The Cholinergic Hypothesis of Geriatric Memory Dysfunction

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Summary. Biochemical, electrophysiological, and pharmacological evidence supporting a role for cholinergic dysfunction in age-related memory disturbances is critically reviewed. An attempt has been made to identify pseudoissues, resolve certain controversies, and clarify misconceptions that have occurred in the literature. Significant cholinergic dysfunctions occur in the aged and demented central nervous system, relationships between these changes and loss of memory exist, similar memory deficits can be artificially induced by blocking cholinergic mechanisms in young subjects, and under certain tightly controlled conditions reliable memory improvements in aged subjects can be achieved after cholinergic stimulation. Conventional attempts to reduce memory impairments in clinical trials have not been therapeutically successful, however. Possible explanations for these disappointments are given and directions for future laboratory and clinical studies are suggested.

Of the many behavioral impairments identified in the elderly, decreased cognition is generally recognized as one of the most severe and consistent. Controlled laboratory studies indicate that the majority of healthy, elderly persons show reliable declines in cognition in the later phase of life (*1*) and that this disturbance is shared by many other mammalian species, including mice (*2, 3*), rats (*4, 5*), and monkeys (*6–8*). In humans, this problem is often exacerbated by the insidious onset of senile dementia, estimated to affect over 2 million persons in the United States alone, and expected to increase to epidemic proportions during the current decade (*9*). In those cases of senile dementia, the cognitive disturbances often require complete and perpetual institutional care of the patient, compromising the quality of life of the patients and placing emotional and financial burdens on families and society.

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Although sociocultural, economic, and psychological factors probably contribute to the cognitive deterioration, the medical community commonly believes that age-related dysfunctions in the central nervous system (CNS) are intimately involved (*10*). Efforts to identify which changes in the CNS play major roles in the cognitive loss have intensified in recent years. Although the specific relationship between age-related CNS dysfunctions and cognitive loss will prove complex, recent evidence suggests that one major factor may be a disruption in the cholinergic neurotransmitter system. This "cholinergic hypothesis" is gaining considerable attention in the geriatric literature and has stimulated clinical trials, which have already attempted to compensate pharmacologically for the presumed cholinergic disturbance. Several paradoxical findings have emerged recently, however, and serious controversies have developed. For this reason, we have attempted to evaluate the available evidence pertinent to this question. We have been guided by three deductive requirements that must be satisfied if the cholinergic hypothesis is to deserve continued attention: (i) specific dysfunctions in cholinergic markers should be found in the brains of subjects suffering from age-related memory loss, (ii) artificial disruption of central cholinergic function in young subjects should induce behavioral impairments that mimic the cognitive loss found naturally in aged subjects, and (iii) appropriately enhancing central cholinergic activity in aged subjects should significantly reduce age-related cognitive deficits. By examining pertinent data from several neurobiological and clinical disciplines within this framework, we have attempted to objectively evaluate the strength of the support for the cholinergic hypothesis. We have also attempted to reconcile certain apparent paradoxes in the literature, identify pseudoissues that have needlessly emerged, and focus on specific critical issues in need of further empirical testing.

Evidence for Age-Related Changes in Central Cholinergic Function

Several neurotransmitter systems undergo reliable changes with advanced age (*11, 12*). Although controversy exists regarding which transmitter systems suffer the most dramatic changes with normal aging and whether this pattern differs in the brains of those with Alzheimer's disease, the basic issue crucial to evaluating the cholinergic hypothesis is whether reliable, functionally relevant changes in the central cholinergic system have been identified in aged brain tissue. In most of the research on human cholinergic mechanisms, comparisons have been restricted to Alzheimer's patients and normal age-matched subjects and have excluded young controls. This limitation makes it difficult to determine which qualitative changes in human brain occur normally with age, which may be exacerbated by the insidious onset of senile dementia, and which might be specific to that age-related disease state. Certain generalizations can be formed, however.

One of the more consistent neurochemical findings in the aged human brain is that the activity of choline acetyltransferase (CAT) is markedly reduced in the brains of Alzheimer's patients when compared with age-matched controls (*13*). Because CAT is far from saturated under normal circumstances (*14*), the functional relevance of these decreases in Alzheimer's disease has been questioned. Acetylcholine synthesis in biopsy samples from Alzheimer's patients, however, has been reported to be less than that in samples from age-matched controls (*15*). Furthermore, comparisons between Alzheimer's pa-

0036-8075/82/0730-0408$01.00/0

tients and age-matched controls revealed a severe loss of neurons in the nucleus basalis of Meynert (located within the substantia innominata) (*16*). Because this brain area is thought to provide the primary cholinergic input to the cortical mantle (*17*), these data offer the possibility that the decrease in cortical CAT in Alzheimer's patients may reflect a specific loss of cholinergic input to the cortex. Further tests are required to determine how characteristic and specific this loss is to patients suffering from senile dementia of the Alzheimer's type. It may be equally important that a positive correlation has been reported between degree of cognitive loss in senile dementia, decreases in CAT activity, and incidence of major neuropathological markers (*18, 19*).

Although a few studies have reported decreases in CAT activity in brains from nondemented (normal) elderly, many more have failed to find any changes (or found much smaller changes) over a range of disease-free age groups (Table 1). This negative trend suggests that the severe and consistent decrease found in Alzheimer's patients may reflect a disease-specific disturbance.

Although some studies comparing brains from animals of different ages report reliable decreases in CAT activity, these changes are typically small (15 to 25 percent). Further, many studies have failed to find similar decreases (Table 1). Thus, most animal aging data agree with the general human literature, failing to demonstrate large or reliable decreases in the activity of CAT as a function of increased (normal) age (Table 1).

