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Differential Response to the Cholinergic Agonist Arecoline among Different Cognitive Modalities in Alzheimer's Disease

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Nine patients with possible or probable dementia of the Alzheimer type were tested on nine cognitive tests prior to (two times) and during continuous intravenous administration of five different doses of the muscarinic cholinergic agonist arecoline (1, 4, 16, 28, and 40 mg/day). The present analysis examined whether improvement on cognitive testing for each patient during arecoline treatment was most likely to occur at the same dose for all tests or whether different test scores improved at different doses of arecoline. Results indicated there were significant differences among tests in the dose at which most patients showed improved cognitive performance. These differences may have therapeutic significance, as verbal ability tended to improve at low doses of arecoline, whereas attention and visuospatial ability tended to improve at higher doses of arecoline. ***[Neuropsychopharmacology 15:163–170, 1996]***

KEY WORDS: *Alzheimer's disease; Arecoline; Acetylcholine; Memory*

One of the earliest deficits detected in the brains of patients with Alzheimer's disease is a decline in the function of the cholinergic neurotransmitter system; the degree of impairment of this system has been correlated with decline in cognitive function (Whitehouse et al. 1982; McGeer 1984; Roth 1986; Geula and Mesulam 1989). Because of the association between the status of the cholinergic system and cognitive abilities in Alzheimer's disease, many studies have examined whether cholinergic replacement therapy can restore cognitive function in patients with Alzheimer's disease (Hollander et al. 1985; Tariot et al. 1988; Thal et al. 1989; Farlow et al. 1992; Knapp et al. 1994; Levy et al. 1994).

We recently completed a study examining cognitive performance of Alzheimer's disease patients receiving a continuous intravenous infusion of arecoline, a direct muscarinic receptor agonist (Raffaele et al. 1991; Soncrant et al. 1993). Because it has been found that not all subjects show improved performance at the same dose of a cholinergic agent and that the dose that optimizes performance may vary (Davis and Mohs 1982), we attempted to determine an optimal dose of arecoline for each individual patient. Arecoline was administered at five different dosing rates in an open-phase rising-dose study. Replication of open-phase results was then attempted during a double-blind placebo-controlled administration of each patient's "best dose," that is, the dose that optimized that patient's overall cognitive performance (Soncrant et al. 1993). Significant improvements were seen on two cognitive tests during the rising-dose study: selective reminding (a verbal memory

Portions of this study were previously presented in abstract form.

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Received July 27, 1994; revised August 11, 1995; accepted September 21, 1995.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/96/$15.00
SSDI 0893-133X(95)00179-4

task) and figure copying (a visuospatial task with no memory component). During the replication study, the performance improvement on the selective reminding task was repeated, but performance on the figure-copying task was not significantly improved. Because of this discrepancy between the results of the rising-dose study and the double-blind replication study, and noting an apparent difference in the dose at which performance improved on the two tasks during the rising-dose administration, we wished to examine whether or not the dose at which performance improved varied among different cognitive tasks. We also sought to determine whether performance on tasks based on similar cognitive functions (such as visual, verbal, or attentional) improved at similar doses.

METHODS

The overall procedures and results for the drug treatment study have been previously published (Soncrant et al. 1993). Briefly, subjects were nine carefully screened healthy subjects (five men and four women; age range 50–81 [mean 63.1 ± 9.4]). All subjects were medication free. All were diagnosed with possible (isolated memory impairment) or probable Alzheimer's disease according to NINCDS/ADRDA criteria (McKhann et al. 1984), and eight of nine subsequently met all criteria for probable Alzheimer's disease. Mini-Mental State Examination (Folstein et al. 1975) scores ranged from 15 to 26 (mean 23.0 ± 3.4). Consent to participate in this investigation was obtained from each patient and/or from an empowered relative. All studies were performed on an inpatient basis. The protocol was approved by the National Institute on Aging Institutional Review Board (protocol number 88-AG100).

