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COMMENTARY

PARKINSON'S DISEASE, ALZHEIMER'S DISEASE AND MOTOR NEURONE DISEASE: IDENTIFYING A COMMON MECHANISM

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Abstract—Although Alzheimer's disease, Parkinson's disease, and motor neurone disease are distinct disorders, there could be a common neurodegenerative mechanism that characterises the death of selective neurone populations in each case. We propose that this mechanism could be an aberrantly activated, developmental process involving a non-classical, non-enzymatic action of acetylcholinesterase mediated via a short linear motif near the C-terminal end of the molecule. Since this motif has a highly conserved homology with part of the amyloid precursor protein, it may be particularly attractive as a target for novel therapeutic strategies in neurodegeneration. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: acetylcholinesterase, neurodegeneration, amyloid precursor protein, peptide, brainstem nuclei.

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Evidence is accumulating that Parkinson's and Alzheimer's diseases and other related disorders, such as Lewy body dementia and Guam syndrome, could be viewed as lying along a spectrum of neurodegeneration, i.e. share a basic underlying common mechanism (Calne et al., 1986) Alzheimer-like dementia and/or the characteristic histopathological markers of plaques and tangles may occur in Parkinson's disease (Aarsland et al., 1996); conversely Parkinson-like movement dysfunction and/or accompanying Lewy body histopathology have been reported in notable numbers of Alzheimer patients (Hulette et al., 1995). Moreover, such patients with overlapping pathology are not suffering from any separate, distinguishable condition (Gearing et al., 1995). This Commentary will consider the question: could there be a common factor that might facilitate the malfunction and/or death of the distinct populations of nerve cells that are affected in these different diseases? The operation of such a factor would be superimposed upon the changes in particular proteins, such as β-amyloid, tau and α-synuclein that characterise these diseases.

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; apoE, apolipoprotein E; APP, β-amyloid precursor protein; BuChE, butyryl cholinesterase; CSF, cerebrospinal fluid.

NEURODEGENERATION AND 'GLOBAL' NEURONES

Twenty years ago, Rossor suggested that both Alzheimer's and Parkinson's diseases could arise from primary disruption to a morphologically homogeneous hub of subcortical neurones, extending from the midbrain (Rossor, 1981) to the top of the spinal cord. In the aetiology of the disorders, the relatively late onset of dysfunctions associated with higher centres could be explained by a transynaptic cell loss triggered initially by a malfunction of these subcortical cells. The involvement in neurodegeneration of this population of neurones, including the locus coeruleus and raphe nuclei, as well as the basal forebrain and substantia nigra, is now widely

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documented (Vickers et al., 2000; DeKosky, 2001). Moreover, these cell groups are selectively affected in the brains of mice deficient in apolipoprotein E (apoE), a protein believed to play a key role in the aetiology of Alzheimer's disease (Chapman and Michaelson, 1998).

Why might this subcortical hub of cells be particularly relevant to the process of neurodegeneration? Interestingly, even though they use a diverse range of transmitters, the distinct brain regions that constitute the hub have critical properties not shared with other neuronal populations. Due to their diffuse projection from brainstem origins to access remote areas of brain and spinal cord, Woolf (1996) has introduced the term 'global neurones'. These global neurones, identical to those subcortical cells implicated in neurodegeneration, differ from the 'serial', amino acid-utilising systems in a variety of features (Table 1).

It is noteworthy that all global neurones contain acetylcholinesterase (AChE) (Smith and Cuello, 1984). At first glance, the presence of this familiar enzyme, responsible for the hydrolysis of acetylcholine (ACh), might not seem remarkable. After all, the most specific medication to date for Alzheimer's disease has been to inhibit the catalytic activity of AChE with tacrine or, more recently, donepezil in an attempt to potentiate the deficient cholinergic transmission at one time thought to be responsible for the dysfunction. Yet the simple form of the 'cholinergic hypothesis' for Alzheimer's disease can readily be discounted, due to a double dissociation. First, not all cholinergic cell groups in the brain are lost in Alzheimer's disease, more specifically the posterior Ch5 and Ch6 neuronal groups of the pedunculopontine nuclei are spared (Smith and Cuello, 1984). Conversely, not all neurones lost in Alzheimer's disease are cholinergic: the global neurones comprise not only the cholinergic neurones of the basal forebrain, but also include the serotonergic cells of the raphe nuclei, the noradrenergic cells of the locus coeruleus, and the dopaminergic cells of the midbrain (Table 1). Even though there is little of its conventional substrate, there are large amounts of AChE co-stored with these amines (Holmes et al., 1997). Just such a disparity has prompted the question whether AChE might have a novel function, completely independent of its conventional enzymatic role in cholinergic transmission.

