the FEBS Journal

MINIREVIEW

Non-hydrolytic functions of acetylcholinesterase

The significance of C-terminal peptides

Susan A. Greenfield, Martina Zimmermann and Cherie E. Bond

The Institute for the Future of the Mind, Oxford University, UK

Keywords
Alzheimer's; degeneration; development; global neurons; motor neuron disease; nicotinic receptor; oxidative stress; Parkinson's; substrate inhibition; trophic-toxic axis

Correspondence
M. Zimmermann, Max-von-Laue Strasse 9, Biocenter N260, Johann Wolfgang Goethe University, 60438 Frankfurt am Main, Germany
E-mail: martina.zimmermann@pharm.ox.ac.uk

(Received 12 September 2007, accepted 12 December 2007)

doi:10.1111/j.1742-4658.2007.06235.x

This review explores the possibility that acetylcholinesterase may play a pivotal, non-hydrolytic role in neurodegeneration. More specifically, C-terminal sequences of acetylcholinesterase may act as signalling molecules in key brain regions characteristically vulnerable to Alzheimer's, Parkinson's and motor neuron disease.

The idea that acetylcholinesterase might have actions independent of the hydrolysis of its familiar substrate acetylcholine is far from new: the evidence subsequently supporting this suggestion is comprehensively reviewed elsewhere in this minireview series and, thus, need not be reiterated here. Nonetheless, two particular features of a non-enzymatic role need noting. First, acetylcholinesterase is not only present in neurons using transmitters such as dopamine, noradrenaline and serotonin, but, second, is actually secreted in a soluble form from these cells [1,2]. What might be its function, therefore, as an intercellular messenger in its own right?

Interestingly enough, the groups of aminergic neurons characterized by the storage and release of acetylcholinesterase cluster together in a continuous hub extending the length of the brainstem – motor neurons, locus coeruleus, raphe nuclei and substantia nigra/ventral tegmental area up to the basal forebrain. Despite the heterogeneity in transmitters, these different nuclei all have the common feature of sending diffuse projections to the outer reaches of the brain. The neurobiologist Nancy Woolf classed these particular groups as 'global' neurons to distinguish them from the more familiar localized circuitry of the neurons in cerebellum, thalamus, cortex, etc., i.e. 'serial' cells [3]. Moreover, global and serial neurons differ in some fundamental ways, for example, their embryonic provenance, basal and alar plates. However, the difference that is perhaps most relevant to this minireview is that global neurons selectively retain a robust plasticity into and throughout adulthood, accompanied by a specific sensitivity to trophic factors. Could the distinguishing developmental feature of these neurons be linked to

Abbreviations
R-AChE, readthrough form of acetylcholinesterase; T-AChE, tailed form of acetylcholinesterase; α7-nAChR, nicotinic acetylcholine receptor alpha-7 subunit.

their other distinguishing feature of secreting 'non-hydrolytic' acetylcholinesterase?

Exogenous application of acetylcholinesterase does, indeed, have a non-hydrolytic action in enhancing neurite outgrowth, by inducing an influx of calcium [4–7]. However, at higher doses, or with longer exposure, sustained calcium entry can be toxic to neurons [8–10]. Notably, a further determining factor in whether calcium entry triggers trophic or toxic effects, is age; as neurons mature, an erstwhile trophic level of intracellular calcium becomes lethal [11]. It is possible that, within global neurons, acetylcholinesterase has a dual non-hydrolytic action that ranges along a trophic–toxic axis, depending on the amount, duration of availability and age.

It may be no coincidence that the global neuron populations are the very nuclei linked to primary vulnerability in the neurodegenerative diseases: Alzheimer's disease (basal forebrain, raphe nuclei, locus coeruleus); Parkinson's disease (substantia nigra, raphe nuclei, locus coeruleus); motor neuron disease/ALS (motor neurons) [12,13]. One possibility is that these neurons specifically will embark on the remorseless cycle of neurodegeneration, precisely because of their persistent developmental mechanism. If serial neurons are damaged in adulthood, other neurons will compensate functionally. By contrast, global neurons will respond to stroke/oxidative stress/mechanical injury by calling on their trophic resources in an attempt to regenerate: but as the subsequent calcium influx is lethal in the mature cells, the resulting damage will trigger further attempts to compensate in a pernicious cycle that arguably characterizes neurodegeneration. Neurodegenerative diseases may, therefore, be viewed as aberrant activation of developmental mechanisms, with the key trophic agent responsible as 'non-hydrolytic' acetylcholinesterase [14].

