The Cholinergic Hypothesis of Age and Alzheimer’s Disease-Related Cognitive Deficits: Recent Challenges and Their Implications for Novel Drug Development

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The Cholinergic Hypothesis

A variety of studies in humans indicate that basal forebrain and rostral forebrain cholinergic pathways including converging projections to the thalamus serve important functional roles in conscious awareness, attention, working memory, and a number of additional mnemonic processes (Perry et al., 1999). For more than 20 years, studies of the brains of those with advanced age and Alzheimer’s disease (AD) have consistently found damage or abnormalities in these pathways (particularly basal forebrain projections) that appeared to correlate well with the level of cognitive decline. As a result, the so-called “cholinergic hypothesis” was developed, which essentially states that a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age and AD (reviewed, Bartus, 2000). Extensive literature from animal experiments supports the human data described above. In fact, the importance of cholinergic function in the brain to learning and memory was first recognized more than 30 years ago after cholinergic antagonists (specifically antimuscarinic agents) were found to impair memory in rats (Deutsch, 1971). Considerable additional evidence now supports this early work, and antimuscarinic agents such as scopolamine and atropine have been shown to impair memory...
performance in a variety of behavioral paradigms in rodents. Such tests include passive (inhibitory) avoidance procedures, operant (matching and nonmatching) tasks, and spatial learning (and working memory) procedures such as water maze and radial arm maze tasks (reviewed, Decker and McGaugh, 1991). These data have been further extended to include selective muscarinic (i.e., M1) antagonists such as pirenzepine (Hunter and Roberts, 1988) as well as centrally acting nicotinic-cholinergic antagonists such as mecamylamine (Levin, 1992). Both muscarinic antagonists (Terry et al., 1993a; Vitiello et al., 1997) and nicotinic antagonists (Elrod and Buccafusco, 1991; Newhouse et al., 1994) have also been shown to impair memory performance in monkeys and humans. Furthermore, lesions in animals that damage cholinergic input to the neocortex or hippocampus from the basal forebrain (e.g., nucleus basalis magnocellularis and medial septum/diagonal band) disrupt performance of the same memory tasks that are impaired with cholinergic blockade (reviewed in Decker and McGaugh, 1991). It should be noted that damage to similar basal forebrain regions in humans (as a result of arterial aneurysms, or resection of an arteriovenous malformation) has also been associated with severe memory deficits (Damasio et al., 1985).

As a result of the findings cited above (i.e., in both humans and animals), the primary therapeutic approach to date to address the cognitive loss associated with AD has been that of a cholinergic replacement strategy. This approach has been attempted using muscarinic and nicotinic-cholinergic ligands and acetylcholinesterase inhibitors (reviewed in Buccafusco and Terry, 2000). To date, however, only the data derived from clinical trials with acetylcholinesterase inhibitors (e.g., tacrine, donepezil, rivastigmine, and galantamine) have provided convincing evidence of an adequate level of efficacy and reliability in AD balanced with an acceptable burden of side effects. Accordingly, these agents are the only drugs currently approved by the United States for clinical use in AD. Due to the modest risk of hepatotoxicity associated with tacrine, the latter three compounds listed above are generally preferred. Agents such as the glutamate antagonist memantine have recently been associated with improvements in advanced AD symptomatology and may suggest one new approach to therapy.

