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Antagonism of Scopolamine-Induced Memory Impairments in Rats by the Muscarinic Agonist RU 35 926 (CI-979)

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M'HARZI, M., A.-M. PALOU, C. OBERLANDER AND F. BARZAGHI. *Antagonism of scopolamine-induced memory impairments in rats by the muscarinic agonist RU 35 926 (CI-979).* PHARMACOL BIOCHEM BEHAV **51**(1) 119–124, 1995. – The promnesic effects of RU 35 926 (CI-979), a muscarinic receptor agonist, were evaluated on memory impairments induced by the muscarinic antagonist scopolamine, using a radial arm maze task, in comparison with tetrahydroaminoacridine (THA), a cholinesterase inhibitor. Groups of rats were trained in a standard version of the radial maze until they had attained an asymptotic level of performance. The animals were then retested with one trial a day. Twenty minutes before each retest, the rats were given subcutaneous administration of 0.1 mg/kg scopolamine. Oral administration of RU 35 926 (0.02, 0.05, 0.1, 0.2, and 0.5 mg/kg) 30 min before memory retest markedly reduced or suppressed the scopolamine-induced deficit. This reduction was evidenced by a significant decrease in the different types of errors and an increase in the number of correct responses. THA (3 mg/kg, intraperitoneally or orally) given 20 min to testing also significantly reduced or suppressed the scopolamine-induced deficits. These results show that RU 35 926 possesses the capacity to reduce memory impairments induced by a deficit of cholinergic transmission in the rat.

RU 35 926 (CI-979) Muscarinic receptor agonists Working memory Transient amnesia Radial maze THA

DECLINE in memory is one of the main symptoms of Alzheimer's disease. Numerous studies have related memory and other cognitive dysfunctions to central cholinergic pathology (3,4,8,17,21,25). One of the models used to mimic aspects of the cognitive dysfunctions in demented patients is based on cholinergic blockade by anticholinergic drugs (e.g., scopolamine, a competitive blocker of muscarinic receptors) in animals (1,5,7,16) and in human volunteers (6,13,22). In animal models, enhancing cholinergic transmission with cholinomimetic agents or acetylcholinesterase inhibitors reduces or suppresses experimental memory deficits (20).

The present experiments were conducted to investigate the capacity of RU 35926 (CI-979) (1-methyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime hydrochloride), a new, orally active, muscarinic agonist (14,24) to reverse scopolamine-induced transient memory impairments. [The cholinergic muscarinic agonist is co-developped by Roussel UCLAF (RU 35926) and Warner Lambert-Park Davis (CI-979).] For this, the working memory (WM) of the rat was studied in the radial maze. Numerous data indicate that this form of memory is particularly affected in subjects suffering from Alzheimer's disease (12). Tetrahydroaminoacridine (THA), an acetylcholinesterase inhibitor, the efficacy of which in alleviating memory impairments was recently demonstrated in patients with dementia of Alzheimer's type (9,11,23), was used here as the reference compound. A preliminary abstract of the present experiments was published elsewhere (19).

MATERIALS AND METHODS

Subjects

The experiments were conducted in male rats of the Wistar strain, about 2 mo old, weighing 180–200 g at the beginning of the experiments, supplied by Iffa Credo. They were housed in individual cages and kept in an air-conditioned animal room at a temperature of 23 ± 1°C with artificial light on a

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24-h cycle (light phase, 0700–1900 h). During the 7–10 days preceding the beginning of the experiment the animals were handled individually in the animal house once a day for 5 min. During the test the animals were placed on a restricted diet with their weight maintained at 80–85% of its normal value. For this the daily diet (Ref. A04; UAR France) was adjusted to about 5% of the animal's body weight and adapted when necessary.

Apparatus

The animals were trained in an elevated eight-arm radial maze, 70 cm above the floor. It was composed of an octagonal central platform 30 cm wide with eight identical arms, 84 cm long and 10 cm wide, radiating out from the center at an angle of 45° from each other. The central platform and arms were equipped with transparent plastic parapets high enough to prevent the animal from falling, but not from seeing all the surrounding cues. The apparatus was automated and controlled by microcomputer, permitting: a) the programmed distribution of 45 mg food pellets (Bio-Serv DPP) to act as positive reinforcers at the far end of the arms; b) the opening and closing of the doors, either to keep the animal at the centre or to allow it access to the arms; c) the recording of all choices and patterns and the real-time monitoring of the animal's progress. The testing room was adjacent to the animal house. The room lighting was adjusted to obtain 10 lx in the maze. Unwanted noises were masked by a white noise of 60 dB. The experiments were conducted between 0800 and 1800 h.

