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Effect of Scopolamine and Nootropic Drugs on Rewarded Alternation in a T-Maze

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BARTOLINI L., R. RISALITI AND G. PEPEU. *Effect of scopolamine and nootropic drugs on rewarded alternation in a T-maze.* PHARMACOL BIOCHEM BEHAV 43(4) 1161–1164, 1992.—The effects of different doses of scopolamine, and of the nootropic drugs oxiracetam and aniracetam, were investigated on the performance of male Wistar rats in a T-maze requiring a spatial discrimination in the stem (reference memory) and an alternate discrimination in the arms (working memory). Criterion (90% correct responses) was reached within 3 days of daily training for stem and 9 days for arm discrimination. Scopolamine (0.1, 0.2, 0.6, and 1.0 mg/kg, SC, 60 min before session) significantly impaired working memory, as shown by a decrease in the number of correct alternations, without affecting reference memory. Both nootropic drugs (25–50 and 100 mg/kg PO) 30 min before scopolamine) attenuated the working memory impairment induced by scopolamine.

Nootropic drugs Oxiracetam Aniracetam Scopolamine T-maze Alternation

NOOTROPICS are a defined groups of drugs that, in animals, enhances information acquisition, protects against learning and memory impairing agents, lacks sedative or stimulating effect on gross behavior, and shows low toxicity (4,12). One of the most distinctive actions of nootropic drugs is their ability to antagonize learning and memory impairment induced by anticholinergic agents (10). This effect is usually investigated on passive avoidance conditioned response, although prevention of scopolamine-induced amnesia in an eight-arm maze by oxiracetam has also been demonstrated (11). The pyrrolidinone derivatives represent the main group of nootropics. They include piracetam, oxiracetam, aniracetam, pramiracetam, and many others. (8). In this work, the ability of oxiracetam and aniracetam to antagonize the amnesic effect of scopolamine on rewarded alternation in a T-maze, with a stem left/right discrimination, has been studied. By this maze task, two types of memory can be evaluated: long-term reference memory and short-term working memory (7).

METHOD

Subjects

Forty male Charles River Wistar rats, 300–350 g body weight at the beginning of the experiments, were used. Two rats were housed in each plastic cage with free access to water and were maintained on a 16 L : 8 D cycle with light on at 7:00 a.m. Rats were deprived of food to 80% of their ad lib weight and maintained at this weight for the duration of the experiment.

Apparatus

A wooden T-maze similar to that described by Hepler et al. (6), has been used. The stem was 100 cm long and 20 cm large, the arms 40 cm long and 12 cm large, and the wall 12 cm high. A guillotine door separates a starting box from the stem. Eighty centimeters from the starting point the stem is divided by a wooden wall into two halves. The entrance to each half of the stem is covered by a cloth curtain. A Plexiglas barrier can be inserted in a slot 5 cm behind the curtain on one side to block access to the arms on that side. A food cup 2.5 cm wide and 2 cm deep is placed at the distant end of the arms.

Procedure

The procedure previously described (6,7) was modified by reducing the number of trials per session.

Shaping. Each rat was trained for 10 days to explore the maze for food reward for 15 min. For the first 3 days, small pieces (70 mg) of food (Altromin, Rapier, Bolzano) were liberally scattered throughout the maze. During the following 7 days, the amount of food was reduced and its location was restricted until a single pellet was placed in each food cup. During the shaping period, free access to food in the home cage was given for 1 h daily, at 4:00 p.m., at the end of the trial.

Training. Rats were presented with two discrimination choices. The first consisted of a left-right discrimination in the stem of the T-maze and involved reference memory. One

