CHAPTER 1

Introduction to Alzheimer’s Disease
Dr. ALOIS ALZHEIMER

1864 To 1915
D. Auguste

Alois Alzheimer’s First Patient (1906)
1.1 INTRODUCTION

Ageing is often recorded as the main factor in memory, impairment and decline in other mental functions or dementia. Memory impairment is normal part of the ageing process\(^1\). Memory loss and other neuropsychological symptoms such as impairments of judgements, language, learning and abstract thinking may be attributed to normal ageing. However Alzheimer’s disease, (AD) is the dementia and other neuropsychology manifesting are progressive increasingly severe and non-reversible. The first patient recorded to be identified with AD was Mrs. Auguste D of ages 51 by Dr. Alois Alzheimer a German psychiatrist. Dr. Alzheimer later worked in the laboratory of esteemed Emil Kraepelin in Munich Germany. Kraepelin the author of a leading textbook in psychiatry was a strong believer that neuropathology could be linked to clinical psychiatric function. Early in April 1906, when Mrs. Auguste D died, Dr. Alzheimer worked with two Italian physicians to examine her anatomy and neuropathology. On November 3, 1906, he presented Auguste D’s case to the 37th Assembly of Southwest German psychiatrists and described the neurofibrillary tangles, amyloid plaques which were the hallmark of the disease. Kraepelin later wrote about this case and others in his “Textbook for Students & Doctors” and indexed these under AD. By 1910, the name of the disease was well established among the specialist community\(^2\).

AD is a progressive, irreversible brain disorder with no known cause or cure. It attacks and slowly steals the minds of its victims. Symptoms of the disease include memory loss, confusion, impaired judgement, personality changes, disorientation and loss of language skills. Always fatal, AD is the most common form of irreversible dementia.

More than 4.5 million Americans are believed to be carriers of AD and by 2050, the number could rise to 13.2 million. Approximately 59,000 victims die and 350,000 new cases of AD are diagnosed every year. America is not alone in dealing with this terrible affliction. In every nation where life expectancy has increased, so has the incidence of AD. Tragically, AD has become common and worldwide. Currently 18 million people are suffering from this dreaded disease. This figure is projected to double to 34 million by 2025. The cause of AD was unclear, until the end of 1970’s.
The degeneration of the cholinergic system of the AD patients was observed by Davies and Maloney in 1976. Several studies have revealed evidence to support the theory that the loss of cholinergic function contributes to the cognitive deficits of AD. First, the amount of cholinergic neurons in the nucleus basalis (NB) and medial septum (MS) that project to the hippocampus, amygdala and cortex are reduced in AD patients. Second, the amount of choline acetyltransferase in the cortex and hippocampus is also reduced in AD patients (Perry et al. 1977). Third, drugs that block the effects of acetylcholine in the brain cause cognitive impairment in young control patients. Finally, and most convincingly, drugs that increase the effects of acetylcholine in the brain, acetylcholinesterase inhibitors, alleviate the symptoms of AD patients.

Amyloid precursor protein (APP) is proteolytically processed via alternative pathways. The cleavage of APP by the α-secretase occurs within the Aβ (amyloid β) domain, generating a large secretory form of APP (sAPP-α) and leaving a 83 amino acid long membrane-retained C-terminal fragment, and this therefore preclude the formation of Aβ. The Aβ peptides are produced when APP has undergone two sequential cleavages by the β- and γ-secretase, and this also produces a secreted APP derivative (sAPP-β). Multiple lines of evidence indicate that an aberrant APP metabolism leads to overproduction and, eventually, deposition of Aβ in the brain, the process that is considered to be central to the pathogenesis of AD.

In recent years, a number of studies have suggested that cholinergic neurotransmission may be involved in the regulation of APP metabolism (reviewed by Roberson and Harrell, 1997, and Rossner et al., 1998). It has been demonstrated in vitro that stimulation of M1 and M3 acetylcholine receptors with muscarinic receptor agonists or treatment with cholinesterase inhibitor (ChEI) greatly increases the release of secreted APP from cultured cells or brain slices, thereby leading to reduced formation of Aβ, presumably by the activation of the α-secretase pathway. On the other hand, cholinergic deafferentation in animals was shown to result in an elevated APP expression or production in the cerebral cortex or hippocampus, though whether this would lead to increased production of Aβ, and Aβ deposition has not yet been clarified due to lack of in vivo AD models. Together, these findings imply a role of cholinergic dysfunction in the promotion of Aβ production, and, further, a potential neuroprotective effect of ChEIs or muscarinic receptor agonists in preventing the
disease progress by restoring or enhancing cholinergic activity; these questions warrant further investigation in a suitable in vivo model of AD.

Aggregated Aβ has been suggested to be neurotoxic by many studies\(^\text{11}\). However, the relation between amyloid deposition and cognitive impairments in AD is still controversial. It was shown by several studies that the loss of synapses but not the number of amyloid plaques correlates with cognitive deficits in AD (reviewed by Terry et al., 1999). In contrast, several recent studies have suggested that the number of amyloid plaques or the Aβ load correlate with cognitive dysfunction\(^\text{12}\). In addition, the role of soluble Aβ behind the dementia symptoms was also emphasized by recent human neuropathological data\(^\text{13}\). With the advent of new therapeutic approaches, such as immunization against Aβ (Schenk et al., 2001), treatment with amyloid β-sheet breakers (Soto et al., 1998), and with β- and γ-secretase inhibitors\(^\text{14}\), it becomes essential to determine the role of Aβ in the development of cognitive impairments in AD.

**Symptoms of Alzheimer’s Disease**

Common early symptoms of Alzheimer's disease are,

1. Confusion
2. Disturbances in short-term memory
3. Problems with attention and spatial orientation
4. Personality changes
5. Language difficulties
6. Unexplained mood swings

It is important to understand that AD does not affect every patient in the same way. The stages listed below represent the general progression of the disease:

**Stage 1:** Early in the illness, Alzheimer's patients tend to have less energy and spontaneity, though often no one notices anything unusual. They exhibit minor memory loss and mood swings, and are slow to learn and react. After a while they start to shy
away from anything new and prefer the familiar. Memory loss begins to affect job performance. The patient is confused, gets lost easily, and exercises poor judgement.

**Stage 2:** In this stage, the Alzheimer's victim can still perform tasks independently, but may need assistance with more complicated activities. Speech and understanding becomes slower, and patients often lose their train of thought in mid-sentence. They may also get lost while traveling or forget to pay bills. As Alzheimer's victims become aware of this loss of control, they may become depressed, irritable and restless. The individual is clearly becoming disabled. The distant past may be recalled, while recent events are difficult to remember. Advancing Alzheimer's disease affects the victims' ability to comprehend where they are, the day and the time. Caregivers must give clear instructions and repeat them often. As the Alzheimer's victims mind continues to slip away, the patient may invent words and not recognize familiar faces.

**Stage 3:** During the final stage, patients lose the ability to chew and swallow. The very essence of the person is vanishing. Memory is now very poor and no one is recognizable. Patients lose bowel and bladder control, and eventually need constant care. They become vulnerable to pneumonia, infection and other illnesses. Respiratory problems worsen, particularly when the patient becomes bedridden. This terminal stage eventually leads to the tragic death.

