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MEETING REPORT

Advances in Alzheimer Therapy: Cholinesterase Inhibitors

J. WESSON ASHFORD,* KATHLEEN A. SHERMAN† AND VINOD KUMAR*

**Department of Psychiatry and †Department of Pharmacology*
Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794-9230

AN international symposium on the use of cholinesterase (ChE) inhibitors as treatment for Alzheimer dementia (AD) was held on March 19 and 20, 1988 at the Southern Illinois University School of Medicine in Springfield, Illinois, sponsored by the World Foundation of Neurology Research Group on Dementia, and the Institute of Developmental Neuroscience and Aging. This meeting provided the first opportunity for many researchers who are pursuing similar approaches for treatment of AD to present and discuss their efforts.

The symposium also provided a unique forum for clinical investigators and basic scientists to have formal and informal discussions and critique relevant issues regarding the cholinomimetic strategy for treating AD. The rationale for cholinomimetic therapy is based on discoveries from the late 1970's, which have subsequently been confirmed and extended. First, cholinergic innervation to cortex and hippocampus is profoundly reduced in Alzheimer patients, and the cholinergic loss correlates with both neuropathological lesions and severity of cognitive impairment. Second, pharmacological evidence indicates that a cholinergic system plays an important role in memory: anticholinergic drugs selectively impair memory, whereas cholinomimetics improve memory in young adults. Although several other neurotransmitter systems can be affected in Alzheimer patients, the cholinergic system is consistently perturbed, and cholinergic markers are affected early in the course of the disease when short-term or recent memory is most selectively impaired. Yet, despite this rationale for the cholinergic replacement approach to Alzheimer therapy, a decade of investigation had produced no clinically relevant treatments for Alzheimer patients. However, in 1986, the New England Journal of Medicine (315:1241–1245) published a report by the group of William Summers, stating that the anticholinesterase agent, tetrahydroaminoacridine (THA), produced marked clinical improvement in Alzheimer patients. Attention focused on this symposium for scrutiny of the work on THA and the controversy it has generated, especially since the potential for hepatotoxicity emerged. Not only this work, but an array of studies on cholinergic agents and Alzheimer treatment were presented, examined and discussed.

PHARMACOLOGY OF ANTICHOLINESTERASE AGENTS

A. G. Karczmar (Loyola University, Maywood, IL) introduced the preclinical session by emphasizing the numerous consequences of the cholinesterase inhibitors and differences in pharmacological profile of drugs in this class. He illustrated that effects of anticholinesterase drugs on the neuromuscular junction can block as well as facilitate cholinergic transmission; and that particular agents such as physostigmine and soman differ in the mechanism of action. Karczmar raised the issues of: 1) presynaptic inhibitory effects, which were discussed further in several of the subsequent talks; 2) direct actions of cholinergic receptors; and effects not related to inhibition of acetylcholine metabolism, such as: 3) direct channel actions; 4) inhibition of potassium currents; and 5) actions on other transmitters. Karczmar summarized the literature on behavioral and mental effects of cholinesterase inhibitors, noting that many of their behavioral actions, including dysphoria, disturbed affect and impaired memory, are not desirable characteristics of putative treatments for AD.

Two reports described the effects of THA at the mammalian neuromuscular junction. R. J. Bradly (University of Alabama-Birmingham, Birmingham, AL) characterized the effect of THA on the compound nerve action potential and antagonism of curare in rat phrenic nerve-diaphragm preparations. THA at concentrations of 12.5 μM reduced the amplitude of the compound action potential which progressively faded to near zero with successive stimulation in a frequency-dependent manner. These effects are thought to be due to depolarization by excessive synaptic acetylcholine (ACh) after acetylcholinesterase (AChE) inhibition. The ED_{50} for effects of THA on transmission at 100 Hz was 1 μM. THA also produced a dose-dependent reversal of curare which was maximal at 1 μM; further increase in concentration actually produced less curare antagonism. THA did not alter the shape of the compound nerve action potential or the muscle action potential at 7.5 μM, indicating that at the concentrations of THA which alter neurotransmission and reverse curare in this model of the nicotinic synapse, K^+ channels are not affected.

In mouse triangularis sterni nerve muscle preparation, A. L. Harvey (University Strathclyde, Glasgow, U.K.) showed that 1 μM THA is effective in prolonging the duration of the miniature endplate potential (MEPP), an action attributed to AChE inhibition; physostigmine at 15 μM had a greater effect and increased the amplitude of the MEPP. Perineural waveforms were recorded during stimulation of intercostal nerves in the presence of alpha-bungarotoxin to block postsynaptic activity. The classic K^+ channel blocker 3,4 diaminopyridine (3,4 DAP)

reduced or abolished a second negative component and revealed a positive component associated with inward Ca^{++} currents and a late negative component due to Ca^{++} activated K^+ current. At 10–100 μM, THA caused a concentration-dependent decrease in the second negative component reflecting blockade of K^+ channels, but at higher concentrations THA also caused a broadening of the signal. Thus, like the aminopyridines, THA does block K^+ channels, which may facilitate release of acetylcholine. However, this action occurred only at 10-fold higher concentrations than that required for the inhibition of AChE or the effects on cholinergic transmission described by R. J. Bradley. Moreover, at very high concentrations THA has additional actions on the perineural signal, perhaps reflecting slowing of Na^+ channel inactivation. Physostigmine also inhibited the second negative perineural wave at high concentrations (75–300 μM), but appears to be less potent than THA in this respect. Further, physostigmine did not cause broadening of the signal.

