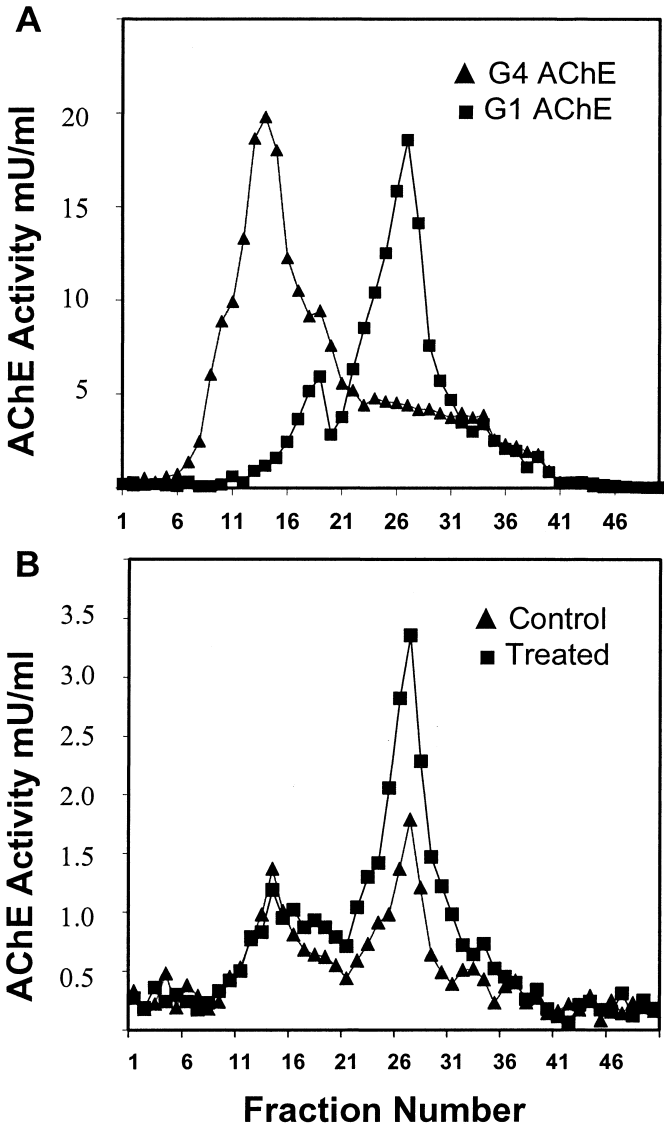


FIG. 4. Sucrose gradient profiles of AChE in cell culture medium. A 5–50% sucrose gradient was prepared in 50 mM Tris buffer containing 1 M NaCl and 0.2 mM EDTA, then loaded with 2 mL serum-free medium and centrifuged at 165 000*g* (35 000 r.p.m.), 4 °C for 18 h. Fractions of 250 µL were collected and analysed for AChE activity as described. (A) 200 mU/mL commercial eel tetrameric AChE (▲) and purified monomeric AChE (■) added to serum-free medium. (B) Medium collected from control astroglial cultures (▲) and glia exposed to 0.5 mM t-BHP for 1 h, then allowed to recover for 1 h (■).

(0.1 mM), presumably due to the glutathione oxidation mechanism present in astroglia, which has a high capacity to detoxify reactive oxygen species rapidly (Dringen *et al.*, 1998). Intracellular AChE activity, measured at 1 h post-treatment, was consistent with the observed increase in released AChE (Fig. 2B).

To confirm that this activity was exclusively attributable to AChE, rather than the alternative esterase butyrylcholinesterase (BuChE), the specific AChE inhibitor BW284c51 and the specific BuChE antagonist Iso-OMPA were also tested (Fig. 2C). The presence of BW284c51 abolished the observed AChE activity, whereas addition of Iso-OMPA had no significant effect. LDH release from cultures exposed to 0.5 or 1.0 mM t-BHP for 1 h was not significantly different from control cultures at 1 or 24 h post-treatment (Fig. 2D), but a trend towards increased cell death with increasing t-BHP concentration was observed.

Transcription of a soluble monomeric AChE isoform is up-regulated in response to oxidative stress

In order to determine if the release of AChE following oxidative stress was the result of an increase in the soluble R-AChE form, we examined mRNA splice variants from control astroglia, and those exposed to oxidative stress. Taqman technology was used to determine mRNA levels of the readthrough transcript (R-AChE) and the more common transcript (T-AChE) (Fig. 3). At the 1-h peak release response, there was a non-significant tendency to decrease the expression of T-AChE ($P = 0.54$) and a significant 240% increase ($P < 0.001$) in R-AChE expression in astroglial cells exposed to t-BHP as compared with control glial cells.

