Pharmacological Drug Treatment of Alzheimer Disease: The Cholinergic Hypothesis Revisited

CHRISTOPHER J. LADNER, PhD, AND JOHN M. LEE, MD, PhD

INTRODUCTION

Alzheimer disease (AD) is a neurodegenerative disease affecting between 5% and 10% of the population (1) over the age of 65. Individuals with AD exhibit profound and disabling cognitive impairments (2) as well as disturbances of affect (3, 4). While the disease itself devastates the brain and mind, an individual with AD can live for years beyond the diagnosis of the disease. Although the emotional toll on the families and friends of AD patients is incalculable, conservative estimates place AD among the more costly diseases to society, at more than $47,000 in direct costs per case annually (1). At minimum, the total cost of AD to the United States economy is one-half trillion dollars (1). Given the current unprecedented, progressive extension of human life, AD is a significant hindrance to enjoyment of the full benefits of medical advances. Despite the prevalence of this devastating disorder, there is no cure and the treatments that are available provide temporary relief for only a fraction of AD victims.

There are 2 basic types of pharmacological treatments for any disease (including AD): symptomatic and preventative drug therapy. Both involve knowledge of the pathophysiology of a patient, but the preventative drug targets, particularly relevant to neurodegenerative diseases, are based primarily on an understanding of the etiology of the disease. Even though the etiology of AD is still debated, there are emerging clues which may prove helpful in developing appropriate drug targets. As of 1998, there are 2 FDA approved drugs on the market for the specific treatment of AD, tacrine and donepezil. Both were originally developed for the symptomatic treatment of AD based on their ability to increase CNS acetylcholine levels by blocking acetylcholinesterase. Interestingly, although the development of this class of compounds was for symptomatic treatment, they may also theoretically prevent and/or slow the progression of the disease, since activation of muscarinic cholinergic receptors, which are involved in behavioral effects as well as learning and memory, may also involved in the processing of the amyloid-β precursor protein (ABPP). In this review, we will revisit the cholinergic hypothesis of AD with regard to the rational development of therapeutic agents for the treatment of this devastating disease.

The Cholinergic System and Alzheimer Disease: An Historical Note

Alzheimer disease (AD) is characterized symptomatically by a progressive decline in cognitive function (2). Individuals with AD exhibit memory deficits that affect a number of tasks, including verbal recall (5), classical conditioning (6), and spatial memory (7). Changes in affect and personality also occur (3, 4). Brain areas implicated as neural substrates of learning and memory, including the entorhinal cortex, hippocampus, and amygdala, are severely affected in AD (8–10). Likewise, association neocortical areas involved in higher cognitive functions such as visual representations and judgment are also severely affected in the disease (11). An early and consistent biochemical finding in the brains of individuals with AD was a reduction in acetylcholine (Ach) neurotransmitter levels (12–15) and a loss of cholinergic innervation from the basal forebrain to the cerebral cortex, hippocampus, and amygdala (9, 10, 16, 17). These brain regions, in which the affected cholinergic projections terminate (15, 18, 19, 20), show the most extensive pathology in AD (21, 22). In addition, an early and consistently reproduced finding in AD is a profound reduction in the activity of the acetylcholine synthesizing enzyme, choline acetyltransferase (CHAT), in the cerebral cortex (13, 23, 24). An inverse relationship between premonobid cognitive impairment and CHAT activity in postmortem brain extracts of individuals with AD has been well established (24). Animal models have substantiated a primary role of acetylcholine in learning, since lesioning of cholinergic brain nuclei in rodents impairs learning and memory and replicates several of the biochemical and behavioral features of AD (25–28).

