Published in final edited form as:

Acta Neuropathol. 2011 July ; 122(1): 49–60. doi:10.1007/s00401-011-0831-1.

PRE- AND POST-SYNAPTIC CORTICAL CHOLINERGIC DEFICITS ARE PROPORTIONAL TO AMYLOID PLAQUE PRESENCE AND DENSITY AT PRECLINICAL STAGES OF ALZHEIMER'S DISEASE

Pamela E. Potter^a, Paula K. Rauschkolb^a, Yoga Pandya^b, Lucia I. Sue^b, Marwan N. Sabbagh^b, Douglas G. Walker^b, and Thomas G. Beach^b

^aMidwestern University, Glendale, AZ

^bBanner Sun Health Research Institute, Sun City, AZ

Abstract

Amyloid imaging has identified cognitively normal older people with plaques as a group possibly at increased risk for developing Alzheimer's disease-related dementia. It is important to begin to thoroughly characterize this group so that preventative therapies might be tested. Existing cholinotropic agents are a logical choice for preventative therapy as experimental evidence suggests that they are anti-amyloidogenic and clinical trials have shown that they delay progression of mild cognitive impairment to dementia. A detailed understanding of the status of the cortical cholinergic system in preclinical AD is still lacking, however. For more than 30 years, depletion of the cortical cholinergic system has been known to be one of the characteristic features of AD. Reports to date have suggested that some cholinergic markers are altered prior to cognitive impairment while others may show changes only at later stages of dementia. These studies have generally been limited by relatively small sample sizes, long postmortem intervals and insufficient definition of control and AD subjects by the defining histopathology. We therefore examined pre- and post-synaptic elements of the cortical cholinergic system in frontal and parietal cortex in 87 deceased subjects, including non-demented elderly with and without amyloid plaques as well as demented persons with neuropathologically-confirmed AD. Choline acetyltransferase (ChAT) activity was used as a presynaptic marker while displacement of ³H-pirenzepine binding by oxotremorine-M in the presence and absence of GppNHp was used to assess postsynaptic M1 receptor coupling. The results indicate that cortical ChAT activity as well as M1 receptor coupling are both significantly decreased in non-demented elderly subjects with amyloid plaques and are more pronounced in subjects with AD and dementia. These findings confirm that cortical cholinergic dysfunction in AD begins at the preclinical stage of disease and suggest that cholinotropic agents currently used for AD treatment are a logical choice for preventative therapy.

Keywords

Alzheimer's disease; cholinergic; muscarinic receptor; G-protein; amyloid imaging; preventative therapy; asymptomatic

Introduction

In the early years of the last century, Alzheimer's disease was defined by the presence of microscopic lesions known as senile (amyloid) plaques and neurofibrillary tangles.

Pioneering biochemical investigations in the 1970s identified that AD subjects had significant losses of basal forebrain cholinergic neurons along with their cortically-projecting axons, leading to cholinergic replacement therapy, which remains the major treatment option. There has been a long-standing controversy, however, over where cholinergic degeneration occurs within the pathogenic sequence leading to AD. That cholinergic depletion is a primary pathogenic event was supported by multiple early studies showing that depletion of choline acetyltransferase (ChAT), the enzyme that converts choline to acetylcholine in presynaptic nerve terminals, could be detected soon after the onset of cognitive dysfunction, in some studies utilizing cerebral cortex biopsies (12,68,66). The findings that several other neurotransmitters were also depleted in AD cortex, and the failure of cholinergic therapy to reverse the disease course, changed this conception. The primary change in AD came to be accepted as death or dysfunction of cerebral cortex neurons due to local plaque and/or tangle burden while loss of basal forebrain cholinergic neurons and their cortically-projecting axons was relegated to a secondary, retrograde status (67). Subsequently, some research groups reported that decreases in cortical cholinergic presynaptic markers occurred only in the later clinical stages of AD (33,38,22,23), after the appearance of dementia. Other published studies indicated a more complex status, with some cortical and/or basal forebrain cholinergic markers being altered prior to the onset of mild cognitive impairment, whereas others showed changes only in later stages of dementia (3,4,83,57,58,31,32,14,39). Recent imaging investigations of the cortical cholinergic system in living human subjects have generally supported an early situation of the deficit, at the stage of mild cognitive impairment or mild dementia (50,60,77). Taken together, these findings suggest that the cortical cholinergic deficit begins at the preclinical stage of AD, which, as suggested by recent amyloid imaging results (75,53,56,73), may tentatively be defined as occurring in non-demented elderly individuals with cortical amyloid plaques.

Determining the stage of AD at which cholinergic deficits appear is not just an academic exercise. Events that occur near the beginning of disease pathogenesis are likely to be better therapeutic targets, as treatment at an earlier stage of disease will presumably prevent more of the downstream consequences of the disease process than treatment at a later stage. Despite an existing dogma that cholinergic agents do not modify the biological disease course, considerable evidence has accumulated to the contrary and has largely been ignored. *In vitro* studies have shown that M1 muscarinic receptor activation leads to changes in the cleavage pattern of the β -amyloid precursor protein (β APP) that favor decreased production of A β (21,27,16,17,25,62). This effect has been confirmed *in vivo*, in both animals (9,48) and humans (2,37,61). Conversely, decreased activation of M1 receptors leads to increased expression of β APP and evidence of increased amyloidogenic cleavage or increased accumulation of A β (5,8,21,79). Additionally, there is now considerable clinical data, derived from both cognitive and imaging measures, to suggest that therapy with cholinergic agents delay disease progression, and are not merely palliative, as has long been assumed (11,15,46,40,72,49,55). Most recently, a large authoritative trial by the Alzheimer's Disease Cooperative Study showed that some MCI subjects that were treated with donepezil, the major acetylcholinesterase inhibitor used for AD therapy, had a delayed conversion to dementia (49,72).

While the loss of cortical cholinergic presynaptic terminal markers in AD is well established, the status of post-synaptic receptors is more complicated. Although there may be little or no change, or possibly a compensatory increase, in the number of cortical M1 receptors in AD (84,65,64,70), M1 receptor signal transduction-related markers or function are reportedly decreased or impaired in AD (85,84,26). Muscarinic agonist stimulation of responses such as binding of [³⁵S]GTP γ S to G-proteins, GTPase activity, phosphoinositide hydrolysis and protein kinase C activity, are markedly decreased in AD (80,81,59,34). These

studies indicate that the M1 receptor becomes uncoupled from its G-protein in Alzheimer's disease. Coupling of M1 receptors to G-proteins has not been quantitatively investigated in preclinical AD.

Previous studies of presynaptic and postsynaptic cholinergic markers in AD have been limited by relatively small sample sizes, long postmortem intervals and insufficient subdivision of controls and AD subjects by the defining histopathology. We therefore undertook a re-assessment of the status of cortical cholinergic synaptic components utilizing a large number of neuropathologically-characterized subjects from a rapid autopsy program.