In order to understand the precise molecular events underlying such a scenario, and, hence, prompt novel forms of treatment for neurodegeneration, the next step clearly is to identify that part of the acetylcholinesterase molecule responsible for this trophic–toxic action. Towards the C-terminus of the tailed form of acetylcholinesterase (T-AChE), two peptides of, respectively, 14 and 30 amino acids (T14 and T30) have clear cleavage points, and bear a strong homology to an equivalent part of the amyloid precursor protein (Fig. 1A) [14]. When synthetic T14 and T30 are applied to a variety of preparations, they exhibit a clear similarity to the trophic–toxic effects already seen for non-hydrolytic acetylcholinesterase, by opening specifically and selectively the L-type calcium channel [4,5,15,16]. However, the L-channel is voltage-gated, and the effect of the peptides, and acetylcholinesterase itself, must be indirect, via a receptor that, in turn, triggers sustained and significant depolarization.

Arguably the most powerful calcium ionophore in the brain is the nicotinic alpha 7 acetylcholine receptor (α7-nAChR) [17]. This receptor would also be an attractive candidate target for the acetylcholinesterase peptides, because it is co-expressed along with acetylcholinesterase in precisely the same highly transient period in various brain regions during development [18]. Moreover, α7-nAChR can bind amyloid [19–24] and has already been implicated in neurodegenerative diseases [22,25–27].

Indirect evidence using a range of diverse nAChR blockers has suggested that T14 binds selectively to an allosteric site specifically on α7-nAChR in oocytes, brain slices and cell cultures, modulating calcium influx underlying short-term plasticity, and chronic, long-term trophic and toxic effects (Fig. 1B,C). These actions were sensitive to blockade of α7-nAChR, in the nanomolar range [28], prior to non-specific effects in the micromolar range and upwards, when non-physiological effects are observed due to fibril formation [29,30].

More recently, we obtained direct evidence (C. E. Bond, M. Zimmerman & S. A. Greenfield, unpublished results) that the target for the acetylcholinesterase-peptides is an allosteric site on α7-nAChR. In a cell line (GH4) stably expressing the receptor, we have shown high-affinity displacement of alpha-bungarotoxin by both peptides (Fig. 1D). Moreover, RT-PCR and western blot analysis reveal that GH4 cells treated for 24 h with T14/T30 increase α7-nAChR mRNA expression and protein levels at the plasma membrane. Could this highly novel signalling mechanism also operate in non-neuronal systems [31], where acetylcholinesterase might also have non-hydrolytic actions? We studied two possible instances: breast cancer cell lines [32] and glial cells [33].

In breast cancer cell lines, we found that T14, but not its scrambled analogue, had a selective action in the strongly metastatic cell line MDA-MB-231. This action was selectively blocked by the α7-nAChR antagonist methylycaconitine, but not the α4-nAChR blocker, dihydro-β-ethroidine (Fig. 2A). It may well be that the mechanism for cell division applicable to neurogenesis might also be extended to tumorigenesis [32].

In cultures of glial cells, oxidative stress of the type thought to occur as the final common path in neurodegeneration, increases the influx of calcium through L-type calcium channels [16] which, in turn, leads to enhanced acetylcholinesterase secretion (Fig. 2B): because we also observed a switching in mRNA from

