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Minaprine Cancels Scopolamine Effects on the Rat's Acquisition of Passive Avoidance Responses in Two Multitrial Paradigms

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AMBROGI LORENZINI, C., E. BALDI, C. BUCHERELLI AND G. TASSONI. *Minaprine cancels scopolamine effects on the rat's acquisition of passive avoidance responses in two multitrial paradigms.* PHARMACOL BIOCHEM BEHAV **41**(4) 715–718, 1992.—The antiamnesic activity of minaprine has been studied in male Wistar rats. Two multitrial paradigms were employed: the light–dark box test (aversive stimulus: 0.6-mA foot-shocks) and the tail-handling test (aversive stimulus: manual tail-handling). In both paradigms, intraperitoneal scopolamine administration 30 min before testing significantly impaired the acquisition of the passive avoidance conditioned response. There were no significant differences in either paradigm between control rats and those to whom scopolamine and minaprine were simultaneously administered. These results show that minaprine fully protects the acquisition process of conditioned responses against scopolamine impairment not only in one-trial tests but also in multitrial paradigms. The effects of minaprine in reversing memory deficits are discussed in relation to its stimulating activity on central cholinergic systems.

Minaprine Scopolamine Amnesia Multitrial Passive avoidance tests Cholinergic systems and memory

3-(2-MORPHOLINO-ethylamino)-4-methyl-6-phenyl-pyridazine dihydrochloride) (minaprine) has been described as an atypical antidepressant drug that is effective on several animal models of depression and on human patients (8,10). Besides this pharmacological activity, it has repeatedly been shown that this compound exerts a protective effect against mnemonic damage caused in experimental animals by diverse procedures (3,6,9,12–14,18,19). In the reported experiments, an amnesic deficit was caused either negatively interfering with the central muscarinic cholinergic system by means of an antagonist like scopolamine (6,18,19), by cerebral ischemia (3,18), or by the administration of a protein synthesis inhibitor like cycloexhimide (9,12,13). The diminution of the reduced deficit after minaprine administration was assessed. This protective activity has been understood as due to the facilitating neurochemical effects of minaprine on cholinergic and dopaminergic neurons and systems (7,11,17), generally thought to be involved in memory processing (5,15). It can be emphasized that most of the published findings were obtained from "one-trial" experimental paradigms in which both the amnesic agent and minaprine were employed only once.

The aim of the present work is to ascertain if, and how much, minaprine exerts its antiamnesic activity even in multiple-trial conditioned learning paradigms. To do this, Wistar rats will be employed in two experimental paradigms, the light–dark box test and the tail-handling test. Of these, the first one—a classical avoidance learning multiple-trial paradigm—has repeatedly been employed by us to assess strain learning differences (1) and the second (which is also a multiple-trial conditioned avoidance paradigm) has recently been reported by us (2).

Although both tests can be defined as conditioned avoidance learning paradigms entailing the performance of a sequence of trials, they are nevertheless quite diverse. Their diversity derives from the stimulus employed for conditioning the rats. In the light–dark box test, it is the electrical foot-shock, a typical nociceptive stimulus, while in the tail-handling test it is a painless (or almost) "situation" stimulus. The repetition every 24 h of the learning sessions will enable us to follow the time course of the learning process, its impairment due to amnesic agents (e.g., scopolamine), and its protection against amnesia due to minaprine.

METHOD

Naive, male Wistar rats, aged 60 days (Morini, Italy) were employed. Rats were housed individually in stainless steel

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cages at a room temperature of 20 ± 1°C, natural illumination. The animals received food and water ad lib.

Scopolamine hydrobromide (Sigma) and minaprine (Sanofi, Midy) were employed in both experiments.

Experiment 1

A total of 108 rats were employed. Animals were randomly divided in 9 groups of 12 subjects each. Thirty minutes before each testing session, animals received an intraperitoneal injection of 1 ml of one of the following solutions: Group 1, saline (NaCl 0.9%); Group 2, scopolamine 0.4 mg/kg in saline; Group 3, scopolamine 0.8 mg/kg in saline; Group 4, minaprine 10 mg/kg in saline; Group 5, minaprine 25 mg/kg in saline; Group 6, scopolamine 0.4 mg/kg plus minaprine 10 mg/kg in saline; Group 7, scopolamine 0.4 mg/kg plus minaprine 25 mg/kg in saline; Group 8, scopolamine 0.8 mg/kg minaprine 10 mg/kg in saline; Group 9, scopolamine 0.8 mg/kg plus minaprine 25 mg/kg in saline.

