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# Effects of RU 52583, an α2-antagonist, on Memory in Rats With Excitotoxic Damage to the Septal Area

M. M'HARZI,[1] F. WILLIG, C. BARDELAY, A.-M. PALOU AND C. OBERLANDER

*Centre de Recherches Roussel UCLAF, Pharmacologie Générale 1, 111, route de Noisy, 93235 Romainville Cedex, France*

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M'HARZI, M., F. WILLIG, C. BARDELAY, A.-M. PALOU AND C. OBERLANDER. *Effects of RU 52583, an α2-antagonist, on memory in rats with excitotoxic damage to the septal area.* PHARMACOL BIOCHEM BEHAV **56**(4) 649–655, 1997.—The anti-amnesic action of RU 52583, an α2-adrenergic receptor antagonist, was evaluated through performance of spatial tasks in a radial maze by rats with *N*-methyl-D-aspartic acid (NMDA) lesion of the medial septal (MS) nuclei. Memory performance of lesioned or sham-operated rats was evaluated by measuring reference memory as long-term maintenance of an acquired performance and working memory or memory for recent events. The lesion: a produced significant impairments of the animals' memory performance, b) significantly reduced the sodium-dependent high-affinity choline uptake in the hippocampal formation, and c) deeply disrupted cholinergic hippocampal theta waves. Oral administration of RU 52583 at 1 and 2 mg/kg (tested doses: 1–5 mg/kg) prior to performance of the task markedly reduced memory impairments, whereas idazoxan, another α2-adrenergic receptor antagonist, had no effect at tested doses (2–5 mg/kg). Cholinergic drugs—arecoline at 0.1 and 1 mg/kg (tested doses: 0.05–1 mg/kg) and physostigmine at 0.02 and 0.1 mg/kg (tested doses: 1, 2, and 5 mg/kg)—administered intraperitoneally showed a tendency to alleviate memory deficits. The present results show that the α2-adrenergic antagonist RU 52583 possesses cognition-enhancing properties in rats with damage to the septohippocampal system. © 1997 Elsevier Science Inc.

Adrenergic receptors Cholinergic Hippocampal theta Radial maze Rat Memory impairments

DECLINE in memory is one of the main symptoms of Alzheimer's disease. Numerous studies have related memory impairment and other cognitive dysfunction to central cholinergic (2,31) and noradrenergic (20,32,40) pathology. One of the models used to mimic aspects of the cognitive dysfunction observed in demented patients is based on experimental damage to one of the central cholinergic systems, the septohippocampal components [e.g., (22,25)]. The main subcortical source of projections to the hippocampus originates in the medial septum (MS) and diagonal band of Broca (21). It is well established that these septohippocampal projections are primarily cholinergic (39). Lesions of the MS not only lower hippocampal cholinergic activity (26) but also disrupt hippocampal theta waves (26,34).

One role of interactions between cholinergic and noradrenergic systems in memory in rats has been demonstrated by Decker and McGaugh (8). Although memory decline may be associated with central noradrenergic deficits [see (40) for review], there is no satisfactory model to date of cognitive impairments in rats following selective lesions of central noradrenergic systems. Because cognitive dysfunction in humans may be caused by damage to central cholinergic and noradrenergic systems, it remains of interest to study the possibility of reducing cognitive impairments by activating the latter system in animals. It has been shown that α2 agents alleviate memory deficits in lesioned and aged animals [see (6) for review] and in patients who suffer frontal lobe dysfunction (33).

The aim of the studies reported herein was to investigate the capacity of an α2-adrenergic receptor antagonist, RU 52583 (9,27), to reduce memory impairment resulting mainly from central cholinergic dysfunction, assuming that doses of α2-adrenergic receptor antagonists might facilitate noradrenaline release from terminals (29). To achieve this, reference memory (RM) as long-term maintenance of an acquired performance and working memory (WM) or memory for recent events (16) were assessed using the radial maze in rats with

[1] To whom requests for reprints should be addressed. E-mail: mharzi@mac.rousseluclaf.fr

lesion of the medial septal nuclei. Numerous data indicate that WM is particularly affected in subjects suffering from Alzheimer's disease (11). The place version of the eight-arm radial maze is used here to study two kinds of memory errors: RM and WM errors. Arecoline (AR), a cholinergic muscarinic agonist; physostigmine (PH), an acetylcholinesterase inhibitor; and idazoxan (ID), an α2-adrenergic receptor antagonist, were used as reference compounds. A preliminary abstract of the present study has been published elsewhere (27).

