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Minaprine Facilitates Acquisition and Retrieval of an Active Avoidance Response in the Rat

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AMBROGI LORENZINI, C., E. BALDI, C. BUCHERELLI AND G. TASSONI. *Minaprine facilitates acquisition and retrieval of an active avoidance response in the rat.* PHARMACOL BIOCHEM BEHAV 45(2) 481–485, 1993.—The nootropic activity of 3-(2-morpholino-ethylamino)-4-methyl-6-phenyl-pyridazine dihydrochloride (minaprine) has been investigated in intact male, adult Long Evans rats by means of an active avoidance paradigm. In the light–dark box apparatus, the rat had to learn the active avoidance response of going out of the normally preferred dark chamber to avoid electric foot-shocks. These were administered during one trial per day for 3 consecutive days (acquisition period). After a 72-h interval, rats underwent, for 3 consecutive days, one trial per day in which punishments were omitted (retrieval period). In the first experiment, rats were injected IP with minaprine (5, 10, and 25 mg/kg b.w.) 30 min before each trial of both periods. Rats injected with the two lower dosages showed better responding during the retrieval period than controls (saline). On the contrary, the highest dosage impaired active avoidance during both periods. In Experiment 2, minaprine (10 mg/kg b.w.) was administered either only during the acquisition or only during the retrieval period. In both instances, active avoidance was equally enhanced, if compared to controls (saline), only during the retrieval period. The results are discussed on the basis of the known facilitating activity on cholinergic systems of this compound. It is concluded that minaprine acts positively both on acquisition and retrieval of mnemonic traces.

Minaprine Memory trace consolidation Memory trace retrieval One-way active avoidance

MINAPRINE (3-(2-morpholino-ethylamino)-4-methyl-6-phenylpyridazine dihydrochloride) has been shown to be effective in several animal models of depression and in human patients (11,14). This compound, described as an atypical antidepressant drug, has facilitating neurochemical effects on cholinergic and dopaminergic neurons and systems (9,15,26) presumably involved in memory processing (7,10,12). In fact, it has been repeatedly shown that in rodents minaprine exerts a protective effect against memory impairment caused by diverse amnesic procedures, like scopolamine administration (1,18, 27), cerebral ischemia (27), or protein synthesis inhibition (16). The peculiar pharmacological properties of this compound suggest that minaprine may also improve acquisition and retention even without previous impairment of higher nervous functions. In fact, this has been shown to be the case for other (so-called nootropic) drugs, like acetyl-*l*-carnitine and pyrrolidones, which besides protecting animals from experimentally induced retrograde amnesia (4,8,17,22,23) improve the cognitive performance of intact subjects (8,17,21,23).

The aim of the present study is to assess whether minaprine enhances learning and memory in normal intact animals. A multiple-trial, one-way active avoidance paradigm will be employed to study the effect of the drug on the acquisition and retrieval of the conditioned response.

EXPERIMENT 1

This experiment was designed to assess whether minaprine influences the learning and memory of an active avoidance response in the rat. Several dosages were employed with the aim of defining a dose–effect relationship.

METHOD

Naive male, adult Long Evans rats, aged 60 days, were employed (Morini, Italy). Animals were individually housed in stainless steel cages at a room temperature of 20 ± 1°C under natural illumination. Rats received food and water ad lib. The one-way active avoidance conditioned response was acquired in the light–dark box (2). The apparatus consisted of two chambers of equal dimensions (30 × 21 × 15 cm), one with white plastic walls and transparent lid and the other with black plastic walls and ceiling. In both chambers, the floor was made of stainless steel rods (2 mm diameter) spaced 1 cm apart. The floor of the black chamber could be electrified.

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The two chambers were connected by a rectangular opening (8 × 6 cm), which could be closed with a guillotine door. The apparatus was placed in an acoustically insulated room kept at a constant temperature (20 ± 1°C). Lighting inside the light chamber of the apparatus was 60 lux.

Rats were tested during two 3-day periods, separated by a 72-h interval. Rats underwent one single trial per day. In trial 1 of the first period (acquisition), the rat was placed by hand into the light chamber with the connecting door open. After the animal had spontaneously entered the dark chamber, the door was closed and the animal received one unavoidable foot-shock (1.2 mA, 3 s). Immediately afterward, the door was opened so that the rat could leave the dark chamber. If the rat was still inside the dark chamber 10 s later, up to five (avoidable) shocks were administered (1.2 mA, 1 s, 0.2-s intervals). As soon as the animal entered the light chamber, the door was closed and the rat was returned to its home cage. Rats that did not leave the dark chamber were taken directly from it to the home cage. In the second and third trials of the acquisition period, rats were directly placed in the dark chamber with the door shut. Immediately after the lid was closed, they underwent the same procedure as in trial 1. The second testing period (retrieval) started 72 h after the third acquisition trial. The procedure used in the three retrieval trials was the same as in the second and third acquisition trials except that no electric foot-shocks were administered.