Arecoline hydrobromide (Regis Chemical, Morton Grove, IL), dissolved in normal saline, was administered by continuous intravenous infusion over the course of the study, using CADD-1 Ambulatory infusion pumps (Pharmacia Deltec, St. Paul, MN). During all infusion periods, methscopolamine bromide (Upjohn, Kalamazoo, MI), a muscarinic receptor antagonist that does not enter the brain, was administered at a dose of 2.5 mg orally every 8 hours (to block the peripheral autonomic actions of arecoline).

Arecoline was administered by continuous intravenous infusion at doses of 0.5, 1, 2, 4, 8, 16, 22, 28, 34, and 40 mg/day. Doses were escalated on a daily basis following completion of psychological and/or physiological testing. Neuropsychological testing was conducted twice prior to the initiation of drug administration (3 days and 3 hours prior to initiation of arecoline treatment), during the infusion of every other dose (1, 4, 16, 28, and 40 mg/day), and twice after cessation of drug treatment (sessions were separated by 1 day without testing and were separated from the drug treatment period by at least 1 week).

A total of nine cognitive tests were administered during two 1- to 2-hour test sessions conducted between 9:00 A.M. and 1:00 P.M. Tests administered were as follows: (1) calculation ability (ability to correctly perform mathematical operations of varying difficulty); (2) visual and verbal continuous recognition memory (ability to recognize faces or words previously presented during sequential presentation on a computer screen [Berardi et al. 1991]); (3) figure-copying (ability to copy accurately a series of geometric drawings [Haxby et al. 1985]); (4) Stroop color-word interference (a test of attention [Golden 1978]); (5) verbal fluency (letter [FAS] and category fluency [Benton and Hamsher 1976; Butters et al. 1987]); (6) the Token test (a test of ability to follow verbal commands [Boller and Vignolo 1966]); (7) Benton Visual Retention Test (a test of ability to accurately draw a series of line drawings from memory [Benton 1974]); (8) Digit/Symbol Substitution Test (a test of attention/concentration [Wechsler 1955]); (9) Buschke Selective Reminding Test (a test of verbal memory [Buschke 1973]). Details of the tests and their administration have been described previously (Soncrant et al. 1993).

For each patient, a "best dose" was determined for each task by selecting the dose of arecoline on which that patient achieved his or her best performance on that task. These individual "best doses" were then compared across tasks using one-way analysis of variance (ANOVA) and least significant difference (LSD) multiple comparison procedures (SAS Institute 1988). A total of 16 score parameters were included in this analysis: Benton Visual Retention Test—total errors (Benton Errors); Buschke Selective Reminding—total recall (Buschke TR) and long-term recall (Buschke LTR); Calculations—total number correct; category fluency—total words named; figure copying—error score; Digit symbol test—number correctly completed; FAS verbal letter fluency—total words named (FAS); Stroop interference task—words, colors, and interference colors named; Token Task—number correct; continuous recognition memory—verbal long-term memory (CR-Word Long), verbal short-term memory (CR-Word Short), visual long-term memory (CR-Face Long), and visual short-term memory (CR-Face Short).

RESULTS

There was a significant overall effect of test ($F = 2.57$, $p = .0022$, df = 15,128), indicating (1) that the dose at which performance improved most differed among tests, and (2) that this difference was not random among subjects but that performance on a particular test improved at similar doses across subjects. Using the

multiple comparison procedure, significant differences between specific tests were identified (see Figure 1). In particular, performance on tests involving verbal function (verbal memory, verbal comprehension, and verbal fluency) improved at low doses of arecoline (mean doses ranging from 4.3 to 12.7 mg/day), and performance on tests involving attentional function (digit/symbol substitution and Stroop interference) improved at higher doses of arecoline (mean dose of 26.7 mg/day for both tasks). Tests involving visuospatial function also improved at higher doses of arecoline (mean doses ranging from 19.7 for Benton retention test to 26.7 for figure copying).