There is no apparent explanation for the success of some authors in finding reliable changes in this enzyme marker with normal aging and the failure of many others. Although differences in assay technique, species, and age of subjects may have contributed to the variability of these results, these variables alone may not adequately explain all the discrepancies reported. Another possibility is that only very small decreases in CAT activity (or number of cholinergic neurons) occur naturally with age and that these changes are difficult to measure consistently. Accordingly, this mild decrease might become greatly exacerbated with senile dementia of the Alzheimer's type, especially in certain brain regions that are particularly vulnerable to the effects of the disease. Also, it seems likely that variations within subregions of certain large brain sites could contribute to differential findings be-

Table 1. Summary of choline acetyltransferase activity (aged rodents compared with young rodents, elderly humans compared with young humans, and Alzheimer's patients compared with age-matched, elderly humans).

Brain area	Decreased activity	
	Yes	No
	Aged rodents	
Cortex	Strong *et al.*, 1980 (Rat) (*3*) Unsworth *et al.*, 1980 (*91*)	Timiras and Vernadakis, 1972 (*91*) Meek *et al.*, 1977 (*91*) Reis *et al.*, 1977 (*91*) Strong *et al.*, 1980 (Mouse) (*3*) Ingram *et al.*, 1981 (*91*)
Striatum	McGeer *et al.*, 1971 (*91*) Meek *et al.*, 1977 (*91*) Strong *et al.*, 1980 (*3*)	Reis *et al.*, 1977 (*91*)
Hippocampus	Vijayan, 1977 (*91*)	Meek *et al.*, 1977 (*91*) Lippa *et al.*, 1980 (*5*) Strong *et al.*, 1980 (*3*) Ingram *et al.*, 1981 (*91*) Sherman *et al.*, 1981 (*20*)
Other areas	Unsworth *et al.*, 1980 (*91*)	McGeer *et al.*, 1971 (*91*) Meek *et al.*, 1977 (*91*) Reis *et al.*, 1977 (*91*) Vijayan, 1977 (*91*) Timiras and Vernadakis, 1972 (*91*)
	Elderly humans	
Cortex	McGeer and McGeer, 1975 (*11*) Perry *et al.*, 1977b, 1977c (*91*) Davies, 1978a (*91*) Perry, 1980 (*91*)	Bowen *et al.*, 1976 (*91*) Spillane *et al.*, 1977 (*73*) White *et al.*, 1977 (*91*) Spokes, 1979 (*91*) Carlsson *et al.*, 1980 (*29*) Yates *et al.*, 1980 (*91*)
Striatum		
Caudate nucleus	McGeer and McGeer, 1975 (*11*) McGeer and McGeer, 1976 (*91*)	Bowen *et al.*, 1976 (*91*) Perry *et al.*, 1977b, 1977c (*91*) Davies, 1978b (*91*) Carlsson *et al.*, 1980 (*29*) Yates *et al.*, 1980 (*91*)
Putamen	McGeer and McGeer, 1975 (*11*) McGeer and McGeer, 1976 (*91*)	Bird and Iverson, 1974 (*91*) McGeer and McGeer, 1975 (*11*) McGeer and McGeer, 1976 (*91*)
Hippocampus	Davies, 1978a, 1978b (*91*) Perry *et al.*, 1977b, 1977c (*91*)	Bowen *et al.*, 1976 (*91*) Carlsson *et al.*, 1980 (*29*) McGeer and McGeer, 1975 (*11*) Spokes, 1979 (*91*)
Other areas	McGeer and McGeer, 1975 (*11*) McGeer and McGeer, 1976 (*91*)	McGeer and McGeer, 1976 (*91*) Davies, 1978a, (*91*) Davies, 1978b (*91*) Spokes, 1979 (*91*) Carlsson *et al.*, 1980 (*29*) Yates *et al.*, 1980 (*91*)
	Alzheimer's patients	
Cortex, striatum and/or hippocampus:	*Bowen *et al.*, 1976 (*91*) Davies and Maloney, 1976 (*91*) Perry *et al.*, 1977a, 1977c (*91*) *Perry *et al.*, 1977b (*91*) Spillane *et al.*, 1977 (*73*) White *et al.*, 1977 (*91*) Davies, 1978a (*91*) *Davies, 1978b (*91*) Perry *et al.*, 1978 (*18*) Reisine *et al.*, 1978 (*24*) Yates *et al.*, 1979 (*91*) Antuono *et al.*, 1980 (*91*) Bowen and Davison, 1980 (*91*) Carlsson *et al.*, 1980 (*29*) Nordberg *et al.*, 1980 (*91*) †Rossor *et al.*, 1980a (*91*) *Rossor *et al.*, 1980b (*91*) Sims *et al.*, 1980 (*83*) Yates *et al.*, 1980 (*91*) Davies and Feisullin, 1981 (*23*) Davies and Terry, 1981 (*91*) Perry *et al.*, 1981 (*32*) Perry *et al.*, 1981 (*19*) Rossor *et al.*, 1981 (*91*)	

*Except caudate nucleus. †Except anterior hippocampus and caudate nucleus.

tween investigators. Although these possibilities cannot be objectively evaluated from existing data, future studies carefully specifying tissue origin and location and directly comparing young control subjects with aged subjects and Alzheimer's patients should help resolve this issue. Of course, the question of the functional significance of these subtle ($\leq$ 25 percent) decreases still has to be addressed.

Recent studies in aged animals reveal additional alterations in biochemical measures that suggest presynaptic dysfunctions. Sodium-dependent, high-affinity choline uptake has been reported to be decreased approximately 20 percent under basal conditions in the hippocampus of aged rats (*20*). Under conditions of potassium stimulation, however, choline uptake did not differ in young and aged hippocampus. Further, no age-related differences were observed in either choline or acetylcholine levels. The age-related difference in basal choline uptake was due to changes in the maximum velocity of the enzyme reaction (V_{max}) and not in the Michaelis constant (K_m). Since the V_{max} for high-affinity uptake is regulated by the activity of cholinergic neurons (*20*), these results suggest a decrease in the activity of septo-hippocampal cholinergic neurons. This possibility has recently received independent corroboration by reports of an age-related decrease in the synthesis of acetylcholine when measured in vivo in two strains of mice (*21*), whereas only marginal decreases were observed in in vitro prisms (*22*) and no loss of synthesis was observed in slices (*20*).

