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# Ameliorating Effects of RU 47213, A Novel Oral and Long-lasting Cholinomimetic Agent, on Working Memory Impairments in Rats

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M'HARZI, M., F. WILLIG, C. GIEULES, A.-M. PALOU, C. OBERLANDER AND F. BARZAGHI. *Ameliorating effects of RU 47213, a novel oral and long-lasting cholinomimetic agent, on working memory impairments in rats.* PHARMACOL BIOCHEM BEHAV **56**(4) 663–668, 1997.—The anti-amnesic effects of RU 47213 [1-(4-chlorophenoxycarbonyl)-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime], a prodrug with oral and long-lasting cholinergic activity, were evaluated on working memory impairments, using tasks of unequal levels of difficulty involving the same reinforcement and motivation in rats: a spatial-based task in a radial maze and a delayed reinforced alternation task in a T-maze. Tetrahydroaminoacridine (THA; tacrine), a cholinesterase inhibitor, was used as a reference. Groups of rats were trained in an automated radial maze or T-maze until they had attained an asymptotic level of performance. On test days, memory impairment was produced by administration of scopolamine (0.1 mg/kg SC) 15 min prior to testing. Both THA (1, 3, and 5 mg/kg) and RU 47213 (0.2, 0.5, 1, and 2 mg/kg) given prior to testing markedly reduced or suppressed the scopolamine-induced working memory deficits in both tasks. This activity was evidenced by either a significant decrease in the number of errors or an increase in the number of correct responses.These results show that RU 47213 possesses the capacity to reduce memory deficits induced by an impairment of cholinergic transmission in the rat © 1997 Elsevier Science Inc.

RU 47213 Muscarinic receptor agonists Scopolamine THA Working memory Radial maze T-maze

DECLINE in memory is one of the main symptoms of Alzheimer's disease. Numerous studies have related loss of memory and cognitive dysfunction to central cholinergic pathology (3,7,12). One of the models used to mimic aspects of the cognitive dysfunction in demented patients is based on cholinergic blockade by anticholinergic drugs (e.g., scopolamine, a competitive blocker of muscarinic receptors) in animals (1,6) and in human volunteers (5,10,20). In animal models, enhancing cholinergic transmission with cholinomimetic agents or acetylcholinesterase inhibitors reduces or suppresses experimental memory deficits [e.g., (18)].

At present, a number of cholinergic agents are being studied for their anti-amnesia efficacy in animal models of cognitive dysfunction and in clinical trials (22). One approach is the indirect stimulation of cholinergic receptors by the use of tetrahydroaminoacridine (THA; tacrine), a potent reversible cholinesterase inhibitor, the efficacy of which in alleviating memory impairments was recently demonstrated in patients with dementia of the Alzheimer type (11,21). Based on clinical observations that show significant degeneration of cortical cholinergic endings but relatively preserved postsynaptic muscarinic receptors (16), the other promising approach is aimed at the direct stimulation of these receptors. It has recently been reported that continuous intravenous infusion of arecoline, a muscarinic receptor agonist at tolerated doses, improves memory in Alzheimer patients (23). Barzaghi et al. (4) and Toja et al. (24) have recently introduced RU 47213, a novel tetrahydropyridine-oxime that is cleaved in vivo to form an active metabolite, RU 35963, a muscarinic receptor agonist that is nonselective with respect to receptor subtypes (4,13). RU 47213 showed a long-lasting central muscarinic activity (as evaluated by the time course of the hypothermic effect),

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induced synchronization of scopolamine-sensitive, rhythmical slow-wave activity, and increased cortical blood flow in rats. Moreover, RU 47213 was able to antagonize the scopolamine-induced deficit of a passive avoidance task in mice. It was also shown that after oral administration, RU 47213 is superior to arecoline in terms of potency, central selectivity, and duration of action (4).

The aim of the present experiments was to study the capacity of RU 47213 to reverse scopolamine-induced working memory (WM) impairments in rats, using the most manipulated tests of WM for rats, the spatial-based task in an eight-arm radial maze and the delayed reinforced alternation task in a T-maze. Numerous data indicate that this form of memory is particularly affected in subjects suffering from Alzheimer's disease (9). Tacrine, approved by the FDA for the palliative treatment of cognitive dysfunction in Alzheimer's disease, was used here as the clinical reference compound.

