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Assessment of a Cholinergic Contribution to Chlordiazepoxide-Induced Deficits of Place Learning in the Morris Water Maze

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McNAMARA, R. K. AND R. W. SKELTON. *Assessment of a cholinergic contribution to chlordiazepoxide-induced deficits of place learning in the Morris water maze.* PHARMACOL BIOCHEM BEHAV 41(3) 529–538, 1992.—This investigation sought to characterize the interaction between benzodiazepine and cholinergic systems in place learning in the Morris water maze. In the first experiment, rats were treated with scopolamine (1 mg/kg) alone or concomitantly with one of two doses of flumazenil (15 and 30 mg/kg) or with chlordiazepoxide (5 mg/kg) alone or concomitantly with flumazenil (15 mg/kg). Chlordiazepoxide and scopolamine severely impaired place learning but not cue learning. The low dose of flumazenil completely reversed the impairment produced by chlordiazepoxide and both high and low doses of flumazenil attenuated the place learning deficit produced by scopolamine. Neither dose of flumazenil affected place learning when administered alone. In the second experiment, rats were administered chlordiazepoxide (5 mg/kg) or scopolamine (1 mg/kg) alone or concomitantly with one of four doses of physostigmine (0.05, 0.10, 0.25, and 0.5 mg/kg). Once again, both chlordiazepoxide and scopolamine impaired place but not cue learning. Physostigmine reversed the impairment produced by scopolamine in a dose-dependent manner but failed at every dose to attenuate the impairment produced by chlordiazepoxide. The higher doses of physostigmine impaired place learning when administered alone. None of the drug treatments impaired cue learning. Together, these results suggest that the scopolamine-induced impairment of place learning is due to an increase in benzodiazepine/GABA activity, and contradict the notion that benzodiazepines impair memory by cholinergic mechanisms.

Chlordiazepoxide Scopolamine Physostigmine Flumazenil Place learning Cue learning
Morris water maze Benzodiazepines Acetylcholine (ACh) Antagonism Rat

A considerable body of research has implicated acetylcholine (ACh) as an important neurotransmitter in learning and memory processes. A major catalyst for this effort was the finding that patients with Alzheimer's disease show a marked depletion of choline acetyltransferase in several cortical areas, as well as cell loss in the nucleus basalis, the source of forebrain ACh [see (10) for a review]. These findings led to the "cholinergic hypothesis" of geriatric memory disorders [e.g., (2)] and to several psychopharmacological models of ACh hypofunction. For example, ACh receptor (muscarinic) antagonists have been found to produce mnemonic deficits in normal human subjects (12,15,16), nonhuman primates (45), and rodents (21). In the Morris water maze, an aversively motivated spatial memory task (37), cholinergic blockade impairs place learning while sparing retention of a previously acquired location and simple associative learning (51). Place learning is also impaired by lesions of either the medial septum, the primary source of hippocampal ACh (22,26,36), or the nucleus basalis (36,52). Together, these findings show that forebrain and hippocampal ACh are important for mnemonic processes and that the Morris water maze is sensitive to the cognitive deficits associated with ACh hypofunction in the rat.

Similar to ACh antagonists, benzodiazepine (BZ) agonists, such as diazepam and chlordiazepoxide, impair learning and memory processes in normal humans (18), nonhuman primates (45), and rodents (9). In the Morris water maze, BZ's impair place learning but not retention or simple associative learning (32,33). Indeed, ACh blockers and BZ agonists produce qualitatively similar impairments in the Morris water maze (33), suggesting that both agents may act upon the same circuit.

There is neurochemical and behavioral evidence that BZ/$GABA_A$ agonists inhibit ACh systems [see (47) for a review]. Neurochemical studies have shown that: 1) the systemic administration of BZ/$GABA_A$ agonists reduce ACh activity in the hippocampus and forebrain region, as determined by high-affinity choline uptake (35,42) and ACh turnover rates (55,56); 2) infusions of BZ/$GABA_A$ agonists into the medial

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septum reduce (4,11,53), while the $GABA_A$ antagonist bicuculline increases (57), ACh activity in the hippocampus, whereas infusions of $BZ/GABA_A$ agonists into the nucleus basalis region reduce forebrain ACh turnover (7,50,53,54); and 3) BZ agonists decrease muscarinic binding affinity and capacity (36). Behavioral studies have shown that: 1) ACh blockers and $BZ/GABA_A$ agonists produce a similar pattern of memory impairment [as above; (12)], 2) infusions of $BZ/GABA_A$ agonists into either the medial septum (5,8,19) or nucleus basalis (14,30) impair acquisition in rats, and 3) BZ antagonists attenuate scopolamine-induced acquisition deficits on passive avoidance (25,29) and spontaneous alternation tasks (46). Together, these results suggest that $BZ/GABA_A$ agonists inhibit the release of ACh, which in turn results in an impairment of mnemonic processes (47).

Alternatively, there is also neurochemical evidence that ACh activity inhibits GABA release. For example, ACh disinhibits neurons in the dorsolateral septal nucleus (23) and dorsal hippocampus in a manner comparable to $GABA_A$ antagonists (28). This disinhibitory action of ACh (23,44) is blocked by pirenzepine, a selective muscarinic M_1 receptor blocker, atropine, and scopolamine, nonspecific M_1 and M_2 receptor blockers. Since ACh does not affect the postsynaptic inhibitory actions of GABA (3), it appears that ACh inhibits the release of GABA from presynaptic terminals. Together, these results suggest that ACh reduces GABA release via muscarinic receptors located on the presynaptic terminals of inhibitory interneurons.

The present experiments sought to better characterize the interaction between $BZ/GABA_A$ and ACh systems in place learning in the Morris water maze. In Experiment 1, the effects of flumazenil (Ro 15-1788), a selective BZ receptor antagonist, on chlordiazepoxide- and scopolamine-induced impairments of place learning were assessed. In Experiment 2, the effects of physostigmine, an acetylcholinesterase inhibitor, on chlordiazepoxide- and scopolamine-induced impairments of place learning were assessed.

EXPERIMENT 1

METHOD

Animals

Forty-six male Long-Evans rats (Charles-River, Quebec, Canada) served as subjects. They were housed in pairs and maintained on a 12 L: 12 D cycle. All testing was conducted during the light phase of the cycle. Rats weighed 350–450 g at the beginning of testing. Food and water were available ad lib.