Experimental apparatus. The light–dark box consisted of two chambers of equal dimensions (30 × 21 × 15 cm), one made of white plastic with a transparent lid, the other made on five sides of dark opaque plastic. In both chambers, the floor was made of stainless steel rods (2 mm diameter) spaced 1 cm. The floor of the dark chamber could be electrified. The two chambers were connected by a guillotine door (8 × 6 cm). 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.

Procedure. In all testing sessions, rats were manually placed, one by one, in the light chamber of the apparatus; the door between the two chambers was open. After animals had either spontaneously gone into the dark chamber (after which the gate was closed), or after having been placed inside it (with the gate closed), whenever the step-through latency values had reached the 180-s duration animals received an electric foot-shock (0.6 mA, 1 s). Immediately following it, rats were taken out of the dark chamber and returned to the home cage.

Manual step-through latency measurements started immediately after closing the lid of the starting chamber, and were taken up to 180 s. Step-through latency was measured as the time taken by the animal to place all four paws in the dark chamber.

The experiment consisted of six consecutive single daily trials.

The data of Trials 2–6 were employed for step-through latency analysis. The data of Trial 1 were subjected to independent analysis since they were obtained before foot-shock administration.

Analysis of variance (ANOVA) and two-way ANOVA with repeated measures on trials were employed. Paired comparisons between groups were performed by means of the post-hoc Tukey test (17).

Results. As shown in Fig. 1, in all groups of rats there is a progressive increase in step-through latency values from the second to the sixth trial. Step-through values of Groups 2 and 3 (scopolamine 0.4 and 0.8 mg/kg) are lower in all trials than those of all the other groups. There are significant differences between trials, $F(4,396) = 121.96$, $p < 0.001$, and between groups, $F(8,99) = 270$, $p < 0.025$. The posthoc Tukey test shows that there are significant differences between Groups 2 and 3 and all other groups ($p < 0.05$ in all instances).

In all groups, step-through latencies measured on Trial 1 are quite short, and there are no significant differences between groups, $F(8,99) = 0.81$, NS.

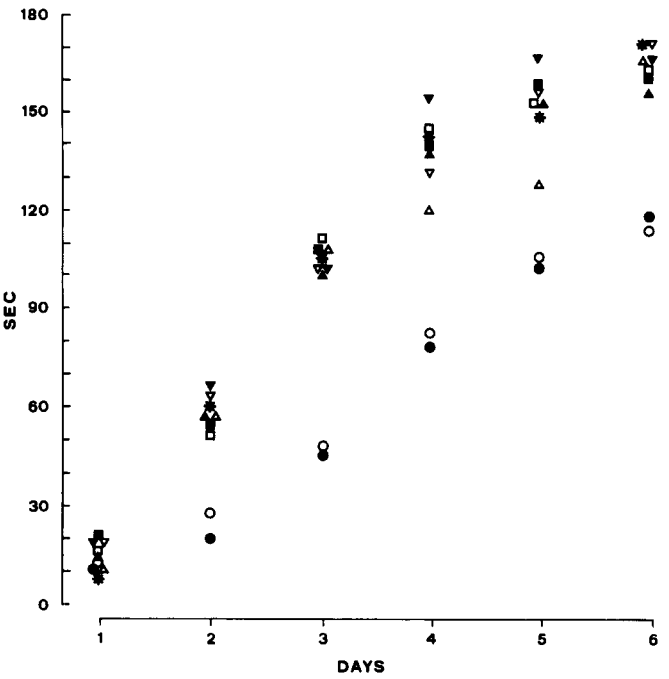

FIG. 1. Mean daily step-through latencies of the groups in Experiment 1. S, scopolamine; M, minaprine. *, Group 1 (saline); ○, Group 2 (S 0.4 mg); ●, Group 3 (S 0.8 mg); □, Group 4 (M 10 mg); ■, Group 5 (M 25 mg); △, Group 6 (S 0.4 mg + M 10 mg); ▲, Group 7 (S 0.4 mg + M 25 mg); ▽, Group 8 (S 0.8 mg + M 10 mg); ▼, Group 9 (S 0.8 mg + M 25 mg).

Experiment 2

For this, 48 rats were employed. Animals were randomly divided in 4 groups of 12 subjects each. Thirty minutes before each testing session, animals received an intraperitoneal injection of 1 ml of one of the following solutions: Group 1, saline (0.9% NaCl); Group 2, scopolamine 0.8 mg/kg in saline; Group 3, minaprine 10 mg/kg in saline; Group 4, scopolamine 0.8 mg/kg plus minaprine 10 mg/kg in saline.