Procedures

The behavioural procedures used in the experiments reported here have been described in detail elsewhere (18). Several extramaze cues (crosses, circles, horizontal or vertical lines, etc.) were present on the walls; there were, however, no explicit intramaze cues. Throughout the experiments, the internal layout in the testing room remained unchanged and the maze remained in the same position with regard to surrounding cues. Prior to acquisition, the animals underwent three habituation sessions of 5 min, one a day. During this time, food pellets were available on the maze, scattered on the arms, and the animal could explore the maze. The procedure for each learning trial was as follows: Each rat was placed on the central platform; each day, the test was continued until the eight reinforcers had been obtained, or the animal had made 16 choices (correct or incorrect), or a period of 8 min had elapsed. Arms from which food had already been taken were no longer rewarded. A response was automatically counted when the animal entered (or engaged its four paws in) an arm. The first entrance into an arm was counted as a correct choice and a return to an arm already visited as a WM error. The daily food ration was given to each animal 15–30 min after being returned to the home cage. In both experiments, prior training under drug conditions, the rats were given a minimum of 20 trials so that they attained the criterion of not more than one WM error to the first eight choices throughout five consecutive trials.

Transient memory impairment was induced by administration of 0.1 mg/kg scopolamine, chosen on the basis of a pilot study in separate animals; lower doses showed only slight amnesic effects and higher doses produced motivational deficits for searching for food [see also (10)]. The selected dose proved its efficacy in producing significant memory deficits [see also (27)]. Data collected from previous behavioral trials (not shown here) revealed that RU 35926 (0.02–2.5 mg/kg) was devoid of peripheral side effects such as hypotonia, diarrhea, lacrimation, and salivation, except for the higher tested dose. Also, in the present study RU 35926 was tested in a range of doses between 0.02 and 0.5 mg/kg. THA was used at a dose of 3 mg/kg, which causes few peripheral symptoms in the rat (pilot experiments not shown here).

RU 35926 and THA (Sigma) were dissolved in distilled water, and scopolamine (hydrobromide; Sigma) in 0.9% NaCl solution. RU 35926 was administered orally and scopolamine-injected subcutaneously (SC). THA was either administred orally (Experiment 1) or intraperitoneally (IP) (Experiment 2); the volumes were 5 ml/kg for the oral route and 2 ml/kg for the IP and SC routes. RU 35926 was administered 30 min, and THA and scopolamine 20 min prior to testing. The controls received the same volumes of corresponding vehicles.

In both experiments, trained rats were assigned to subgroups and treatments according to a pseudo-Latin-square design, such that all subjects received the six treatments in a counterbalanced order. The rats were submitted to one trial per day and the compounds were administered every 2 days. On the day on which the animals were not treated they received the vehicle but were still tested in the maze.

The animals' performance in the radial maze was evaluated by recording the different types of errors committed and the correct responses made during a trial, namely: a) the number of errors to the first eight choices: b) the number of errors to eight arms; c) the number of error perseverations, i.e., a reentry to an arm already revisited; d) the number of correct choices before the first error; and e) the percentage of reinforced choices, as an index of efficiency: (number of reinforced arm entries × 100)/total number of arm entries. Re-

TABLE 1

EFFECTS OF RU 35 926 AND THA ON SCOPOLAMINE-INCREASED RESPONSE LATENCIES

	VEH + VEH	SCO + VEH	THA + SCO	RU 35926 + SCO		
Experiment 1		0.1 mg/kg	3 mg/kg	0.1 mg/kg	0.2 mg/kg	0.5 mg/kg
	12 ± 1	19 ± 3*	19 ± 3*	15 ± 2	20 ± 3*	21 ± 3*
Experiment 2		0.1 mg/kg	3 mg/kg	0.02 mg/kg	0.05 mg/kg	0.1 mg/kg
	9 ± 1	18 ± 2†	15 ± 1†	12 ± 1‡	13 ± 1*§	16 ± 2†

Data represent means ± SEM duration (s) to complete an arm in the maze. The route for THA was oral in Experiment 1 and IP in Experiment 2. VEH, vehicle; SCO, scopolamine. *,†$p < 0.05$ and 0.01 vs. VEH. ‡,§$p < 0.05$ and 0.01 vs. SCO.

sponse latencies under each treatments were also evaluated (Table 1).