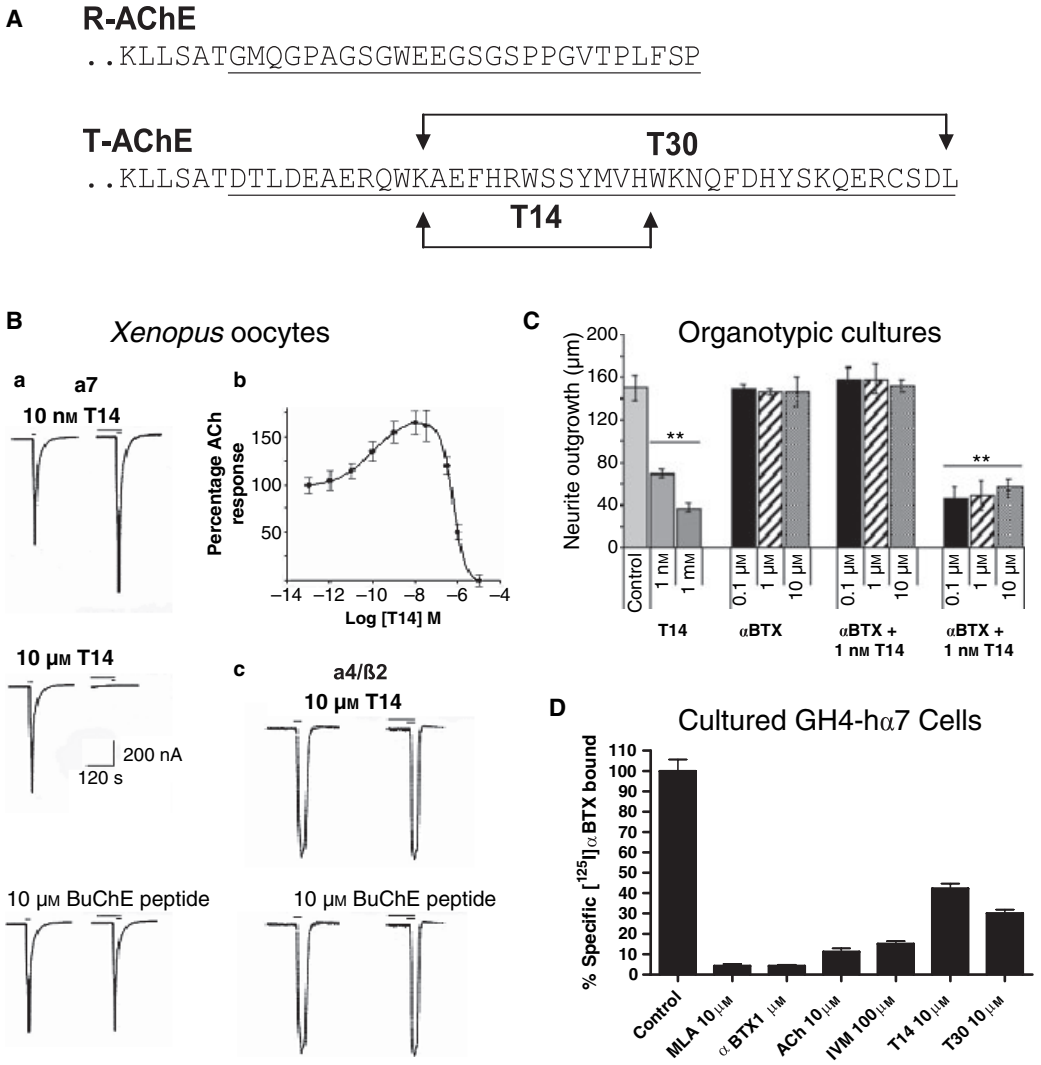

Fig. 1. Effects of T-AChE C-terminal peptides on α7-nAChR. (A) Comparison of C-terminal amino acid sequences of R- and T-AChE isoforms. Unique isoform sequences are underlined; arrows indicate the sequence and location of T14 and T30 peptides. (B) (a) Current response of human α7-nAChR expressing *Xenopus* oocytes to 100 µM acetylcholine before and during co-application of peptides. Upper, 10 nM T14; middle, 10 µM T14; lower, 10 µM butyrylcholinesterase 14-amino acid peptide. (b) Effects of T14 on EC_{50} acetylcholine-induced current responses in human α7-nAChR-expressing oocytes were plotted as a percentage of the response of acetylcholine alone (mean ± SEM, 10 oocytes). Data were fitted as described previously [28]. (c) Current responses of human α4β2-nAChR-expressing oocytes to 30 µM acetylcholine before and during co-application of 10 µM T14 (upper) or 10 µM butyrylcholinesterase peptide (lower). Figure modified from Greenfield *et al.* [28]. (C) Quantification of effects of α7-nAChR antagonism on *in vitro* T14-induced toxicity in rat hippocampal organotypic cultures. Cultures were maintained in serum-free medium in the presence of indicated concentrations of T14 and alpha-bungarotoxin for 14 days and then processed for microtubule-associated protein 2 immunochemistry. Neurite outgrowth was measured by selecting cells in a non-biased manner and using camera Lucida drawings. Experiments were repeated a minimum of three times with separate culture groups; $n = 131–134$; $^{**}P < 0.01$. Figure modified from Greenfield *et al.* [28]. (D) Comparison of acetylcholinesterase C-terminal peptides T14 and T30 with known α7-nAChR ligands at concentrations indicated in live cell binding to GH_4 cells stably expressing the α7-nAChR; $n = 6$; MLA, methylylcaconitine; α-BTX, alpha-bungarotoxin; ACh, acetylcholine; IVM, ivermectin (C. E. Bond, M. Zimmerman & S. A. Greenfield, unpublished data).

the classical membrane-bound 'T-AChE' to a preferential increase in the splice variant for the soluble readthrough form of acetylcholinesterase (R-AChE; Fig. 2C) [33], it seems reasonable to conclude that R-AChE is released in response to stress, in a fashion comparable with the stress-induced release reported for neurons [34,35].