In addition to examining the brains of aged subjects for changes in presynaptic activity, several research groups have investigated postsynaptic muscarinic receptors using radioligand receptor-binding techniques (*23*). Because the majority of the human studies were concerned with changes that might occur specifically with Alzheimer's disease, however, most comparisons were made between brains from Alzheimer's patients and age-matched controls (normal elderly). Although a definitive answer is not yet possible, these studies generally agree that no major difference in receptor binding exists between normal aging and Alzheimer's disease (Table 2). Unfortunately, this comparison cannot address the question of what changes might occur during normal aging. Of the three studies that specifically evaluated changes in muscarinic binding over a range of ages in the nondiseased human brain, two reported significant decreases in binding of muscarinic antagonists in the cortex of the older brains. The receptor densities reported in these elderly subjects were not substantially different from those in Alzheimer's patients, confirming the majority opinion that receptor alterations in the cholinergic system do not occur with Alzheimer's disease to any further extent than that which occurs with natural aging. On the other hand, Reisine *et al.* have reported that the hippocampus of Alzheimer's patients does endure exaggerated loss of muscarinic receptors when compared with that of normal, age-matched controls (*24*). Others have failed to observe this change in the hippocampus (Table 2). The possibility that regional sampling differences within the hippocampus may be responsible for this discrepancy needs to be explored systematically.

Determinations of muscarinic binding have also been performed in aged rodents. Results from these animal studies seem reasonably consistent; of the six reports that have been published, all but one (*3*) reported age-related decreases (20 to 50 percent) in the density of muscarinic receptors with no change in affinity (Table 2). Although perfect agreement does not exist concerning which brain regions exhibit the most reliable changes, the hippocampus, cortex, and striatum have attracted the greatest attention. Once more, the lack of clear definition or identification of what tissue was included when a particular brain site was assayed probably explains many of the apparently contradictory effects in specific brain areas. This problem would seem particularly important when one considers the wide variation in tissue that might be affected when relatively large heterogeneous areas such as cortex and hippocampus are dissected out and the fact that certain regions may be altered by age at different rates. In other words, one major factor for many of the discrepancies in neurochemical changes reported with aging, as well as with dementia, may involve indiscriminate pooling of heterogeneous subregions which exist within classically defined brain sites. Despite these apparent discrepancies, experimental destruction of these same areas in young animals induces specific behavioral deficits similar to many of those found in aged subjects (*25, 26*).

Collectively, there exists good evidence for decreased muscarinic receptor density with normal aging, although little evidence indicates that these changes are more severe in the brains of Alzheimer's patients. This conclusion should not be interpreted to mean that there is no decrease in muscarinic receptors in the brains of Alzheimer's patients. Rather, there appears to be no further loss of muscarinic receptors in Alzheimer's patients beyond that found in age-matched controls. If the decrease in muscarinic receptors is indeed relevant to decreased

Table 2. Summary of muscarinic receptor binding (aged rodents compared with young rodents, elderly humans compared with young humans, and Alzheimer's patients compared with age-matched, elderly humans).

Brain area	Decrease in receptor density	
	Yes	No
	Aged rodents	
Cortex	James and Kanungo, 1976 (*92*) Strong *et al.*, 1980 (*3*)	Morin and Wasterlaine, 1980 (*92*)
Striatum	Morin and Wasterlaine, 1980 (*92*) Strong *et al.*, 1980 (*3*)	
Hippocampus	Lippa *et al.*, 1980 (*5*) Lippa *et al.*, 1981 (*27*)	Morin and Wasterlaine, 1980 (*92*) Strong *et al.*, 1980 (*3*)
Other areas	James and Kanungo, 1976 (*92*) Freund, 1980 (*92*) Morin and Wasterlaine, 1980 (*92*)	Morin and Wasterlaine, 1980 (*92*)
	Elderly humans	
Cortex	White *et al.*, 1977 (*91*) Perry, 1980 (*91*)	Davies and Verth, 1978 (*92*)
	Alzheimer's patients	
	*Reisine *et al.*, 1978 (*24*)	†Perry *et al.*, 1977 (*91*) White *et al.*, 1977 (*91*) Davies, 1978 (*91*) †Davies and Verth, 1978 (*92*) Perry *et al.*, 1978 (*18*) Reisine *et al.*, 1978 (*24*) †Antuono *et al.*, 1980 (*91*) Bowen and Davison, 1980 (*91*) †Nordberg *et al.*, 1980 (*91*) †Perry, 1980 (*91*)

*Hippocampus only. †Including hippocampus.

cholinergic function in normal aging, the persistence of the decrease in Alzheimer's patients must play an equally important role in this disease state.

Although the functional significance of these subtle (and sometimes inconsistent) decreases in receptor density requires further investigation, it has recently been demonstrated that functional disturbances in postsynaptic mechanisms occur in aged animals exhibiting receptor loss and memory impairment (*5, 27*). This was accomplished by applying microiontophoretic techniques to study responsiveness of hippocampal muscarinic receptors in young and aged Fischer 344 rats. Single-cell recordings revealed that both acetylcholine and glutamic acid iontophoretically applied stimulated pyramidal cell firing rate in proportion to ejection current. However, aged brains became significantly less sensitive to acetylcholine but not to glutamic acid, whereas γ-aminobutyric acid inhibited firing in aged cells slightly more (*27*). This ability of glutamic acid to stimulate cells argues against a generalized age-related decrease in neuronal sensitivity. Rather, these results may be considered direct evidence for a selective impairment of hippocampal cholinergic function in surviving neurons from aged (nonhuman) brains.