## MATERIALS AND METHODS

### *Animals*

The experiments were conducted in male rats ($n = 213$) of the Long–Evans strain that were about 2 months old at the start of the experiments (supplied by Charles River). They were housed in individual cages and kept in an air-conditioned animal room at a temperature of 23 ± 1°C with artificial lighting on a 24-h cycle (light phase 0700–1900 h). During behavioral testing, the animals were submitted to a restricted diet such that their weight was maintained at 80–85% of its normal value. The daily diet (UAR France, Ref. A04) was adjusted to about 5% of the animal's body weight and adapted as necessary.

### *Surgery*

Brain lesions that produce cell death and spare fibers of passage can be made in rats with excitotoxic amino acids (7,37). The surgical procedures were similar to those described by M'Harzi et al. (23). Anesthetized animals were placed in a Kopf stereotaxic apparatus with the incisive bar set at −3.5 mm below the horizontal plane (30). *N*-Methyl-D-aspartic acid (NMDA $H_2O$; Tocris Neuramin) was injected at a concentration of 15 μg/μl, pH 7.1 (adjusted with phosphate buffer). Injections were made with a thin needle (o.d. 300 μm) mounted on the stereotaxic frame and attached with a catheter to a 10-μl Hamilton syringe, which was mounted on a microinjection pump. Injection of 1 μl was made over 5 min, and the needle was left in place for an additional 5 min to prevent spreading up along the tract. Coordinates for the lesions were as follows: 1.0 mm anterior to the bregma, 0.0 mm lateral to the midline, and 5.5 mm ventral to the dura. In the operated control (SH) rats, the needle was lowered to 1.0 mm above the intended structure and no injection was made. Surgery was performed under a microscope; the superior sagittal sinus and cortex underwent no damage. Behavioral testing took place 10–14 days after surgery, during the light cycle. Because damage to the MS lowers hippocampal cholinergic activity (26) and disrupts hippocampal theta activity (26,34), some medial septal lesioned (MS) and SH rats were submitted at the end of the experiments either to hippocampal theta recording (after 6 weeks survival, $n = 16$ rats) or to neurochemical assays (after 6–11 weeks survival, $n = 44$ rats) in order to check the adequacy of the septal lesions used in the present experiments.

At the end of the experiments, the animals were deeply anesthetized and decapitated. The brains were removed and stored frozen. They were then cut at 40 μm in the coronal plane. Sections showing lesions or recording electrode traces were stained with cresyl violet stain and examined under a microscope and the limits of the lesions were defined. After this histological check, brains that did not show properly localized lesions ($n = 41$) were discarded, and data from those subjects were not included in the postoperative study.

### *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 at different 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 center 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 progress. The maze was located in a soundproof air-conditioned (22 ± 2°C) testing room adjacent to the animal house. Unwanted noises were masked by white noise of 60 dB. The room lighting was adjusted to obtain 5–10 lux in the maze. The internal arrangement and the apparatus in the testing room remained unchanged throughout the experiments.

### *Behavioral Procedures*

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.

*Experiment 1.* The animals were trained in the maze using a procedure that involves placing the reinforcement at the end of each of the eight arms. For each trial, the rat was placed on the central platform and testing was continued until the eight reinforcers had been obtained or until the animal had made 16 choices (correct or incorrect) or a period of 10 min had elapsed. The animal was confined to the center of the maze for an additional period of 10 s at the start of a trial and for periods of 5 s between arm entries. 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 animals were given 15 trials, one per day, between 0800 and 1900 h. The daily food ration was given to each animal 15–30 min after its return to the home cage. At the end of a trial, droppings and urine puddles were removed by the experimenter; no additional effort was made to ensure absence of odor cues.

*Experiment 2.* One problem in interpreting impaired performance following drug or brain manipulations is that errors may reflect a problem in remembering the general procedures of the task (RM) or impaired ability to remember which arms have already been visited (WM). A procedure that might determine more precisely the nature of the memory impairments found in the radial maze was employed. In the present experiment, we used a limited baiting procedure, e.g., four out of eight arms are consistently baited over trials (19). In this procedure, RM errors are operationally defined as choices of arms that are never baited, while WM errors are defined as repeated entries into baited and unbaited arms that had already been visited within the trial. WM errors can be divided further into repeated entries into baited arms (working mem-

ory correct, or WMc) and reentries into arms that have never been baited (working memory incorrect, or WMi). Eight equivalent combinations of arms were used, e.g., the arms numbered 1, 2, 4, and 7 were consistently reinforced and the arms numbered 3, 5, 6, and 8 were never reinforced. In each trial, a rat was placed on the central platform and testing was continued until the four reinforcers had been obtained or until the animal had made 16 choices (correct or incorrect) or a period of 10 min had elapsed. The animal was confined to the center of the maze for an additional period of 10 s at the start of a trial and for periods of 5 s between arm entries. Arms from which food had already been taken were no longer rewarded. The animals were given 30 trials, one per day, between 0800 and 1900 h. The daily food ration was given to each animal 15–30 min after its return to the home cage.