A. Enz (Sandoz, Basel, Switzerland) presented neurochemical data comparing presynaptic actions of THA, physostigmine and RA7. All three compounds exert presynaptic inhibitory actions as indicated by reduced ACh turnover (as measured using deuterated choline incorporation), and each agent elevated brain ACh levels. These effects did not occur equally in all brain regions: ponsmedulla was not affected and RA7 had the greatest effect in cortex. The inhibition of ACh turnover after physostigmine was detectable after a relatively low dose (100 μg/kg) and was well correlated with the inhibition of brain AChE. In the discussion, concern was raised about the use of labeled choline as a precursor since cholinesterase inhibitors are likely to affect choline flux in cholinergic neurons.

K. A. Sherman (Southern Illinois University, School of Medicine, Springfield, IL) reported on the results of animal studies comparing the in vivo actions of THA and physostigmine. The onset of THA's behavioral effect (inhibition of locomotor activity and induction of splay posture) was gradual (reaching a maximum at 3 hr after SC treatment), but the effects were long lasting (>6 hr after 10 mg/kg THA) compared to physostigmine (which lasted less than 2 hr). Brain AChE was maximally inhibited from 1 to 3 hr after THA. AChE inhibition was detectable after doses as low as 0.625 mg/kg and dose dependent to 15 mg/kg. The degree of brain and blood cholinesterase inhibition observed in these studies (maximum 75–80%) was greater than in many previous reports, which Sherman attributed to the use of minimally diluted tissue homogenates in her radiometric assay. She showed that the degree of enzyme inhibition decreases linearly as a log function of tissue dilution, which may account for the difficulty in estimating THA's effect by the ex vivo assays reported by A. Enz and S. Gauthier at this meeting and by others. A close relationship between plasma and brain cholinesterase inhibition was shown after THA treatment when dose or time were varied. Consistent with the results of physostigmine, these THA results indicate that inhibition of blood cholinesterase activity may provide a valuable and convenient measure of biological efficacy for comparing patients in clinical trials. In addition, Sherman reported on the pharmacodynamic factors which may limit the therapeutic window for cholinomimetics: inhibition of presynaptic neuronal activity as reflected in reduced high affinity choline uptake and tolerance with repeated administration.

A. Nordberg (Uppsala University, Uppsala, Sweden) reported her investigations of the biochemical actions of physostigmine and THA in human post-mortem brain tissue. Nordberg argued that in the absence of a reliable animal model for Alzheimer's disease, comparison of drug actions on control and patient brain tissue obtained with short (≤14 hr) post-mortem delay is the most appropriate avenue. When tissue is preserved by slow freezing in sucrose followed by rapid thaw, calcium-dependent release of labeled ACh synthesized from tritiated choline can be measured during high potassium depolarization. Evoked release of ACh is markedly reduced in Alzheimer brain, as expected. Nordberg's most interesting observation is that while THA or physostigmine reduced evoked release in control brain (consistent with findings in rat brain), these drugs caused an increase in the evoked release of ACh from Alzheimer brain tissue up to untreated control levels.

Nordberg considered that some of the effects of the cholinesterase inhibitors, especially THA, may be related to their ability to interact with nicotinic and/or muscarinic cholinergic receptors. In μM concentration, THA displaced both nicotinic and muscarinic ligands. Further, THA has greater affinity for cholinergic receptors than physostigmine. Nordberg noted that 7 groups in addition to her own have found a decreased number of nicotine receptors in Alzheimer brain (which corresponds to the decrease of ACh release), but this finding may represent a transformation of high affinity nicotinic binding sites into low affinity binding sites. Thus, she suggested that THA may exert its beneficial effect in AD not only through an anticholinesterase effect, but also by a direct effect on both nicotinic and muscarinic receptors.

R. Wurtman (MIT, Cambridge, MA) proposed that cholinesterase inhibitors increase the brain's need for free choline. He first pointed out that every cell in the body uses choline via the enzyme choline kinase for making phospholipids, which are critical for membrane production. Cholinergic neurons use choline for a second purpose, to make ACh, via the enzyme choline acetyltransferase (CAT). Neither enzyme is saturated at physiological concentrations of choline. Therefore, changes in choline availability affect the activity of both enzymes. Repeated neuronal depolarization leads to continued production of ACh at the expense of the choline reservoir in membranes. To support this notion, Wurtman presented studies of LAN-2 neuroblastoma cells in which 20% of ACh formed in 1 hour of incubation came from the membrane source. Stimulation of striatal brain slices (with no choline in the medium) for up to 3 hours led to continued release of ACh. Membrane phospholipid depletion occurred only in the presence of physostigmine, which blocked the breakdown of ACh and thus removed the availability of this usual source of choline. Addition of choline increased the release of ACh and protected against the depletion of membrane phospholipids in a dose related fashion. If learning also involves synaptic remodeling (neural plasticity), which requires high membrane turnover, then adequate choline must be available for making phospholipids as well as ACh. Further, abnormalities in phospholipid breakdown have been found in Alzheimer brain. Specifically, levels of glycerylphosphoethanolamine are approximately double those in control brain, and there are similar changes in glycerylphosphocholine. Thus, Wurtman concluded that treatments which will further impair the availability of choline in the brain should not be used chronically. He later argued that high doses of choline (administered in the form of phosphatidylcholine) would supplement the choline stores of the brain, enhancing both synaptic plasticity (for learning/memory) and the cholinomimetic effects of anticholinesterase drugs.