It remains to be determined (i) to what extent this decrease in responsiveness to acetylcholine directly reflects the loss of muscarinic receptors, (ii) what other factors (membrane alterations, receptor-effector coupling, and so forth) may also be involved, and (iii) whether they indeed relate to changes in the aging human brain. At the same time, these neurophysiological data, when considered with other neurochemical findings in animals and humans, satisfy an important prerequisite for the cholinergic hypothesis: changes do occur in the cholinergic system with age, and these changes are reflected in decreased functional activity of cholinoreceptive neurons.

Simply demonstrating that age-related changes in the cholinergic system occur does not address the question of whether these changes might be related to the memory loss observed in aged subjects. Age-related changes in the CNS have been observed in many other neurotransmitter systems as well. In certain brain areas, neurochemical markers for other transmitter systems exhibit much more robust changes with normal aging than those reviewed here for the cholinergic system. For example, substantial age-related changes in catecholamines have been reported in the hypothalamus and striatum (*28*). The relationship between these changes and the specific memory loss observed in aged subjects has yet to be addressed systematically. Although some investigators have also reported alterations in catecholamines in Alzheimer's patients (*24, 29, 30*), these data have been disputed by other groups and remain controversial (*31*). Finally, certain subpopulations of Alzheimer's patients have been reported to exhibit substantial cell loss in the locus coeruleus (*32, 33*). Because the locus coeruleus is rich in catecholamine projections to the cortex, differences in degree of locus coeruleus degeneration between undefined subpopulations of Alzheimer's patients might explain the conflicting results regarding catecholamine alterations with senility. However, a recent evaluation of this possibility failed to demonstrate any apparent relationship between changes in cortical activity of dopamine β-hydroxylase and number of locus coeruleus neurons in Alzheimer's patients (*19, 32*). Further, no correlation was found between loss of dopamine β-hydroxylase activity and the major neuropathological marker (plaque counts) and clinical measures of dementia (*19*). Thus, the role that changes in catecholamines may play in the memory loss of old age and dementia remains uncertain.

One method of gaining additional information about the extent to which changes in various neurochemical systems contribute to the memory loss associated with age and dementia would be to pharmacologically impair function in various neurotransmitter systems in young subjects and compare the changes in memory ability with those occurring naturally in aged subjects. If age-related changes in the cholinergic or any other system contribute to the memory loss observed in old age, pharmacological disruption of that system should induce similar changes in behavior of young subjects.

Cognitive Effects of Pharmacological Disruption of Cholinergic Function

Deutsch advanced the idea of the role of the cholinergic system in the storage and retrieval of information during new learning (*34*), which has become increasingly accepted. However, the alteration of retention of newly acquired behaviors by pharmacologically manipulating many other neurotransmitter systems (*35*) raises the question of whether the role of the cholinergic system in retention of learned events is any greater than that of other neurotransmitter systems. Moreover, since many of the tasks used in these early studies (such as multiple-trial learning tasks and tests of long-term retrieval) do not display severe age-related deficits (*25, 36, 37*), one must question the relevance of these earlier learning and memory studies to the behavioral deficits associated with old age.

More recent studies have directly addressed these issues. Collectively, they provide circumstantial evidence for a role of the cholinergic system in age-related memory deficits. The deficits observed in aged subjects typically occur in situations requiring relatively recent events to be remembered, usually without the benefit of extensive rehearsal or practice (*6, 7, 38*). The primary pharmacological data supporting an important cholinergic involvement in this deficit is that blockade of central muscarinic receptors induce a deficit in young subjects which is qualitatively similar to that occurring naturally in aged subjects. In human studies, Drachman *et al.* used a number of clinical measures to find that young subjects tested under a low dose of scopolamine exhibited memory (*39*) and other cognitive (*40*) deficits similar to those found naturally in aged subjects tested on the same clinical battery. The tests which revealed the most severe deficits in both cases involved memory for recent (but not immediate) events.

Aged monkeys tested on a number of different behavioral tasks suffer a very consistent and severe deficit on tasks requiring memory for recent sensory events (*6, 7*), with greatest deficits under those conditions requiring longest retention of recent information. This deficit shares many conceptual and operational similarities with that suffered by elderly and demented humans (*41*). One of the most consistent and robust pharmacological phenomena observed on this memory task is that young monkeys injected with the central cholinergic receptor blocker scopolamine (but not the peripheral blocker methylscopolamine) exhibited a deficit strikingly similar to that occurring naturally in the aged monkeys (*42*).

Subsequent studies demonstrated that the deficit produced by scopolamine can be partially, but reliably, reduced by the anticholinesterase physostigmine in both humans (*43*) and monkeys (*44*). Similar beneficial effects were not observed with the CNS stimulants methylphenidate (*9*) or amphetamine (*43*). It is therefore unlikely that the retention deficit induced by scopolamine in either human or nonhuman primates can be related to its more general effects on arousal, attention, or similar sedative-like properties.

These data provide additional support

for the possibility that the amnesia induced by scopolamine is due to a specific disruption of cholinergic mechanisms that are important to the behavioral expression of memory. As such, they suggest that an important functional relationship may exist between normal aging, cholinergic malfunctioning, and loss of memory.