*Drug Administration*

RU 52583 (batch no. 3, Roussel UCLAF, Romainville, France) and idazoxan (batch no. 2, Roussel UCLAF) were dissolved in distilled water. Physostigmine salicylate (Sigma) and arecoline hydrobromide (Fluka) were dissolved in 0.9% NaCl solution. The volumes were 5 ml/kg of body weight for RU 52583 and ID administered orally 30 min before testing, and 2 ml/kg for AR and PH injected IP 30 min and 15 min, respectively, before behavioral testing. The controls received the same volume of the corresponding vehicle. Tested doses were as follows: 1, 2, and 5 mg/kg of RU 52583, 2 and 5 mg/kg of idazoxan, and 0.05, 0.1, 0.3, 0.5, and 1 mg/kg of arecoline in Experiment 1; and 2 and 3 mg/kg of RU 52583 and 0.02, 0.05, and 0.1 mg/kg of physostigmine in Experiment 2.

*Hippocampal Theta Recording*

On completion of the experiments, 16 animals (4 SH and 12 SM rats) were submitted to hippocampal theta activity (θ) recording. Recording procedures were similar to those previously described (24). Briefly, bipolar recording electrodes insulated except at the tips, 200 μm in diameter, were stereotaxically implanted in the right hippocampal formation (4.3 mm anterior and 1.7 mm lateral to lambda), with the incisor bar set at −3.5 mm below the horizontal plane (30). One electrode (the shorter one) was aimed at the suprapyramidal region of CA1; the other (the longer one) was aimed at the stratum moleculare of the dentate gyrus (DG). Implantation and recording were performed under urethane anesthesia, a condition during which atropine-sensitive (presumably cholinergic) hippocampal theta activity can be seen (3,36). Both electrodes were lowered to the position at which they showed the maximum amplitude of spontaneous θ (if any) and at which the waves recorded from CA1 and DG, respectively, were about 180° phase reversed.

*Neurochemical Assays*

In parallel with the behavioral studies, groups of rats that underwent excitotoxic damage to the MS nuclei under the same conditions as mentioned above and SH rats were submitted to neurochemical assays. The animals were killed by decapitation and the brains were quickly removed. The two hippocampi were dissected at 4°C and immediately homogenized. Cholinergic activity was quantified by measuring the velocity of the sodium-dependent high-affinity choline uptake (SDHACU) mechanism in crude synaptosomal (P2) fractions using the method of Atweh at al. (1) as modified by Durkin et al. (10). The velocity of SDHACU was expressed as picomoles of $^{3}$H-choline captured in 4 min per milligram protein. Choline acetyltransferase (ChAT) activity was measured according to the procedure described by Fonnum (12). ChAT activity was expressed as picomoles of acetylcholine (ACh) synthesized per hour at 37°C per milligram protein in the presence of choline (10 mM) and $^{14}$C-AcetylCoA (170 μM). Noradrenaline (NA) was separated on an alumina microcolumn by a method adapted from Gaushy et al. (14) and then analyzed by reversed-phase high-performance liquid chromatography with electrochemical detection. NA concentration was expressed as nanograms per milligram protein.

*Data analysis*

Statistical analysis was performed by analysis of variance (ANOVA) and, whenever statistical ANOVAs were obtained, multiple comparisons among means were performed by Duncan's multiple-range test. Normal distribution of the data was not checked. Nevertheless, to make the variances more homogeneous and avoid measurements that were equal or close to zero, data were transformed according to the formula $(X + 3/8)^{0.5}$ (19) before being analyzed. The sample sizes ($n$) per group were as follows: 20 SH, 14 MS, 6 RU 1 mg/kg, 18 RU 2 mg/kg, 8 RU 5 mg/kg, 7 AR 0.05 mg/kg, 7 AR 0.1 mg/kg, 8 AR 0.3 mg/kg, 14 AR 0.5 mg/kg, 8 AR 1 mg/kg, 9 ID 2 mg/kg, and 9 ID 2 mg/kg in Experiment 1, and 8 SH, 6 MS, 6 RU 2 mg/kg, 7 RU 3 mg/kg, 5 PH 0.02 mg/kg, 6 PH 0.05 mg/kg, and 6 PH 0.1 mg/kg in Experiment 2. Significant differences between groups are given for $p < 0.05$ and $p < 0.01$. The statistical analyses were performed on the following variables: a) those that might reflect memory performance, namely, the number of errors (to eight choices, to eight arms, and error perseverations in Experiment 1) and the number of RM and WM errors in Experiment 2; and b) the number of correct responses before a WM error was made, considered as an index of memory integrity in both experiments. In Experiment 2, WM errors will refer to WMc errors + WMi errors because few reentries were made in never-baited arms (WMi errors).