Apparatus and Procedure

The Morris water maze (37) consisted of a circular pool (diameter: 150 cm, height: 45 cm), with a featureless white inner surface. The pool was filled to a height of 25 cm with 22°C (±1°C) water, in which 1500 ml powdered skim milk was dissolved. The hidden escape platform was a clear Plexiglas stand (13 × 13 cm) submerged 2 cm below the water surface so that it was invisible at water level. The visible platform was a black stand (13 × 13 cm) that protruded 5 cm above the surface of the water.

During initial acquisition, the submerged escape platform was located in the center of the northwest quadrant. All groups were given four trials each day for six consecutive days. For each trial, the rat was placed in the water facing the pool wall at one of four randomly determined starting locations (north, south, east, or west pole). During each trial, the rat's swim path, drawn on a map of the pool, and escape latency, measured with a stop watch, were recorded. Once the rat located the platform, it was permitted to remain on it for 10 s. If the rat did not locate the platform within 60 s, it was placed on the platform for 10 s. After each trial, the rat was returned to a holding cage positioned 90 cm under a 250-W brooding lamp (for warmth) and allowed to remain there for the intertrial interval, approximately 4 min.

On the day following the last day of training, a drug-free probe trial was given to assess the strength and accuracy of initial learning, as well as the nature of the strategy adopted by the rat to locate the platform. Rats were required to swim in the pool without an escape platform for 60 s. All rats were released from the southern pole and the distance spent in each quadrant was recorded. The following day a cue task was given to assess simple associative learning; drugged rats were required to navigate to a visible platform located in a different quadrant on each trial (to prevent the accurate use of extramaze cues). Swim path lengths and latencies were recorded.

Drugs and Group Assignment

Rats were divided into one of the following eight treatment groups. The first group received chlordiazepoxide hydrochloride + saline (n = 5; 5 mg/kg; dissolved in 0.9% NaCl; Hoffmann La Roche, Inc.). The second group received scopolamine hydrobromide + saline (n = 5; 1 mg/kg; dissolved in 0.9% NaCl; Sigma Chemical Co.). The third and fourth groups received one of two doses of flumazenil (n = 5/group; 15 or 30 mg/kg; suspended in 0.9% NaCl with a drop of Tween 80; Hoffmann La Roche, Inc.). The fifth group received chlordiazepoxide hydrochloride (5 mg/kg) + 15 mg/kg flumazenil (n = 5). The sixth and seventh groups received scopolamine hydrobromide (1 mg/kg) + 15 mg/kg flumazenil (n = 8) or 30 mg/kg flumazenil (n = 8). The eighth group received saline (n = 5; 1 mg/ml; 0.9% NaCl) and served as controls. All injections were administered in a volume of 1 ml/kg and administered in the rat's home cage. Chlordiazepoxide, scopolamine, and saline were administered 30 min prior to testing and flumazenil was administered 15 min prior to testing.

Data Analysis

Escape latencies and swim path lengths were assessed using an analysis of variance (ANOVA) procedure with repeated measures. Posthoc comparisons were assessed using Tukey's (HSD) method. In every case, the acceptable level for statistical significance was $p < 0.05$.

RESULTS

Chlordiazepoxide increased the distances required by rats to locate the submerged platform. The low dose of flumazenil (15 mg/kg) reversed this deficit while having little effect when administered alone (Fig. 1A). The high does of flumazenil (30 mg/kg) also had no effect when administered alone (Fig. 1A). Scopolamine drastically increased the distance taken to reach the submerged platform (Fig. 1B). Both doses of flumazenil attenuated, but did not reverse, the distance increase produced by scopolamine (Fig. 1B). An overall ANOVA on the swim path lengths of groups treated with chlordiazepoxide and flumazenil or flumazenil alone revealed a significant group dif-

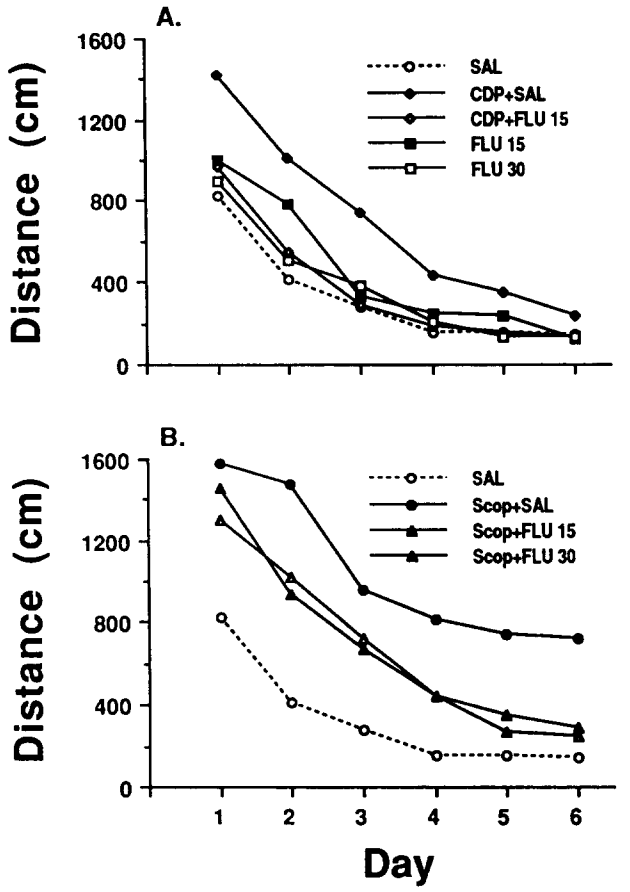

FIG. 1. Effects of (A) chlordiazepoxide and flumazenil and (B) scopolamine and flumazenil on the distance taken to locate the submerged platform over the 6 days of training. Note that the low dose of flumazenil reversed the place learning deficit produced by chlordiazepoxide, while both doses of flumazenil attenuated the place learning deficit produced by scopolamine. SAL, saline; CDP, chlordiazepoxide; FLU, flumazenil; Scop, scopoloamine; numbers, drug dose in mg/kg.

