ACETYLCHOLINESTERASE: THE KEY TO UNDERSTANDING ETIOLOGY AND THERAPY OF ALZHEIMER'S DISEASE ?

Anna STRUNECKÁ
Department of Physiology and Developmental Biology Faculty of Sciences, Charles University, Prague

Summary

The etiology of Alzheimer's disease, neurodegenerative disorder with impairment of cognitive functions and the loss of memory, is unknown. The deficit of the brain acetylcholine and cholinergic neurotransmission insufficence in Alzheimer's patient is commonly accepted now. The key role of acetylcholinesterase, an important enzyme of cholinergic transmission in the etiology as well as in the therapy of Alzheimer's disease is discussed in this paper.

KEY WORDS: Alzheimer's disease; Acetylcholinesterase; Nervous system; Etiopathology.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder with impairment of cognitive function and the loss of memory. Deficits in language and visuospacial skills also emerge in time. Since first characterization of Alzheimer's disease (2), the number of patients has risen dramatically in industrialized world as well as in developing countries (23). AD is among the most frequent obstacles of healthy aging. The increasing size of very old population has led to increasing cost of social and health care. The scientific world and pharmaceutical companies are searching mechanism of the disease for better understanding pathogenesis and means to oppose it. In spite of accumulated knowledge of cellular and molecular aspects as well as genetic risk factors, it is not known specifically how to arrest or delay the course of this disease.

AD is characterized by senile plaques and neurofibrillary tangles in the brain and loss of cholinergic neurons in the basal forebrain. The depletion of choline acetyltransferase in the cortex and in the limbic structures of patients with AD is one of the most consistent and profound changes as early as one year after onset of the disease (6). The cortex of patients also shows decreased rates of acetylcholine synthesis and choline uptake. The basal forebrain nuclei that give rise to cholinergic fibers also show cell degeneration in brains of AD patients (28).

The cholinergic hypothesis therefore suggests that the reduction of cholinergic neurotransmission could explain the most important cognitive deficit in AD (4). Among different drug strategies, the cholinergic approach has gained a great interest (22). Inhibitors of acetylcholinesterase sustain the availability of the natural transmitter by limiting its removal from the synapse. Pharmaceutical companies are working on the development of new drugs from the group of cholinesterase inhibitors (tacrine, donepezil, metrifonate, galanthamine and analogues, huperzin A, eptastigmine and others) (23).

Research on the role of cholinergic transmission in AD patients

The new era of biochemical research of AD, started in late 1970s, is characterized with the aim to search such changes in the processes of neurotransmission which could contribute to understanding a pathophysiological basis for cognitive defects of the aging brain, and simultaneously to develop target drugs for therapy.

The cholinergic hypothesis and the crucial role of acetylcholine in cognitive function attracted attention to study of cholinergic transmission. There are two basic principles of cholinergic therapy: first, stimulation of various cholinergic receptors with selective agonists; second, reduction of acetylcholine hydrolysis by cholinesterase inhibitors.

The cholinergic receptors in the brain are mostly muscarinic and their activation has been connected with the cognitive function. The discovery of different muscarinic receptor subtypes has offered new opportunities to develop specific inhibitors of the M1 postsynaptic receptor subtypes. However, the therapeutic strategy of direct pharmacological activation of muscarinic receptors is somehow questionable since an additional effects could be derived from muscarinic receptor antagonism. At postsynaptic muscarinic receptor sites, acetylcholine acts by reducing potassium conductance making the cholinoreceptive neuron more susceptible to other excitatory inputs (19). It is unclear e.g. whether a treatment should enhance or diminish glutamatergic transmission, since a positive modula-

tion could facilitate learning while increasing glutamate function may enhance excitoxicity and neuronal death (15).

According to experimental observations, the number of presynaptic M2 receptors is diminished, whereas the postsynaptic M1 receptors are relatively preserved (16). The decreased number of nicotinic acetylcholine receptors in the cortex and hippocampus associated with AD and the beneficial effects of nicotine observed in AD patients suggests the important role of nicotinic receptors (3). Presynaptic nicotinic receptors have the key role in the regulation of release of many transmitters such as acetylcholine, glutamate, serotonine and GABA. However, AD involves more than cholinergic deficiency. In brains of patients with AD, several other transmitters are depleted.

