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Physostigmine, But Not 3,4-Diaminopyridine, Improves Radial Maze Performance in Memory-Impaired Rats

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BENINGER, R. J., B. A. WIRSCHING, P. E. MALLET, K. JHAMANDAS AND R. J. BOEGMAN. *Physostigmine, but not 3,4-diaminopyridine, improves radial maze performance in memory-impaired rats.* PHARMACOL BIOCHEM BEHAV **51**(4) 739–746, 1995. – The results of some studies suggest that 3,4-diaminopyridine (3,4-DAP), a drug that enhances the release of acetylcholine, may improve memory. The present study examined the ability of 3,4-DAP to reverse the memory impairment produced by scopolamine and the ability of 3,4-DAP and physostigmine to reverse the memory impairment produced by quinolinic acid lesions of the nucleus basalis magnocellularis (nbm) in rats. Mnemonic functioning was assessed with the use of a partially baited eight-arm radial maze. Entries into arms that were never baited were defined as reference memory errors; entries into baited arms from which the food already had been eaten were defined as working memory errors. In Experiment 1, 0.1 mg/kg scopolamine produced a significant increase in working and reference memory errors. Various doses of 3,4-DAP had no significant ameliorative effect on the mnemonic deficit. In Experiment 2, cholinergic function was impaired using a unilateral intra-nbm injection of quinolinic acid (120 nmol in 1.0 μl). These lesions reduced the levels of the cholinergic marker, choline acetyltransferase, in the cortex by more than 40%. Results showed that the nbm lesion animals were significantly more impaired on the working than reference memory component of the task. Physostigmine (0.01, 0.05, 0.10, 0.20, 0.50 mg/kg) dose-dependently decreased the number of working but not reference memory errors. 3,4-DAP (10^{-8}, 10^{-6}, 10^{-4}, 10^{-2}, 10^{0} mg/kg) had no reliable effect. It was concluded that physostigmine, but not 3,4-DAP, ameliorates memory impairments following decreases in cholinergic function.

3,4-Diaminopyridine Physostigmine Scopolamine Quinolinic acid Memory Working memory
Reference memory Radial maze Acetylcholine Nucleus basalis magnocellularis Excitotoxic lesions

THE AMINOPYRIDINES have been classified as the monoamino and diamino derivatives of pyridine (25). These compounds have the interesting property of blocking neuronal K^+ channels and facilitating neurotransmitter release (25). Recent studies have confirmed that various aminopyridines have these effects on central cholinergic neurons. Thus, 4-aminopyridine blocked K^+ release from basal forebrain cholinergic neurons in brain slices (26) and 2,4-diaminopyridine, when injected directly into the striatum, stimulated acetylcholine release from striatal neurons, detected with intracerebral dialysis (14). The cholinergic action of aminopyridines has made them useful pharmacotherapies for the treatment of disorders requiring increased cholinergic neurotransmission [e.g., myasthenia gravis (30)] and has made them candidates for use as memory-enhancing compounds.

Many data have implicated central cholinergic neurotransmission in memory. Decreased cholinergic function is seen with normal aging and is associated with age-related memory impairments (4). Similar memory impairments can be induced in young people treated with anticholinergic drugs (19). Moreover, memory impairment is the earliest clinical sign of Alzheimer's disease, characterized by an extensive loss of basal forebrain cholinergic neurons (13).

In animal studies, excitotoxic lesions of the basal forebrain that damage cholinergic neurons of the nucleus basalis magnocellularis (nbm) produced memory impairments in a number

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of paradigms (9,20,28,47). Recently, we have shown that different excitotoxins differentially damage cortico- and amygdalopetal cholinergic neurons of the nbm (10). Those excitotoxins producing the greatest damage to amygdalopetal cholinergic neurons (e.g., ibotenic, quinolinic, phthalic, and *N*-methyl-D-aspartic acids) have been reported to produce the greatest mnemonic impairments (12,32,33). Significant but less severe mnemonic impairments have been reported following intra-nbm injections of quisqualic acid, an excitotoxin that is relatively selective for corticopetal cholinergic projections of the nbm (9). Thus, there is strong evidence from animal research supporting a role for nbm cholinergic cells in the control of memory.

Few previous studies have evaluated the behavioral and/or mnemonic effects of aminopyridines. Peterson and Gibson (41) reported that 3,4-diaminopyridine (3,4-DAP), given systemically in nanogram doses, improved motor performance of aged mice. Haroutunian et al. (27) found that posttraining injections of 4-aminopyridine improved memory of rats in a passive avoidance task. Conflicting results have been reported for 3,4-DAP; it improved memory of aged rats in the radial maze (16) but had no significant effect on memory of aged primates (2). Similarly, in humans, Wessling et al. (45) found improvement in Alzheimer patients given 4-aminopyridine whereas Davidson et al. (15) did not. Thus, at present, the possible memory-enhancing effects of aminopyridines remain in question.