The step-through latency was measured in the first trial of the acquisition period and the performance of the active avoidance response was recorded in all six trials. This was done by measuring the time that had elapsed between the end of the unavoidable shock administration and the exit from the dark chamber. All exits within 10 s (i.e., effectively and completely avoiding the further escapable foot-shocks) were counted as active avoidance responses. Manual stopwatch measurements started immediately after closing the lid of the respective compartment. Latencies were measured up to the time when the animal placed all four paws in the other compartment.

Fifty rats were randomly divided into four groups of 12–13 each. Thirty minutes before every trial, animals of each group received an IP injection (1 ml) of one of the following solutions: a) physiological saline (S); b) 5 mg/kg b.w. minaprine in saline (M 5); c) 10 mg/kg b.w. minaprine in saline (M 10); d) 25 mg/kg b.w. minaprine in saline (M 25).

χ^2, one-way analysis of variance (ANOVA), and the Newman–Keuls multiple comparisons test were employed for statistical evaluation of the data.

RESULTS

The step-through latency duration, measured in trial 1 of the acquisition period, was short in all groups [range from 10.9 ± 3.1 s (mean ± SE) to 18.0 ± 4.5 s]. There were no significant differences between groups, $F(3, 46) = 0.69$, n.s.

Figure 1 shows the mean percentages of active avoidance responses exhibited by all groups of rats during the acquisition and retrieval periods. For all four groups of rats, the percentages of correct conditioned responses in the three trials of each period were similar. In fact, there were no significant differences between trials either during the acquisition period [S group, $\chi^2(2) = 0.67$, n.s.; M 5 group, $\chi^2(2) = 2.67$, n.s.; M 10 group, $\chi^2(2) = 1.48$, n.s.; M 25 group, $\chi^2(2) = 1.11$, n.s.] or during the retrieval period [S group, $\chi^2(2) = 0.67$, n.s.; M 5 group, $\chi^2(2) = 0.27$, n.s.; M 10 group, $\chi^2(2) = 1.07$, n.s.; M 25 group, $\chi^2(2) = 0.56$, n.s.]. Absence of significant between-trial differences allowed us to calculate the average percentages of active avoidance responses for each rat in either period. From these data, mean group percentages were calculated and the overall results were analyzed by means of one-way ANOVA.

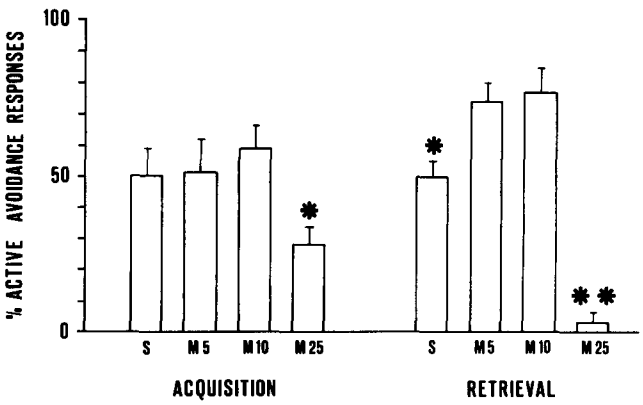

FIG. 1. Mean group percentages (±SEM) of active avoidance responses during acquisition and retrieval periods of Experiment 1. S, rats injected with saline; M 5, rats injected with minaprine 5 mg/kg b.w.; M 10, rats injected with minaprine 10 mg/kg b.w.; M 25, rats injected with minaprine 25 mg/kg b.w. Newman–Keuls comparisons (*$p < 0.05$; **$p < 0.01$): during acquisition, M 25 vs. S, M 5, and M 10 ($p < 0.05$); during retrieval, M 25 vs. S, M 5, and M 10 ($p < 0.01$) and S vs. M 5 and M 10 ($p < 0.05$).

There were significant differences between groups during the acquisition period, $F(3, 46) = 3.54$, $p < 0.025$. This was due solely to the M 25 group, the performance of which was worse than that of all other groups, as shown by the Newman–Keuls test ($p < 0.05$ in all three instances). On the other hand, there were no significant differences between the other three groups: S, M 5, and M 10.