## MATERIALS AND METHODS

### *Radial Maze Task*

*Subjects.* The experiments were conducted in male rats of the Wistar strain supplied by Iffa Credo; the rats were 7 weeks old and weighed 180–200 g at the beginning of the experiments. They were housed in individual cages and kept in an air-conditioned animal room at a temperature of 23 ± 2°C with artificial lighting on a 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 behavioral testing, the animals were placed on a restricted diet and kept at 80–85% of their free-feeding weight and allowed to gain 5 g body weight per week. To achieve this, the daily diet (UAR France, Ref. A04) was adjusted to about 5% of the animal's body weight and adapted as 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 one another. Vertical sliding doors were placed at the entrance to each arm. The central platform and the arms were equipped with transparent plastic parapets high enough to prevent the animal from falling but not from seeing surrounding cues. Infrared cells were installed atdifferent levels of the maze. 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/closing of the doors (lowering/raising of doors, respectively), either in order to keep the animal at the centre or to allow it access to the arms; c) the recording of all the choices and patterns and the real-time monitoring of the animal's behavior. The maze was located in a soundproof air-conditioned (22 ± 2°C) testing room adjacent to the animal house. The room lighting was adjusted to obtain 10 lux in the maze. Unwanted noises were masked by white noise of 60 dB. The internal arrangement and the apparatus in the testing room remained unchanged throughout the experiments.

*Procedures.* The behavioural procedures used in the experiments reported here have been described previously (16). 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 per day. During this time, food pellets were available in the maze, scattered on the arms, and the animal could explore. The procedure for each learning trial was as follows. The rat was placed on the central platform, and the test was continued until the eight reinforcers had been obtained or until 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 to 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 in the first eight choices throughout five consecutive trials. The animals were tested during the daylight phase of the cycle.

Memory impairment was induced by administration of 0.1 mg/kg of scopolamine. This selected dose proved its efficacy in producing significant memory deficits (17). Data from previous reports (4,24) revealed that RU 47213 (0.2–4 mg/kg) administered orally is devoid of peripheral side effects such as hypotonia, diarrhoea, lacrimation, or salivation. THA was used at doses ranging from 1 to 5 mg/kg, administered orally, which causes few peripheral symptoms in rats (17).

RU 47213 [1-(4-chlorophenoxycarbonyl)-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-*O*-methyloxime] (batch no. 4, Roussel UCLAF, Romainville, France) was suspended in a methylcellulose solution (0.5%) supplemented with a few drops of Tween 80. THA (batch no. 117F3657, Sigma, St. Louis, MO, USA) was dissolved in distilled water, and scopolamine hydrobromide (batch no. 39F0846, Sigma) was dissolved in 0.9% aqueous NaCl solution. The volumes were 5 ml/kg of body weight for RU 47213 and THA administered orally, and 2 ml/kg for scopolamine injected subcutaneously. RU 47213, THA, and scopolamine were administered 120, 20, and 20 min, respectively, before memory testing. The controls received the same volume of the corresponding vehicles. All doses refer to the salt. The pH of the solutions was between 6 and 6.5.

Two experiments were carried out. In Experiment 1, trained rats were assigned to subgroups and treatments according to a pseudo Latin square design so 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 and were still tested in the maze (wash-out day). Experiment 2 was performed to eliminate any effects that might be associated with administration of several compounds to the same animal: the rats from the same shipment of animals were divided into control and treated groups, each of them receiving a single compound. They performed two trials, one per day.

Rats given vehicle made very few, if any, errors in the first eight choices. Memory performance was therefore evaluated by recording the total number of errors, i.e., errors to complete eight arms, and the number of correct choices until the first error, for one trial in Experiment 1 and for two trials in Experiment 2.

