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Milacemide Enhances Memory Storage and Alleviates Spontaneous Forgetting in Mice[1]

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QUARTERMAIN, D., T. NUYGEN, J. SHEU AND R. L. HERTING. *Milacemide enhances memory storage and alleviates spontaneous forgetting in mice.* PHARMACOL BIOCHEM BEHAV **39**(1) 31–35, 1991.—The objective of this study was to evaluate the effectiveness of milacemide as a memory-enhancing drug in mice. Experiment 1 showed that forgetting of active avoidance learning produced by a 14-day training to test delay could be alleviated by milacemide (10 mg/kg) administered before the retention test. Experiment 2 demonstrated that the same dose of milacemide could also attenuate spontaneous forgetting of passive avoidance learning, thereby ruling out nonspecific effects as an explanation for the enhancement of performance following pretesting drug administration. A third experiment showed that the facilitation of retrieval induced by milacemide could be blocked by the NMDA receptor antagonist AP-7, suggesting that the effects of milacemide on memory may be mediated by NMDA receptor activation. A final experiment demonstrated that retention was improved when milacemide was administered immediately following active avoidance training, indicating that the drug can also facilitate remembering by its actions on consolidation and storage processes.

Milacemide Glycine Excitatory amino acid Memory retrieval Passive avoidance Active avoidance Learning enhancement NMDA receptor

RECENT evidence has demonstrated that activation of the excitatory amino acid neurotransmitter glutamate and its N-methyl D-aspartate (NMDA) receptor may be an important step in the sequence of neural events which lead to the storage of information in the brain (2, 4, 5). For example, pharmacological agents which antagonize the effects of glutamate at NMDA receptors block hippocampal long-term potentiation (LTP) and disrupt the learning and remembering of certain tasks (6, 7, 9, 13–15, 22, 23). Conversely, drugs which potentiate the action of glutamate at NMDA receptors may facilitate remembering in animals and man. This is suggested by the results of experiments using the novel glycine prodrug milacemide. This drug is metabolized into glycinamide via MAO-B and can produce increases in brain glycine levels (1). Glycine in turn potentiates NMDA receptor activity by activating the strychnine-insensitive recognition site (glycine B receptor) located on the NMDA receptor complex (10). This activation induces changes in the receptor which stimulate increased binding of glutamate (11) resulting in a marked increase in ion channel opening frequency (11,21). Recent experiments with milacemide have shown that it can ameliorate the memory impairing effects of scopolamine, diazepam and AP-7 on spontaneous alternation in mice (8) and improve selective attention, numerical memory and vigilance in human subjects (19,20).

The purpose of the present experiment was to obtain further information on the memory enhancing capability of milacemide by determining whether the agent can attenuate spontaneous forgetting. We have selected this paradigm because we believe that the ability of a drug to reverse spontaneous forgetting in animals may be a useful indication of its potential to alleviate benign senescent forgetfulness in man.

METHOD

Subjects

Male Swiss-Webster mice (Taconic, NY) 10 weeks of age and approximately 35 grams body weight were the subjects for these experiments. Animals were housed 5 per cage with food and water available ad lib.

Behavioral Tasks and Apparatus

Two behavioral tasks were employed in this study: one-way active avoidance learning and single-trial passive avoidance. In both tasks training parameters were adjusted so that a significant amount of spontaneous forgetting occurred within a 14-day period. The effectiveness of milacemide as a memory-enhancing

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agent was evaluated by the extent to which the drug could attenuate this forgetting.

Apparatus was a two-compartment mouse shuttle chamber (LVE No. MSC-022). Each compartment was 23 cm long, 9 cm wide and 11 cm high with a floor constructed from stainless steel rods (3 mm diameter, 7 mm between rods). Each floor could be independently connected to a Coulbourn constant current shocker. The two compartments were separated by a dividing wall in which there was a 4 × 6 cm aperture and a guillotine door. One compartment was painted black and the other white. The white compartment was brightly lit by a 5-watt miniature lamp set in the center of a transparent lid while the dark compartment was enclosed by a black Plexiglas cover.

Procedure

Active avoidance. A trial was begun by placing the mouse in the white compartment facing away from the door. After 5 s the door was raised which initiated the CS (lamp) and activated the latency timer. After 10 s the UCS (0.3 mA scrambled shock) was automatically initiated. When the mouse crossed into the dark compartment the CS, UCS and timer were automatically terminated. Mice were removed and transferred to a holding cage for a 30-s intertrial interval (ITI). Training was continued until mice completed 4 consecutive avoidances (cross-over latencies shorter than 10 s). Retention was tested using the same procedure as described above except that no shock was programmed on the test trials. Mice failing to cross into the dark compartment within 60 s were assigned the maximum latency as a test score. The test session consisted of 5 trials each separated by a 30-s ITI.