During the retrieval period, there were significant between-group differences, $F(3, 46) = 11.81$, $p < 0.0001$, that were due not only to the poor performance of the M 25 group but also to the fact that both M 5 and M 10 groups performed better than the S group. In fact, the Newman–Keuls test showed that the performance of the M 25 group was worse than that of any other group ($p < 0.01$ in all three instances). Moreover, S group performance was significantly worse than that of M 5 and M 10 groups ($p < 0.05$ in both instances). There was no significant difference between M 5 and M 10 groups.

DISCUSSION

The results of this experiment show that the highest dosage of minaprine impairs active avoidance responding during both acquisition and retrieval periods. On the contrary, lower dosages of minaprine improve active avoidance responding only during the retrieval period.

Because in this experiment minaprine was administered during both periods, it was not possible to ascertain whether the improvement observed in the M 5 and M 10 groups was due to a better consolidation or to a more efficient retrieval of the memory trace. This point was addressed in Experiment 2.

EXPERIMENT 2

To demonstrate a differential effect of minaprine on formation or retrieval of the active avoidance memory trace,

the drug was administered to different groups of rats either only during the acquisition period or only during the retrieval period. The 10-mg/kg b.w. dosage was employed in this experiment because it had been shown to be effective in Experiment 1.

METHOD

Methods and procedure were the same as in Experiment 1. Twenty-seven rats were randomly divided into two groups of 14 and 13 animals. Rats of the first group (M 10-S) were injected with minaprine (10 mg/kg b.w. in saline) during the first 3 days (acquisition period) and were administered saline only during the second period (retrieval). The other group (S-M 10) received saline during the acquisition period and minaprine (10 mg/kg b.w. in saline) during the retrieval period.

RESULTS

The behavior of the M 10-S and S-M 10 groups was compared with the behavior of the groups of Experiment 1 that received saline (S) or the same dosage of minaprine (10 mg/kg b.w.) throughout both periods (M 10). These two groups were the appropriate controls of those of Experiment 2. The step-through latencies measured during the first trial of the four compared groups were again short, ranging from 8.0 ± 2.5 s (mean ± SE) to 18.0 ± 4.5 s. There were no significant differences between these groups, $F(3,48) = 1.69$, n.s.

Figure 2 shows mean active avoidance responses percentages of the four groups during acquisition and retrieval periods. As for all groups of Experiment 1, also the S-M 10 and M 10-S percentages of correct conditioned responses in the three trials of each period were similar. In fact, statistical analysis did not reveal significant differences between trials either during the acquisition period [S-M 10 group, $\chi^2(2) = 4.33$, n.s.; M 10-S group, $\chi^2(2) = 0.81$, n.s.] or during the retrieval period [S-M 10 group, $\chi^2(2) = 0.25$, n.s.; M 10-S group, $\chi^2(2) = 0.7$, n.s.]. Because of these results, we could use the same statistical procedure employed in Experiment 1.

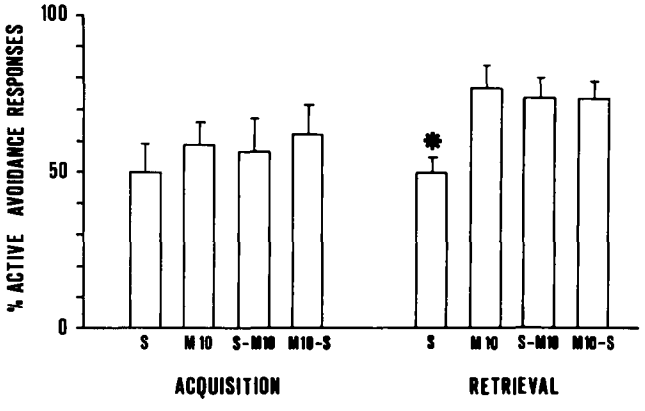

FIG. 2. Mean group percentages (±SEM) of active avoidance responses during acquisition and retrieval periods of groups S (saline) and M 10 (minaprine 10 mg/kg b.w.) of Experiment 1 and groups S-M 10 (saline during acquisition and minaprine 10 mg/kg b.w. during retrieval) and M 10-S (minaprine 10 mg/kg b.w. during acquisition and saline during retrieval) of Experiment 2. Newman-Keuls comparisons (*$p < 0.05$): during retrieval, S vs. M 10, S-M 10, and M 10-S ($p < 0.05$).

The four compared groups did not differ during the acquisition period, $F(3, 48) = 0.35$, n.s., but were significantly different during retrieval, $F(3, 48) = 2.97$, $p < 0.05$. The Newman-Keuls test showed that the difference was due to the poorer response of the S group, which differed significantly from all of the other three groups: M 10-S, S-M 10, and M 10 ($p < 0.05$ in all three instances). On the other hand, the differences between these three groups were not significant.