Experimental apparatus. The two-box apparatus consisted of two Plexiglas chambers of equal dimensions (30 × 21 × 15 cm) with stainless steel bar floors. Chambers were connected by a guillotine door (8 × 6 cm). The walls of one of the chambers were solid white; those of the other chamber were vertically striped black and white (black stripe, 1.4 cm; white stripe, 3 cm). Both chambers were covered by transparent lids. The apparatus was placed in an acoustically insulated room kept at a constant temperature (20 ± 1°C). Lighting inside the apparatus was 60 lux.

Procedure. Subjects were placed inside a plastic container and slipped gently into the starting (solid white) box of the apparatus. As soon as rats had gone into the goal (striped) chamber, the guillotine door was closed and animals were taken out of it by the tail (close to the root), replaced in the plastic container, and finally returned to the home cage. Manual step-through latency measurements started immediately after closing the lid of the starting chamber and were taken up to 180 s. Step-through latency was measured as the time taken by the animal to place all four paws in the goal chamber.

The experiment consisted of five consecutive daily single trials.

Statistical analysis was performed as in Experiment 1.

Results. As shown in Fig. 2, in all groups of rats there is a progressive increase of step-through latency values from the second to the fifth trial. Step-through values of Group 2 (scopolamine 0.8 mg/kg) are lower in Trials 2, 3, and 4 than those of all other groups. There were no differences between groups in Trial 5. Two-way ANOVA showed that in Trials 2-4 there are significant differences between trials, $F(2,88) = 52.06$, $p < 0.001$, and between groups, $F(3,44) = 3.11$, $p < 0.05$. The post-hoc Tukey test shows that there are significant differences between Group 2 (scopolamine) and all the other groups ($p < 0.05$ in all three instances). In Trial 5, differences between groups disappear, $F(3,44) = 0.66$, NS.

In all groups, step-through latencies measured on Trial 1 are quite short and there are no significant differences between groups, $F(3,44) = 0.82$, NS.

DISCUSSION

In both experiments, step-through latency values of Trial 1 were low and were quite similar in all groups of rats, that is, there were no differences in the spontaneous explorative behavior of naive subjects. This finding is important on several counts. First, one may safely assume that in both experiments all between-groups differences observed during the successive trials were due to the effects of scopolamine and minaprine on the acquisition of the conditioned responses since these same compounds did not influence spontaneous behavior. In other words, the reported findings were due to modifications of the learned response and not to alterations of the spontaneous behavior of rats. Second, this finding shows that the handling of subjects and the intraperitoneal injection, as such, did not negatively influence the spontaneous activity of rats. In fact, step-through latency values measured in Trial 1 of both experiments of the present work are almost identical to those measured in previous experiments in which rats of the same strain were employed (1,2).

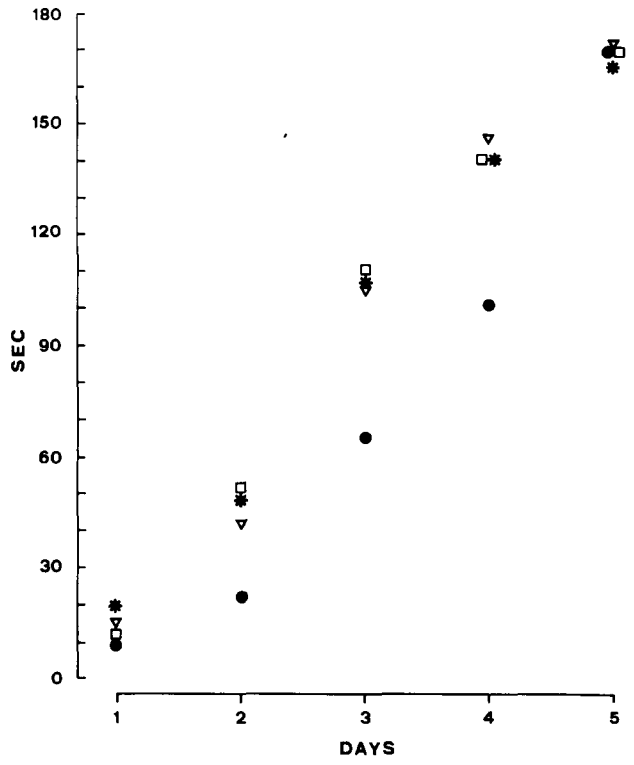

FIG. 2. Mean daily step-through latencies of the groups in Experiment 2. For explanations, see Fig. 1.

As regards the results of the subsequent trials, in both experiments the administration of scopolamine was followed by significant amnesic effects, thus confirming the disruptive effect of scopolamine on memory (5). It is also true that in successive trials subjects receiving scopolamine exhibited a progressive increase in step-through latency, but in these subjects step-through values were always lower than those of control animals (with the sole exception of the results of Trial 5, Experiment 2). This means that scopolamine administration significantly impaired the acquisition of the conditioned passive avoidance responses.