## RESULTS

*Behavioral*

*Experiment 1 (Fig. 1).* A number of animals ($n$ = 5 SH, 8 MS, 3 RU 1 mg/kg, 7 RU 2 mg/kg, 2 RU 5 mg/kg, 4 ID 2 mg/kg, 5 ID 5 mg/kg, 6 AR 0.05 mg/kg, 4 AR 0.1 mg/kg, 7 AR 0.3 mg/kg, 4 AR 0.5 mg/kg, and 4 AR 1 mg/kg) did not complete some of the first five trials within the allowed period of 10 min. These trials were not included in data analysis. The 10 remaining trials that were analysed showed no significant lesion or compound effect on the animal's mobility in the maze [$F(11, 116) = 1.46, p > 0.15$]. The mean response latencies [i.e., duration (s) to complete a choice] were as follows for each group: SH, 12 ± 1; MS, 12 ± 2; RU 1 mg/kg, 12 ± 4; RU 2 mg/kg, 13 ± 2; RU 5 mg/kg, 14 ± 3; AR 0.05 mg/kg, 23 ± 4; AR 0.1 mg/kg, 13 ± 3; AR 0.3 mg/kg, 20 ± 6; AR 0.5 mg/kg, 10 ± 1; AR 1 mg/kg, 12 ± 2; ID 2 mg/kg, 16 ± 5; and ID 5 mg/kg, 11 ± 2. Due to the animal's confinement to the center of the maze between choices, very few trials (group means = 0.0–1.1) were solved by adopting strategy selection of arms. Within a trial, a strategy was arbitrary defined as selecting arms by making efficient transitions (45° or 135°) or inefficient transitions (90° or 180°) in a clockwise or counterclockwise direction for at least five consecutive choices. There was no significant lesion or compound effect on the animal's strategy, as defined above [$F(11, 116) = 1.5, p > 0.1$].