GENERAL DISCUSSION

Pigmented rats of the Long Evans strain were employed in the present experiments because previous work had shown that subjects of this strain acquire and retrieve the active avoidance response in the light–dark box paradigm more easily than albino rats (2). All animals employed entered the dark chamber of the apparatus during the first experimental session fairly quickly. This means that the spontaneous behavior of the animal population employed was homogeneous and that it was not influenced by minaprine administration.

In the paradigm employed, one unavoidable shock preceded avoidance response testing. In this way, it is likely that innate aversive reactions like immobility (freezing) may occur (2). However, this possibility was deemed less important than the advantages deriving from administration of an effective nociceptive stimulation in the most uniform way to the entire population. To achieve a better memory trace consolidation, the acquisition trial was repeated three times. The effectiveness of the procedure was shown by response resistance to extinction (no significant progressive diminution of the response during the three retrieval trials).

The results of Experiment 1 clearly show that during the acquisition period administration to Long Evans rats of the highest minaprine dosage (25 mg/kg) causes a marked impairment of active avoidance responding. During the retrieval period, avoidance responding disappeared almost completely in this group. Thus, the highest dosage was shown to impair acquisition significantly and perhaps also the retrieval of the conditioned response. On the other hand, the two lower dosages (5 and 10 mg/kg) did not induce acquisition impairment and, moreover, during retrieval testing both groups performed better than the control S group. Thus, during the retrieval period minaprine showed an inverted U-shaped dose-dependent effect curve. A high minaprine dosage did not elicit negative effects in an earlier study, but it may be pointed out that a diverse rat strain (Wistar albinos) was employed in a different paradigm (passive avoidance conditioning) (1).

Although it is not easy to determine precisely the time course of memory trace formation, passive avoidance conditioning results, obtained using similar intensity foot-shocks, show that memory trace consolidation requires between 24 and 48 h (5,24). Even if these times were obtained from a different paradigm, the 72-h interval between the last acquisition trial and the first retrieval trial can be considered sufficient for completion of memory trace formation. If this is the case, minaprine effects during the second period of both experiments must be due solely to its influence on memory trace retrieval mechanisms. Another important consideration is that active avoidance is performed under different circumstances during the two periods. During the first period, rats leave the dark chamber after one unavoidable shock to avoid further escapable aversive stimuli. During the second period, rats leave the dark chamber having received neither the unavoidable nor the avoidable foot-shocks. When foot-shocks

are administered, rats may exhibit freezing, which hinders active avoidance responding (2). In consequence, even if minaprine at the lower dosages had had a positive effect on memory trace formation and consolidation differences between M 5, M 10, and S groups would be hardly appreciable whereas these might become evident during the retrieval period. On the other hand, during this second period the observed facilitatory minaprine effects could also solely be due to selective positive effects of the drug on memory trace retrieval. Thus, Experiment 1 results show that during retrieval testing there is a facilitatory effect of minaprine but cannot answer whether this effect is due to a betterment of response acquisition or to a betterment of response retrieval mechanisms. This point was addressed by Experiment 2.

In Experiment 2, no differences between groups were expected during the acquisition period because the two new groups of rats did not differ respectively from the S and M 10 groups of Experiment 1. On the other hand, retrieval period results of Experiment 2 lead to several conclusions. First, the improved performance of group S-M 10 indicates that minaprine acts on memory trace retrieval. Conversely, the similar retrieval improvement observed in the M 10-S group indicates a minaprine-induced facilitation of learning. That this facilitation becomes evident only during the retrieval period may be explained by foot-shock interference with active avoidance responding during the acquisition period. Finally, the absence of significant differences between M 10-S, S-M 10, and M 10 groups during the retrieval period may be due to the high incidence of correct conditioned responses (almost 80%) approaching a behavioral asymptote hard to be improved by the drug. Experiment 2 also shows that minaprine effects cannot be explained by "state-dependent learning" (13,19). In fact, the similar improvement of performance in the M 10-S and S-M 10 groups is a result that conflicts with a state-dependent explanation.

The results of the two experiments show that minaprine positively affects both memory trace acquisition and retrieval. Minaprine influences cholinergic and dopaminergic systems, systems reported to be involved in aversively motivated behaviors (3,20). It must also be borne in mind that the acquisition and retrieval of aversive responses may involve diverse cerebral circuits or systems (6). Possibly, minaprine exerts a facilitatory influence on several phases of learning and memory by means of its various distinct, albeit often convergent, mechanisms of action (9,15,25–27). The results of the present work show that minaprine, at least for active avoidance responding in the rat, improves the cognitive performance of intact subjects, thus suggesting that this compound may be useful not only as an antidepressant but also as a nootropic drug.

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