ANTICHOLINESTERASE EFFECTS OF ANIMAL BEHAVIOR

Cholinergic agents seem to be able to produce a clear augmentation of memory in mice, rats and monkeys. M. J. Forster (Texas College of Osteopathic Medicine, Fort Worth, TX)

presented data supporting the use of autoimmune mouse strains (such as NZB/BINJ) as a model of aging. In these strains, an increase in brain reactive antibodies occurs at 1–3 months as opposed to 6–9 months in C57BL/6NNia mice. The immunodeficient mice also show impaired memory retention at an earlier age compared to C57 mice. In tests of habituation to a novel environment, 3–4-month-old autoimmune mice were as impaired by 3–4 months as senescent (24 months) C57 mice. When physostigmine (0.04–0.16 mg/kg) was administered to the autoimmune-strain immediately postsession, retention was similar to that of normal mice.

V. H. Haroutunian (Bronx VA Medical Center, Bronx, NY) reported data involving lesions of the cholinergic neurons in the basal forebrain and the ascending noradrenergic bundle in rats. These studies address in this rat model the issue of variable responsiveness to anticholinesterase therapy among Alzheimer patients. Reasoning that loss of noradrenergic innervation is often severe in Alzheimer patients, although not as universal as cholinergic loss, Haroutunian compared the effect of ibotenic acid lesions of nucleus basalis (which affects cholinergic neurons but may involve other systems, too) alone and combined with 6-hydroxydopamine (6-OHDA) lesions of the ascending noradrenergic bundle (which projects to the cortex as well as other regions). The nucleus basalis lesions produced a 32% decrease of cortical CAT. A deficit in passive avoidance retention was improved with 4-aminopyridine (which releases acetylcholine), physostigmine or oxotremorine. The lesions induced by 6-OHDA produced no behavioral deficit by themselves, but they did prevent the ability of cholinergic agents to ameliorate the deficits due to nucleus basalis lesions. Clonidine, in combination with a cholinergic agent, reversed the retention deficit in rats with combined lesions. These results suggest that Alzheimer patients who fail to respond to cholinomimetics alone may benefit from combined cholinergic and noradrenergic drugs. The Bronx VA-Mount Sinai group is beginning a clinical trial with the combination of clonidine and physostigmine in Alzheimer patients.

R. T. Bartus (Lederle Laboratories, Pearl River, NY) compared the effects of 3 anticholinesterase drugs: physostigmine, THA and 3,4 diaminopyridine (DAP) in aged monkeys performing on an automated delayed response task. There is an age-related deficit in recent memory demonstrated by this task: the longer the delay, the more severe the deficit. Using a comparable task in humans (young, aged and Alzheimer patients with varying degrees of dementia), a similar age-related phenomenon is seen with a floor effect for severely demented patients. Thus, this animal model can be easily applied to the study of human aging and may be relevant to the study of early Alzheimer's disease, where a conceptually similar recent memory deficit is seen. Bartus proposed, based on the cholinergic hypothesis of AD and age-related cognitive impairments, that cholinomimetic drugs can improve memory and that drugs with better pharmacodynamic effects can be developed. Bartus directly compared physostigmine, THA and 3,4 DAP (the latter substance because it has been shown to enhance ACh release and that it may have a second mechanism of action similar to that of THA). He used a minimum of 3 different oral doses of each drug 1 hour before testing in 10 aged cebus monkeys (20–30 years old). A significant positive effect on memory was found for physostigmine 11/14 times (27% of doses, $p<0.005$), for THA 6/12 times (17% of doses, $p<0.05$), for 3,4 DAP 4/9 times (11% of doses, $p=0.09$). Using the best dose, 7/10 monkeys demonstrated a reliable, replicable, positive effect to physostigmine (21% improvement for responders), 4/10 for THA (18% improvement for the responders) and 4/10 for 3,4 DAP (18% improvement for the responders). Bartus discussed the numerous limitations for applying these results to the clinic, but based on his comparison, he concluded that THA was no better than physostigmine for the single time interval tested in the setting of acute treatment. He suggested that THA may have a therapeutic benefit because of its advantageous pharmacokinetic profile. Further, it should be possible to develop other cholinomimetic drugs with even better efficacy.

