IMPROVEMENT OF COGNITIVE FUNCTIONS BY THE ACETYLCHOLINE RELEASER SM21

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Research Article

Improvement of Cognitive Functions by the Acetylcholine Releaser SM 21

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ABSTRACT The effect of administration of SM 21 on memory processes was evaluated in the mouse passive avoidance and in the rat social learning tests. SM 21 (10–20 mg kg^{-1} i.p.) prevented amnesia induced by scopolamine and dicyclomine as tested by the mouse passive avoidance test and prevented memory disruption by AF-64A and benehexol ascertained by the rat passive avoidance test. Both SM 21 enantiomers were able to abolish dicyclomine-induced amnesia in mice. SM 21, starting from the dose of 10 mg kg^{-1} i.p., antagonized the memory impairment produced by mecamylamine, baclofen, and diphenhydramine in mice, as well as amnesia induced by diazepam in rats. SM 21, at doses ranging between 10 and 30 mg kg^{-1} i.p., prevented memory reduction in mice by hypoxia in the passive avoidance test. In the social learning test, SM 21 (10 mg kg^{-1} i.p.) injected in adult rats reduced the duration of active exploration of a familiar partner in the second session of the test. SM 21 prevented amnesia in both mice and rats comparable to that of the cholinesterase inhibitor physostigmine (0.2 mg kg^{-1} i.p.), the M_1 selective agonist AF-102B (10 mg kg^{-1} i.p.), and the nootropic drug piracetam (30 mg kg^{-1} i.p.). These results demonstrated the ability of SM 21 to modulate memory functions and suggests that SM 21 could be useful in the treatment of cognitive deficits. Drug Dev. Res. 47:118–126, 1999.

Key words: SM 21; ACh releaser; memory; amnesia; cholinergic system

INTRODUCTION

Cholinergic activity has long been associated with memory processes. Morphological and neurochemical studies of Alzheimer's disease, the major type of dementia, have revealed marked decreases in the cholinergic innervation of the cortex and hippocampus [Bartus et al., 1982; Mash et al., 1985; Whitehouse, 1986]. Drugs involving cholinergic stimulation alleviate cognitive dysfunctions in Alzheimer's disease [Bartus et al., 1982] and, in particular, M_1-selective agonists have been proposed as a promising treatment strategy in this pathology [Mash et al., 1985; Whitehouse, 1986; Fisher et al., 1989; Gualtieri et al., 1995]. On the other hand, cholinergic blockade produces significant impairments of cognitive functions. A delay-dependent disruption following treatment with scopolamine and atropine, which appeared to resemble that occurring spontaneously in aged subjects and in Alzheimer patients, has been reported [Deutsch, 1971; Bartus and Johnson, 1976]. Ghelardini et al. [1997b] reported that the antimuscarinic compound atropine, at very low doses, was able to prevent amnesia in mice and in particular this beneficial effect was attributable to the R-(+)-enantiomer of atropine, R-(+)-hyoscyamine, since S-(–)-hyoscyamine was ineffective. It is interesting to note that this antiamnesiac activity, different from that produced by direct muscarinic agonists and cholinesterase inhibitors, was not accompanied by typical cholinergic symptomatology, such as tremors, lacrimation, sialorrhea,

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diarrhea etc., Bartolini et al. [1994], investigating the paradoxical effect of atropine, using microdialysis techniques demonstrated that R-(+)-hyoscyamine, at antiamnesic doses, produced an increase in acetylcholine (ACh) release from the rat cerebral cortex in vivo. On these bases, a synthetic program to modify the chemical structure of atropine was started, aimed at developing cholinergic amplifiers as endowed with ameliorated antiamnesic activity as atropine, but lacking the cholinergic side effects of atropine. These compounds would, therefore, be potentially useful in pathological conditions such as Alzheimer's disease, that are characterized by cholinergic deficit. Of the many compounds synthesized and studied, the racemate [Gualtieri et al., 1994] and the enantiomers [Romanelli et al., 1996] of the compound labeled SM 21 (3-α-tropanyl 2-[4-(Cl-phenoxy)] butanoate) showed the best pharmacological profile.