**Clinical Features**

The usual first symptom noticed is memory loss which progresses from seemingly simple and often fluctuating forgetfulness (with which the disease should not be confused) to a more pervasive loss of recent memory, then of familiar and well-known skills or objects or persons. Aphasia, disorientation and disinhibition usually accompany the loss of memory. Alzheimer's disease may also include behavioural changes, such as outbursts of violence or excessive passivity in people who have no previous history of such behaviour. In the later stages, deterioration of musculature and mobility, leading to bed fastness, inability to feed, and incontinence will be seen if death from some external cause (e.g. heart attack or pneumonia) does not intervene. Average duration of the disease is approximately 7-10 years, although cases are known where reaching the final stage occurs within 4-5 years, or up to 25 years.
Diagnosis

There is no single test for Alzheimer’s disease, and diagnosis depends in part on excluding other potential causes of dementia. These include vascular dementia (often known as multi-infarct dementia, or MID), dementia with Lewy bodies (DLB), fronto temporal dementia (including Pick’s disease), Parkinson’s disease, and alcohol-related dementia (Korsakoff’s syndrome). GP suspects Alzheimer’s, symptoms as memory loss and verbal (speech) impairment. Physical examination and blood and urine tests can be carried out to help exclude other causes of confusion. If GP suspects Alzheimer’s disease but can’t show a positive diagnosis, the patient can be referred to a specialist (a neurologist, elderly physician or a psychiatrist) for more specialist tests. These may include the mini-mental state examination (MMSE), which includes a series of questions and tests which investigate memory, language and mathematical skills.

Other investigations may include a brain scan, typically magnetic resonance imaging (MRI). Some people may also be referred to a "memory clinic" specializing in mental state assessments.

1.1.1 Biochemical Characteristics of Alzheimer’s Disease

Alzheimer's disease has been identified as a protein misfolding disease due to the accumulation of abnormally folded amyloid beta protein in the brains of AD patients. Amyloid beta, also written Aβ, is a short peptide that is an abnormal proteolytic by product of the transmembrane protein amyloid precursor protein (APP), whose function is unclear but thought to be involved in neuronal development. The presenilins are components of proteolytic complex involved in APP processing and degradation. Although amyloid beta monomers are soluble and harmless, they undergo a dramatic conformational change at sufficiently high concentration to form a beta sheet-rich tertiary structure that aggregates to form amyloid fibrils that deposit outside neurons in dense formations known as senile plaques or neuritic plaques, in less dense aggregates as diffuse plaques, and sometimes in the walls of small blood vessels in the brain in a process called amyloid angiopathy or congophilic angiopathy.

AD is also considered a tauopathy due to abnormal aggregation of the tau protein, a microtubule-associated protein expressed in neurons that normally acts to
stabilize microtubules in the cell cytoskeleton. Like most microtubule-associated proteins, tau is normally regulated by phosphorylation. However, in AD patients, hyperphosphorylated tau are accumulated as paired helical filaments\(^\text{18}\) that in turn aggregate into masses inside nerve cell bodies known as neurofibrillary tangles and as dystrophic neuritis associated with amyloid plaques.

1.2 BASIC TYPES OF ALZHEIMER’S DISEASE

1.2.1 Familial Alzheimer’s Disease (FAD)

Familial AD (FAD) is a rare form of AD, affecting less than 10 percent of AD patients. It is associated with gene mutations on chromosomes 1, 14, and 21. In 1992, researchers at the University of Washington at Alzheimer’s disease Center (ADC) in Seattle, supported by NIA and the National Institute of Neurological Disorders and Stroke (NINDS), discovered a link between FAD cases and genes in a particular region of chromosome 14. They were then able to identify the defective gene which they named presenilin 1. Many cases of FAD are caused by presenilin 1. In FAD, all offspring in the same generation have a 50/50 chance of developing AD if 1 of their parents had it. FAD occurs in younger people (usually before age 60) than sporadic AD does. Almost all FAD cases are known so far with an early onset and tend to progress more quickly than the late-onset form of AD.

1.2.2 Sporadic Alzheimer’s Disease (SAD)

Sporadic AD (SAD) is far more common than FAD. It generally occurs later in life and appears to be related to the apoE gene found on chromosome 19. ApoE comes in several different forms or alleles, but three occur most frequently. People inherit one allele (apoE2, apoE3, or apoE4) of the apoE gene from each parent. Having one or two copies of the E4 allele increases a person's risk of getting AD. Having one or two E4 alleles of the apoE gene maximize a person's risk of AD, but not to 100 percent. AD researchers are studying people who inherit different forms of this gene to learn more about risk factors for AD. Scientists are yet to determine the exact degree of risk of AD for any given person based on apoE status.
Treatment of Alzheimer’s Disease

Unfortunately, so far there is no permanent cure for Alzheimer’s disease. However, the disease can be managed with drugs, and other treatments with support from a range of services.

Drug Treatment

Recently-available drugs called cholinesterase inhibitors are effective for drug treatment of Alzheimer’s in people suffering with Alzheimer’s, the cholinesterase breaks down and destroys acetylcholine, the neurotransmitter chemical. Cholinesterase inhibitors help to prevent this breakdown and so promote a more plentiful supply of acetylcholine. There are three such drugs available: donepezil hydrochloride (Aricept), rivastigmine (Exelon) and galantamine (Reminyl). In people in the early to middle stages of the disease, these drugs may slow down the progression of symptoms.

A newer treatment called memantine (Ebixa) has recently been launched for people in the middle to late stages of Alzheimer’s. It is not fully understood how well this drug works in practice, and it may not work for everyone. As a new drug that has not been reviewed by the National Institute of Clinical Excellence, memantine may not be readily available on NHS prescription.

Sometimes anti-depressants are prescribed to help treat the depression that can be associated with Alzheimer’s disease.

Other Treatments

There are psychological techniques for helping to cope with Alzheimer’s disease. These include techniques known as reality orientation, reminiscence therapy and validation therapy. Art and music therapies are also used, but their effectiveness is yet to be proven.

1.3 THE ALZHEIMER’S DISEASE BRAIN

AD is marked by a breakdown in communication among nerve cells. This breakdown results in a loss of function in the neurons and eventually cell death. Cell death is predominant in the region of the brain that is responsible for controlling
memory. In this region is a structure known as the hippocampus. When nerve cells die in the hippocampus short-term memory fails and a decline is seen in an individual’s ability to perform familiar tasks. Even greater damage is witnessed in the areas of the cerebral cortex that control reason and language. Gradually language skills and judgement becomes impaired and personality changes, such as emotional outbursts, wandering, agitation, and other disturbing behaviors, occur.

Figure 1: Normal Brain and Alzheimer’s Disease Brain

![Normal Brain and Alzheimer’s Disease Brain](image)

Figure 2: Normal Brain and Alzheimer’s Disease Brain
The presence of two abnormal structures in the brain, amyloid plaques and neurofibrillary tangles, have long been markers of AD (Figure 1 and 2). The formation of amyloid plaques and neurofibrillary tangles are thought to contribute to the degradation of the neurons (nerve cells) in the brain and the subsequent symptoms of Alzheimer's disease.

1.3.1 Amyloid Plaques

One of the hallmarks of Alzheimer's disease is the accumulation of amyloid plaques between nerve cells (neurons) in the brain. Amyloid is a general term for protein fragments that the body produces normally. Beta-amyloid is a fragment of a protein that is snipped from another protein called amyloid precursor protein (APP). In a healthy brain, these protein fragments would be broken down and eliminated. In Alzheimer's disease, the fragments accumulate to form hard, insoluble plaques.

1.3.2 Neurofibrillary Tangles

Neurofibrillary tangles consist of insoluble twisted fibers that are found inside the brain's cells. They primarily consist of a protein called tau, which forms part of a structure called a microtubule. The microtubule helps transport nutrients and other important substances from one part of the nerve cell to another. In Alzheimer's disease, however, the tau protein is abnormal and the microtubule structures collapse. The formation of amyloid plaques and neurofibrillary tangles are thought to contribute to the degradation of the neurons (nerve cells) in the brain and the subsequent symptoms of Alzheimer's disease.