Pre- and postsynaptic cholinergic status was examined in brains from 59 non-demented elderly controls with varying amounts of cortical amyloid plaques. Young and AD groups were included for comparison. ChAT activity was used as a presynaptic marker and was measured in frontal and parietal cortices. Displacement of ^3H -pirenzepine binding by oxotremorine-M in the presence and absence of GppNHp was measured in frontal cortex to assess the functional coupling of the postsynaptic M1 receptor with its G-protein (G_{q11}).

Our results indicate that cortical ChAT activity is significantly decreased in non-demented elderly subjects with plaques, as compared to non-demented elderly subjects without plaques, with the severity of ChAT activity loss being proportionate to the plaque density. Coupling of M1 receptors to G-proteins is also impaired in non-demented controls with plaques compared to that in non-demented controls without plaques. These findings suggest that cortical cholinergic degeneration and dysfunction in AD begins at the preclinical stages of disease, before the onset of dementia, in parallel with the accumulation of cortical amyloid plaques.

Materials and Methods

Human subjects

The study took place at Banner Sun Health Research Institute (BSHRI), located in the Sun Cities retirement communities of northwest metropolitan Phoenix, Arizona. The Institute is part of Banner Health, a regional not-for-profit health care provider centered in Arizona. Brain necropsies were performed on elderly subjects who had volunteered for the BSHRI Brain Donation Program, a longitudinal clinicopathologic study of normal aging, dementia and parkinsonism (7). The operations of the Brain Donation Program have been approved by the Institutional Review Board of Banner Health. Subjects were chosen by searching the Brain Donation Program database. A young control group was derived from a hospital autopsy series. Non-demented control subjects were defined as those that had not had a clinical diagnosis of dementia or parkinsonism. Subjects with AD were defined as those that had clinically-documented dementia and a neuropathological diagnosis of AD. Additionally, both AD and control subjects were chosen on the basis of availability of short post mortem interval (PMI < 8 hours) fresh-frozen cerebral cortex for biochemical analysis. Subjects were clinically characterized by standardized periodic neurological and neuropsychological assessments, review of medical records, self-report and telephone interviews with spouses and/or caregivers. A subset of subjects in this study received at least one Mini Mental State Examination (MMSE).

As part of the Brain Donation Program's standard protocol, two years of private medical records are obtained from the subjects' private physicians, both at the time of enrollment and at the time of death. Subjects also fill out a medical history questionnaire in which they are asked to report any significant neurological conditions. Additionally, at the time of death, a telephone interview is conducted with the spouse and/or caregiver in which the presence of any neurological conditions, symptoms or signs are again queried.

For the purpose of this study the subjects were divided into groups on the basis of age, presence or absence of dementia and senile plaque density. The elderly non-demented controls were divided into three subgroups; those without plaques (no plaques, NPL), those with plaque density scores in the lower 50% of the plaque score range (sparse plaques, SPL) and those with plaque scores in the upper 50% of the range (many plaques, MPL) (see Methods section below for details of plaque scoring method). Two other groups were composed of young normal subjects and subjects who had dementia during life as well as a neuropathological diagnosis of Alzheimer's disease.

Tissue processing and histological methods

The cerebrum was cut in the coronal plane into 1 cm thick slices while the brainstem was sliced axially at 1 cm intervals. The entire brain was then divided sagittally into left and right halves. The slices from the right half were frozen between slabs of dry ice while the slices from the left half were fixed by immersion in buffered 4% formaldehyde for 48 hours at 4 degrees C. Following cryoprotection in ethylene glycol and glycerol, selected 3 x 4 cm cerebral, cerebellar and brainstem blocks were sectioned at 40 μ m thickness on a sliding freezing microtome. The cerebral sections were taken at standard coronal levels as follows. For frontal lobe, the entire frontal cortical ribbon from cingulate gyrus to the middle frontal gyrus was assessed at the level of the genu of the corpus callosum. For temporal lobe, the entire neocortical ribbon was evaluated at the level of the amygdala, level of the pes hippocampus and level of the lateral geniculate nucleus. For parietal lobe, the entire parietal cortical ribbon from the cingulate gyrus to the inferior parietal lobule was assessed at a level 1–2 cm posterior to the trigone. For occipital lobe, the entire cortical ribbon, including primary and association cortical regions, was assessed at a level 2–3 cm anterior to the pole. Entorhinal cortex was assessed at the two anterior temporal lobe levels as described above. For hippocampus, the CA1 field was assessed at the two posterior temporal lobe levels as described above.

Additionally, a standard set of 24 brainstem, cerebellar and cerebral blocks were embedded in paraffin, cut at 6 μ m and stained with hematoxylin and eosin. Free-floating 40 μ m sections were stained with H & E, thioflavine S and enhanced silver methods for amyloid plaques and neurofibrillary tangles (Campbell-Switzer and Gallyas methods, respectively). Immunostaining for A β was not performed. Amyloid plaques and neurofibrillary tangles were graded and staged based on the aggregate impression from 40 μ m sections stained with thioflavine S, Campbell-Switzer and Gallyas methods. Scores for total plaque density ("plaque score" in Table 2 and Figure 3; neuritic and diffuse plaques considered together) and neurofibrillary tangle density ("tangle score" in Table 2 and Figure 4) were obtained with reference to the CERAD templates (54), assigning scores of zero, sparse, moderate and frequent to the standard regions within frontal cortex, temporal cortex, parietal cortex, hippocampus and entorhinal area as described above. Conversion of these to numerical values results in a score of 0–3 for each area, with a maximum score of 15 for all five areas combined. Neurofibrillary tangle abundance and distribution was also graded in these thick sections according to the original Braak protocol (13, 20) ("Braak Stage" in Table 2). Diagnostic criteria for AD were those established by CERAD and NIA-Reagan consortia (54, 1). All subjects except the young controls were genotyped for apolipoprotein E (ApoE) using a modification of a standard method (36).

Measurement of ChAT activity

ChAT was measured in frozen cortical tissue from frontal and parietal cortex using a standard method (28). Tissues were stored at -80°C, then thawed and homogenized by sonication in 19 volumes of 75 mM potassium phosphate buffer, pH 7.4. Equal aliquots of homogenate and buffered substrate solution (75 mM potassium phosphate solution, pH 7.4,

NaCl 0.6 mM, MgCl₂ 40 mM, physostigmine 2 mM, bovine serum albumin 0.05%, choline iodide 10 mM, acetyl CoA, 0.87 mM, ³H-acetyl CoA, 4.35 µCi) were incubated for 30 min at 37°C. Radiolabelled acetylcholine (ACh) was extracted by mixing with 150 µl of 3-heptanone: 75 mg/ml sodium tetraphenylboron. Organic and aqueous phases were separated by centrifugation and ³H-ACh was determined in the organic phase by liquid scintillation spectrometry. Each sample was assayed in triplicate. Protein was measured by the method of Lowry. Data were expressed as pmoles acetylcholine (ACh) formed/mg protein/minute.