In connection with the pathogenesis of AD, the noncatalytic and nonspecific role of acetylcholinesterase is being discussed.

Much experimental evidence shows that one of the earliest changes in the pathogenesis of AD is the formation of β amyloid protein in brain neurophil (27). Acetylcholinesterase colocalizes with β amyloid deposits in AD brains. This has led to the suggestion that senile plaques may represent degenerated endings of the cholinergic fibers arising in the nucleus basalis (28).

Acetylcholinesterase accelerates amyloid formation (1) and these authors suggest that it may therefore act as a pathological chaperone inducing a conformational transition of β amyloid protein.

Moreover, acetylcholinesterase activity associated with plaques, tangles and amyloid angiopathies possesses different enzymatic properties and quite possibly is of a different source as compared with the enzyme associated with normal neurons and axons. The postulated function includes acting as proteases/peptidases. It can thus participate directly not only in the processing of amyloid protein but also in causing aberrant growth and development of neurons (9).

Pathological lesions characteristic for AD are observed mostly during post mortem examinations of brains of AD patients. Neurochemical studies of post mortem brains contributed to the knowledge about the pathophysiology of AD, but simultaneously they shown that such measurements are affected by the clinical state of the patient before death, by medication and by the post mortem interval. The levels of many neurotransmitters and the activities of many enzymes change very rapidly after death. The nerve tissue of patients is not usually available for laboratory studies during the patient's life, the models of other peripheral cells are used. The strategy of such approach suggests that the molecular signal leading to the development of characteristic lesions does exist. The discovery of genes involved in the pathogenesis of AD (10) and the ability of thrombocytes to produce amyloid precursor protein - the source of β amyloid (7) - supports this approach.

Acetylcholinesterase is present in the plasma membrane of blood cells, despite the fact that its role in the hematopoietic cells is not explained.

Therapeutic application of acetylcholinesterase inhibitors requires the need to monitor the activity of this enzyme on the periphery. Since the brain is the target organ for all potential cholinergic drugs, any peripheral measures can provide important information about a compound efficacy and mechanism of action. Such studies are relatively non-invasive, and simple. They also bring a hope for the discovery of biological markers for the diagnosis of the early and middle stages of AD. Some hypotheses suggest that pathogenesis of AD may be connected with a systemic cholinesterase abnormality (12) or with damage to the blood-brain barrier, allowing acetylcholinesterase to leak from the brain to the plasma (25).

Cholinesterase in blood and blood elements

The high activity of cholinesterase in human blood was described in 1928 (8), although the physiological meaning of this activity is still unclear. Many studies reveal the different but overlapping substrate specificity of two main types of cholinesterase: the specific acetylcholinesterase (EC 3.1.1.7) and the non-specific butyrylcholinesterase (EC 3.1.1.8), which are inhibited differentlly by various cholinesterase inhibitors including both organophosphate poisons (26) and therapeutically used drugs. The most selective inhibitor of butyrylcholinesterase seems to be ethopropazine (20); acetylcholinesterase is relatively selectively inhibited by edrophonium. The levels of butyrylcholinesterase in human blood plasma are about 1000 times greater than acetylcholinesterase levels, while rodents have high levels of acetylcholinesterase (34). Skau concluded that it is unlikely that the function of blood-borne cholinesterase is the hydrolysis of acetylcholine, since other tissues contain more than sufficient amounts of this enzyme. Butyrylcholinesterase is synthesized in the liver, and its level of plasma may be affected by liver diseases (40).

These two distinct enzymes are coded by two different, but related, genes. At least 11 silent variants of human butyrylcholinesterase have been identified, but until now, no real evidence has been provided for clinical value for their use in diagnosis of AD (17) and it seems that healthy people with no detectable plasma cholinesterase activity appear to be at no disadvantage. In a study of physostigmine in senile dementia patients, no relationship between plasma cholinesterase activity and memory tests performance was found (32).Our study of measurement of butyrylcholinesterase in human blood during

aging and in patients with AD did not reveal any differences (36).