However, it is important to note here that R-AChE is an alternatively spliced form of acetylcholinesterase that omits exon 6, and does not contain either T14 or

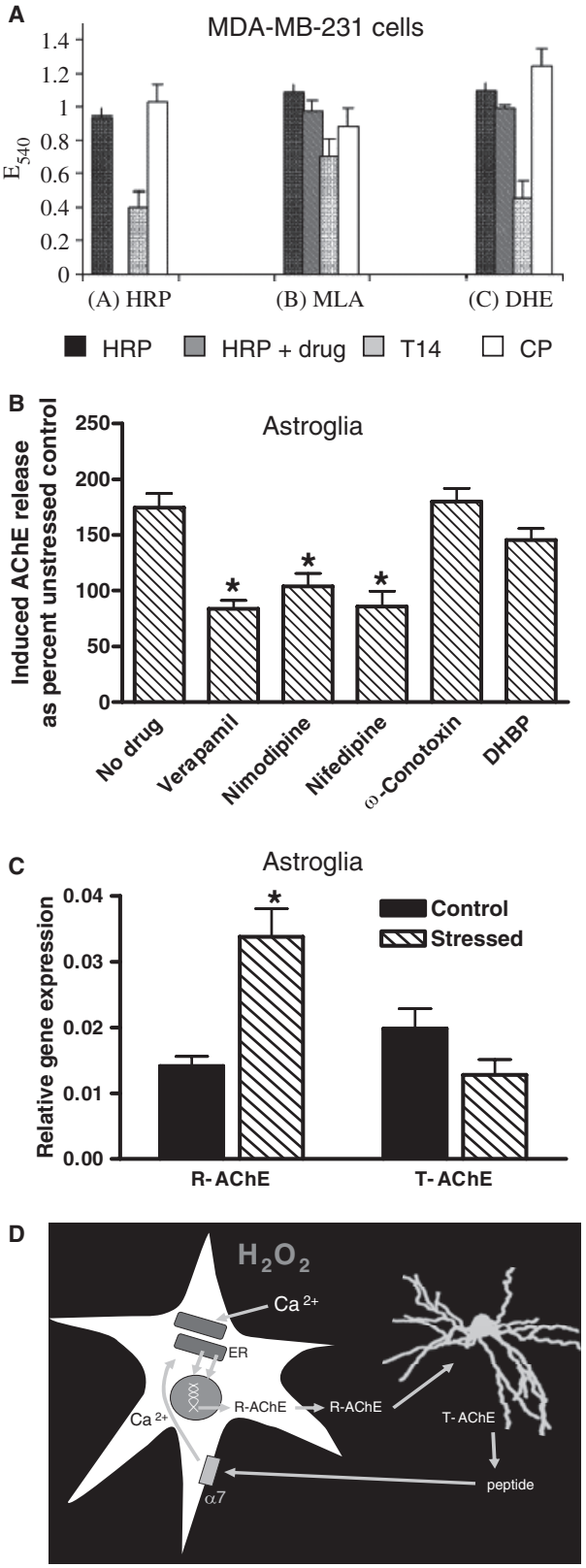

Fig. 2. Potential signalling mechanism involving T-AChE C-terminal peptides. (A) Effects of two cholinergic antagonists, methyllycaconitine and dihydro-β-erythroidine, 100 nM each, on horseradish peroxidase uptake with endogenous peroxidase activity subtracted (denoted by E_{540}). CP, control/scrambled-peptide. The effect of each drug was determined by co-incubation during horseradish peroxidase uptake. Figure modified from Onganer *et al.* [32]. (B) Effect of calcium channel blockers on oxidative stress-induced acetylcholinesterase release. Astroglia were exposed to 0.5 mM *tert*-butyl hydroperoxide for 1 h in the presence and absence of verapamil (10 μM), nimodipine (10 μM), nifedipine (10 μM), ω-conotoxin MVIIC (100 μM) and 1,1′-diheptyl-4,4′-bipyridinium dibromide (10 μM). Cells were recovered for 1 h, and the medium was sampled and assayed for acetylcholinesterase activity. Asterisks indicate values significantly different from controls ($P < 0.005$; $n = 6$). Figure modified from Bond and Greenfield [16]. (C) Quantitative RT-PCR analysis of acetylcholinesterase isoform expression 1 h post-treatment in control and *tert*-butyl hydroperoxide-treated (0.5 mM, 1 h) astroglia. Average R-AChE expression increased 240% ($P < 0.001$), whereas T-AChE expression decreased by 35% ($P = 0.054$) in treated cells compared with controls. Results were obtained from 10 experiments each performed in triplicate. Values were normalized to internal TATA-binding protein controls, which showed no variability between control and treated samples. Figure modified from Bond *et al.* [33]. (D) Schematic depicting the proposed short-circuit positive-feedback mechanism between astroglia and neurons involving different acetylcholinesterase isoforms.

T30 within its C-terminus (Fig. 1A). However, preliminary data from our laboratory suggest that glial cells will express α7-nAChR in response to the same oxidative stress that triggers expression and release of R-AChE. Indeed, increased α7-nAChR protein expression in glia in Alzheimer's disease has already been reported [36]. What would be the point of co-expression of a receptor with the variant of an agent that lacked the ability to bind to it?

