Pharmacology Biochemistry and Behavior, Vol. 44, pp. 37–43, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Linopirdine (DuP996) Facilitates the Retention of Avoidance Training and Improves Performance of Septal-Lesioned Rats in the Water Maze

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Received 28 April 1992

BRIONI, J. D., P. CURZON, M. J. BUCKLEY, S. P. ARNERIC AND M. W. DECKER. *Linopirdine (DuP996) facilitates the retention of avoidance training and improves performance of septal-lesioned rats in the water maze.* PHARMACOL BIOCHEM BEHAV **44**(1) 37–43, 1993.—The behavioral effects of 3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one [linopirdine (DuP996)] were investigated on retention of the inhibitory avoidance test in normal mice and acquisition of spatial discrimination in the two-platform water maze task in septal-lesioned rats (a model of cholinergic dysfunction characteristic of Alzheimer's disease). Linopirdine significantly enhanced retention of the inhibitory avoidance response in mice (0.026 μmol/kg) and also reduced the number of errors made by septal-lesioned rats in the water maze to a level comparable to sham-operated animals. At this dose, no effects were observed on septal-lesion-induced hyperactivity in an open field or in unoperated rats tested in the elevated plus-maze anxiolytic test. This study extends previous findings of facilitatory effects of linopirdine on memory and demonstrates improved spatial learning in septal-lesioned rats. As the facilitatory effects on memory are not accompanied by a reduction in the hyperactive state present in septal-lesioned animals, a dissociation between cognitive and noncognitive effects of linopirdine can be differentiated in septal-lesioned rats.

Linopirdine DuP996 Memory Spatial learning Alzheimer's disease

3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one [linopirdine (DuP996)] is a novel compound that facilitates the performance of experimental animals on memory tests. Linopirdine improves the acquisition and retention of the inhibitory avoidance (IA) response in the hypoxia- or CO_2-induced amnesia model in rats, enhances the acquisition of the active avoidance response in mice and rats, and increases the rate of acquisition of a lever-pressing response for food in rats (5,10). At the biochemical level, linopirdine enhances the release of acetylcholine, dopamine, and serotonin from rat striatal, hippocampal, and cortical slices, and it enhances stimulation-induced acetylcholine (ACh) release in vivo without increasing baseline levels of ACh release (18). It has recently been demonstrated that linopirdine binds to a novel receptor in the rat brain (25); binding to this novel site is specific, reversible, saturable, and potent (K_d = 19 nM). This compound does not have anticholinesterase activity and does not compete for the binding to muscarinic or nicotinic cholinergic receptors (18).

In Phase I clinical trials in healthy young volunteers, linopirdine induced significant changes in the electroencephalograph (EEG) consistent with an increase in vigilance (23), and it is currently being evaluated in Phase III clinical trials for the treatment of Alzheimer's disease (AD). To further characterize the behavioral actions of linopirdine at the experimental level, the effects of linopirdine were evaluated in the inhibitory avoidance in mice and in the two-platform spatial discrimination task in septal-lesioned rats, a lesion model of the cholinergic hypofunction characteristic of AD. To dissociate the cognitive and noncognitive effects of linopirdine, we also investigated its effect on locomotion in septal-lesioned and normal rats in the elevated plus-maze anxiolytic test.

METHOD

Mouse Studies

Animals. Male CD1 mice (weighing 25–30 g upon arrival) supplied by Charles River were used. They were housed in groups of 14 per cage with food and water available ad lib.

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Mice were acclimated to laboratory conditions for 1 week before the training session and maintained on a 12 L : 12 D cycle.

Locomotor activity. Mice were given saline or linopirdine (0.08, 0.26, 0.8, and 2.6 μmol/kg, IP) injections immediately before being placed in a 41 × 41-cm open field. Horizontal activity was measured in 10-min bins for 60 min using Digiscan activity monitors (Omnitech Electronics, Columbus, OH).