Verbal Tasks

The mean best doses for all verbal tasks, other than the continuous recognition tasks, were tightly clustered at the lower end of the dose range. The mean best dose ranged from 4.3 mg/day for Buschke total recall to 14.7 mg/day for Stroop Words. As there was a significant overall improvement in performance on the Buschke Selective Reminding task (Buschke TR) at these doses ($p < .05$ vs. baseline at 4 mg/day; see Soncrant et al. 1993), this clustering does not simply represent impairment of performance by arecoline at the higher dose levels (there was in fact no significant impairment of baseline performance at any dose level). The two verbal fluency tasks, FAS and category fluency, which had identical designs and differed only in the type of verbal fluency measured (letter or category fluency), had almost identical mean best doses (12.7 and 12.3 mg/day, respectively) which would be unlikely if the differences in mean best dose across tasks were simply a random finding.

The "verbal" portion of the continuous recognition task (CR Word) had mean best doses for both the long- and short-term memory components that were not as low as the mean best doses found for other verbal tasks. This result may be due to a general lack of sensitivity of the continuous recognition test to arecoline under the testing conditions (mean best doses for the two short-term memory measures for this task were not significantly different from the mean best dose for any other task; this would imply a lack of drug effect on at least this component of the task). It is also possible that the higher mean best doses are due to a visuospatial com-

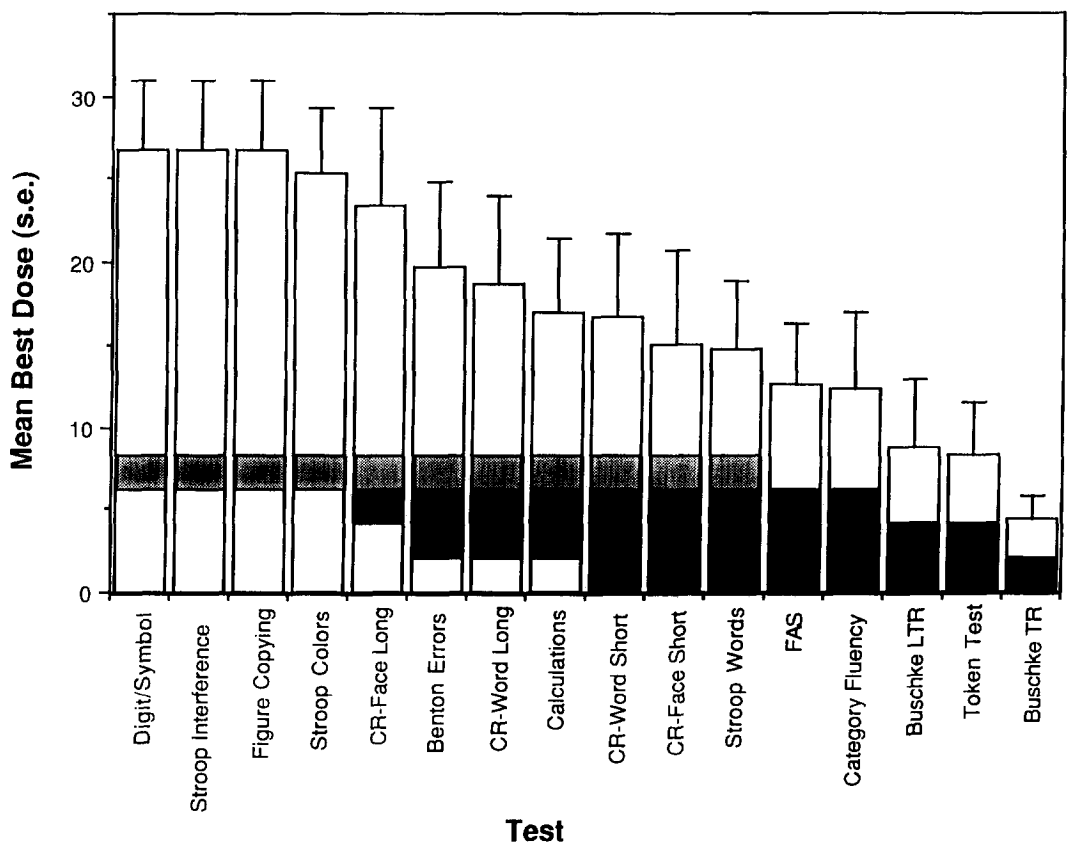

Figure 1. Mean best dose for each test (error bars indicate standard error). *Shaded* bars indicate significance groupings (i.e., groups with the same shade bars are not significantly different from each other).

ponent of the "words" continuous recognition task, because it involves reading the word (Peterson et al. 1988).