SM 21 antiamnesic properties were investigated in mice and rats using the passive avoidance and social learning tests, whereas the incidence of behavioral side effects was detected by the rota-rod test and Animex apparatus.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (23–30 g) and Wistar rats (90–110 g, 200–300 g, 350–450 g) from Morini (San Polo d'Enza, Italy) breeding farms were used. Fifteen mice and four rats were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were kept at 23 ± 1°C with a 12-h light/dark cycle, lights on at 7 AM, with food and water ad libitum. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

Intracerebroventricular Injection Technique

Intracerebroventricular (i.c.v.) administration was performed under ether anesthesia using isotonic saline as solvent, according to the method described by Haley and McCormick [1957] for mice and which we adapted for rats. Briefly, during anesthesia, mice and rats were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle attached to a 10 μl syringe was inserted perpendicularly through the skull at a depth of no more than 2 mm into the brain of the mouse and 4 mm into the brain of the rat, where 5 μl (mice) or 10 μl (rats) were then administered. The injection site was 1.5 mm (mice) or 2.5 mm (rats) from either side of the midline on a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice and rats were i.c.v. injected with 5–10 μl of diluted 1:10 India ink and their brains examined macroscopically after sectioning. The accuracy of the injection technique was evaluated and 95% correct.

Passive-Avoidance Test

The test was performed according to the step-through method described by Jarvik and Kopp [1967]. The apparatus consisted of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. As soon as they entered the dark compartment, mice received a punishing electrical shock (0.5 mA, 1 sec). The latency times for entering the dark compartment were measured in the training test and after 24 h in the retention test. For memory disruption, animals were either exposed to a hypoxic environment (5% O_2 in water-saturated nitrogen) for 8 min up to 30 sec before passive avoidance training or injected with amnesic drugs. Scopolamine, dicyclomine, diazepam, benzhexol, baclofen, mecamylamine, and diphenhydramine were i.p. injected immediately after the training session, whereas AF-64A was i.c.v. injected 4 h before the training session. To improve memory, animals were treated 20 min before the training session with SM 21, piracetam, AF-102B, or physostigmine. The drug administration schedule was chosen on the basis of preliminary experiments in which the time-course for every compound was determined. The maximum entry latency allowed in the training session was 30 sec for mice and 150 sec for rats, whereas in the retention session the entrance latency allowed was 120 sec and 20 min, respectively, for mice and rats. The memory degree of received punishment was expressed as latencies recorded in the retention and training sessions.

Social Learning Test

The social learning test was performed according to Mondadori et al. [1992]. Male Wistar rats (350–450 g) were used throughout the experiments and juvenile males (90–110 g) were used as social stimuli. All the adult animals were housed individually and placed in the testing room at least 24 h before the experiment. On the day preceding the experiment, adult rats were handled to become familiar with the operator. Juvenile rats were housed four per cage and brought into the testing room on the day of the experiment. Experimental sessions were always conducted between 10 AM and 2 PM. Each mature male rat was tested in its home cage. The first day of the experiment, a juvenile rat was introduced into the adult male's cage and the time spent in social investigatory behavior by the adult male within a 5-min fixed interval was recorded. Social investigatory behavior was defined as being proximally oriented to the juvenile or in direct contact while sniffing, following, nosing, grooming, or generally inspecting any body surface of the juvenile.

After 24 h, either the same juvenile or an unfamiliar one was placed again into the mature male's cage and social investigatory behavior was recorded in a 5-min interval. SM 21 and piracetam were i.p. injected 20 min before the first session of the experiment.

Spontaneous Activity Meter (Animex)

Locomotor activity in rats was quantified using an Animex activity meter Type S (LKB, Farad, Sweden) set to maximum sensitivity. Every movement of rats, which were placed on the top of the Animex activity meter, produced a signal due to variation in inductance and capacity of the apparatus resonance circuit. Signals were then automatically converted to numbers. On the day of the experiment, the rats were treated and the cage, containing three rats, was put on the measuring platform. Activity counts were made for 5 min at 15-min intervals for 45 min (total of three sessions) starting immediately after injection of the drug. Because of the arbitrary scale adopted to quantify movements, drug-treated rats were always compared with saline-treated ones.