CNS Cholinergic Pharmacology

Acetylcholine exerts its CNS effects via its interaction with 2 classes of receptor proteins, the nicotinic and muscarinic receptors. While the nicotinic receptor acts as a ligand-gated cation channel, the muscarinic receptor mediates its effects through heterotrimeric guanine nucleotide-binding proteins (G proteins). These 2 classes of receptors were first distinguished by their responsiveness to different ligands, nicotine and muscarine, as well as the differential sensitivity of biological responses in different organs. The development of radiolabeled ligands revealed additional subtypes of both the nicotinic...
and muscarinic receptor families. Subsequently, the cloning of the genes encoding these 2 classes of receptors provided an additional layer of complexity. There are at least 5 genes encoding distinct muscarinic receptor proteins (29, 30). These studies confirmed that the pharmacologically defined M1, M2, and M3 receptors corresponded to the m1, m2 and m3 gene products. The m4 receptor exhibited properties of both M1 and M2 receptor subtypes (31). The m5 receptor, which is not expressed to any significant degree in the brain, has binding properties similar to that of the m3 Ach receptor (31). Nevertheless, the antagonist pirenzepine exhibited the greatest receptor subtype selectivity of all the muscarinic ligands tested to date, with the following rank order potency: m1 > m4 > m5 = m3 = m2 (31). While studies of ligand binding explain tissue differences in sensitivity to various antagonists, studies of the coupling of the various muscarinic receptors to the activation of G protein second messengers and effectors provide a further explanation for the diversity of responses to acetylcholine. On the other hand, nicotinic receptors in the CNS are primarily the α4β2 subtype and are outnumbered by muscarinic receptors 100- to 150-fold (14). Nicotinic receptors are primarily located on the presynaptic cholinergic terminals. In animal models, activation of nicotinic receptors causes an increase in presynaptic release of Ach. Therefore, nicotinic agonists have shown a positive feed forward effect on cholinergic function.

However, the predominant cholinergic receptor subtype in brain regions involved in learning and memory is the M1 Ach receptor (M1AChR). It has long been known that the pharmacological disruption of this cholinergic system by muscarinic antagonists produces impairments of short term memory in mice (32) and in humans (33). For example, the muscarinic antagonist scopolamine impairs human memory (33), while the acetylcholinesterase (AchE) inhibitor physostigmine, which elevates the synaptic concentrations of acetylcholine, enhances memory in normal human subjects (34). Other studies have shown that CNS administration of the M1AChR-selective antagonist pirenzepine impairs working memory in rats (35, 36, 37). These cognitive deficits were prevented by coadministration of muscarinic agonists (37). Collectively, these studies underscore the possibility that the deficiencies in the muscarinic cholinergic system might be involved in the mnemonic manifestations of AD.

Pharmacotherapeutic Approaches to the Treatment of Alzheimer Disease

Given the cholinergic system deficits in AD and the role of the cholinergic system in the processes of learning and memory, the development of therapeutics for AD has primarily focused on the cholinergic synapse. A simplified cholinergic synapse model is depicted in Figure 1. This therapeutic approach has led to the development of 2 basic classes of cholinomimetics: compounds that indirectly stimulate postsynaptic receptors by increasing the synaptic concentration of acetylcholine via inhibition of acetylcholinesterase (AchE) (e.g. tacrine, donepezil/E-200/Aricept, phystostigmine), direct muscarinic agonists (e.g. arecoline, milameline, bethanechol, xanomeline, talsicline), or direct nicotine agonists. A double-blind study of phystostigmine showed no beneficial effect in AD patients (38), while other studies of phystostigmine have shown small but significant improvements in cognition in some patients, particularly those early in the course of the disease (39, 40). Studies with the AchE inhibitor tacrine have generally yielded similar results. In a double-blind, multicenter, controlled clinical trial, tacrine used in combination with the choline precursor lecithin, produced minor, insignificant improvements in cognitive measures (41). Although this study used a relatively low dose of tacrine (100 mg/d), other studies with higher doses have shown small but significant effects on the cognitive symptoms of AD (42, 43). Additional studies suggest that tacrine is beneficial to a subset of individuals with AD depending on apo E genotype and gender. In general, the patients who respond best to tacrine are females of the apo E 2-3 genotype status compared with patients with apo E 4 genotype (44-46). In addition, patients with the Lewy Body variant of AD may be a subset of patients who respond better to tacrine (47). It has been reported that there is a greater cholinergic deficit in the Lewy body variant of AD compared with typical AD (47). This points out a vital role of the neuropsychologist in the evaluation of clinical drug trials. If the patients who respond best to current and new drugs are a particular pathological subgroup, this would be important information for both pharmacologists and clinicians.