1.4 MECHANISM OF ALZHEIMER'S DISEASE

Three major competing hypotheses exist to explain the cause of the disease. The oldest, on which most currently available drug therapies are based, is known as the "cholinergic hypothesis" and suggests that AD begins as a deficiency in the production of the neurotransmitter acetylcholine. The medications that treat acetylcholine deficiency have served to only treat symptoms of the disease and have neither halted nor reversed it. The cholinergic hypothesis has not maintained widespread support in the face of this evidence, although cholinergic effects have been proposed to initiate large-scale aggregation leading to generalized neuroinflammation.
Resent research hypotheses centered around the effects of the misfolded and aggregated proteins, amyloid beta and tau. The two positions are lightheartedly described as "ba-ptist" and "tau-ist" viewpoints in scientific publications by Alzheimer's disease researchers. "Tau-ists" believe that the tau protein abnormalities initiate the disease cascade, while "ba-ptists" believe that beta amyloid deposits are the causative factor in the disease. The tau hypothesis is supported by the long-standing observation that deposition of amyloid plaques do not correlate well with neuron loss. However, a majority of researchers support the alternative hypothesis that amyloid is the primary causative agent.

The amyloid hypothesis is initially compelling because the gene for the amyloid beta precursor APP is located on chromosome 21, and patients with trisomy 21 - better known as Down’s syndrome - who thus have an extra gene copy almost universally exhibit AD-like disorders by 40 years of age. The traditional formulation of the amyloid hypothesis points to the cytotoxicity of mature aggregated amyloid fibrils, which are believed to be the toxic form of the protein responsible for disrupting the cell's calcium ion homeostasis and thus inducing apoptosis. A more recent and widely supported hypothesis suggests that the cytotoxic species is an intermediate misfolded form of amyloid beta, neither a soluble monomer nor a mature aggregated polymer but an oligomeric species. Relevantly, much early development work on lead compounds has focused on the inhibition of fibrillization, but the toxic-oligomer theory would imply that prevention of oligomeric assembly is the more important process or that a better target lies upstream, for example in the inhibition of APP processing to amyloid beta.

It should be noted further that ApoE4, the major genetic risk factor for AD, leads to excess amyloid build up in the brain before arising of AD symptoms. Thus, beta-amyloid deposition precedes clinical AD. Another strong support for the amyloid hypothesis, which looks at the beta-amyloid as the common initiating factor for the Alzheimer’s disease, is that transgenic mice solely expressing a mutant human APP gene develop first diffuse and then fibrillar beta-amyloid plaques, associated with neuronal and microglial damage. And yet another support for the amyloid hypothesis comes from the knowledge of other amyloid diseases. Humans get many amyloid diseases, generally referred to as amyloidosis. Blocking the production of the
responsible amyloid protein (e.g., beta-amyloid in AD) can successfully treat these diseases.

![Figure 3: How the Brain and Nerve Cells Change During Alzheimer's Disease](image)

One of the hallmarks of Alzheimer's disease is the accumulation of amyloid plaques *between* nerve cells (neurons) in the brain. Amyloid is a general term for protein fragments that the body produces normally. Beta-amyloid is a fragment of a protein that is snipped from another protein called amyloid precursor protein (APP). In a healthy brain, these protein fragments would be broken down and eliminated. In Alzheimer's disease, the fragments accumulate to form hard, insoluble plaques.

Neurofibrillary tangles consist of insoluble twisted fibers that are found *inside* of the brain's cells. They primarily consist of a protein called tau, which forms part of a structure called a microtubule (Figure 3). The microtubule helps transport nutrients and other important substances from one part of the nerve cell to another (the axon is the long threadlike extension that conducts nerve impulses *away* from the body of a nerve cell, and dendrites are any of the short branched threadlike extensions that conduct nerve impulses *towards* the nerve cell body. In Alzheimer's disease the tau protein is abnormal and the microtubule structures collapse.

There is an overall shrinkage of brain tissue as Alzheimer's disease progresses. In addition, the ventricles, or chambers within the brain that contain cerebrospinal fluid,
are noticeably enlarged. In the early stages of Alzheimer's disease, short-term memory begins to decline when the cells in the hippocampus, which is part of the limbic system, degenerate. The ability to perform routine tasks also declines. As Alzheimer's disease spreads through the cerebral cortex (the outer layer of the brain), judgment declines, and emotional outbursts may occur and language is impaired. Progression of the disease leads to the death of more nerve cells and subsequent behaviour changes, such as wandering and agitation. The ability to recognize faces and to communicate is completely lost in the final stages. Patients lose bowel and bladder controls, and eventually need constant care. This stage of complete dependency may last for years before the patient dies. The average length of time from diagnosis to death is 4 to 8 years, although it can take 20 years or more for the disease to run its course.

1.5 CHOLINERGIC SYSTEM IN AGEING AND ALZHEIMER'S DISEASE

1.5.1 Cholinergic System in Ageing

The available data on age related changes in the cholinergic markers or neuronal counts from the basal forebrain of rodents are inconclusive. Discrepancies between data in several studies have resulted from the various methodological and animal species differences. This may also be compounded by other factors such as different age, gender and strain. For example, a reduction in size and number of ChAT/NGF receptor positive cells in the basal forebrain during ageing was reported in one study, while swelling of ChAT immuno positive neurons and no significant changes in the cholinergic cell numbers of MS and NbM during ageing were observed by other group (Armstrong et al., 1993). Although both these studies were conducted on rats, the animals were from different inbred strains. Similarly, inconsistent findings were reported from other cholinergic parameters as well. Significant age related reductions in ChAT activity of frontal and cerebral cortices were observed in aged rodents. However, changes in ChAT activity during ageing might also be sex dependent. Luine et al. (1986) showed that ChAT and AChE activity may differentially decrease in aged male and female rats than that in young ones.

Consistent results have been reported from sodium dependent high affinity choline uptake (HACU) studies. HACU shows the ability of cortical cholinergic synapses to absorb choline. As a matter of fact, HACU is the rate limiting step in ACh
synthesis. Therefore, this marker reflects the functional activity of the cholinergic system. Experimental studies showed that HACU could remain unaltered during ageing in rodents (Lebrun et al., 1990; Meyer et al., 1984; Sirviö et al., 1988).

1.5.2 Cholinergic System in Alzheimer's Disease

In 1974, Drachman and Leavitt demonstrated that the blockade of the cholinergic receptors in young healthy individuals produce a memory deficit, which is similar to that seen in AD patients\(^{38}\). Subsequently, a severe loss (up to 95%) of cholinergic markers in the cerebral cortex in AD subjects was independently reported by two research groups\(^{39}\). Later studies showed significant decreases (of varying extents, ranging between 15% and 95%) in the number of cholinergic neurons in the NbM of AD patients\(^{40}\). Furthermore, the severity of the cholinergic deficits in AD was found to be positively correlated with the severity and duration of the AD\(^{41}\). This encouraged the development and introduction of pharmacotherapies that would involve the cholinergic system modulating agents such as inhibitors of AChE (Orgogozo, 2003). However, the enthusiasm that cholinergic therapy may be used to eliminate memory and cognitive deficits in demented patients soon decreased. Clinical trials using these cholinergic drugs showed only modest improvements and could not restore cognitive function\(^{42}\). There are several factors that could influence such an outcome. First, cholinergic degeneration is not apparent in cases with mild cognitive impairment\(^{43}\). These individuals are the main target group for the disease prevention. Moreover, there is no general brain cholinergic system lesion in AD\(^{44}\). The cholinergic nuclei in the brainstem remain relatively intact in contrast to the basal forebrain cholinergic neurons co-expressing p75NTR. Finally, catecholaminergic neurons show even more prominent losses in activity at early stages of the disease\(^{45}\) than cholinergic cells. Therefore, the current treatment strategies that use cholinomimetics at preclinical or early stages of the disease might prove to be productive when combined with other therapeutic approaches than when used alone.