In a review of possible cholinergic pharmacotherapies for the treatment of memory disorders, Bartus et al. (5) suggested that more attention should be given to the aminopyridines. As the effects of these compounds remain in question, the present experiments were undertaken to further evaluate the possible memory-improving effects of 3,4-DAP. In two experiments, rats were trained in a radial maze task requiring reference and working memory (39). In Experiment 1, trained rats were treated with the anticholinergic agent scopolamine and in Experiment 2, trained rats received quinolinic acid lesions of the nbm; both treatments produced memory impairments. The ability of a wide range of doses of 3,4-DAP to reverse mnemonic impairments in these animals was tested. Additionally, in Experiment 2, the anticholinesterase physostigmine was tested as a reference compound that would improve memory (27,34).

METHOD

Subjects

Treatment of the subjects used in the present experiment was in accordance with the guidelines of the Canadian Council on Animal Care, the Animals for Research Act, and relevant university policies and was reviewed and approved by the Queen's University Animal Care Committee.

Eighty-eight (13 in Experiment 1 and 75 in Experiment 2) male Sprague-Dawley rats (Charles River Canada) weighing 250–350 g at the beginning of the experiment were individually housed in wire mesh cages in a temperature-controlled (21 ± 1°C) colony room kept on a 12L : 12D (0700–1900 h) cycle. Rats were deprived and maintained at 80% of their free-feeding weights, adjusted for growth, by daily feeding with measured rations of chow (Purina); water was always available in the home cage.

Animals from Experiment 2 that failed to learn the task to criterion (n = 19), died during training for reasons unrelated to the experiment (n = 1), died during surgery (n = 3), failed to perform the task postoperatively (n = 3), did not perform on at least two of the drug sessions (n = 2), or had their cannulae placed outside of the target zone (n = 3) were excluded from the experiment.

Preparation for Surgery

Because of the anorexic effects of nbm lesions (6), rats in the quinolinic acid group of Experiment 2 (see below) received a highly palatable diet of wet mash, chocolate chip cookies, and Eagle brand milk beginning 3–4 days prior to surgery and continuing during the postoperative recovery period until they attained 80% of their free-feeding weights (adjusted for growth).

Surgery

Rats from Experiment 2 were anesthetized using an oxygen flow containing 2% halothane (Halocarbon, Malton, Ont.) and positioned in a Narashighe stereotaxic frame. With the incisor bar set at 3.3 mm below the horizontal plane passing through the interaural line, unilateral microinjections were aimed at the right or left nbm with coordinates from bregma being 0.8 mm posterior, 2.6 mm lateral to the midline, and 8.0 mm ventral to the surface of the skull. All rats received 1.0 μl infused over 2.5 min, and the cannula (Hamilton, 0.35 mm o.d.) was left in place for an additional 3 min to promote diffusion. Quinolinic acid (Sigma) was injected in a dose of 120 nmol titrated to pH 7.4 with 1 M NaOH. Sham rats received 0.9% saline.

Apparatus

The radial maze, elevated 60 cm above the floor, consisted of a circular platform (32 cm diameter) surrounded by eight equally spaced radial arms (68 cm long × 10 cm wide). Food wells, located 6 cm from the end of each arm were 1.5 cm deep and 2.5 cm in diameter. Testing was carried out in a white painted room lit by 70-W fluorescent tubes. Several visually distinct cues (e.g., door, shelf, chair) were present in the room and remained in the same position with respect to the maze.

Experiment 1

Pretraining. During the first 2 weeks in the colony, rats were handled daily, exposed to the reinforcer for the maze task (pieces of Froot Loops cereal), and deprivation was begun. Maze training began by placing each rat on the maze for 20 min each day for 2 days and then for 10 min on each of the following 2 days. During the first two of these sessions, reinforcers were scattered about all arms of the maze and during the second 2 days reinforcers were placed along a randomly selected four arms that would subsequently constitute the baited arms for that rat.

Training. Formal training began the next day and all rats were tested for one session each day, 5 days a week. At the beginning of each session the appropriate four arms were baited by placing a reinforcer in the food well and the rat was placed on the center platform such that it faced arm 1. The session was terminated when the four baited arms had been entered, 14 choices had been made, or 10 min elapsed, whichever came first. After each session the maze was cleaned with a dilute cider vinegar solution. Working memory errors and reference memory errors were recorded for each rat. Entries into arms that were never baited were defined as reference memory errors; entries into baited arms from which the food already had been eaten were defined as working memory errors. All rats were tested until they achieved a criterion of two consecutive sessions with a total of seven out of eight correct choices, summing the first four choices from each session.

Drug testing. Once criterion was reached, drug sessions were begun. Drugs were always given in two-session blocks. The two drug sessions were never separated by a weekend, nor were the two criterion sessions and the two drug sessions separated by a weekend. Thus, the two-session criterion could only be met on a Monday-Tuesday, Tuesday-Wednesday, or Friday-Monday.