One possibility is that such a scenario would be effectively a short circuit, and that the stress-induced switching to R-AChE allows communication with other types of cells. It has been acknowledged for several years that astroglia induce neurogenesis from adult neural stem cells [37], yet the signalling molecule has not been identified. However, Coleman and Taylor [38] reported earlier that only when stem cells are adopting the neural cell line, do they transiently express acetylcholinesterase. It is tempting to suggest that oxidative stress has a preferential effect, first, on glial cells, which are known to be more responsive than neurons to changing conditions in the local environment [39]. Such conditions trigger influx of calcium through voltage-gated L-channels which, in turn, leads to a switching to expression and release of R-AChE and concomitant expression of α7-nAChR in readiness for the indirect effect of R-AChE acting on

other cell types. The cell type in question may well be stem cells, which convert to neurons once modulated by the released R-AChE. The new neurons are then able to express their own, standard (T) form of acetylcholinesterase containing T14 and T30 which, under appropriate conditions, would be cleaved to feedback on the original glial cells, via the stress-induced expression of α7-nAChR. As a consequence, calcium would enter the glial cell and the cycle would start again (Fig. 2D).

In this way, a relatively short duration of oxidative stress could be amplified into a sustained process for neurogenesis. Such a system could be valuable in, say, the hippocampus, where adult neurogenesis has been reported as a basis for cognitive prowess [40,41]. However, within the global neuron population the generation of still higher levels of acetylcholinesterase-peptides may shift trophic levels of calcium into the toxic range, with resultant neurodegeneration.

Although both T14 and T30 clearly have intriguing actions and possible interactions, in cancer cells, glia and neurons, the vital question remains as to whether either or both peptides are cleaved from the acetylcholinesterase molecule in true physiological or pathological conditions.

Saxena *et al.* [42] suggested that, indeed, in the fetus, T-AChE is cleaved to yield a truncated form that lacks both peptides. Interestingly, this truncated acetylcholinesterase (T548-acetylcholinesterase) might also predominate in Alzheimer's disease where, as in the fetal brain [43], there is loss of substrate inhibition [44]. As well as indicating a further possible link between neurodegeneration and development, the existence of the truncated T548-acetylcholinesterase