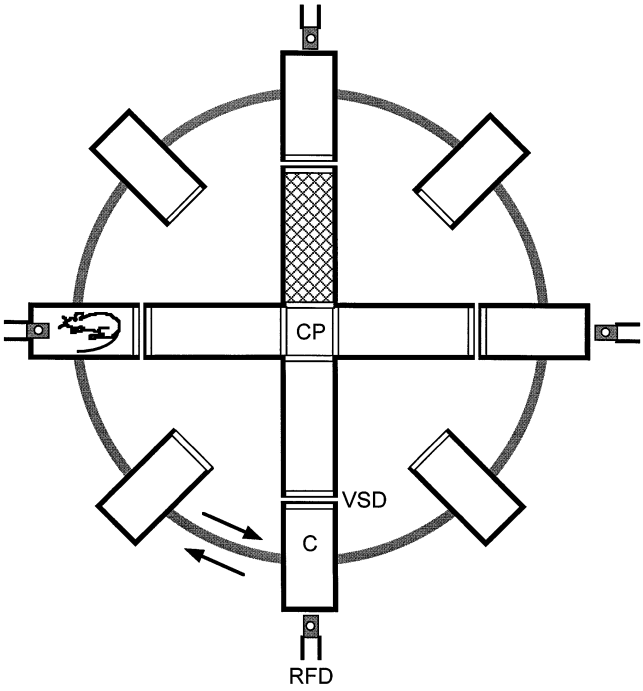


FIG. 1. Schematic drawing of the automated T-maze. The cross-hatched arm was not used. Arrows indicate carriage rotation. C, carriage that serves as start box or goal box; CP, choice point; RFD, retractable food dispenser; VSD, vertical sliding door.

*Delayed Reinforced Alternation*

*Subjects.* The experiment was conducted in male rats of the Long–Evans strain (supplied by Charles River) that were 2 months old and weighed 230–250 g at the beginning of the experiments. The other conditions were the same as described above.

*Apparatus.* The maze, made of grey PVC, consisted of a 15-cm square central platform, designated as the choice area, and four identical arms, 40 cm long and 15 cm wide, that radiated out from this central platform at right angles to one another. At the periphery of the maze, eight carriages, 30 cm long and 15 cm wide and at an angle of 45° to one another relative to the choice area, served either as start or goal box (Fig. 1). This allowed the animal to be transported from the end of one arm to that of another in a circular movement. The maze had grey PVC walls 40 cm high and transparent lids. A vertical sliding door positioned at the entry to each carriage and on each side of the choice area enabled access to certain arms to be cut off and the animal to be confined in the carriage, particularly while being transported from one point of the maze to another. A food pellet dispenser located at the end of the carriages opposite each of the four arms gave the animal access to its food reinforcement.

The apparatus was automated and controlled by microcomputer, using a program developed jointly by F. Willig and the Imetronic Company (33600 Pessac, France). The program permitted all the events of an experiment to be monitored in real time and recorded. The maze was located in a room similar to that described above. The internal arrangement and the whole of the experimental apparatus in the experimental room remained unchanged throughout the experiment. For the behavioral procedure used in the experiments reported here, the maze was configured in the shape of a T-maze, i.e., three of the four available arms were used, with one arm being used as the starting point and the two others as the goal arms.

*Procedure.* The behavioural procedures used here were described elsewhere (25). Each rat underwent a habituation session lasting 5–10 min daily for 5 days, during which it was familiarised with the apparatus. The rats were then given about 20 learning sessions with one session daily except on the weekend. The rats were tested individually, in pairs. For each of them, a session comprised 10 trials separated from one another by an intertrial interval of 75 s, during which time the other animal was tested. Each trial was itself divided into two parts, a forced choice followed by a free choice, separated from one another by an intratrial interval of 15 s.

At the beginning of each session, two rats were placed individually in adjacent carriages with the doors closed. The carriage containing the first animal to be tested was transported to the start point. Fifteen seconds later the door of the carriage was opened: this was the forced choice in which access to one of the arrival arms (right or left depending on a pseudo-randomly determined sequence) was blocked off. The rat had to explore the accessible arm and the carriage at its end. Once the rat entered the carriage, it was confined for 2 s and allowed to eat the food pellet. This carriage then moved to the start point. Fifteen seconds (intratrial interval) after transportation had started, the rat was allowed to explore freely one of the two arms and carriages of the maze. The procedure used was noncorrective. The animal was considered to have chosen an arm when its four paws had crossed into it. Entry into the arm not visited during the preceding forced choice was considered as correct response and the rat was given three food pellets. Conversely, entry into the already-visited arm was counted as an error and the rat was not reinforced. Two seconds after the arrival of the animal in the choice point, the intertrial interval for this animal began, during which time the carriage containing the other rat was transported to the starting point to be tested for the first trial. When the second animal had completed its first trial, the second trial began for the first animal. The procedure used was the same for the following trials.