NON-CLASSICAL FUNCTIONS OF ACHE

Not only are there such regions of brain where AChE exists in disproportionate excess to ACh and its synthesising enzyme choline acetyltransferase, but AChE can also display a further property not immediately nor obviously relevant to cholinergic transmission. As well as existing in its familiar membrane form, AChE is expressed as several soluble forms, one of which can be secreted, and which is electrophoretically distinct from the membrane-bound form. Stimulus-linked secretion of AChE has been described in a wide variety of tissues, namely the adrenal medulla (Chubb and Smith, 1975), the superior cervical ganglion (Somogyi and Chubb, 1976), the nerve–diaphragm junction (Brimijoin, 1983), the ileum (Appleyard and Smith, 1989) and in the invertebrate *Aplysia* (Srivatsan and Peretz, 1997), as well as in the mammalian brain, more specifically the cerebellum (Appleyard et al., 1988), the hypothalamus (Romero and Smith, 1979), the hippocampus (Appleyard, 1995), the striatum (Greenfield et al., 1980), and the substantia nigra (Greenfield et al., 1980).

Since secretion of AChE is not a requirement for cholinergic transmission, and since enhanced secretion can be acutely and transiently evoked by a range of drugs, it has been tempting to suggest that secreted AChE might underlie a novel form of neuronal signaling (Greenfield, 1996). This notion has now been validated directly, by manipulation of splice variants of AChE and subsequent differentiation of the catalytic action from roles in neuritogenesis, cell adhesion, synaptogenesis, amyloid fibre assembly, haematopoiesis and thrombopoiesis (Greenfield, 1996).

Table 1. Serial or global characterisation of central neurones (Woolf, 1996)

	Serial systems[a]	Global systems[b]
Brain regions:	Primary sensory neurones, sensory relays, cerebellum, thalamus, hypothalamus, hippocampus, cortex	Motor neurones, raphe nuclei, locus coeruleus, mesopontine tegmentum, substantia nigra, neostriatum, basal forebrain
Neurotransmitters:	Glutamate, aspartate, GABA	Acetylcholine, serotonin, norepinephrine, dopamine
Embryological origin:	Alar plate derivatives	Basal plate derivatives
Axonal plasticity:	Central axons largely lack regenerative capacity in adult	Central axons exhibit robust plasticity in adult
Neurotrophism:	Adult central neurones decrease sensitivity to NGF, BDNF, neurotrophin-3 and bFGF	Adult neurones maintain or increase sensitivity to NGF, BDNF, neurotrophin-3, bFGF and insulin-like growth factors
Physiological response pattern:	Isolated circuits are electrically silent unless stimulated directly	Isolated cells continue to fire spontaneously in rhythmic patterns
Specialised functions:	Relay data from external and internal milieu; repository for neural representations encoded by reorganisation of global afferents	Control overt behaviour; regulate EEG; focus attention; modulate potentiation or depression; enhance signal-to-noise ratio of responses in retrieval and recognition

NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; EEG, electroencephalogram.
[a]Neurones arranged in ordered series which participate in the relay of information ultimately derived from a sensory receptor to ascending centres, generally preserving some degree of modality and receptive field specificity.
[b]Constellations of neurones which are highly interconnected and physiologically interactive, and which determine the pattern of activity in their targets.

Evidence from electrophysiological studies (Webb and Greenfield, 1992) and from organotypic cultures (Day and Greenfield, 2002) suggests that the non-classical action of AChE involves the enhancement of calcium entry into neurones. Since calcium entry is a frequent trigger for instigating changes within neurones, particularly during development, it is perhaps not surprising that AChE has been implicated in development: not only do certain (Robertson and Yu, 1993) if not most (Soreq and Seidman, 2001) neurones transiently stain for AChE in development, but the appearance of the protein can be uncoupled from its normal catalytic function (Ling et al., 1995): indeed commitment of stem cells to a neuronal differentiation pathway is marked by the appearance of AChE mRNA, and subsequent secretion of AChE into the culture medium (Coleman and Taylor, 1996).

We propose that this developmental, non-classical role of AChE might be a key factor in the degeneration of global neurones. Unlike all other neurones in the adult mammalian brain, the global neurones retain a sensitivity to growth factors, as well as exhibiting axonal regeneration following injury (Table 1). However, the unexpected ability of this neuronal group to 'compensate' might actually lead to deleterious consequences. It has already been suggested that a process of 'dysdifferentiation' might underlie the pathology seen in Alzheimer's disease, such that exuberant sprouting might lead to the attraction of astroglia synthesising neurotrophic agents to which the global neurones remain sensitive (Woolf and Butcher, 1991), as well as acting as a binding matrix for immunoglobulins and aluminosilicates (Woolf and Butcher, 1991). The presence of neurotrophic factors would lead to yet further sprouting in a destructive cycle whereby the aberrantly grown neurites lead to the formation of tangles and neuritic plaques (Woolf and Butcher, 1991). Moreover, this idea, that neurodegeneration is an aberrant form of development (Greenfield, 1996; Arendt, 2001), is not only validated by a compensatory increase in axonal regeneration, but in addition, by the observation that the cell cycle is reactivated in Alzheimer's disease (Nagy et al., 1998), in key brain areas including the global cell population, i.e. the locus coeruleus and raphe nuclei (Busser et al., 1998). In animal models, aberrant axonal sprouting is seen in β-amyloid precursor protein (APP) transgenic mice (Phinney et al., 1999), whilst Alzheimer-like lesions within the rat brain can result in hyperinnervation of the basal forebrain (Henderson, 1996). A similar hypertrophy is apparent in the brains of those presenting with a co-pathology of Alzheimer's and Parkinson's diseases (Bowser et al., 1997).