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half of the divided part of the stem beyond the curtain was blocked by a Plexiglas barrier. The other half was open. If the rat chose the open side, a correct response was recorded and the rat was allowed to proceed to the arm. If the rat chose the blocked side, an incorrect response was recorded. The rat was permitted to explore the stem until it found the open side. The second discrimination choice was a rewarded alternation in the arm of the T-maze, involving working memory. It consisted of two runs requiring a forced and a free choice. In the first run, when the rat reached the arms a wooden block forced it to enter the opposite arm, at the end of which it was allowed to eat the food. The rat was then removed from the arm and placed immediately in the starting box. In the subsequent free choice run, the wooden block was removed and the rat, after entering the open half of the stem and reaching the arms, had to enter the previously blocked arm to obtain food. If the rat chose the arm that had been blocked during the forced run, a correct arm response was recorded; if the rat chose the arm in which it had entered in the forced run, an incorrect arm response was recorded. Rats were then returned for 5 min to the home cage before the second trials in which the wooden block was placed in the opposite arm. At the end of the second trial, rats were returned to the home cage. Each day, the rats were given two sessions of two trials, the first in the morning and the second 3–4 h later. Criterion was reached when 90% of the arm responses made by the group of rats in training were correct. Drugs were tested at least 1 week after criterion was reached.

Statistical Analysis

The correct responses performed in each two-trial session were recorded. The accuracy of the performance was expressed as percent of correct responses over the number of trials made by the different groups of rats. The statistical analysis of percent values was performed using the χ^2 test.

Drugs

The following drugs were used: scopolamine HBr (Sigma Chemical Co., St. Louis, MO) oxiracetam (ISF, Trezzano sul Naviglio, Italy), and aniracetam (Roche, Basel, Switzerland). Scopolamine was dissolved in saline and injected SC in a volume of 0.3 ml 60 min before the session; oxiracetam and aniracetam were suspended in methylcellulose 0.5% and administered orally in a volume of 0.3 ml 90 min before the session. Control rats received saline IP and orally.

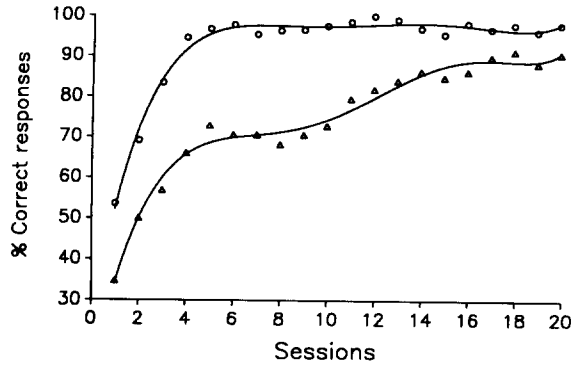

FIG. 1. Rate of acquisition of stem (○ – ○) and arm (△ – △) discrimination in the T-maze.

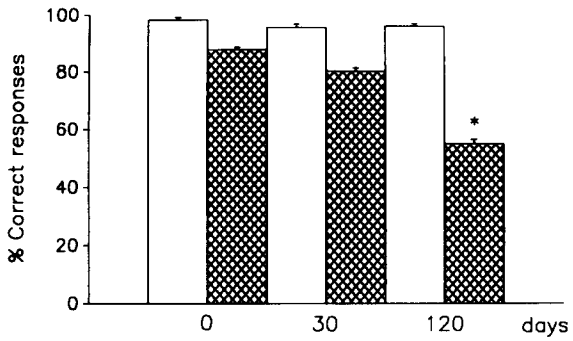

FIG. 2. Rate of extinction of stem (□) and arm (▨) discrimination in the T-maze. Bars indicate SEM. *$p < 0.05$ versus 0 and 30 days of the arm discrimination.