Receptor concentration and binding assays

Preparation of P2 membranes was performed as described previously (82). Tissues were homogenized in 10 volumes of ice-cold 0.32 M sucrose, containing 5 mM EDTA, 0.1 mM PMSF, and 0.02% azide. The sample was centrifuged at 1000 *g* for 10 minutes, the supernatant was decanted and kept on ice. The pellet was resuspended and recentrifuged. The combined supernatants were centrifuged at 15,000 *g* for 20 minutes, and the resulting pellet resuspended in 50 mM potassium phosphate buffer containing 5 mM EDTA, 0.1 mM PMSF, and 0.02% NaN₃. This was centrifuged at 15,000 *g* for 20 min, resuspended and centrifuged again at 15,000 *g*. The pellet was suspended in phosphate buffer (2.5 ml per gram wet weight of the tissue).

For determination of M1 muscarinic receptor binding, the membranes were diluted 1:50 with 20 mM Tris buffer, pH 7.4, containing 1 mM MnCl₂ (protein concentration, 0.4 mg/ml), ³H-pirenzepine (NEN, sp. act. 70 Ci/mmol) was added to a final concentration of 4 nM and oxotremorine-M was added in concentrations from 10⁻¹² to 3x 10⁻⁶M. GppNHp (0.2mM) was added to half of the samples. The samples were kept at room temperature for 1 hour, then placed on ice and filtered through Whatman GF/C filters presoaked with 0.3% polyethenimine, and washed with ice-cold Tris in a Brandel cell harvester. The filters were dissolved overnight in scintillation fluid, then radioactivity determined by liquid scintillation spectroscopy. Specific binding is defined as total binding minus that in the presence of 1 µM atropine (non-specific binding). Binding curves were analyzed using GraphPad Prism software for one-site and two-site competition. The cases from the young control subject group were used only for ChAT activity determinations and not for receptor binding assays.

Statistical Analyses

Statistical analyses for comparing group means consisted of one-way analysis of variance (ANOVA) followed by post-hoc pairwise significance testing. Corrections were made for multiple comparisons. Proportions were compared using chi-square tests. Correlations were done using Spearman's rank correlation method. Best fits for the displacement curves were obtained using non-linear regression for one-and two-site competition using Graph Pad Prism software. For all tests, results were considered significant for *p* < 0.05.

Results

Characteristics of study subjects

The basic characteristics of the study subjects are shown in Table 1. The young control (YG) group differed significantly from all other groups with respect to age and postmortem interval. The groups differed in their percentages carrying the apoE-ε 4 allele, with the MPL and AD groups having significantly higher percentages (45 and 68%, respectively) than the NPL and SPL groups (11% and 18%, respectively). Table 2 shows mean plaque and tangle density scores for the groups; these differed as expected based on group definitions. The NPL group differed significantly from the MPL and AD groups in terms of Braak neurofibrillary tangle stage. In terms of incidental neuropathological findings, Lewy bodies were present in 8 of the AD subjects, 5 of the NPL group, 5 of the SPL group and 1 of the

MPL group while cerebral infarcts were present in 8 of the AD group (median infarct volume 1 cc), 8 of the NPL group (median 2.2 cc), 7 of the SPL group (median 2 cc) and 4 of the MPL group (median 1 cc).

Cortical ChAT activity in the study groups

Figures 1 and 2 show mean frontal and parietal ChAT activity in the study groups. There is a progressive decline in ChAT activity proceeding from YG through NPL, SPL, MPL and AD groups. In both frontal and parietal cortex, the groups were found to be significantly different (ANOVA, $p < 0.0001$) and pairwise significance testing showed that the YG, NPL and SPL groups differed significantly from the MPL and AD groups. With the YG group values defined as 100%, ChAT activity declined sequentially in the NPL, SPL, MPL and AD groups to 96%, 84%, 71% and 57%, respectively.

Figures 3 and 4 show correlations of ChAT activity with plaque and tangle density scores. Correlation coefficients were 0.48 and 0.57 for ChAT vs plaque score in frontal and parietal cortex respectively. The correlations between ChAT and tangles were also significant, although the correlation coefficients were somewhat lower: 0.41 in frontal cortex and 0.42 in parietal cortex. Figure 5 shows correlations of ChAT activity with MMSE scores. These correlations were significant ($p < 0.01$) in both frontal and parietal cortex, with correlation coefficients of 0.51 and 0.44, respectively.

ChAT activity did not differ when groups were divided by the presence or absence of the apolipoprotein E-- $\epsilon 4$ allele.

Receptor binding measures in the study groups

There was no significant difference in specific binding of ^3H -pirenzepine in the different groups, indicating that the number of M1 receptors does not differ between groups (data not shown). However, when displacement of the antagonist ^3H -pirenzepine by the agonist oxotremorine-M was examined, uncoupling of the M1 receptor from its G-protein complex was seen in all of the groups with plaques. Coupling of M1 muscarinic receptors can be demonstrated by assessing displacement curves of an antagonist by an agonist, in the presence and absence of the non-hydrolyzable GTP analogue GppNHp. Addition of GppNHp shifts the displacement curve for antagonist by agonist to the right, indicating functional uncoupling of the M1 receptor/G protein complex (74). Figure 6 shows curves for displacement of ^3H -pirenzepine by oxotremorine-M in the three non-demented groups with varying plaque levels, and in the AD group. The curve was shifted significantly to the right by addition of GppNHp in the non-demented control group with no plaques (NPL), indicating that the M1 receptor was coupled to the G-protein in this group. However, addition of GppNHp produced little change in the displacement curves in the non-demented samples with plaques (SPL, MPL) or in the AD group, indicating that the receptors had become uncoupled. The K_i value was significantly different in the absence and presence of GppNHp in the non-demented control group without plaques, but there were no significant differences in the other groups. Additionally, the proportion of M1 receptors in the high-affinity binding state was determined by examining two-site competition, and was found to be reduced in the non-demented control groups with plaques, compared to that without plaques (Figure 7). A reduction in high-affinity binding sites was also observed in the samples from AD subjects. These reductions correlated significantly with plaque score (Spearman correlation, $r = 0.36$).

Discussion

The results of this investigation indicate that cortical ChAT activity is progressively decreased in non-demented elderly subjects in concert with the progressive accumulation of cortical amyloid plaques and this decrease is further accentuated in subjects with dementia associated with neuropathologically-confirmed AD. This firmly places the presynaptic cholinergic deficit at a very early stage of AD. The association of a cortical cholinergic presynaptic deficit with amyloid plaques in non-demented subjects has been previously reported (3,4,43); the present study confirms these and also documents, for the first time, a postsynaptic cholinergic abnormality in non-demented subjects with plaques, consisting of uncoupling of cortical M1 receptors from their G-proteins as well as a reduction in high-affinity M1 binding sites. This work supports a growing agreement that several different markers of the cortical cholinergic system are affected early in the disease, at the stage of mild cognitive impairment or even prior to this (50,60,77,14,57,58).

Recent amyloid imaging results (75,53,56,73) have confirmed the longstanding neuropathological documentation of the frequent presence of cortical amyloid plaques in the non-demented elderly (10,19,20,24). It seems increasingly likely that non-demented subjects with cortical amyloid plaques represent a preclinical stage of AD. The results presented here strongly support this developing consensus, as our group of non-demented elderly subjects with plaques have plaques, tangles, a cholinergic deficit and an increased prevalence of the apoE— ϵ 4 allele.