In contrast, similar deficits have not been observed with analogous pharmacological blockade of dopamine or β-adrenergic receptors (*45*), supporting the notion that the role of the cholinergic system is somewhat specific. It has been suggested that depletion of dopamine in young monkey frontal cortex by 6-hydroxydopamine induces a cognitive deficit (*46*) qualitatively similar to that observed with aged monkeys (*6*). However, contrary to the effects in aged monkeys and those injected with scopolamine (*42*), the deficit observed with dopamine depletion resembles that found with haloperidol injections (*45*), showing clear deficits on the task but lacking the necessary selectivity on longer delay intervals. Because performance was not differentially affected on long versus short delay intervals, one cannot rule out the possi-

Table 3. Summary of clinical cholinergic precursor studies.

Study	Dose (g/day)	Substance	Duration	Procedure	Subject population	Effects
Boyd *et al.*, 1977 (*93*)	5 to 10	Choline	2 to 4 weeks	Open	Alzheimer's (70 to 80 years)	No measurable improvement
Etienne *et al.*, 1978a (*93*)	8	Choline	4 weeks	Open	Moderate Alzheimer's (76 to 88 years)	One of three possibly improved
Signoret *et al.*, 1978 (*93*)	9	Choline	4 weeks	Open	Early Alzheimer's (59 to 78 years)	Claim some improvement, but little data shown
Etienne *et al.*, 1978b (*93*)	25	Lecithin	4 weeks	Open	Alzheimer's (42 to 81 years)	No effects on memory scores; three of seven improved on learning rate
Smith *et al.*, 1978 (*93*)	9	Choline	2 weeks	Double-blind	Alzheimer's (mean age 77)	No effects on cognitive scores
Peters and Levin, 1978 (*64*)	3.6	Lecithin	1 day	Double-blind	Alzheimer's (58 to 79 years)	No effects on memory scores
Renvoize and Jerram, 1979 (*93*)	15	Choline	2 months	Double-blind	Alzheimer's (57 to 78 years)	No differences in communication skills
Ferris *et al.*, 1979 (*93*)	12 to 20	Choline	4 weeks	Open	Elderly outpatients	No effects on cognitive test scores, including memory
Mohs *et al.*, 1979 (*93*)	16	Choline	7 days	Double-blind	Healthy elderly with memory impairment (64 to 86 years)	No effects on any test scores, including memory
Whitely *et al.*, 1973 (*93*)	9	Choline	3 weeks	Open	Early Alzheimer's (50 to 58 years)	No effects on cognitive test score; two of eight reported improved on recall test
Christie *et al.*, 1979 (*93*)	2 to 5	Choline	9 days	Open	Alzheimer's (53 to 67 years)	No measurable improvement; trend in mild dementia
	28 to 100	Lecithin	3 months	Open	Same	No further deterioration after 3 months, compared with patients terminating treatment
Mohs *et al.*, 1980 (*93*)	8	Choline	3 weeks	Double-blind	Healthy elderly (62 to 83 years)	No effects on memory scores
Fovall *et al.*, 1980 (*93*)	8 to 16	Choline	2 weeks	Double-blind	Early Alzheimer's (55 to 77 years)	Improvement in word recognition only
Vroulis *et al.*, 1981 (*93*)	70	Lecithin	2 to 8 weeks	Double-blind	Early-severe Alzheimer's	Improvement in short-term (6 of 15) and long-term (8 of 15) recall and long-term storage (10 of 15). Improvement in EEG frequency (10 of 18)
Thal *et al.*, 1981 (*93*)	4 to 16	Choline	2 weeks	Double-blind	Mild to moderate Alzheimer's (49 to 80 years)	No subjective functional improvement nor enhancement of objective cognitive scores, despite doubling of plasma choline concentrations
Etienne *et al.*, 1981 (*93*)	30	Lecithin	3 months	Double-blind	Moderate Alzheimer's outpatients (47 to 85 years)	No improvement on any test measures
Brinkman *et al.*, 1982 (*93*)	35	Lecithin	2 weeks	Double-blind	Mild to moderate Alzheimer's patients	No improvement in memory

bility that disturbances in important nonmemory functions (those not directly involved with the storage, maintenance, or retrieval of information in memory), are responsible for the behavioral impairment (*42, 47*).

Certainly, future research can be expected to identify other neurotransmitter systems playing important roles. In fact, other pharmacological agents (most notably benzodiazepines) can induce similar amnestic performance deficits (*48*). These selective effects are the exception rather than the rule, however, and they emphasize the important role cholinergic mechanisms apparently play in helping to mediate this behavior.

The question of what role the age-related changes in other neurochemical systems, particularly the catecholamines, may play in aged behavior again arises. The high correlation between extrapyramidal Parkinson symptoms and loss of cognitive function (*49*), as well as depression and age, attests (*50*) to the likelihood that these systems are involved in important age-related changes in brain function and behavior. Changes in these systems may also be involved in cognitive dysfunctions related to but different from the memory impairments discussed here. Recent evidence for cell loss in the locus coeruleus with normal aging (*51*) and subgroups of senile patients (*19, 33, 52*) supports this possibility. Given that the locus coeruleus provides a major norepinephrine input to the cortex and has independently been associated with performance of learned tasks in rodents (*53*), it is conceivable that age-related declines in locus coeruleus neurons and concomitant catecholamine dysfunction might contribute significantly to the cognitive deterioration of the elderly. To date, however, empirical support is lacking.

Another consequence of age-related changes in catecholamine markers might be to further exacerbate the neurochemical inbalance associated with the cholinergic disturbances, producing greater functional loss. Age-related changes in catecholamines (and other neurotransmitters) could then play a necessary, but not sufficient, role in the memory disorders of the aged. This possibility might explain why pharmacological blockade of these systems fails to induce specific memory impairments similar to those seen in aged subjects and young subjects given central cholinergic blockers.