While higher doses of tacrine are associated with greater effects on cognition, tacrine has several undesirable side effects, such as nausea and vomiting. Tacrine is also associated with significant hepatotoxicity (41). A recent 24-week, double-blind, placebo-controlled study of donepezil (Aricept), also a AchE inhibitor, shows that it is a well-tolerated drug that can slightly but significantly improve cognition and generalized function in mild to moderate AD patients (48). One of the differences between tacrine and donepezil is the relative selectivity of donepezil for acetylcholinesterases vs butyrylcholinesterases (49). This difference may be important, given that acetylcholinesterases are decreased in AD, whereas the butyrylcholinesterases are maintained. In addition, both drugs are reversible inhibitors of cholinesterases. There are now clinical trials of irreversible cholinesterase inhibitors such as ENA-713 (49) and metrifonate (49).

The AchE inhibition-based cholinomimetic strategy makes 2 basic assumptions: (a) sufficient cholinergic terminals are present to release acetylcholine into the syn-
meline, a selective agonist at the M1AchR, have shown some beneficial effects in the treatment of AD (53). While small but significant improvements in cognition were noted at the highest doses tested, xanomeline appeared to be most effective at treating the behavioral symptoms of AD (i.e. vocal outbursts, agitation, and hallucinations) (53). More than half of the patients receiving the highest doses withdrew from the study because of adverse gastrointestinal side effects. These side effects are not surprising, since agonist stimulation of M1AchRs outside the CNS results in increased acid secretion from parietal cells in the stomach. In fact, the M1AchR-selective antagonist pirenzepine is used clinically to treat excessive acid secretion in the gastrointestinal system. Xanomeline is the only M1AchR-selective agonist to reach clinical trials, although several others are reportedly in development (54). Even at the highest doses, xanomeline did not significantly ameliorate the memory deficits associated with AD. Agonist stimulation of the M1AchR suppresses the M current (55, 56) mediated by voltage-gated potassium channels (55–59). Inhibition of the M-current produces a slow excitatory postsynaptic voltage potentiation in these neurons (58) that facilitates the induction of long term potentiation (LTP) (60), a putative cellular mechanism of memory (61). Activation of M1AchRs has been shown to directly promote induction of LTP (62). Therefore, the M1AchR remains an attractive target for AD therapeutics, given its role in learning and memory.

The Molecular Mechanisms Underlying the Development of Alzheimer Disease Neuropathology and their Regulation by the Cholinergic System

Over the past few years, studies indicate that activation of the M1AchR in vivo may have an "anti-Alzheimer" effect by retarding production of the neurotoxic Aβ fragment of APP (63, 64). Amyloid-β peptide (Aβ), a major component of amyloid plaques, is derived from a larger integral membrane protein, amyloid-β precursor protein (AβPP) (Fig. 2) (65). The major Aβ-producing pathway is intracellular, occurring in the endosomal-lysosomal compartment (66), while a second, minor pathway involves the secretion of soluble, but potentially amyloidogenic, Aβ into the extracellular medium (67, 68). Despite the characterization of the substrates, intermediates, and products of AβPP metabolism, the isolation and identification of the specific proteases responsible for the various APP endoproteolytic activities has yet to be accomplished.

Aβ is a 39- to 42-amino acid fragment deposited in plaques (69), representing an internal sequence of AβPP. Cell culture studies examining the processing of AβPP have demonstrated that activation of protein kinase C (PKC) through M1AchRs or M3AchRs enhances the non-amyloidogenic processing of AβPP, thereby attenuating
the production of Aβ (64, 70). Direct stimulation of M1AchRs with carbachol produced a 3- to 5-fold increase in the secretion of a 90–100 kDa, soluble fragment (APPs) corresponding to the N-terminal of AβPP (64). APPs is produced from cleavage within the Aβ sequence, precluding the formation of Aβ. Additionally, indirect stimulation of muscarinic receptors via AChE inhibitors (e.g. physostigmine) produced a reduction in Aβ secretion in metabolically intact hippocampal slices (71).