What underlying mechanism might developmental processes and neurodegeneration have in common? Dickie et al. (1996) have shown that *N*-methyl-D-aspartate, which enhances calcium entry, can be beneficial in terms of neurite outgrowth in cultures of cells from the substantia nigra, so long as the dose is relatively low, but that this agent becomes toxic as the dose increases. It is notable that neurones containing high levels of calcium binding proteins are relatively spared in neurodegenerative (Yamada et al., 1990) diseases, whilst excitotoxicity due to excessive calcium entry has been widely considered as a final common path in the degenerative process (Small et al., 2001). Yet it is not immediately obvious why a developmental process could, at certain times, be toxic, if the amount of calcium entry was indeed physiological. A further, crucial factor, however, appears to be age: cytosolic calcium buffering can decline dramatically with age (Clementi and Meldolesi, 1996) and even in cultures differing by only 7 days, the tolerance to calcium influx can decline by a third (Eimerl and Schramm, 1994).

Hence it is possible that some trigger, be it a blow to the head, genetic predisposition, ischaemia or local toxin, causes damage to part of the global neurone population, either to nuclei associated with Parkinson's disease or with Alzheimer's diseases or, if the lesion is extensive, with a co-pathology. Unlike in serial cells, which have lost the ability to activate developmental processes, the global cell population will react with an attempt to compensate for the loss, by exuberant sprouting. Indeed, damage to certain neuronal populations in maturity can lead to the reappearance of an intensity in AChE staining, reminiscent of development (Farris et al., 1995). The subsequent release of AChE, acting in a non-cholinergic capacity, would trigger an enhanced calcium influx into the global neurone populations themselves, as well as into the target serial cells of hippocampus and cortex. Unlike in immature cells, the non-cholinergic AChE would now have a toxic effect. In view of the ubiquity of AChE throughout the global cell population, and in the light of the non-classical, trophic actions of AChE described above, and considering the ability of AChE to seemingly enhance calcium entry, we suggest that secreted AChE might be a critical agent in mediating the neurodegenerative process (Fig. 1).

Several lines of evidence already suggest that AChE per se could be pivotal in Alzheimer's disease (Greenfield, 1996). As we have already seen, all the primary, subcortical groups showing Alzheimer pathology, irrespective of whether they are cholinergic, express AChE (Greenfield, 1996; Smith and Cuello, 1984). Secondly, AChE is decreased in the cerebrospinal fluid (CSF) of Alzheimer patients where an anomalous form is also present (Navaratnam et al., 1991). Thirdly, in Alzheimer patients, there is the selective loss by some 50% of the soluble, secretable form of AChE from the adrenal gland, an organ not usually implicated in CNS disorders (Appleyard and McDonald, 1991). Hence it appears that AChE could indeed be a pivotal factor in the aetiology of the disease. Fourthly, in post-mortem samples of Alzheimer brain tissue, it was found that AChE reverted to the kinetics and configuration of subunits seen only otherwise in the embryo (Arendt et al., 1992). This finding corroborates further the idea that AChE could well be the crucial factor in a degeneration attributable to an aberrant form of development. Fifthly, there is a relative increase in AChE in CSF, according to the number of apoE4 alleles (Soininen et al., 1995), which none the less bears no relation to central cholinergic activity (Anderson and Higgins, 1997). Sixthly, transgenic mice