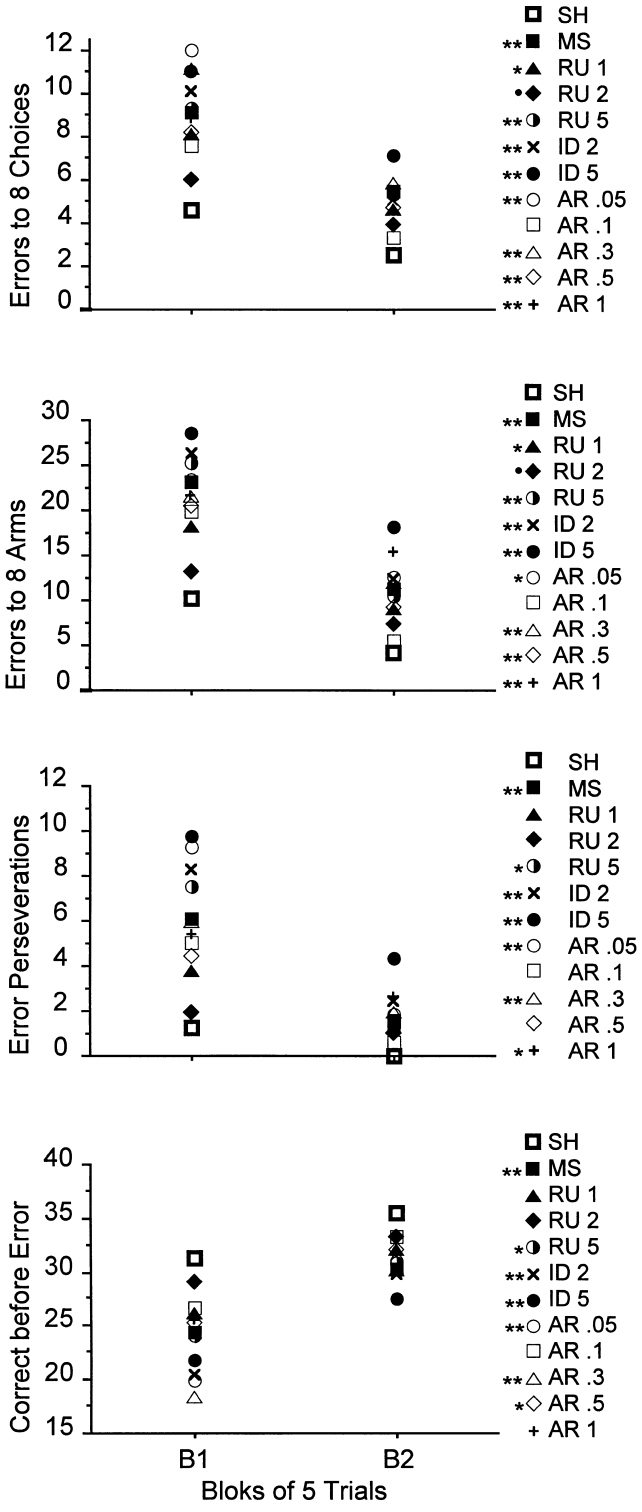


FIG. 1. Effects of pharmacological treatments on WM performance of rats with lesion of the medial septal nucleus. SH, sham-operated treated with vehicle; MS, MS-lesioned treated with vehicle; RU, MS-lesioned treated with RU 52583 (1, 2, or 5 mg/kg); ID, MS-lesioned treated with idazoxan (2 or 5 mg/kg); AR, MS-lesioned treated with arecoline (0.05, 0.1, 0.3, 0.5, or 1 mg/kg). •, ••: $p < 0.05$, 0.01 vs. MS. *, **: $p < 0.05$, 0.01 vs. SH. Significant differences among groups are indicated on the legend for the total number of errors and correct responses.

A 12 × 2 (12 groups as the between-subject factor by 2 blocks of five trials as the within-subject factor) mixed design ANOVA was used to analyse the following four variables. a) *Errors to complete eight choices*: Data analysis showed significant differences among groups for this variable [$F(11, 116) = 5.68$, $p < 0.0001$]. Multiple comparisons amongst the groups revealed significant WM impairments in rats with damage to the medial septal nuclei ($p < 0.01$). RU 52583 at 2 mg/kg significantly alleviated this deficit ($p < 0.05$). AR at 0.1 mg/kg also had a mild beneficial effect, since no significant differences were observed in comparison with the SH group. However, ID and the other doses of AR failed to improve memory performance in lesioned rats. There was no significant interaction of groups by blocks of trials [$F(11, 116) = 1.0$, $p > 0.4$]. b) When considering the total number of WM errors, i.e., the number of *WM errors to complete eight arms*, data analysis showed about the same significant differences among groups as mentioned above [$F(11, 116) = 5.12$, $p < 0.0001$]. There was, however, no significant interaction of groups by blocks of trials [$F(11, 116) = 1.0$, $p > 0.4$]. c) The groups also differed in the number of *error perseverations* [$F(11, 116) = 4.51$, $p < 0.0001$]. MS animals made more error perseverations than SH rats ($p < 0.01$). While the results for rats treated with RU 52583 (1 or 2 mg/kg) and AR (0.1 or 0.5 mg/kg) did not significantly differ from those of SH animals, rats that received ID and the other doses of AR and RU 52583 remained significantly impaired. The interaction of groups by blocks of trials fell close to significance [$F(11, 116) = 1.79$, $p > 0.06$]. d) Finally, the acquisition of the radial maze tasks was impaired by MS lesions in that the number of *correct choices until the first error* was significantly lower in MS than in SH rats [$F(11, 116) = 5.09$, $p < 0.0001$]. As can be expected from the results above, RU 52583 (1 and 2 mg/kg) and AR (0.1 and 1 mg/kg), but not ID, enhanced the number of correct responses in MS-damaged rats. Also there was a significant interaction of groups by blocks of trials [$F(11, 116) = 2.11$, $p$ 0.025]. While lesioned rats made significantly fewer correct responses than SH rats in the first block of five trials ($p < 0.05$), AR at 0.1, 0.5, and 1 mg/kg and RU 52583 at 1 and 2 mg/kg enhanced this choice accuracy. In the second block of trials, only rats treated with ID showed poor choice accuracy as compared with SH rats.