Inhibitory (passive) avoidance test. Mice were trained according to classic IA procedures (4) in an automated apparatus (San Diego Instruments, San Diego, CA). On the training day, each mouse was placed in the lighted compartment and after 10 s the door leading to the dark side was automatically opened. When the mouse crossed with all four feet into the dark side, the door closed and the mouse received a 0.35-mA foot-shock (2 s). The latency to step-through was recorded by the instrument. The mouse was then removed from the apparatus and returned to the home cage. Retention was evaluated 24 h later following a similar procedure. A maximum step-through latency of 300 s was recorded on the testing day. Drugs were administered IP 15 min before the training session. No drugs were injected on the test day.

Rat Studies

Animals. Male Wistar rats (250–275 g upon arrival) from Sasco Laboratories were used. They were individually housed and maintained on a 12 L:12 D schedule, with food and water available ad lib. They were acclimated to laboratory conditions for 1 week before the start of experiments.

Surgery. Medial septal lesions were produced in rats under pentobarbital anesthesia (55 mg/kg, IP). An electrode was inserted into the medial septum under stereotaxic control (0.5 mm anterior to bregma, 0.0 mm lateral to the midline, and 6.5 mm ventral to the skull surface) and radiofrequency current sufficient to maintain an electrode tip temperature of 63°C was passed for 60 s. Sham lesions were produced by lowering the electrode to a point 1 mm above the target location but passing no current. Animals were allowed to recover for at least 1 week before behavioral testing. Lesions were verified with standard histological techniques after the behavioral studies were completed.

Two-platform water maze. Medial septal-lesioned and sham-lesioned control rats were trained to discriminate two visible platforms in a water tank as previously described (8,17). A cylindrical water tank (60 cm high and 180 cm in diameter) was filled to a depth of 37 cm with 26 ± 1°C water rendered opaque by the addition of dry milk. Rats were initially trained to escape to a circular platform (13 cm in diameter) positioned so that its upper surface was 1.5 cm above the surface of the water. Four such trials, using four different start locations and four different platform locations, were conducted on each of 2 consecutive days. Three days later, spatial discrimination training was begun. For spatial discrimination training, two platforms were present in the pool. One of these platforms was that used during cue training and the other a similar looking platform constructed of expanded polystyrene. Thus, one of the platforms was stable and provided a means to escape while the other was unstable and sank when the rat climbed onto it. The platforms could only be distinguished by their spatial locations. The visible platforms remained in the same spatial locations throughout the spatial discrimination training. During this phase of training, each trial was initiated by placing the rat in the water at one of the two locations along the perimeter of the pool that were equidistant from the two visible platforms. The trial ended when the rat climbed onto the escape platform or did not escape within 60 s and was placed on the platform by the experimenter. At the end of each trial, the rat was allowed to remain on the platform for 20 s before being removed from the maze. Each time the rat contacted the incorrect platform with its head or forepaws, an error was recorded. Each rat received six training trials per day with three trials starting from each of the two start positions. During the first 4 days of spatial discrimination training, saline or linopirdine was administered IP 15 min before training. On the day following the fourth training session, a retention test was conducted using the same procedures used during training except no injection was administered.

Locomotor activity. Those rats that received saline treatment during training on the two-platform water maze were used in this experiment. Septal-lesioned rats and their corresponding sham-operated animals were injected IP with saline or linopirdine (0.026 μmol/kg) and 15 min later placed in a 41 × 41-cm open field. Locomotor activity was measured in 5-min bins for 30 min using Digiscan activity monitors (Omnitech). Each rat participated in two test sessions, one under saline and one under drug. The sessions were separated by 48 h and treatment was counterbalanced.

Elevated plus-maze. The procedure originally described by Pellow et al. was used with minor modifications (22). The apparatus consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) extending from a central platform (10 × 10 cm). It was mounted on a base raising 50 cm above the floor. Light levels on the open and enclosed arms were similar. Unoperated rats were injected IP with saline or the different drug doses and 15 min later submitted to the test. Rats were placed in the center of the maze and the following variables scored: a) time spent in the open arms; b) number of entries to the open arms; c) total distance traveled by the rat. These variables were automatically recorded by a camera mounted above the apparatus and analyzed by computer software (Videomex, Columbus Instrument, Columbus, OH). The test lasted 5 min. All animals used were naive to the apparatus.