Thirty minutes prior to each drug session, each rat was injected IP with 0.1 mg/kg scopolamine hydrobromide (Sigma) dissolved in 0.9% saline at a concentration of 0.1 mg/ml. Fifteen minutes later a second IP injection was given consisting of 0.9% saline or one of the following doses of 3,4-DAP (Aldrich) adjusted to a pH of 6.5-7.5 in a volume of 1.0 ml/kg: 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{0} mg/kg. Each rat received scopolamine followed by saline for the first two drug sessions. Drug-free trials were then conducted until criterion was again established. For each rat the first dose of 3,4-DAP was randomly selected and administered after scopolamine for the next two drug sessions. Criterion was again established in drug-free sessions and then the next dose of 3,4-DAP was selected, etc. After all doses of 3,4-DAP had been tested for a rat, criterion was again established and then a final pair of drug sessions with scopolamine followed by saline was given. Table 1 shows the order of 3,4-DAP doses for each rat as well as the number of drug-free sessions required to establish criterion prior to each pair of drug sessions.

Experiment 2

Training. Rats were trained to perform the radial maze task in a manner similar to that described in Experiment 1. Following training, rats were matched according to the number of days to criterion and randomly assigned to either the quinolinic acid (n = 37) or sham (n = 12) group. The quinolinic acid group was further subdivided into a nondrug (n = 11), a physostigmine (n = 13), and a 3,4-DAP (n = 13) group.

Postoperative testing. Following at least 1 week of recovery animals were tested on the radial maze for 32 consecutive days. The physostigmine and 3,4-DAP groups were tested for 8 consecutive days prior to beginning drug testing.

Drug testing. This phase consisted of six 4-day blocks of treatment with one session per day. Each treatment block was preceded by four nondrug sessions. In each group, every rat received every dose.

Rats in the physostigmine group received either an IP injection (1 ml/kg) of 0.9% saline or physostigmine (Sigma) dissolved in distilled water at doses of 0.01, 0.05, 0.10, 0.20, or 0.50 mg/kg, 30 min prior to a session. For treatment blocks 1-3, six animals received either saline, 0.05, or 0.20 mg/kg of physostigmine in a counterbalanced order. During treatment blocks 4-6 these animals received the remaining doses of 0.01, 0.10, or 0.50 mg/kg, which were also administered in a counterbalanced order. The remaining six animals received the reverse order of administration: 0.01, 0.10, or 0.50 mg/kg during treatment blocks 1-3, followed by either saline, 0.05, or 0.20 mg/kg during treatment blocks 4-6. Animals were randomly assigned to the orders.

Rats in the 3,4-DAP group received either an IP injection (1 mg/kg) of 0.9% saline or 3,4-DAP (Aldrich) at doses of 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2}, or 10^{0} mg/kg, 15 min prior to a session. The doses of 3,4-DAP were dissolved in 0.9% saline and the pH was adjusted to 6.5-7.5 by adding a small quantity of 0.1 molar HCl. Six animals received either saline, 10^{-6}, or 10^{-2} mg/kg of 3,4-DAP in a counterbalanced order during treatment blocks 1-3, followed by 10^{-8}, 10^{-4}, or 10^{0} mg/kg, which were also administered in a counterbalanced order during treatment blocks 4-6. The remaining animals received the drug in the reverse order: 10^{-8}, 10^{-4}, or 10^{0} mg/kg during treatment blocks 1-3, followed by either saline, 10^{-6}, or 10^{-2} mg/kg during treatment blocks 4-6. Animals were randomly assigned to the order.

ChAT assay. Following behavioral testing, animals were killed by decapitation and their brains rapidly removed and rinsed in ice-cold saline for 15 s. ChAT assays were then carried out using a modification of the procedure described by Fonnum (23). A sample of frontoparietal cortex was obtained from a coronal slice taken 3 mm caudal to the posterior aspect of the optic chiasm. Tissue was dissected on ice and homogenized in ChAT homogenizing buffer before being stored at −75°C. Protein assays (29) were then carried out within 1 week of freezing and the amount of ChAT activity was ex-

TABLE 1

DRUG DOSES IN CHRONOLOGICAL ORDER FOR EACH RAT AND SESSIONS REQUIRED TO ESTABLISH CRITERION PRIOR TO EACH DOSE IN EXPERIMENT 1