It might be expected that, since the ascending cholinergic system has been thought to have a stimulatory effect on cerebral metabolism (63,42), that the decreases in cortical cholinergic afferentation that are present in plaque-positive non-demented elderly should be accompanied by decreases in cerebral glucose metabolism, however, imaging studies to date have largely failed to find a correlation between measures of amyloid load and glucose metabolism in such subjects (18). However, some evidence indicates that while the basal forebrain cholinergic system has great influence over cerebral blood flow, its effects on cerebral glucose metabolism may be comparatively small (47).

Some studies of cortical cholinergic markers (33,38,22,23) have been interpreted as evidence that the cortical cholinergic deficit is a later-stage event in AD. These studies, however, defined their study groups by clinical dementia staging rather than by histopathologic criteria, thereby including subjects in the dementia category that did not meet histopathologic diagnostic criteria for AD. Similarly, some subjects within the normal control categories had high densities of cortical neuritic plaques as well as Braak III or IV neurofibrillary tangle stages and would have met consensus clinicopathologic criteria for AD had they been demented. The resultant mixing of cases with and without histopathologic AD would have impaired the statistical power of these studies and increased the probability of false-negative results. In the present study, the diagnosis of AD was based on the clinical presence of dementia in association with neuropathological criteria for AD. Furthermore, non-demented elderly control subjects were subdivided on the basis of neocortical plaque densities into “true” controls (without plaques) and putative preclinical AD (with plaques).

It is known that astrocytes have M1 receptors and therefore may have influenced our measurements. We have not quantified the astrocytes in our subjects but there is a well-documented astrocytic reaction in brain tissue affected by Alzheimer’s disease histopathology, especially around neuritic plaques. Therefore it is almost certain that our diagnostic groups would have an astrocyte reaction that was proportional to the density of neuritic plaques. As a literature search revealed no published data regarding activation-

related changes in astrocytic M1 receptor numbers or characteristics, it is not possible to know if such changes exist and affected our study.

It is also possible that medications being taken in the perimortem period may have affected some of our measurements. Unfortunately, we do not have the details, for all of our subjects, of the medications being taken in the immediate postmortem period, but most elderly subjects have their AD-related medications withdrawn as they enter hospice care.

It is logical that, like other chronic diseases, AD has a prolonged preclinical period and that the disease does not begin only when it becomes symptomatic. We have considered that, since the cortical cholinergic deficit in non-demented aging humans begins when subjects are in their 50s, at about the same time at which cortical A β concentrations start to become elevated (29,51,69), and since both the cholinergic deficit and A β accumulation are accentuated in AD, that both of these changes are prodromal events in AD. In non-demented subjects with plaques, previous reports have already indicated that indices of cortical pre-synaptic cholinergic elements are decreased relative to age-similar subjects without plaques (3,4,43). The cause of both the cholinergic deficit and cortical plaque formation may be neurofibrillary degeneration of the nucleus basalis of Meynert, as this neuronal group is one of the first affected by this age-related change (6,78). Additionally, we and others have causally linked cortical cholinergic deafferentation or dysfunction to A β deposition in animal models, through immunotoxin lesions of the basal forebrain cholinergic neurons (5,8) and M1 receptor knockout of amyloid precursor protein (APP) transgenic mice (21). Other animal models do not support the competing retrograde theory of cholinergic degeneration, as it has been shown that neither cortical lesions (52) or marked A β deposition (30,35,86) necessarily result in significant losses of cortical cholinergic markers.

The results presented here are the first report of a preclinical alteration of post-synaptic function in AD. The M1 muscarinic receptor displays both a high affinity and a low affinity binding site when displacement of an antagonist by an agonist is measured. Addition of a non-hydrolyzable GTP analogue such as GppNHp causes the complex to dissociate, shifting the binding towards the lower-affinity site (uncoupling from the G-protein). Non-demented elderly subjects with plaques show a shift of M1 muscarinic receptor binding from high affinity to low affinity, and a decrease in the displacement of competition binding curves with GppNHp, consistent with uncoupling of M1 receptors from their G-proteins. While the loss of cortical cholinergic presynaptic terminal markers in AD has been extensively documented, much less attention has been paid to the status of postsynaptic receptors, and, possibly because of the greater complexity of the post-synaptic apparatus, there is not yet a complete picture of cholinergic postsynaptic function in the disease. Although there may be little or no change, or even a small increase in the number of M1 muscarinic receptors (84,65,64,70), multiple measures of M1 receptor signal transduction-related markers or function are reportedly decreased or impaired in AD (85,84,26,80,81,59,34).

The cause of receptor uncoupling in the presence of plaques or AD is unclear. Uncoupling could result from increased concentrations of soluble A β , as studies in animals have demonstrated that A β exposure can lead to uncoupling of the M1 receptor (44). Receptor uncoupling may also be secondary to loss of cholinergic presynaptic terminals, as we have observed this in rats with depleted cortical cholinergic afferents due to intracerebroventricular injection of the immunotoxin IgG 192-saporin (unpublished results). Uncoupling in these animals was prevented by treatment with a muscarinic receptor agonist. Additionally, treatment of AD subjects with an acetylcholinesterase inhibitor has been reported to increase cortical uptake of ¹²³I quinuclidinylbenzilate (QNB), consistent with an increase in high affinity binding sites (45). These data suggest that cholinergic therapy at the

preclinical stages of AD may be beneficial by augmenting or preserving postsynaptic cholinergic function.

The early situation of the cortical cholinergic deficit in AD indicates that it could have a critical causative or accelerating effect on AD pathogenesis and thus be an important target for AD prevention therapy. There is mounting evidence, derived from both basic science and clinical studies, consistent with a disease-modifying effect for cholinergic therapy. *In vitro*, animal and human studies have linked M1 muscarinic receptor activation status with the metabolism of β APP and A β (21,27,16,17,25,62). The results of multiple clinical trials as well as some imaging studies are also consistent with disease modification (11,15,46,40,72,49,55). Recent trials of acetylcholinesterase inhibitors in subjects with mild cognitive impairment (MCI) showed definite effects on the rate of cognitive deterioration and/or conversion to AD (49,72). These effects may have been underestimated as recent clinicopathological studies (41,76,71) have demonstrated that up to one-third to one-half of MCI cases are not pathologically AD, so that any disease-specific effects of the medication may have been diluted by the inclusion of subjects that did not have AD. In any case, as both amyloid plaque deposition and the cholinergic deficit are already likely to be well advanced even by the time mild cognitive impairment appears, primary prevention trials may be necessary in order to adequately test whether cholinergic therapy can slow or halt disease progression. The results shown here suggest that cholinotropic agents are a logical choice for primary prevention trials. These trials would be optimized by enhanced selection of high-risk subjects using amyloid imaging in combination with in vivo imaging of cortical cholinergic afferents.

Acknowledgments

The Banner Sun Health Research Institute Brain Donation Program is supported by the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research.