Although the specific relationship between age-related changes in catecholaminergic function and possible behavioral impairments await further study, an important role for cholinergic dysfunctions in age-related memory deficits has begun to emerge. The human and nonhuman primate studies reviewed corroborate each other and demonstrate that one of the most severe and consistent deficits observed with age occurs on tasks requiring memory for relatively recent events. At the same time, of all the classes of drugs tested on these memory tasks, drugs having anticholinergic effects seem to produce deficits most closely mimicking the natural, age-related memory impairments, satisfying another logical prerequisite for the cholinergic hypothesis. Although more research is needed, particularly concerning the possibility that other neurotransmitter systems may play equally important roles in this impairment, these pharmacological data support a cholinergic role. When these pharmacological data are considered with the correlative neurochemical and neurophysiological changes discussed earlier, this cholinergic interpretation has even greater appeal.

Facilitation of Geriatric Memory by Cholinomimetics

A question not yet addressed is whether enhancing central cholinergic function can reduce age-related memory deficits. Although neither a necessary nor a sufficient test of the cholinergic hypothesis, studies directed toward this issue may nevertheless provide information useful for determining the overall strength of the evidence for and against the idea. The vast majority of clinical studies concerned with this problem can be classified as one of two types: (i) those attempting to enhance the synthesis and release of acetylcholine by providing abundant amounts of the precursor substances choline or lecithin and (ii) those attempting to enhance cholinergic activity by pharmacological intervention within the synapse or at the receptor site.

The rationale for attempting to improve geriatric cognition with increased amounts of cholinergic precursors is simple. A number of in vitro studies indicate that under certain conditions, increases in brain choline (or lecithin, a normal dietary source of choline) can induce a concomitant increase in the synthesis (and presumably release) of acetylcholine (*54*). Although these findings continue to generate controversy (*55*), recent surveys offer explanations for these discrepancies and conclude that under appropriate conditions (such as increased neuronal stimulation) certain brain regions do increase their rate of acetylcholine synthesis when extra precursor is available (*56, 57*). Since increased precursor availability may stimulate cholinergic function, cognitive loss might be reduced when abundant quantities of precursor are administered.

Of the 17 studies of either choline or lecithin (Table 3), only one claims substantial improvement (about 60 percent of patients tested). Ten did not obtain facilitative effects on the cognitive tasks (*58*). Although some investigators claim that positive trends seemed to exist in some small subpopulation of the subjects, the effects of the precursors are far from impressive, particularly in well-controlled, double-blind studies (Table 3). The lack of consistent group effects seems particularly striking in view of the wide range of doses tested in these studies and the long-term treatment (of many months) used in many studies. Although it is possible that still undefined subpopulations of patients may benefit from precursor loading, the results to date are disappointing.

The use of cholinomimetic drugs to enhance cholinergic activity as a way of improving geriatric memory has not been as extensive as precursor therapy, but has apparently been somewhat more successful. To date, the most popular cholinomimetic has been the anticholinesterase physostigmine. Early studies with young adults reported moderate improvement on cognitive tests within a very restricted range of single doses (*59*). Doses outside this narrow range produced either no change in performance or marked impairment (*60*). Similar effects have also been reported with young rhesus monkeys (*61*).

Recent studies with physostigmine in aged subjects have also demonstrated reliable facilitation of performance on memory tasks (*61–64*). Contrary to the effects of physostigmine in young subjects, however, the optimal acute dose seems to vary dramatically among individual aged subjects [rhesus monkeys (*61*), Cebus monkeys (*7*), and humans (*63*)]. Although there exist many possible explanations for this phenomenon, the marked improvement on memory tasks achieved with an anticholinesterase is consistent with a cholinergic role in the age-related memory disorders.

In addition to physostigmine, the muscarinic agonist arecoline has been evaluated for effects on performance in memory tasks. After receiving a single injection of arecoline, young adult volunteers exhibited significant improvement in ability to recall recently learned verbal material (*65*). Short-term doses of arecoline can also enhance performance on a

memory task in aged monkeys (*7*) and Alzheimer's patients (*62*). In the monkey study, direct comparisons revealed that the effects of arecoline were more robust and less variable than when the same monkeys were tested under either physostigmine or choline (*66*).

Although additional tests of cholinomimetics in aged subjects (including humans) are needed, it is already apparent that reliable improvement on tasks intended to measure memory can be obtained in the laboratory and clinic by pharmacologically manipulating the cholinergic system. Thus, another important prerequisite of the cholinergic hypothesis has been satisfied. Although the effects observed to date may not be therapeutically outstanding, one must recognize that the ability of physostigmine and arecoline to measurably improve performance must certainly be tempered by the adverse side effects, short half-life, and narrow effective dose range, which are hallmarks of both of these drugs. Further, the specific effects of physostigmine and arecoline on the cholinergic system may not be most consistent with the particular aspects of cholinergic function needed to maximize improvement in cognition. It has been suggested that some other aspect of cholinergic function, or more than a single point in the metabolic pathway, may have to be improved before significant clinical effects are obtained (*67*). Similarly, it may also be necessary to simultaneously improve the function of other undefined systems or affect the balance between the cholinergic and other neurotransmitter systems in order to substantially reduce the behavioral impairments. Presumably, as more is learned about the specific nature of the cholinergic deficiency and its relation to other neurotransmitter systems, drugs with more specific and appropriate actions may be developed, leading to greater therapeutic effects. At the same time, the positive results obtained with current cholinomimetics corroborate the pharmacological, biochemical, and electrophysiological data; together they support an important cholinergic role in age-related memory loss.

These studies have demonstrated that (i) significant changes in cholinergic markers occur in the brains of aged animals and humans; (ii) these changes can be related to a loss of cholinergic function at the neuronal level; (iii) relationships can be established between these changes in the cholinergic system and the loss of memory that occurs with age; (iv) artificial disruption of cholinergic mechanisms in young subjects impairs memory tasks in ways strikingly similar to those that occur naturally in old age and dementia; and (v) a narrow range of doses of certain cholinomimetics can significantly reduce the memory impairments in aged subjects. Although it might be premature to draw any final conclusions from this circumstantial evidence, the data demonstrate that certain logical criteria, prerequisite for accepting the cholinergic hypothesis, have been satisfied and that continued empirical and therapeutic interest is therefore justified.