In addition, a reciprocal relationship between M1AchRs-mediated increases in APPs release and reduction in Aβ has been demonstrated (72). Therefore, the net effect of muscarinic receptor activation is a stimulated α secretase activity and a potential decrease in the formation of amyloidogenic APP derivatives. This is schematically depicted in the top panel of Figure 3. In AD, a loss of cholinergic terminals in conjunction with the deficits in M1AchR-mediated signal transduction, see below, depicted in the lower panel of Figure 3, favors the processing of APP towards Aβ.

Besides muscarinic receptors, other G protein–coupled receptors also accelerate APPs production, including bradykinin (73), metabotropic glutamate (74), and serotonin 5HT2A and 5HT2C receptors (75). The effect of M1AchRs most likely occurs via Gα11-mediated activation of phospholipase C β and the liberation of diacylglyceride and Ca2+, both of which activate PKC. Direct activators of protein kinase C, such as phorbol esters, enhance APPs secretion (70, 72, 76) and inhibit Aβ production (72). Conversely, phosphatase inhibitors, such as okadaic acid and cyclosporin A (a specific inhibitor of calcineurin, protein phosphatase 2B), promote APPs secretion (76, 77).

In addition to potentially influencing the development of the amyloid pathology in AD, signal transduction at the M1AchR may play a role in the processes underlying NFT formation as well. The M1AchR has been shown to regulate the activity of both kinases that phosphorylate and phosphatases that dephosphorylate tau (Fig. 4). Activation of M1AchRs inhibits the activity of mitogen-activated protein (MAP) kinases (78). This class of kinases is capable of phosphorylating tau (79–81). A more direct role for M1AchR-mediated signal transduction in the regulation of tau phosphorylation has been shown in PC12 cells transfected with the M1AchR. Incubation with the muscarinic agonist carbachol resulted in a rapid and sustained reduction in the phosphorylation of tau (82). One possible mechanism for this finding is that stimulation of M1AchRs results in increased intracellular Ca2+ via activation of phospholipase Cβ and the generation of inositol phosphate 3 (IP3). This could result in the activation of protein phosphatase 2B/calcineurin via interactions with Ca2+ and calmodulin. Direct coupling of muscarinic receptor stimulation to calcineurin activation has been demonstrated (83). Therefore, a reduction in the efficiency of M1AchR signal transduction would be expected to favor increased phosphorylation of tau. While a role for M1AchRs in the regulation of tau phosphorylation is suggested by these studies, other processes may also be involved in the formation of NFT and dystrophic neurites.

Alterations in Cholinergic Muscarinic and Nicotinic Receptors in Alzheimer Disease

Cholinergic receptors have been studied extensively in postmortem brain tissue using receptor-selective radiolabeled ligands. While some discrepancies in the literature exist, in general nicotinic cholinergic receptor density is reduced in the cerebral cortex and hippocampus of individuals with AD relative to age-matched controls (14, 84–86). Most studies, including ours, have shown that there is a decrease in the number of nicotinic receptors in brain regions that contain the most AD lesions (J. Lee; personal observation). The decrease (20% to 50%) depends on the region studied. However, just like the dopaminergic system in Parkinson disease, if the presynaptic cholinergic fibers are being continuously lost, then nicotinic agonist effectiveness may be limited by the stage of the disease. The number of M2AchRs are also reduced in AD cerebral cortex (87). Like nicotinic receptors, M2AchRs are primarily located on presynaptic cholinergic terminals. On the other hand, M1AchR density measured with the M1AchR-selective antagonist [3H]pirenzepine is not changed in AD (14, 88, 89).

Although studies using the M1AchR selective antagonist pirenzepine found no difference in the absolute number of receptors between aged controls and AD
**Fig. 3.** M1AchR regulation of APP proteolytic processing. The top panel depicts the α-secretase pathway that is activated by stimulation of M1 and M3 muscarinic receptor subtypes. This pathway results in the cleavage of APP within the sequence that gives rise to formation of Aβ, thus forming nonamyloidogenic derivatives of APP. The bottom panel depicts the β- and γ-secretase activities that are thought to underlie the formation of Aβ, the primary component of neuritic plaques found in the brains of individuals with AD.