Human erythrocytes and other hematopoietic cells contain mostly specific acetylcholinesterase. This enzyme has been found also in erythrocytes of the guinea pig, horse, rabbit, and rat, the cat has none (41, 34). In this connection it is interesting that the animal studies of effects of cholinesterase inhibitors physostigmine and tacrine found a linear correlation between the cholinesterase activity in the brain, plasma, and erythrocytes (11, 31).

On the other hand, the reliable correlations between peripheral and central cholinesterase inhibition in humans depend on many factors and clearly vary from drug to drug. and require detailed pharmacokinetic studies. While the measurement of plasma butyrylcholinesterase does not seem to be a useful marker of acetylcholinesterase in the brain, there is still some hope that the measurement of erythrocyte acetylcholinesterase may be used as a marker of brain inhibition. However, the interpretation requires the standardisation of acetylcholinesterase measurements (26).

Molécular forms of acetylcholinesterase in the brain and in red blood cells

Detailed studies of the molecular structure of acetylcholinesterase have shown that this enzyme exists in many molecular forms. There are globular (G) and asymmetric (A) classes which (5) are further subdivided. A form consisting of one, two or three tetramers joined to three collagen-like tails is found predominantly at skeletal neuromuscular junctions anchored to the extracellular basal laminar membrane (13).

The globular forms predominate in mammalian brain. Most brain acetylcholinesterase consists of assemblies of four identical 77 kDa catalytic subunits linked by disulfidic bridges (G_4). The brain contains a small amount of monomeric G_1 form with a small amount of G_2 form (5). G_4 forms are localized presynaptically while the G_1 forms are postsynaptic (18). The only acetylcholinesterase molecular form found in erythrocytes is globular dimer G_2 (30). The enzyme is anchored in the membrane by a glycosylated phosphatidylinositol (PI). PI-specific phospholipase C releases acetylcholinesterase from porcine, bovine, and rat erythrocytes but not from human or murine red blood cells. This resistance of human erythrocyte acetylcholinesterase results from the presence of additional acyl chain on the inositol ring of PI (for a review see 29). Serum anchor-specific phospholipase D cleaves the human erythrocyte acetylcholinesterase anchor, but this treatment does not release hydrophilic acetylcholinesterase (38).

Despite this multiplicity all acetylcholinesterase molecular forms have equivalent catalytic activities (18), but some inhibitors affect them differently. For example, it is known that galanthamine produces greater inhibition in human erythrocytes than in human brain tissue.

The decrease of presynaptically bound G_4 and the increase of the soluble G_1 and G_2 forms was observed in the brains of aging rats (18). Similar changes were found in frontal and parietal cortex, in the hippocampus and in cholinergic projection nuclei in brains of AD patients (33, 21). In this connection, the therapeutic implication of G_1selective inhibitor eptastigmine (heptylphysostigmine) is suggested.

What can the study of erythrocyte acetylcholinesterase tell us?

The therapeutic use of acetylcholinesterase inhibitors directed the research on a catalytic function of this enzyme, and the membrane bound acetylcholinesterase in human red blood cells provide an accessible model. However, the different sensitivity of erythrocyte acetylcholinesterase to inhibitors should be taken into account for conclusions about the central effects.

However, the presence of acetylcholinesterase in the plasma membrane of erythrocytes does not seem to be connected with the processes of neurotransmission. Understanding the role of acetylcholinesterase could draw us nearer to understanding the dual role of acetylcholinesterase in the pathogenesis of AD.

The results of intensive research on acetylcholinesterase as a therapeutic target provide us with many parts of a puzzle and lead us to postulate many further questions. Is the degeneration of cholinergic neurons and the reduction of acetylcholine the primary change or is it evoked by the action of soluble acetylcholinesterase?

Abnormal expression of this enzyme has been detected around the amyloid plaques and neurofibrillary tangles in the brains of AD patients and accelerates amyloid formation. This action of acetylcholinesterase was not affected by edrophonium, an active site inhibitor, but it was affected by propidium, a peripheral anionic binding site ligand. Butyrylcholinesterase did not affect amyloid formation (13).