Minaprine administration did not modify the acquisition of the conditioned avoidance responses in either experiment since there were no significant differences between these groups of rats and the control ones.

There were no differences, in either experiment, between the conditioned avoidance behavior of rats contemporaneously receiving scopolamine and minaprine and control animals. This finding indicates that minaprine exerts full protection against the amnesic effects of scopolamine coherently with the well-described facilitatory effects of minaprine on cholinergic systems.

In Experiment 1, two dosages of scopolamine (0.4 and 0.8 mg/kg) and two dosages of minaprine (10 and 25 mg/kg) were used. These were chosen on the basis of the mean dosages reported in previous published papers on this topic. Since there were no significant differences between Groups 6, 7, 8, and 9 of Experiment 1, it may be concluded that even at the lower dosage minaprine efficiently counteracts the effects of the higher amount of scopolamine.

On this finding rests the choice of dosages and consequently the number of experimental groups of rats employed in Experiment 2. We felt that to test the protective effects of minaprine in this second paradigm it would be sufficient to start by administering the lower dosage of minaprine and the higher one of scopolamine. Should minaprine exert its protective influence in these conditions, it would be unnecessary to test its action in other symmetric groups. If not, we could expand the protocol. The results of Experiment 2 did not call for expansion.

The paradigm of both experiments enables us to follow the time course of the acquisition of the passive avoidance responses learned after exposure to qualitatively and quantitatively diverse stimuli.

The results show that the conditioned passive avoidance response was acquired by subjects of all groups and that scopolamine significantly impaired the learning process in a fairly constant measure. This shows that scopolamine exerts its amnesic effect even in a series of repeated trials.

The action of minaprine is equally evident in both experiments. There were no differences between control animals and those to which minaprine and scopolamine had contemporaneously been administered. This means that minaprine provides complete protection against memory impairment caused by scopolamine without any signs of habituation. It should be remembered that minaprine alone does not impair either spontaneous behavior or learned responses.

Some words may be spent on the characteristics of the aversive stimuli employed in the two experiments. In Experiment 1, electric foot-shocks, a classic type of nociceptive stimulation of current usage in avoidance learning paradigms, were administered.

Instead, in Experiment 2 rats acquired the passive avoidance response to avoid caudal handling, an aversive stimulus of a peculiar type. This may be considered less painful than electric foot-shocks and may possibly not be at all painful. Indeed, caudal handling could be better described as an event rather than a stimulus. In fact, as discussed elsewhere (2), caudal handling may vividly simulate predation (4), a very stressful and therefore aversive experience for the rat.

Minaprine's action mechanisms appear to be rather complex. In fact, minaprine is reported to possess direct cholinergic and dopaminergic activities and would also interfere with the serotonergic systems. As regards cholinomimetic activity, thought to be closely related to the mnesic facilitatory effects of this compound, it has been proposed that these effects would be due to the direct stimulation of M1 muscarinic receptors (and not of M2 receptors, thus justifying the absence of motor-stimulating effects) either by minaprine as such or by some of its metabolites (17). On the other hand, minaprine has been reported to act as a dopaminergic agonist, increasing striatal acetylcholine content (7). Furthermore, it has been suggested that minaprine may decrease the inhibitory effects exerted by serotonin on medial septal cholinergic neurons terminating in the hippocampus (11). So, the mechanisms attributed to minaprine, which may act contemporaneously, are consensual in finally increasing cholinergic systems' activity.

Our results, which show the protective action of minaprine on scopolamine-induced amnesia, fully agree with the above-reported mechanisms of action of this compound and confirm the recently expressed opinion that the direct or indirect stimulant action of minaprine on central cholinergic systems may play an important role in the reversal of memory deficits (20).

For the acquisition of the aversive response, the two qualitatively diverse stimuli may involve diverse cerebral circuits or systems. If such were the case, the results of the present work would suggest that scopolamine exerts a generalized amnesic effect, while minaprine protects more than one learning model, possibly because of its several distinct but consensual mechanisms of action.

In conclusion, the results of the present work confirm and amplify our knowledge of the protective, antiamnesic properties of minaprine. The results may further support the hypothesis that minaprine's stimulatory action on central cholinergic neurons may contribute to its protective action against scopolamine-induced amnesia (19). In our opinion, it must also be emphasized that the protective action of minaprine does not appear to decrease after a series of administrations. Finally, the fact that in both paradigms rats underwent a series of trials in which they repeatedly and serially received the active compounds may exclude false-positive results associated with the "drug dissociation" phenomena.

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