In cases, antagonist binding alone does not provide any information regarding the functional status of these receptors. A critical assumption of any therapeutic strategy targeting postsynaptic cholinergic receptors is that their signal transduction systems remain intact. As stated above, the M1AchR belongs to the family of metabotropic cholinergic neurotransmitter receptors that have a complex cellular signal transduction system. The 5 subtypes have been cloned and all possess the now classic 7 transmembrane helical domains of metabotropic receptors (30, 90). The portion of the receptor between the third and fourth, as well as fifth and sixth, transmembrane helical segments make up the second and third intracytoplasmic loops, respectively (i2 and i3). These cytoplasmic loops of the receptor interact with the heterotrimeric G proteins and are believed to represent the physical locus of receptor-G protein coupling (29). M1AchRs are functionally coupled to the activation of the Gs11 isoform of G proteins (91), which can then activate effector systems such as phospholipase C (92) and ion channels (93). Agonist affinity at the brain muscarinic receptors exists in 2 affinity states (high and low affinity) that are subject to modulation by guanine nucleotides (94, 95). In the presence of Mg2+ and low concentrations of guanine nucleotides, M1AchRs exhibit both low and high affinity sites for agonist binding with approximately one third of
Fig. 4. Role of M1AchR regulation of protein kinases and protein phosphatases in the development of the neurofibrillary pathology of AD. Activation of M1AchRs inhibits the activity of kinases which phosphorylate tau, as well as activating protein phosphatases that dephosphorylate tau.

all receptors in the high agonist affinity state. The presence of high concentrations of GTP (and GTP-analogs) favors the binding of GTP to G protein alpha subunits, and receptors exist predominately in the low-affinity state. These findings are consistent with a ternary complex model of ligand-receptor–G protein interactions (Fig. 5).

Although the M1AchR density is not changed, functional studies of M1AchR-mediated signal transduction have shown impairments in the AD brain. Studies examining M1AchR-G protein coupling in AD have demonstrated both a loss of high affinity agonist M1AchR sites as well as an insensitivity of the M1AchR to the effects of guanine nucleotides on agonist binding (81, 95, and Table 1). The reduction in high affinity agonist binding to the M1AchR most likely reflects a deficiency in receptor-G protein coupling and signifies an impairment in the initial step of signal transduction. Further evidence of impaired M1AchR–G protein coupling is evidenced by diminished sensitivity of agonist binding to the inhibitory effects of guanine nucleotides (95). The localization of this uncoupling at the receptor–G protein interface of the signal transduction cascade is supported by the finding of decreased carbachol-stimulated M1AchR-mediated activation of PLC in AD frontal cortex relative to controls without a change in the expression levels of Gq protein (96–98) or a change in the maximal PLC activity elicited by Ca2+ (97). We have also found that the ability of carbachol (a muscarinic agonist) to promote the binding of the radiolabeled hydrolysis-resistant GTP analogue [32P]azidoanilidoGTP ([32P]AAGTP) to the α subunit of Gq/11 in control and AD superior frontal cortex membrane preparations was changed. There was a decrease in the amount of [32P]AAGTP bound to G protein αq subunits obtained from moderate to severe AD brains relative to controls (Fig. 6). Western blots quantifying Gq/11 showed no difference in absolute protein levels (data not shown). Furthermore, we found a reduction of basal labeling of Gq/11 in AD compared with controls, which suggests an intrinsic defect in G protein function independent of agonist-induced coupling. This could also be the mechanism for the changes in high affinity agonist binding at the M1AchR. In addition, microscopic examination of sections from the regions used in our binding studies showed that the loss of high-affinity agonist binding at the M1AchR is associated with the presence of neuritic/core plaques, but not diffuse plaques or neurofibrillary tangles (Table 2).