J. W. Ashford and J. Bice (Southern Illinois University, Springfield, IL) reported the effects of physostigmine and scopolamine on simple and choice reaction time in elderly monkeys (approximately 23 years old). Since choice reaction time is more affected in AD than simple reaction time, the choice measure was expected to show sensitivity to cholinergic agents. However, in titrating the drugs to find doses affecting reaction time, both agents disrupted animal performance of the task before specific effects on correctness or reaction time could be demonstrated. To further investigate the effects of such drugs on information processing in the primate, it is necessary to improve the motivational aspects of the tasks and develop paradigms which present single stimuli under computer control. Discrete stimuli will allow the analysis of memory retrieval functions which may be more selectively sensitive to the effects of cholinergic agents than reaction time.

CLINICAL STUDIES OF ALZHEIMER PATIENTS

P. Riekkinen (University of Kuopio Medical School, Kuopio, Finland) discussed the multiplicity of deficits seen in demented patients. He pointed out that only 50% of Alzheimer patients may have a selective cholinergic deficit while an additional 30% also have a norepinephrine deficit. Examining the EEG's of Alzheimer patients, a decrease of occipital frequency was found which correlated with a decline of cognitive scoring. Following patients on a yearly basis, half of the patients showed a progression in the EEG (increase in delta or theta), but the other half of the patients did not, even though dementia was progressing in both groups. The delta power increase correlated with a decline of AChE in CSF (no correlation with CSF-HVA, 5HIAA, MHPG). This data (along with a study by L. J. Thal showing that n. basalis lesions in the rat increases delta power on the ipsilateral side of the lesion) suggest that EEG delta power can indicate the importance of the cholinergic deficit of the forebrain in Alzheimer patients. This measure may identify patients who would respond to cholinergic agents and could be used to evaluate such agents for potential therapeutic efficacy.

EFFICACY OF PHYSOSTIGMINE IN ALZHEIMER PATIENTS

Physostigmine has consistently shown a small, but usually positive effect on memory in Alzheimer patients. New routes of administration may improve the efficacy of this drug. L. J. Thal (VA Medical Center, San Diego, CA) reviewed data from the literature on both intravenous and oral physostigmine for treating Alzheimer patients. He stressed the importance of finding the intermediate effective doses in successful studies (high enough to show an effect and low enough to avoid disabling side effects), an "extraordinarily short" time-course of effect and entry of the active agent into the CNS. Thal's own data with long-term effects of physostigmine showed an improved retrieval from long-term memory which "varied very, very widely from patient to patient." Similar results were found with and without coadministration of lecithin. Considering that the wide variation of response could be related to absorption, CSF cholinesterase inhibition was measured and found to correlate with the physostigmine-induced improvement. In a study administering oral physostigmine to outpatients on a qid basis,

improvement was found in 7 of 10 patients, but only in verbal learning (recall and intrusions) and family reports. Activity of Daily Living (ADL) ratings or other areas of cognition were not improved. In order to overcome the short pharmacokinetic time course of physostigmine, time-release preparations are being developed which extend the half-life. Thal also presented data on using these new, long-acting preparations. In measuring physostigmine blood levels, the variance in the area under the time curve was only 12%. Comparison of plasma physostigmine and cholinesterase levels suggested that the two measures had a good correspondence, indicating that plasma cholinesterase levels are an excellent guide to actual drug levels.

D. M. Masur (Albert Einstein College of Medicine, Bronx, NY) further elaborated on the psychological tests administered to patients during the oral physostigmine study presented by Thal. Selection criteria included the Blessed Information-Memory-Concentration test, the Buschke Selective Reminding Test (BSRT) and the ability to place at least one item into long-term memory. Several other neuropsychological tests were also administered. A considerable degree of variability was found in the placebo and drug groups. Therefore, the percent improvement (a nonparametric analysis) was examined, and 7 of 10 patients improved on drug while none of the 6 patients on placebo reached criteria for improvement. Though a Fisher exact probability test showed a significant effect ($p<0.01$), there was still a wide variation of responses. Only 2 patients showed consistent improvement in retrieval on the best dose, and only 1 showed a reduction of intrusion errors. Though all of the subjects were mildly demented, the neuropsychological baseline tests did not predict performance on the drug. The possibility of subtyping Alzheimer patients for predicting drug response was discussed.

R. J. Elble (Southern Illinois University, Springfield, IL) presented the data from the SIU group on the effect of prolonged intravenous physostigmine infusions. The results of 17 patients [age=51–81 years old and Folstein Mini-Mental State (MMS) scores of 5–28] showed exceedingly variable behavioral effects even though a stable level of plasma cholinesterase inhibition was achieved. Scopolamine (IV bolus of 0.161 mg/m^2) produced a significant (9%) decrement of performances, while the physostigmine produced a nonsignificant (4%) improvement. The response during the dose-finding phase of physostigmine did not predict performance during the replication phase. In fact, none of the factors studied was found to predict a positive physostigmine response.