RESULTS

The difference in the rate of acquisition of stem and arm discrimination is illustrated by Fig. 1. In three days, after six sessions of two trials each, rats made more than 90% correct responses in stem discrimination and their performance remained steady in the following days. Conversely, 90% correct responses in the arm discrimination was reached after 18 sessions in 9 days. Even if we consider that each rat made the stem discrimination twice as often as the arm discrimination, the rate of acquisition of stem discrimination was much faster than arm discrimination. At the end of the training sessions and drug experiments, rats were kept without training for 120 days. As shown in Fig. 2, when tested in the T-maze 30 days after the last training session rats showed no or little decrease in the number of correct stem and arm responses; 120 days after training, stem discrimination was still maintained while only 50% of arm responses were correct, indicating a random alternation. No doses of scopolamine, injected SC 60 min before the session, impaired the stem discrimination response. Conversely, injection of 0.1 mg/kg SC scopolamine significantly reduced the number of correct arm discrimination responses, as shown in Fig. 3. With the dose of 0.2 mg/kg random alternation was observed, and this dose was therefore used for the experiments with the nootropic drugs. To ascertain whether the performance was affected by peripheral effects of scopolamine, *N*-methylscopolamine was also tested at doses of 0.2 and 1.0 mg/kg SC. As also shown in Fig. 3, it did not decrease the number of correct arm discrimination responses. Figures 4 and 5 show that both oxiracetam and aniracetam administered orally 30 min before scopolamine significantly prevented the disruption of arm discrimination. A tendency to a bell-shaped dose–effect relationship was seen because the dose of 50 mg oxiracetam and aniracetam was more effective than those of 25 and 100 mg/kg.

DISCUSSION

In our experiments, the maze is identical to that described by Hepler et al. (6) and Lowy et al. (7), but two modifications were introduced in the training procedure to reduce the time spent daily in the training. Namely, rats were given two sessions of two trials per day and the time between the trials of each session was 5 min, while in other experiments (6) rats were given one session of eight trials separated by an intertrial interval of 2 min. Similarities in age, weight, and strain of rats make it possible to compare our experiments with those

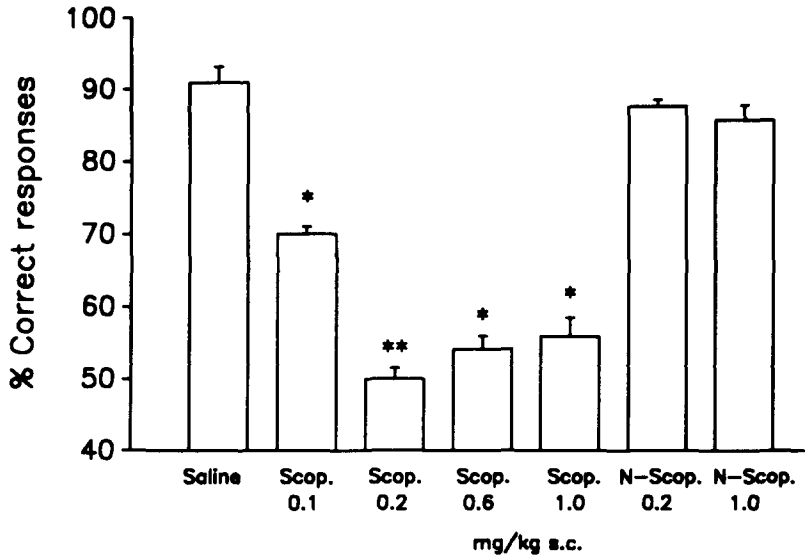

FIG. 3. Effect of scopolamine and *N*-methylscopolamine on arm discrimination in a T-maze. Note the disrupting effect of scopolamine and the lack of effect of *N*-methylscopolamine. Bars indicate SEM. $*p < 0.05$, $**p < 0.01$ versus saline and *N*-methylscopolamine.

of Hepler and colleagues (6). In the latter, both stem and arm discrimination criteria were attained in 5 days. In our experiments, 2 and 9 days were needed to reach stem and arm discrimination criteria, respectively. Stem discrimination was neither impaired by scopolamine administration, in our experiments, nor by lesion of the nucleus basalis and septum, which destroys the cortical and hippocampal cholinergic systems, respectively (6). These findings indicate that formation and recall of reference memory, which is involved in stem discrimination, do not require the integrity of the cholinergic system. On the contrary, the working memory used in arm discrimination is impaired by scopolamine administration in the present experiments and by lesions affecting the cholinergic pathways (6). The impairment of working memory by anticholinergic agents has been repeatedly demonstrated in the rat using a variety of tasks (3,5). Oxiracetam and aniracetam are equally effective in reducing the working memory impairment induced by scopolamine administration. The difference in the effect of the three doses used for each drug is small, with a tendency to the bell-shaped dose-effect relationship, a feature of nootropics (12). In a radial maze, a reduction of the disrupting effect of scopolamine, given at the same dose used in the present experiments, was obtained with 30 mg/kg SC oxiracetam while no effect was seen with 100 mg/kg (11). Rewarded