ference, $F(4,95) = 9.17$, $p < 0.001$, day difference, $F(5,475) = 84.3$, $p < 0.001$, but not a significant interaction between groups and day, $F(20,475) = 1.30$, $p = 0.19$. Posthoc tests revealed that rats treated with chlordiazepoxide had significantly longer swim paths relative to both controls ($p < 0.01$) and rats treated with chlordiazepoxide + flumazenil ($p < 0.01$). The swim path lengths of controls and rats treated with chlordiazepoxide + flumazenil did not differ significantly ($p > 0.05$). An overall ANOVA on the swim path lengths of groups treated with scopolamine and flumazenil revealed a significant group difference, $F(3,96) = 33.38$, $p < 0.001$, day difference, $F(5,480) = 61.4$, $p < 0.001$, but not a significant interaction between groups and day, $F(15,480) = 1.27$, $p = 0.22$. Posthoc tests revealed that rats treated with scopolamine, scopolamine + flumazenil (15 mg/kg), and scopolamine + flumazenil (30 mg/kg) had significantly shorter swim paths relative to rats treated with scopolamine alone ($p < 0.01$) but still had significantly longer swim paths relative to controls ($p < 0.01$). Latency data (not shown) showed the same pattern of impairments and statistical significances.

Data from the drug-free probe trial confirmed the pattern of impairments found during training. Rats treated with saline demonstrated a significant bias for the quadrant that had contained the platform during training [$p < .0.01$, relative to chance (25%); Fig. 2], revealing their accurate knowledge of the platform's location and their use of a spatial strategy. Rats treated with chlordiazepoxide failed to demonstrate a quadrant bias, revealing their failure to accurately learn the platform's location in space, while rats treated with chlordiazepoxide + flumazenil, or either dose of flumazenil alone, demonstrated a significant bias for the correct quadrant. Rats treated with scopolamine alone failed to demonstrate a bias for the correct quadrant while rats treated with scopolamine and either dose of flumazenil did show a bias for the correct quadrant ($p < 0.01$). The quadrant bias demonstrated by rats treated with scopolamine plus the high dose of flumazenil (30 mg/kg) was significantly less than the bias demonstrated by controls, showing that flumazenil attenuated but did not completely reverse the scopolamine-induced deficit.

Swim speed, averaged over the 6 days of training, was affected only by scopolamine (Fig. 3). Rats treated with chlordiazepoxide, chlordiazepoxide + flumazenil, or either dose of flumazenil swam at approximately the same speed as controls (28 ± 1.7 cm/s; Fig. 3A). Surprisingly, rats treated with

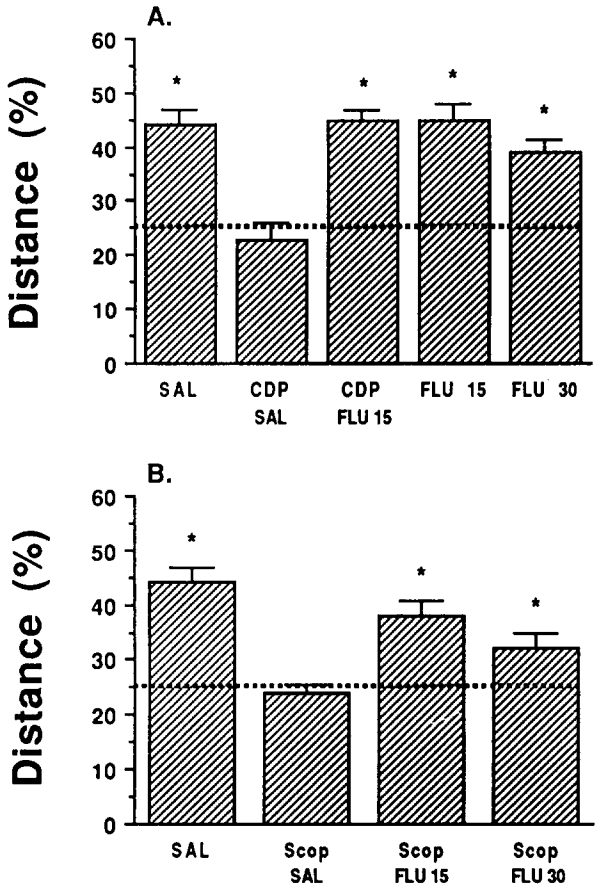

FIG. 2. Effects of (A) chlordiazepoxide and flumazenil and (B) scopolamine and flumazenil on the percentage distance spent in the correct quadrant during the 60-s probe trial. Note that rats treated with scopolamine plus either dose of flumazenil demonstrate a bias for the quadrant that had contained the platform. *$p < 0.01$ compared to chance level (25%) represented by dotted line.

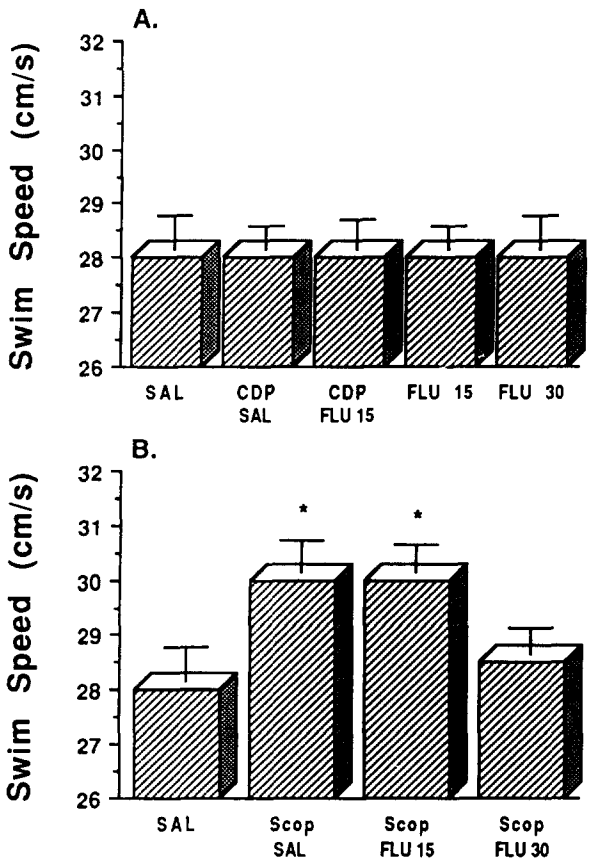

FIG. 3. Effects of (A) chlordiazepoxide and flumazenil and (B) scopolamine and flumazenil on swim speed averaged over the 6 days of training. Note that the increased swim speed produced by scopolamine is reversed by the high dose of flumazenil. *$p < 0.01$ compared to saline-treated controls.

scopolamine swam significantly faster than controls ($p < 0.01$; Fig. 3B), as did rats treated with scopolamine and the low dose of flumazenil (15 mg/kg; $p < 0.01$). The highest dose of flumazenil (30 mg/kg) reversed the increased swim speed produced by scopolamine.

