Discovering and targeting the basic mechanism of neurodegeneration: The role of peptides from the C-terminus of acetylcholinesterase Non-hydrolytic effects of ache: The actions of peptides derived from the C-terminal and their relevance to neurodegeneration

Susan Greenfield *

University Dept. of Pharmacology, Oxford OX1 3QT, United Kingdom

ARTICLE INFO

ABSTRACT

Acetylcholinesterase (AChE) is now well-established widely as a signalling molecule with non-hydrolytic functions including trophic activity in a diverse variety of situations in both neural and non-neural tissues. We have focussed on the observation that AChE, operating as a trophic agent independent of its enzymatic action, does indeed trigger calcium entry into neurons. It is possible that AChE has a dual non-classical action that ranges along a trophic-toxic axis, depending on amount, duration of availability and, most significantly, age. The neurodegenerative diseases could therefore be viewed as aberrant activation of developmental mechanisms with 'non-cholinergic' AChE as a, perhaps the, pivotal molecule. We have identified two peptides that could be cleaved from the C-terminus of T-AChE, one (T14), within the other (T30), and which have a strong sequence homology to the comparable region of beta-amyloid whilst the inert residue within the T30 sequence ('T15') acts as a control, and is without effect. We have subsequently been able to ascribe the trophic-toxic actions of the both T14 and T30 peptides to the modulation of calcium influx via an allosteric site on the alpha-7 nicotinic acetylcholine receptor (α7-nAChR). If the scenario described here is indeed the primary mechanism of neurodegeneration, then interception of the actions of the 'non-cholinergic' AChE-peptides T14 and T30 at the α7-nAChR, would be a promising novel therapy for arresting and stabilising cell loss in Alzheimer's disease, whereas detection of the peptides ideally in the blood, could provide a sensitive surrogate marker. If the marker was sensitive enough to be detected pre-symptomatically in a routine blood test, then the medication for arresting further cell loss could be initiated at that time, and the symptoms would never appear. This dual approach of identifying the marker and then intercepting its further action, could thus amount to an effective treatment for Alzheimer's and other neurodegenerative diseases.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

There has been no new drug introduced to combat Alzheimer's disease specifically, nor neurodegeneration more generally, over the last 15 years. The reason is that as yet, there is no accepted nor proven basic mechanism that could consequently be targeted pharmaceutically. The 'cholinergic hypothesis' posits that the key issue is a deficit of acetylcholine, due to death of cholinergic neurons. However this theory fails to account for a well know discrepancy: not all areas of the brain affected by Alzheimer's are cholinergic, nor are all cholinergic areas of the brain affected in the disease. It is perhaps not surprising therefore that the current treatment of choice, the AChE inhibitor Aricept, does not prevent the continuing death of cells, since it merely tackles a symptom, i.e. the dwindling availability of a transmitter.

The other main contender for accounting for the process of neurodegeneration is the 'amyloid hypothesis', where neuronal death is attributed to disruption of the cell membrane by toxic deposits of amyloid, a characteristic feature of the post-mortem Alzheimer brain, and resulting from abnormal cleavage of amyloid precursor protein. However, the 'amyloid hypothesis' does not explain the neuronal selectivity for death seen in neurodegenerative diseases nor the absence of amyloid deposits in animal models of dementia, nor indeed the occurrence of amyloid in certain brain regions e.g. cerebellum, where cognitive deficits are not apparent. Moreover there is a lack of correlation between amyloid plaques, neurodegeneration and cognitive impairment [17].

Where is our approach different? First, our starting point is based not on discrepancies in post-mortem brains, but on a

* Tel.: +44 01865 271 628; fax: +44 01865 271 853.
E-mail address: susan.greenfield@pharm.ox.ac.uk

hypothesis for the basic mechanism of neurodegeneration [12,14]. We shall suggest a strategy for neurodegenerative diseases in general, although examples may well be drawn from Alzheimer's Disease specifically since it has been so intensively studied. Secondly, our approach will explain previously puzzling facts, i.e. neuronal selectivity where only certain brain cells seem prone to neurodegeneration, and the frequent co-pathology of Alzheimer's with Parkinson's disease. Thirdly, by identifying the potential pivotal mechanism of neurodegeneration, a twin-pronged strategy will be possible: the identification of a biomarker in blood that could be used to detect disease before symptoms present coupled with the development of an oral medication for stopping any further cell loss. Each goal on its own, if successful, would be a great advance: but in combination, this two stage approach would amount to an effective 'cure'.