Rota-Rod Test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a nonslippery surface. This rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of endurance time of the animals on the rotating rod. One day before the test, the animals were trained twice. On the day of the test, only the mice that were able to stay balanced on the rotating rod between 70 and 120 sec (cut-off time) were selected for testing. The performance time was measured before and at various times after treatment.

Reagents and Compounds

The following drugs were used: SM 21 racemate was prepared according to Gualtieri et al. [1994]; R-(+)-SM 21 and S-(–)-SM 21 were prepared according to Romanelli et al. [1996]; dicyclomine hydrochloride (Le Petit, Italy); benzhexol (Cyanamid); diazepam (Valium, Roche); mecamylamine hydrochloride; acetylethylcholine mustard hydrochloride (RBI; Natick, MA); AF-102B (Inst. for Neurobiol. Res., Bruxelles, Belgium); diphenhydramine hydrochloride (De Angeli); scopolamine hydrobromide; baclofen, piracetam; and physostigmine hemisulphate (all Sigma Chemical Co., St. Louis, MO).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution, with the exception of diazepam, which was dissolved in a water and dimethyl sulphoxide (DMSO) (3:1) vehicle immediately before use. AF-64A was prepared from 10 mM acetylethylcholine mustard hydrochloride. It was dissolved in distilled water (pH 11.5–11.7), and the solution was vigorously stirred for 20 min. This solution was diluted with 0.9% NaCl and finally titrated with HCl to adjust the pH to 7.3–7.4. The cerebral ventricles were then bilaterally infused with 2.0 nmol of AF-64A. A total volume of 10 µl was delivered to each side. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 5 µl per mouse by i.c.v. injection and 10 ml kg^{-1} by i.p. injection.

Statistical Analysis

All experimental results are given as the mean ± SEM. Analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, was used to verify significance between two means. Data were analyzed with the StatView software for the Macintosh. *P* values less than 0.05 were considered significant.

RESULTS

Prevention by SM 21 of Amnesia Induced by Antimuscarinic Drugs

Pretreatment of ACh releaser SM 21 prevented amnesia induced by the administration of antimuscarinic drugs in both mice and rats (Figs. 1, 2).

SM 21 dose-dependently prevented scopolamine (1 mg kg^{-1} i.p.; Fig. 1A) and dicyclomine (10 mg kg^{-1} i.p.; Fig. 1B) in the mouse passive avoidance test. SM 21, at the dose of 1 and 5 mg kg^{-1} i.p. was completely ineffective, whereas starting from a dose of 10 mg kg^{-1} i.p. it prevented antimuscarinic amnesia, reaching entrance latency values comparable to those produced by saline-treated mice. SM 21 was also able to prevent amnesia induced by AF-64A (2 nmol per rat i.c.v.; Fig. 2A) and benzhexol (10 mg kg^{-1} i.p.; Fig. 2B), as demonstrated by the rat passive avoidance test. Similar to the profile observed in mice, the antiamnesic effect of SM 21 reached statistical significance at 10 mg kg^{-1} i.p..

SM 21 prevented antimuscarinic amnesia in both mice and rats comparably to that exerted by the cholinesterase inhibitor physostigmine (0.2 mg kg^{-1} i.p.; Figs. 1A,B, 2B) and the M_1 selective agonist AF-102B (10 mg kg^{-1} i.p.; Fig. 2A). The maximum antiamnesic effect of SM 21 (20 mg kg^{-1} i.p.) was also equal to that produced by the well-known nootropic drug piracetam (30 mg kg^{-1} i.p.) (Fig. 1A,B). However, at active doses SM 21 did not enhance the entrance latency in unamnesic mice in comparison with the control group (Figs. 1A, 2). There were no differences observed in the various entrance latencies of every group in the training session of the passive avoidance test (Figs. 1, 2).