Can erythrocytes or thrombocytes release acetylcholinesterase into the brain?

Why there are so many forms of acetylcholinesterase?

From molecule to integrative view

The attempts to find one enzyme or one receptor which could explain the pathophysiological basis of disease or to be targeted by therapy do not seem to be realistic solution in case of AD. Strategies to affect one enzyme pharmaco-logically are followed by many side effects.

For example the initial studies of the effect of tacrine showed its hepatotoxicity. The AD patients with two epsilon 4 alleles do not respond to such therapy and had higher acetylcholinesterase activity in cerebrospinal fluid than controls and AD patients with one or no epsilon 4 (35). Agonists of muscarinic receptors stimulate the formation of β amyloid whose presence in the membranes induces the uncoupling of muscarinic receptors from phospholipase C.

The diverzity of molecules is expressed on all levels of signal transduction: in the multiplicity of receptor molecules and molecules of effectors. Why there are so many receptors? Molecular biology shows that such diversity is coded genetically. E.g. 12 genes were found for nicotinic cholinergic receptors; the synthesis of muscarinic receptors is coded by five separate genes (39). Different subtypes of these receptors have different physiological and pharmacological characteristics. Muscarinic cholinergic receptors M1, M3, and M5 stimulate the generation of cAMP, but in the case of M1 such stimulation is secondary via the activation of phospholipase C, production of inositol 1,4,5 trisphosphate and calcium mobilization. These receptors also stimulate the activation of phospholipase A_2 phospholipase D, tyrosine kinase and activate voltage gated calcium channels. On the other hand, muscarinic receptors m2 and m4 inhibit the activity of adenylate cyclase, calcium influx and activity of phospholipase A_2. Many reactions proceed simultaneously or are overlapping.

At present, we have no conceptual framework which could allow us to integrate all knowledge about the regulatory mechanisms. The discovery of AD prevention probably waits for a change of a concept and a strategy of scientific research from the reductionistic approach, which supplies us with the parts of the puzzle, to the integration of these parts into a multidimensional and nonlinear whole. Attempts to interfere in the whole pharmacologically may find out large problems. Experience with the therapeutic use of acetylcholinesterase inhibitors demonstrates the variability of adverse effects.

AD is probably an example of a multifactorial disease whose etiology cannot be understood on the basis of one altered molecule, but we can come to the complex integrative view through the detailed study of individual molecules.

Clinical experience with the therapy of AD seems to be paradoxical and anecdotal in comparison with our knowledge of a healthy way of life (37). Phosphatidylcholin and cholesterol from egg yolk may be beneficial for patients with AD. Smoking may reduce the incidence of AD and the administration of nicotine enhances the cognitive functions which are impaired in AD. Indebenon has nootropic effects in aging brains despite the fact that its reduced forms generate superoxid free radicals. And finally, if we want to reduce acetylcholinesterase activity, let us add, few aluminium with fluoride. Moreover, there have been reports of AD patients who having lost their ability to speak and recognize familiar faces regained these functions during the process of dying. This suggests that neuronal death is not so important for human consciousness.

AD is the new threat of civilization epidemy for the next century. Shall we find the highest regulatory factor which determines the program shifting the body to the symptoms of AD?

Our compassion and respect for the elderly may help us in our efforts to find the way to prevention of this devastating disease.

This work has been supported by grant from the Charles University, Prague (No. 113/1998/BBio/PřfF).