Attention and Visuospatial Tasks

Overall, visuospatial tasks improved at a higher range of doses than did verbal tasks. For example, the mean best doses were 26.7 for figure copying and 19.7 for Benton Visual Retention. There were no visuospatial tasks with mean best doses at the low end of the dose range.

The mean best doses for the two attentional tasks were toward the top of the dose range (mean best dose was 26.7 mg/day for both digit/symbol substitution and stroop interference) but did not include the highest dose administered (40 mg/day). No mean best dose was higher than 28 mg/day (the second highest administered dose). The improvement in attention and visuospatial function found at high doses is unlikely to be due solely to a practice effect, for the following reasons. (1) Although the improvement occurred toward the end of testing, it was not greatest at the highest tested dose. In fact, the difference between the calculated mean best dose for figure copying, stroop interference, and digit symbol substitution (26.7 mg/d) and the highest administered dose (40 mg/d) was 13.3, greater than the least significant difference of 12.4 (by the LSD multiple comparison procedure). (2) Mean post-test scores were not different from baseline scores for Stroop interference or figure copying; there was some improvement over baseline in post-test scores for digit-symbol substitution, but the improvement was less than that achieved on the highest arecoline dose (Soncrant et al. 1993). (3) Although similar tests were used, a similar distribution of mean best dose levels was not seen in a second study in which physostigmine (which acts by a different mechanism, inhibiting acetylcholinesterase as opposed to directly activating receptors) was given during a similar rising dose administration period. If the effects seen following arecoline administration were due solely to a practice effect, the same tests should have been subject to practice effects, and therefore have shown the highest mean best dose, in the physostigmine study. This was not the case (Asthana et al. in press).

Memory Tasks

There was no clear best dose range for memory as a unitary function. Within a functional category (such as visuospatial or verbal), tasks that also included a memory component tended to improve most at the lower end of the best dose range for that function. Thus, Buschke total recall (mean best dose 4.3 mg/day) and long-term recall (mean best dose 8.7 mg/day) had lower mean best doses than the verbal fluency tasks. The mean best dose for the token task (8.3 mg/day) was similar to that for Buschke total recall. Within the group of visuospatial tasks, the Benton Visual Retention Task, a visual memory task, had a lower mean best dose (19.7 mg/day) than did the figure-copying task (mean best dose 26.7 mg/day), which included no memory component. It is possible that the overall cognitive environment of a task, such as the verbal or visuospatial context, is more critical in determining the optimal level of cholinergic activation, and that other aspects of task, such as memory, may lead to a finer level of distinction within the general range.

DISCUSSION

These results demonstrate that during the administration of arecoline by continuous intravenous infusion to patients with possible or probable Alzheimer's disease, different aspects of cognition were improved by differing doses of arecoline. These findings may in part explain the variability of the dose-response curve for cholinergic drugs among individuals, and perhaps the mixed results on effectiveness of cholinergic drugs in treating symptoms of Alzheimer's disease (Becker and Giacobini 1988; Brinkman and Gershon 1983). In addition, they indicate that even finely tuned cholinergic stimulation may not achieve simultaneous optimization of all cognitive modalities.

The limitations in our original arecoline studies, especially the open design during the escalating-dose phase and the potential for confounding by practice effects (especially with regard to tasks with a higher mean best dose), accentuate the need for replication of these results before firm conclusions can be made based on these findings. However, the present findings may explain the failure to replicate, during double-blind testing, the significant improvement found in figure copying during an open escalating-dose study (see Soncrant et al. 1993 for details). The mean optimal dose used during the replication study [7.7 $\pm$ 6.3 (sd) mg/day] was within the range of maximal improvement for verbal functions, particularly verbal memory, but substantially lower than that producing maximal improvement on visuospatial tests.