Recently, Kelly et al (99) demonstrated that addition of Aβ to a primary rat cortical neuron culture directly impaired muscarinic receptor (most likely M1AchR) signal transduction. This effect of Aβ was ascribed to increased free radical formation as preincubation with antioxidants prevented uncoupling of muscarinic receptor stimulation by Aβ. These studies suggest impaired M1AchR-mediated signal transduction in AD might be a consequence of Aβ deposition. However, we have found a trend toward impaired M1AchR-stimulated labeling of Gq/11 even in several cases with mild neuropathological changes (Fig. 6). One interpretation of these findings is that the alteration in M1AchR-G protein coupling in AD precedes the development of the neuropathological changes. Taken together with Kelly et al (99), impaired M1AchR-G protein coupling would tend to decrease the metabolism of APP via the α-secretase pathway, potentially increasing the availability of APP as a substrate for the β/γ secretases. The increased formation of Aβ might then exert an additional inhibitory effect on M1AchR-
Fig. 5. Ternary complex model of M1 AchR-G protein coupling. Interactions between the M1 AchR and Gαq/11 stabilize a conformation of the receptor with relatively high affinity for agonist. This allows for the formation of the activated signal transducing unit consisting of a ternary complex of agonist-receptor-G protein. This complex can be measured by assaying for high-affinity agonist binding, measure of receptor-G protein coupling from the receptor perspective. In the presence of high concentrations of guanine nucleotides, such as GTP or nonhydrolyzable analogs such as GppNHp, the interaction between M1 AchR and G protein is hampered. These changes are attributable to reduced association of the α and βγ G protein subunits secondary to the binding of guanine nucleotides to the α subunits, resulting in decreased high-affinity agonist binding. The inhibitory effects of guanine nucleotides on high-affinity agonist binding to the receptor provide a measure of receptor-G protein coupling.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Group</th>
<th>log Kd_{low}</th>
<th>log Kd_{high}</th>
<th>% High Affinity Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbachol</td>
<td>Controls (n = 6)</td>
<td>-3.91 ± 0.08</td>
<td>-5.35 ± 0.16</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>O [\begin{array}{c} CH_3 \ H_2N-C-OCH_2CH_2-N-CH_3 \</td>
<td>AD (n = 8)</td>
<td>-4.01 ± 0.19</td>
<td>-5.94 ± 0.24</td>
<td>24 ± 5**</td>
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<td>\end{array} )</td>
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<tr>
<td>Acetylcholine</td>
<td>Controls (n = 6)</td>
<td>-4.13 ± 0.16</td>
<td>-5.49 ± 0.10</td>
<td>62 ± 4</td>
</tr>
<tr>
<td>O [\begin{array}{c} CH_3 \ CH_3-C-OCH_2CH_2-N-CH_3 \</td>
<td>AD (n = 8)</td>
<td>-4.31 ± 0.12</td>
<td>-5.84 ± 0.23</td>
<td>42 ± 6*</td>
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Mean values for Kd low and Kd high are the log molar (M) values ± S.E.M. Percent high affinity represents the percentage of M1 receptors in the high-affinity state (% high affinity = 1 - % low affinity). Group comparisons between controls and AD were done using a one-factor ANOVA. * indicates p < 0.05, ** indicates p < 0.01.
mediated signal transduction, resulting in a cycle of reduced signal transmission at M1AChRs and increasing deposition of Aβ. In addition, it appears that the impaired coupling between the M1AChR and Gq/11 appears to be specific for the dementia of AD. Cases with dementia attributable to other causes, such as Pick disease, progressive supranuclear palsy, and diffuse Lewy body disease, exhibited [32P]AAGTP-labeling comparable to age-matched controls.