R. F. Zec (Southern Illinois University, Springfield, IL) compared and contrasted the positive results of the Mohs and Davis (American Journal of Psychiatry 142:28–33) physostigmine study with the SIU group study. The age range of patients in the SIU study was about a decade older than the Mohs and Davis study, but the results on memory tests indicated that the patients had similar levels of dementia. The SIU study gave higher doses of physostigmine (300, 600 and 900 μg-m^2), but those doses were administered over a longer period of time (30 minutes vs. 135 minutes). Analysis of the Mohs and Davis study suggested that the primary effect of physostigmine was to decrease false alarms, perhaps an indicator of behavioral impairment, rather than a memory improvement. The SIU study did find a trend towards a decrease of false alarms. Clearly, a wide variety of methodological issues remain unresolved. Even the distinction of responders and nonresponders has not been settled. In the literature, 14 studies using physostigmine have indicated negative effects while 23 studies have shown some type of positive effects. The strength of the cholinergic model and the frequent findings of beneficial effects have kept research on this classical drug active.

E. Giacobini (Southern Illinois University, Springfield, IL) discussed initial studies at SIU involving intracerebroventricular (ICV) injections of physostigmine to avoid peripheral side effects. Research in the rat and dog demonstrated that physostigmine, when injected ICV, diffuses throughout the brain. In the human, an 8 μg dose of physostigmine ICV: 1) produced a 70–90% inhibition of CSF cholinesterase; 2) a peak of CSF ACh at 3 hours of 5 nmol/ml; and 3) remained in the CSF for 6 hours. An elevation of plasma cortisol was also produced. It was suggested that the brain may store physostigmine, thus prolongating its kinetics. Giacobini emphasized that neurosurgical approach offered the benefit of both histological confirmation of the diagnosis and neurochemical research on brain tissue from early stage AD patients. In the biopsy specimens, a 70% decrease of nicotine binding sites was found. He also stressed the benefit of the ICV approach for potential future therapeutic agents.

THA TREATMENT OF ALZHEIMER PATIENTS

Although not all of the attempts to replicate the initial report of Summers *et al.* with THA have been positive, the focus on clinical protocols and side effects revealed continued optimism about the potential benefit of THA.

L. J. Fitten (Sepulveda VA Medical Center, Los Angeles, CA) reported his work on THA, which was administered to mice, monkeys and humans in various studies. Eight-week-old C57BL/Nia mice were given THA or placebo for 4 to 6 months. When THA and placebo groups were compared, the THA group had better retention than the placebo group ($p<0.001$) on a shock-motivated T-maze task. Significant improvement with THA administration was also seen on a task requiring monkeys to learn four color pair discriminations. THA (25–250 mg/day with lecithin or only placebo for a week in a double blind crossover design) was administered to 10 patients meeting NINCDS-ADRDA criteria for probable AD. Overall results suggested that there was no significant difference between the drug and nondrug condition. Only one patient of the ten could have been classified as a mild responder. Three of the ten patients (30%) showed significant liver enzyme abnormalities. This study contradicts the earlier findings reported by Summers. However, the duration of drug treatment was very short and the number of patients relatively small.

H. Nyback and G. Ohman (Karolinska Institute and Hospital, Stockholm, Sweden) presented their results with ten patients given THA (25–250 mg) each day for 1–2 week periods in an open trial. Five patients (50%) showed improvement in verbal memory, but 70% of the patients experienced various side effects. CSF HVA, MHPG, 5HIAA and ACh were measured predrug and during the drug period. There was a noticeable increase in CSF HVA, 5HIAA and ACh caused by THA administration in Alzheimer patients. ACh and plasma THA concentrations were elevated in the responders, and the THA levels correlated with the presence of side effects. Though it is interesting to note these changes, the sample size was small, and the dose of THA was varied between patients. Thus, it is difficult to draw any firm conclusions from these findings at the moment.

S. Gauthier (McGill University Center for Studies in Aging, Montreal, Canada) presented the efficacy data of 19 patients who completed six weeks of THA and lecithin in various doses and two weeks of lecithin only. The results showed statistically significant improvements of self care, ADL ratings, scores on the MMS, the BSRT and on verbal word fluency. The dose of THA varied a great deal, and the results presented at the conference did not separate out those patients who responded to

smaller doses or larger doses. There were questions about the double blindness of the dose finding phase of the study. The frequency of side effects was reported for a group of 51 patients, and 80% of the patients in this group experienced various side effects in spite of the administration of glycopyrrolate (1 mg tid). Reversible elevations in liver enzymes were found in 34% (17/50) of the patients. These findings support the hypothesis that THA is efficacious in the symptomatic treatment of some Alzheimer patients, but confirm the occurrence of frequent side effects including hepatotoxicity.