Rat	T	Scop + D1	T	Scop + D2	T	Scop + D3	T	Scop + D4	T	Scop + D5	T	Scop + D6	T	Scop + D7	T	Scop + D8	T	Scop + D9
3	25	Sal	9	10^{-3}	2	10^{-2}	3	10^{-1}	3	10^{-4}	4	10^{0}	3	10^{-5}	3	10^{-6}	7	Sal
6	25	Sal	8	10^{-1}	3	10^{-3}	3	10^{-2}	3	10^{0}	3	10^{-6}	4	10^{-4}	3	10^{-5}	7	Sal
7	35	Sal	3	10^{-3}	3	10^{-1}	32	10^{-6}	3	10^{-2}	5	10^{0}	3	10^{-4}	6	10^{-5}	3	Sal
8	35	Sal	3	10^{-1}	3	10^{-5}	8	10^{0}	3	10^{-3}	4	10^{-4}	7	10^{-2}	26	10^{-6}	3	Sal
10	25	Sal	8	10^{-2}	3	10^{-1}	3	10^{-3}	32	10^{-5}	14	10^{-6}	7	10^{0}	3	10^{-4}	5	Sal
12	36	Sal	2	10^{-3}	2	10^{-5}	5	10^{0}	2	10^{-1}	3	10^{-4}	3	10^{-6}	8	10^{-2}	2	Sal
14	12	Sal	11	10^{0}	3	10^{-2}	4	10^{-1}	3	10^{-4}	6	10^{-6}	2	10^{-5}	4	10^{-3}	3	Sal
15	15	Sal	37	10^{-5}	10	10^{0}	11	10^{-2}	3	10^{-1}	3	10^{-4}	5	10^{-6}	3	10^{-3}	4	Sal
16	17	Sal	7	10^{-6}	2	10^{0}	19	10^{-5}	18	10^{-4}	6	10^{-3}	3	10^{-2}	19	10^{-1}	3	Sal
18	12	Sal	6	10^{-4}	3	10^{-3}	3	10^{-2}	4	10^{-5}	3	10^{-6}	7	10^{-1}	2	10^{0}	4	Sal
19	15	Sal	9	10^{-1}	4	10^{-2}	17	10^{-3}	2	10^{0}	3	10^{-6}	8	10^{-5}	3	10^{-4}	2	Sal
20	20	Sal	8	10^{-2}	33	10^{-3}	3	10^{-5}	4	10^{0}	2	10^{-4}	4	10^{-6}	2	10^{-1}	2	Sal
23	16	Sal	7	10^{-4}	3	10^{-1}	4	10^{-3}	3	10^{-2}	6	10^{-6}	2	10^{0}	4	10^{-5}	3	Sal

T: number of sessions required to reach criterion of seven out of eight correct choices summing the first four choices over two consecutive sessions.

D1-D9: injection following scopolamine (Scop; 0.1 mg/kg): either saline (Sal) or a dose of 3,4-DAP (mg/kg).

pressed in nmol of acetylcholine formed per mg of protein per hour in each brain sample.

RESULTS

Experiment 1

Variables analyzed were working memory errors and reference memory errors. In each case, errors were summed over the two drug sessions or two criterion sessions. The purpose of conducting scopolamine followed by saline sessions at the beginning and end of the drug series was to assess the effects of scopolamine alone on memory and to assess the possibility that the response to scopolamine changed with repeated testing. As shown in Fig. 1 (top), it appeared that the effects of scopolamine on working memory errors were greater at the end of drug testing whereas the effects on reference memory errors (Fig. 1, bottom) were about the same in the initial and final block. Comparing the two scopolamine sessions, *t*-tests for related measures revealed no significant differences in reference memory errors, $t(12) = 0.77$, $p > 0.05$, but a difference in working memory errors, $t(12) = 2.30$, $p < 0.05$. To assess the possibility that there were systematic changes in the effects of scopolamine on working and reference memory, errors over each of the nine pairs of scopolamine sessions, *disregarding dose* of 3,4-DAP, were analyzed with one-way repeated-measures analyses of variance (ANOVA) using Geisser-Greenhouse adjusted degrees of freedom for repeated measures to reduce type I errors related to violations of the sphericity assumption. Although the effects of scopolamine on working memory errors appeared to increase across blocks of sessions (Fig. 1, top) and effects on reference memory errors appeared to decrease (Fig. 1, bottom), results revealed no significant effect of drug session for working memory errors, $F(3.68, 44.17) < 1.0$, $p > 0.05$, or for reference memory errors, $F(3.41, 40.87) < 1.0$, $p > 0.05$. Because repeated dosing with scopolamine had no significant effect, data for the two scopolamine plus saline sessions were averaged across each of the dependent variables.