Drugs

Linopirdine was kindly provided by DuPont-Merck Pharmaceutical Co. (Wilmington, DE). It was dissolved in 0.1 N

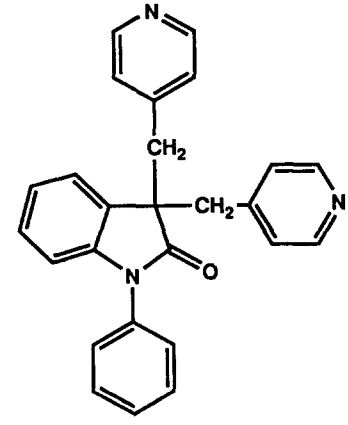

FIG. 1. Structure of linopirdine.

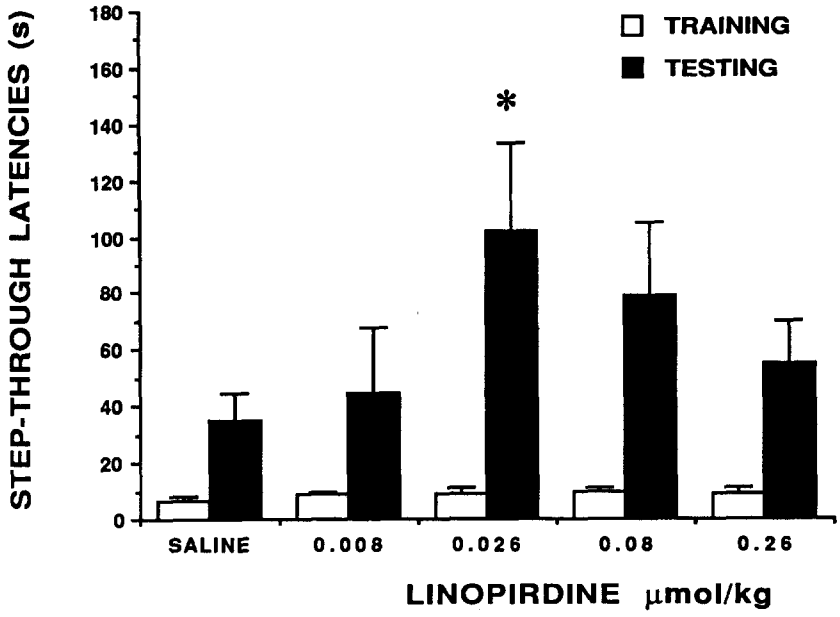

FIG. 2. Effect of linopirdine on the inhibitory avoidance in mice. Data represent the mean ± SEM latency to step-through on training and testing (n = 12 mice). The drug was injected IP 15 min before training. Control animals received a saline injection. *p = 0.03 compared to saline-treated animals (Mann-Whitney U-test).

HCl and diluted with saline solution to the desired concentrations.

Statistics

The mouse locomotor activity data and the rat water maze and elevated plus-maze data were analyzed by one- and two-way analysis of variance (ANOVA) followed by Fisher's probable least significant difference (PLSD) test for individual comparisons. Because rats served as their own controls in the locomotor activity experiment, these data were analyzed using a three-way, repeated-measures ANOVA with lesion as the between-group factor and time interval and drug dose as the repeated measures. Inhibitory avoidance data were analyzed nonparametrically using the Mann-Whitney U-test.

RESULTS

Figure 1 shows the structure of linopirdine. It is a novel compound with no structural similarity to nootropics like piracetam or aniracetam. Evaluation of the effect of linopirdine (0.08–2.6 μmol/kg) on the locomotor activity in mice revealed a small but significant reduction in horizontal activity during the 60-min observation period [drug × time interaction, $F(20, 290) = 2.4$, $p = 0.0008$] (data not shown). Based upon these findings, we decided to carry out the IA studies in a lower dose range. Figure 2 shows the facilitatory effect of linopirdine on the IA test in mice. Linopirdine enhanced retention and a significant effect was observed at the 0.026-μmol/kg dose ($U = 35$, $p = 0.03$). The drug did not affect the performance of animals on the training day as step-through latencies were similar among all groups of animals.