2. A new approach

One clue for identifying the basic mechanism of neurodegeneration, could be that only certain neuronal groups are primarily vulnerable. Moreover, the different cell groups prone to degeneration in Alzheimer's, Parkinson's and Motor Neurone Diseases nonetheless form a continuous 'hub' extending from brainstem to forebrain that send diffuse projections upwards and outwards to higher cerebral centres. Hence, despite their heterogeneity in transmitters, these neuronal groups have been collectively dubbed 'Global' neurons to distinguish them from the more familiar and localised circuits of cells in most other parts of the brain, such as the cerebellum, thalamus, cortex etc. (Fig. 1). These selectively vulnerable Global neurons have a critical feature in common that might explain the puzzling and as yet unanswered question as to why only these cells succumb to progressive death whilst their counterparts elsewhere in the brain, even when damaged by stroke, do not: they have retained a developmental mechanism for regrowth. Interestingly enough these same groups of cells had been previously identified by Michael Rossor some thirty years ago when he suggested that neurodegenerative diseases could be viewed as dysfunctions in the 'isodendritic core' [18].

Global neurons differ in some very fundamental and telling ways, for example their embryonic provenance – the basal rather than the alar plate. However, perhaps the difference of most relevance here, is that Global neurons selectively retain a robust plasticity into and throughout adulthood, accompanied by a specific sensitivity to substances aiding and sustaining growth – 'trophic factors'. In the developing brain, trophic factors work by stimulating calcium influx, which triggers a cascade of events within the cell, eventually resulting in selective differentiation and growth. However, in higher doses or with longer exposures, sustained calcium entry can be toxic to neurons [8]. Most significantly, a further determining factor in whether or not calcium entry triggers trophic or toxic effects, is age: as neurons mature, an erstwhile trophic level of intracellular calcium becomes lethal [9].

We are therefore proposing that the neurodegenerative process is in fact an aberrantly activated process of development: in support of this hypothesis, a hyper-trophy of the brainstem 'hub' neurons has actually been reported in Alzheimer brains [5]. If large areas of this hub are damaged, then more than one neurodegenerative disease will present, as occurs in the frequently seen but never as yet explained cases of co-pathology with Alzheimer's and Parkinson's diseases. Could there be a common developmental mechanism restricted specifically to all Global neurons irrespective of the different transmitters they use? If so, and if indeed it accounted for the vulnerability of these cells to neurodegeneration, then identification and characterisation of such a mechanism would be the first step towards a novel approach to combating neurodegenerative diseases.

Interestingly, all the neurons within the vulnerable hub, despite transmitter heterogeneity, all contain the familiar enzyme acetylcholinesterase (AChE). AChE is therefore present in neurons where it would be unable to perform its normal function, since such cells as the noradrenergic locus coeruleus, the dopaminergic substantia nigra, or the serotonergic raphe nuclei, in no cases contain the usual substrate, acetylcholine. A further unexpected deviation from its normal, enzymatic role is that the AChE is actually released from Global neurons, presumably as some kind of inter-cellular messenger in its own right. Our group have been among the first to pioneer the concept, and provide substantial evidence, that AChE does indeed have novel, non-classical actions including a trophic one of enhancement of neurite outgrowth.

In general, AChE is now widely and well-established as a signalling molecule that has trophic activity in a diverse variety of situations in both neural and non-neural tissue. One particular contribution of our group has been to show that AChE, operating as a trophic agent independent of its enzymatic action, does indeed trigger calcium entry into neurons. It is possible therefore that within Global neurons, AChE has a dual non-classical action that ranges along a trophic-toxic axis, depending on amount, duration of availability and, most significantly, age. If standard neurons are damaged in adulthood, as in a stroke, others will compensate functionally. In contrast, Global neurons will respond by calling on their trophic resources in an attempt to regenerate. But because the subsequent calcium influx will be lethal in the older, mature cells, the resulting damage will trigger further attempts to compensate in a pernicious cycle that characterises neurodegeneration. The neurodegenerative diseases could therefore be viewed as aberrant activation of developmental mechanisms with 'non-cholinergic' AChE as a, perhaps the, pivotal molecule (Fig. 2).