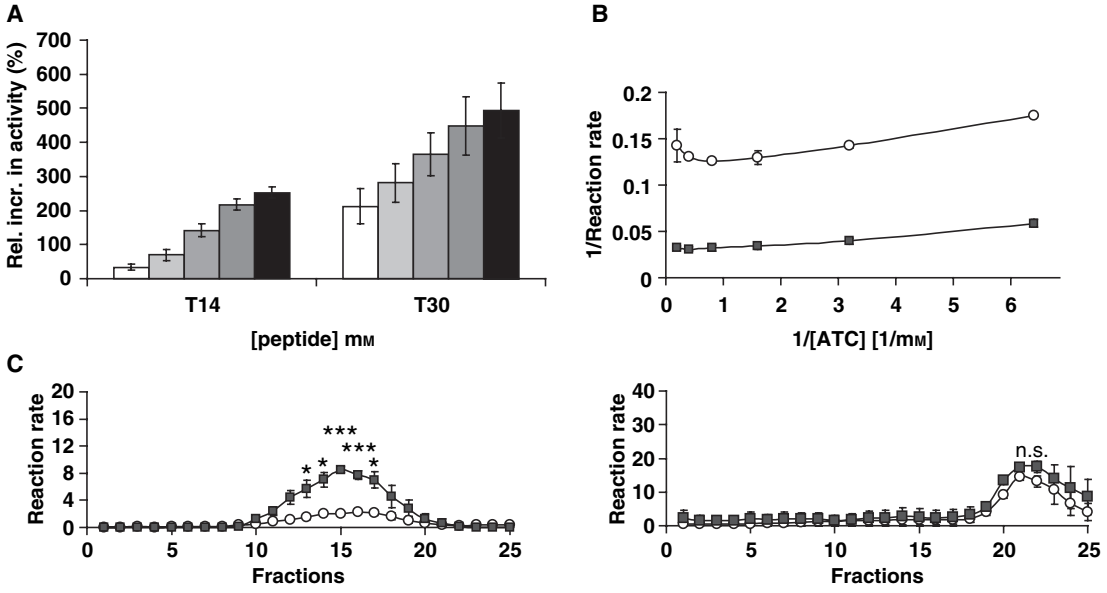

Fig. 3. Effects of T14 and T30 on T-AChE. (A) Dose–response curves for the T14 and T30 peptides enhancing T548-acetylcholinesterase activity. T548-acetylcholinesterase activity enhancement is displayed as the relative increase in activity with the activity of non-boosted T548-acetylcholinesterase, therefore, being equal to zero. The final peptide concentrations (mM) are as follows: 0.014, 0.028, 0.055, 0.111, 0.222 and represented by bar fillings progressing through white, light, medium and dark grey to black, respectively; $n = 3$. (B) Substrate inhibition delay for Triton X-100 enhanced T548-acetylcholinesterase (filled rectangle) versus buffer-incubated T548-acetylcholinesterase (empty circle). Lineweaver–Burk plot for the reciprocal of the rate of reaction (1/reaction rate) versus the reciprocal of substrate (acetylthiocholine) concentration (1/[ATC]). By observation, substrate inhibition is seen only at higher substrate concentrations (2.5 mM acetylthiocholine compared with 1.25 mM) when the activity of T548-acetylcholinesterase is enhanced with Triton X-100. Acetylthiocholine was used in concentrations ranging from 0.3125 to 20 mM. Figure modified from Zimmermann *et al.* [45]. (C) (Left) Enhancement of sucrose-density gradient separated T548-acetylcholinesterase: T548-acetylcholinesterase is clearly monomeric with this fact being represented by one single peak of acetylcholinesterase activity. The activity displayed corresponds to the absolute activity measured for T548-acetylcholinesterase alone (empty circle) and for Triton X-100-boosted T548-acetylcholinesterase (filled rectangle). The activity of detergent-boosted T548-acetylcholinesterase is significantly higher than the activity of not-enhanced T548-acetylcholinesterase. (Right) Enhancement of sucrose density gradient separated full-length T-AChE: The activity of full-length T-AChE is not significantly enhanced for any of its oligomers. The activity displayed corresponds to the absolute activity measured for full-length T-AChE alone (empty circle) and for Triton X-100-boosted full-length T-AChE (filled rectangle). Error bars reflect standard error, $n = 3$. Statistical analysis was performed using one-way ANOVA comparison of means (*$P < 0.05$; ***$P < 0.005$). Figure modified from Zimmermann *et al.* [45].

form has prompted investigation of whether its particular properties could be exploited as an eventual tool for detecting free acetylcholinesterase-peptides. Might incubation of acetylcholinesterase-peptides with exogenous T548-acetylcholinesterase result in an interaction that may, in turn, modify the activity of the enzyme?

Zimmermann *et al*. [45] have been able to answer in the affirmative. We have shown that, due to a high net positive charge, incubation of T548-acetylcholinesterase with both T14 and T30 results in a dose-dependent enhancement of catalytic activity by up to 600%, with T30 the more potent compound (Fig. 3A). In addition, incubation of T548-acetylcholinesterase with activity-enhancing molecules leads to a delay of substrate inhibition (Fig. 3B) that is most likely indicative of involvement of the peripheral anionic site, which is unobstructed only in the monomer [46], and which is readily receptive to specific positively charged peptides. Importantly, all T548-acetylcholinesterase molecular mass species are significantly enhanced in their activity, whereas the activity of full-length species is not markedly changed upon incubation (Fig. 3C).

As yet, however, despite circumstantial evidence and promising tools, a definitive and direct demonstration of free peptides T14 or T30 in brain tissue, under either physiological or pathological conditions, remains an urgent goal. If, however, the processes described here do take place in the human brain, then they might offer a highly novel, yet, attractive approach to neurodegeneration. If detection of peptide(s) could serve as a surrogate marker, then the course of an individual's aetiology could be monitored in a bespoke fashion and treated accordingly: if detection of early stages of the disease were possible even presymptomatically, then early medication might slow the course of deterioration or, at least, give the patient and carer the maximal time to prepare for what lies ahead.

Moreover, if the allosteric site of α7-nAChR is, indeed, a good target for modulating calcium entry, selective blockade might shift the trophic–toxic axis back in the desired direction. Such medication could, therefore, break the pernicious cycle of neuronal self-destruction. Best of all, however, would be to combine these two prospects. If it were possible to detect neurodegeneration before onset of symptoms, and then administer a treatment that arrested further cell death, the symptoms would never appear – an effective 'cure'. Such a prospect remains, of course, purely speculative; but the more we can characterize non-hydrolytic functions of acetylcholinesterase and understand their significance, the more likely it may be that the dream could become a reality.

Acknowledgements

MZ and CEB are James Martin fellows and The Institute for the Future of the Mind is part of the James Martin 21st Century School at Oxford University, UK.