In the two-platform water maze, saline-treated septal-lesioned rats made significantly more errors than sham animals throughout the study, $F(1, 15) = 5.2$, $p = 0.04$ (Fig. 3). On day 1, there were no differences in the number of errors made by saline-treated sham or lesioned rats, $F(1, 15) = 0.02$, NS, but a significant difference between these groups became evident on the remaining 3 days of training, $F(1, 15) = 4.9$, $p = 0.04$. Administration of linopirdine (0.026 and 0.08 μmol/kg) to septal-lesioned rats significantly reduced the number of errors during the training period, $F(2, 22) = 3.4$, $p = 0.049$. A higher dose of linopirdine (0.26 μmol/kg) was also tested and found ineffective.

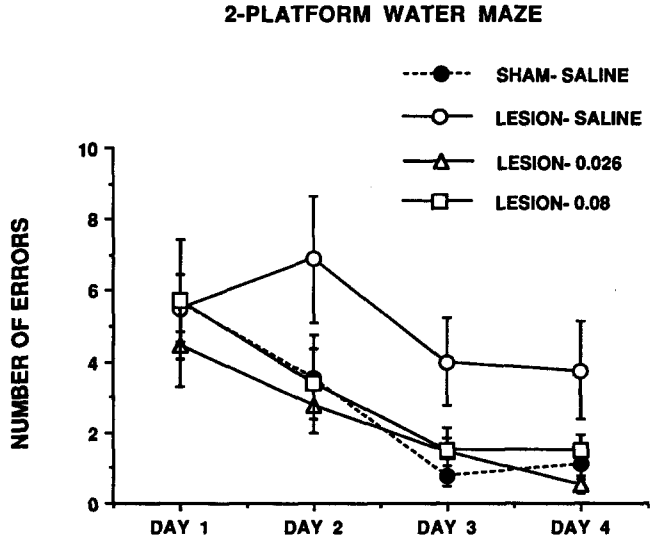

FIG. 3. Effect of linopirdine on acquisition of the spatial discrimination (two-platform) water maze in medial septal-lesioned rats (days 1–4). The drug was injected IP 15 min before the test. Eight to nine animals were included in each group.

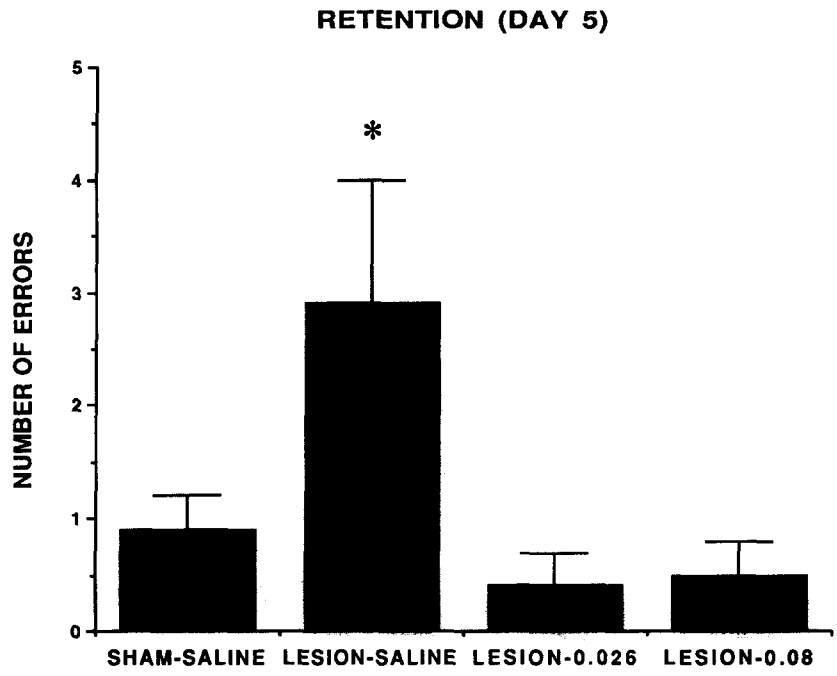

FIG. 4. Retention of the spatial discrimination task (day 5) 24 h after the 4-day training period. Animals received no drug injections before this test session. *$p < 0.05$ compared to the remaining three groups.