Each session ended when each of the animals had performed 10 trials. Thirty minutes after the end of the session, each animal received its daily food ration. At the end of this learning phase, the animals that had achieved the learning criterion (90% correct responses in three consecutive sessions) were retained for the study phase of the compounds. WM performance was assessed by scoring the number of correct responses made during each session of 10 trials.

RU 47213 and THA were administered orally and scopolamine SC 120, 30, and 15 min, respectively, before memory testing. The doses used were 0.2, 0.5, 1, and 2 mg/kg for RU 47213; 1, 3, and 5 mg/kg for THA; and 0.1 mg/kg for scopolamine. The other conditions concerning the drugs were the same as those described for the radial maze. The trained rats ($n = 11$) were given the treatments according to a pseudo Latin square design, such that all subjects received all the treatments twice in a counterbalanced order.

*Data analysis.* Data analysis was performed on the number of errors to eight arms and the number of correct responses until the first error in the radial maze task (Experiments 1 and 2), and on the number of correct responses in the T-maze task (Experiment 3). Subject-by-treatment (Experiments 1 and 3) and group-by-treatment (Experiment 2) ANOVA designs (26) were used. Whenever statistically significant ANOVAs were obtained, the pairwise comparisons were per-

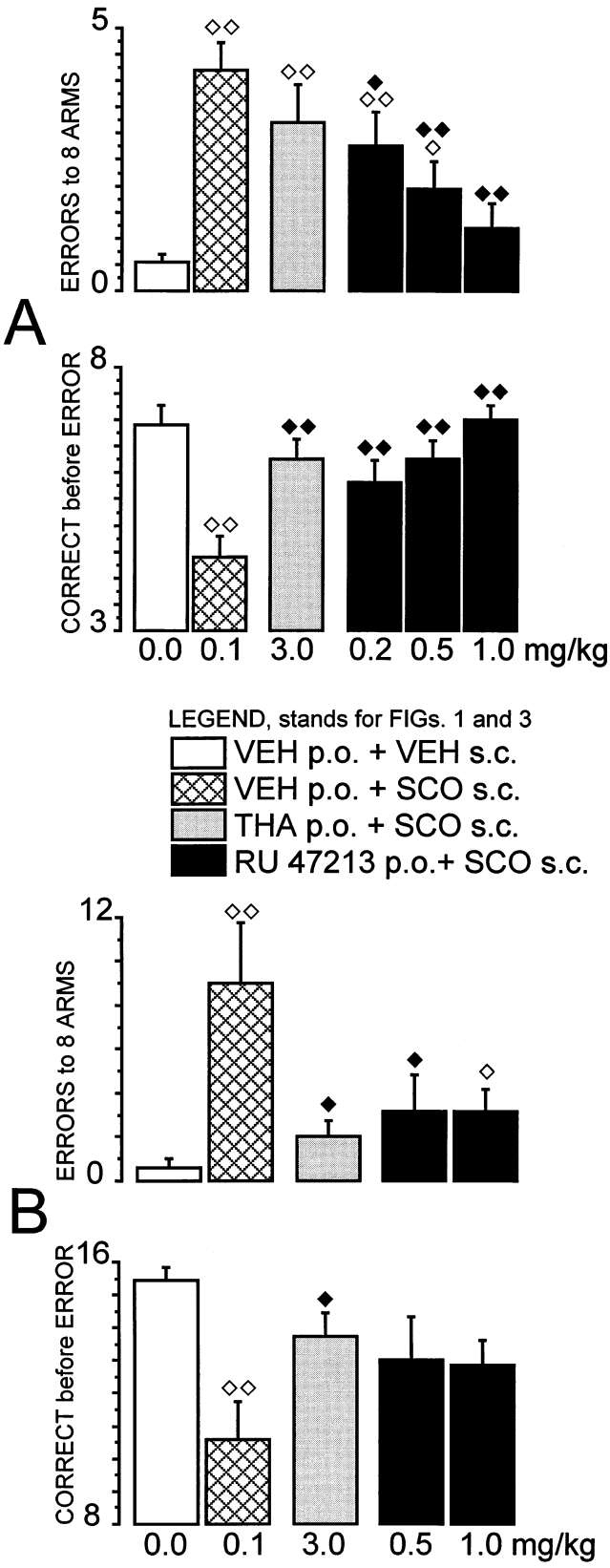


FIG. 2. Effects of RU 47213 and THA on scopolamine-induced memory impairments (radial maze task). (A) and (B) refer to Experiments 1 and 2, respectively. Each bar represents mean value (+SEM) following vehicle (VEH), scopolamine (SCO), SCO plus THA, or one of the three doses of RU 47213 for one trial in A and two trials in B. ◆, ◆◆: $p < 0.05$ and $p < 0.01$, respectively, vs. SCO; ◇, ◇◇: $p < 0.05$ and $p < 0.01$, respectively, vs. VEH; ANOVA followed by Duncan's multiple-range test. Sample sizes: $n = 20$ rats (subject-by-treatment design) in Experiment 1 and $n = 7$ rats per group in Experiment 2 (group-by-treatment design).

formed by Duncan's multiple-range test. To make the variances more homogeneous and reduce the measurements (number of errors) that were equal or close to zero (26), adequate transformations were made as follows:

$$\log(X + 1)$$

for the number of errors, and

$$(X + 0.5)^{0.5}$$