3. A bioactive peptide

The next crucial issue has been to identify the salient part of the AChE molecule, responsible for these non-cholinergic actions, i.e. the trophic-toxic effects. We have identified two peptides that could be cleaved from the C-terminus of T-AChE, one (T14), within the other (T30), and which have a strong sequence homology to the comparable region of beta-amyloid (Fig. 3).
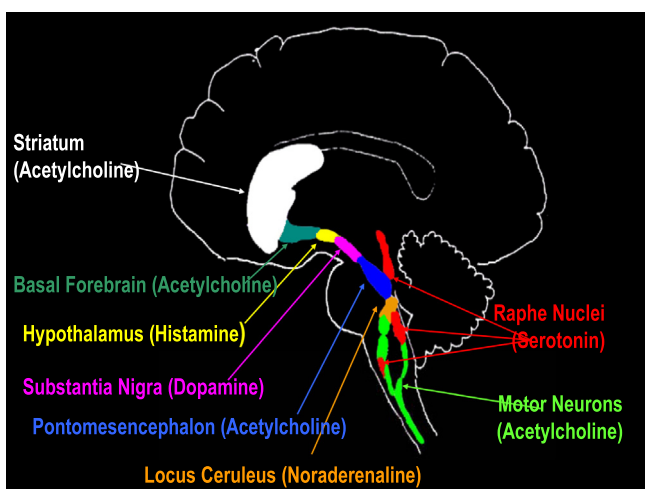
Fig. 1. The 'hub' of neurons primarily affected in neurodegenerative disorders. Whilst the basal forebrain is the primary site of lesion in Alzheimer's, the substantia nigra in Parkinson's, and motor neurons in Motor Neuron Disease, other areas such as the raphe nuclei, the locus coeruleus, and the histamine-containing areas can also be affected in any of these disorders.
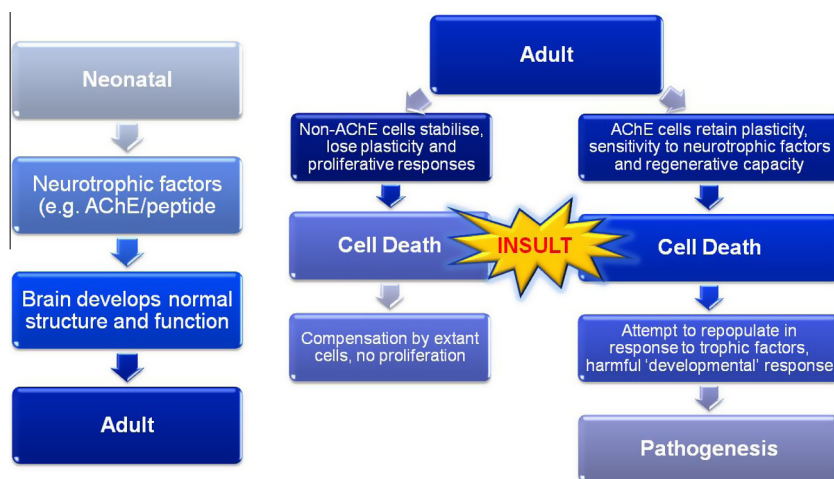
Fig. 2. A Novel Model: Neurodegeneration is an aberrant form of development where by key neurons retain mechanisms of plasticity which in turn make them selectively vulnerable, when activated inappropriately in the context of the mature brain (see [12]).
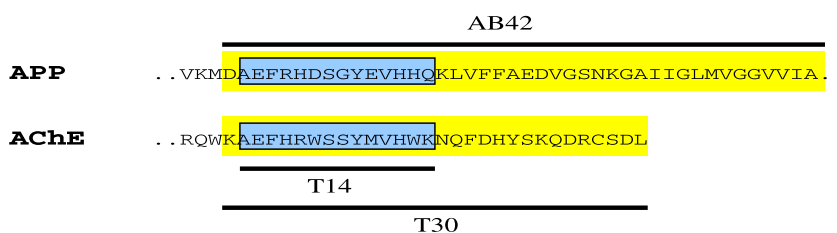
Fig. 3. In the form of AChE found in adult brains, two fragments towards the end of the molecule, 'peptides', of respectively 14 and 30 amino acids (T14 and T30) have clear cleavage points and bear a strong homology to an equivalent part of the amyloid precursor protein, a naturally occurring compound closely linked to Alzheimer's disease.

Table 1
Effects of synthetic AChE C-terminal peptides.