Data Analysis

Data analysis was performed on the six behavioral variables described earlier, using a subjects × treatments ANOVA design (26). Whenever statistical ANOVAs are obtained, the pairwise comparisons were performed by Duncan's multiple-range test. To make the variances more homogeneous and avoid the measurements (number of errors) that were equal or close to zero (26), adequate transformations were made as follows: $\text{Log}(X + 1)$ for the three types of errors; $(X + 0.5)^{0.5}$ for the correct choices before error and response latencies; $(X)^{0.5}$ for the index of efficiency. Differences were considered significant at $p < 0.05$. Results are expressed in bars as means ± SEM and differences indicated at the $p < 0.05$ and $p < 0.01$ levels.

RESULTS

As expected, in both experiments, at 0.1 mg/kg, scopolamine caused marked memory impairments, as reflected by a significant increase in the different types of errors and a marked decrease in correct choices and in efficiency. The results are shown in Figs. 1 and 2, with $p < 0.01$ in all comparisons. Although scopolamine significantly slowed the animals' running speed to complete the trial in the maze (Table 1), doses of RU 35926 antagonized this effect to some degree. A total of six rats in both experiments were unable to complete testing under scopolamine and were therefore dropped from the study. Data analysis was carried out for 19 rats in Experiment 1 and 29 rats in Experiment 2.

Experiment 1

Number of errors to the first eight choices. (Fig. 1) Significant differences among treatments were observed [$F(5, 108) = 5.98$, $p < 0.0001$]. THA and the 0.1-mg/kg dose of RU 35926 significantly reduced the memory deficit ($p < 0.05$). However, under RU 35926 at higher doses no significant improvement of performances was observed.

Errors to eight arms. There was a significant differences among treatments [$F(5, 108) = 8.67$, $p < 0.0001$]. Both THA ($p < 0.01$) and RU 35926 ($p < 0.05$ and 0.01) significantly reduced the deficits.

Total number of error perseverations. This type of error was lowered by THA and the three doses of RU 35926; the animals under these treatments were not significantly different compared either to VEH or SCO conditions [$F(5, 108) = 2.3$, $p < 0.05$].

Number of correct choices before the first error. Although the overall analysis revealed significant differences among treatments [$F(5, 108) = 4.25$, $p < 0.01$], only RU 35926 (0.1-mg/kg dose) significantly reduced the incapacitating effect of scopolamine ($p < 0.05$). There were, however, no significant improvement with either THA or the higher doses of RU 35926.

Percent efficiency. Significantly reduced by scopolamine [$F(5, 108) = 7.36$, $p < 0.0001$] (95.5% in VEH vs. 66.3% in SCO), this index of efficiency was significanly improved by both THA and the three doses of RU 35926 in scopolamine treated rats ($p < 0.5$–0.01).

Experiment 2

(Fig. 2)The main results from Experiment 1 show that the three doses (0.1–0.5 mg/kg) of RU 35926 significantly allevi-