In addition, to confirm at the protein level that the soluble AChE released was in the monomeric form, we performed sucrose density gradient sedimentation analysis of media samples from control and t-BHP-treated astroglia (Fig. 4). Commercial eel AChE was used as a control marker for the tetrameric (G4) form of the enzyme; purified C-terminal truncated G1 AChE (gift from Palmer Taylor, Department of Pharmacology, UCSD) was used as a control marker for the monomeric form (Fig. 4A). Sucrose gradient profiles of media samples taken at 1 h post-treatment indicate that only secreted monomeric AChE increases in response to oxidative stress (Fig. 4B).

Discussion

The exclusivity or otherwise of R-AChE as a signalling molecule from astroglia during conditions simulating brain damage is currently an open question. Similarly, although consistent with the expanded role of astrocytes to include other more dynamic functions such as neural repair, we have yet to discover how widespread such a system would be, or whether only astroglia from specific brain regions would respond in this way. Certainly the mechanism described here would explain the observation, under ischaemic conditions, of enhanced AChE release from organotypic rat hippocampal slice cultures which contain glia as well as neurons (Sáez-Valero *et al*., 2003).

Following oxidative stress, the consequent release of AChE seen here could not be attributed to passive leakage from dying cells, as release of the soluble cytoplasmic enzyme LDH, an indicator of compromised plasma membrane integrity, remained at control levels after t-BHP treatment. Additionally, increased intracellular AChE activity was consistent with both increased activity of released AChE and with changes in R-AChE expression. These results support the conclusion that the stress-induced increase in AChE release is due to up-regulation of functional R-AChE isoform expression, although it is possible that this may be attributed to increased R-AChE mRNA transcript stability, as well as an increase in the rate of AChE transcription.

The surprisingly rapid change in astroglial AChE gene expression (two- to three-fold increase in R-AChE within 1 h post-stress) is supported by earlier *in vivo* work showing c-fos-promoted up-regulation of R-AChE mRNA within 30 min of acute stress or AChE inhibition (Kaufer *et al*., 1998). *In vivo* induced R-AChE up-regulation can persist for more than 3 days post-stress, but a gradual decline in measured AChE activity over the 24 h subsequent to t-BHP treatment was observed in glial cultures. This decrease in activity most probably reflects both degradation of the initial spike of released R-AChE in the media and down-regulation of the induced response as cells recover. It is also possible that in the absence of feedback signals from neurons in pure astroglial cultures, the response is not sustained to the same extent as *in vivo*.

In previous studies on AChE release, the observed response was attributed to neuronal origin, as astroglia normally exhibit comparatively very low levels of AChE activity. However, our results in astroglia alone matched those from *in vivo* studies, both temporally and in degree, provocatively suggesting that astroglia may be the predominant source of stimulus-induced R-AChE secretion. The sequence of events described here, in conditions similar to the early stages of neurodegeneration, might well be key in subsequent pathology, and would be consistent with a more general response by astrocytes to neuronal insult (Desagher *et al*., 1996).

Because stem cells transiently express monomeric R-AChE selectively when adopting the neuronal cell line (Coleman & Taylor, 1996), the phenomenon described here might be a critical intermediary in neurogenesis. However, aberrant activation of the system could lead to excessive levels of R-AChE, i.e. the monomeric form. Such a scenario does indeed characterize the Alzheimer brain, in which the level of monomeric AChE increases disproportionately to its tetrameric counterpart (Arendt *et al*., 1992; Toiber & Soreq, 2005), suggesting an inappropriate recapitulation of developmental mechanisms. The finely tuned astroglial expression and secretion of a specific isoform of AChE reported here could be a pivotal mechanism in any situation attempting to exploit, aberrantly or otherwise, neurogenesis in the adult brain.

Acknowledgements

Special thanks are due to P. Wendy Tynan for her technical assistance with histochemistry. This work was supported in part by a grant from Synaptica, Ltd.

Abbreviations

AChE, acetylcholinesterase; BSA, bovine serum albumin; BW284c51, 1,5-bis(4-allydimethylammoniumphenyl)-pentan-3-one dibromide; DMEM, Dulbecco's minimal essential medium; FAM, 6-carboxyfluorescein; GFAP, glial fibrillary acidic protein; Iso-OMPA, tetra-isopropyl pyrophosphoramide; LDH, lactate dehydrogenase; Neu-N, neuron-specific nuclear protein; PBS, phosphate-buffered saline; TAMRA: 6-carboxytetramethylrhodamine; t-BHP: *tert*-butyl hydroperoxide.

References

Allen, M., Millett, P., Dawes, E. & Rushton, N. (1994) Lactate dehydrogenase activity as a rapid and sensitive test for the quantification of cell numbers in vitro. *Clin. Mater.*, **16**, 189–194.

Arendt, T., Bruckner, M.K., Lange, M. & Bigl, V. (1992) Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development – a study of molecular forms. *Neurochem. Int.*, **21**, 381–396.