Passive avoidance. Mice were placed in the white compartment facing away from the door. After 5 s the door was opened and the CS and the latency timer were automatically turned on. When the mouse crossed into the dark compartment the door was closed, the CS and latency timer were terminated, and a 0.2 mA foot shock was automatically administered for a duration of 1.2 s. Retention was tested on a single trial by placing the mouse in the white compartment and measuring latency to cross into the dark side. Animals failing to respond within 300 s were assigned this latency as a test score.

Experiment 1

The objective of this experiment was to determine whether milacemide could attenuate spontaneous forgetting of an active avoidance response induced by a 14-day interval between training and testing. Milacemide was administered 1 h prior to the retention test with the objective of attempting to facilitate retrieval processes.

Procedure. Mice were trained as described above. One day following training, one group of 13 mice was tested for retention to establish a baseline against which to measure spontaneous forgetting. Fourteen days after training, groups of mice were injected subcutaneously with either saline (N = 11) or milacemide 3 mg/kg (N = 9), 10 mg/kg (N = 11) and 30 mg/kg (N = 10).

Results. The results of this experiment are shown in Fig. 1. It can be seen from the first two bars at the left of the figure that a substantial amount of forgetting occurred in the 14-day period. The mean test latency of the 1-day group was significantly faster than that of the 14-day control group, $t(22) = 3.34$, $p = 0.003$. A one-way ANOVA carried out on the scores of the 3 milacemide-treated groups shows that there was a significant overall effect of dose level, $F(2,27) = 8.26$, $p = 0.001$. Inspection of the figure reveals that while neither 3 nor 30 mg/kg doses improved remembering, the 10 mg/kg dose produced a significant facilitation of retrieval. The 10 mg/kg group exhibited significantly faster latencies than the 14-day control group, $t(20) = 3.26$, $p = .004$, and showed approximately the same level of retention as the control group tested 1 day after learning.

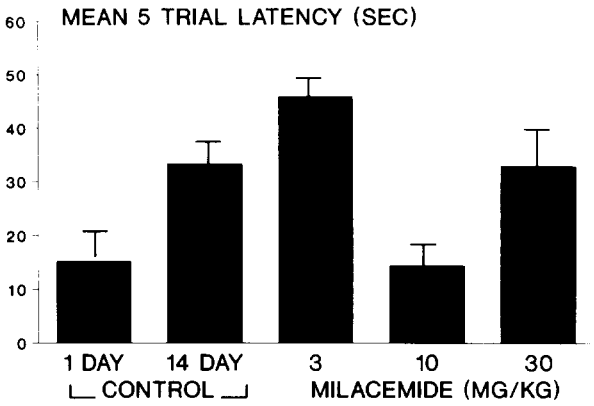

FIG. 1. Mean (+SEM) of 5 test trials for mice treated with milacemide 1 h before the retention test. The 14-day control group was given saline.

Experiment 2

The results of the first experiment demonstrated that milacemide administered prior to the retention test can improve active avoidance test performance. The hypothesis that this improvement in performance was the result of facilitated memory retrieval would be strengthened if it could be demonstrated that milacemide would also improve performance in a task where memory is indexed by an increase in latency rather than a decrease in response time. When animals are tested following treatment with any pharmacological agent there is always the possibility that nonspecific (i.e., nonmemorial) factors such as activity alterations, changes in motor behavior, etc., may be producing the behavioral change. These factors are usually evaluated by the use of sham trained control groups [e.g., (17)], but a more satisfactory solution is to employ a behavioral task where retention can be measured by opposing response requirements. The purpose of this experiment is to determine if milacemide can facilitate remembering when good performance requires a decrease rather than an increase in the rate of responding.

Procedure. Mice were trained in the passive avoidance task using the methods described above. One day following training, one group (N = 13) was tested to establish a baseline against which to measure forgetting. The remaining animals were tested 14 days later. One group (N = 15) was injected with saline while the other (N = 15) was injected with 10 mg/kg milacemide 1 h before the retention test.

Results. The test scores of the three groups are shown in Fig. 2. A one-way ANOVA carried out on these data indicated that there was a significant difference among the three treatment groups, $F(2,40) = 5.97$, $p = 0.005$. Post hoc t-tests revealed that the 14-day control group had statistically significant shorter test latencies than the 1-day group, $t(26) = 3.45$, $p = 0.002$, thereby demonstrating that significant memory loss was produced by a 14-day training to test interval. The group which was treated with milacemide exhibited statistically significant longer latencies, $t(28) = 2.63$, $p = 0.013$, than the saline control group thus