**Challenges to the Cholinergic Hypothesis**

Investigations focused on the brains of those with mild cognitive impairment (MCI) or the very early stages of AD are becoming increasingly important as diagnostic methods for these conditions become more refined and accurate. Such investigations may aid the development and/or identification of neuroprotective strategies as well as more specific disease management approaches. In most previous studies (that have attempted to correlate the level of cognitive decline with disease neuropathology), the brains of patients with end stage disease were analyzed and therefore may not be particularly helpful for new investigative efforts aimed at altering disease progression if the disease is diagnosed at a very early stage. The results of the small number of published reports available in which the brains of patients diagnosed with MCI and/or mild AD were analyzed have led some to begin to challenge the validity of the cholinergic hypothesis. For example, Davis and colleagues (1999) reported that the activity of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) was not reduced in post mortem neocortical tissues of those recently diagnosed with mild AD. As a result, the authors suggested that: 1) it is unlikely that a cholinergic marker could be used as an early indicator of AD; 2) it is unlikely that a cholinergic deficit could be identified prior to the patient becoming symptomatic; and 3) only the patients with more severe disease should be a target for cholinergic treatment. In addition, DeKosky and colleagues (DeKosky et al., 2002) failed to detect any reduction in ChAT activity in a number of cortical regions studied in patients diagnosed with MCI or mild AD, and in fact, activity was actually up-regulated in the frontal cortex and hippocampus of those with MCI. In another study, neurons containing ChAT and the vesicular acetylcholine transporter protein were preserved in the nucleus basalis in individuals with MCI and early AD (Gilmor et al., 1999). Collectively, the articles cited here have led to editorials (e.g., Morris, 2002) that have further challenged the assumptions and validity of the cholinergic hypothesis as it applies to AD (particularly in the early stages).

It should be noted that while the aforementioned studies and subsequent editorials provide valuable data and discussion, some of the conclusions appear somewhat premature. Since neither ChAT nor AChE are rate-limiting cholinergic enzymes, they are unlikely to accurately reflect cholinergic function in the living patient, and a host of factors that were not assessed (or even mentioned in these studies) could be compromised in cholinergic neurons before changes in these enzymes would be observed. Examples from the post mortem AD literature include alterations in high-affinity choline uptake, impaired acetylcholine release, deficits in the expression of nicotinic and muscarinic receptors, and dysfunctional neurotrophin support (reviewed in Auld et al., 2002). Each of these important factors deserves further discussion. Alterations in high-affinity choline transport (i.e., the rate-limiting process for acetylcholine synthesis) have been observed in post mortem AD brains (Slotkin et al., 1990) as well as in the brains of transgenic mice that exhibit AD-like amyloid pathology (Apelt et al., 2002). An increase in choline flux across the membranes of neuronal cells exposed to β-amyloid has also been hypothesized to contribute to the selective vulnerability of cholinergic neurons in AD (Allen et al., 1997). The results of experiments by Kar and colleagues (Kar et al., 1998) using rat hippocampal slices indicated that under acute conditions, amyloid peptides could inhibit the uptake of choline and decrease endogenous acetylcholine release without exhibiting effects on ChAT activity. Interestingly, in earlier studies, Nilsson and colleagues (Nilsson et al., 1986) detected a deficit in potassium evoked acetylcholine release in post mortem cortical tissue from AD patients. A variety of studies have reported reductions in central nicotinic receptors in aged subjects and those who suffered from AD or other age-related disease in which dementia was present (e.g., Lewy Body disease and Parkinson’s disease; see Perry et al., 2000). In AD, high-affinity α4 containing nicotinic receptors appear to be more significantly reduced than either α3 or α7 containing receptors, although decreases in α7 binding sites have been observed in Lewy Body disease (see review, Picciotto and Zoli, 2002). This finding suggests that nicotinic receptor subtypes may be differentially reduced in different forms of dementia.

There is a considerable amount of evidence to suggest that
nerve growth factor (NGF) support to cholinergic neurons in the basal forebrain of AD patients is deficient leading to atrophy and possibly cell death. While there does not appear to be a deficiency in the synthesis or availability of NGF protein in the hippocampus or neocortex in AD brains, substantial evidence suggests that retrograde transport of the neurotrophin and signal transduction via the high-affinity tyrosine receptor kinase (TrkA receptor) is compromised (see Mufson, 1999). While not demonstrated specifically in the cholinergic phenotype, deficits in axonal transport in cortical neurons have also been reported to occur in AD (Dai et al., 2002). This finding highlights a fundamental cellular process that may be disrupted in many neuronal populations in AD brains including cholinergic neurons, and further, deficits in axonal transport could underlie the deficits of retrograde transport of NGF. It is certainly conceivable that early subtle deficits in both retrograde and anterograde axonal transport could precede measurable deficits in many cholinergic markers including ChAT or AChE. For a graphic summary of the information provided in this section, please refer to Fig. 1.