Coleman, B.A. & Taylor, P. (1996) Regulation of acetylcholinesterase expression during neuronal differentiation. *J. Biol. Chem.*, **271**, 4410–4416.

Day, T. & Greenfield, S.A. (2002) A non-cholinergic, trophic action of acetylcholinesterase on hippocampal neurons *in vitro*: molecular mechanisms. *Neuroscience*, **111**, 649–656.

Desagher, S., Glowinski, J. & Premont, J. (1996) Astrocytes protect neurons from hydrogen peroxide toxicity. *J. Neurosci.*, **16**, 2553–2562.

Dori, A., Cohen, J., Silverman, W.F., Pollack, Y. & Soreq, H. (2005) Functional manipulations of acetylcholinesterase splice variants highlight alternative splicing contributions to murine neocortical development. *Cerebral Cortex*, **15**, 419–430.

Dringen, R., Kussmaul, L. & Hamprecht, B. (1998) Rapid clearance of tertiary butyl hydroperoxide by cultured astroglial cells via oxidation of glutathione. *Glia*, **23**, 139–145.

Ellman, G.L., Courtney, K.D., Andres, V. & Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88–95.

Eriksson, M., Ansved, T., Edstrom, L., Wells, D.J., Watt, D.J., Anvret, M. & Carey, N. (2000) Independent regulation of the myotonic dystrophy locus genes postnatally and during adult skeletal muscle regeneration. *J. Biol. Chem.*, **275**, 19964–19969.

Greenfield, S. & Vaux, D.J. (2002) Parkinson's disease, Alzheimer's disease and motor neurone disease: identifying a common mechanism. *Neuroscience*, **113**, 485–492.

Grisaru, D., Sternfeld, M., Eldor, A., Glick, D. & Soreq, H. (1999) Structural roles of acetylcholinesterase variants in biology and pathology. *Eur. J. Biochem.*, **264**, 672–686.

Hedreen, J.C., Bacon, S.J. & Price, D.L. (1985) A modified histochemical technique to visualize acetylthiocholinesterase-containing axons. *J. Histochem. Cytochem.*, **35**, 134–140.

Kaufer, D., Friedman, A., Seidman, S. & Soreq, H. (1998) Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature*, **93**, 373–377.

Keymer, J.E., Gaete, J., Kameid, G. & Alvarez, J. (1999) Acetylcholinesterase and inhibitors: effects upon normal and regenerating nerves of the rat. *Eur. J. Neurosci.*, **11**, 1049–1057.

Marchetti, B., Kettenmann, H. & Streit, W.J. (2005) Glia–neuron crosstalk in neuroinflammation, neurodegeneration, and neuroprotection. *Brain Res. Rev.*, **48**, 129–132.

Meshorer, F., Toiber, D., Zurel, D., Sahly, I., Dori, A., Cagnano, E., Schreiber, L., Grisaru, D., Tronche, F. & Soreq, H. (2004) Combinatorial complexity of 5′-alternative AChE transcripts and protein products. *J. Biol. Chem.*, **279**, 29740–29751.

Patel, P., Weerasekara, N., Hitchins, M., Boyd, C.A.R., Johnston, D.G. & Williamson, C. (2003) Semi quantitative expression analysis of *MDR3*, *FIC1*, *BSEP*, *OATP-A*, *OATP-C*, *OATP-D*, *OATP-E* and *NTCP* gene transcripts in 1st and 3rd trimester human placenta. *Placenta*, **24**, 39–44.

Pick, M., Flores-Flores, C. & Soreq, H. (2004) From brain to blood: alternative splicing evidence for the cholinergic basis of mammalian stress responses. *Ann. NY Acad. Sci.*, **1018**, 85–98.

Sáez-Valero, J., Gonzalez-Garcia, C. & Cena, V. (2003) Acetylcholinesterase activation in organotypic rat hippocampal slice cultures deprived of oxygen and glucose. *Neurosci. Lett.*, **348**, 123–125.

Shohami, E., Gati, I., Beit-Yannai, E., Trembovler, V. & Kohen, R. (1999) Closed head injury in the rat induces whole body oxidative stress: overall reducing antioxidant profile. *J. Neurotrauma*, **6**, 365–376.

Soreq, H. & Seidman, S. (2001) Acetylcholinesterase – new roles for an old actor. *Nat. Rev. Neurosci.*, **2**, 294–302.

Toiber, D. & Soreq, H. (2005) Cellular stress reactions as putative cholinergic links in Alzheimer's disease. *Neurochem. Res.*, **30**, 909–919.

Volterra, A. & Meldolesi, J. (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nature Rev.*, **6**, 626–640.

Whyte, K.A. & Greenfield, S.A. (2003) Effects of acetylcholinesterase and butyrylcholinesterase on cell survival, neurite outgrowth, and voltage-dependent calcium currents of embryonic ventral mesencephalic neurons. *Exp. Neurol.*, **184**, 496–509.