Directions for Future Research

A question that is beginning to emerge is why different cholinomimetics seem to produce different results on memory in geriatric subjects. The absence of clear positive effects of choline and lecithin on geriatric patients is also perplexing. Among the many possible explanations, one that is consistent with all available data is that the more directly one stimulates the muscarinic receptor, the more robust and consistent are the effects on memory performance in aged subjects (*7*). Accordingly, even if choline and lecithin increase acetylcholine release, they may have relatively little effect in geriatric subjects because the aged brain may be functionally disturbed at the receptor or coupling mechanism of the cholinoceptive neuron (*5, 27, 68*). Such a disturbance might then be most effectively treated by stimulating receptors or the secondary messenger on the effector side of the synapse. Increasing acetylcholine synthesis might do little to alleviate the functional loss since that aspect of cholinergic activity is still relatively intact. Similarly, inhibiting the degradation of acetylcholine released into the synapse may be more effective than that, but still less so than direct agonist stimulation.

Further, drugs that bypass a probable effective link in transmission somewhere beyond the actual binding site might improve performance even more effectively. Research evaluating the effects of different cholinergic agonists and agents in aged humans would be useful, as would that with new classes of drugs to improve cholinergic function in currently unimagined ways.

Other testable possibilities also exist for the inability of choline and lecithin to enhance geriatric memory. One may simply be that peripherally administered precursors do not effectively stimulate cholinergic activity. Although it is becoming accepted that choline has weak muscarinic agonist effects (*69*), its ability to enhance acetylcholine synthesis and release remains controversial (*55–57*). Every study attempting to improve geriatric cognition by precursor loading depends on the validity of this assumption, and thus the data supporting and contradicting this notion must continue to be critically evaluated until a common consensus develops.

Another reason percursors have failed to improve geriatric patients may be that the neurochemical changes are insufficient to produce measurable behavioral effects, particularly on tasks intended to measure memory and other cognitive skills. However, choline induces changes in less complex behaviors in both animals (*70*) and humans (*71*), and there is no a priori reason to expect that the presumed neurochemical factors may be less effective for memory-related tasks. Additionally, a single published account demonstrated increased memory performance when young subjects were administered choline (*72*). Although this question remains open to future experimentation, it seems reasonable that still other factors may be involved.

A third possibility for the apparent paradox may be that the cholinergic dysfunction that contributes to the age-related memory deficit may prevent choline from being effectively converted into acetylcholine in the aged brain. This may be even more true in Alzheimer's disease, where the majority of cholinergic neurons projecting to the cortex (and possibly hippocampus) may be lost, and therefore the machinery to incorporate extra precursor into acetylcholine is no longer intact. Even in the normal aging brain, however, serious deficiencies could impair conversion of plasma choline to intraneuronal acetylcholine. For example, choline uptake (*20, 73*), choline acetyltransferase activity (Table 1), and oxidative metabolism (*68, 74*) have all been reported to be decreased in the brains of aged and demented subjects. Since these factors all contribute to normal acetylcholine synthesis, deficiencies in them may not allow choline to be incorporated into acetylcholine as easily as in the brains of younger subjects.

Further, although acetyl coenzyme A (CoA) is normally synthesized de novo in the CNS (*75*), decreases in glucose utilization and oxidative metabolism may decrease the ability of the aged brain to synthesize acetyl CoA, thus making its availability a rate-limiting fac-

tor in acetylcholine synthesis in the aged brain (*21, 76*). Despite the interest this area of research has recently generated, no studies have directly compared young with aged brain to determine if similar changes in acetylcholine synthesis can be induced with precursor loading, and only one study has evaluated the effects of precursor loading in the aged brain (*77*). Similarly, few systematic studies have yet been performed to determine how the influence of variables such as choline uptake and acetyl CoA may change with age and alter the effects of choline loading (*78*).

These questions raise the possibility that choline is relatively ineffective in stimulating cholinergic activity, particularly when given to aged subjects already suffering deficiencies in the cholinergic system. Although this question needs direct empirical investigation, two recent studies attempted to circumvent problems associated with it while studying the possible beneficial effects of precursor loading.

In the first, the effects of choline were evaluated before the onset of age-related neurobehavioral disturbances occurred (*79*). If age-related changes in the cholinergic system are at least partially responsible for memory impairments, and if dietary manipulation of choline significantly affects cholinergic function, it might be possible to modulate the rate at which memory impairments occur with age by varying the availability of dietary choline. Retired breeder mice (8.5 months old) were placed on purified diets that were either deficient in or enriched with choline. Because life-span tests indicated that reliable deficits in retention of a passive avoidance task are not apparent at this age, it seemed reasonable to assume that the major neurochemical alterations responsible for the deficits were not yet severe in these mice. After 4.5 months the mice were trained on a single-trial passive avoidance task and tested for retention either 24 hours or 120 hours later. Their performance was compared with that of mice of various ages that were maintained on a control diet. Two salient findings were observed: (i) a dramatic decrease in retention of the task was observed in the senescent mice (23 months and older) and (ii) marked differences occurred between the choline-deficient and choline-enriched groups (13 months old). The choline-enriched mice performed as well as 3-month-old mice, whereas the choline-deficient mice performed as poorly as the senescent mice.

This study demonstrated that dietary manipulation of choline can significantly alter behavior in ways that are qualitatively and quantitatively similar to those occurring across the life-span of the mouse. Whether or not these behavioral changes are due to alterations in cholinergic function, per se, remain to be seen. Choline has many important functions in the nervous system, including roles in phospholipid metabolism (*80*). Thus, more general changes in neuronal membranes (or their functions) could have contributed to the deficits. Nevertheless, the data do offer the possibility that certain age-related changes in behavior can be modulated by long-term control of precursor availability.