Preparation	Methods	Effect observed	Refs.
Acute effects			
Guinea pig brain slice	Electrophysiology	Modulates calcium entry into neurons	Bon and Greenfield [2]
Rat brain hippocampus	Tissue culture Immunohistochemistry	Trophic – ↑ neuron outgrowth	Day and Greenfield [6]
Rat brain subst. nigra	Tissue punch	Inhibits calcium-induced AChE release	Emmett and Greenfield [10]
Human breast cancer cells	Cell culture	Trophic – increases metastasis	Onganer et al. [16]
Frog oocytes	Electrophysiology	Potentiates responseInhibits response	Greenfield et al. [13]
Rat astroglia	Cell culture	Trophic – ↑ AChE	Bond et al. [3]
	Enzyme assay	Toxic – ↑ cell death	
Chronic effects			
Rat brain hippocampus	Tissue culture Immunohistochemistry	↓ neuron growth 50%	Day and Greenfield [6]
		Induces apoptosis	
		↓ neuron growth 75%	
		Toxic – necrosis	
Rat brain hippocampus	Tissue culture Immunohistochemistry	α7-receptor modulator mitigates AChE action	Zbarsky et al. [19]
Rat astroglia	Cell culture/Live cellradioligand binding	↓ labelled binding to α7-receptor	Bond et al. [4]
GH4-hα7 cells	Cell culture	↑ AChE releaseRNA and protein expression changes	Bond and Greenfield (2007)
	Enzyme assay		
	Molecular biology		
PC12 cells	Cell culture	↑ AChE release	Garcia-Rates & Greenfield
	Enzyme Assay	↓ cell growth 30%	(In preparation)

Why should this peptide be viewed as the more salient, over amyloid itself? First, unlike amyloid, AChE and its peptide are secreted directly from within the cell into the extracellular space, hence acting as a signalling molecule; secondly AChE peptide wwis a selective signalling molecule for certain cell groups, the very same ones primarily vulnerable in neurodegeneration; thirdly, unlike the ACxhE-peptide, amyloid has a much wider, less specific distribution in the brain and is even found in non-neurodegenerative brain tissue – and hence unlikely to be the triggering compound.

The AChE C-terminal peptide 'T14' has been originally identified as being the salient part of the AChE molecule responsible for its range of non-hydrolytic actions; the synthetic 14mer peptide analogue, and subsequently the larger, more stable, and more potent amino acid sequence in which it is embedded (T30) display actions comparable to those reported for 'non-cholinergic' AChE, where scrambled peptide variants, the butyrylcholinesterase equivalent, or the inert residue within the T30 sequence ('T15') are all without effect (Table 1). It is hard to view the peptide as operating other than completely independently of its parent AChE molecule: in

normal adult brain, the 'G4' tetramer of four catalytic subunits, whilst in development and Alzheimer's there is a disproportionate level of 'G1' [1], the monomer lacking a disulphide bond within which the peptide would reside. As well as validating the concept that neurodegeneration is an aberrant form of development, this finding suggests that the peptide is indeed cleaved in certain scenarios. Moreover, the entire ACXhE molecule is unlikely to bind to the receptor in its entirety, as documented for the peptide itself.

4. The peptide target

We have suggested that the all-important molecular target for AChE-peptide is a secondary, modulatory ('allosteric') site on a specific receptor (the nicotinic alpha-7 receptor). This receptor would be an attractive candidate for therapeutic intervention, since (i) it is one of the most powerful calcium ionophores in the brain; (ii) it is co-expressed along with AChE in precisely the same highly transient time period in various brain regions during development, (iii) it can bind the amyloid peptide already related to Alzheimer's disease and has already been implicated in the neurodegenerative diseases; (iv) it can operate independent of ACh, with choline as an alternative primary ligand. Since choline would be derived from the diet, the binding of AChE-peptides at an allosteric site on the α7-nAChR would be a potent means for modulating calcium into neurons, no longer requiring any classic cholinergic transmission at all, and available throughout the 'hub' of the vulnerable Global neurons. Moreover, the neuronal death seen in Alzheimer's disease has been linked to a dysfunction in choline uptake mechanisms (Novakova et al. [15]). We have shown [19] that the actions of the peptide are indeed sensitive to blockade of the alpha-7 receptor, in the nanomolar range, but such evidence could only be regarded as indirect. Subsequently, we obtained evidence that the peptides directly impact on receptor binding and expression: indeed, within 24 h, application of AChE peptide leads to a marked enhancement of the alpha-7 receptor on the external plasmamembrane [4]. If indeed neurodegeneration is an aberrant form of development, and the C-terminal AChE peptides are pivotal in the trophic-toxic action via an identified receptor site, then new avenues could open for devising a new approach to neurodegeration: identification of a pre-symptomatic biomarker (the peptide) coupled with interception at the alpha-7 receptor thereby stabilising any further cell loss, an effective 'cure'.