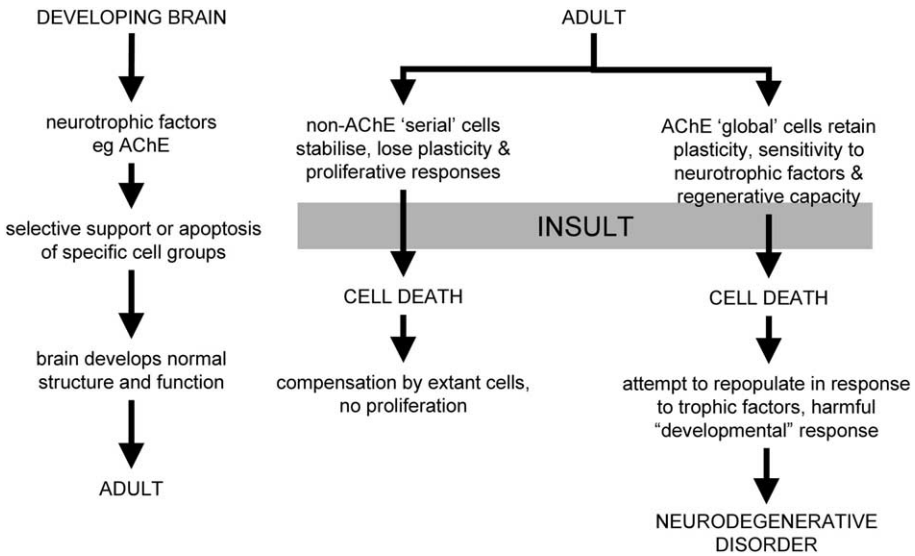

Fig. 1. Scheme for selective degeneration of certain groups of neurones in Alzheimer's, Parkinson's and motor neurone disease. In the developing brain most neuronal groups will transiently express neurotrophic factors such as AChE, which will ensure the development of specific cell circuitry that characterises the adult brain. Once mature, the majority of these serial circuits will no longer express AChE and, indeed, lose much of their plasticity and proliferative responses. If there is an insult, the cell death that ensues will ensure a functional compensation that characterises, in many cases, recovery or partial recovery in stroke. However, the global AChE-containing neurones, because they have retained their ability to regenerate and their sensitivity to trophic factors, will respond to a local insult in a different fashion. In this case there will be an exuberant axonal regeneration in an attempt to repopulate leading to enhanced release of AChE. AChE, acting in a non-cholinergic capacity, or a fragment derived from AChE, will then trigger calcium entry, both within the local global cell population as well as on to target serial cells of hippocampus and cortex. Whereas a certain degree of calcium would be beneficial in development, the mature cell is less able to handle intracellular calcium levels which therefore become toxic. The death of the cells will then constitute a further insult, leading to a pernicious cycle of events, neurodegeneration.

expressing twice as much AChE as normal show cognitive deficits reminiscent of Alzheimer's disease (Beeri et al., 1995). Since the catalysis of ACh by AChE is one of the most efficient enzyme reactions known, it is unlikely that a doubling of AChE levels would have a significant effect on the removal of ACh within the mouse brain: hence the effects seen are more likely to be attributable to non-cholinergic actions of AChE. Seventhly, AChE has non-cholinergic, dose-dependent toxic actions in neurones associated with neurodegeneration (Webb and Greenfield, 1992; Calderon et al., 1998). Finally, independent of the conventional catalytic site, AChE can accelerate the assembly of amyloid-β peptide into fibrils (Inestrosa et al., 1996). However, if the scheme proposed in Fig. 1 is to have any therapeutic value, the salient part of the molecule, responsible for its non-classical and potentially toxic action, needs to be identified.

SIMILARITIES BETWEEN ACHE AND APP

There are diverse parallels between 'non-cholinergic' AChE and APP. First, both AChE (Greenfield, 1996) and APP (de Sauvage and Octave, 1989) are secreted from neurones in CSF. Secondly, levels of both AChE (Navaratnam et al., 1991) and APP (Farlow et al., 1992) decrease in Alzheimer CSF. Thirdly, both AChE (Soreq and Seidman, 2001) and APP (de Sauvage and Octave, 1989) can have trophic functions where APP (Moreno et al., 1996), as for AChE (Soreq and Seidman, 2001), can trigger exuberant outgrowth, and where, in a neuronal cell line, an APP fragment and AChE can increase in parallel with cell confluence and differentiation (Bronfman et al., 1996). Fourthly, both AChE (Jones et al., 1995) and overexpression of APP (Fukuchi et al., 1996) enhance neuronal survival in developing systems. Fifthly, both AChE (Klegeris et al., 1994a,b) and β-amyloid (Klegeris et al., 1994a,b) activate macrophages. Finally, both AChE (Webb and Greenfield, 1992; Day and Greenfield, 2002) and β-amyloid (Wu et al., 1995) can enhance calcium influx into neurones. Therefore taken together the possible pathological consequences of aberrant activation of a non-classical developmental action for AChE, the intimacy of this protein with various aspects of Alzheimer's disease, and indeed its close parallels with APP have prompted us to examine any molecular similarity between the two proteins.

The predominant form of human brain APP is a protein of 695 residues encoded by a gene on the long arm of chromosome 21, while human AChE is a protein of 615 residues encoded by a gene on chromosome 7 (7q22). Both proteins can exist as membrane proteins; in the case of AChE, alternative splicing and post-translational processing and oligomerisation result in monomeric and oligomeric (dimer and tetramer) soluble forms, and forms bound to the plasma membrane either with a transmembrane domain or via a glyco-phospho-inositol linkage. In the case of APP, the parent protein is found as a type I membrane protein at the plasma membrane, with a short cytoplasmic domain containing residues important for intracellular trafficking.

The parallels between AChE and APP, and the possi-

A

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                        *           *   *               *
Human AChE      582  E R Q W K A  E  F H R W  S S  Y M  V H  W K N Q F  604
Rabbit AChE     552  E R Q W K A  E  F H R W  S S  Y M  V H  W K N Q F  574
Mouse AChE      582  E R Q W K A  E  F H R W  S S  Y M  V H  W K N Q F  604
Rat AChE        582  E R Q W K A  E  F H R W  S S  Y M  V H  W K N Q F  604
Bovine AChE     581  E R Q W K A  E  F H R W  S S  Y M  V H  W K N Q F  603
Human BuChE     568  E W E W K A  G  F H R W  N N  Y M  M D  W K N Q F  590
Rabbit BuChE    547  E Q E W K A  G  F H R W  N N  Y M  M A  W K N H F  569
Mouse BuChE     569  E Q E W K A  G  F H R W  S N  Y M  M D  W Q N Q F  591
Human APP       669  E V K M D A  E  F R H D  S G  Y E  V H  H Q K L V  691
                                 *           *          * *
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B