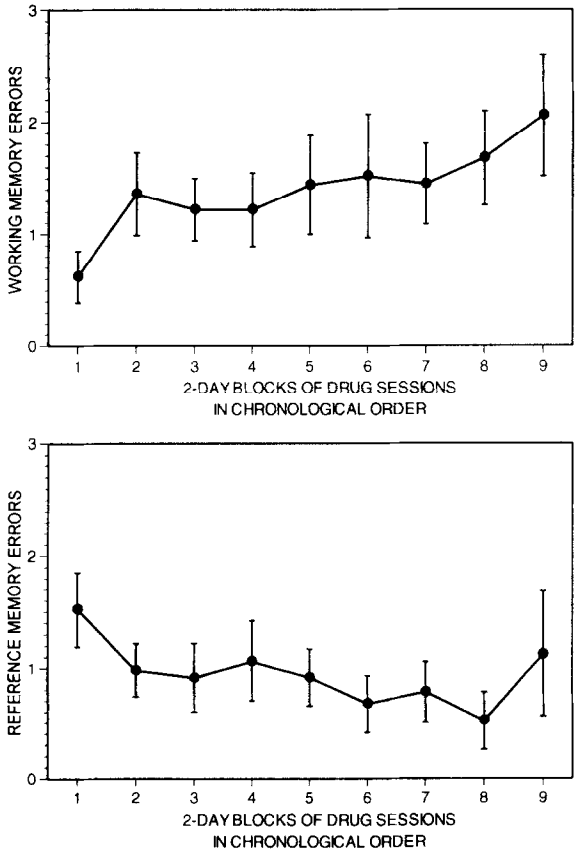

FIG. 1. Mean ± SEM working and reference memory errors summed over 2-day blocks of drug sessions with scopolamine (0.1 mg/kg) followed by saline (1, 9) or scopolamine followed by 3,4-DAP (2–8) in chronological order, disregarding dose of 3,4-DAP.

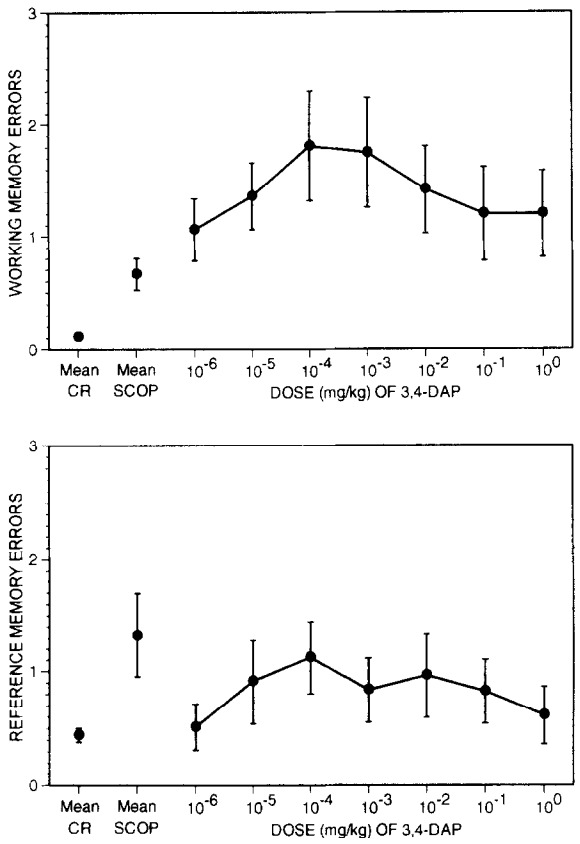

FIG. 2. Mean ± SEM working and reference memory errors summed over 2-day blocks of all criterion sessions (mean CR), the first and last scopolamine (0.1 mg/kg) plus saline sessions (mean SCOP), or the scopolamine plus 3,4-DAP sessions for each dose of 3,4-DAP.

To assess the effects of scopolamine on memory, error scores for the combined scopolamine plus saline sessions were compared to criterion sessions. Criterion error scores for each rat were computed by averaging errors across the nine pairs of criterion sessions (Table 1). Scopolamine appeared to impair both working and reference memory (Fig. 2). Results of *t*-tests for related measures revealed that scopolamine led to increases in working memory errors, $t(12) = 4.11$, $p < 0.01$, and reference memory errors, $t(12) = 2.39$, $p < 0.05$.

The possible ameliorative effects of 3,4-DAP on scopolamine-induced memory impairments were analyzed with the use of repeated measures one-way ANOVAs involving the mean criterion error scores, the combined scopolamine plus saline error scores, and the error scores from the pair of ses-

sions with each dose of 3,4-DAP (Fig. 2). The dose-effect profile of 3,4-DAP appeared to be an inverted U for both working and reference memory. For working memory, the middle doses (10^{-4}, 10^{-3} mg/kg) when combined with scopolamine appeared to further increase errors (Fig. 2, top). For reference memory, the lowest (10^{-6} mg/kg) and highest (10^{0} mg/kg) doses of 3,4-DAP appeared to improve memory (Fig. 2, bottom). However, results revealed no significant treatment effect for working memory errors, $F(4.61, 55.28) = 2.06$, $p > 0.05$, or reference memory errors, $F(4.30, 51.63) = 1.24$, $p > 0.05$. Thus, although scopolamine alone led to a memory impairment, scopolamine followed by 3,4-DAP failed to reverse this effect.

Experiment 2

Groups were matched on the number of days it took to acquire the task prior to surgery. Hence, the mean ± SEM number of days to criterion for the nondrug quinolinic acid (26.88 ± 2.9), physostigmine (28.80 ± 4.1), 3,4-DAP (28.66 ± 3.9), and sham (31.00 ± 2.8) groups were not significantly different, $F(3, 34) < 1.0, p > 0.05$.