for the correct choices before error (radial maze) and for the number of correct responses (T-maze). Differences were considered significant at $p < 0.05$. Results are expressed in bars as mean ± SEM, and significant differences are indicated at $p < 0.05$ and $p < 0.01$ levels.

## RESULTS

In the three experiments, 0.1 mg/kg scopolamine caused marked memory impairments, as reflected by a significant increase of errors and a marked decrease in correct choices. The results are shown in Figs. 2 and 3, with $p < 0.01$ in all comparisons. Data analysis was carried out on 20 rats in Experiment 1, 35 rats (seven animals per group) in Experiment 2, and 11 rats in Experiment 3.

### *Experiment 1*

There was a significant difference among treatments for the number of errors [$F(5, 114) = 9.15$, $p < 0.0001$] (Fig. 2A). There was a significant reduction of induced memory impairment by 0.5 mg/kg and 1 mg/kg ($p < 0.01$) and by 0.2 mg ($p < 0.05$) of RU 47213. For number of correct choices before the first error, differences among treatments were highly significant [$F(5, 114) = 6.206$, $p < 0.0001$]. THA and the three doses of RU 47213 significantly reduced the incapacitating effect of scopolamine ($p < 0.01$).

### *Experiment 2*

There was a significant difference among the groups for the number of errors [$F(4, 30) = 4.29$, $p < 0.01$]; scopolamine produced significant memory impairments, and both THA and RU 47213 at 0.5 mg/kg significantly reduced this deficit ($p < 0.05$) (Fig. 2B). While scopolamine significantly lowered the number of correct responses [$F(4, 30) = 3.187$, $p < 0.03$], THA reduced the memory impairments ($p < 0.05$). There was no significant difference between vehicle- and RU 47213-treated groups.

### *Experiment 3*

Although scopolamine caused marked memory impairments, as reflected by a significant decrease in the number of correct responses [$F(8, 90) = 3.47$, $p < 0.002$] (Fig. 3), these deficits were significantly reduced following administration of 1 mg/kg ($p < 0.01$) and 2 mg/kg ($p < 0.05$) of RU 47213 and 5 mg/kg ($p < 0.05$) of THA. Doses of 0.2 and 0.5 mg/kg of

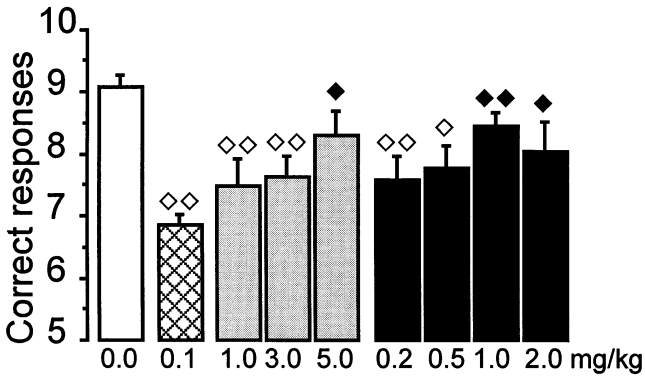


FIG. 3. Effects of RU 47213 and THA on scopolamine-induced memory impairments (delayed reinforced alternation task). Abbreviations, legend, and symbols are as in Fig. 3. Sample size: $n = 11$ rats. ANOVA followed by Duncan's multiple-range test was applied for two sessions of 10 trials.

