Alzheimer’s disease and the non-cholinergic activities of cholinesterases – A possible relationship

A. S. Balasubramanian
Division of Medical Biotechnology, Tamil Nadu Hospital Academic Trust Research Council, Perumbakkam, Chennai 601 302, India

Three of the major features of Alzheimer’s disease are, a cholinergic deficit, amyloid beta peptide deposition in plaques and abnormalities in zinc status. A unified hypothesis linking these findings to the non-cholinergic activities of cholinesterases demonstrated by work in the author’s laboratory is presented in this article.

Alzheimer’s disease (AD) begins with the loss of short-term memory, followed by progressive dementia which involves memory loss, impairment of intellectual functioning, judgement and decision making. Most patients have steadily progressive disease with survival of 5 to 12 years after onset1. It is the most common cause of dementia in adults in north America and Europe. Exact data on the prevalence of the disease in India are not available primarily because of difficulties in the accurate diagnosis of the disease. Most of the affected patients are above the age of 60. With the increase in ageing population all over the world, the disease has become an important issue.

Many isolated, but well-confirmed, biochemical and histopathological findings have been made in AD from different laboratories (Figure 1). It is not known whether these findings are the cause or consequence of the disease process. In this article we provide a unified hypothesis linking some of the major abnormalities noticed in AD based on our own work and those of others.

Cholinergic transmission in AD

There is a deficit in cholinergic neurotransmission in AD. A loss of cholinergic cell bodies in the cerebral cortex and a reduction in the activity of cerebral cortical choline acetyl transferase, the enzyme responsible for the synthesis of acetylcholine is reported2. Variations in the activity of acetylcholinesterase (AChE), the enzyme which catalyses the degradation of acetylcholine, in the cerebrospinal fluid and in the brain of Alzheimer patients have also been reported by several investigators3-10. Although AD is now believed to be more than the result of a cholinergic deficit, the treatment of choice for AD continues to be the potentiation of central cholinergic function. The three main cholinergic approaches to AD treatment are acetylcholine precursor loading, cholinesterase inhibition and direct cholinergic receptor stimulation11. Administration of cholinesterase inhibitors has been the most frequently used experimental treatment for AD. Modest but reliable improvements in memory have been produced by the AChE inhibitor phystostigmine12. Two aminoacridines, tacrine and velnacrine, which are inhibitors of AChE, have been more recently used in a multicentric study for the treatment of AD13.

Amyloid beta peptide and neurofibrillary tangles in AD

Reliable diagnosis of AD comes from histopathological observations made on brain biopsy samples or autopsy specimens. The numerous plaques and neurofibrillary tangles found in the hippocampus and surrounding areas

![Figure 1. Factors associated with Alzheimer’s disease.](image-url)
in the central cortex are hallmarks of AD. Recent advances made in studying the constituents of plaques and tangles show that the plaques are enriched in a peptide of approximately 40 to 42 amino acids, called beta amyloid peptide, and the tangles are made of paired helical filaments of which the microtubule protein Tau (in a hyperphosphorylated form) is a major constituent.  

The beta amyloid peptide is shown to arise from a larger protein known as amyloid precursor protein, which is made of about 560 to 770 amino acids and which can exist in different forms. At least six forms of the precursor protein are known to exist by alternative mRNA splicing of the gene responsible for this protein. Four of these forms contain the sequence of the amyloid beta peptide. The most likely possibility for the abnormal extracellular accumulation of beta amyloid peptide in the plaques is an abnormal proteolytic cleavage of the amyloid precursor protein due to altered proteolytic enzymes or their activities influenced by unknown activators or inhibitors and/or due to mutation in the amyloid precursor protein which in turn alters its susceptibility to cleavage by proteases.

Amyloid peptide fibril formation, deposition as plaques and the role of zinc

Amyloid peptide is found at very low levels (microgram quantities) in the cerebrospinal fluid in normal individuals. Its harmful effects appear to be due to its deposition as insoluble fibrils in the plaques found in the brain of AD patients. Whether an altered conformation of the peptide confers the property of insolubility or whether additional factors are involved is not clearly known. Metals like aluminium and zinc are suggested to be causative factors of AD by promoting amyloid peptide fibrillation. In particular, an association of AD with abnormal zinc metabolism has been observed in several laboratories. Decreased levels of zinc in temporal lobe of the brain and in cerebrospinal fluid and enhanced hepatic zinc with reduced zinc bound to metallothionein are some of the observations made in AD. Clinical zinc deficiency is common in Down’s syndrome, a disease which shows AD pathology. A novel zinc-binding site in amyloid precursor protein that modulates its function has also been reported.