To assess postoperative performance of the nondrug quinolinic acid and sham groups, a two-way ANOVA with one factor repeated (block) was performed for both working and reference memory errors (Fig. 3). As is evident (Fig. 3, top),

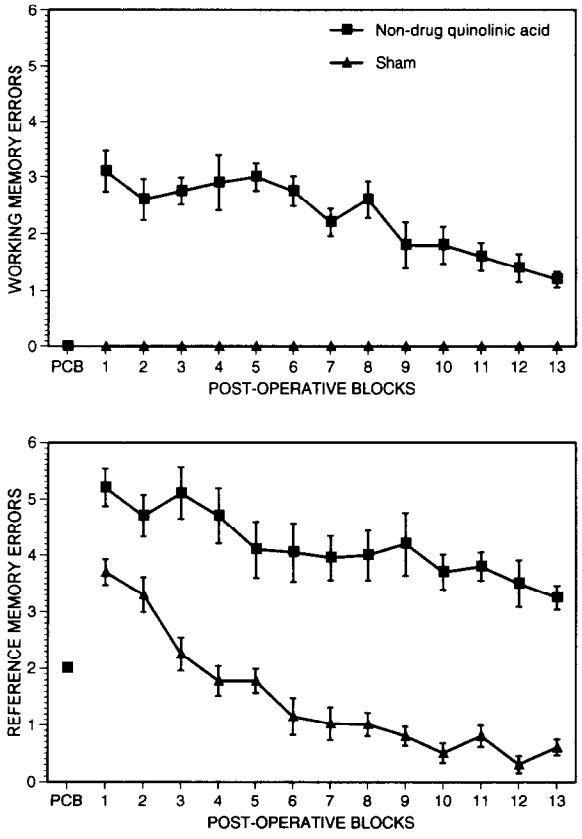

FIG. 3. Mean ± SEM total number of working (top) and reference (bottom) memory errors as a function of the preoperative criterion block (PCB) and postoperative blocks for the nondrug quinolinic acid and sham groups. Errors are based on the first four choices and summed over 4-day blocks.

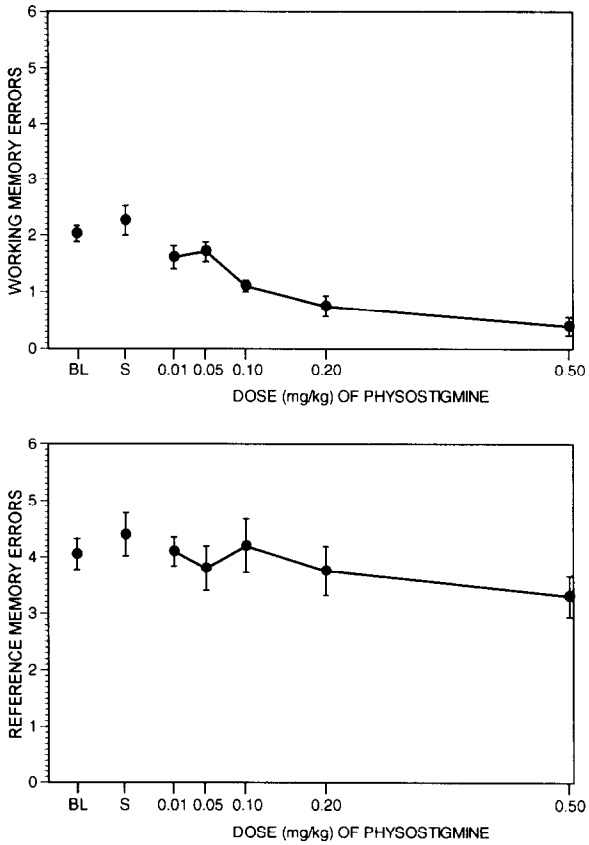

FIG. 4. Mean ± SEM total number of working (top) and reference (bottom) memory errors within the first four choices summed over 4 days at the various doses of physostigmine. Baseline (BL), saline (S).

only the quinolinic acid group made working memory errors. However, although the working memory performance of the nondrug quinolinic acid group was impaired relative to the sham controls, it showed improvement with repeated testing, $F(4.64, 37.13) = 4.88$, $p < 0.01$. For reference memory errors (Fig. 3, bottom), there were significant main effects of group, $F(1, 17) = 104.29$, $p < 0.001$, and block, $F(5.89, 100.05) = 16.55$, $p < 0.001$. The block by group interaction was not significant, $F(5.89, 100.05) = 2.17$, $p = 0.0532$. Inspection of Fig. 3 (bottom) suggests that the rate of improvement in the sham group was more rapid across blocks than in the lesion group. Tests of simple main effects of the interaction indicated that reference memory errors decreased for both the nondrug quinolinic acid, $F(12, 204) = 4.54$, $p < 0.0001$, and sham $F(12, 204) = 14.71$, $p < 0.0001$, groups. In summary, the analyses suggest that the quinolinic acid lesions impaired both the working and reference memory components of the radial maze task. However, with repeated testing these impairments diminished.