*Experiment 2 (Fig. 2).* As in Experiment 1, a number of animals ($n$ = 4 SH, 3 MS, 4 RU 2 mg/kg, 6 RU 3 mg/kg, 4 PH 0.02 mg/kg, 5 PH 0.05 mg/kg, and 5 PH 0.1 mg/kg) failed to complete some of the first 10 trials within the allowed period of 10 min. These trials were not included in data analysis. The remaining 20 trials that were analyzed showed no significant lesion or compound effect on the animal's mobility in the maze [$F(6, 37) = 1.05$, $p > 0.4$]. The mean response latencies [i.e., duration (s) to complete a choice] were as follows for each group: SH, 13 ± 1; MS, 10 ± 2; RU 2 mg/kg, 14 ± 2; RU 3 mg/kg, 11 ± 1; PH 0.02 mg/kg, 13 ± 2; PH 0.05 mg/kg, 15 ± 3; and PH 0.1 mg/kg, 13 ± 1. Few trials (group means = 1.2–3.1) were solved by adopting strategy selection of arms as defined in Experiment 1. There was no significant lesion or compound effect on the animal's strategy [$F(6, 37) = 1.5$, $p > 0.2$].

A 7 × 4 (7 groups as the between-subject factor by 4 blocks of five trials as the within-subject factor) mixed design ANOVA was used to analyse the following three variables. a) The groups did not differ significantly for the overall number of *RM errors* made throughout testing [$F(6, 111) = 1.79$, $p > 0.1$]. Note that, though not statistically significant, RU 52583 at 2 mg/kg and PH at 0.02 mg/kg reduced the number

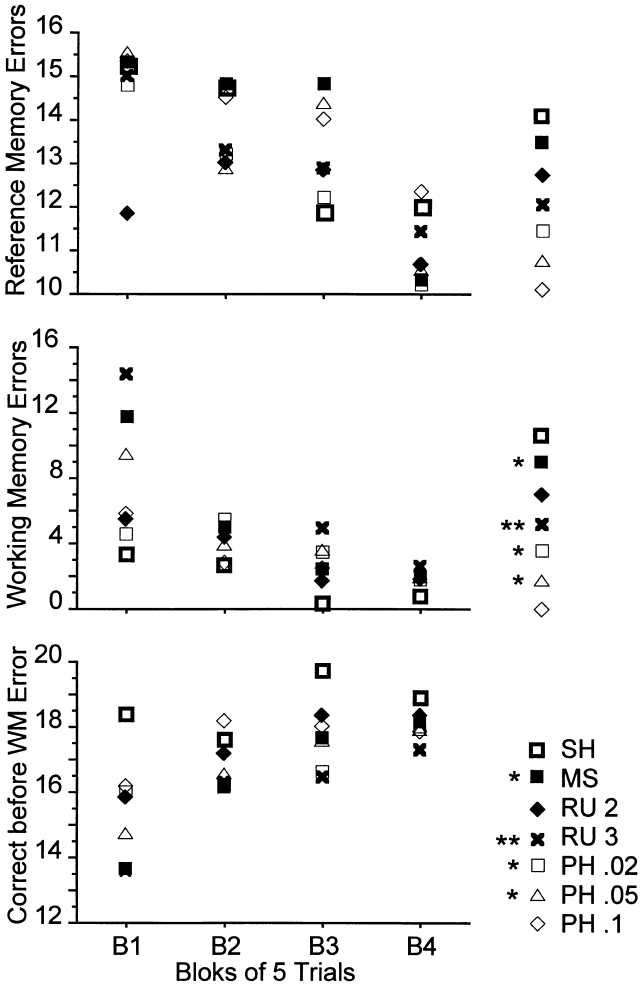


FIG. 2. Effects of pharmacological treatments on RM and WM performance of rats with lesion of the medial septal nucleus. PH, MS-lesioned treated with physostigmine (0.02, 0.05, or 0.1 mg/kg). Other abbreviations and significant differences are as in Fig. 1.