References

1 Dally JJ & Greenfield SA (1994) The release of acetylcholinesterase *in vivo* is regulated by dopaminergic systems in the guinea-pig substantia nigra. *Neurochem Int* **25**, 339–344.
2 Greenfield SA & Shaw SG (1982) Release of acetylcholinesterase and aminopeptidase *in vivo* following infusion of amphetamine into the substantia nigra. *Neuroscience* **7**, 2883–2893.
3 Woolf NJ (1996) Global and serial neurons form a hierarchically arranged interface proposed to underlie memory and cognition. *Neuroscience* **74**, 625–651.
4 Day T & Greenfield SA (2002) A non-cholinergic, trophic action of acetylcholinesterase on hippocampal neurones *in vitro*: molecular mechanisms. *Neuroscience* **111**, 649–656.
5 Day T & Greenfield SA (2003) A peptide derived from acetylcholinesterase induces neuronal cell death: characterisation of possible mechanisms. *Exp Brain Res* **153**, 334–342.
6 Taylor SJ, Bartlett MJ & Greenfield SA (1988) Release of acetylcholinesterase within the guinea-pig substantia nigra: effects of 5-hydroxytryptamine and amphetamine. *Neuropharmacology* **27**, 507–514.
7 Webb CP, Nedergaard S, Giles K & Greenfield SA (1996) Involvement of the NMDA receptor in a non-cholinergic action of acetylcholinesterase in guinea-pig substantia nigra pars compacta neurons. *Eur J Neurosci* **8**, 837–841.
8 Brorson JR, Manzolillo PA & Miller RJ (1994) Ca^{2+} entry via AMPA/KA receptors and excitotoxicity in cultured cerebellar Purkinje cells. *J Neurosci* **14**, 187–197.
9 Ferreira IL, Duarte CB & Carvalho AP (1996) Ca^{2+} influx through glutamate receptor-associated channels in retina cells correlates with neuronal cell death. *Eur J Pharmacol* **302**, 153–162.
10 Van Reempts J & Borgers M (1985) Ischemic brain injury and cell calcium: morphologic and therapeutic aspects. *Ann Emerg Med* **14**, 736–742.
11 Eimerl S & Schramm M (1994) The quantity of calcium that appears to induce neuronal death. *J Neurochem* **62**, 1223–1226.
12 Schmitt HP (2005) Neuro-modulation, aminergic neurodisinhibition and neuro-degeneration. Draft of a comprehensive theory for Alzheimer disease. *Med Hyp* **65**, 1106–1119.

13 Rossor MN (1981) Parkinson's disease and Alzheimer's disease as disorders of the isodendritic core. *Br Med J (Clin Res Ed)* **283**, 1588–1590.
14 Greenfield S & Vaux DJ (2002) Parkinson's disease, Alzheimer's disease and motor neurone disease: identifying a common mechanism. *Neuroscience* **113**, 485–492.
15 Bon CL & Greenfield SA (2003) Bioactivity of a peptide derived from acetylcholinesterase: electrophysiological characterization in guinea-pig hippocampus. *Eur J Neurosci* **17**, 1991–1995.
16 Bond CE & Greenfield SA (2007) Multiple cascade effects of oxidative stress on astroglia. *Glia* **55**, 1348–1361.
17 Patrick J, Sequela P, Vernino S, Amador M, Luetje C & Dani JA (1993) Functional diversity of neuronal nicotinic acetylcholine receptors. *Prog Brain Res* **98**, 113–120.
18 Broide RS, Robertson RT & Leslie FM (1996) Regulation of alpha 7 nicotinic acetylcholine receptors in the developing rat somatosensory cortex by thalamocortical afferents. *J Neurosci* **16**, 2956–2971.
19 Dineley KT (2007) Beta-amyloid peptide–nicotinic acetylcholine receptor interaction: the two faces of health and disease. *Front Biosci* **12**, 5030–5038.
20 Dineley KT, Bell KA, Bui D & Sweatt JD (2002) Beta-amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in *Xenopus oocytes*. *J Biol Chem* **277**, 25056–25061.
21 Lain E, Penke B, Delacourte A, Gundisch D, Schroder H & Witter B (2005) Effects of Abeta1–42 fibrils and of the tetrapeptide Pr-IIGL on the phosphorylation state of the tau-protein and on the alpha7 nicotinic acetylcholine receptor *in vitro*. *Eur J Neurosci* **21**, 879–888.
22 Gotti C, Moretti M, Bohr I, Ziabreva I, Vailati S, Longhi R, Riganti L, Gaimarri A, McKeith IG, Perry RH *et al.* (2006) Selective nicotinic acetylcholine receptor subunit deficits identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies by immunoprecipitation. *Neurobiol Dis* **23**, 481–489.
23 Wang HY, Lee DH, Davis CB & Shank RP (2000) Amyloid peptide Abeta(1–42) binds selectively and with picomolar affinity to alpha7 nicotinic acetylcholine receptors. *J Neurochem* **75**, 1155–1161.
24 Fodero LR, Mok SS, Losic D, Martin LL, Aguilar MI, Barrow CJ, Livett BG & Small DH (2004) Alpha7-nicotinic acetylcholine receptors mediate an Abeta(1–42)-induced increase in the level of acetylcholinesterase in primary cortical neurones. *J Neurochem* **88**, 1186–1193.
25 Dineley KT, Westerman M, Bui D, Bell K, Ashe KH & Sweatt JD (2001) Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: *in vitro* and *in vivo* mechanisms related to Alzheimer's disease. *J Neurosci* **21**, 4125–4133.
26 Duda JE (2004) Pathology and neurotransmitter abnormalities of dementia with Lewy bodies. *Dement Geriatr Cogn Disord* **17** (Suppl. 1), 3–14.
27 Nagele RG, D'Andrea MR, Anderson WJ & Wang HY (2002) Intracellular accumulation of beta-amyloid(1–42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease. *Neuroscience* **110**, 199–211.
28 Greenfield SA, Day T, Mann EO & Bermudez I (2004) A novel peptide modulates alpha7 nicotinic receptor responses: implications for a possible trophic–toxic mechanism within the brain. *J Neurochem* **90**, 325–331.
29 Cottingham MG, Hollinshead MS & Vaux DJ (2002) Amyloid fibril formation by a synthetic peptide from a region of human acetylcholinesterase that is homologous to the Alzheimer's amyloid-beta peptide. *Biochemistry* **41**, 13539–13547.
30 Cottingham MG, Voskuil JL & Vaux DJ (2003) The intact human acetylcholinesterase C-terminal oligomerization domain is alpha-helical *in situ* and in isolation, but a shorter fragment forms beta-sheet-rich amyloid fibrils and protofibrillar oligomers. *Biochemistry* **42**, 10863–10873.
31 Small DH, Michaelson S & Sberna G (1996) Non-classical actions of cholinesterases: role in cellular differentiation, tumorigenesis and Alzheimer's disease. *Neurochem Int* **28**, 453–483.
32 Onganer PU, Djamgoz MB, Whyte K & Greenfield SA (2006) An acetylcholinesterase-derived peptide inhibits endocytic membrane activity in a human metastatic breast cancer cell line. *Biochim Biophys Acta* **1760**, 415–420.
33 Bond CE, Patel P, Crouch L, Tetlow N, Day T, Abu-Hayyeh S, Williamson C & Greenfield SA (2006) Astroglia up-regulate transcription and secretion of 'readthrough' acetylcholinesterase following oxidative stress. *Eur J Neurosci* **24**, 381–386.
34 Deutsch VR, Pick M, Perry C, Grisaru D, Hemo Y, Golan-Hadari D, Grant A, Eldor A & Soreq H (2002) The stress-associated acetylcholinesterase variant AChE-R is expressed in human CD34(+) hematopoietic progenitors and its C-terminal peptide ARP promotes their proliferation. *Exp Hematol* **30**, 1153–1161.
35 Nijholt I, Farchi N, Kye M, Sklan EH, Shoham S, Verbeure B, Owen D, Hochner B, Spiess J, Soreq H *et al.* (2004) Stress-induced alternative splicing of acetylcholinesterase results in enhanced fear memory and long-term potentiation. *Mol Psychiatry* **9**, 174–183.
36 Teaktong T, Graham A, Court J, Perry R, Jaros E, Johnson M, Hall R & Perry E (2003) Alzheimer's disease is associated with a selective increase in alpha7 nicotinic acetylcholine receptor immunoreactivity in astrocytes. *Glia* **41**, 207–211.