In clinical settings, AD patients demonstrate changes in M1AChR function as well. In 1994, Scinto et al (100) reported that AD patients exhibited increased sensitivity to pupillary dilation in response to the muscarinic antagonist tropicamide. The receptor thought to mediate this response is the M1AChR and/or M3AChR. This study was also able to identify an individual who was not demented at the time of the test but who subsequently developed AD within a year after the test. These findings point to a deficiency in ternary complex formation of M1AChR and G proteins considering the following scenario. The addition of muscarinic antagonists promotes the dissociation of the ternary complex (101). Alzheimer disease patients presumably have fewer functional agonist ternary complexes of M1AChR-G protein. If so, then AD patients' pupils would be more sensitive to the effects of antagonists, which promote the dissociation of the ternary complex, of which the AD patients have fewer at the onset of their disease. While this interpretation is speculative, it is consistent with the in vitro data described above. Unfortunately, several groups have been unable to replicate this finding (102, 103), although these effects may be influenced by the apolipoprotein E genotype (104).

A recent study examining the effects of xanomeline, a selective M1AChR agonist, on blood pressure regulation in controls and AD patients also suggested changes in M1AChR function in AD (105). In healthy controls, xanomeline increased sympathetic tone as measured by an increase in blood pressure, heart rate, and plasma norepinephrine concentrations. In the AD patients, increases in heart rate and norepinephrine were observed; however, there was no significant increase in blood pressure in response to xanomeline. Furthermore, the AD patients were relatively insensitive to the orthostatic effects of an upright tilt test. In this test, subjects remain in a supine position for a period of time and are tilted at 60° for 45 minutes or until the individual faints. All 8 AD subjects tested tolerated the full 45 minutes of the tilt test, whereas a third of the healthy controls fainted during the tilt test. In addition, controls exhibited a significantly greater degree of orthostatic hypotension than did the AD cases.

In summary, several studies examining the G protein-coupled M1AChR have demonstrated both a loss of high-affinity agonist M1AChR sites in AD and an apparent insensitivity of M1AChR agonist binding to guanine nucleotides (88, 95, 97, 106, 107; see Table 3). A reduction in high-affinity agonist binding and a reduction in sensitivity of agonist binding to guanine nucleotides both indicate a loss of receptor-G protein coupling. This uncoupling of receptor stimulation to G protein activation signifies an impairment in the initial step of signal transduction. The localization of uncoupling at the receptor-G protein interface of the signal transduction cascade is supported by the finding of decreased carbachol-stimulated M1AChR-mediated activation of phosphatidylinositol 4,5 bisphosphate (PIP2) hydrolysis in AD frontal cortex relative to controls without a change in the expression levels of Gq protein (96, 97).

Currently, the mechanism(s) underlying the deficiency in M1AChR-mediated signal transduction in AD brain is not known. One general phenomenon that can occur at
TABLE 3
Experimental Evidence for Cholinergic Receptor on Coupling