None of the drug treatments resulted in a significant impairment of visible platform training (data not shown). Rats in each treatment learned on the first trial to escape to the visible platform and continued to swim directly to it on subsequent trials.

DISCUSSION

The results of Experiment 1 replicate previous findings that both chlordiazepoxide and scopolamine produce a severe impairment of place learning in the Morris water maze (32,33), as evidenced by longer distances taken to locate the submerged platform during training and a failure to show a quadrant bias during the probe trial. A novel finding of the present experiment was that flumazenil (15 mg/kg) completely reversed the impairment produced by chlordiazepoxide, suggesting that this deficit was mediated through endogenous BZ receptors. When administered alone, neither dose of flumazenil affected place learning. This latter result suggests either that the optimal performance by controls concealed an enhancing effect of flumazenil or that endogenous BZ ligands are not activated by the present procedures in a quantity detrimental to learning. The most important finding of Experiment 1 was that both doses of flumazenil attenuated the scopolamine-induced deficit, shortening the distance required to find the submerged platform and increasing the quadrant bias during the probe trial. The degree of attenuation produced by flumazenil was not dose dependent, suggesting that the lowest dose of flumazenil produced a maximal attenuation of the place learning deficit produced by scopolamine. Since only a portion of the scopolamine-induced deficit, albeit a significant one, was attenuated by both doses of flumazenil, it would appear that only a portion of the deficit was mediated by BZ/$GABA_A$ receptors.

The finding that BZ receptor antagonists attenuated the place learning deficit produced by scopolamine replicates findings in other learning paradigms (25,29,46) and suggests that BZ and ACh systems interact in a manner consequential to place learning. The nature of this interaction, however, is unknown. One possibility is that flumazenil increases arousal/vigilance (by preventing endogenous BZ activity), thereby overcoming the sedative effects of scopolamine [e.g., (34)]. This interpretation seems unlikely, however, since rats treated with scopolamine alone swam faster than controls, suggesting that scopolamine did not induce sedation or reduce motivation in the present paradigm. Further, the increased swim speed produced by scopolamine was antagonized (not enhanced) by the high dose of flumazenil. A second possible interpretation is that scopolamine blocked the inhibitory actions of ACh on BZ/$GABA_A$ systems, thereby increasing BZ/GABA-mediated inhibition (28). The resulting increase of endogenous BZ/GABA activity could be blocked by flumazenil. A third interpretation is that flumazenil blocked the inhibitory actions of BZ/$GABA_A$ receptors on cholinergic neurons, thereby disinhibiting ACh release, which then competed with scopolamine for receptor sites (47). The results of Experiment 1 support both of the latter two interpretations.

EXPERIMENT 2

The results of Experiment 1 confirm that BZ and ACh systems interact in some manner to impair place learning. However, the nature of this interaction remains uncertain. In Experiment 2, it was reasoned that if chlordiazepoxide and scopolamine both impair place learning by reducing ACh release, then prolonging ACh activity, by reducing its catabolism with physostigmine, should attenuate both impairments. Indeed, cholinesterase inhibitors attenuate place learning deficits produced by ACh depletion (20) and by lesions of the nucleus basalis (13,31,38,49) or medial septum (43). Alternatively, if ACh is inhibiting the presynaptic release of GABA, but not its postsynaptic actions, physostigmine would fail to block the place deficit produced by chlordiazepoxide but would attenuate the deficit produced by scopolamine. In Experiment 2, rats were treated with amnesic doses of either chlordiazepoxide or scopolamine, alone or in combination with one of four doses of physostigmine, and trained in the Morris water maze.

METHOD

Animals, Apparatus, and Procedure

Ninety naive, hooded, male rats of the Long-Evans strain served as subjects. Rats weighed between 350–450 g at the beginning of the experiment and food and water were available ad lib. Rats were maintained as described in Experiment

1. The same Morris water maze and procedures used in Experiment 1 were used in Experiment 2.

Drugs and Group Assignment

Prior to experimentation, rats were divided into 1 of 15 treatment groups. Five groups of rats were administered chlordiazepoxide hydrochloride (5 mg/kg; dissolved in 0.9% NaCl; Hoffmann-La Roche, Inc.) concomitantly with either saline (n = 10; 0.9% NaCl) or one of four doses of physostigmine hemisulfate (n = 5/group; 0.05, 0.10, 0.25, or 0.50 mg/kg; dissolved in saline; Sigma Chemical Co.). An additional five groups of rats were administered scopolamine hydrobromide (1 mg/kg; dissolved in saline; Sigma) concomitantly with saline (n = 10) or one of four doses of physostigmine hemisulfate (n = 5/group; 0.05, 0.10, 0.25, or 0.50 mg/kg). Another four groups of rats were administered one of four doses of physostigmine hemisulfate (n = 5/group; 0.05, 0.10. 0.25, and 0.50 mg/kg). The control group was administered saline (n = 10; 0.9% NaCl). All injections were administered in a volume of 1 ml/kg and administered in the rat's home cage. Chlordiazepoxide, scopolamine, and saline were administered 30 min prior to testing and physostigmine was administered 15 min prior to testing.

