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Milacemide Treatment in Mice Enhances Acquisition of a Morris-Type Water Maze Task

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FINKELSTEIN, J. E., J. M. HENGEMIHLE, D. K. INGRAM AND H. L. PETRI. *Milacemide treatment in mice enhances acquisition of a Morris-type water maze task.* PHARMACOL BIOCHEM BEHAV **49**(3) 707–710, 1994.—The *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor appears to be involved with processes of learning and memory. A neutral amino acid binding site is known to exist on the NMDA complex. Glycine binds with high affinity to this site and has been found to potentiate NMDA activity. 2-*N*-Pentylaminoacetamide HCl (milacemide) is a glycine agonist that has been found to enhance performance of rodents in passive and active avoidance tasks and has improved the performance of humans in several word retrieval tasks. We evaluated the effects of milacemide on the performance of male C57BL/6J mice in a complex spatial task, the Morris water maze. Because NMDA receptor activation appears involved in induction of long-term potentiation, it was hypothesized that milacemide administration would be involved in task acquisition. Therefore, mice were treated with either milacemide (10 mg/kg) or vehicle 1 h prior to training on each of 4 consecutive days. Results indicated that mice treated with milacemide learned the task significantly faster than controls over 4 days of training, as measured by mean distance (cm) to reach the goal platform. Therefore, agonism of the glycine site on the NMDA receptor appears to facilitate performance of learning in a spatial memory task.

Glycine receptor NMDA receptor Memory Long-term potentiation Spatial learning

THE *N*-METHYL-D-ASPARTATE (NMDA) receptor subtype of the glutamate receptor has been linked to neurobiological processes underlying formation of new memories (3,5). Activation of this receptor-cationic channnel complex appears to be associated with hippocampal long-term potentiation (LTP) (4). Pharmacological antagonism of the NMDA receptor has been found to effectively block LTP (18) and, specifically, its induction (4,8). Competitive NMDA antagonists (AP5, AP7) and noncompetitive antagonists (phencyclidine, MK-801) are known to impair memory for a learned task (26), as well as to impair acquisition of a novel task in rats (9,18,14,25). NMDA antagonists have been shown to preferentially impair acquisition rather than retention of spatial tasks (9,14,25). Thus, potentiation of NMDA activity has been linked primarily to the acquisition of information.

It is known that a binding site for glycine exists on the NMDA receptor (11). NMDA receptor-mediated neurotransmission can be modulated by glycine binding (1,11,15) at the strichnine-insensitive recognition site (7). In the absence of glycine, or in the presence of a glycine antagonist, electrophysiological responses to NMDA agonists are blocked (12). The requirement of glycine binding for NMDA-mediated neurotransmission and subsequent effects on LTP induction has raised interest in the exogenous application of glycine agonists as a mechanism for enhancing learning and memory (6).

The glycine prodrug milacemide (2-*N*-pentylaminoacetamide HCl) has been shown to facilitate learning and memory by potentiating NMDA activity (21). Milacemide readily crosses the blood–brain barrier and is metabolized to glycinamide through the action of monoamine oxidase B (19). Glycinamide is then rapidly converted to glycine, which may act as a positive modulator of the NMDA complex by activating its strychnine-insensitive glycine B receptor. It is thought that glycine binding potentiates the opening of magnesium$^+$ gated calcium channels to subsequently increase NMDA-associated glutamate binding (22).

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The potential cognitive-enhancing effects of milacemide have stimulated several studies using a variety of learning and memory tasks in rodents. Milacemide was found to improve performance of rats in a shock-motivated passive avoidance paradigm and to overcome the memory-impairing effects of scopolamine, diazepam, and AP7 in mice (7). Milacemide treatment was also found to improve the retrieval and storage of an active avoidance task and the retrieval of a passive avoidance task in mice (21).