The neurotoxicity in AD

Neurotoxicity in AD may result from multiple factors such as the enhanced cellular processing of the amyloid precursor protein and the consequent deposition of the amyloid beta peptide, interaction of the peptide with cell membranes, macromolecules producing modified structures that can form crosslinks and generate reactive oxygen intermediates and abnormality in the protective response to oxidant stress and/or susceptibility to apoptotic stimuli. An example of this multifaceted damage comes from a recent work in which it is demonstrated that the amyloid beta peptide interacts with an intracellular-binding protein which mediates the cellular toxicity of the peptide. The intracellular amyloid beta peptide in this case can be that generated within the cell by cellular processing or from uptake of released amyloid beta peptide.

Other factors associated with AD

Additional factors that are unrelated to one another but found associated with AD are apolipoprotein E gene, mutant presenilins and synelmin.

Apolipoprotein E is involved in cholesterol transport. Apolipoprotein E gene has three alleles E2, E3, and E4. Apolipoprotein E3 is the most common in all populations (nearly 80%). Many investigators have found that apolipoprotein E4 is a major risk factor for AD. Interestingly, E4 is also known as a risk factor for coronary heart disease. It has, however, been cautioned that although E4 is a risk factor, it is not a predictive test for AD in an asymptomatic individual. It will be a useful test to differentiate AD from other dementias.

Mutated genes linked with most familial forms of AD, known as presenilins 1 and 2, have been identified. Increased production of amyloid beta peptide occurs in transgenic mice overexpressing mutant presenilins. Presenilin 2, which is homologous to ALG-3, a protein involved in programmed cell death, can function as a susceptibility factor for apoptosis in differentiated PC12 cells in response to growth factor/serum withdrawal or to addition of amyloid beta peptide. The physiological role of proteins encoded by presenilins is not known.

A protein named synelmin associated with auditory imprint is found in the brain of the singing bird zebra finch. Interestingly, proteins of a similar sequence are also found in the plaques of AD patients.

Noncholinergic activities of cholinesterases

The basic abnormality in AD is a deficiency of acetylcholine, the neurotransmitter required for memory. This is also exemplified by the fact that drugs that are used for improvement of AD patients are mild AChE inhibitors that preserve acetylcholine. The role of acetylcholine in memory is known and transgenic mice that overexpress human AChE in brain neurons show impairment of memory. In view of these, a consideration of the functions of the enzymes cholinesterases in AD will be relevant.
Cholinesterases are now known to belong to a family of alpha/beta hydrolases. AChE, the most important in the family is known for its role in cholinergic transmission from 1914 and it is enriched in excitable areas like nerve endings and neuromuscular junctions and the electric organ of the electric fish. A closely related enzyme with 53% amino acid sequence identity to AChE is butyrylcholinesterase (BChE) present in abundance in human serum whose physiological function is not entirely clear. The amino acid sequence at the active site serine residue is identical for both AChE and BChE and both show similar response to classical cholinesterase inhibitors. The preferred substrates for AChE and BChE are acetylcholine and butyrylcholine respectively.

Apart from the esterase activity, both AChE and BChE exhibit an aryl acylamidase (AAA) activity and a peptidase activity (Figure 2). The initial detailed studies on the identity of AAA with AChE and BChE have been confirmed in other laboratories. AAA activity of AChE and BChE is sensitive to inhibition by 5-hydroxy tryptamine and AAA activity of human serum BChE is activated several fold by tyramine. The synthetic substrate used for AAA assay is α-nitroacetanilide and the physiological substrate is elusive.

A carboxypeptidase-like activity has been identified in both AChE and BChE. Detailed studies by the author's group using purified human serum BChE have shown that AAA and the carboxypeptidase are part of the same polypeptide chain of BChE. Further, it was shown that the carboxypeptidase was stimulated by the metal ions zinc, cobalt and manganese and inhibited by metal chelators, identifying it as a metallo-carboxypeptidase. Additional evidence for this was provided by showing that BChE has zinc-binding site(s).

1. Amine sensitive arylaclylamidase activity.
2. Zinc-stimulated metallo-carboxypeptidase activity.
3. Cocaine hydrolysing activity.

Figure 2. Non-cholinergic enzymatic activities of butyrylcholinesterase.