References

1. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging*. 1997; 18:S1-S2. [PubMed: 9330978]
2. Basun H, Nilsberth C, Eckman C, Lannfelt L, Younkin S. Plasma Levels of Abeta42 and Abeta40 in Alzheimer Patients during Treatment with the Acetylcholinesterase Inhibitor Tacrine. *Dement Geriatr Cogn Disord*. 2002; 14:156-160. [PubMed: 12218259]
3. Beach TG, Honer WG, Hughes LH. Cholinergic fibre loss associated with diffuse plaques in the non-demented elderly: the preclinical stage of Alzheimer's disease? *Acta Neuropathol (Berl)*. 1997; 93:146-153. [PubMed: 9039461]
4. Beach TG, Kuo YM, Spiegel K, Emmerling MR, Sue LI, Kokjohn K, Roher AE. The cholinergic deficit coincides with Abeta deposition at the earliest histopathologic stages of Alzheimer disease. *J Neuropathol Exp Neurol*. 2000; 59:308-313. [PubMed: 10759186]
5. Beach TG, Potter PE, Kuo YM, Emmerling MR, Durham RA, Webster SD, Walker DG, Sue LI, Scott S, Layne KJ, Roher AE. Cholinergic deafferentation of the rabbit cortex: a new animal model of Abeta deposition. *Neurosci Lett*. 2000; 283:9-12. [PubMed: 10729621]
6. Beach TG, Sue LI, Scott S, Sparks DL. Neurofibrillary tangles are constant in aging human nucleus basalis. *Alzheimer's Reports*. 1998; 1:375-380.
7. Beach TG, Sue LI, Walker DG, Roher AE, Lue L, Vedders L, Connor DJ, Sabbagh MN, Rogers J. The Sun Health Research Institute Brain Donation Program: description and experience, 1987-2007. *Cell Tissue Bank*. 2008; 9:229-245. [PubMed: 18347928]

8. Beach TG, Walker D, Sue L, Scott S, Layne K, Newell A, Potter P, Durham RA, Emmerling M, Webster SD, Honer W, Fisher A, Roher A. Immunotoxin lesion of the cholinergic nucleus basalis causes A β deposition: towards a physiologic animal model of Alzheimer's disease. *Curr Med Chem.* 2003; 3:57–75.
9. Beach TG, Walker DG, Potter PE, Sue LI, Fisher A. Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. *Brain Res.* 2001; 905:220–223. [PubMed: 11423097]
10. Berg L, McKeel DW Jr, Miller JP, Baty J, Morris JC. Neuropathological indexes of Alzheimer's disease in demented and nondemented persons aged 80 years and older. *Arch Neurol.* 1993; 50:349–358. [PubMed: 8460956]
11. Birks J, Grimley EJ, Iakovidou V, Tsolaki M, Holt FE. Rivastigmine for Alzheimer's disease. *Cochrane Database Syst Rev.* 2009:CD001191. [PubMed: 19370562]
12. Bowen DM, Benton JS, Spillane JA, Smith CC, Allen SJ. Choline acetyltransferase activity and histopathology of frontal neocortex from biopsies of demented patients. *J Neurol Sci.* 1982; 57:191–202. [PubMed: 7161618]
13. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl).* 1991; 82:239–259. [PubMed: 1759558]
14. Bruno MA, Mufson EJ, Wu J, Cuellar AC. Increased matrix metalloproteinase 9 activity in mild cognitive impairment. *J Neuropathol Exp Neurol.* 2009; 68:1309–1318. [PubMed: 19915485]
15. Bullock R, Dengiz A. Cognitive performance in patients with Alzheimer's disease receiving cholinesterase inhibitors for up to 5 years. *Int J Clin Pract.* 2005; 59:817–822. [PubMed: 15963209]
16. Buxbaum JD, Oishi M, Chen HI, Pinkas-Kramarski R, Jaffe EA, Gandy SE, Greengard P. Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc Natl Acad Sci U S A.* 1992; 89:10075–10078. [PubMed: 1359534]
17. Bymaster FP, Carter PA, Peters SC, Zhang W, Ward JS, Mitch CH, Calligaro DO, Whitesitt CA, DeLapp N, Shannon HE, Rimvall K, Jeppesen L, Sheardown MJ, Fink-Jensen A, Sauerberg P. Xanomeline compared to other muscarinic agents on stimulation of phosphoinositide hydrolysis in vivo and other cholinomimetic effects. *Brain Res.* 1998; 795:179–190. [PubMed: 9622623]
18. Cohen AD, Price JC, Weissfeld LA, James J, Rosario BL, Bi W, Nebes RD, Saxton JA, Snitz BE, Aizenstein HA, Wolk DA, DeKosky ST, Mathis CA, Klunk WE. Basal cerebral metabolism may modulate the cognitive effects of A β in mild cognitive impairment: an example of brain reserve. *J Neurosci.* 2009; 29:14770–14778. [PubMed: 19940172]
19. Crystal H, Dickson D, Fuld P, Masur D, Scott R, Mehler M, Masdeu J, Kawas C, Aronson M, Wolfson L. Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology.* 1988; 38:1682–1687. [PubMed: 3185902]
20. Davies L, Wolska B, Hilbich C, Multhaup G, Martins R, Simms G, Beyreuther K, Masters CL. A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. *Neurology.* 1988; 38:1688–1693. [PubMed: 3054625]
21. Davis AA, Fritz JJ, Wess J, Lah JJ, Levey AI. Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology in vitro and in vivo. *J Neurosci.* 2010; 30:4190–4196. [PubMed: 20335454]
22. Davis KL, Mohs RC, Marin D, Purohit DP, Perl DP, Lantz M, Austin G, Haroutunian V. Cholinergic markers in elderly patients with early signs of Alzheimer disease. *JAMA.* 1999; 281:1401–1406. [PubMed: 10217056]
23. DeKosky ST, Ikonovic MD, Styren SD, Beckett L, Wisniewski S, Bennett DA, Cochran EJ, Kordower JH, Mufson EJ. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Ann Neurol.* 2002; 51:145–155. [PubMed: 11835370]
24. Dickson DW, Crystal HA, Mattiace LA, Masur DM, Blau AD, Davies P, Yen SH, Aronson MK. Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiol Aging.* 1992; 13:179–189. [PubMed: 1311804]