The activation of NMDA receptors, which is associated with the induction of LTP, suggests that milacemide should be most greatly involved with initial task acquisition. Speed of maze learning in rats has been found to be correlated with the magnitude of LTP (13). LTP is readily manifested in the hippocampus, an area of the brain related to spatial abilities (20), and in areas containing high concentrations of NMDA receptors (16). This evidence is taken to suggest that milacemide effects will be strongest in the acquisition of a spatial task. Acquisition performance in a Morris maze has been shown to involve spatial abilities (2,17,18). Thus, the objective of the current study was to expand behavioral studies using milacemide into a spatial learning paradigm. Specifically, we investigated whether milacemide treatment in mice could facilitate acquisition performance in a Morris maze.

METHOD

Subjects

Eighty virgin male C57BL/6J mice, 6 months of age and approximately 30–40 g body weight, served as subjects. Mice were obtained from Jackson Laboratories, Bar Harbor, ME, at 6 weeks of age and were maintained until testing at the Gerontology Research Center (GRC). All animals were prescreened for signs of disease or physical abnormality. Animals were housed in groups of five in a 30 × 19 × 13 cm plastic cage with corncob bedding and access to food (NIH formula 07, 24% protein) and filtered water ad lib. Mice were maintained on a 12 L : 12 D cycle and were tested during the light cycle. Pilot research conducted at the GRC demonstrated that C57BL/6J mice successfully learn a Morris-type water maze during the light cycle (unpublished data).

Apparatus

A Morris-type water maze served as the apparatus. The maze was a white, circular plastic pool, 73 cm in diameter and 48 cm in height, filled with clear tap water to a depth of 22 cm. Water was maintained at 19–20°C. A transparent circular platform, 7 cm in diameter, was fixed in the center of one quadrant of the maze, 2 cm below the water surface. The water maze was always maintained in the same position in the room, as were all visible extramaze cues. A white cloth curtain was draped around all sides of the maze to allow for adequate video camera tracking of mice within the maze. Pilot research conducted in our laboratory determined that C57BL/6J mice learn the Morris-type water maze significantly faster in the presence of multiple three-dimensional cues in close proximity to the maze. Therefore, seven three-dimensional objects (soda can, toy mouse, floppy disk box, nondescript foam rubber cutout, origami crane, light bulb, and rubber stopper) were anchored at equidistances around the top inside wall of the maze and served as cues in this study. The cues varied in shape, size, color, and were no larger than 15 × 15 cm. Activity of mice within the swim maze was monitored by a Videomex tracking system (Columbus Instruments, Columbus, OH). Distance traveled (cm) to reach the platform was recorded as the dependent variable.

Procedure

To examine the effects of milacemide on acquisition, mice were treated with the drug prior to training in the water maze. Mice were randomly assigned to either a control group or treatment group, to be injected IP with either milacemide (10mg/kg) (obtained from G. D. Searle and Co., Skokie, IL) or phosphate-buffered saline (PBS: 0.99% NaCl, pH 7.4) 1 h prior to training on each day, in the order that they were to be tested. The dose of 10 mg/kg was selected based on pilot studies that we conducted using a range of doses (0, 5, 10, and 20 mg/kg IP). In the Morris-type water maze task, mice must learn to escape by swimming to a concealed platform within the circular pool. Before the first trial on day 1, each animal was placed onto the platform in the water maze for 90 s as pretraining. A trial was begun by placing the mouse into a counterbalanced starting quadrant (either N, W, E, or S) facing the wall. The computer then began recording time and distance traveled. Each animal had a maximum of 90 s to swim to the platform, at which point activity tracking was terminated. When the mouse reached the platform, it was permitted to remain there for 30 s before being removed from the maze. When the mouse failed to find the platform within 90 s, it was placed onto the platform by the experimenter and was removed after 30 s. Following a trial, the mouse was returned its home cage with free access to food and water between trials. Following completion of a trial for all 24 animals, a 15-min rest interval was provided before the start of a new trial. Each mouse received four trials/day for four consecutive days (one trial/day from each of the four counterbalanced starting quadrants).

An additional trial, designated as the probe trial, was given to all animals following their final trial on the last day of training. The probe trial was identical to previously described trials, except that the goal platform was removed, and the percent of total distance traveled in the training quadrant (the quadrant where the platform had been located throughout the experiment) was calculated. This variable was used to determine the amount of spatial bias that mice developed for the location of the platform, and was an additional indicator of the degree of task learning and memory. Acquisition was measured as mean group distance traveled each day (four trials/day) as well as mean group distance traveled collapsed across all trials.