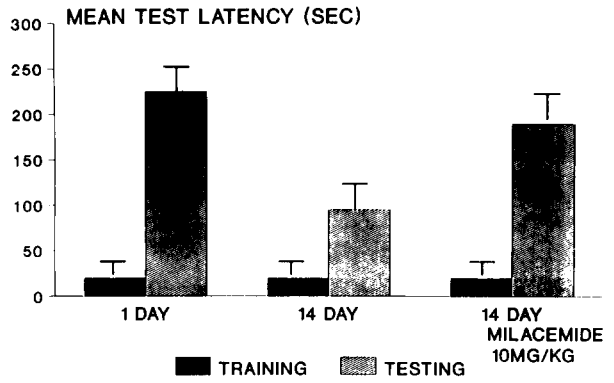

FIG. 2. Mean (+SEM) latencies of mice tested 1 and 14 days after passive avoidance training. Milacemide was given 1 h before the 14-day test.

demonstrating that spontaneous forgetting of a passive avoidance response can also be eliminated by milacemide.

Experiment 3

Milacemide is metabolized by MAO-B to glycinamide which is converted to glycine which can act at a glycine B receptor to potentiate the effects of NMDA agonists (1, 10, 21). This suggests that the effect of milacemide on memory may be mediated by the modulatory effect of glycine on the NMDA receptor. The aim of this experiment was to determine if pretreatment with the NMDA receptor antagonist 2-amino-7-phosphonoheptanoic acid (AP-7) would block the facilitation of memory retrieval induced by milacemide.

Procedure. Twelve mice were trained to a criterion of 4 consecutive avoidance responses as previously described. Fourteen days later they were injected with 50 mg/kg AP-7 and 30 min later treated with the effective dose of milacemide (10 mg/kg). Retention was tested 1 hour after the milacemide treatment. The test performance of these animals was compared with the milacemide 10 mg and control group from Experiment 1.

Results. The test latencies of the three groups are shown in Fig. 3. The latencies of the milacemide + AP-7 group were significantly longer than those of the group which received milacemide alone, $t(21)=4.52$, $p=0.001$. Before concluding that

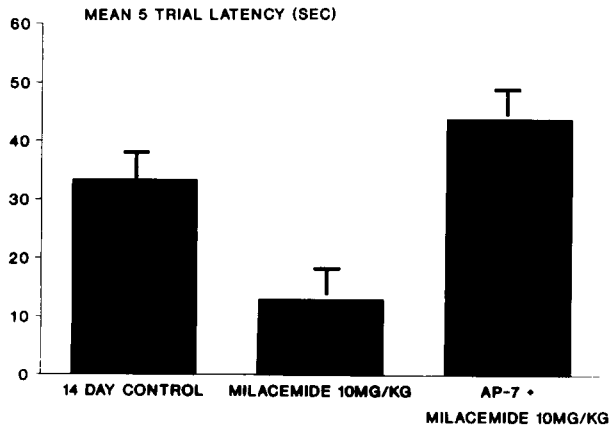

FIG. 3. Mean (+SEM) of 5 trial test latencies for mice treated with AP-7 prior to milacemide.

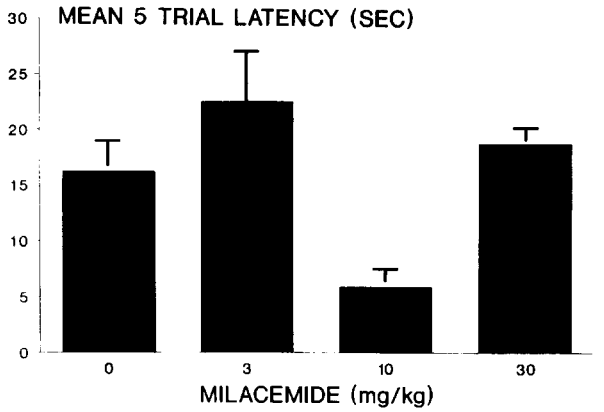

FIG. 4. Mean (+SEM) of 5 trial test latencies for mice treated immediately after active avoidance training with different doses of milacemide.

AP-7 blocked the effect of milacemide on memory retrieval, it is necessary to demonstrate that the antagonist did not induce nonspecific behavioral depression. To evaluate this possibility, two groups of mice (N = 12/group) were trained in the active avoidance task and tested 24 hours later, a time at which test latencies are typically similar to those exhibited by mice treated with milacemide and tested 14 days after training. One and one-half hours prior to the test one group was injected with 50 mg/kg AP-7, while the other group was injected with the vehicle. The mean 5 trial test latency for the AP-7 group was 10.9 s and for the vehicle group 16.3 s. This difference was not statistically significant. There is thus no evidence that AP-7 exerts any nonspecific behavioral effects which impair test performance, and it is, therefore, appropriate to conclude that the memory enhancing effects of milacemide can be completely blocked by the NMDA antagonist AP-7.