Figure 4 shows the performance of the different groups of animals 24 h after the training period. Animals received six trials to evaluate the retention of the spatial task, and no drug was administered before the test. Saline-treated lesioned rats made significantly more errors than sham animals ($p < 0.05$ as compared to the remaining three groups), and the improved performance of lesioned rats that received linopirdine during training was still present on the retention day, $F(3, 30) = 3.5$, $p = 0.027$.

The improved water-maze performance observed in septal-lesioned rats given linopirdine could have been related to effects on noncognitive features of the septal syndrome (irritability, hyperactivity, etc.). To test this hypothesis, we assessed the effect of linopirdine on septal lesion-induced locomotor hyperactivity. Rats with septal lesions exhibited increased locomotor activity than corresponding sham-operated rats (Fig. 5), demonstrated by a significant time × lesion interaction, $F(5, 70) = 3.5$, $p = 0.007$. This hyperactivity of septal-lesioned rats was not modified by linopirdine at the 0.026-μmol/kg dose, which had been effective in the water maze.

The effect of linopirdine on a rodent test of anxiety was also evaluated in unoperated Wistar rats. Figure 6 shows that linopirdine did not modify the time spent by rats in the open arms, a measure of anxiolytic activity, $F(3, 25) = 0.9$, NS. Linopirdine also did not affect the number of entries to the open arm, $F(3, 25) = 0.6$, NS, or the total distance traveled by the rat, $F(3, 25) = 0.9$, NS.

DISCUSSION

The findings of this study provide additional evidence that linopirdine facilitates the performance of mice and rats in different tests that evaluate memory function. Linopirdine induced a dose-dependent facilitation of retention of the IA response in mice at doses lower than those that reduce locomotor activity in an open field. The latencies to step-through on the training day were similar between all groups, and as it has already been demonstrated that linopirdine does not alter foot-shock sensitivity (5) the increased latencies on the test day can be interpreted as an effect of linopirdine on associative

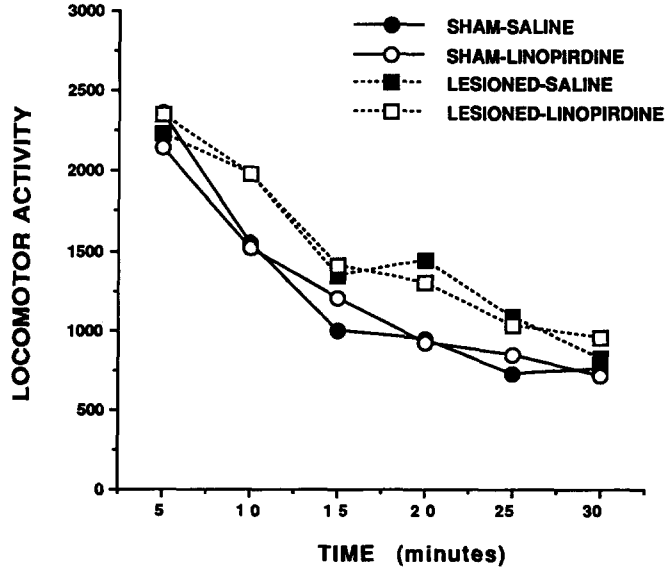

FIG. 5. Effect of linopirdine (0.026 μmol/kg) on locomotor activity in medial septal-lesioned and sham rats. The drug was injected IP 15 min before the test. Data represent the mean counts of seven rats.