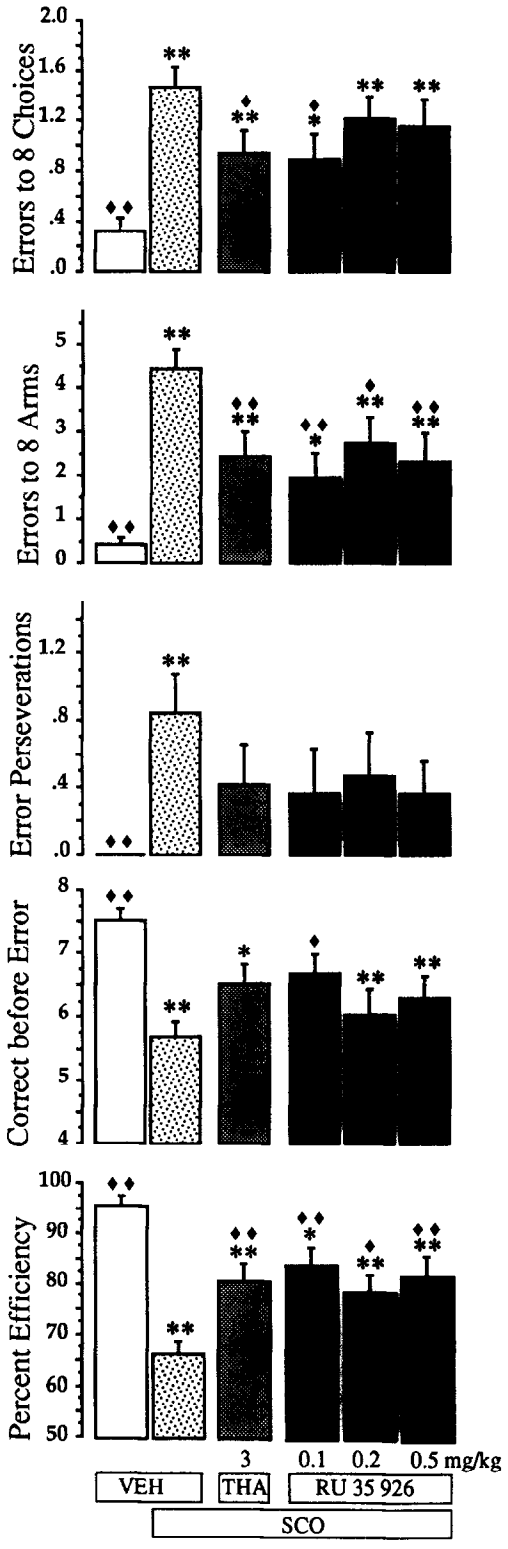

FIG. 1. Effects of RU 35 926 and THA on scopolamine-induced working memory impairments. Each bar represents mean values (± SEM) following vehicle (VEH), scopolamine (SCO), and scopolamine plus THA or one of the three doses of RU 35 926. ♦, ♦♦: $p <$ 0.05 and 0.01, respectively, vs. SCO; * and **$p < 0.05$ and 0.01, respectively, vs. VEH.

ate scopolamine-induced memory deficits. THA at 3 mg/kg administred orally had similar effects. Experiment 2 investigated the effects on scopolamine-induced memory deficits of lower doses (0.05 and 0.02 mg/kg) of RU 35926; the 0.1-mg/kg dose was used as the most effective one in Experiment 1. THA was administred IP at the same dose as in Experiment 1.

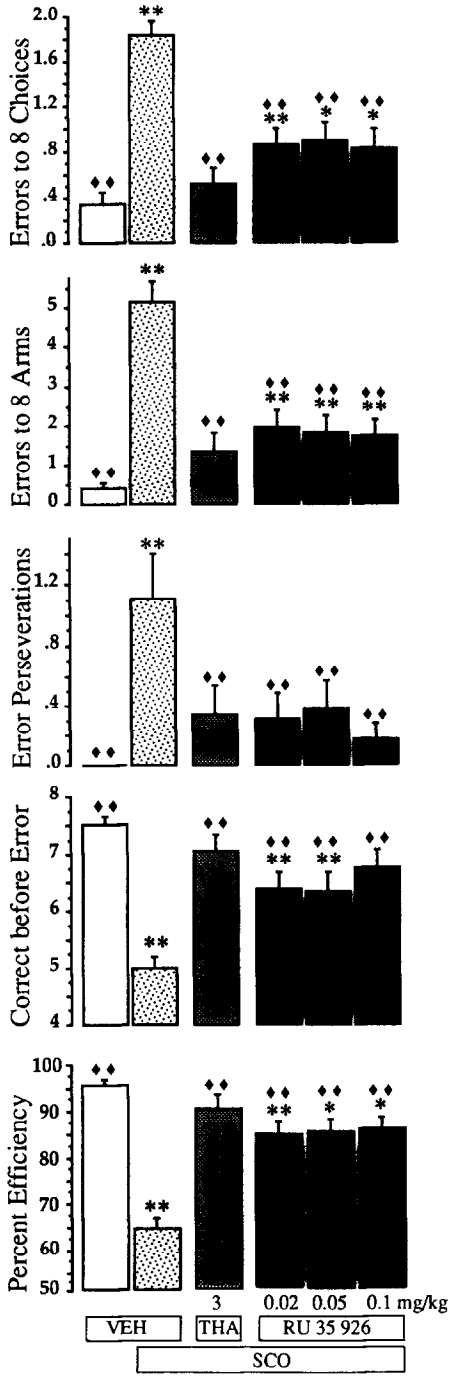

FIG. 2. Effects of RU 35 926 and THA on scopolamine-induced working memory impairments. Abbreviations and symbols as in Fig. 1.