An additional issue that is important to address in regard to the recent challenges to the cholinergic hypothesis (as well as many of the reports that support the hypothesis) relates to the condition of the tissue samples studied. It should not be understated that the collection of post mortem human tissues for neurochemical analysis involves unavoidable delays that can compromise the viability of the tissues analyzed. While the studies cited above report impressive post mortem intervals ranging from approximately 4 to 12 h, this contrasts with animal studies in which post mortem intervals often involve a matter of minutes. Therefore, unavoidable tissue deterioration and variability in the data associated with post mortem AD brains pose a significant challenge, an issue that underscores the importance of the development and use of appropriate animal models of AD. As better in vivo imaging methods become more widely available, ambiguities related to cholinergic function in the central nervous system of living patients suffering from MCI or early AD will likely become better elucidated. Interestingly, several in vivo imaging studies conducted to date in AD patients appear to support the cholinergic hypothesis. For example, PET studies using $^{[11C]}N$-methylpiperidin-4-yl-propionate indicate that cortical acetylcholinesterase activity is indeed reduced in AD patients (Kuhl et al., 1999). $^{[11C]}$Nicotine-based PET studies indicate that nicotinic receptor deficits are in fact an early phenomenon in AD, and these reports further suggest that cortical nicotinic receptor deficits significantly correlate with the level of cognitive impairment (Nordberg, 2001). Other PET studies employing the nonselective muscarinic ligands $^{[123I]}$quinuclidinyl benzilate and $^{[11C]}N$-methyl-4-piperidyl benzilate indicate both age- and AD-related decreases in binding in neocortical regions (see Zubieta et al., 2001). Moreover, single photon emission computerized tomography (SPECT) studies using $^{[123I]}$benzovesamacol binding indicate that the vesicular acetylcholine transporter is reduced throughout the entire cerebral cortex and hippocampus in early onset AD patients (Kuhl et al., 1996).

### Aging and Brain Cholinergic Neurons

As age currently represents the most potent of the known risk factors for AD, it seems relevant to ask whether the...
function of central cholinergic neurons is impaired in the aged. Challenges to the cholinergic hypothesis of AD appear to ignore the body of evidence in support of the relationships between aging, cholinergic impairment, and cognitive decline. A study of the effect of advanced age on brain cholinergic function began in earnest in the early 1980's when chemical enzymatic methods were developed with the specificity and sensitivity to measure the dynamic aspects of transmitter function. Methods for the rapid stabilization of brain levels of acetylcholine and choline by rapid freezing or focused microwave irradiation were also introduced for routine use. For example, Gibson and coworkers (1981) examined whole brain synthesis of acetylcholine in aged mice from 3 to 30 months of age. They reported that the biosynthesis of acetylcholine (measured by injection of a radio-labeled precursor) declined by up to 75% in the 30 month-old animals. Mild hypoxia further decreased acetylcholine synthesis by 90%. Moreover, aged cholinergic neurons were more impaired in their ability to release acetylcholine following potassium stimulation than they were in their ability to synthesize the transmitter (Gibson and Peterson, 1981). Subsequent in vivo microdialysis methods largely confirmed these early findings (e.g., Wu et al., 1988). The concept that aged brain cholinergic neurons function relatively normally until stressed has been supported through experiments that used various methods to increase acetylcholine output (Meyer et al., 1986; Gilad et al., 1987; Moore et al., 1996) or that damage cholinergic neurons (Burk et al., 2002). It seems reasonable to conclude, therefore, that any sustained insult to forebrain cholinergic neurons could interfere with the ability of these cells to provide sufficient transmitter release for normal function. Sarter and his coworkers (Burk et al., 2002) tested this possibility directly in a longitudinal series of experiments in which chemical lesions of basal forebrain cholinergic neurons were created in young rats with the aim of producing only limited loss of basal forebrain cholinergic cells. The rats had been previously well trained in the performance of a sustained attention task. Whereas initially there was a similar degree of task performance in both experimental groups, a significant dissociation between lesioned and control rats (in terms of task efficiency) did not occur until the animals reached 31 months of age, when the lesioned group exhibited significant task impairment. The results of these studies in aged rodents become more relevant to the topic of this article considering the observation that most of the age-related changes pertained specifically to dynamic aspects of brain cholinergic neurons. In many of the studies cited above and in many other reports, indirect measures of standard cholinergic markers (as might be determined from autopsied tissues) often do not show such dramatic age-related differences.