RESULTS

The effects of each drug treatment on the distance taken to reach the submerged platform are illustrated in Figs. 4 and 5. Saline-treated rats rapidly acquired the location of the submerged platform, reaching asymptotic levels by the fourth day of testing. As in Experiment 1, both chlordiazepoxide- and scopolamine-treated rats demonstrated severe place learning deficits, as evidenced by longer distances to locate the platform. Physostigmine failed to attenuate the place learning deficit produced by chlordiazepoxide at any dose (Figs. 4A and 5A). An overall ANOVA on the swim path lengths of groups treated with chlordiazepoxide and physostigmine revealed a significant group difference, $F(5,154) = 12.98$, $p < 0.001$, day difference, $F(5,770) = 74.61$, $p < .0.001$, and a significant interaction between groups and day, $F(25,770) = 2.13$, $p < 0.001$. Posthoc tests revealed that all rats treated with chlordiazepoxide, including those treated concomitantly with physostigmine, had significantly longer swim paths relative to saline-treated controls ($p < 0.01$). Further, physostigmine failed, at any dose, to reduce swim path lengths relative to rats treated with chlordiazepoxide alone ($p > 0.05$).

Physostigmine attenuated the place learning deficit produced by scopolamine in a U-shaped manner (see Fig. 5B). The smallest doses of physostigmine (0.05 and 0.10 mg/kg) attenuated the scopolamine deficit, while the 0.25-mg/kg dose of physostigmine completely reversed the deficit. The highest dose of physostigmine (0.50 mg/kg) failed to attenuate the scopolamine-induced deficit. An overall ANOVA on the swim path lengths of groups treated with scopolamine and physostigmine revealed a significant group differences, $F(5,154) = 24.02$, $p < 0.001$, day difference, $F(5,570) = 67.75$, $p < 0.001$, and a significant interaction between groups and day, $F(25,570) = 3.51$, $p < 0.001$. Posthoc tests revealed that rats treated with scopolamine or scopolamine + physostigmine (0.05, 0.10, and 0.50 mg/kg) had significantly longer swim paths relative to saline-treated controls ($p < 0.01$). Rats treated with scopolamine plus the second-highest dose of physostigmine (0.25 mg/kg) had swim distances comparable to controls ($p > 0.05$). Rats treated with scopolamine plus the two medium doses of physostigmine (0.10 and 0.25 mg/kg) had significantly shorter swim path lengths relative to rats treated with scopolamine + saline ($p < 0.01$). Latency data (not shown) followed the same pattern of deficits and statistical significances.

The two largest doses of physostigmine (0.25 and 0.50 mg/kg) increased swim path lengths, while the two smallest doses (0.05 and 0.10 mg/kg) had little effect (Fig. 4C). An overall ANOVA on the swim path lengths of groups treated with physostigmine revealed a significant group difference,

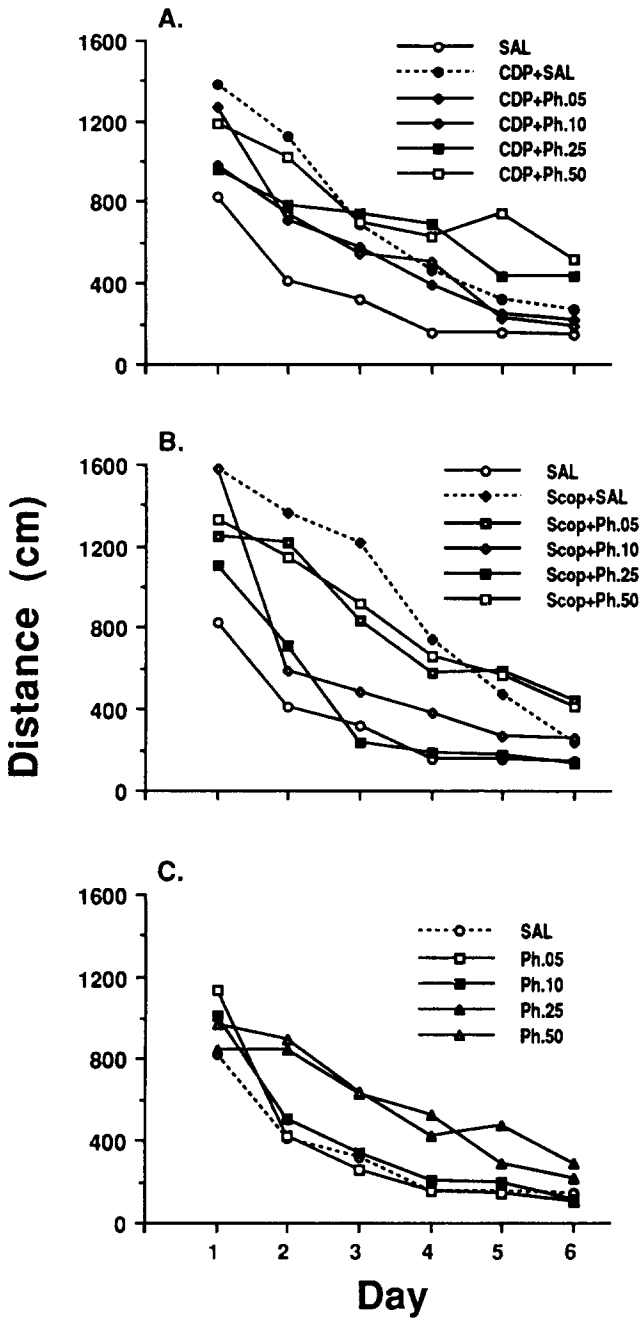

FIG. 4. Effects of (A) chlordiazepoxide and physostigmine, (B) scopolamine and physostigmine, and (C) physostigmine alone on the distance taken to locate the submerged platform over the 6 days of training. Ph, physostgimine.