Species and protein	Z score
Polar bear APP	19.85
Pig APP	19.85
Sheep APP	19.83
Dog APP	19.83
Rabbit APP	19.83
Bovine APP	19.82
Rabbit AChE	5.40
Cat AChE	15.27
Bovine AChE	15.26
Rat AChE	15.25
Human AChE	15.25
Mouse AChE	15.25
Monkey APP	14.56
Human APP	14.49
Eel AChE	13.97
Zebrafish AChE	12.39
Rat APP	8.45
Mouse APP	8.45

Fig. 2. (A) AChE peptide (plus APP and BuChE) multiple sequence alignments. Asterisks above the alignment identify residues common to AChE, BuChE and APP, while bold text and asterisks below the alignment identify residues shared by AChE and APP but not BuChE. The start of β-amyloid is underlined in the APP sequence. (B) Matches to profile search showing absence of other proteins with significant match/similar motif.

bility of a causal role for both in neurodegenerative disorders led us to search for a common domain shared between these two previously unrelated proteins, a domain that could serve as a ligand for a common downstream receptor. Initially, we sought such a putative common ligand domain by sequence comparison; a simple pairwise comparison of the protein sequences of human APP and AChE shows that there is little obvious sequence similarity between the two proteins over most of their length. However, a small region of 12 amino acids with seven identical and three conservatively substituted residues is observed. This region consists of residues 587–598 of human AChE and residues 598–609 of human APP. This region of similarity becomes much more striking when a multiple sequence alignment is carried out on human, bovine, canine, ursine, porcine and ovine APP sequences, together with human, mouse, rabbit and rat AChE sequences. As a negative control for the significance of this alignment, the related enzyme butyrylcholinesterase (BuChE) can be used. This enzyme shares with AChE the ability to hydrolyse acetylcholine, using a highly related active site, but it does not have the non-cholinergic effects attributable to AChE. When mammalian BuChE sequences are included in the multiple alignment, then the similarities resolve into two classes: those that are common to all APP sequences and all cholinesterase sequences, and those that are shared between AChEs and APPs, but not BuChEs (Fig. 2A).

The APP–AChE alignment can be used to construct a profile in which the conserved residues are given a higher weighting for sequence comparisons (Fig. 2B). When the SwissProt database (96 841 protein sequences at September 2001) is searched using such a profile, the highest scoring matches are either mammalian APPs (with Z scores of 19.8 or greater) or AChEs (Z score between 12.4 and 15.4). There are no other proteins with Z scores above 8.0, and the majority of proteins with Z scores above 5.0 are prokaryotic sequences. No BuChE sequences occur in this search. This observation suggests that the alignment has revealed a significant, short, evolutionarily conserved domain that is shared between APP and AChE. Could this domain account for the apparent similarities in the behaviour of the two proteins?

If sequence analysis singles out this region, and structural determinations using fragments of β-amyloid suggest that the region forms a plausible domain, does any evidence exist to link this domain to biological function,

either in APP or in AChE? In the case of AChE, the peptide sequence shown in the alignment is contained within exon 6 of the human AChE gene. Experiments involving the expression of AChE mini-gene constructs in a rat glioma cell line reveal morphological changes in culture consistent with differentiation that occur only when the AChE produced by the transfected construct contained expressed exon 6 (Soreq and Seidman, 2001). In contrast, expression of AChE lacking exon 6 polypeptides resulted in a different morphology, with small rounded cells reminiscent of malignant glioma cells, which have been shown to produce a read-through transcript lacking expression of exon 6 (Karpel et al., 1996). In the case of APP, the region of interest forms the amino-terminus of the highly studied 39–43-residue β-amyloid peptide, the excessive extracellular accumulation and filamentous assembly of which is a hallmark of Alzheimer's disease. This sequence, acting either as the salient part of the intact AChE molecule, or perhaps as an independent, cleaved peptide, could be the pivotal agent that mediates the scheme for neurodegeneration outlined in Fig. 1.

MOTOR NEURONE DISEASE

Not only might 'non-cholinergic' AChE, by virtue of this C-terminal peptide, play a pivotal role in the aberrantly activated developmental mechanism that could characterise Alzheimer's and Parkinson's diseases, but the proposed scenario might be extended to motor neurone disease too. Motor neurone disease can, like Parkinson's disease, co-exist with Alzheimer-like features (Massman et al., 1996), whilst mRNA for APP is up-regulated in dying motor neurones (Barnes et al., 1998). Motor neurones comprise part of the 'global' cell population (Table 1) and hence they too presumably exhibit the same persistent regenerative capacity that we have postulated is aberrantly recapitulated during neurodegeneration. Indeed the current, most widely used medication, Riluzole, is predicated on a presumed underlying excitotoxic mechanism. Moreover, motor neurones too secrete AChE, and this secretion actually precedes the neurotoxicity triggered by excitatory amino acids (Rodriguez-Ithurralde et al., 1998). In fact, the particular vulnerability of α motor neurones in motor neurone disease might be due to their turnover of AChE, and to autoantibodies to AChE seen in motor neurone disease patients (Conradi and Ronnevi, 1993). Moreover, in patients with motor neurone disease, there are increased titres of IgG and IgA antibodies towards AChE (Sindhuphak et al., 1988), whilst in transgenic mice over-expressing AChE, there are aberrations in neuromuscular structure and pronounced amyotrophy (Andres et al., 1998). The basis for this pathological association might therefore be, as we have postulated for Alzheimer's disease and Parkinson's disease, an aberrant action of a non-classical, trophic action, that has also been demonstrated in motor neurones (Bataille et al., 1998).