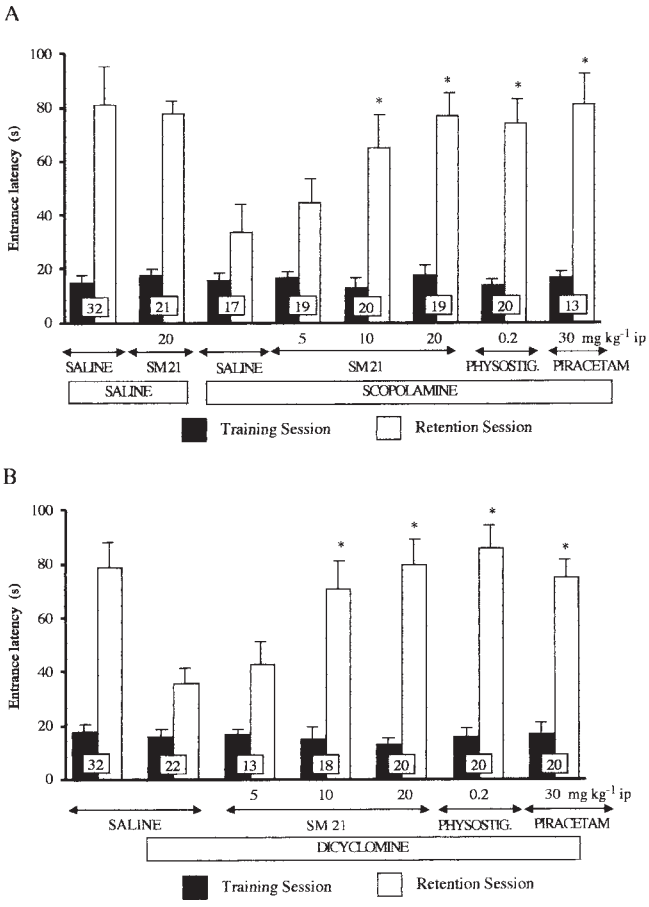

Fig. 1. Dose–response curves of SM 21 in comparison with piracetam and physostigmine on amnesia induced by scopolamine (1.5 mg kg i.p.) (**A**) and dicyclomine (10 mg kg^{-1} i.p.) (**B**) in mouse passive avoidance test. SM 21, piracetam, and physostigmine were administered 20 min before training session, while scopolamine and dicyclomine were injected immediately after. The number of mice is inside the column. *$P < 0.01$ in comparison with antimuscarinic-treated mice.