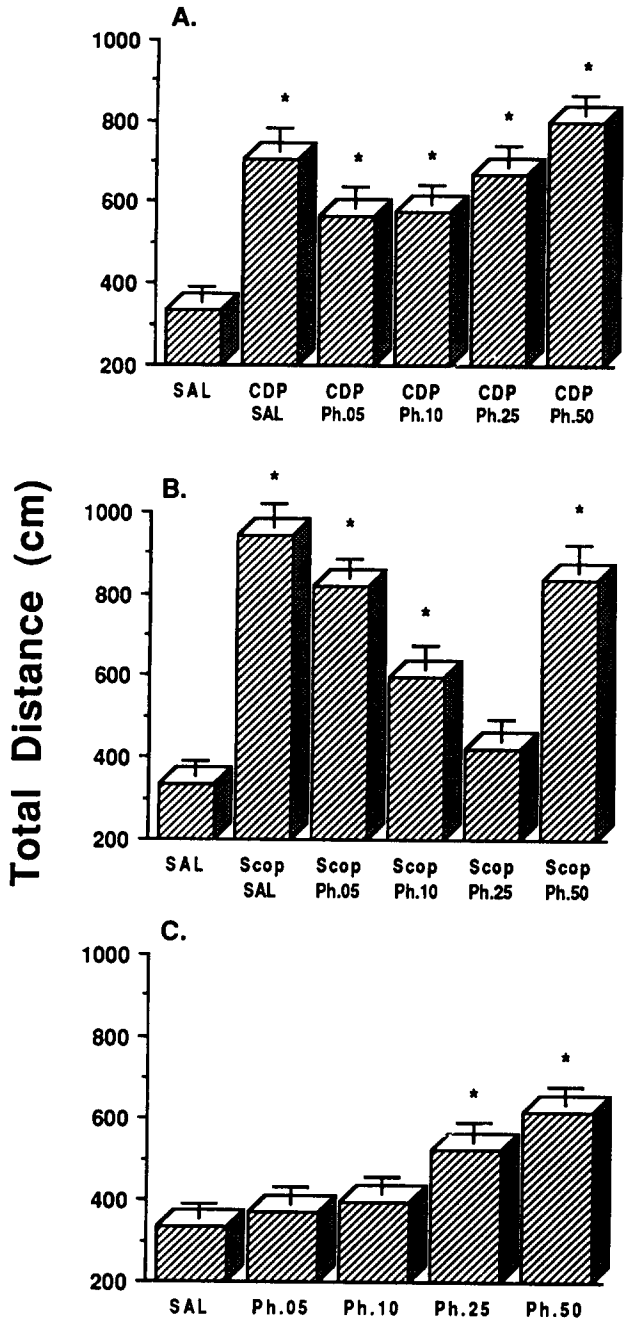

FIG. 5. Effects of (A) chlordiazepoxide and physostigmine, (B) scopolamine and physostigmine, or (C) physostigmine alone on the distance taken to locate the submerged platform averaged over the 6 days of training. Note: 1) the U-shape dose–response curve of distances taken by rats treated with scopolamine plus physostigmine and 2) the failure of physostigmine to significantly attenuate the place learning deficit produced by chlordiazepoxide. *$p < 0.01$ compared to control (SAL) levels.

$F(4,95) = 10.08$, $p < 0.001$, day difference, $F(5,475) = 61.30$, $p < 0.001$, and a significant interaction between groups and day, $F(20,475) = 2.23$, $p < 0.001$. Posthoc tests revealed that rats treated with the two largest doses of physostigmine had significantly longer swim paths relative to controls ($p < 0.01$).

The pattern of impairments observed during the drug-free probe trial tended to confirm the pattern of impairments observed during training (Fig. 6). Rats treated with saline demonstrated a robust bias for the correct quadrant [44%; $p < 0.01$ compared to chance (25%) levels; Fig. 6], indicating that they had acquired the spatial location of the platform. Conversely, rats treated with chlordiazepoxide or chlordiazepoxide + physostigmine (all doses) failed to show a quadrant bias (Fig. 6A), indicating that they had not acquired the spatial location of the platform. Rats treated with scopolamine + saline similarly failed to show a quadrant bias (Fig. 6B). Rats treated with scopolamine and the three highest doses of physostigmine showed a quadrant bias ($p < 0.01$), while rats treated with scopolamine and the lowest dose of physostigmine (0.05 mg/kg) failed to show a quadrant bias ($p > 0.05$). Rats treated with the three lower doses of physostigmine showed a quadrant bias ($p < 0.01$), while rats treated with the highest dose of physostigmine (0.50 mg/kg) did not (Fig. 6C).

Some of the drug treatments also affected motor and motivational performance as indexed by swim speed (Fig. 7). Rats treated with saline swam at an average speed of 28 ± 1 cm/s over the course of training. Rats treated with chlordiazepoxide swam at approximately the same speed as controls, although rats treated with chlordiazepoxide and the two highest doses of physostigmine swam significantly slower than controls ($p < 0.01$). Rats treated with scopolamine swam significantly faster than controls ($p < 0.01$), while rats treated with scopolamine and the two highest doses of physostigmine swam at the same speed as controls. Rats treated with the two highest doses of physostigmine alone (0.25 and 0.50 mg/kg) swam significantly slower than controls ($p < 0.01$).

Unlike the deficits seen during training with the submerged platform, none of the drug treatments produced a significant impairment of visible platform training (data not shown). Each treatment group learned to navigate to the platform within the first trial and continued to swim directly to it for the remainder of training.

DISCUSSION

The results of Experiment 2 demonstrate once again that chlordiazepoxide- and scopolamine-treated rats have severe place learning impairments. Although chlordiazepoxide- and scopolamine-treated rats eventually became proficient at locating the submerged escape platform, as evidenced by the gradual decrease in swim path lengths over training, it is unlikely that such a reduction reflected acquisition of the spatial location of the platform since both scopolamine- and chlordiazepoxide-treated rats failed to show a preference for the correct quadrant during the subsequent probe trial. Rather, scopolamine- and chlordiazepoxide-treated rats appeared to adopt an efficient response strategy, such as swimming toward or away from a particular cue or circling the pool a particular distance from the wall. Indeed, the control-level performance on the visible platform task in Experiments 1 and 2 suggests that scopolamine-and chlordiazepoxide-treated rats are capable of accurately navigating to a single cue. Furthermore, good performance on the visible platform task and normal swim speeds suggests that scopolamine- and chlordiazepoxide-treated rats were motivated to escape from the water and did not suffer from sensorimotor impairments. Indeed, chlordiazepoxide-treated rats swam as fast as controls and scopolamine-treated rats actually swam faster than controls, suggesting that neither drug interfered with motorical proficiency or escape motivation.