Characteristics of the cholinesterases found in the plaques and tangles in AD

Cholinesterases have been found in the plaques and tangles of AD brain. In histopathological studies it was reported that these cholinesterases seen in AD were significantly susceptible to inhibition by 5-hydroxy tryptamine and a carboxypeptidase inhibitor as well as the general protease inhibitor bacitracin. More than 500 tissue sections were examined in these studies and similar results were seen in all of the areas and all specimens that were examined. In addition to AChE reactivity, prominent BChE activity was also present in the senile plaques and tangles of AD brain. It appeared that instead of the normal AChE, new cholinesterases (more similar to BChE) exhibiting distinguishing properties of inhibition by 5-hydroxy tryptamine and carboxypeptidase inhibitor emerge in the amyloid lesions of AD brain. Geula and Mesulam, who made these observations called them 'AD cholinesterases'. Whether these cholinesterases are aberrant forms and present at very low levels in the normal brain but increase in AD are questions that remain to be answered.

The indoleamine and carboxypeptidase inhibitor sensitivity exhibited by cholinesterases found in AD brain described above is reminiscent of the 5-hydroxytryptamine sensitive AAA activity and carboxypeptidase activity found in cholinesterases.

An altered cholinesterase in AD?

It seems possible to enunciate a unified hypothesis in AD, linking cholinesterases and their non-cholinergic activities, zinc, amyloid beta peptide and protease activity based on our own work and those of others (Figure 3). The hypothesis starts on the premise that altered (mutated or conformationally altered) AChE and BChE are present in AD. A genetic predisposition for this altered cholinesterase can be envisaged in AD patients. Under the influence of this altered cholinesterase action (this could be a hyperactivity of the enzyme or activity stimulated by other stimulants or effectors) there is an excessive loss of acetylcholine which is the primary finding in
AD. Simultaneously, the carboxypeptidase-like activity present both in AChE and BChE also exhibit unusual activity to cleave the amyloid precursor protein and release the amyloid beta peptide extracellularly. A mutated amyloid precursor protein in AD that is more susceptible to the action of the proteases is also a possibility. The proteolytic cleavage by cholinesterases can happen in conjunction with other proteases present in the brain also. In fact, many proteases including metalloproteases have been shown to cleave beta amyloid precursor protein. The stimulation by zinc of the carboxypeptidase-like activity in cholinesterases emphasizes the role of zinc status in AD.

Some of the experimental observations that support the above hypothesis have already been mentioned. Although genetic variants of BChE determining its activity in plasma are known, there is yet no direct evidence to demonstrate a genetically mutated form of AChE or BChE in AD. However as indicated by the work of Mesulam and his group, cholinesterases exhibiting unique properties have been found in AD. Additionally, an anomalous form of AChE present in 19 out of 23 AD brains and absent in all of the 19 non-demented brains has been reported. This new molecular form of AChE has been detected in the lumbar cerebrospinal fluid of living demented individuals. After death, 13 out of these 15 patients were diagnosed as AD cases. This unusual form of AChE is characterized as a new band on isoelectric focusing gels between pH 5 and 7.

Conclusions

The foregoing discussions are limited to the major abnormalities found in AD, namely the cholinergic deficit and the beta amyloid peptide deposition. The neurotoxicity resulting from these abnormalities may find its route through many intermediate events. In fact, alterations in neurotransmitters other than acetylcholine, free radical-induced damage and apoptosis are implicated to play a role in AD. Cholinesterase inhibitors continue to be the drugs of choice for treatment of the disease. Huperzine, derived from a Chinese herbal plant, is claimed to be a useful drug for AD because of its ability to inhibit AChE. Several plant sources and products in India are claimed to enhance memory and it would be worthwhile to make a systematic study of the anticholinesterase properties of these plants.
MEETINGS/SYMPATHIES/SEMINARS

International Seminar on Applied Hydrogeochemistry

Date: 18–20 November 1998
Place: Annamalai Nagar, India

Themes: Aquatic geochemistry and analytical methods; Geochemistry of fluoride-bearing waters; Biogeochemical cycling of metals in the aquatic environments; Hydrogeochemistry and human health; Hydrogeochemistry applied to agriculture, industry and mining activities; Application of hydrogeochemical tracers; EIA studies based on hydrogeochemistry and by remote sensing techniques; Models and computation of hydrogeochemical data; Salination and salt water intrusion; Natural and anthropogenic pollution of hydrosphere; Recycling of waste water in urban areas.

Contact: Dr AL. Ramanathan
Convenor
International Seminar on Applied Hydrogeochemistry
Department of Geology
Annamalai University
Annamalainagar 608002, India
Phone: 0091-04144-22749/22282
Fax: 0091-04144-23080/22027