The cholinergic system is not the only neurotransmitter system that degenerates in AD. Other systems, such as the serotonergic and noradrenergic systems, are also affected by the disease (Mann 1983, Palmer et al. 1987). Indeed, the hallmarks of AD pathology, beta amyloid plaques and neurofibrillary tangles, are associated with most neuron types independent of the transmitter. Therefore, the basis of the cholinergic
therapy for AD is mainly symptomatic. However, some reports have suggested that the cholinergic therapy may also reduce amyloid accumulation. Nevertheless, since the cell loss becomes very substantial at the later stage of the disease, the beneficial symptomatic effects of cholinergic therapy are likely to be achieved only at the early stages of AD. Even though the effects of cholinergic therapy are modest so far, they are still important as even small improvements in the symptoms can significantly improve the quality of life and postpone the institutionalization.

The basic mechanisms by which the cholinergic therapy affects cognition are still poorly understood. Therefore, this study was designed to give valuable information about the mechanisms of cholinergic system and cholinergic therapy in cognitive processes.

1.5.3 Cholinergic Neurotransmission and APP Metabolism

Several lines of evidence suggest that modulating cholinergic neurotransmission can influence the metabolism of APP.

First, stimulation of M1 and M3 receptors with muscarinic receptor agonists or ChEI greatly increases the production of secreted APP with concomitant decreases in Aβ generation. It was first reported by Nitsch et al., 1992, using cells that were stably transfected with human mAChRs (M1, M2, M3, M4), that carbachol, a nonselective muscarinic receptor agonist, increased the amount of secreted APP released in cells expressing M1 and M3 receptors, but not in cells expressing the M2 or M4 subtypes. The increase of APP secretion could be blocked by the muscarinic antagonist atropine or by protein kinase C (PKC) inhibitors, suggesting that PKC mediates the receptor-controlled APP secretion. In addition, increased APP secretion from rat brain slices and cell cultures was also observed after indirect activation of muscarinic ACh receptors via the inhibition of AChE. Further, increased APP secretion has been demonstrated to be associated with a reduction of Aβ generation. Hung et al. (1993) have shown that in cell lines over expressing mAChR and huAPP with AD-associated mutations, the increase in APP secretion after mAChR stimulation is accompanied by a 50% reduction in the release of soluble Aβ and by an increase in the generation of the non-amyloidogenic p3 fragment. Based on these findings, it was postulated that the activation of M1/M3 mAChR-associated signaling pathways enhances α-secretase activity but decreases β-
secretase processing of APP (Nitsch and Growdon, 1994). In addition, it should be noted that few studies reported that treatment with nicotine, both in vitro and in vivo, could also modify APP processing by increasing the release of secretary form of APP\textsuperscript{48}.

Second, a reduction in cholinergic neurotransmission has been linked to enhanced APP expression/production, which may potentially lead to an increase in Aβ production. In rats, excitotoxic lesions of the cholinergic basal forebrain or transection of fimbria-fornix have been reported to increase the levels of APP mRNA or APP immuno reactivity in the cerebral cortex, or hippocampus\textsuperscript{49}. Similarly, selective cholinergic lesions of basal forebrain have also been shown to increase APP gene expression or protein level in rats and in marmosets. However, whether an enhanced APP expression/production would lead to increased production of Aβ, and further the deposition of Aβ has not yet been clarified due to a lack of in vivo AD models (Beach et al., 2000; Boncristiano et al., 2002). On the other hand, it should be noted that several studies have reported contrasting results showing a decrease or no changes in the APP levels after lesion of the cholinergic basal forebrain\textsuperscript{50}.

Together, these findings imply a role of cholinergic dysfunction in the promotion of Aβ production, and, further, support the notion that treatment with ChEIs may slow down the disease progression by modulating APP processing into the less amyloidogenic direction\textsuperscript{51}.

1.5.4 Cholinomimetic Therapy in Alzheimer’s Disease

A prediction of the cholinergic hypothesis is that drugs that potentiate central cholinergic function should improve cognition and perhaps even some of the behavioral problems experienced with Alzheimer’s disease. There are a number of approaches to the treatment of the cholinergic deficit in Alzheimer’s disease, most of which have initially focused on the replacement of ACh precursors (choline or lecithin) but these agents failed to increase central cholinergic activity. Other studies have investigated the use of ChE inhibitors that reduce the hydrolysis of ACh (figure, B)—for example, physostigmine. More recent investigational compounds include specific M1 muscarinic or nicotinic agonists, M2 muscarinic antagonists, or improved “second generation” ChE inhibitors.
Additional potential symptomatic therapeutic avenues relevant to the cholinergic hypothesis of Alzheimer's disease have resulted from the rapid development in the understanding of the molecular pathology of the disease. For example, during the development of cholinergic neurons in the basal forebrain, they express functional nerve growth factor (NGF) receptors. In adult life, these neurons seem to remain responsive to NGF. Consequently, intraventricular administration of NGF has been shown to prevent the lesion induced loss of cholinergic neuronal cell bodies and to accelerate the recovery of behavioral deficits in learning. Another approach is the transplantation of ACh rich foetal tissue grafts, which has been shown to improve the cognitive performance of primates after excitotoxic lesions of cholinergic nuclei. Thus, although such approaches may provide additional future possibilities for the palliative treatment for Alzheimer's disease, the use of ChE inhibitors is the most well developed approach for the treatment as on date.

1.6 MUSCARINIC RECEPTEORS

Acetylcholine and carbamylcholine can bind to both muscarinic and nicotinic receptors, yet the responses elicited by activating each receptor differ in several ways. Muscarinic responses are slower, and produce excitation or inhibitions which involve second messenger systems, rather than the direct opening of an ion channel. Muscarinic receptors are G protein-coupled receptors and mediate their responses by activating a cascade of intracellular pathways. Muscarine is the prototypical muscarinic agonist and derives from the fly agaric mushroom Amanita muscaria. Like acetylcholine, muscarine contains quaternary nitrogen important for action at the anionic site of the receptor (an aspartate residue in transmembrane domain III). Most muscarinic agonists obey the "rule of five" atoms from the quaternary ammonium moiety to the terminal atom.

Muscarinic receptors are found in the parasympathetic nervous system. Muscarinic receptors in smooth muscle regulate cardiac contractions, gut motility and bronchial constriction. Muscarinic receptors in exocrine glands stimulate gastric acid secretion, salivation and lacrimation. Muscarinic receptors also are found in the superior cervical ganglion where they can produce at least two physiologically distinct responses. In addition, muscarinic receptors are found throughout the brain, including the cerebral cortex, the striatum, the hippocampus, the thalamus and brainstem.
There are two different types of acetylcholine receptors – the nicotinic and muscarinic receptors. The muscarinic receptors are further classified into M₁–M₅ subtypes. The M₁ receptors are widely distributed throughout the neurons of the central nervous system. Coupled to stimulatory G-proteins (Figure 4), M₁ muscarinic acetylcholine receptors have a stimulatory effect on neurotransmission when bound by an agonist. M₃ and M₅ receptor subtypes also have a stimulatory effect on the target tissue, whereas the M₂ and M₄ subtypes are inhibitory.