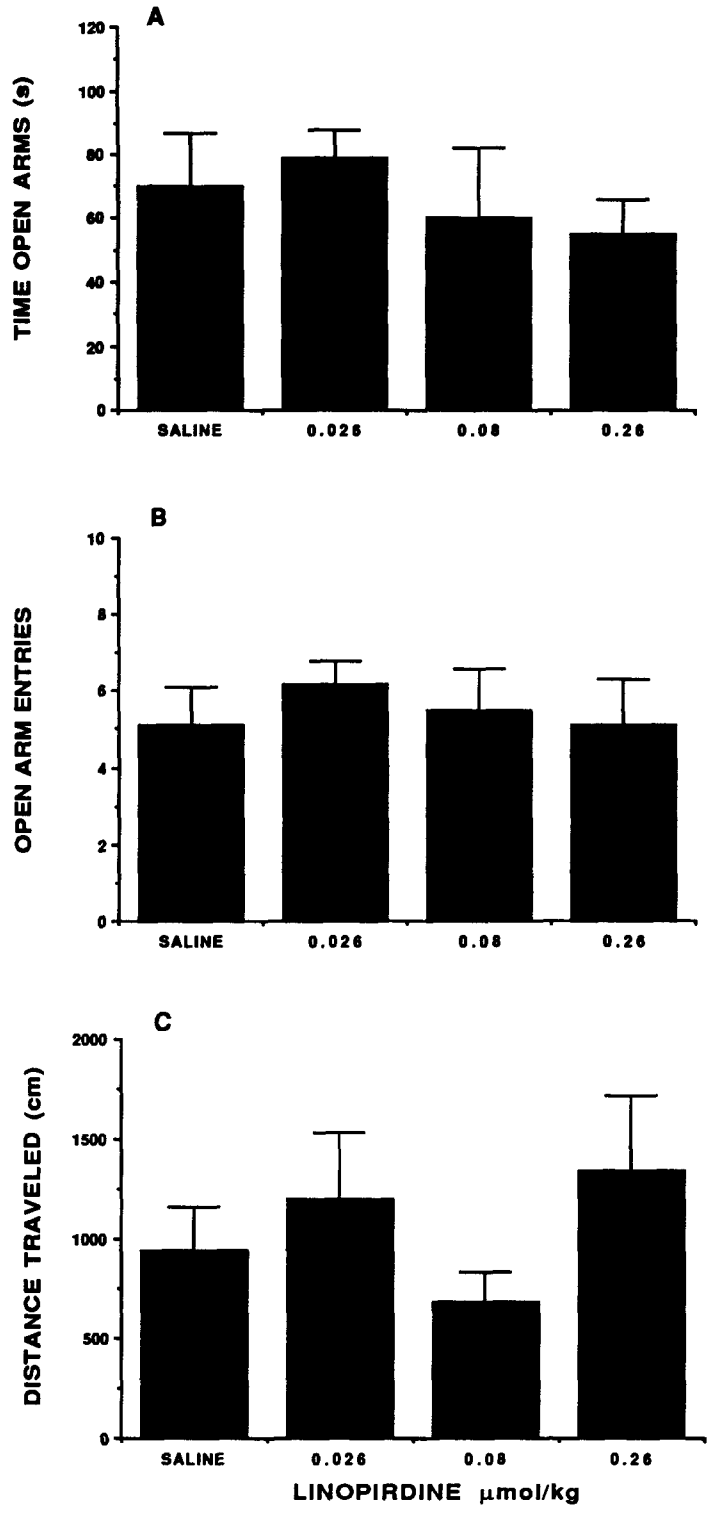

FIG. 6. Effect of linopirdine on the elevated plus-maze in unoperated rats. The drug was injected IP 15 min before the test. Data represent the mean ± SEM time spent by rats in the open arms (A), the number of entries to the open arms (B), and the total distance traveled during the 5-min test (C). Seven rats were included in each group.

processes. Our data are also in accordance with the effect and potency of linopirdine against hypoxia-induced amnesia in rats (10).

In the two-platform water maze test, linopirdine significantly reduced the number of errors exhibited by septal-lesioned rats at doses similar to those effective in the IA. Moreover, this improved performance was maintained during a subsequent retention test day when no additional drug was administered to animals. Thus, it would appear that information learned under linopirdine remains accessible when the drug is not present.

It has been suggested that the septal area is a central target for the action of different drugs that modulate spatial memory (1,2), and the demonstration of enhanced performance in rats with septal lesions is of particular importance given that the septal-lesioned rat model has been postulated as an animal model of the cholinergic deficiency found in AD (13,15). Cholinergic neurons in the nucleus basalis magnocellularis (NBM) and septum that provide input to the cortex, amygdala, and hippocampus are severely damaged in Alzheimer's patients (6). Lesions of these structures in experimental animals impair performance in a wide variety of memory tests (9,12,20), suggesting that damage to the NBM and/or the septum may play a role in producing memory impairments associated with AD. Although NBM lesions are more frequently used to model the memory deficits associated with AD, recent findings suggest that septal lesions may actually more accurately reflect the role of cholinergic dysfunction in memory deficits found in AD patients (11).