25. Farber SA, Nitsch RM, Schulz JG, Wurtman RJ. Regulated secretion of beta-amyloid precursor protein in rat brain. *J Neurosci.* 1995; 15:7442–7451. [PubMed: 7472496]
26. Ferrari-DiLeo G, Mash DC, Flynn DD. Attenuation of muscarinic receptor-G-protein interaction in Alzheimer disease. *Mol Chem Neuropathol.* 1995; 24:69–91. [PubMed: 7755848]
27. Fisher A. Cholinergic treatments with emphasis on m1 muscarinic agonists as potential disease-modifying agents for Alzheimer's disease. *Neurotherapeutics.* 2008; 5:433–442. [PubMed: 18625455]
28. Fonnum F. Radiochemical micro assays for the determination of choline acetyltransferase and acetylcholinesterase activities. *Biochem J.* 1969; 115:465–472. [PubMed: 4982085]
29. Funato H, Yoshimura M, Kusui K, Tamaoka A, Ishikawa K, Ohkoshi N, Namekata K, Okeda R, Ihara Y. Quantitation of amyloid beta-protein (A beta) in the cortex during aging and in Alzheimer's disease. *Am J Pathol.* 1998; 152:1633–1640. [PubMed: 9626067]
30. Gau JT, Steinhilb ML, Kao TC, D'Amato CJ, Gaut JR, Frey KA, Turner RS. Stable beta-secretase activity and presynaptic cholinergic markers during progressive central nervous system amyloidogenesis in Tg2576 mice. *Am J Pathol.* 2002; 160:731–738. [PubMed: 11839594]
31. Geula C, Mesulam MM. Cortical cholinergic fibers in aging and Alzheimer's disease: a morphometric study. *Neuroscience.* 1989; 33:469–481. [PubMed: 2636703]
32. Geula C, Nagykerly N, Wu CK, Bu J. Loss of calbindin-D28K from aging human cholinergic basal forebrain: relation to plaques and tangles. *J Neuropathol Exp Neurol.* 2003; 62:605–616. [PubMed: 12834105]
33. Gilmor ML, Erickson JD, Varoqui H, Hersh LB, Bennett DA, Cochran EJ, Mufson EJ, Levey AI. Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine transporter in the elderly with mild cognitive impairment and early Alzheimer's disease. *J Comp Neurol.* 1999; 411:693–704. [PubMed: 10421878]
34. Greenwood AF, Powers RE, Jope RS. Phosphoinositide hydrolysis, G alpha q, phospholipase C, and protein kinase C in post mortem human brain: effects of post mortem interval, subject age, and Alzheimer's disease. *Neuroscience.* 1995; 69:125–138. [PubMed: 8637611]
35. Hernandez D, Sugaya K, Qu T, McGowan E, Duff K, McKinney M. Survival and plasticity of basal forebrain cholinergic systems in mice transgenic for presenilin-1 and amyloid precursor protein mutant genes. *Neuroreport.* 2001; 12:1377–1384. [PubMed: 11388415]
36. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res.* 1990; 31:545–548. [PubMed: 2341813]
37. Hock C, Maddalena A, Heuser I, Naber D, Oertel W, von der KH, Wienrich M, Raschig A, Deng M, Growdon JH, Nitsch RM. Treatment with the selective muscarinic agonist talsaclidine decreases cerebrospinal fluid levels of total amyloid beta-peptide in patients with Alzheimer's disease. *Ann N Y Acad Sci.* 2000; 920:285–291. [PubMed: 11193166]
38. Ikonomic MD, Abrahamson EE, Isanski BA, Wu J, Mufson EJ, DeKosky ST. Superior frontal cortex cholinergic axon density in mild cognitive impairment and early Alzheimer disease. *Arch Neurol.* 2007; 64:1312–1317. [PubMed: 17846271]
39. Ikonomic MD, Wecker L, Abrahamson EE, Wu J, Counts SE, Ginsberg SD, Mufson EJ, DeKosky ST. Cortical alpha7 nicotinic acetylcholine receptor and beta-amyloid levels in early Alzheimer disease. *Arch Neurol.* 2009; 66:646–651. [PubMed: 19433665]
40. Jack CR Jr, Petersen RC, Grundman M, Jin S, Gamst A, Ward CP, Sencakova D, Doody RS, Thal LJ. Longitudinal MRI findings from the vitamin E and donepezil treatment study for MCI. *Neurobiol Aging.* 2008; 29:1285–1295. [PubMed: 17452062]
41. Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, Tangalos EG, Boeve BF, Knopman DS, Braak H, Petersen RC. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Arch Neurol.* 2006; 63:674–681. [PubMed: 16682537]
42. Katsumi Y, Hanakawa T, Fukuyama H, Hayashi T, Nagahama Y, Yamauchi H, Ouchi Y, Tsukada H, Shibasaki H. The effect of sequential lesioning in the basal forebrain on cerebral cortical glucose metabolism in rats. An animal positron emission tomography study. *Brain Res.* 1999; 837:75–82. [PubMed: 10433990]

43. Katzman R, Terry R, DeTeresa R, Brown T, Davies P, Fuld P, Renbing X, Peck A. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol*. 1988; 23:138–144. [PubMed: 2897823]
44. Kelly JF, Storie K, Skamra C, Bienias J, Beck T, Bennett DA. Relationship between Alzheimer's disease clinical stage and Gq/11 in subcellular fractions of frontal cortex. *J Neural Transm*. 2005; 112:1049–1056. [PubMed: 15583959]
45. Kemp PM, Holmes C, Hoffmann S, Wilkinson S, Zivanovic M, Thom J, Bolt L, Fleming J, Wilkinson DG. A randomised placebo controlled study to assess the effects of cholinergic treatment on muscarinic receptors in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2003; 74:1567–1570. [PubMed: 14617718]
46. Krishnan KR, Charles HC, Doraiswamy PM, Mintzer J, Weisler R, Yu X, Perdomo C, Ieni JR, Rogers S. Randomized, placebo-controlled trial of the effects of donepezil on neuronal markers and hippocampal volumes in Alzheimer's disease. *Am J Psychiatry*. 2003; 160:2003–2011. [PubMed: 14594748]
47. Le MC, Chavoix C, Chapon F, Mezenge F, Epelbaum J, Baron JC. Effects of damage to the basal forebrain on brain glucose utilization: a reevaluation using positron emission tomography in baboons with extensive unilateral excitotoxic lesion. *J Cereb Blood Flow Metab*. 1998; 18:476–490. [PubMed: 9591840]
48. Lin L, Georgievska B, Mattsson A, Isacson O. Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapse loss and muscarinic receptor activation. *Proc Natl Acad Sci U S A*. 1999; 96:12108–12113. [PubMed: 10518584]
49. Lu PH, Teng E, Tingus K, Petersen RC, Cummings JL. Donepezil delays progression to AD in MCI subjects with depressive symptoms. *Neurology*. 2009; 72:2115–2121. [PubMed: 19528519]
50. Mazere J, Prunier C, Barret O, Guyot M, Hommet C, Guilloteau D, Dartigues JF, Auriacombe S, Fabrigoule C, Allard M. In vivo SPECT imaging of vesicular acetylcholine transporter using [(123)I]-IBVM in early Alzheimer's disease. *Neuroimage*. 2008; 40:280–288. [PubMed: 18191587]
51. McGeer PL, McGeer EG, Suzuki J, Dolman CE, Nagai T. Aging, Alzheimer's disease, and the cholinergic system of the basal forebrain. *Neurology*. 1984; 34:741–745. [PubMed: 6539435]
52. Minger SL, Davies P. Persistent innervation of the rat neocortex by basal forebrain cholinergic neurons despite the massive reduction of cortical target neurons. I. Morphometric analysis. *Exp Neurol*. 1992; 117:124–138. [PubMed: 1499691]
53. Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, Klunk WE, Mathis CA, DeKosky ST, Morris JC. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. *Neurology*. 2006; 67:446–452. [PubMed: 16894106]
54. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991; 41:479–486. [PubMed: 2011243]
55. Mori E, Hashimoto M, Krishnan KR, Doraiswamy PM. What constitutes clinical evidence for neuroprotection in Alzheimer disease: support for the cholinesterase inhibitors? *Alzheimer Dis Assoc Disord*. 2006; 20:S19–S26. [PubMed: 16772752]
56. Mormino EC, Kluth JT, Madison CM, Rabinovici GD, Baker SL, Miller BL, Koeppe RA, Mathis CA, Weiner MW, Jagust WJ. Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. *Brain*. 2009; 132:1310–1323. [PubMed: 19042931]
57. Mufson EJ, Ma SY, Cochran EJ, Bennett DA, Beckett LA, Jaffar S, Saragovi HU, Kordower JH. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. *J Comp Neurol*. 2000; 427:19–30. [PubMed: 11042589]
58. Mufson EJ, Ma SY, Dills J, Cochran EJ, Leurgans S, Wu J, Bennett DA, Jaffar S, Gilmore ML, Levey AI, Kordower JH. Loss of basal forebrain P75(NTR) immunoreactivity in subjects with mild cognitive impairment and Alzheimer's disease. *J Comp Neurol*. 2002; 443:136–153. [PubMed: 11793352]