**Figure 4: Mechanism and Action of Muscarinic Receptor**

In general the classical muscarinic antagonists such as atropine recognize a single class of binding sites as determined in binding assays. In the 1980’s, several selective muscarinic antagonists were identified. Pirenzepine 1 was very useful in the characterization of M₁ muscarinic receptors, while AF-DX 116 2 was used to identify M₂ receptors in the heart. M₃ receptors are found in smooth muscle and in both exocrine glands (e.g., lacrimal glands) and endocrine glands (e.g., pancreas). Muscarinic agonists bind heterogeneously to receptors in both the brain and peripheral nervous system.
In the late 1980’s, molecular cloning techniques identified five different subtypes of muscarinic receptors. Each receptor shares common features including specificity of binding for the agonists acetylcholine and carbamylcholine and the classical antagonists atropine and quinuclidinyl benzilate. Each receptor subtype couples to a second messenger system through an intervening G-protein. M1, M3 and M5 receptors stimulate phosphoinositide metabolism while M2 and M4 receptors inhibit adenylate cyclase. The tissue distribution differs for each subtype. M1 receptors are found in the forebrain, especially in the hippocampus and cerebral cortex. M2 receptors are found in the heart and brainstem while M3 receptors are found in smooth muscle, exocrine glands and the cerebral cortex. M4 receptors are found in the neostriatum and M5 receptor mRNA is found in the substantianigra, suggesting that M5 receptors may regulate dopamine release at terminals within the striatum. The structural requirements for activation of each subtype remain to be elucidated (Table 1).
The following is the summary of muscarinic acetylcholine receptor:

<table>
<thead>
<tr>
<th>Receptors</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
</tr>
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<tbody>
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<td>Cortex, Hippocampus</td>
<td>Herat</td>
<td>Exocrine glands, GI tract</td>
<td>Neostritum</td>
<td>Subtantianigra</td>
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<tr>
<td>Antagonists</td>
<td>Pirenzepine</td>
<td>AF-DX116</td>
<td>pF-HHSiD</td>
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<td>-</td>
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<td>Agonists</td>
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</tr>
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<td>Gαq/11</td>
<td>Gαi/o</td>
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<td>Adenylyl cyclase inhibition</td>
<td>Phospholipase Cβ</td>
<td>Adenylyl cyclase inhibition</td>
<td>Phospholipase Cβ</td>
</tr>
</tbody>
</table>

Table 1: Muscarinic Receptor Acetylcholine Receptor

1.6.1 Muscarinic Receptor Agonist

A muscarinic receptor agonist is an agent that enhances the activity of the muscarinic acetylcholine receptor. The muscarinic receptor has different has subtypes, labeled M1-m5, allowing for further differentiation.

In the form of pilocarpine muscarinic receptor agonist have been used medically for a long time. A number of muscarinic agonists have been developed and are under investigations to treat AD. These agents show promise as they are neurotropic, decrease amyloid deposition, and improve damage due to oxidative stress. Tau phosphorylation is decreased and cholinergic function enhanced. Notably several agents of the AF series of muscarinic agonists have become the focus of such research. AF102B, AF150(S), AS267B. in animal models that are mimicking the damage of AD, these agents appear promising

The ability for the quaternary ammonium group to fit in to anionic site on muscarinic receptor may be an important factor for the binding of a ligand to muscarinic receptor. For an example of the requirement of the quaternary amine moiety, consider that dimethylaminomethylacetate (the tertiary form of acetylcholine) is 1000-fold less than acetylcholine, in than acetylcholine, in part due to a lower affinity for the receptor.
The molecule of acetylcholine is flexible and may an infinite number of conformations from the extended to the quasi-ring structure. The three-membered ring of acetoxycyclopropyl-trimethylammonium iodide 3 demonstrates the concept that the extended form of acetylcholine exhibits the highest intrinsic activity. The trans isomer has much higher activity than the cis isomer which orients the ester and the quaternary amine together.

While the quaternary nitrogen is essential for eliciting full muscarinic responses with muscarinic agonists, there are a few potent muscarinic agents which contain tertiary amines (e.g., arecoline, oxotremorine and pilocarpine). They are potent both peripherally and centrally although they are of limited therapeutic value because of the wide range of cholinergic responses that they elicit. Oxotremorine 5 is of interest because of its ability to produce tremors, thereby providing an early model for Parkinson's disease.

Simple tertiary amines do not show considerable potency for the receptor, but this can be counteracted if the rest of the molecule binds potently to the receptor (e.g., through an ester bioisostere). Oxotremorine fills this role with an amide group in a
pyrrolidone ring as the nitrogen replaces oxygen in a hydrogen bond acceptor role. Arecoline (isolated originally from the betel nut) has a reversed ester acetylcholine profile, while Pilocarpine 6 has its ester in the cyclic form of a lactam ring, which may help increase the binding interaction. In general, it is important to have two sites for hydrogen bond acceptance in the ester isostere. The orientation of the ester isostere may be important for selective action as well.

![Pilocarpine](image)

The events associated with G protein-coupled receptor activation are as follows.

1. Agonist binds to the receptor, which has a high affinity for agonists at rest.

2. The binding of the agonist stabilizes a receptor conformation promotes receptor/G protein coupling and allows GTP to exchange for GDP on the G protein α subunit.

3. The binding of GTP leads to the dissociation of the G protein from the receptor, thereby lowering agonist affinity. The agonist then dissociates from the activated receptor.

4. The G protein consists of three subunits (α, β and γ) which also dissociate. The subunit activates the appropriate second messenger system (e.g., phospholipase C for M1 receptors). The β and γ subunits can exert independent actions.

5. The α subunit is inactivated by the hydrolysis of GTP to form GDP by a GTPase intrinsic to the G protein (GTPase activity may be activated by other intracellular proteins called GTPase activating proteins [GAPs]).
6. The α subunit (with GDP bound) can then recombine with the β and γ subunits. The receptor is then in a high affinity state and ready for the binding of another agonist.

1.6.2 Cognitive Functions Mediated by Muscarinic Receptor

Muscarinic receptors in the central nervous system (CNS) are involved in the regulation of learning and memory functions. Originally, Drachman and Leavitt (Drachman and Leavitt, 1974) found that the muscarinic antagonist, scopolamine, produced amnesia in young volunteers similar to that observed in aged non-demented people (Bartus et al., 1982). In rodents, scopolamine is known to disrupt performance in spatial learning and memory. However, the cognitive effects mediated by muscarinic receptors are not specific to learning and memory, since muscarinic receptors also mediate processes that are needed in the regulation of attention and arousal. It is possible, that disruption of attentional and other non-cognitive processes in the CNS is the actual cause of the learning and memory deficits caused by muscarinic antagonists.

Classically, muscarinic receptors have been divided into M1 and M2 receptors, with high and low affinities to muscarinic receptor antagonist pirenzepine, respectively. Muscarinic receptor subtypes mediate different aspects of behaviour, and there are reports indicating that postsynaptic M1 receptors mediate the performance-disrupting effects of scopolamine. For example, in the water maze and radial arm maze tasks, pirenzepine, a selective M1 antagonist, causes performance disturbance similar to that with scopolamine or atropine. In addition, pirenzepine has been reported to cause more specific effects on spatial short-term memory performance than scopolamine in the delayed non-matching to position task (DNMTP). In this study, the result of pirenzepine administration was a delay-dependent disruption of performance, whereas scopolamine induced a non-specific and delay-independent disruption of all task parameters, including motivation and motor performance (Andrews et al., 1994). In the same study, an M2 receptor antagonist (AFDX 116) had no effect on DNMTP performance, suggesting that the M2 receptors do not mediate the disrupting effect of muscarinic antagonists on spatial short-term memory. In theory, it is possible that blockade of presynaptic M2 receptors enhance the function of ACh system by...
increasing the release of ACh, which may lead to beneficial effects on cognitive behaviour.