Septal lesions reduce both electrophysiological θ-rhythm and cholinergic markers in the hippocampus (8,26) and similarities between the behavioral effects of hippocampal and septal lesions suggest that disruption of septal input to the hippocampus mediates many of the behavioral effects of septal damage. Two important theories have been postulated to understand the role of the hippocampus in learning and memory. O'Keefe and Nadel emphasize that the rat constructs a spatial map using distal cues from the environment to reach its goal (19); the second theory emphasizes that the hippocampus is involved in working memory processes, a short-term memory that requires flexible stimulus–response associations irrespective of the type of information, spatial or nonspatial (21). As is true with hippocampal lesions, septal lesions disrupt performance of working memory tasks, as well as memory tasks that involve the acquisition of spatial information, such as the two-platform spatial discrimination task used in the present experiment (7).

In addition to effects on memory processing, septal lesions induce a variety of behavioral changes in the rat. Two of the most prominent noncognitive features of the "septal syndrome" are irritability and hyperactivity (12,24). Based upon pharmacological and behavioral data, Gray postulated that the septohippocampal circuitry is part of the behavioral inhibition system and is important in the control of emotional behavior (12). Thus, the improved water-maze performance observed in septal-lesioned rats given linopirdine could have been related to an effect of the drug on these noncognitive features of the septal syndrome. One situation in which the reduction in behavioral inhibition and increased reactivity characteristic of septal rats is in particular prominent is in open-field behavior. Hyperactivity in septal-lesioned rats appears to be related to a reduced rate of habituation to the novel environment (7,12). As in these previous reports, septal lesions reduced habituation of locomotor activity in the current experiment. However, a dose of linopirdine that enhanced water maze performance in septal animals was devoid of any effect in either septal- or sham-lesioned animals, suggesting that linopirdine did not improve water maze performance through some nonspecific, general effect on septal-lesion-induced hyperemotionality. Because only animals that previously had received saline treatment in the water maze were included in the locomotor activity experiment, our results cannot be attributed to the development of tolerance to linopirdine.

While open-field activity provides suggestive evidence regarding emotional behavior, it does not provide a specific test of emotionality. Thus, the anxiolytic effect of linopirdine using the elevated plus-maze in unoperated rats was determined. This procedure has been validated physiologically and pharmacologically as an appropriate test to detect anxiolytic drugs in rodents (14,16,22). Rats are allowed to explore an apparatus consisting of two open and two enclosed arms, and a conflict situation is generated as exposure to the open arms leads to an avoidance response stronger than that evoked by the enclosed arms. Classic anxiolytic agents (diazepam, ethanol, etc.) significantly increase the exploration of the open arms by the rat. Linopirdine did not affect either of the two measures of anxiolytic activity—time spent by rats in the open arms or number of arm entries; it also did not affect the locomotor activity of rats. These data demonstrate that these doses of linopirdine are devoid of anxiolytic properties in a standard test in rodents.

The mechanism of action by which linopirdine produces its cognitive-enhancing effects is unknown, but it should be noted that (-)nicotine, which like linopirdine releases several neurotransmitters, produces effects similar to those reported here in both IA and spatial discrimination (3,8). The facilitatory effect on release of acetylcholine, dopamine, and serotonin might be responsible of the cognitive effects of linopirdine, and studies with selective antagonists of each system will help us understand their relative role in cognition.

In summary, linopirdine facilitates the retention of the IA task in normal mice and reduces the number of errors made by septal-lesioned rats in the two-platform water maze. The effect of linopirdine on the performance of lesioned rats does not appear to be a result of an anxiolytic action or a reduction of the hyperactive state present in septal-lesioned rats but rather an effect on associative processes.

ACKNOWLEDGEMENTS

Linopirdine was kindly provided by Dr. Leonard Cook, DuPont-Merck Pharmaceutical Co.

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