Anticholinergic Drugs in Elderly Subjects and AD Patients

The body of evidence in support of the role for central cholinergic neurons, particularly nucleus basalis and septo-hippocampal projections, in learning and memory is too vast to discuss here. As indicated previously in this article, however, the cholinergic hypothesis pertaining to memory loss in AD was largely derived initially from reports of decreased cholinergic markers in post mortem AD brains and several antecedent animal studies citing amnestic properties of centrally acting muscarinic receptor antagonists such as atropine and scopolamine. If the hypothesis that decrements in the functional integrity of cholinergic neurons underlie (or at least significantly contribute to) diminished cognitive function in aged subjects and those with AD is correct, then one would expect such individuals to be especially sensitive to the memory-impairing effects of anticholinergic drugs. Indeed, substantial data to support this premise have been published. For example, scopolamine evokes significantly more profound amnestic effects in elderly subjects when compared with younger subjects (Zemishlany and Thorne, 1991; Flicker et al., 1992) and in subjects with AD when compared with nondemented elderly subjects (Sunderland et al., 1987).

Some years ago we also investigated the issue of age-related sensitivity to anticholinergics and compared the effects of scopolamine and the selective peripherally acting muscarinic antagonist glycopyrrolate on a series of cognitive paradigms administered to healthy elderly (55–67 years old) and young (28–47 years old) volunteer subjects (Ray et al., 1992). For each test administered, the results were compared directly to those produced by glycopyrrolate. The scopolamine data are reorganized and reproduced in Fig. 2, A and B. Glycopyrrolate did not significantly affect baseline test scores (data not shown). We also measured drug levels in the subjects and found them to be similar between the two groups. As indicated in Fig. 2A, the elderly subjects were impaired relative to their younger cohort in their performance of the selective reminding task, both for the consistent long-term retrieval and the delayed versions. In particular, elderly subjects exhibited a rapid decline in task performance with dose in the delayed version of the task that involved the learning and recall of new words in the selective reminding task. Elderly subjects also were impaired by the highest dose of scopolamine on their performance of the paired associates learning task. Younger subjects were not affected by this less cognitively demanding task (associated word pairs are formed during rehearsal). Performance of the symbol digit modality task was also impaired in the elderly subjects after receiving the highest dose of scopolamine (Fig. 2B). This task requires the subject to use a key to substitute numbers for meaningless geometric designs and requires the efficiency of multiple mechanisms in both hemispheres. As with the symbol digit modality task, the digit span task was performed less efficiently by the elderly cohort at baseline, but unlike the symbol digit modality task, there was no further decrement with scopolamine. Digit span is a crude measure of attention or immediate memory. The inability of scopolamine to further impact this aspect of cognition was confirmed by the lack of effect of the drug in the continuous performance task, which requires sustained vigilance. In the more difficult CPT-AX version (see Fig. 2B) of the continuous performance task, the elderly appeared to be more affected by scopolamine, but relative to the effects of glycopyrrolate, there were no significant differences between the two groups. Overall, results of this study are consistent with the general impairment of elderly subjects at baseline on certain cognitive tasks. They are also consistent with the marked sensitivity to muscarinic receptor blockade exhibited by the elderly. Surprisingly, tasks of attention and sustained vigilance were affected to a much lower degree than were tasks of immediate recall and delayed recall.
Amyloid and the Cholinergic System