1.6.3 Muscarinic Receptors in Ageing and Alzheimer’s Disease

Aged rats, spatial learning deficit in aged rats has been used as a model for the cognitive decline related to ageing and AD (Barnes, 1994). The anatomical and physiological changes occurring in the aged rodent brain resemble those occurring in AD. For example, the decrease in the ACh synthesizing enzyme ChAT during ageing may reflect degeneration of cholinergic cells in the basal forebrain, although the evidence supporting a ChAT decrease in the rodent brain is somewhat contradictory for both the cortex and HC. In addition, AChE activity is decreased in both AD (Namba et al., 2002) and rodent ageing. Aged rats, like young rats with hippocampus damage, show greater impairment in water maze learning than do young controls. Those aged rats that are most severely impaired in spatial tasks also have the greatest amount of degeneration in cholinergic cells in the basal forebrain, which further supports the view that the degeneration of the septohippocampal cholinergic system is linked to age-related learning impairment. Furthermore, the function of muscarinic receptors may be disrupted over the course of ageing, since both memory-impaired and memory-intact aged rats are more sensitive to the disrupting effects of scopolamine than young rats (Gallagher et al., 1994). The spatial learning deficit of aged rats can be used for investigating the efficacy of drugs in improving memory function, and the results from such studies may help in finding new treatments for the cognitive decline seen in AD.

1.6.4 Muscarinic Receptor 1 (M1 receptor) and Alzheimer’s Disease

M1 receptor is a G protein coupled receptor, located on outer surface of the cell membrane of neurons in the brain. It is a glycoprotein with molecular weight approximately 64 KD. Stimulation of the same will subside the formation of neurotoxic β amyloid via secondary messengers. Amyloid formation is an early event in brain’s of AD patients and defines much of the histopathology of AD. β amyloid is deposited in cerebral blood vessels, as they diffuse to extra cellular space may trigger neuritic reaction. The α β amyloid fragment deposited in AD brains is neurotoxic where as the N-terminal portion of APP may have neuroprotective and neurophilic effects formed by stimulation of M1 receptor.
1.6.5 Different Class of Arecoline Molecules in Research and their Muscarinic Activity

Arecoline stimulate muscarinic receptor because of its structural similarity with that of acetylcholine as shown below 7 and 8.

![Chemical structures of Arecoline and acetylcholine](image)

Arecoline bioisosters have general formulae as shown in 9 and the basic nucleus is tetrahydropyridine.

![Chemical structure of a general tetrahydropyridine](image)

Both affinity and efficacy are significantly enhanced by tetrahydropyridine series to M1 receptor, provides semirigid template, which has good affinity for the muscarinic receptor. If the molecule is flexible as that of acetylcholine, it interacts with different class of muscarinic receptors and lacks the specificity to interact with a specific muscarinic receptor. If the molecule is rigid, it is unable to stimulate different class of muscarinic receptors. Tetrahydropyridines are semirigid class which can bind specifically to M1 receptor and also provide some kind of flexibility to stimulate M1
receptor. N-methyl group on tetrahydropyridines makes the molecule selective towards M1 than M2 receptor.

1.6.7 General Structure Activity Relationship Studies of Arecoline Bioisosters

Extensive database have been developed and continued for better pharmacodynamic and pharmacokinetic parameters. Database developed is as follows:

1. In arecoline (10) ester group is prone to acid hydrolysis in stomach, it lacks specificity to M1 receptor and also it is carcinogenic according to studies reported64.

2. Quaternization of nitrogen of the arecoline produces equipotent M1 receptor agonist as compared to arecoline itself 65.

3. The secondary amine of nor arecoline (absence of CH3 group on ester of arecoline) is weaker muscarinic agonist (Bieger et al. 1970; Sauerberg et al. 1986).

4. In case of ester substituent on ester (-COOR), the affinity and biological activity increases in this order. Where, the triple bond of propargyl ester contributes to the receptor binding (Lambrecht and Mutschler 1981). R = CH3 < C2H5 < nC3H7 < -CH2 - CH = CH2 < = CH2 - C´CH

5. Reduction or removal of the ring double bond (between three and four position) reduces the muscarinic agonist activity by 250 to 1000 times (In 1, if the nitrogen of the arecoline is substituted by sulphur (bioisoster, in 1 where N= S), activity is retained, but not active as nitrogen in arecoline (Moser et al. 1983).

6. Introduction of another nitrogen in the ring of arecoline, to produce basic structures that is pyrimidine analogue, which gives less potent derivatives than arecoline itself (Messer et al. 1992).

7. N - CH3 group of arecoline produces selectivity of the basic structure to M1 receptor (Moltzen et al. 1994). Substitution at 3rd position of the ring increases the biological activities but at 4th substitution antagonizes M1 receptor activity and other substitution doesn’t have significant effect. The ligand is designed as tertiary nitrogen, which facilitates bioavailability (passage through blood brain
barrier), and after the passage the ligand is expected to be convert positive nitrogen (Nitrenium ion) in vivo oxidation in presence of mono amino oxidase, as supported by structurally related drugs or ligands (Eg. MPTP or Arecoline) and hence the, molecules will be highly reactive. Due to of the above structural and functional relations to M1 receptor, this basic structure and its analogues are selected for study. 3-Acetoxy quinuclidines are potent muscarinic and also thianium, piperidine derivatives of quinuclidine also provides potent muscarinic activity (11).

Where \( R = \text{CH}_3 \) for arecoline and \( X \) may be 11a and 11b

An alternative and better strategy to design arecoline derivatives, is by substituting the ester (because of non-specificity to the receptor, hydrolysis in the body and carcinogenicity in nature) by five or six membered heterocyclic ring (Lambrecht and Mutschler 1981) to produce better muscarinic agonist:-
a) In Five Membered Heterocycles, (11a)

1. Electronegative atom at 1st position increases the biological activity. Order of biological activity with respect to hetero atom, is as follows (Sauerberg et al. 1991). N > S > O.

2. Presence or absence of electronegative atom at 5th position of the arecoline ring doesn’t change the biological activity.

3. Electronegative atom as a part of the ring at 4th position increases the biological activity, in the order. N > S > O. d. SR, OR, group attached to 3rd position increases the biological activity. As the increase in the carbon number of R in SR or OR up to 6 number, increases the biological activity. [Hydrophobic nature increases binding to receptor, and avoids being washed out from receptor]

b) In Six Membered Heterocycles, (11b)

1. Electronegative atom at 1st position as part of ring of sublead increases the biological activity, in the order, N > S > O (Ward et al. 1992).

2. Electronegative atom at 4th position as a part of ring of sublead increases the biological activity, in the order, N > S > O.

3. SR, OR attached at 3rd position increases the biological activity. As the increase in the carbon number of R in SR or OR at 2nd position of sublead up to 6 numbers, increases the agonistic activity.
1.6.8 SAR of Arecoline Bioisosteres in which Arecoline Nucleus Linked to Different Substituents (R) through Various Functional Groups

**Ester linkage (12)**

![Ester linkage](image)

Ester linkage is prone to hydrolysis and some of its derivatives are carcinogenic.

1. When R substituent is H, straight or branched alkyl from one to six carbon atoms or cycloalkyl from four to eight carbon atoms, muscarinic activity increases up to two carbon atoms, beyond which the activity decreases (Butler et al. 1988).

2. When R substituent is straight or branched alkenyl from one to six carbon atoms, as carbon number increases, correspondingly activity also decreases.

3. When R is phenyl alkyl where in, the alkyl portion is straight or branched from one to six carbons and the phenyl ring may be unsubstituted or substituted with halogen, hydroxy, alkyl from one to six carbon atoms, or alkylxy from one to four carbon atoms, muscarinic activity decreases as the carbon length increases, as the substituted group on phenyl ring become electronegative, agonistic activity also increases.

**Amide Linkage (13)**

![Amide linkage](image)
1. When R1 and R2 are independently hydrogen or alkyl from one to four carbon atoms, muscarinic activity decreases as carbon length increases in R1 and R2 independently (Butler et al. 1988).

2. When group R1 is hydrogen and R2 is cycloalkyl from three to eight carbon atoms, muscarinic activity decreases as the carbon length increases in cycloalkyl ring.