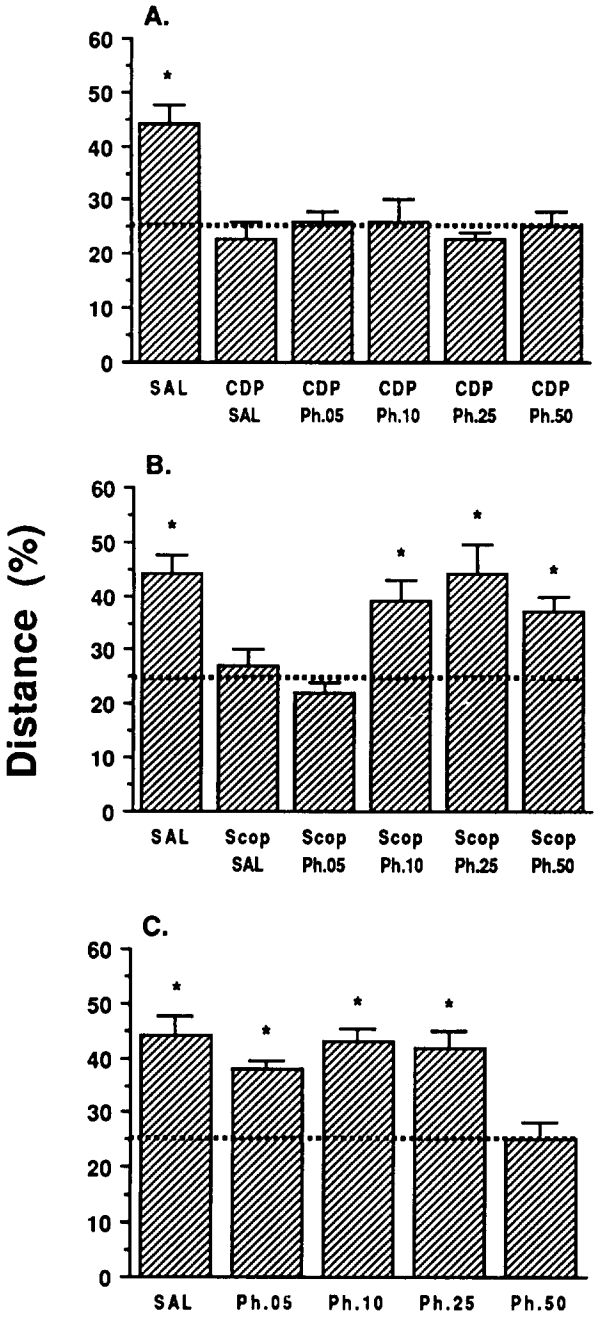

FIG. 6. Effects of (A) chlordiazepoxide and physostigmine, (B) scopolamine and physotigmine, and (C) physostigmine alone on the percentage distance spent in the correct quadrant during the 60-s probe trial. *$p < 0.01$ compared to chance level (25%) represented by dotted line.

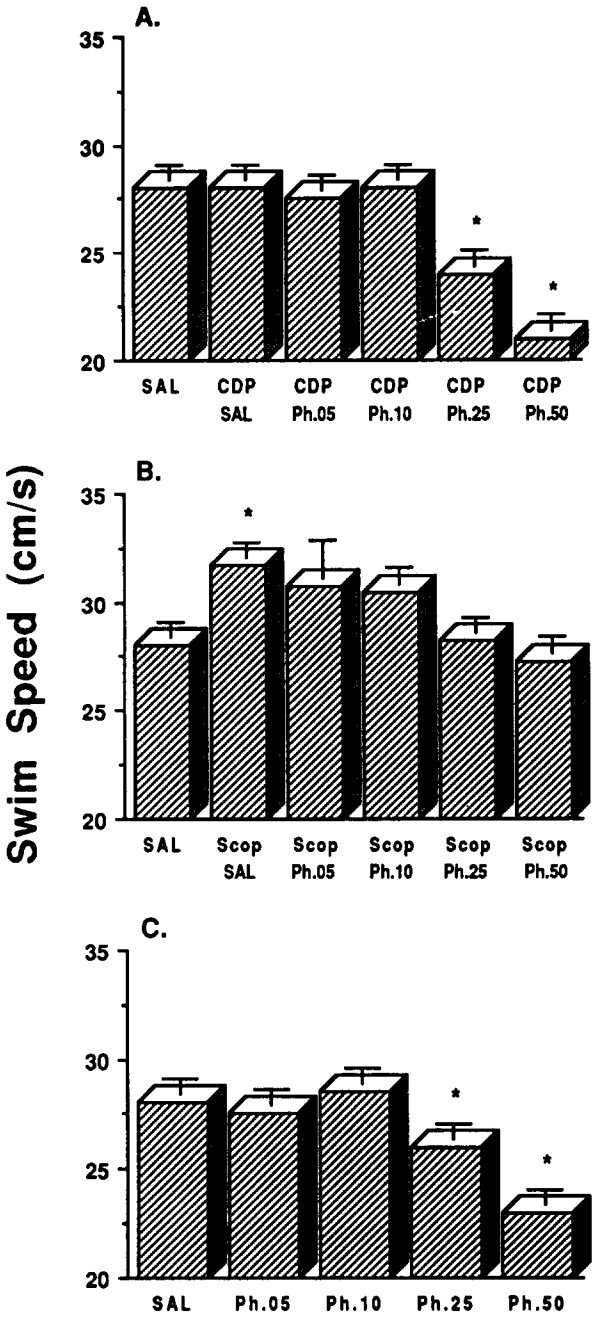

FIG. 7. Effects of (A) chlordiazepoxide and physostigmine, (B) scopolamine and physostigmine, and (C) physostigmine alone on swim speed averaged over the 6 days of training. *$p < 0.01$ compared to saline-treated controls.

Physostigmine also produced a dose-dependent impairment of place learning. Swim speeds of rats treated with the two highest doses of physostigmine were significantly reduced, suggesting that impairments of motorical proficiency may have contributed to the place learning deficit. Additionally or alternatively, it is possible that the higher doses of physostigmine impaired place learning by increasing synaptic ACh levels to the extent that presynaptic autoreceptors reduced subsequent ACh release (27). The latter actions would be expected to have the same consequences as scopolamine. A third alternative is that the high doses of physostigmine impaired place learning by overstimulating postsynaptic ACh receptors. Whatever the mechanism, the failure to observe a facilitatory effect with the low doses of physostigmine contrasts with previous reports [see (21) for review], while the impairment produced by the large dose is consistent with these previous reports.