CONCLUSION

We propose that all three major neurodegenerative disorders can be linked by a common, pivotal mechanism: an insult to any part, and to any extent, of the population of cells including motor neurones, the raphe nuclei, the locus coeruleus, the substantia nigra, and the basal forebrain, will result, unlike elsewhere in the brain, in an aberrant activation of compensatory neurite outgrowth. This outgrowth will be mediated by the release of AChE which, via a short linear C-terminal signalling motif, will enhance calcium entry into local neurones, as well as target serial cells of hippocampus and cortex. Since these adult neurones can withstand only a fraction of the calcium entry compared to immature cells they will die, and lead in a vicious cycle to further 'compensation'. If this scenario does indeed form the basis of neurodegeneration, it would explain the anomalies in our current understanding, in particular the cell selectivity that is discrepant with the 'cholinergic' and amyloid hypotheses of neurodegeneration. It might, in addition, offer a new therapeutic target, namely the relevant C-terminal portion of AChE.

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REFERENCES

Aarsland, D., Tandberg, E., Larsen, J.P., Cummings, J.L., 1996. Frequency of dementia in Parkinson disease. Arch. Neurol. 53, 538–542.
Anderson, R., Higgins, G.A., 1997. Absence of central cholinergic deficits in ApoE knockout mice. Psychopharmacology (Berlin) 132, 135–144.
Andres, C., Seidman, S., Beeri, R., Timberg, R., Soreq, H., 1998. Transgenic acetylcholinesterase induces enlargement of murine neuromuscular junctions but leaves spinal cord synapses intact. Neurochem. Int. 32, 449–456.
Appleyard, M.E., 1995. Acetylcholinesterase induces long-term potentiation in CA1 pyramidal cells by a mechanism dependent on metabotropic glutamate receptors. Neurosci. Lett. 190, 25–28.
Appleyard, M.E., McDonald, B., 1991. Reduced adrenal gland acetylcholinesterase activity in Alzheimer's disease. Lancet 338, 1085–1086.
Appleyard, M.E., Smith, A.D., 1989. Secretion of acetylcholinesterase and butyrylcholinesterase from the guinea-pig isolated ileum. Br. J. Pharmacol. 97, 490–498.
Appleyard, M.E., Vercher, J.L., Greenfield, S.A., 1988. Release of acetylcholinesterase from the guinea-pig cerebellum *in vivo*. Neuroscience 25, 133–138.
Arendt, T., 2001. Alzheimer's disease as a disorder of mechanisms underlying structural brain self-organisation. Neuroscience 102, 723–765.
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.

Barnes, N.Y., Li, L., Yoshikawa, K., Schwartz, L.M., Oppenheim, R.W., Milligan, C.E., 1998. Increased production of amyloid precursor protein provides a substrate for caspase-3 in dying motoneurons. J. Neurosci. 18, 5869–5880.

Bataille, S., Portalier, P., Coulon, P., Ternaux, J.P., 1998. Influence of acetylcholinesterase on embryonic spinal rat motoneurones growth in culture: a quantitative morphometric study. Eur. J. Neurosci. 10, 560–572.

Beeri, R., Andres, C., Lev-Lehman, E., Timberg, R., Huberman, T., Shani, M., Soreq, H., 1995. Transgenic expression of human acetylcholinesterase induces progressive cognitive deterioration in mice. Curr. Biol. 5, 1063–1071.

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

Brimijoin, S., 1983. Molecular forms of acetylcholinesterase in brain, nerve and muscle: nature, localization and dynamics. Prog. Neurobiol. 21, 291–322.

Bronfman, F.C., Fernandez, H.L., Inestrosa, N.C., 1996. Amyloid precursor protein fragment and acetylcholinesterase increase with cell confluence and differentiation in a neuronal cell line. Exp. Cell Res. 229, 93–99.

Busser, J., Geldmacher, D.S., Herrup, K., 1998. Ectopic cell cycle proteins predict the sites of neuronal cell death in Alzheimer's disease brain. J. Neurosci. 18, 2801–2807.

Calderon, F.H., von Bernhardi, R., De Ferrari, G., Luza, S., Aldunate, R., Inestrosa, N.C., 1998. Toxic effects of acetylcholinesterase on neuronal and glial-like cells *in vitro*. Mol. Psychiatry 3, 247–255.

Calne, D.B., Eisen, A., McGeer, E., Spencer, P., 1986. Alzheimer's disease, Parkinson's disease, and motorneurone disease: abiotropic interaction between ageing and environment? Lancet 2 (8515), 1067–1070.