3. If Group R1 is H and group R2 is benzyloxy, it increases the activity (Kelly et al. 2001).

4. Group R1 is H and group R2 is phenyl alkyl where in, the alkyl portion is straight or branched from one to six carbon atoms, phenyl ring may be unsubstituted or substituted with halogen, hydroxy, alkyl from one to six carbon atoms or alkyloxy from one to four carbon atoms, as carbon length decreases in alkyl portion and electronegativity of substituent on phenyl ring increases, proportionately muscarinic activity increases.

**Ketone Linkage (14)**

1. When R is pyrrolidinyl, piperidinyl, 4-diphenyl methylene piperziny1, azepinyl, morpholiny1, thiomorpholiny1, isoxazoly1, piperaziny1, pyrrolidiny1 and isoxzoly1 rings show good Muscarinic action than six numbered heterocyclic rings.

2. When R is 4-alkyl piperaziny1 ring where the alkyl group may be straight or branched alkyl from one to six carbon atoms, as the carbon length of alkyl chain increases, muscarinic activity decreases (Bergmeir et al. 1995).
Oxime Ether Linkage (15)

1. When R is straight or branched alkyl chain having one to four carbon atoms, muscarinic activity decreases as carbon length increases (Bergmeir et al. 1995).

2. When R1 substituent is straight or branched alkyl from one to six carbon atoms optionally substituted with hydroxyl or alkoxy from one to four carbon atoms, as carbon length of alkyl chain increases and electronegativity of group attached to alkyl chain increases, proportionally muscarinic activity increases.

3. When R1 is cycloalkyl of from three to eight carbon atoms where hydrocarbon chain of from one to four carbon atoms, muscarinic activity decreases as the carbon number increases in cycloalkyl ring.

1.7 Acetylcholinesterase Inhibitors

A variety of neurological and neuromuscular disorders involve a diminution of cholinergic activity. Often the most effective treatments are ligands, which inhibit the breakdown of acetylcholine. Acetylcholinesterase inhibitors have been used clinically in the treatment of myasthenia gravis, a degenerative neuromuscular disorder, glaucoma and more recently Alzheimer’s disease. In addition, cholinesterase inhibitors are widely utilized as pesticides and, if misused, can produce toxic responses in mammals and man.

1.7.1 Acetylcholinesterase: Structure, Function & Inhibition

AChE exists in two general classes of molecular forms: simple homomeromic oligomers of catalytic subunits (i.e., monomers, dimers, and tetramers) and
heteromeric association of catalytic subunits with structural subunits. The homomeric forms are found as soluble species in the cell, presumably destined for export, or associated with the outer membrane of the cell through either an intrinsic hydrophobic amino acid sequence or an attached glycopropholid. The other consists of tetramers of catalytic subunits, disulfide linked to each of three strands of a collagen-like structural subunit. This molecular species, whose molecular mass approaches 10^6 daltons, is associated with the basal lamina of junctional area of skeletal muscle.

Acetylcholinesterase is one of the most crucial enzymes for nerve response and function. Acetylcholinesterase (AChE) catalyzes the hydrolysis of acylcholinesters with a relative specificity for acetylcholine. The hydrolysis reaction proceeds by nucleophilic attack of the carbonyl carbon, acylating the enzyme and liberating choline.

Scheme 1

This is followed by a rapid hydrolysis of the acylated enzyme yielding acetic acid, and the restoration of the esteratic site. The pathway is similar to a pipeline, where the substrate goes in one end and the products come out the other through conformational changes, along with the hydrophobic and electrostatic forces (Scheme 1). Acetylcholine is the neurotransmitter common to many synapses throughout mammalian nervous systems.

Acetylcholinesterase is a tetrameric protein, which catalyzes the hydrolysis of acetylcholine. The active site of AChEase includes a serine hydroxyl group, which is rendered more nucleophilic through the proton-acceptor action of a nearby histidine residue. The serine residue exerts a nucleophilic attack on the carbonyl carbon of ACh. A tetrahedral transition state is reached, which results in serine acetylation and the loss
of free choline. The acetyl group binds to histidine as an N-acetate, but is hydrolyzed rapidly to yield free choline, acetate, and the free enzyme.

1.7.2 Mechanism of Action of Acetylcholinesterase Inhibitors

There are 3 different types of acetylcholinesterase inhibitors – short-acting, medium-duration and irreversible inhibitors, which differ in their interactions with the active site of acetylcholinesterase. Neostigmine is a medium-duration acetylcholinesterase inhibitor that enhances cholinergic transmission in the central nervous system, autonomic nervous system and at neuromuscular junctions. Acetylcholinesterase inhibitors provide an established therapy for Alzheimer’s disease and dementia (Figure 5).

![Diagram of Mechanism of Action of Acetylcholinesterase Inhibitors](image)

**Figure 5: Mechanism of Action of Acetylcholinesterase Inhibitors**
The mechanisms of action of compounds that typify the three classes of anti-chE agents. Three distinct domains on AChE constitute binding sites for inhibitory ligands and form the basis for specificity difference between AChE and butyrylcholinesterase; the acyl pocket of the peripheral amnioic site. Reversible inhibitors such as edrophonium and tacrine bind to the choline subside in the vicinity of tryptophan 86 and glutamate 202 (Silman and Sussman, 2000). Additional reversible inhibitors, such as donepezil, bind with higher affinity to the active centre. Drugs that have carbamoyl ester linkage, such as physostigmine and neostigmine, are hydrolysed by AChE, but much more slowly than is ACh. Both the quaternary amine neostigmine and the tertiary amine physostigmine exist as cation at physiological PH. By serving as an alternate substrate with a similar binding orientation as an acetylcholine attack by the active centre serine gives rise to the carbamoylated enzyme (Figure 6).

Quaternary compounds inhibit the enzyme reversibly by either binding with the esteratic site, or with a site spatially removed, termed the peripheral anionic site. Edrophonium binds reversibly and selectively to the active centre; this reversible binding and its rapid renal elimination result in its short duration of action.
Acetylcholinesterase (AChE) inhibitor indicated for the treatment of AD. Unlike currently marketed AChE inhibitors, it has a dual mechanism of action that also includes anti-amyloid activity, which may confer disease-modifying effects in patients with AD. If this is substantiated in an ongoing clinical trial then phenserine may open the door to an entirely new type of treatment for AD (Figure 7).

**Figure 7: Site of Action of AChE Inhibitors, the Major Class of Agents Currently Used in the Treatment of Alzheimer's Disease (AD)**

**1.7.3 Pharmacological Properties of Acetylcholinesterase**

Generally, the pharmacological properties of anti-ChE agents can be predicted by knowing those loci where ACh is released physiologically by nerve impulses, the degree of nerve impulse activity, and the response of the corresponding effector organs to ACh. The anti-ChE agents potentially can produce all the following effects:

1. Stimulation of muscarinic receptor responses at autonomic effector organs.
2. Stimulation, followed by depression or paralysis, of all autonomic ganglia and skeletal muscle (nicotinic actions).

3. Stimulation with occasional subsequent depression, of cholinergic receptor sites in the CNS.

In general compounds containing a quaternary ammonium group do not penetrate cell membrane readily; hence, anti-ChE agents in this category are absorbed poorly from the gastrointestinal tract or across the skin and are excluded from the CNS by the blood-brain barrier after moderate dose. On the other hand, such compounds act preferentially at the neuromuscular junctions of skeletal muscle, exerting their action both as anti-ChE agents and as direct agonist.