Notwithstanding the recent challenges to the cholinergic hypothesis cited above, it has been generally promoted that basal forebrain cholinergic neurons constitute an early target for toxicity associated with the disease. Cholinergic neurons arising from the nucleus basalis and from the medial septum appear to be significantly more vulnerable than even the nearby neostriatal cholinergic neurons (Jhamandas et al., 2001). Therefore, theories concerning the proximal cause of AD should account for this selective vulnerability of neurons comprising basal forebrain cholinergic pathways. Since the overexpression and deposition of brain amyloid probably plays some role in the neurodegeneration associated with AD, the relationship between amyloid deposition and cholinergic neuron activity is certainly of interest. Interestingly, agonists partially selective for the M1 subtype of the muscarinic-cholinergic receptor have been reported to elevate the nonamyloidogenic amyloid precursor protein (α-APPs) and decrease amyloid-β (Aβ) levels (Muller et al., 1997; Fisher et al., 2002). This effect on APP processing appears to occur via the ability of these drugs to use downstream signaling pathways that involve the activation of protein kinase C and mitogen-activated protein kinase (Haring et al., 1998). M1 agonists also may decrease tau protein phosphorylation in vitro and in vivo (Sadot et al., 1996; Genis et al., 1999), another potential disease-modifying effect of this class of compounds, as hyperphosphorylated tau protein is linked to cellular disruption by neurofibrillary tangles.

The activation of nicotinic acetylcholine receptors also may produce disease-modifying actions in AD. For example, the ability of nicotine to evoke neuroprotective effects has been demonstrated in both in vitro and in vivo models of neural toxicity (Owman et al., 1989; Kihara et al., 1997). The mechanism for nicotine’s neuroprotective actions may involve the drug’s ability to transiently increase intracellular calcium with downstream actions to increase the synthesis of various neurotropic factors and their receptors (e.g., Dajas-Bailador et al., 2000; Jonnala et al., 2002). In fact, nicotine has been shown to inhibit the development of cellular toxicity induced by Aβ peptides (see Woodruff-Pak et al., 2002). Clearly, the degeneration of basal forebrain cholinergic neurons, which depend for their viability on continuous neurotrophic influence, could lead to both a cycle of decreasing stimuli for factors associated with cell survival and for emphasis of the production of neurotoxic forms of Aβ peptides. These characteristics of basal forebrain cholinergic neurons fail to provide...
an explanation as to their selective vulnerability to the disease process. Nevertheless, it has been shown that α7 nicotinic acetylcholine receptors can serve as high-affinity binding sites for Aβ peptides (Wang et al., 2000). Moreover, Aβ peptides can block the functional interaction of nicotinic agonists with their receptors on hippocampal neurons (Liu et al., 2001). The potential blockade of basal forebrain and hippocampal nicotinic receptors by endogenous Aβ peptides has implications not only for the cognitive decline associated with early stages of the disease process but also suggests a mechanism for the targeting of the AD-related toxic peptides to neural cells expressing α7 nicotinic receptors.

Thus, failure of the dynamics of cholinergic neurotransmission that is associated with aging and with early stages of AD could contribute to a cycle of neurotoxicity in advance of any detectable change in standard cholinergic marker enzymes or even before the deposition of amyloid plaques (Selkoe, 2002; Woodruff-Pak et al., 2002). This possibility is suggested by the finding that in certain transgenic strains of mice that overexpress mutated human APP, cognitive decline occurs in advance of the deposition of significant amounts of amyloid material (Holcomb et al., 1999; Kotilinek et al., 2002).