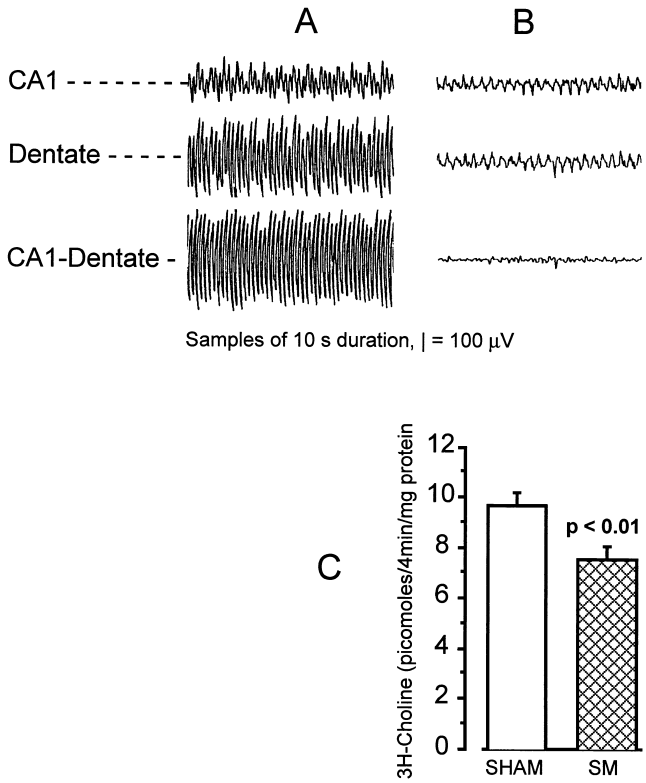


FIG. 3. Atropine-sensitive theta waves recorded from the CA1 and the dentate generators of the dorsal hippocampal formation 6 weeks after NMDA infusion in the medial septal nucleus (B) or sham operation (A). The bar graph (C) shows sodium-dependent high-affinity choline uptake (pmol/4 min/mg protein) in the hippocampus 6–11 weeks after MS lesion. SHAM, sham-operated; SM, MS-lesioned.

of RM errors in MS rats. b) Of more interest is the significant difference among groups for the number of *WM (WMc + WMi) errors* [$F(6, 111) = 4.01$, $p < 0.005$]. Among-group comparisons showed that damage to the MS significantly impaired WM ($p < 0.05$), and that RU 52583 at 2 mg/kg and PH at 0.1 mg/kg enhanced WM performance in MS-damaged animals. No significant interaction of groups by blocks of trials was found [$F(18, 111) = 1.45$, $p > 0.1$]. c) Finally, statistical analysis of the number of *correct choices before a WM error occurred* revealed interesting group differences [$F(6, 111) = 3.25$, $p < 0.02$]. RU 52583 at 2 mg/kg and PH at 0.1 mg/kg enhanced correct choices in MS-lesioned rats. No significant interaction of groups by blocks of trials was found [$F(18, 111) = 1.06$, $p > 0.4$].

*Summary of behavioral results.* Overall, the results described above revealed that rats with NMDA lesion of the septal nuclei were generally impaired when repeating previously visited arms (WM errors, Experiments 1 and 2), but not when choosing never-baited arms (RM errors, Experiment 2), and that RU 52583, AR, and PH significantly reduced this impairment.

*Histology control.* In all animals that were included in the final analysis, visual inspection showed that the lesion destroyed almost the entire medial septal nucleus. In most brains, the damage extended to include portions of the vertical limb of the diagonal band of Broca and of the dorsolateral septal nuclei. Examination of the stained brain sections showed an extensive loss of somas in the area of interest. Significant atrophy of tissue was observed, as evidenced by the enlargement of lateral ventricles. Hippocampal theta activity was significantly disrupted in brains that showed properly localized lesions (see below).

*Hippocampal theta waves.* In SH rats, fully developed hippocampal theta activity was recorded from both CA1 (about 250 μV) and dentate (about 500 μV) (Fig. 3A). As can be seen from recordings of representative animals shown in Fig. 3B, medial septal lesions strongly reduced the postulated cholinergic theta activity in both the hippocampal CA1 pyramidal cell field and the dentate gyrus. In 11 of the 12 lesioned rats, the EEG patterns were altered, essentially by significant reduction of amplitude or its suppression, depending on the individual. The remaining rat showed almost normal theta activity, associated with slight damage to the MS nucleus. It should be noted that in this experiment only the atropine-sensitive form of the hippocampal theta activity was recorded under urethane [see (3,36) for relevant reviews].

*Neurochemical data.* Neurochemical findings can be summarized as follows: excitotoxic lesions of the septal nuclei

caused a significant decrease, about 22%, of cholinergic activity in the hippocampal formation as evidenced by the levels of SDHACU [$F(1, 42) = 8.01, p < 0.01$] (Fig. 3C). However, ChAT and NA activities in the hippocampal formation were not significantly altered: 10% and 0.0% decreases, respectively [$F(1, 9) = 0.01, p > 0.9$ and $F(1, 26) = 2.09, p > 0.1$].