The results obtained in Experiment 2 corroborate those described earlier in that RU 35926 significantly alleviated scopolamine-induced memory impairments. The main results are as follows: a) ANOVA comparing the treatments reached high significance on the number of errors to the first eight choices [$F(5, 168) = 12.50, p < 0.0001$]. The three doses (0.02, 0.05, and 0.1 mg/kg) of RU 35926 significantly reduced the memory deficit ($p < 0.01$). THA (3 mg/kg, IP) completely reversed this deficit. b) In terms of errors to eight arms, there was again a highly significant alleviation of the memory deficits by both THA and the three doses of RU 35926 ($p < 0.01$) [among treatments differences, $F(5, 168) = 16.73, p < 0.0001$]. c) For the error perseverations, although scopolamine induced significant memory impairment [$F(1, 168) = 5.37, p < 0.0001$], THA and the three doses of RU 35926 almost completely reversed this deficit; the animals under these treatments were not significantly impaired, compared to VEH conditions. d) Although comparisons among treatments showed significant differences [$F(5, 168) = 8.29, p < 0.0001$], both THA and the three doses of RU 35926 significantly enhanced the number of correct choices before error ($p < 0.01$). THA and RU 35926 (0.1 mg/kg) abolished the incapacitating effect of scopolamine; the animals were not impaired, compared to VEH conditions. e) Percent efficiency was significantly reduced by scopolamine [$F(5, 168) = 16.74, p < 0.0001$] (95.6% in VEH vs. 63.2% in SCO); this index of efficiency was significantly improved by both THA and the three doses of RU 35926 in scopolamine-treated rats ($p < 0.01$). It should be noted that THA (3 mg/kg, IP) completely reversed the effects of scopolamine on the aspects of memory measured in the present experiment.

DISCUSSION

Our results clearly show that RU 35926 and THA antagonised the deleterious effects of scopolamine on memory, probably by enhancing cholinergic transmission. THA administred orally exhibited less efficacy compared with the same dose administered IP. RU 35926 showed evidence of promnesic effects at very low doses (0.02 mg/kg) compared with those of THA [1-3 mg/kg; see, for example, (20)]. When considering unwanted cholinergic side-effects (hypotonia, diarrhea, lacrimation, salivation, etc.), no such symptoms were observed following administration of the promnesic doses of RU 35926 used in the present experiments. As mentioned earlier, these side-effects appeared following oral administration of 2.5 mg/kg of RU 35926 to scopolamine-untreated rats. It should be noted that THA (3 mg/kg, IP) almost fully counteracted the deleterious effects of scopolamine (Fig. 2). It would appear from the present data that the lower doses (ranging from 0.02–0.1 mg/kg orally) of RU 35926 were more effective than higher doses (0.2 and 0.5 mg/kg orally) in antagonizing the effects of the muscarinic antagonist scopolamine on behavior. Further experiments with larger range of doses are needed to determine the dose response of RU 35926.

Scopolamine did not produce global incapacitation in the animals that were included in the study, because the animals, although admittedly very impaired, remained capable of making correct responses and accomplishing the memory test. Although the animals under scopolamine were significantly slower in completing the task, they were still motivated to search for food pellets [see also (10) and (16)] and able to use the general rules concerning the radial maze task. Moreover, even doses (Experiment 1) of RU 35926 that did not signifi-

cantly antagonize the effects of scopolamine on animals' mobility in the maze nevertheless improved memory performance significantly. This suggests that the transient amnesia did not result from a sensory or motor or motivational incapacity, or if so only to a small extent, but rather from difficulty in using the visuospatial information relevant to each stage of the test. Therefore, RU 35926 might act, with a degree of selectivity, on memory functions.

The form of memory involved in the radial maze test as employed here is the working memory. It might be considered as the equivalent of recent memory in humans, a type of memory that is impaired following cholinergic dysfunctioning produced by either scopolamine or ageing. In a specific temporospatial context the working memory enables the animal to organise its own responses by bringing relevant information to the task in hand. It "temporarily stores information as part of the performance of complex cognitive tasks . . . " (2). The enhancing effect of RU 35926 on performances is particularly marked when the improvement in memory integrity is considered, as measured by the number of correct choices before error, by error perseverations, or by efficiency of performance. One of the factors that can disrupt the animal's memory during a trial is proactive interference. Examination of all the variables measured indicates that RU 35926 improves the animal's overall capacity, under the effect of scopolamine, to organize and use the information contained in recent memory, probably by rendering the animal less vulnerable to this type of interference. It is also possible that RU 35926 enhances the animal's performance by alleviating the disturbing effects of scopolamine on sustained attention (6,23).

Finally, the improvement in the performances of animals rendered deficient by scopolamine strongly suggests that RU 35926 exerts its cholinomimetic action on the central cholinergic systems involved in memory. The present pharmacologic findings support the general studies from both humans and animals attributing to the cholinergic systems an important role in learning and memory. In conclusion, RU 35926 possesses a multifaceted promnesic action, and is therefore of potential interest for the treatment of the symptoms characteristic of the deterioration of cognitive functions in Alzheimer's disease (9,23).

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