<table>
<thead>
<tr>
<th>Reference</th>
<th>Measure of receptor-G protein coupling</th>
<th>Findings in Alzheimer disease</th>
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<tr>
<td>Smith et al, 1987 (95)</td>
<td>Inhibition of agonist binding at M1 receptor by GppNHp</td>
<td>Reduced sensitivity of agonist binding at M1 receptors to inhibitory effects in parietal lobe samples from AD patients</td>
</tr>
<tr>
<td>Flynn et al, 1991 (88)</td>
<td>High-affinity agonist binding at the M1 receptor</td>
<td>Reduction in high-affinity agonist binding in superior frontal cortex, which correlates with reduced ChAT activity.</td>
</tr>
<tr>
<td>Jope et al, 1994 (97)</td>
<td>Muscarinic receptor and GTPγS stimulation of phosphoinositide hydrolysis.</td>
<td>Reduced muscarinic and GTPγS stimulation of phospholipase C in AD prefrontal cortex without a change in Gq protein levels.</td>
</tr>
<tr>
<td>Ladner et al, 1995 (107)</td>
<td>High-affinity agonist binding at the M1 receptor</td>
<td>Reduction in high-affinity agonist binding in superior frontal and occipital cortex, which correlates with neuritic/core plaque density.</td>
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G protein-coupled receptors is desensitization (108, 109). Desensitization represents a decrease in the efficacy of receptor-mediated signal transduction and can be classified into homologous or heterologous desensitization. Homologous desensitization involves phosphorylation of receptor by a specific receptor kinase induced by continuous agonist exposure, while heterologous desensitization involves agonist independent phosphorylation of a receptor either via protein kinase C or through activation of a kinase through a different receptor. This phenomenon has been particularly well characterized for several receptor types, including muscarinic receptors, which are substrates for several kinases including PKC, calmodulin-dependent kinase II, and G protein receptor kinases (108, 110–112). The addition of a phosphate group to a serine and/or threonine residue(s) within the third intracytoplasmic loop and carboxy tail of the receptor may interfere with the ability of the G protein to interact with the receptor and produce a conformational change to reduce high-affinity agonist binding (see Fig. 5). Conversely, exposure of phosphorylated, desensitized muscarinic receptors to calcineurin dephosphorylates and resestilizes muscarinic receptors (112–114). Thus, it appears that phosphorylation imposes a barrier on receptor-G protein interactions to reduce signal transduction. While the concept of desensitization classically refers to the actions of a kinase, an impairment of a phosphatase functionally would have the same net effect. Preincubation of neuroblastoma cells with calmidazolium (an inhibitor of calcineurin) uncoupled muscarinic receptors from the stimulation of phospholipase C (115). Furthermore, a recent paper demonstrated that inhibition of calcineurin activity in adipocytes resulted in the desensitization and hyperphosphorylation of the β1-adrenergic receptor (116). Thus, inhibition of calcineurin activity has been shown to produce a desensitization of G protein–coupled receptors in a phosphorylation-dependent manner. This could be a mechanism, since we have found a reduction in calcineurin activity in brain areas that have uncoupled cholinergic receptors (107). Understanding the processes that render the M1AcHR insensitive to agonist stimulation would provide insight into the disease process and also suggest alternative pharmacotherapeutic targets for the treatment of AD.

Common Effector Systems/Common Effects

In addition to the cholinergic system, receptor-G-protein uncoupling has been reported in other neurotransmitter systems in AD. Uncoupling has been reported at D1 dopamine receptors in human frontal cortex from AD cases (117) and β-adrenergic receptors in cultured skin fibroblasts from AD patients (118). These receptors are coupled to the Gs isoform of G proteins, which stimulate adenyl cyclase. It has been demonstrated that a significant reduction in G protein activation of adenyl cyclase occurs in the frontal cortex in AD (119). On the other hand, serotonergic 5-HT1A and adrenergic α2 receptors in the frontal cortex remain coupled to Gi proteins (120, 121). Studies examining the levels of α G protein subunits in general have failed to detect AD-associated changes (97, 122). Thus, receptor–G protein uncoupling does not appear to be related to a particular neurotransmitter system, but to coupling of specific G protein systems. The adenyl cyclase (via Gs) and phosphoinositide hydrolysis (via Gq/11) systems appear to be particularly affected in AD. Therefore, it should be pointed out that the effects on M1 receptor coupling to Gq/11 may be generalized to any of the Gq/11 linked receptors, including the 5HT2 receptors, which are altered in AD as well.

CONCLUSION

Uncoupling of the M1AcHR from its respective G protein provides an explanation for the marginal success of
cholinomimetic pharmacotherapy in AD. It may also represent an underlying mechanism of the memory loss associated with AD. Our findings of an apparent relationship between M1AcHR-G protein uncoupling and neuritic amyloid core plaques suggests a possible pathological relationship. The restoration of M1AcHR-G protein coupling would have very practical consequences from a pharmacotherapeutic perspective. In addition, restoration of coupling or developing specific cholinergic drugs that may be able to overcome this blockade may enhance the effectiveness of cholinomimetic agents that are currently in use as well as those being developed. Furthermore, the M1AcHR and related Gq/11 linked receptor uncoupling may provide a unifying link between the symptomatology and pathology of this devastating disease.

Finally, it is important to emphasize the unique role that the neuropathologist should play in any assessment of clinical trials. Since there are pathological variants and overlapping syndromes in AD and many different responses to antemortem drug therapy, clinicopathological correlation of therapeutic responders and nonresponders will provide important information necessary for any future rational drug development.

REFERENCES


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