37 Song H, Stevens CF & Gage FH (2002) Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417**, 39–44.
38 Coleman BA & Taylor P (1996) Regulation of acetylcholinesterase expression during neuronal differentiation. *J Biol Chem* **271**, 4410–4416.
39 Marchetti B, Kettenmann H & Streit WJ (2005) Glia–neuron crosstalk in neuroinflammation, neurodegeneration and neuroprotection – introduction. *Brain Res Rev* **48**, 129–132.
40 Kempermann G & Gage FH (1999) Experience-dependent regulation of adult hippocampal neurogenesis: effects of long-term stimulation and stimulus withdrawal. *Hippocampus* **9**, 321–332.
41 Kempermann G, Gast D & Gage FH (2002) Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol* **52**, 135–143.
42 Saxena A, Hur RS, Luo C & Doctor BP (2003) Natural monomeric form of fetal bovine serum acetylcholinesterase lacks the C-terminal tetramerization domain. *Biochemistry* **42**, 15292–15299.
43 Moreno RD, Campos FO, Dajas F & Inestrosa NC (1998) Developmental regulation of mouse brain monomeric acetylcholinesterase. *Int J Dev Neurosci* **16**, 123–134.
44 Arendt T, Bruckner MK, Lange M & Bigl V (1992) Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development–a study of molecular forms. *Neurochem In* **21**, 381–396.
45 Zimmermann M, Groesgen S, Westwell MS & Greenfield SA (2007) Selective enhancement of the activity of C-terminally truncated, but not intact, acetylcholinesterase. *J Neurochem* **104**, 221–232.
46 Bourne Y, Taylor P, Bougis PE & Marchot P (1999) Crystal structure of mouse acetylcholinesterase, a peripheral site-occluding loop in a tetrameric assembly. *J Biol Che* **274**, 2963–2970.