59. Muma NA, Mariyappa R, Williams K, Lee JM. Differences in regional and subcellular localization of G(q/11) and RGS4 protein levels in Alzheimer's disease: correlation with muscarinic M1 receptor binding parameters. *Synapse*. 2003; 47:58–65. [PubMed: 12422374]
60. Muth K, Schonmeyer R, Matura S, Haenschel C, Schroder J, Pantel J. Mild cognitive impairment in the elderly is associated with volume loss of the cholinergic basal forebrain region. *Biol Psychiatry*. 2010; 67:588–591. [PubMed: 19375072]
61. Nitsch RM, Deng M, Tennis M, Schoenfeld D, Growdon JH. The selective muscarinic M1 agonist AF102B decreases levels of total Abeta in cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol*. 2000; 48:913–918. [PubMed: 11117548]
62. Nitsch RM, Wurtman RJ, Growdon JH. Regulation of APP processing. Potential for the therapeutical reduction of brain amyloid burden. *Ann N Y Acad Sci*. 1996; 777:175–182. [PubMed: 8624081]
63. Nordberg A. PET studies and cholinergic therapy in Alzheimer's disease. *Rev Neurol (Paris)*. 1999; 155(Suppl 4):S53–S63. [PubMed: 10637939]
64. Nordberg A, Winblad B. Reduced number of [3H]nicotine and [3H]acetylcholine binding sites in the frontal cortex of Alzheimer brains. *Neurosci Lett*. 1986; 72:115–119. [PubMed: 3808458]
65. Overk CR, Felder CC, Tu Y, Schober DA, Bales KR, Wu J, Mufson EJ. Cortical M1 receptor concentration increases without a concomitant change in function in Alzheimer's disease. *J Chem Neuroanat*. 2010; 40:63–70. [PubMed: 20347961]
66. Palmer AM. Neurochemical studies of Alzheimer's disease. *Neurodegeneration*. 1996; 5:381–391. [PubMed: 9117551]
67. Pearson RC, Powell TP. Anterograde vs. retrograde degeneration of the nucleus basalis medialis in Alzheimer's disease. *J Neural Transm Suppl*. 1987; 24:139–146. [PubMed: 3119773]
68. Perry EK, Blessed G, Tomlinson BE, Perry RH, Crow TJ, Cross AJ, Dockray GJ, Dimaline R, Arregui A. Neurochemical activities in human temporal lobe related to aging and Alzheimer-type changes. *Neurobiol Aging*. 1981; 2:251–256. [PubMed: 6174877]
69. Perry EK, Johnson M, Kerwin JM, Piggott MA, Court JA, Shaw PJ, Ince PG, Brown A, Perry RH. Convergent cholinergic activities in aging and Alzheimer's disease. *Neurobiol Aging*. 1992; 13:393–400. [PubMed: 1625768]
70. Perry EK, Perry RH, Smith CJ, Dick DJ, Candy JM, Edwardson JA, Fairbairn A, Blessed G. Nicotinic receptor abnormalities in Alzheimer's and Parkinson's diseases. *J Neurol Neurosurg Psychiatry*. 1987; 50:806–809. [PubMed: 2956364]
71. Petersen RC, Parisi JE, Dickson DW, Johnson KA, Knopman DS, Boeve BF, Jicha GA, Ivnik RJ, Smith GE, Tangalos EG, Braak H, Kokmen E. Neuropathologic features of amnesic mild cognitive impairment. *Arch Neurol*. 2006; 63:665–672. [PubMed: 16682536]
72. Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, Galasko D, Jin S, Kaye J, Levey A, Pfeiffer E, Sano M, van Dyck CH, Thal LJ. Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med*. 2005; 352:2379–2388. [PubMed: 15829527]
73. Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, Mathis CA, Klunk WE, Masters CL, Rowe CC. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. *Brain*. 2007; 130:2837–2844. [PubMed: 17928318]
74. Potter PE, Tedford CE, Kindel G, Hanin I. Inhibition of high affinity choline transport attenuates both cholinergic and non-cholinergic effects of ethylcholine aziridinium (AF64A). *Brain Res*. 1989; 487:238–244. [PubMed: 2525061]
75. Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, Ayutyanont N, Keppler J, Reeder SA, Langbaum JB, Alexander GE, Klunk WE, Mathis CA, Price JC, Aizenstein HJ, DeKosky ST, Caselli RJ. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009; 106:6820–6825. [PubMed: 19346482]
76. Sabbagh MN, Shah F, Reid RT, Sue L, Connor DJ, Peterson LK, Beach TG. Pathologic and nicotinic receptor binding differences between mild cognitive impairment, Alzheimer disease, and normal aging. *Arch Neurol*. 2006; 63:1771–1776. [PubMed: 17172618]
77. Sabri O, Kendziorra K, Wolf H, Gertz HJ, Brust P. Acetylcholine receptors in dementia and mild cognitive impairment. *Eur J Nucl Med Mol Imaging*. 2008; 35(Suppl 1):S30–S45. [PubMed: 18228017]