W. K. Summers (Arcadia, CA) reviewed the history of the development of the diaminoacridine agents originally used as anthelmintics. This class of drugs did have antiseptic effect, but was discovered to have hepatotoxicity in the 1930's. Adrian Albert, working with the monoamine acridine compounds, synthesized THA in 1945 and noted that it had a marked capacity to "arouse" the central nervous system. It had anticholinesterase effects and became known for its ability to reverse anticholinergic delirium. In the 1960's, S. Gershon reversed phencyclidine induced delirium with THA, but Summers was not able to replicate this finding in 13 patients. Summers' experience with AD began with studies of 12 patients and now includes 45 patients. Two points in his New England Journal of Medicine paper were clarified. First, the screening psychiatric instrument was a list of tests, some of which were not used on all of the patients. The purpose of the list was only to confirm the diagnosis. Second, the "Daily Global Assessment" was a sheet which summarized the daily state of each patient, including lab tests, which was observed clinically. Summers pointed out differences between his study and the NIA/ADRDA/Warner-Lambert national replication study. First, he coadministered lecithin. Second, the THA dosage used in his subjects was based on an open clinical adjustment period. Of his patients, 57% were exposed to a dose of 200 mg/day, but only 16% of them ended up on his highest maintenance dose of THA (150 mg/day).

Summers reported his experience with the chronic side effects of THA including elevated liver enzymes. Overall, 8 of 45 patients showed elevations in liver function studies, though only 3 of the 8 had elevations on rechallenge. Liver biopsy on 2 patients showed hepatotoxicity; the primary pathological finding was peri-portal inflammation. Summers claims that the pattern of liver enzyme elevation was a "classic chlorpromazine type reaction." He noted that chlorpromazine produces liver enzyme elevation in 50% of patients (R. K. Okner, in Hepatology, eds., D. Zakim and T. D. Boyer, Saunders, Philadelphia, 1982). Since THA produces fewer side effects than chlorpromazine, he considers the hepatotoxic effect of THA not that alarming. Summers concluded by suggesting that the acceptance of THA will be related to the tolerance of its side effects.

V. Kumar (Southern Illinois University, Springfield, IL) described the initiation and subsequent suspension of the U.S. multicenter THA study. He commented on the promptness of the National Institute of Aging, ADRDA, medical community and Warner-Lambert, a pharmaceutical company, in organizing and starting the multicenter trial. It was indicated that after the suspension of the trial, the available behavioral outcome measures were analyzed. However, these data should not be considered as evidence of efficacy since patients were only exposed to one week at each treatment level. Nevertheless, the data from the titration phase, which included a blinded placebo treatment, were sufficient to justify continued evaluation of THA despite the obvious elevated liver enzyme side effects profile. The study has been recently restarted with a modified protocol using smaller doses of THA. Results of this study are expected to be available at the end of 1988 or early 1989.

OTHER ANTICHOLINESTERASE AGENTS POSSIBLE FOR ALZHEIMER TREATMENT

There is an extensive array of cholinomimetic agents available which may turn out to be of benefit for Alzheimer patients.

E. F. Domino (University of Michigan, Ann Arbor, MI) reviewed the literature on galanthamine, a reversible cholinesterase inhibitor which readily crosses the blood-brain barrier. This drug has been well characterized by Eastern Europeans and has been used as an adjunct in surgical anesthesia. Galanthamine is the active ingredient of *Galanthus nivalis*, which was used to antagonize the central anticholinergic syndrome in Homer's Odyssey. Subsequent experiments have confirmed this action. Galanthamine also has anticurare action but is 1/10th as potent as neostigmine. Galanthamine has been used clinically to reverse nondepolarization blockade and to treat myasthenia gravis. Galanthamine has about 65% oral bioavailability and an elimination half-life of 40–50 min in rats. Two phase elimination was observed after IV galanthamine in anesthetized patients, and the $t_{1/2}$ in plasma of the slower phase was 264 min. He concluded that galanthamine has a similar duration of action as physostigmine, but may produce fewer side effects.

M. Pomponi (Catholic University, Rome, Italy) studied heptylphysostigmine (heptyl-phy), a physostigmine analog modified according to structure-activity relationships. This analog is more potent than physostigmine in rats and mice and produces a long-lasting inhibition of both brain and serum ChE. At doses between 0.1 and 0.3 mg/kg, heptyl-phy did not affect spontaneous activity in mice, though higher doses (3.0 mg/kg) antagonized the increased activity induced with scopolamine (1.0 mg/kg). When mice were injected posttraining with different doses of heptyl-phy on a passive avoidance task, step-through latencies were significantly longer at 24 and 48 hours following the injection compared to controls. These results indicate that heptyl-phy exerts its beneficial anti-ChE activity at doses which are significantly less toxic. Further, this drug may facilitate memory by acting on the consolidating mechanism.

P. G. Waser (Pharmakologisches Institut der Universitat, Zurich, Switzerland) discussed the idea of using long-acting organophosphates, including nerve gases, for the treatment of AD. Waser emphasized a considerable data base on the pharmacokinetics of compounds, such as sarin, gained in radiolabeled tracer studies. He also noted that overdosage in humans can be safely managed with atropine.