Experiment 4

The previous experiments have demonstrated that milacemide can improve remembering when the drug is given before the retention test. This finding indicates that milacemide is capable of facilitating retrieval processes. In this experiment we sought to determine whether milacemide can enhance retention when it is administered immediately after training. A positive result would indicate that milacemide was also capable of influencing the neural processes underlying the consolidation and storage of information.

Procedure. Forty mice were trained in the active avoidance task to a criterion of 4 consecutive avoidances as described above. Immediately after attainment of this training criterion, mice were assigned to 1 of 4 groups and treated with 0 (saline), 3, 10, or 30 mg/kg milacemide. Animals were assigned to groups by the use of a matching procedure which resulted in approximately equivalent mean trials to criterion in the 4 groups. Retention was tested 24 hours after training. No injections were given before the test.

Results. The results are shown in Fig. 4. A one-way ANOVA carried out on these data indicated a significant difference in mean 5 trial latency among the 4 groups, $F(3,38)=4.24$, $p=0.011$. Post hoc comparisons using t-tests showed that the 10 mg/kg milacemide group had significantly faster test latencies ($p=<0.01$) than each of the other 3 groups.

DISCUSSION

The results of this study demonstrate that milacemide can both enhance learning and alleviate spontaneous forgetting. The

demonstration that posttraining injections of milacemide can improve retention 24 h later suggests that the drug can amplify the neural processes which are triggered by the training experience to produce a stronger and more durable memory. This action of milacemide can be plausibly interpreted in terms of the effect of glycine on NMDA receptors resulting in enhancing LTP. However, such posttraining modulation of consolidation has been demonstrated for a number of pharmacologic agents which activate a variety of neurotransmitter and hormonal systems [see (12) for a recent review of these studies]. The possibility that the enhancement of memory consolidation in this experiment merely reflects a carryover effect on retrieval cannot be ruled out.

The improvement of memory which follows pretesting administration of milacemide indicates that this agent can also influence memory retrieval processes. The interpretation of this finding in terms of NMDA receptor function is less clear. Milacemide provides a reservoir of glycinamide in the brain and its action is presumably mediated by slow release of glycine. Since glycinamide per se does not bind to the glycine B site (A. A. Cordi, personal communication) it is unlikely to be an intermediate candidate for NMDA agonist activity, though action of glycinamide on other systems cannot be ruled out. A possible explanation for the facilitation of retrieval is that milacemide administered before testing reactivates NMDA receptors that were active during training, thereby reinstating some aspects of the neural state which was present during initial learning.

It has been noted that when drugs are active while the animal is performing the test response, it is frequently difficult to distinguish effects of the drug on retrieval from nonspecific effects on performance (16). The present study eliminates this source of ambiguity by showing that the same dose of milacemide can improve remembering for both active and passive avoidance learning. When a drug which is administered before testing can induce behavioral changes in opposite directions depending on the requirements of the memory task, the results are more plausibly attributed to a direct effect on retrieval processes than to nonspecific performance changes.

A notable feature of the results of this study is the U-shaped dose-response curves. In all of the experiments in this study, the intermediate dose (10 mg/kg) was consistently effective in enhancing remembering, while the high and the low doses were repeatedly ineffective. Such dose-response curves, while uncommon in classical pharmacology, are frequently observed in behavioral pharmacology especially in memory enhancement experiments. We have recently shown that salbutamol sulphate, phenylephrine hydrochloride and the serotonin agonist 5-methoxy-N,N-dimethyl-tryptamine also facilitate retrieval at intermediate doses, but are ineffective at higher doses (17). Such findings indicate that moderate levels of neural activation are more likely to facilitate memory processing than are high levels which can frequently lead to memory disruption (12).

The demonstration that the memory facilitating effects of milacemide can be blocked by the NMDA antagonist AP-7 suggests that the drug may be influencing remembering via its effects on the NMDA receptor. As noted in the introduction, milacemide appears to influence the NMDA receptor complex indirectly by increasing the supply of glycine to the glycine B receptor (1). Glycine has been shown to potentiate the action of glutamate on the NMDA receptor by enhancing its ability to open channels (10). The activation of NMDA receptors allows calcium to enter the postsynaptic cell which promotes neurotransmitter release and may also trigger changes in pre- and postsynaptic terminals (5). In addition, calcium induces biochemical reactions such as the activation of protein kinase C which has been hypothesized to play a role in memory storage (18). Further research will be necessary to establish a direct link between milacemide's effect on remembering and activation of the NMDA receptor complex. However, the present demonstration that NMDA receptor blockade abolishes the memory enhancement, taken together with the finding that prevention of the metabolism of milacemide to glycine also eliminates memory facilitation (8), provide strong circumstantial evidence that the behavioral effects are mediated through NMDA receptor activation.

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