Chapman, S., Michaelson, D.M., 1998. Specific neurochemical derangements of brain projecting neurons in apolipoprotein E-deficient mice. J. Neurochem. 70, 708–714.

Chubb, I.W., Smith, t., 1975. Release of acetylcholinesterase into the perfusate from the ox adrenal gland. Proc. R Soc. London B Biol. Sci. 191, 263–269.

Clementi, E., Meldolesi, J., 1996. Pharmacological and functional properties of voltage-independent Ca2+ channels. Cell Calcium 19, 269–279.

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

Conradi, S., Ronnevi, L.O., 1993. Selective vulnerability of alpha motor neurons in ALS: relation to autoantibodies toward acetylcholinesterase (AChE) in ALS patients. Brain Res. Bull. 30, 369–371.

Day, T., Greenfield, S.A., 2002. A non-cholinergic, trophic action of acetylcholinesterase on hippocampal neurons *in vitro*: molecular mechanisms. Neuroscience 1–8.

de Sauvage, F., Octave, J.N., 1989. A novel mRNA of the A4 amyloid precursor gene coding for a possibly secreted protein. Science 245, 651–653.

DeKosky, S.T., 2001. Epidemiology and pathophysiology of Alzheimer's disease. Clin. Cornerstone 3, 15–26.

Dickie, B.G., Holmes, C., Greenfield, S.A., 1996. Neurotoxic and neurotrophic effects of chronic N-methyl-D-aspartate exposure upon mesencephalic dopaminergic neurons in organotypic culture. Neuroscience 72, 731–741.

Eimerl, S., Schramm, M., 1994. The quantity of calcium that appears to induce neuronal death. J. Neurochem. 62, 1223–1226.

Farlow, M., Ghetti, B., Benson, M.D., Farrow, J.S., van Nostrand, W.E., Wagner, S.L., 1992. Low cerebrospinal-fluid concentrations of soluble amyloid beta-protein precursor in hereditary Alzheimer's disease. Lancet 340, 453–454.

Farris, T.W., Butcher, L.L., Oh, J.D., Woolf, N.J., 1995. Trophic-factor modulation of cortical acetylcholinesterase reappearance following transection of the medial cholinergic pathway in the adult rat. Exp. Neurol. 131, 180–192.

Fukuchi, K., Ohman, T., Dang, N., Smith, A.C., Furlong, C.E., Martin, G.M., 1996. Overexpressions of cDNAs for beta-amyloid precursor proteins 695, 751, and 770 enhance the secretion of beta-amyloid precursor protein derivatives and the survival of P19-derived neurons. J. Neurochem. 66, 2201–2204.

Gearing, M., Schneider, J.A., Rebeck, G.W., Hyman, B.T., Mirra, S.S., 1995. Alzheimer's disease with and without coexisting Parkinson's disease changes: apolipoprotein E genotype and neuropathologic correlates. Neurology 45, 1985–1990.

Greenfield, S., 1996. Non-classical actions of cholinesterases: role in cellular differentiation, tumorigenesis and Alzheimer's disease. Neurochem. Int. 28, 485–490.

Greenfield, S., Cheramy, A., Leviel, V., Glowinski, J., 1980. *In vivo* release of acetylcholinesterase in cat substantia nigra and caudate nucleus. Nature 284, 355–357.

Henderson, Z., 1996. Responses of basal forebrain cholinergic neurons to damage in the adult brain. Prog. Neurobiol. 48, 219–254.

Holmes, C., Jones, S.A., Budd, T.C., Greenfield, S.A., 1997. Non-cholinergic, trophic action of recombinant acetylcholinesterase on mid-brain dopaminergic neurons. J. Neurosci. Res. 49, 207–218.

Hulette, C., Mirra, S., Wilkinson, W., Heyman, A., Fillenbaum, G., Clark, C., 1995. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part IX. A prospective cliniconeuropathologic study of Parkinson's features in Alzheimer's disease. Neurology 45, 1991–1995.

Inestrosa, N.C., Alvarez, A., Perez, C.A., Moreno, R.D., Vicente, M., Linker, C., Casanueva, O.I., Soto, C., Garrido, J., 1996. Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. Neuron 16, 881–891.

Jones, S.A., Holmes, C., Budd, T.C., Greenfield, S.A., 1995. The effect of acetylcholinesterase on outgrowth of dopaminergic neurons in organotypic slice culture of rat mid-brain. Cell Tissue Res. 279, 323–330.

Karpel, R., Sternfeld, M., Ginzberg, D., Guhl, E., Graessmann, A., Soreq, H., 1996. Overexpression of alternative human acetylcholinesterase forms modulates process extensions in cultured glioma cells. J. Neurochem. 66, 114–123.

Klegeris, A., Budd, T.C., Greenfield, S.A., 1994a. Acetylcholinesterase activation of peritoneal macrophages is independent of catalytic activity. Cell. Mol. Neurobiol. 14, 89–98.

Klegeris, A., Walker, D.G., McGeer, P.L., 1994b. Activation of macrophages by Alzheimer beta-amyloid peptide. Biochem. Biophys. Res. Commun. 199, 984–991.