References

1. ALVAREZ, A., et al.: Acetylcholinesterase, a senile plaque component, affects the fibrillogenesis of amyloid-beta-peptides. Neurosci. Lett., 1995, vol. 201, no. 1, p. 49-52.
2. ALZHEIMER, A.: Uber eine eigenartige Erkrankung der Hirnrinde. Allg. Z. f. Psychiatrie, 1907, Jg. 151, S. 1105-1113.
3. AMERIC, SP. - WILLIAM, M.: Neuronal nicotinic acetylcholine receptors: novel targets for CNS therapeutics. In: Psychopharmacology: the fourth generation of progress. New York, Raven Press, 1995, p. 1001-1016.
4. BARTUS, RT., et al.: The cholinergic hypothesis of geriatric memory dysfunction. Science, 1982, vol. 217, p. 408-417.
5. BON, S. - VIGNY, M. - MASSOULIE, J.: Assymetric and globular forms of acetylcholinesterase in mammals and birds. Proc. Natl. Acad. Sci. USA, 1979, vol. 76, p. 2546-2550.
6. BOWEN, DM.: Biochemical assessment of neurotransmitter and metabolic dysfunction and cerebral atrophy in Alzheimer's disease. Banbury Rep., 1983, vol. 15, p. 219-230.
7. BUSH, AL., et al.: The amyloid precursor protein of Alzheimer's disease is released by human platelets. J. Biol. Chem., 1990, vol. 265, p. 15977-15983.
8. GALEHR, O. - PLATTNER, F.: Über das Schicksal des Acetylcholins im Blute. Pflugers Arch., 1928, Jg. 218, S. 488-505.
9. GEULA, C. - MESULAM, MM.: Cholinesterases and the pathology of Alzheimer's disease. Alzheimer Dis. Assoc. Disord., 1995, vol. 9, Suppl. 2, p. 23-28.
10. GOATE, AM.: Genetics of AD: where are we now? The News, 1996, vol. 3, p. 1-2.
11. HALLAK, M. - GIACOBINI, E.: A comparison of the effects of two inhibitors on brain cholinesterase. Neuropharmacol., 1987, vol. 26, p. 521-530.
12. HANIN, I., et al.: Blood choline and its meaning in psychiatric