Statistical Analysis

Data were analyzed using Student independent groups *t*-test ($p < 0.05$). Separate analyses were computed on the overall scores of both groups collapsed across 16 trials, as well as the mean scores for both groups during each block (1 day of four trials) and the individual probe trial. A square root transformation was computed on all data points to maintain equality of variances for groups.

RESULTS

The mean distance to find the platform averaged over 16 trials was significantly less for mice treated with milacemide than for those treated with PBS (See Fig. 1A). The overall difference between groups on the dependent measure was

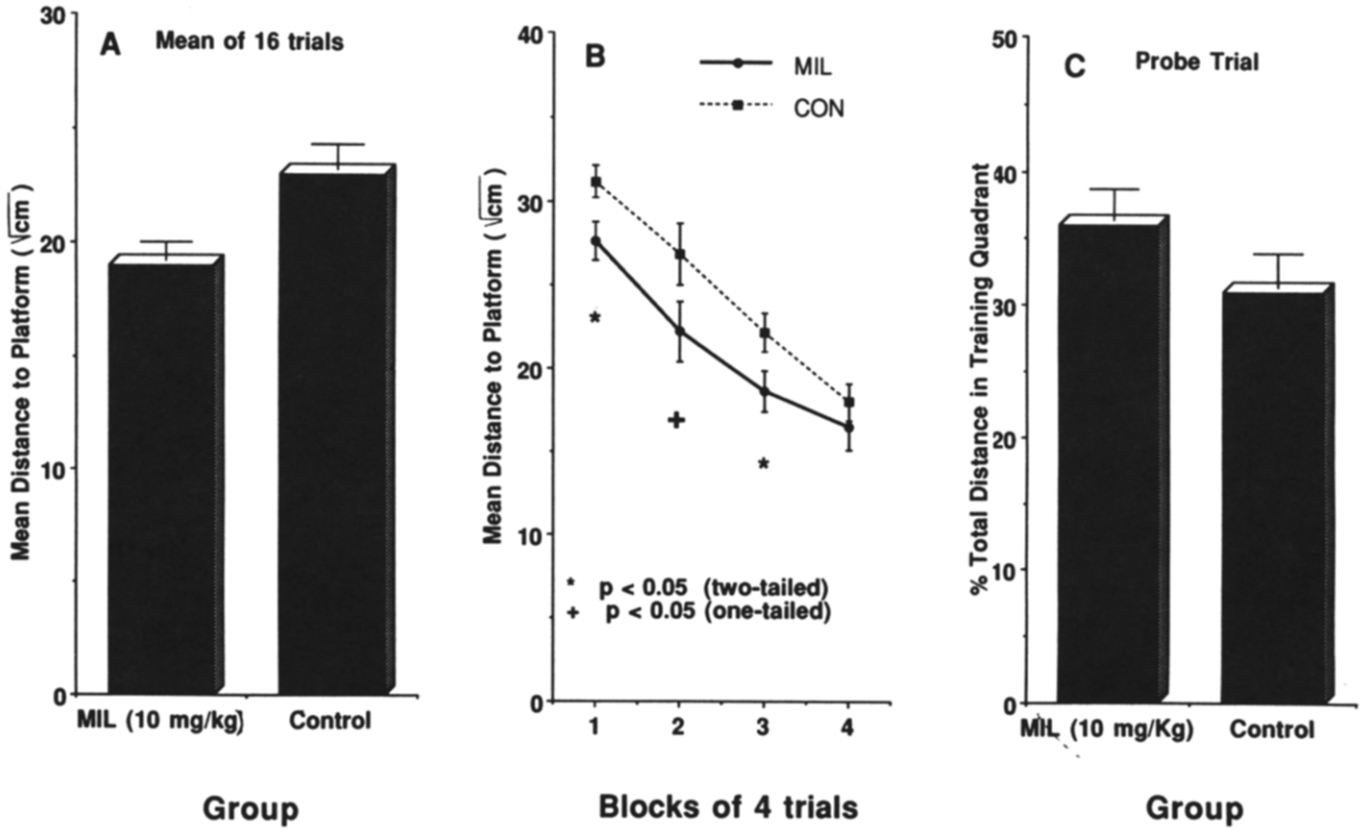

FIG. 1. Mean (SEM) distance to platform (square root transformation) of milacemide (MIL)- and PBS control (CON)-treated male C57BL/6J mice as (A) collapsed across training trials and (B) a function of training trials. (C) Mean (SEM) percent total distance traveled in training quadrant during probe trial.

32%. During the first block of trials, milacemide-treated mice traveled significantly less distance than did controls (two-tailed *t*-test). This difference was also found to be significant at block 3 (two-tailed *t*-test), and at block 2 (one-tailed *t*-test), but not at block 4 (see Fig. 1B). Analysis of the probe trial data indicated that, following 16 training trials, there was no significant difference between groups in the percent total distance traveled in the training quadrant (see Fig. 1C).

DISCUSSION

The results of this experiment indicated that milacemide treatment provided 1 h prior to training significantly improved acquisition performance of mice in the water maze task, as compared to controls. These results are consistent with past studies demonstrating a positive effect of milacemide on memory processes (7,21), and this study extends knowledge of the effectiveness of the compound to acquisition of a spatial task. Milacemide-treated mice performed better than controls, beginning on the first day and then during each subsequent day of training, except the last day. The difference between groups decreased with each day of training. This is probably the result of control animals "catching up" to milacemide-treated mice through permanent storage of the task following successive days of training.

The probe trial was run immediately following the sixteenth trial on the last day of training. This trial was utilized to assess the degree of spatial bias the mice attained over successive trials in searching for the platform in a fixed location. The analysis found no difference between groups in the percent of total distance traveled in the training quadrant (the quadrant where the platform was located). This