Physostigmine attenuated the place learning deficit pro-

duced by scopolamine in a U-shaped manner, suggesting that there is an optimal level of ACh activity. Rats treated with scopolamine and the highest dose of physostigmine were impaired during initial training, but, surprisingly, demonstrated a quadrant bias during the probe trail, suggesting that these rats had adopted a spatial strategy. The slowed acquisition may have resulted from a nonspecific performance impairment produced by the high dose of physostigmine. Together, these findings are consistent with previous reports that mnemonic deficits produced by scopolamine in human subjects can be attenuated by physostigmine (17,34,40) and confirm that place learning deficits produced by ACh blockade can be overcome by prolonging ACh activity.

Physostigmine failed, at every dose, to attenuate the place learning deficits produced by chlordiazepoxide, a finding consistent with previous human studies in which memory deficits produced by BZ's were not attenuated by physostigmine (34,41). Rats treated with chlordiazepoxide and any of the doses of physostigmine demonstrated severe place learning deficits, as revealed by longer swim distances and a failure to demonstrate a quadrant bias. Given that place learning deficits produced by ACh blockade (present results), depletion (20), nucleus basalis lesions (13,31,38,49), and septal lesions (43) are attenuated by inhibiting cholinesterase, the failure of physostigmine to attenuate the place learning deficits produced by chlordiazepoxide suggests that the impairment is not mediated by GABA-induced reductions of ACh.

GENERAL DISCUSSION

The results of Experiments 1 and 2 demonstrate that the blockade of ACh receptors with scopolamine and activation of BZ receptors with chlordiazepoxide impairs place learning, but not cue learning, in the Morris water maze. Experiment 1 demonstrated that flumazenil, a BZ receptor blocker, could reverse the place learning deficit produced by both chlordiazepoxide and attenuate the deficit produced by scopolamine while failing to affect place or cue learning when given alone. Experiment 2 showed that physostigmine, in a dose-dependent manner, could attenuate the place learning deficit produced by scopolamine but not chlordiazepoxide and impair place learning at high doses. Together, these results suggest that both BZ and ACh systems are important modulators of rodent spatial memory and that a specific interaction between the two systems is responsible for the observed place learning deficits.

Recently, Sarter et al. (47) proposed that the cognitive decline associated with ACh hypofunction may be partly due to (or exacerbated by) tonic inhibition of surviving cholinergic cells by endogenous activity at the BZ/$GABA_A$ receptors on cholinergic neurons. Sarter et al. (47) further proposed that the disinhibition of these cholinergic neurons with the BZ antagonist/inverse agonist ZK 93 426 could ameliorate the cognitive deficit. BZ/$GABA_A$ receptor agonists are known to reduce hippocampal and forebrain ACh activity (35,42,55,56) and also impair place learning [present findings; (32,33)], but it is not clear that the ACh reduction causes the place learning deficit. According to the proposal of Sarter et al. (46), prolonging ACh activity with physostigmine should have compensated for chlordiazepoxide-induced ACh hypofunction and resulting place learning deficit, just as prolonging ACh activity compensates for memory deficits associated with ACh hypofunction produced by global (20) or selective hippocampal (43) or forebrain (13,31,38,49) ACh depletion. However, physostigmine failed to attenuate the chlordiazepoxide-induced deficit, thereby contradicting the notion that the place learning deficit was due to chlordiazepoxide-induced reductions of ACh activity.

The present results provide support for the alternative notion that the place learning deficits produced by ACh antagonists and BZ/$GABA_A$ agonists are both mediated by activity at BZ/$GABA_A$ receptors. One mechanism by which ACh is thought to produce excitation in the hippocampus is by inhibiting GABA release presynaptically while sparing the inhibitory postsynaptic actions of GABA (3,28). Thus, blocking the inhibitory actions of ACh on GABAergic neurons would increase the amount of GABA-mediated inhibition in a manner similar to activation of BZ/$GABA_A$ receptors (1,44). By this model, physostigmine would attenuate the scopolamine-induced disinhibition of GABA systems by restoring cholinergic inhibition of GABA release. However, physostigmine would not be expected to affect the actions of a BZ/$GABA_A$ receptor agonist like chlordiazepoxide since the inhibitory postsynaptic actions of GABA are not affected by ACh (3,28). However, flumazenil would be expected to block the actions of both chlordiazepoxide and scopolamine since both drugs act ultimately through the BZ/$GABA_A$ receptor. The results obtained in the present study argue in favor of the latter model.

One mechanism by which scopolamine and chlordiazepoxide may both be acting to impair place learning is by reducing the hippocampal excitability. Normal place learning requires the functioning of both the hippocampus (48) and the medial septum (22), the main source of hippocampal ACh. Scopolamine may reduce hippocampal activity by blocking the direct excitatory effects of tonic ACh activity on hippocampal neurons and the indirect effect mediated by presynaptic inhibition of GABA release, thereby mimicking medial septal lesions. Chlordiazepoxide would enhance GABA-mediated inhibition directly at BZ/$GABA_A$ receptors in the hippocampus. Thus, both chlordiazepoxide and scopolamine would reduce the excitability of hippocampal neurons to afferent input. Consistent with this interpretation are the findings that the induction of hippocampal long-term potentiation (a putative mnemonic mechanism) is blocked by both scopolamine (24) and the BZ agonist lorazepam (6).

In sum, the present investigation replicated previous observations that both scopolamine and chlordiazepoxide produce a severe impairment of place learning, but not cue learning, in the Morris water maze (32,33). It extended these results by showing that flumazenil attenuates the place learning deficits produced by both chlordiazepoxide and scopolamine, while physostigmine attenuates the place learning deficits produced by scopolamine but not chlordiazepoxide. Together, these results support the notion that the scopolamine-induced deficit in place learning is due to the disinhibition of GABA release and contradict the notion that the BZ-induced impairment of place learning is mediated by cholinergic systems. It is proposed that cholinergic systems act at least partially through BZ/$GABA_A$ systems, possibly in the septo-hippocampal system, by inhibiting the release of endogenous GABA to permit mnemonic-related activity.

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