An important question not yet answered concerns how long into the life-span increased choline will continue to retard the onset of age-related memory losses. These effects represented a retardation in the development of deficits in middle-aged animals. It remains to be seen whether long-term choline administration might reverse existing cognitive impairments in aged subjects (*81*). If the presumed cholinergic dysfunction renders the aged brain relatively incapable of responding to additional precursor stimulation, it might be necessary, with this precursor approach, to intervene before the behavioral impairments and neurochemical dysfunctions fully develop.

Another recent animal study suggests that certain types of pharmacological intervention may potentiate the effects of choline in the aged brain (*67*). This study was based on the possibility that one reason for the lack of significant precursor effects in the geriatric population may be the inability of the aged brain to incorporate or utilize abundant precursor substance. If so, it may be necessary to improve other factors in aged brains before substantial increases in presynaptic cholinergic effects are obtained with precursor loading. For example, although normal cholinergic activity depends on intact oxidative metabolism, several parameters that reflect energy production are decreased in the aged CNS (*82, 83*). Further, although choline converts into acetylcholine more readily under conditions of increased neuronal activity (*56*), recent circumstantial evidence suggests the activity of certain cholinergic pathways may be reduced in aged subjects (*20*). Thus, either of these (or similar) factors could contribute to a situation in the aged brain that would prohibit extra choline from being effectively utilized for the synthesis of additional acetylcholine and, in turn, would explain the negative results obtained with precursor studies in aged animals and humans.

One way to attempt to compensate for these possible age-related deficits would be to administer abundant amounts of choline while simultaneously giving a drug that might correct other critical age-related neuronal deficiencies. Although no drug yet exists that is recognized as being effective in correcting age-related neuronal dysfunctions, one that is beginning to attract interest for its biochemical and pharmacological properties is piracetam. Several lines of pharmacological evidence indicate that piracetam enables the CNS to function more effectively under hypoxic conditions (*84*) and improves performance in oxygen-deprived (*85*) or aged animals (*86*). Neurochemical determinations suggest piracetam may facilitate conversion of adenosine diphosphate to adenosine triphosphate (*84, 87*). Other tests indicate that piracetam also enhances intercerebral neuronal activity (*88*) and may deplete hippocampal tissue acetylcholine levels, presumably by increasing release (*89*). Given this profile, piracetam might be able to reduce deficiencies in the aged brain that normally contribute to the lack of significant effects observed with choline loading. This possibility was tested with aged Fischer 344 rats administered saline, choline, piracetam, or combinations of each for 1 week; retention of a one-trial passive avoidance task was measured (*67*).

Aged Fisher 344 rats had previously been shown to suffer severe impairments on this task as a natural consequence of aging (*5*). Control studies suggested that a major source of this impairment is loss of memory for the learned event. For example, control tests demonstrate that possible differences in motor activity or shock threshold cannot explain the age-related differences in the test day (*5*). Further, evaluations of performance after various retention intervals demonstrated that the performance of the aged rats was comparable to that of young rats when tested within a hour after training, but decreased sharply, exhibiting severe deficits within 4 hours after training (*5*). These findings strongly suggest a memory-related component of this age deficit.

The scores of those rat administered only choline did not differ from those of control rats given saline. Although the rats administered piracetam were improved subtly over the saline and choline groups, the retention scores of rats administered the choline-piracetam mixture were several times as high as those

of rats given piracetam alone. These data, therefore, provide preliminary evidence that the effects of increased choline availability in aged animals may be greatly enhanced by the simultaneous administration of a pharmacological agent purported to enhance oxidative metabolism. It is encouraging that a recent clinical trial based on these preliminary animal data found significant improvement in three of ten mild to moderate Alzheimer's patients treated for 1 week with combined choline and piracetam, and all three responders exhibited unusually high choline levels in red blood cells (but not plasma) relative to nonresponders (*90*). Further tests with other drugs to ameliorate other neuronal deficiencies may produce even greater improvement.

It should also be useful to determine mechanisms of action of piracetam and the specific neurochemical changes induced by the combined piracetam-choline treatment. Preliminary neurochemical assays performed on the brains from the behaviorally tested animals revealed modest regionally specific changes in choline and acetylcholine with the combination, the most interesting of which occurred in the hippocampus. Whether these subtle changes were responsible for the more robust behavioral effects remains to be determined (*67*). If certain assumptions of the effects of the drugs are correct, these data suggest that choline may not normally be sufficient to induce measurable behavioral (or neurochemical) improvement in aged subjects, but that correcting other aspects of CNS metabolism may allow this precursor to exert reliable, positive effects in each. The most significant improvement in aged memory may be achieved when multiple, interactive neurochemical dysfunctions in the brain are corrected or when activity in more than one aspect of a deficient metabolic pathway is enhanced. These preliminary data from aged rats suggest that solutions to this problem may not be simple, for different physiological functions may have to be affected; alterations may be necessary at more than one point in the cholinergic or other metabolic pathway, or alternatively, the balance or tone between two or more neurotransmitter systems may need to be improved. Future multidisciplinary studies directed toward identifying the specific alterations responsible for these neurobehavioral dysfunctions should greatly facilitate the search for new and truly effective pharmacological treatment for those aged and demented humans suffering cognitive deterioration.

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94. We thank D. Haubrich, C. Finch, and P. Davies for comments and criticism that helped strengthen earlier versions of this manuscript; J. S. Scozzafava for technical assistance; M. Parham for assistance with the literature reviews; B. Dubnick for continuing encouragement and support; and R. Sheppard and R. VanBrunt for assistance in the preparation of this article.