is most likely explained by the fact that performance of control mice had approached the level of the experimental group by the time of the probe trial. Thus, no difference would be expected in spatial performance (or any measure) at that time. Results indicated that both groups performed on par with C57BL/6J mice from previous probe trials in our water maze task (MIL mice: 36%, controls: 31%; average approximately 32% in our task for this strain of mice, unpublished data). These data demonstrate that our water maze paradigm was a successful test of spatial memory.

Previous research has demonstrated that the effects of milacemide are not due to extraneous noncognitive actions, such as generalized increases in motor activity (21). Evaluation of behavior in the present experiment supports this finding as well. In the absence of quantitative data, mice treated with milacemide demonstrated adequate resting behavior on the goal platform, and demonstrated no signs of hyperexcitability at any time. This was observed across trials by the decreasing tendency of mice to jump off the platform after successfully finding it, or following manual placement by the experimenter, and a reduced tendency to swim away from the platform following accidental contact.

Despite success in several rodent studies (7,21), other studies have experienced only limited success with milacemide treatment in humans. In two separate word retrieval tasks, subjects treated with milacemide recalled more previously viewed words than controls (23) and recalled the source of spoken words significantly better than controls (24). In healthy older subjects, milacemide has been found to improve abilities (23) that are commonly impaired in Alzheimer's disease, i.e., naming pictures, objects, and producing words that fit a definition (10). Chronic treatment with milacemide in Alzheimer's patients, however, has produced disappointing results (10). Chronic administration of milacemide may actually negate any cognitive enhancing effect of the drug by inactivating the enzyme needed for its conversion to glycine (10). This, along with behavioral evidence from human and animal studies, suggests that the memory-enhancing effects of milacemide are best applied in acute learning and memory tasks.

Future research into the facilitation of memory processes through glycine agonism should consider the use of D-cycloserine (DCS), a compound that stimulates NMDA receptor activity in a manner similar to milacemide, but which seems less susceptible to tolerance effects (R. L. Herting, personal communication, 1993). DCS, like milacemide, has been found to improve performance in a variety of learning tasks in rats (6).

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