Ling, J.J., Yu, J., Robertson, R.T., 1995. Sustained inhibition of acetylcholinesterase activity does not disrupt early geniculocortical ingrowth to developing rat visual cortex. Dev. Brain Res. 86, 354–358.

Massman, P.J., Sims, J., Cooke, N., Haverkamp, L.J., Appel, V., Appel, S.H., 1996. Prevalence and correlates of neuropsychological deficits in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 61, 450–455.

Moreno, R.D., Inestrosa, N.C., Culwell, A.R. et al., 1996. Sprouting and abnormal contacts of nonmedullated axons, and deposition of extracellular material induced by the amyloid precursor protein (APP) and other protease inhibitors. Brain Res. 718, 13–24.

Nagy, Z., Esiri, M.M., Smith, A.D., 1998. The cell division cycle and the pathophysiology of Alzheimer's disease. Neuroscience 87, 731–739.

Navaratnam, D.S., Priddle, J.D., McDonald, B., Esiri, M.M., Robinson, J.R., Smith, A.D., 1991. Anomalous molecular form of acetylcholinesterase in cerebrospinal fluid in histologically diagnosed Alzheimer's disease. Lancet 337, 447–450.

Phinney, A.L., Deller, T., Stalder, M., Calhoun, M.E., Frotscher, M., Sommer, B., Staufenbiel, M., Jucker, M., 1999. Cerebral amyloid induces aberrant axonal sprouting and ectopic terminal formation in amyloid precursor protein transgenic mice. J. Neurosci. 19, 8552–8559.

Robertson, R.T., Yu, J., 1993. Acetylcholinesterase and neural development – new tricks for an old dog. News Physiol. Sci. 8, 266–272.
Rodriguez-Ithurralde, D., Maruri, A., Rodriguez, X., 1998. Motor neurone acetylcholinesterase release precedes neurotoxicity caused by systemic administration of excitatory amino acids and strychnine. J. Neurol. Sci. 160 (Suppl 1), S80–86.
Romero, E., Smith, A.D., 1979. Release of acetylcholinesterase from superfused slices of rat hypothalamus. J. Physiol. 301, 52P.
Rossor, M.N., 1981. Parkinson's disease and Alzheimer's disease as disorders of the isodendritic core. Br. Med. J. (Clin. Res. Ed. 283, 1588–1590.
Sindhuphak, R., Karlsson, E., Conradi, S. et al., 1988. Immunoglobins from patients with amyotrophic lateral sclerosis affect human-erythrocyte acetylcholinesterase. J. Neurol. Sci. 86, 195–202.
Small, D.H., Mok, S.S., Bornstein, J.C., 2001. Alzheimer's disease and Abeta toxicity: from top to bottom. Nat. Rev. Neurosci. 2, 595–598.
Smith, A.D., Cuello, A.C., 1984. Alzheimer's disease and acetylcholinesterase-containing neurons. Lancet 1 (8375), 513.
Soininen, H., Lehtovirta, M., Helisalmi, S., Linnaranta, K., Heinonen, O., Riekkinen, P., Sr., 1995. Increased acetylcholinesterase activity in the CSF of Alzheimer patients carrying apolipoprotein epsilon4 allele. NeuroReport 6, 2518–2520.
Somogyi, P., Chubb, I.W., 1976. The recovery of acetylcholinesterase activity in the superior cervical ganglion of the rat following its inhibition by diisopropylphosphorofluoridate: a biochemical and cytochemical study. Neuroscience 1, 413–421.
Soreq, H., Seidman, S., 2001. Acetylcholinesterase – new roles for an old actor. Nat. Rev. Neurosci. 2, 294–302.
Srivatsan, M., Peretz, B., 1997. Acetylcholinesterase promotes regeneration of neurites in cultured adult neurons of Aplysia. Neuroscience 77, 921–931.
Vickers, J.C., Dickson, T.C., Adlard, P.A., Saunders, H.L., King, C.E., McCormack, G., 2000. The cause of neuronal degeneration in Alzheimer's disease. Prog. Neurobiol. 60, 139–165.
Webb, C.P., Greenfield, S.A., 1992. Non-cholinergic effects of acetylcholinesterase in the substantia nigra: a possible role for an ATP-sensitive potassium channel. Exp. Brain Res. 89, 49–58.
Woolf, N.J., 1996. Global and serial neurons form a hierarchically arranged interface proposed to underlie memory and cognition. Neuroscience 74, 625–651.
Woolf, N.J., Butcher, L.L., 1991. Dysdifferentiation of structurally plastic neurons initiates the pathologic cascade of Alzheimer's disease: toward a unifying hypothesis. In: M. Steriade, D. Biesold (Eds.), Brain Cholinergic Systems, Oxford University Press, New York, pp. 387–426.
Wu, J., Anwyl, R., Rowan, M.J., 1995. beta-Amyloid selectively augments NMDA receptor-mediated synaptic transmission in rat hippocampus. NeuroReport 6, 2409–2413.
Yamada, T., McGeer, P.L., Baimbridge, K.G., McGeer, E.G., 1990. Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. Brain Res. 526, 303–307.

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