To assess drug effects, one-way repeated-measures ANOVAs were performed on the total number of working memory errors and the total number of reference memory errors. Error scores, based on the first four choices, were summed over 4 days. As can be seen in Fig. 4, physostigmine appeared to improve working memory in a dose-dependent manner while having little effect on reference memory. This was supported by statistical analyses revealing a selective de-

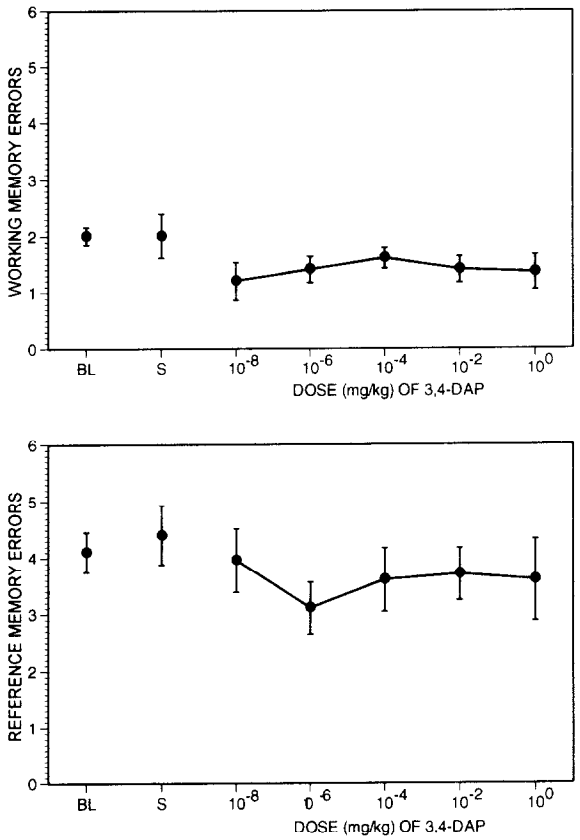

FIG. 5. Mean ± SEM total number of working (top) and reference (bottom) memory errors within the first four choices summed over 4 days at the various doses of 3,4-DAP. Baseline (BL), saline (S).

crease in working memory errors (Fig. 4, top) under physostigmine treatment, $F(2.84, 25.53) = 13.40$, $p < 0.0001$. Reference memory errors (Fig. 4, bottom), on the other hand, did not change significantly, $F(3.66, 32.98) = 1.85$, $p > 0.05$.

3,4-DAP appeared to have little effect on mnemonic performance of quinolinic acid lesion animals (Fig. 5). There were no significant decreases in either working memory errors, $F(3.49, 27.94) = 1.49$, $p > 0.05$, or reference memory errors, $F(3.50, 28.03) < 1.0$, $p > 0.05$.

The cortical ChAT assays were performed on average 12.8 weeks (± 0.15) postlesion. Table 2 shows the results of the ChAT assays. Percent decrease data were analyzed by a one-way ANOVA. The differences among groups were significant, $F(3, 34) = 25.42$, $p < 0.0001$. Post hoc multiple comparisons using the Dunnett test revealed that the three groups differed reliably from the sham group ($p < 0.001$).

DISCUSSION

The finding that treatment with a low dose (0.1 mg/kg) of scopolamine led to significant impairments of performance in the radial maze task is in agreement with our previous findings (46). However, the finding that both working and reference memory errors increased with scopolamine is not consistent with our results; thus, Wirsching et al. (46) found an increase in working, but not reference, memory errors with a dose of 0.1 mg/kg. It may be that the level of training prior to scopolamine tests differed in the two studies. That is, Wirsching et al. trained animals to a criterion of 4 consecutive days with a cumulative score of 14 or more out of 16 correct choices whereas the present study employed a more lenient 2-day criterion of seven out of eight. The level of training hypothesis is supported by previous studies showing that better trained habits are less susceptible to the disruptive effects of anticholinergics (18). As the experiment progressed, repeated training might have been expected to lead to an improvement in reference memory; in parallel, working memory errors might have been seen to increase over the course of training because the opportunity to make them was increased when reference memory errors decreased. This is exactly what Wirsching et al. (46) found (cf., their Fig. 1) and inspection of Fig. 1 here reveals that over time reference memory errors decreased slightly whereas working memory errors increased slightly. Although neither of these effects achieved statistical significance, the effects of the second scopolamine plus saline treatment on working memory were found to be significantly greater than the first. Thus, the present findings are in general agreement with our previous report (46) suggesting that low doses (0.1 mg/kg) of scopolamine have a relatively greater influence on working memory in well-trained animals. It should be noted that Lydon and Nakajima (31) specifically manipulated level of training prior to scopolamine tests in a partially baited radial maze; they found that well-trained animals were more likely to show an increase in reference memory errors when treated with scopolamine. However, they used a dose of 0.5 mg/kg. In previous studies we (46) and others (37,38,43) have found that doses greater than 0.4 mg/kg affect both working and reference memory.