D. E. Moss (University of Texas, El Paso, TX) investigated certain sulfonyl fluorides which are long-acting cholinesterase inhibitors with inherent selectivity for the CNS. Treatment with phenylmethyl sulfonyl fluoride or methane sulfonyl fluoride (MSF) produced 90% inhibition of brain ChE, but less than 35% inhibition in peripheral tissues such as ileum, heart or muscle. In addition to this inherent selectivity, the ChE activity returns to control levels much more rapidly in peripheral tissues than brain due to the slower resynthesis of ChE in brain, where ACh levels were elevated for over 5 days. Doses producing marked CNS ChE inhibition had low toxicity even with chronic treatment. In monkeys, CSF ChE inhibition likewise showed marked and long-lasting inhibition ($t_{1/2}$ was 2.2 days after MSF), and no behavioral evidence of toxicity occurred with long-term high dose administration. These preclinical studies indicate that sulfonyl fluorides have promise as agents for long-lasting central ChE inhibition without the toxicity due to effects on peripheral autonomic and somatic motor cholinergic systems. These effects have been problematic with other cholinergic strategies for treating AD.

X. C. Tang (Shanghai Institute Materia Medica, Chinese

Acad. Sci., Shanghai, China) presented results on huperzines A and B (Hup A and B), alkaloids recently isolated from the Chinese herb *Huperzia serrata*. These alkaloids markedly inhibit AChE in vitro and show greater selectivity for this specific enzyme (than for serum ChE) compared to the carbamates. EEG alerting occurred after IV Hup A (0.05 mg/kg) or Hup B (0.05 mg/kg) through muscarinic stimulation; the effect of Hup B on EEG was more long lasting. Tritiated Hup A was used to show that the elimination half-life is 122 min after IV or 248 min after intragastric administration in rats. Since the huperzines have a superior safety margin compared to physostigmine, the effects on learning and memory were assessed. In rats, Hup A facilitated acquisition in a Y-maze brightness discrimination task, blocked the learning impairment due to hypercapnia and produced a dose-dependent facilitation of performance on a 48 hr retention test (from 36–165 μg/kg, IP). Physostigmine (80–180 μg/kg, IP) produced similar, but less marked, effects. The effect of Hup A on retention was blocked by centrally-acting antimuscarinics or hemicholinium. Similar results were obtained with Hup A or B in mice on a spatial discrimination task. Hup A also improved passive avoidance retention and reversed the amnestic effects of $NaNO_2$, cycloheximide, electroconvulsive shock or scopolamine. In clinical trials, Tang reported that Hup A produced significant improvement in memory in patients with cerebral arteriosclerosis.

RECEPTOR AGONIST TREATMENTS OF ALZHEIMER PATIENTS

With the recognized loss of presynaptic cholinergic neurons in Alzheimer brain tissue, several groups have tried to directly stimulate postsynaptic cholinergic receptors in the brain. One approach has used the muscarinic agonist, bethanecol, injected directly into the brain (since it does not readily pass the blood-brain barrier). Another approach has utilized cholinergic agonists which easily pass the blood-brain barrier. These approaches have encountered difficulties similar to those experienced with anticholinesterase agents.

S. L. Read (John Douglas French Center, Los Alamitos, CA) presented his results with chronic intracerebral injections of bethanecol in Alzheimer patients (in an attempt to replicate the work of R. E. Harbaugh and his group in New Hampshire) in an open study on 5 mildly demented patients. Measures included the MMS and the RANT memory test as well as subjective reports from patients' family members. Two patients showed some persuasive improvement at intermediate drug levels, while high doses impaired function, perhaps due to psychomotor retardation. For the other 3 patients, all had seizures, one had a major cerebral hemorrhage due to the biopsy, another had meningitis with progressive agitation and depression and the third became progressively worse with paranoia and depression. Three patients (including the hemorrhage patient) who stayed in the study for 2 years showed only a small degree of deterioration, and these preliminary data suggest a long-term benefit not apparent from psychometric tests.

R. S. Wilson and J. H. Fox (Rush Alzheimer's Disease Center, Chicago, IL) presented their results with 11 Alzheimer patients (mild to moderately demented) administered intracerebroventricular injections of bethanecol. One patient had a transient postoperative right hemiparesis and was removed from the study. Another patient developed a subdural hematoma but did enter the study. The initial study was a double blind cross-over trial over 24 weeks using 0.35 mg/day of bethanecol (similar to R. E. Harbaugh's study. The second study was an 8-week double-blind cross-over design using the same dose with revised outcome measures, followed by an open escalating dose trial up to 1.75 mg/day. After the second study, patients were maintained chronically on bethanecol. Tests were performed once every 4 weeks, including measures of global mental status, language and memory. Family members completed an ADL scale once per week. In the second study, no significant effects of bethanecol were noted. In the subsequent studies, there was a trend toward decreased abnormal behaviors. Further, 3 of 9 patients improved in 1 or more psychological measures. The therapeutic window, however, seemed quite narrow. MMS scores progressively declined in all patients over a 2-year follow-up period.

M. Davidson (Mt. Sinai, New York, NY) reported on the effects of RS-86, oxotremorine and 4-aminopyridine in Alzheimer patients. The RS-86 (a direct muscarinic agonist) study was completed in 12 of 15 patients (the other three had syncope, abdominal distress or a seizure). Six of those 12 had some drug-induced side effects. The cognitive effects (measured by the Alzheimer's Disease Assessment Scale—ADAS) of the best dose were generally negative, causing as much as an 8% decrement, but two patients showed improvements of 31% and 36%. There was a slight correlation between early a.m. cortisol and cognitive effect. Oxotremorine was studied in 7 patients (0.25–2 mg doses) and side effects were seen in all patients (e.g., anxiousness, depression, crying). Similarly, 4-aminopyridine was given to 14 patients with negative results. Davidson is planning to combine a noradrenergic drug (clonidine) with the cholinergic agents (following the suggestion from a previous publication by A. Cherkin) to replicate the positive effects in AD patients that have been reported in rats with such combinations.