and neurological disease states. In Pepeu, G., Ladinsky, H. (Eds.). Cholinergic mechanisms: Phylogenetic aspects, central and peripheral synapses and clinical significance. New York, Plenum Press, 1981, p. 901-920.
13. INESTROSA, N. C.- PERELMAN, A.: Distribution and anchoring of molecular forms of acetylcholinesterase. Trends Pharmacol. Sci., 1987, vol. 10, p. 325-329.
14. INESTROSA, NC., et al.: Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. Neuron, 1996, vol. 16, no. 4, p. 881-891.
15. KORNHUBER, J.: Paradoxical glutamate: important for memory but a potential killer of neurones. The News, 1996, vol. 3, p. 8-9.
16. MASH, DCC. - FLYNN, DD. - POTTER, LT.: Loss of M2 receptores in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. Science, 1985, vol. 228, p. 1115-1117.
17. McQUEEN, MJ.: Clinical and analytical considerations in the utilization of cholinesterase measurements. Clinica Chimica Acta, 1995, vol. 237, p. 91-105.
18. MENEGUZ, A. - BISSO, GM. - MICHALEK, H.: Agerelated changes in acetylcholinesterase and its molecular forms in various brain areas of rats. Neurochem. Res., 1992, vol. 17, p. 785-790.
19. MESULAM, MM.: The cholinergic connection in Alzheimer's disease. News in physiol. Sci., 1986, vol. 1, p. 107-109.
20. MIKALSEN. A. - ANDERSEN, RA. - ALEXANDER, J.: Use of ethopropazine and BW 284C51 as selective inhibitors for cholinesterases from various species. Comp. Biochem. Physiol. C, 1986, vol. 83, p. 447-449.
21. OGANE, N. - GIACOBINI, E. - STRUBLE, R.: Differential inhibition of acetylcholinesterase molecular forms in normal and Alzheimer's disease brain. Brain Res., 1992, vol. 589, p. 307-312.
22. PATOČKA, J.: Cholinergní hypotéza Alzheimerovy choroby - teoretické východisko pro racionální léčbu. In Sikora, J., et al. (Eds.). Biologické podklady psychických poruch. Praha, Galén, 1997, s. 193-194.
23. PATOČKA, J. - FUSEK, J.: Současné trendy ve vývoji látek ze skupiny inhibitorů cholinesteráz jako léčiv Alzheimerovy choroby. Čs. Psychiatrie, 1992, roč. 88, s. 258-268.
24. PATOČKA, J. - ŘÍPOVÁ, D.: Od objevu Alzheimerovy choroby uplynulo 90 let. Psychiatrie, 1998, roč. 2, č. 1, s. 46-48.
25. PERRY, RH., et al.: Plasma and erythrocyte acetylcholinesterase in senile dementia of Alzheimer type. Lancet, 1982, i, p. 174-175.
26. PORTMAN, R. - HOFMAN, W.: Standardisation of acetylcholinesterase activity measurements. Voj. zdrav. Listy, 1997, roč. 66, č. 2, s. 38.
27. PRICE, DL. - SISODIA, SS. - GANDY, SE.: Curr. Opin. Neurol., 1995, vol. 8, p.268-274.
28. PRICE, DL., et al.: Basal forebrain cholinergic neurons and neuritic plaques in primate brain. In R. Katzman (Ed.), Biological aspects of Alzheimer's disease. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1983, p. 65-76.
29. RICHIER, P. - ARPAGUS, M. - TOUTANT, JP.: Glykolipid-anchored acetylcholinesterases from rabbit lymphocytes and erythrocytes differ in their sensitivity to phosphatidylinositol-specific phospholipase C. Biochim. Biophys. Acta, 1992, vol. 1112, p. 83-88.
30. ROSENBERRY, TL. - SCOGGIN, DM.: Structure of human erythrocyte acetylcholinesterase. J. Biol. Chem., 1984, vol. 259, p. 5643-5652.
31. SHERMAN, KA. - MESSAMORE, E.: Cholinesterase inhibitor therapy for Alzheimer dementia: what do animal models tell us? Prog. Clin. Biol. Res., 1989, vol. 17, p. 1209-1222.
32. SHERMAN, KA., et al.: Effect of oral physostigmine in senile dementia patients: utility of blood cholinesterase inhibition and neuroendocrine responses to define pharmacokinetics and pharmacodynamics. In Strong, R. (Ed), Central nervous system disorders of aging: Strategies for intervention. Vol. 33. New York, Raven Press, 1987, p. 71-90.
33. SCHEGG, KM., et al.: Soluble and membrane-bound forms of brain acetylcholinesterase in Alzheimer's disease. Neurobiol. Aging, 1992,1 vol. 3, p. 697-704.
34. SKAU, KA.: Acetylcholinesterase molecular forms in serum and erythrocytes of laboratory animals. Comp. Biochem. Physiol., 1985, vol. 80C, p. 207-210.
35. SOININEN, H., et al.: Increased acetylcholinesterase activity in the CSF of Alzheimer patients carrying apolipoprotein epsilon 4 allele. Neuroreport, 1995, vol. 6, no. 18, p. 2518-2520.
36. STRUNECKÁ, A. - ŘÍPOVÁ, D.: Strategie výzkumu Alzheimerovy choroby: od redukcionismu k integrativnímu celku. Čes. Slov. Psychiatrie, 1997, vol. 5, p. 245-257.
37. STRUNECKÁ, A., et al.: Serum cholinesterase and red blood cell acetylcholinesterase activity in patients with Alzheimer's disease. Eur. Neuropsychopharmacol., 1998, p. 240.
38. TOUTANT, JP., et al.: Conversion of human erythrocyte acetylcholinesterase from an amphiphilic to a hydrophilic form by phosphatidylinositol-specific phospholipase C and serum phospholipase D. Eur. J. Biochem., 1989, vol. 180, p. 503-508.
39. VIDAL, C. - CHANGEUX, JP.: Neuronal nicotinic acetylcholine receptors in the brain. News in physiol. Sci., 1996, vol. 11, p. 202-207.
40. WHITTAKER, M.: Cholinesterase. In Bergman, E. (Ed), Monographs in human genetics. 1986, vol. 11, p. 81-90.
41. ZAJICEK, J.: Studies on the histogenesis of blood platelets and megakaryocytes. Acta Physiol. Scand., 1957, vol. 40, Suppl. 138.

Correspondence: Prof. RNDr. Anna Strunecká, DrSc.
Přírodovědecká fakulta Karlovy univerzity
Viničná 7
128 00 Praha

Received: 13. 10. 1998