Although it was not planned in Experiment 1, two doses of 3,4-DAP (10^{-4} and 10^{-6}) were evaluated most often in the last three test sessions (see Table 1). This raises the possibility that treatment effects of 3,4-DAP may be confounded with order

TABLE 2

MEAN LEVELS OF CORTICAL CHOLINE ACETYLTRANSFERASE ACTIVITY ON THE NONINJECTED AND INJECTED SIDE

Group	*n*	Noninjected Side	Injected Side	Decrease %
Nondrug quinolinic acid	9	34.1 ± 3.1	19.3 ± 2.3	44.0 ± 3.7
Physostigmine	10	37.9 ± 2.5	22.1 ± 1.3	40.3 ± 3.5
3,4-DAP	9	36.5 ± 3.4	20.8 ± 2.2	42.6 ± 3.8
Sham	10	28.8 ± 2.0	27.3 ± 1.6	3.0 ± 4.6

Choline acetyltransferase activity levels are expressed as mean ± SEM nmol acetylcholine formed per mg protein per h.

of treatments. However, as 3,4-DAP failed to produce significant treatment effects, it is unlikely that order of treatments influenced the results.

Although there was a small improvement in reference memory following the lowest (10^{-6} mg/kg) dose of 3,4-DAP in Experiment 1, a wide range of doses of 3,4-DAP failed to improve significantly the mnemonic performance of scopolamine-treated and nbm-lesioned rats. This finding is not in accord with previous reports showing that 4-AP enhanced recall of a passive avoidance response (27) and that 3,4-DAP improved age-related (40,41) and hypoxic-induced deficits in tight rope performance (24) as well as performance of aged rats in a fully baited eight-arm maze (16). Hypoxia, or low oxygen, is a condition that parallels some of the behavioral and cholinergic alterations that accompany normal aging (i.e., recent memory impairments, inability to perform certain cognitive and motor tasks, as well as impaired synthesis and release of acetylcholine) [cf. (40,41)] and thus has been used to test the effectiveness of cholinomimetics in reversing such deficits. The reasons for the discrepancy between the present results and those using a radial maze task with all arms baited (16) are unclear, especially as their dose was within the dose range employed here. Perhaps the general enhancement of neurotransmitter release produced by 3,4-DAP (25) improved the maze performance of aged rats through combined effects on cholinergic and other (e.g., noradrenergic, dopaminergic) transmitter systems because hypoxia and aging may decrease catecholamine and serotonin as well as acetylcholine (ACh) synthesis (17). Indeed, it has been shown that both cholinergic and noncholinergic cells are sensitive to 4-AP using in vitro brain slice preparations (26). Another possible reason for the discrepancy between present results and those of others is that the latter studies used tasks of a much simpler nature that were perhaps more amenable to amelioration by the aminopyridines (8).

Results provide further evidence that cholinergic neurons of the basal forebrain are involved in memory. This was indicated by a significantly greater disruptive effect on working than reference memory following unilateral quinolinic acid lesions of the nbm. More importantly, the present findings demonstrated that high doses of physostigmine (0.10, 0.20, and 0.50 mg/kg) selectively improved the working memory performance of lesion animals; reference memory was not reliably affected.

The ability of physostigmine to improve the radial maze performance of lesion animals substantiates previous reports showing that treatments that enhance ACh neurotransmission mitigate age-related (3) and scopolamine-induced (1) deficits in primates in the delayed matching-to-sample task and neurotoxin-induced deficits in rats in passive avoidance (27,36), T-maze (35), as well as radial maze tasks (42). The physostigmine data also support the notion that the lesion-induced mnemonic deficits resulted from damage to cholinergic nbm neurons as opposed to damage of noncholinergic neurons in the same region. It does not, however, agree with Murray and Fibiger's (34) report that physostigmine ameliorated the reference memory deficits induced by bilateral ibotenic acid nbm lesions in rats tested in a partially baited 16-arm maze. However, in that study animals were not pretrained and were already showing an improvement in performance when physostigmine was administered at the end of the acquisition period, thereby making an interpretation of the results and a comparison with the present study difficult. Nonetheless, it is worth noting that a slight improvement of reference memory, although not significant, was noted at the lowest dose of 3,4-DAP (Fig. 2).

The present results provide further support for the cholinergic hypothesis of memory. This hypothesis has been challenged recently by the finding of a dissociation between mnemonic deficits vs. cortical ChAT depletion following excitotoxic lesions of the nbm (21,22,33,44). However, we have found recently in biochemical studies that different excitotoxins, when injected into the nbm, differentially affect cholinergic projections to the cortex and amygdala (10,11). In behavioral studies, we have shown that excitotoxins (e.g., ibotenate, phthalate) that produce large decreases in amygdaloid ChAT produce large mnemonic deficits whereas those (e.g., quisqualate) that produce relatively small decreases in amygdaloid ChAT affect memory less (7,32). Thus, there appears to be good evidence for a role for brain ACh in memory and basoamygdaloid cholinergic neurons appear to play a potentially important role. Although the present results provide no evidence that the ACh release-enhancer 3,4-DAP improves memory, these findings continue to encourage the search for agents that will improve cholinergic neuronal function and memory.

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