One previous study investigating arecoline administration to Alzheimer's patients demonstrated an improvement in picture recognition following a dose of 2 mg/hour (the mean best dose for visuospatial tasks in our study was 26.7 mg/day, equivalent to 1.1 mg/hour) and an impairment in category retrieval (similar to our verbal fluency measure) following the same 2 mg/hour dose (Sunderland et al. 1988; Tariot et al. 1988). Qualitative observer ratings of verbal expressiveness and word

finding demonstrated a significant improvement in patients during the 1 mg/hour arecoline infusion. Because the lowest dose administered in this study was 1 mg/hour (a dose at which category retrieval was not changed from baseline), there was no dose comparable to that at which verbal ability was improved in our study (4.3–14.7 mg/day, equivalent to 0.18–0.61 mg/hour). It is possible that these results may be interpreted as providing some support for our findings (i.e., that visuospatial tasks were improved only at a higher dose of agonist, while verbal tasks were improved at a dose (0.18 to 0.61 mg/hour) lower than that administered in the Tariot et al. (Tariot et al. 1988) study (1 mg/hour), but impaired or unchanged at higher doses of arecoline).

Other studies have demonstrated differences in the level of cholinergic activation required for physiological functions (e.g., heart rate, body temperature) compared with cognitive functions (activity levels in animals, memory tests, and other cognitive tests in humans) (Ashe and Weinberger 1991; Becker and Giacobini 1988; Flicker et al. 1990; Russell et al. 1986) and differences in effective doses among similar tasks with varying task parameters (e.g., positive vs. negative reinforcement [Raffaele et al. 1990]; automated vs. manual administration [Rupniak 1992]), but clear distinctions among optimal levels of cholinergic function for specific types of cognitive functions have not been made. Although studies in animals have used many different types of task and many different cholinergic/anticholinergic agents, no clear difference in dose-response parameters for different types of cognitive tasks has been established. This failure may be due to the difficulties in comparing specific tasks and the frequent lack of dose-response information (Fibiger et al. 1991), rather than to a true absence of a dose-response differential across task types. For example, many studies use only one drug dose; thus, even if several tasks are investigated, with some showing sensitivity and others not, it is not possible to say that there is a varying dose-response curve across tasks because it is not known whether performance on unaffected tasks would or would not change with lower or higher drug doses than the one administered.

The mechanism of the apparent differential sensitivity of cognitive functions to muscarinic drugs cannot be determined based on the current study. However, evidence from other studies supports several possible mechanisms. First, there may be differences in overall cholinergic receptor concentrations among different brain regions, or variation of receptor subtypes or receptor affinity among the anatomic structures may be involved in mediating different types of cognitive functions. Differences in the distribution of receptor subtypes among brain regions have been demonstrated (Crews et al. 1986; Ashe and Weinberger 1991). Arecoline is a partial agonist with higher M_2 than M_1 receptor affinity (Messer et al. 1989). Different concentrations of arecoline may alter the relative level of activation of M_1 vs. M_2 receptors (for example, different arecoline concentrations may be required to stimulate different receptor subtypes) (Freedman et al. 1988). Different patterns of relative activation may be required for optimization of different types of cognitive function. This has been shown for physiological functions (e.g., ratios of effective doses for induction of hypothermia and salivation vary among muscarinic agonists; see Freedman et al. 1990).

Second, baseline levels of acetylcholine vary among brain regions (Day et al. 1991), which might imply that in order to change the functionality of acetylcholine among different systems, different levels of stimulation are required, leading to differences in effective doses of pharmacological agent among systems.