## DISCUSSION

NMDA damage of the septal area resulted in impaired acquisition of the spatial radial maze task in rats. The most obvious impairment of the septal-lesioned rats was in WM. This impairment was reflected in the lesioned rats by their repeated choice of arms that had already been entered during the trial. A pattern of results indicating impaired working performance on the spatial task has been reported previously following damage to different components of the hippocampal formation. The pattern of results obtained following excitotoxic damage to the MS nuclei showed that only WM was significantly impaired and does not support the view that there was a general problem in handling spatial information, as was previously suggested based on results in rats with electrolytic lesions of the same structure [see, e.g., (22)]. This discrepancy might be explained by the "mass effects" of large and nonselective lesions as compared with excitotoxic damage. It is important to point out that memory impairment throughout testing was moderate and that the damaged animals were able to overcome WM deficits, that is, they learned to avoid reentering arms that had already been visited. Previous studies [e.g., (13)] reported recovery of function in rats following hippocampal formation lesions. Accordingly, one can hypothesize that in the present experiments, the behavioral recovery, along with the weak (although significant) decrease of the levels of SDHACU as well as the lack of significant decrease in hippocampal ChAT activity, observed 6 weeks after the lesions were made might be due to axonal sprouting of the undamaged septohippocampal and/or intraseptal neurons. Possible contributions of other systems, e.g., the intraseptal GABAergic neurons (4), in the observed memory impairments should not be excluded.

The present results obtained following excitotoxic lesions confirm previous reports showing that lesions of the MS nuclei disrupt hippocampal theta waves (26) and cholinergic activity in the hippocampal formation (21,26), at least as revealed by the reduction in the level of choline uptake activity. Our data support previous demonstration that ibotenate lesions of the MS nuclei disrupt the atropine-sensitive (presumably cholinergic) form of hippocampal theta waves, with, however, little alteration of hippocampal cholinergic activity (18,36). As already suggested by Stewart and Vanderwolf (36), it might be that the excitotoxic lesions alter septal units that play a role in hippocampal theta waves and exert trans-synaptic regulation of the septohippocampal cholinergic neurons without destroying these neurons.

The forms of memory involved in the radial maze test as employed here are the WM (Experiment 1) and RM plus WM (Experiment 2). WM might be considered as the equivalent of recent memory in humans, a type of memory that is impaired by brain damage and in the aged. The results reported here clearly show that RU 52583 antagonized the effects of MS lesions on WM in the radial maze. Paradoxically, AR and PH exhibited less efficacy compared with RU 52583. The dosage (2–5 mg/kg) of ID used in this study was previously found to enhance memory performance (35). However, in the present experiments, the same dosage failed to improve WM performance in MS-lesioned rats. The discrepancies might be attributable to differences in the animal models used. In particular, while the present experiments were carried out on rats with brain damage, most if not all the reported data on the cognition-enhancing effects of idazoxan were obtained in experiments that involved intact adult or aged animals. Whether idazoxan may enhance memory performance in other animal models was also addressed in our laboratory. In particular, it has been reported (27) that both idazoxan and RU 52583: a) enhance memory performance in normal rats trained in a linear maze, b) reduce spontaneous alternation deficits in rats with electrolytic damage to the MS nuclei, and c) reduce WM deficits in rats with fimbria-fornix lesions trained in the radial maze. Also, it might be plausible that the different types of α2-adrenergic receptors (α2A, B, C, D, . . .) may have differential influence on cognition and that RU 52583 and idazoxan act differentially on these receptors. Whatever the explanation, the issue of whether the brain damage-induced memory impairments might be alleviated by adrenoceptor antagonists will require further investigation.

The present findings support the general studies of both humans and animals mainly attributing to the cholinergic system an important role in learning and memory (2). Of more interest is the significant alleviation by RU 52583 of memory impairments caused by cholinergic disruption, whatever the precise mechanisms. The impairments produced by MS lesions might have been compensated for by an increase in attentiveness and wakefulness produced by catecholaminergic stimulation mediated by RU 52853, acting not only at the hippocampal formation but also at other brain structures. Although both idazoxan and RU 52583 do not improve memory performance in normal rats trained in the radial maze (unpubl. data), support for this hypothesis could be provided by the ameliorating effect of both compounds on memory performance of normal rats assessed in a linear maze (27). The enhancing effect of RU 52583 could also be a result of catecholaminergic stimulation interacting with the central cholinergic systems (17). It has, for instance, been shown that release of NA in the septal region enhances cholinergic activity in the hippocampal formation (5,8) and that cortical acetylcholine release may be enhanced in the rat by the α2-adrenoceptor antagonist (+)-efaroxan (38). This idea is also supported by the recent demonstration that activation of beta-adrenergic receptors induces an increase in the excitability of the pyramidal neurons of the CA1 hippocampal region, a neural structure implicated in memory formation (15).

In conclusion, RU 52583 possesses anti-amnesic activity in rats with damage to central cholinergic system, which endows it with the potential to treat the symptoms characterizing the deterioration of cognitive functions in Alzheimer's disease and dementia of the frontal lobe type.

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