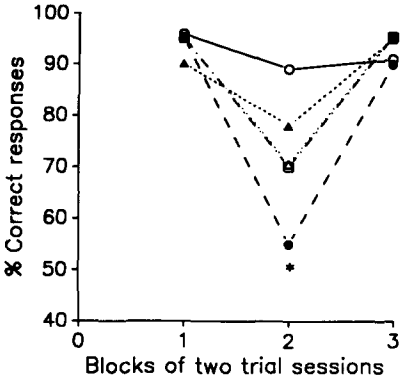

FIG. 4. Oxiracetam antagonizes the disrupting effect of scopolamine on arm discrimination in a T-maze. (○—○), saline PO + saline SC; (●---●), scopolamine 0.2 mg/kg SC; (△ · · · △, oxiracetam 25 mg/kg PO + scopolamine 0.2 mg/kg SC; (▲----▲), oxiracetam 50 mg/kg PO + scopolamine 0.2 mg/kg SC; (□·-·□), oxiracetam 100 mg/kg PO + scopolamine 0.2 mg/kg SC. Drugs were administered before the second block. $*p < 0.05$ versus saline and oxiracetam + scopolamine.

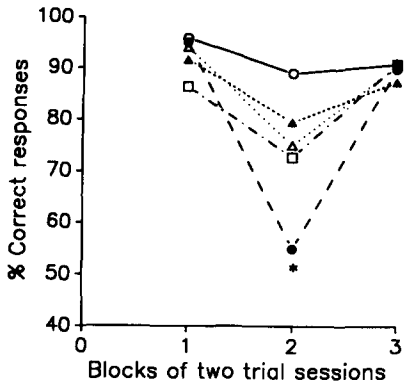

FIG. 5. Aniracetam antagonizes the disrupting effect of scopolamine on arm discrimination in a T-maze. (○—○), saline PO + saline SC; (●---●), scopolamine 0.2 mg/kg SC; (△ · · · △), aniracetam 25 mg/kg PO + scopolamine 0.2 mg/kg SC; (▲---▲), aniracetam 50 mg/kg PO + scopolamine 0.2 mg/kg SC; (□·-·□) aniracetam 100 mg/kg PO + scopolamine 0.2 mg/kg SC. Drugs were administered before the second block. $*p < 0.05$ versus saline and aniracetam + scopolamine.

alternation has not been used frequently for investigating drugs able to reduce the disrupting effect of scopolamine. A reduction of the disrupting effect of scopolamine on alternation performance in a two-lever rat chamber was obtained with physostigmine, while muscarinic cholinergic agonists, including arecoline, and tetrahydroaminoacridine were ineffective (13). In a T-maze rewarded alternation task with a delay of 60 s between forced and free run, idebenone was able to reduce the disrupting effect of scopolamine (9). Similarly, in an elevated T-maze both the 5-hydroxytryptamine$_3$ antagonist ondansetron and arecoline were able to reduce the disrupting effect of subchronic treatment with scopolamine during pretraining and training days (1). Scopolamine disrupts the alternation task by blocking M_1 and $_2$ muscarinic receptors and preventing the action of acetylcholine released from the cholinergic nerve endings in the cerebral cortex and hippocampus. A similar disruption is obtained with excitotoxic lesions destroying the same pathways (6). Physostigmine, by increasing extracellular acetylcholine levels, and arecoline, by acting directly on the receptors, may overcome scopolamine blockade and reduce the disruption of rewarded alternation. Evidence indicates that ondansetron increases cholinergic function by preventing the inhibitory effect of 5-hydroxytryptamine on acetylcholine release occurring in some brain regions (2). An interaction of the pyrrolidinone derivatives (10) and of idebenone (9) with brain cholinergic mechanisms has also been demonstrated, although none of these drugs shows direct cholinomimetic effects. Impairment and restoration of brain cholinergic function seem, therefore, to result in disruption and recovery of the working memory needed for performing correctly the rewarded T-maze alternation.

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