L. Ravizza (University of Turin, Italy) presented the results of 3 studies. In the first, he showed that MMS scores correlated with CSF somatostatin levels. In the second, he examined the cholinergic receptors on lymphocytes with QNB and N-methylscopolamine (NMS). The binding of these receptors increased with age. No saturation could be obtained with QNB in intact lymphocytes or lysed membranes. Saturation could be established with NMS and indicated a single class of binding sites. He found a significant reduction (40%) in the number of NMS receptors on the lymphocytes of AD patients. The age dependent modulation of these presumed muscarinic receptors was not seen in the relatives of Alzheimer patients. In the third study, L-acetylcarnitine, an endogenous compound structurally similar to acetylcholine, was given to 15 Alzheimer patients. A trend toward improvement (on MMS, Blessed Dementia scale and Information-Memory-Concentration test and the Rey test) was seen after the first month of 2 grams/day and became statistically significant by the 4th month.

SUMMARY

After a decade of intense study of cholinergic therapies for Alzheimer's disease, three conditions in this field are apparent:

1) The potential that cholinergic agents will ameliorate the memory dysfunction of Alzheimer patients (as l-dopa benefits Parkinson patients) is still a stimulus for research.

2) Cholinergic neuropharmacology and its impact on the therapy of memory disorders associated with cholinergic dysfunction needs to be further characterized and understood.

3) While there is still a search for a symptomatic treatment for AD, the path to find a treatment for the Alzheimer disease process must first pass through a phase of basic research to find the cause of Alzheimer's disease.

At the meeting, there was an undercurrent of concern that the cholinergic deficit is too severe to be treated, that the cholinergic systems are too complex to respond to a pharmacologic

therapy and that too many other systems are involved in Alzheimer's disease for a cholinergic treatment to be successful. However, this concern was balanced by the evidence of basic scientific experiments which indicate that the central cholinergic system mediating memory can be positively manipulated in animal lesion preparations and Alzheimer tissue. Also there were reports that improved pharmacological approaches and psychological measures are being developed. It appears that Alzheimer therapy is at the stage that cancer chemotherapy was 20 years ago: the promising agents cause nausea without producing clear effects but the basic laboratory studies strongly suggest that substantial benefits are possible and several agents have shown encouraging results. Meanwhile, patients and scientists are becoming increasingly interested in the field. However, several areas need to be more carefully addressed, particularly preclinical determination of drug effects, patient evaluation techniques and experimental design.

At the preclinical stage, many old drugs are being rediscovered, new agents are being synthesized and drugs affecting many systems are being studied. The precise effects of these drugs on the nervous system need to be more clearly characterized and understood. Are they specific? Are they working presynaptically or postsynaptically? Is their net effect on the target systems positive? It is also important to determine the availability of the drug to the receptors and the long-term effects on those systems. Animal models need to be developed which can accurately predict whether these drugs will have any benefit for human memory. Learning and memory functions observed in the rat have been difficult to relate to human capacities. A computerized paradigm for testing memory consolidation in the primate would be useful. A primate model of Alzheimer's disease would offer the best test, but none has yet been validated.

There are serious design problems in studying psychological processes. Memory has been a particularly difficult area and probably involves many brain systems. Memory problems in Alzheimer patients are still more difficult to assess, and the level of function may fluctuate day to day as much as 20%. With similar fluctuations expected in specific measures of cognitive function, a drug effect which only produces a 10% improvement will be hard to detect. Thus, there is a need to develop better methods for reliably and precisely determining dementia severity as well as the permanent level of impairment of specific types of cognitive function.

Experimental design has not successfully met the challenge of evaluating drugs in Alzheimer patients. Clinical pharmacology has long relied on the double-blind cross-over design for comparing drugs. However, even with this time-honored method, many drugs have shown positive effects in some studies while not in others. There was a call during the conference for an open-phase adjustment of medication regimen with repeated measures which would then be followed by the double-blind cross-over phase. Dose effect interactions should also be more carefully considered. However, there is a lack of adequate active placebos and positive control drugs to serve for comparison. Acute studies are confounded by day-to-day fluctuations of dementia severity while longer studies can not adequately account for the variability of disease progression. With so many dependent variables being measured in each case, it is also difficult to assess the claims of statistical significance, especially when conflicting results are found in similar studies. These studies need to have larger numbers of patients in whom the heterogeneity is better characterized both biologically and clinically, especially in terms of the question of responders and non-responders. For progress it is important that investigators meet more often to discuss their approaches and findings. The multicenter THA trial and this conference serve as examples. As the methodological flaws are solved, new experimental systems will lead to the discovery of successful treatments and the eventual prevention of Alzheimer's disease.