RU 47213 and of 1 and 3 mg/kg of THA showed a tendency to reduce this deficit as compared with scopolamine treatment ($0.05 < p < 0.1$).

## DISCUSSION

The results reported here clearly show that RU 47213 and THA, two compounds that enhance cholinergic transmission by different mechanisms of action, antagonized the deleterious effects of scopolamine on WM. These results also indicate that the effective doses of RU 47213 were lower than those of THA. Unwanted cholinergic symptoms (hypotonia, diarrhoea, lacrimation, salivation, etc.) were not observed following administration of the promnesic doses of RU 47213. Although the doses of 0.5 and 1 mg/kg of RU 47213 showed about the same effect on memory performance of scopolamine-treated rats in Experiment 2 (radial maze), it appears from Experiment 1 that the dose of 1 mg/kg of RU 47213 was more effective than the lower doses (0.5 and 0.2 mg/kg), and from Experiment 3 (T-maze) that the dose of 1 mg/kg was more effective than the lower (0.5 and 0.2 mg/kg) and the higher (2 mg/kg) doses.

The animals given scopolamine were still motivated to search for food pellets (8,15) and were able to use the general rules concerning both the radial and T-maze tasks to complete the trials. This suggests that the transient amnesia induced by scopolamine did not result from a sensory or motor or motivational incapacity, or if so only to a small extent, but rather from a difficulty in using the relevant information to run the tasks.

The form of memory involved in the radial maze task, as employed here, and in the delayed reinforced alternation task is working memory. Although both tasks admittedly require WM and involve the same motivation and reinforcement in rats, some differences should however be noted. One difference is inherent to the complexity of the task: in the T-maze task as used here, choice accuracy requires the animal to remember a single item for each trial (the entered arm during forcedchoice), whereas performance of the radial maze task is more complicated because it requires the animal to remember as many items as the number of entered arms during a trial. Another important difference is that the radial maze test is a spatial-based discrimination in which the rats use explicit extramaze cues to solve the task. However, the rats probably use egocentric cues (left and right turns) to solve T-maze tasks when explicit extramaze cues are not available (19). Regardless, the form of memory tested in the present experiments might be considered as the equivalent of recent memory in humans, a type of memory that is impaired following cholinergic dysfunction produced by either ageing or Alzheimer's disease. Working memory "temporarily stores information as part of the performance of complex cognitive tasks" (2).

The enhancing effect of RU 47213 and THA on memory performance of rats given scopolamine is particularly marked when considering the improvement in memory integrity, measured by the number of correct choices before error (radial maze task). One of the factors that can disrupt memory during a trial is proactive interference. The measured variables indicate that RU 47213 and THA improved the capacity of rats given scopolamine to organize and use the information contained in recent memory, probably by rendering the animals less vulnerable to this type of interference (in both radial maze and delayed alternation tasks). However, as various studies have shown a deleterious effect of scopolamine on attention processes, it is also possible that RU 47213 and THA indirectly improved memory performance by antagonizing the negative effects of scopolamine on sustained attention (6,21), which is involved in the process of WM. Whatever the case, insofar as it is accepted that the memory-enhancing activity obtained in this model of memory deficits (14) is predictive of cognition-ameliorating activity in humans, the present results suggest that RU 47213 can improve some cognitive deterioration observed during senescence or Alzheimer's disease.

The improvement in the performance of animals rendered deficient by scopolamine strongly suggests that RU 47213 and THA exert their cholinomimetic action on the central cholinergic systems involved in memory processes. The present pharmacological findings support the general studies from both humans and animals attributing an important role in learning and memory to the cholinergic systems. In conclusion, RU 47213, which possesses a long-lasting central cholinomimetic activity, may be of potential interest for the treatment of the symptoms characteristic of the deterioration of cognitive functions in Alzheimer's disease (21,22).

## ACKNOWLEDGEMENTS

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