Third, differences in the responsiveness of neurons in different brain regions to behavioral or pharmacological stimuli (Crews et al. 1986; Day et al. 1991) and of different cortical neurons to the same pharmacological stimulation (Bassant et al. 1990; Juliano et al. 1990) have been demonstrated. It has also been shown that the effects of arecoline on cerebral brain metabolism in rats vary among brain regions depending on the administered dose of arecoline, with the hippocampus showing increased activation (as measured by local cerebral glucose utilization) at lower doses of arecoline than were required to increase cortical activation (Soncrant et al. 1985). These differences in responsiveness could cause neurons in different brain regions to respond differentially to the same dose of pharmacological agent.

Fourth, there may be differences in cortical stimulation by basal forebrain neurons among different brain regions, implying differences in the levels of activation needed to promote optimal neuronal function for a specific brain region. Projections of basal forebrain neurons are highly localized to small cortical areas, and there are topographical differences in the baseline firing rate of basal forebrain neurons (Richardson and DeLong 1991), differences in firing activity related to physiological state (Woolf and Butcher 1991), and differences in acetylcholine release in the cortex in response to different behavioral or pharmacological states (Day et al. 1991). These mechanisms, individually or in concert, could account for the differences in dose-response curves among tasks shown in our data.

Our results indicate that multiple cognitive abnormalities (e.g., dysfunctions spanning both verbal and visuospatial domains) cannot be simultaneously corrected by systemic administration of cholinergic agonists. They would also imply that cholinergic therapies might be more useful earlier in the disease, when a single dominant performance deficit could be targeted. These findings also raise the question of whether cholinesterase inhibition would be a more physiologically

relevant way to treat this disease (because the endogenous differences in stimulation would be amplified), rather than trying to treat by directly stimulating at a (perhaps inappropriate) generic level. The lack of unitary function in the cholinergic system, both as an explanation for the varying results of Alzheimer's disease treatment trials and as an exposition on the futility of treatment by simply increasing baseline cholinergic stimulation, has been discussed by several authors (Crews et al. 1986; Becker and Giacobini 1988; Durkin 1989; Sarter et al. 1990; Fibiger 1991; Fibiger et al. 1991). Our results are in agreement with their theoretical discussions and (if confirmed) perhaps provide some details of the end points achieved by differential functioning within the cholinergic system. Further studies are needed to determine whether the present findings can be replicated and whether the same types of differential dose-response curves will be found for drugs that act as cholinesterase inhibitors or whether this effect may be specific to those drugs that act directly on muscarinic receptors.

These results also raise questions about the sensitivity of the methods currently used to evaluate drug efficacy in therapeutic trials of cholinergic treatments for Alzheimer's disease. Commonly used evaluations, especially in the large multi-center trials, are based on cognitive scales or measures using a composite score derived from a variety of cognitive components (e.g., the Alzheimer's Disease Assessment Scale [Rosen et al. 1984]). Because different components of these scales may be improved on different drug doses or improvements in some components on a particular dose may be obscured by lack of improvement or impairment in other components at the same dose, drug efficacy evaluations based on these scales may need to be reassessed.

These results may also offer some insight into the functioning of the cholinergic system and its role in cognitive performance. It has previously been suggested that the cholinergic system may be important in maintaining a level of "alertness" or "constraints on information processing" in an organism, and that control of this "alertness" level may be important in memory and attentional function (Callaway et al. 1992). The results of this study support a somewhat different hypothesis, suggesting that optimal modulation of cognitive efficiency within different neural systems requires different concentrations of cholinergic agonists. Thus, the optimal level of functioning for the cholinergic system may be dependent upon the type of task being performed. As discussed above, this hypothesis is supported by a variety of physiological evidence, including differences in type and concentration of muscarinic receptors among brain regions, highly localized cortical projections from the basal forebrain, baseline concentrations of acetylcholine that vary among brain regions, and differences in baseline firing rate among basal forebrain neurons. These factors, in combination with the highly specific types of information and processes involved in cognitive function, argue strongly for much greater degrees of specificity and sensitivity in patterns of cholinergic stimulation than was previously thought.

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