### Cholinergic-Based Therapeutic Strategies (Present and Future Considerations)

The preceding paragraphs summarize data that strongly support the assertion that the use of cholinergic agents remains valid as one strategy to combat the cognitive and neurodegenerative changes of AD. It is also important to note that changes in the central cholinergic system in AD may also contribute to a variety of adverse behavioral symptoms (i.e., in addition to cognitive deficits) such as depression, aggressive behavior, psychosis, and overactivity (Minger et al., 2000). These so-called “nongenetic” symptoms of AD increase caregiver burden, significantly raise the direct costs of care, and result in earlier institutionalization (reviewed Eustace et al., 2002). A number of studies now indicate that the standard therapy for cognitive dysfunction in AD (i.e., the acetylcholinesterase inhibitors) are associated with improvement in a number of behavioral symptoms including depression, psychosis, agitation, and a delay in nursing home placement (reviewed, Cummings, 2003). Such findings provide an additional rationale for the use of cholinergic-based therapies in AD.

Cholinergic abnormalities (which correlate with the degree of memory decline) have also been observed in association with neurodegenerative conditions other than AD such as Parkinson’s disease, dementia with Lewy bodies (reviewed, Perry et al., 1999), and most recently, vascular dementia (reviewed, Grantham and Geerts, 2002). Accordingly, the use of acetylcholinesterase inhibitors has to a limited extent been studied in these patient populations. To date, rivastigmine has been observed to benefit patients suffering from dementia with Lewy Bodies and Parkinson’s disease, and galantamine has been found to benefit those suffering from vascular dementia and AD with cerebrovascular disease (reviewed in Cummings, 2003).

It should also be noted that cholinergic agents including cholinesterase inhibitors (Terry et al., 1999b; Furey et al., 2000), muscarinic agonists (Ruske and White, 1999), and nicotinic agonists (Terry et al., 2002) have been shown to enhance learning and memory and/or attention in young unimpaired subjects. Hence, a cholinergic strategy to memory enhancement may have a wider application than merely the conditions (described above) in which cholinergic function is (significantly) impaired. Schizophrenia and other disorders in which cognitive dysfunction and distractibility are observed (e.g., attention deficit hyperactivity disorder) offer just a couple of examples. Currently, several cholinergic-based treatment strategies are in fact being pursued in the early phases of clinical trials for treatment of the cognitive deficits associated with schizophrenia.

### Concluding Remarks

One of the greatest challenges to the elucidation of AD etiology is the difficulty in studying the earliest changes in neuronal function in the brain and correlating these changes with ante mortem cognitive and behavioral function. Although suitable tissue specimens from patients with MCI and early AD are difficult to obtain, they currently represent the most logical pathway to understanding the most proximal causes of the disease process. The small numbers of studies that have been conducted to date to analyze tissues from patients who had MCI or early AD have certainly created a number of new questions related to the cholinergic hypothesis that may only be adequately addressed after in vivo imaging techniques become more reliable and widely available to study neurotransmitter systems in the brains of living patients. While it is no doubt quite interesting to know that the marked loss of cholinergic enzymes in relevant brain regions well known to occur in late stage AD are not apparent in early stage AD, the finding is perhaps not that surprising. From the above discussion it is evident that there probably exists ongoing targeted neural insults during aging and in the earliest stages of AD that affect the dynamics of cholinergic function without the marked loss of these enzymes. Indeed, the anatomically selective up-regulation of ChAT activity in autopsied brain tissues derived from subjects with mild cognitive impairment (DeKosky et al., 2002) may represent an attempt by cholinergic neurons under stress to compensate for functional impairments in transmitter release. Accordingly, there remains a host of rational drug development approaches related to central cholinergic neuronal function in memory disorders that may indeed pay further dividends in the future. Such approaches involve the development of novel and selective cholinesterase inhibitors (with more innocuous side effect profiles) muscarinic and nicotinic-cholinergic ligands, and methods to enhance growth factor (NGF) support to central cholinergic neurons. Such therapies may not only afford cognitive improvements and the amelioration of adverse behavioral symptoms in AD but also may provide neuroprotective and neurotrophic actions that could be beneficial in several forms of dementia as well as other debilitating conditions such as schizophrenia.

### References