78. Sassin I, Schultz C, Thal DR, Rub U, Arai K, Braak E, Braak H. Evolution of Alzheimer's disease-related cytoskeletal changes in the basal nucleus of Meynert. *Acta Neuropathol (Berl)*. 2000; 100:259–269. [PubMed: 10965795]
79. Seo H, Ferree AW, Isacson O. Cortico-hippocampal APP and NGF levels are dynamically altered by cholinergic muscarinic antagonist or M1 agonist treatment in normal mice. *Eur J Neurosci*. 2002; 15:498–506. [PubMed: 11876777]
80. Shimohama S, Fujimoto S, Matsushima H, Takenawa T, Taniguchi T, Perry G, Whitehouse PJ, Kimura J. Alteration of phospholipase C-delta protein level and specific activity in Alzheimer's disease. *J Neurochem*. 1995; 64:2629–2634. [PubMed: 7760043]
81. Shiozaki K, Iseki E. Decrease in GTP-sensitive high affinity agonist binding of muscarinic acetylcholine receptors in autopsied brains of dementia with Lewy bodies and Alzheimer's disease. *J Neurol Sci*. 2004; 223:145–148. [PubMed: 15337615]
82. Thorne B, Wonnacott S, Dunkley PR. Isolation of hippocampal synaptosomes on Percoll gradients: cholinergic markers and ligand binding sites. *J Neurochem*. 1991; 56:479–484. [PubMed: 1846398]
83. Tiraboschi P, Hansen LA, Alford M, Masliah E, Thal LJ, Corey-Bloom J. The decline in synapses and cholinergic activity is asynchronous in Alzheimer's disease. *Neurology*. 2000; 55:1278–1283. [PubMed: 11087768]
84. Tsang SW, Lai MK, Kirvell S, Francis PT, Esiri MM, Hope T, Chen CP, Wong PT. Impaired coupling of muscarinic M1 receptors to G-proteins in the neocortex is associated with severity of dementia in Alzheimer's disease. *Neurobiol Aging*. 2006; 27:1216–1223. [PubMed: 16129514]
85. Tsang SW, Pomakian J, Marshall GA, Vinters HV, Cummings JL, Chen CP, Wong PT, Lai MK. Disrupted muscarinic M1 receptor signaling correlates with loss of protein kinase C activity and glutamatergic deficit in Alzheimer's disease. *Neurobiol Aging*. 2007; 28:1381–1387. [PubMed: 16828202]
86. Wong TP, Debeir T, Duff K, Cuellar AC. Reorganization of cholinergic terminals in the cerebral cortex and hippocampus in transgenic mice carrying mutated presenilin-1 and amyloid precursor protein transgenes. *J Neurosci*. 1999; 19:2706–2716. [PubMed: 10087083]

Fig. 1. Choline acetyltransferase (ChAT) activity in frontal cortex. ChAT activity is indicated as pmoles/mg protein/hour. NPL = non-demented control with no plaques (N = 26); SPL = non-demented control with sparse plaques (N = 16); MPL = non-demented control with many plaques (N = 13); AD = Alzheimer's disease (N = 21); young control group (N = 5). The groups differ significantly from each other (ANOVA, $p < 0.05$). * These groups are significantly different from NPL ($p < 0.01$).

Fig. 2. Choline acetyltransferase (ChAT) activity in parietal cortex. ChAT activity is indicated as pmoles/mg protein/hour. Abbreviations and sample size as indicated in Fig. 1. The groups differ significantly from each other (ANOVA, $p < 0.05$). *These groups are significantly different from NPL ($p < 0.05$).

Fig. 3. Correlations of ChAT activity (pmoles/mg protein/hr) and plaque score. Plaque score is correlated with ChAT activity in frontal cortex (top panel) and parietal cortex (bottom panel). Abbreviations as in Figure 1. All correlations were significant (r = Spearman correlation coefficient; $p<0.001$).

Fig. 4. Correlations of ChAT activity and tangle score. Tangle score is correlated with ChAT activity in frontal cortex (top panel) and parietal cortex (bottom panel). Abbreviations as in Figure 1. All correlations were significant (r = Spearman correlation coefficient).

Fig. 5. Correlations of ChAT activity with MMSE score. ChAT activity in frontal (top panel) and parietal (bottom panel) is correlated with MMSE score. Both correlations were significant (r = Spearman correlation coefficient).

Fig. 6.

Displacement of pirenzepine by oxotremorine-M. Displacement of ^3H -pirenzepine by the agonist oxotremorine-M was measured in frontal cortex samples. (a) Results for elderly control subjects with no plaques (NPL; $N = 17$). (b) Results for elderly control subjects with sparse plaques (SPL; $N = 10$). (c) Results for elderly control subjects with many plaques (MPL; $N = 5$). (d) Results for AD subjects ($N = 21$). Data shown are mean $\pm$ SEM. Lines shown are best fit from non-linear regression, one-site competition. Only the curve for the NPL group was significantly shifted in the presence of GppNHp, indicating that receptor-G-protein coupling was functional only in this group.

Oxotremorine-M displacement of ^3H -PZ binding

Fig. 7.

Percent of binding sites in high affinity state. Percent of binding sites in the high-affinity state was determined using two-site competition curves for displacement of pirenzepine by oxotremorine-M. The groups differ significantly and all differ in pairwise comparisons with elderly non-demented subjects ($p < 0.05$) with no plaques (NPL). SPL = elderly non-demented subjects with sparse plaques; MPL = elderly non-demented subjects with many plaques; GppNHp = samples to whose samples GppNHp was added (see Materials and Methods section). The sample sizes are as given in Figure 5 except for the GppNHp group, which was composed of samples from all groups combined (N = 17 for NPL group; N = 10 for SPL group; N = 5 for MPL group; N = 21 for AD group).

Table 1

Basic characteristics of study subjects. Values shown are means and standard deviations (SD), and for the apolipoprotein E-- ϵ 4 allele, the ratio of cases carrying and not carrying the ϵ 4 allele.

Group (N)	Age	PMI (hrs)	ApoE-- ϵ 4
Young (5)	44.8 (14.2) ¹	16.8 (13.1) ¹	N/A
No plaques (28)	80 (8.9)	2.24 (0.66)	3:25 ²
Some plaques (17)	84.7 (5.9)	2.59 (0.74)	3:14 ³
Many plaques (12)	81.5 (8.1)	2.68 (0.84)	5:6
AD (25)	80.7 (8.7)	2.78 (1.15)	17:8

PMI = postmortem interval; ApoE-- ϵ 4 = apolipoprotein E-- ϵ 4 allele; NA = not available.

¹Significantly different from all other groups ($p < 0.01$).

²Significantly different from many plaque and AD groups ($p = 0.01$, $p < 0.0001$, respectively).

³Significantly different from AD group ($p = 0.001$).

Amyloid plaque and neurofibrillary tangle scores for study subjects. Values shown are means and standard deviations (SD) except for Braak stage, for which the median is shown.

Table 2

Group	Frontal Plaque Score	Parietal Plaque Score	Frontal Tangle Score	Parietal Tangle Score	Braak Stage
Young	0 ¹	0 ¹	0	0	0
No plaques	0 ¹	0 ¹	0.071 (0.22)	0.071 (0.22)	2
Some plaques	0.84 (0.79) ²	0.82 (0.86) ²	0.12 (0.33)	0.09 (0.36)	2
Many plaques	2.57 (0.62)	2.89 (0.29)	0.15 (0.27)	0.33 (0.54)	3
AD	2.63 (0.67)	2.69 (0.62)	2.04 (1.25) ¹	2.02 (1.19) ¹	5

¹Significantly different from all other groups (p < 0.01).
²Significantly different from many-plaque and AD group (p < 0.01).
³Significantly different from AD group (p < 0.01).