1.7.4 Significance of the AChEI on Alzheimer’s Disease

The alkaloids physostigmine and neostigmine act as metabolic inhibitors of acetylcholinesterase. The carbamyl ester formed by these compounds is much more stable than acetate (half-life measured in minutes as opposed to microseconds). The cholinesterase inhibitors are widely used to treat glaucoma (a disorder characterized by increased intraocular pressure). Acetylcholine reduces intraocular pressure, and cholinesterase inhibitors such as physostigmine are useful in treating the disease. Another major use of cholinesterase inhibitors is for treatment of myasthenia gravis, an autoimmune disease in which antibodies are formed against the nicotinic receptor at the neuromuscular junction. The antibodies bind to nicotinic receptors to cause a profound muscle weakness and paralysis. Cholinesterase inhibitors can alleviate the symptoms of myasthenia by improving muscle strength and endurance.

1.7.5 Reversible AChEIs

In contrast with the three above described classes of AChEIs, reversible AChEIs interact with the enzyme near its catalytic site, without producing a covalently modified complex. Three general structural families can be considered: aminoacridines, N-benzylpiperidines and some alkaloids (Aminoacridines Figure 8). The prototype of centrally active AChEI aminoacridine is tacrine, which was the first drug approved by US FDA for the treatment of AD in 1993. Tacrine is slightly more potent toward BuChE than toward AChE, and has other actions, including blocking sodium and
potassium channels and direct activity at muscarinic receptors and on other neurotransmitter systems, which might contribute to its demonstrated activity on AD. However, several disadvantages, such as short-half life, high incidence of side effects and especially induction of hepatotoxicity, have largely eliminated it from the market. Large numbers of analogues have been designed around tacrine. The hydroxy- and methoxy-derivatives velnacrine.

Figure 8: Structures of Aminoacridines and N-benzylpiperidines
1.8 Therapeutic and Experimental AChE Inhibitors

Recent efforts have been directed towards the development of novel strategies for the treatment of Alzheimer's disease. One strategy for the treatment of Alzheimer's patients has been the use of acetylcholinesterase inhibitors to increase the levels of acetylcholine in the synapse, thereby enhancing cholinergic activity in the affected brain regions. Physostigmine was used in early efforts to enhance cholinergic activity in the central nervous system although results were far from satisfactory.

Particularly relevant to these studies is the fact that therapy currently available for AD patients is limited to drugs which improve central cholinergic neurotransmission such as AChEI. These drugs control the key symptoms of Alzheimer's disease, namely memory, and cognitive impairment; however, since the final effect of these drugs is to improve cholinergic transmission. Number of authors have also sought to investigate whether these molecules may have an effect on APP processing with the potential to modulate the biochemical pathways involved in the pathogenesis of AD. Most of the molecules included in the studies that will be cited below are either currently used in AD pharmacotherapy or investigational drugs that are either under clinical evaluation or did not reach the market for various reasons, but were all tested as therapeutics for AD. This chapter will introduce briefly most of them.

1.8.1 Tacrine

Tacrine was initially synthesized more than 40 years ago, and was the first drug approved by the FDA for the treatment of AD in 1993. Tacrine is a centrally acting reversible cholinesterase inhibitor with additional pharmacological activity on monoamine levels, and ion channels. Tacrine appears to improve cognitive function, and behavioral deficits in patients with AD at doses ranging 80–160 mg/day, with a significant dose–response relationship. However, the molecule shows severe side
effects. In fact, large numbers of patients were withdrawn during the trials, many because of tacrine-associated increases in transaminase levels that in some cases reached clinical significance\textsuperscript{73-74}. These prominent side effects and the availability of other less toxic molecules today limit its usefulness in the treatment of AD.

1.8.2 Donepezil

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\text{\includegraphics[width=0.5\textwidth]{donepezil.png}}
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Donepezil was developed in order to overcome the disadvantages of physostigmine, and tacrine, and later approved by the FDA for treatment of AD. Donepezil is a piperidine-based, reversible acetylcholinesterase inhibitor, which is highly selective for acetylcholinesterase with a significantly lower affinity for butyrylcholinesterase. The clinical trials conducted for the evaluation of the drug provided evidence that donepezil 5 and 10 mg/day significantly improved cognition, and global clinical function compared with placebo. Beneficial effects on cognition were observed in short and long term trials demonstrating that the treatment (10 mg/day for most patients) significantly delayed symptomatic progression of the disease. The drug is generally well tolerated with typical cholinergic side effects including nausea, vomiting, diarrhea, constipation, some cases of headache, dizziness, and sleep disturbance, all of mild nature, and transient. Importantly, there has been no evidence of clinically significant changes in laboratory parameters, including liver function\textsuperscript{75-76}.

1.8.3 Rivastigmine

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\text{\includegraphics[width=0.5\textwidth]{rivastigmine.png}}
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Rivastigmine is a carbamylating, pseudo-reversible acetylcholinesterase inhibitor which in preclinical biochemical studies has shown significant central nervous system selectivity\(^\text{77}\). The preclinical experience has indicated efficacy in improving cognitive functions in rats with forebrain lesions, and led to the characterization of the drug in multiple clinical trials with the major phase III trials including more than 1500 treated patients. Data extracted from these trials indicate that rivastigmine 612 mg/day produces clinically significant benefits in domains related to cognition, daily activities, and behavior in patients with mild-to-moderate AD, with higher doses producing more benefits. The side effects experienced with rivastigmine are those expected from its pharmacological activity mostly affecting the gastrointestinal system. Sometimes, they may be severe enough to result in treatment withdrawal but more often they are mild to moderate, of short duration, and responsive to dosage reduction. They occur mostly during the dosage titration phase, and decrease during the maintenance phase; therefore, it is recommended that patients should always be started at a dose of 1.5 mg taken twice a day, and then have the dosage titrated to their maintenance dose\(^\text{78-80}\).

**1.8.4 Galantamine**

![Galantamine structure](image)

Galantamine is the last drug approved for the treatment of AD. It is a tertiary alkaloid with a unique, dual mode of action. It is a reversible, competitive AChE inhibitor, and also an allosteric modulator of nicotinic acetylcholine receptors (nAChRs)\(^\text{81-82}\). The clinical impact of this latter mechanism of action has not been fully elucidated. The efficacy of galantamine has been extensively studied in clinical trials that have demonstrated that a dosing regimen of 16–24 mg/day consistently produced beneficial effects on cognitive and non-cognitive AD symptoms. Improvements over placebo were observed in cognitive, and global function, ability to perform activities of
daily living (ADL), and behavior. Galantamine exhibits favorable pharmacokinetic characteristics including predictable linear elimination kinetics at the recommended maintenance doses (16 and 24 mg/day), a relatively short half-life (approximately 7 hours), and high bioavailability, and side effects include the predictable gastrointestinal upset which is transient with mild intensity, and easily controllable using the recommended slow dose-escalation scheme (reviewed in\textsuperscript{83-84}).

1.8.5 Metrifonate

\[
\begin{align*}
\text{O} & \text{OH} \text{Cl} \\
\text{O} & \text{Cl} \text{Cl} \\
\end{align*}
\]

Metrifonate (\(O, O\)-dimethyl-(1-hydroxy-2,2,2-trichloroethyl)-phosphonate) is a prodrug that, in aqueous solution spontaneously rearranges to the active organophosphate compound dichlorvos (2,2-dichlorovinyl-dimethyl-phosphate) or DDVP\textsuperscript{85}. The latter compound is an irreversible cholinesterase inhibitor that in preclinical studies showed improvement of cognitive performance in rats with a favorable tolerability. Metrifonate, administered orally to patients with probable Alzheimer’s disease in a once-daily dose, readily enters the brain, and inhibits AChE activity in a dose-dependent fashion. Data from preliminary and pivotal clinical studies performed in mild to moderate AD patients, have shown improvements of cognition or reduced rates of decline of cognition compared with placebo. In addition, the drug also benefited the global function of these patients including domains of cognition, function, activities of daily living, and behavior. The drug was well tolerated in all clinical studies with no significant laboratory abnormalities.
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