References

[1] T. Arendt, M.K. Brückner, M. Lange, V. Bigl, Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development–a study of molecular forms, Neurochem. Int. 3 (1992) 381–396.

[2] C.L. Bon, S.A. Greenfield, Bioactivity of a peptide derived from acetylcholinesterase: electrophysiological characterization in guinea-pig hippocampus, Eur. J. Neurosci. 17 (2003) 1991–1995.

[3] C.E. Bond, P. Patel, L. Crouch, N. Tetlow, T. Day, S. Abu-Hayyeh, C. Williamson, S.A. Greenfield, Astroglia up-regulate transcription and secretion of 'readthrough' acetylcholinesterase following oxidative stress, Eur. J. Neurosci. 24 (2) (2006) 381–386.

[4] C.E. Bond, M. Zimmermann, S.A. Greenfield, Upregulation of alpha7 nicotinic receptors by acetylcholinesterase C-terminal peptides, PLoS One 4 (2009) e4846.

[5] R. Bowser, J.H. Kordower, E.J. Mufson, A confocal microscopic analysis of galaninergic hyperinnervation of cholinergic basal forebrain neurons in Alzheimer's disease, Brain Pathol. 7 (1997) 723–730.

[6] T. Day, S.A. Greenfield, Bioactivity of a peptide derived from acetylcholinesterase in hippocampal organotypic cultures, Exp. Brain Res. 155 (4) (2004) 500–508.

[8] B.G.M. Dickie, C. Holmes, S.A. Greenfield, Neurotoxic and neurotrophic effects of chronic N-Methyl-D-Aspartate exposure upon mesencephalic dopaminergic neurons in organotypic cultures, Neuroscience 72 (3) (1996) 731–741.

[9] S. Eimerl, M. Schramm, The quantity of calcium that appears to induce neuronal death, J. Neurochem. (1994) 1223–1226.

[10] S.R. Emmett, S.A. Greenfield, A peptide derived from the C-terminal region of acetylcholinesterase modulates extracellular concentrations of acetylcholinesterase in the rat substantia nigra, Neurosci. Lett. 358 (2004) 210–214.

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

[13] S.A. Greenfield, T. Day, E.O. Mann, I. Bermudez, A novel peptide modulates alpha7 nicotinic receptor responses: implications for a possible trophic-toxic mechanism within the brain, J. Neurochem. 90 (2004) 325–331.

[14] S.A. Greenfield, M. Zimmermann, C.E. Bond, Non-hydrolytic functions of acetylcholinesterase. The significance of C-terminal peptides, FEBS J. 275 (4) (2008) 604–611.

[15] J. Nováková, L. Mikasová, E. Machová, V. Lisá, V. Dolezal, Chronic treatment with amyloid beta(1–42) inhibits non-cholinergic high-affinity choline transport in NG108-15 cells through protein kinase C signaling, Brain Res. 1062 (1–2) (2005) 101–110.

[16] P.U. Onganer, M.B. Djamgoz, K. Whyte, S.A. Greenfield, An acetylcholinesterase-derived peptide inhibits endocytic membrane activity in a human metastatic breast cancer cell line, Biochim. Biophys. Acta 1760 (2006) 415–420.

[17] R.J. Perrin, A.M. Fagan, D.M. Holtzman, Multimodal techniques for diagnosis and prognosis of Alzheimer's disease, Nature (2009) 916–922.

[18] M.N. Rossor, Parkinson's disease and Alzheimer's disease as disorders of the isodendritic core, Br. Med. J. (Clin. Res. Ed.). 283 (6306) (1981) 1588–1590.

[19] V. Zbarsky, J. Thomas, S. Greenfield, Bioactivity of a peptide derived from acetylcholinesterase: involvement of an ivermectin-sensitive site on the alpha 7 nicotinic receptor, Neurobiol. Dis. 16 (2004) 283–289.