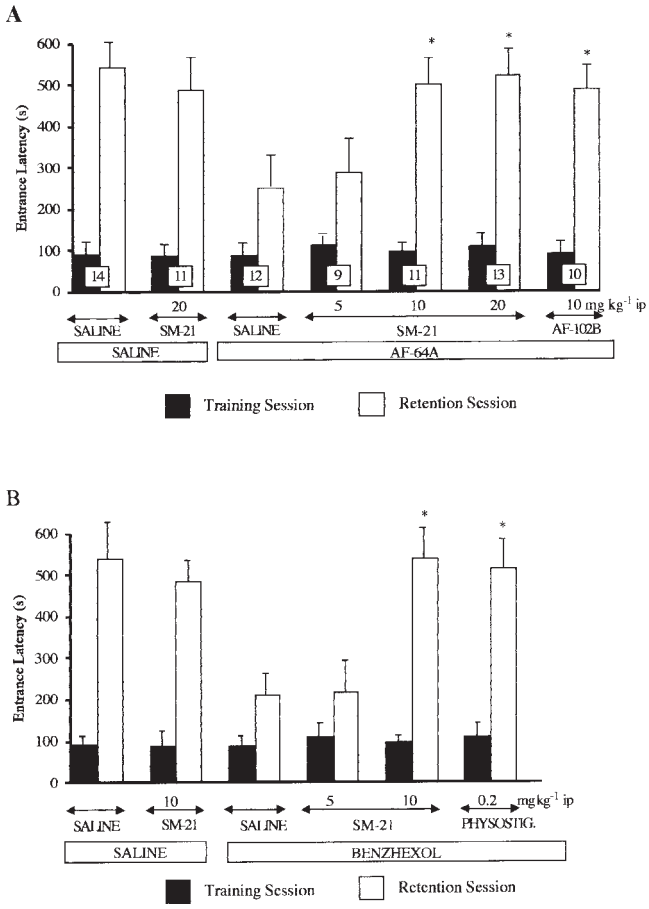

Fig. 2. Prevention by SM 21 of amnesia induced by AF-64A (2 nmol icv) in comparison with AF-102B (**A**) and benzhexol (10 mg kg^{-1} i.p.) in comparison with physostigmine (**B**) in rat passive-avoidance test. AF-64A was injected 4 h before training session, while SM 21, AF-102B, and physostigmine 20 min before training session. Benzhexol was administered immediately after training session. The number of rats is inside the column. *$P <$ 0.01 in comparison with AF-64A or benzhexol-treated rats.

The antiamnesic effect of the two enantiomers of SM 21, R-(+)-SM 21 and S-(–)-SM 21 was evaluated in the mouse passive avoidance test (Fig. 3). Both enantiomers dose-dependently were able to prevent dicyclomine-induced amnesia, even if R-(+)-SM 21 was slightly more effective than S-(–)-SM 21. As a matter of fact, the lowest active doses of R-(+)-SM 21 and S-(–)-SM 21 were, respectively, 10 and 20 mg kg^{-1} i.p. (Fig. 3). For both enantiomers, the maximum antiamnesic effect was reached at 20 mg kg^{-1} i.p., obtaining an entrance latency value comparable to that in saline-treated mice.

Prevention by SM 21 of Amnesia Induced by Mecamylamine, Baclofen, Diphenhydramine, and Diazepam

The administration of SM 21 dose-dependently antagonized the memory disruption produced by mecamylamine (20 mg kg^{-1} i.p.), baclofen (2 mg kg^{-1} i.p.), and diphenhydramine (20 mg kg^{-1} i.p.) in the mouse passive avoidance test (Fig. 4A) as well as the amnesia induced by diazepam (1 mg kg^{-1} i.p.) in the rat passive avoidance test (Fig. 4B).

SM 21, at 10 mg kg^{-1} i.p., partially prevented diazepam-induced amnesia, even if statistical significance was not reached. The dose of 20 mg kg^{-1} i.p. increased the entrance latency in the retention session up to a value comparable to that produced by control animals (Fig. 4B). Conversely, the amnesia induced by mecamylamine, baclofen, and diphenhydramine was prevented by lower doses of SM 21, with 10 mg kg^{-1} i.p. being the first active dose (Fig. 4A). The maximum antiamnesic effect was reached at the dose of 20 mg kg^{-1} i.p.

SM 21 was active in facilitating memory similar to

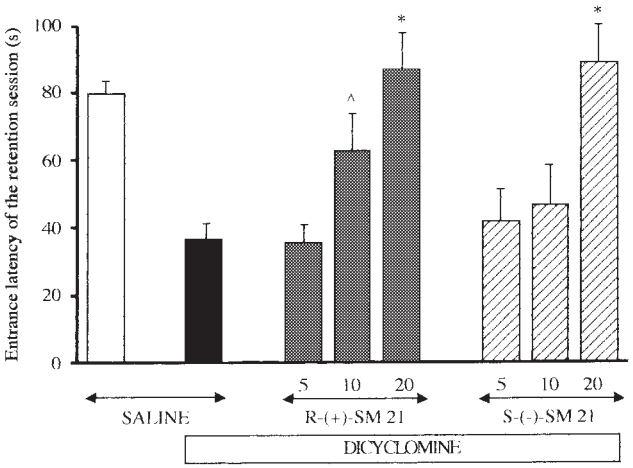

Fig. 3. Dose–response curves of R-(+)- SM 21 and S-(–)- SM 21 on amnesia induced by dicyclomine in the mouse passive avoidance test. Both enantiomers were administered 20 min before training session while dicyclomine was injected immediately after. Each column represents the mean of at least 18 mice. $^P < 0.05$; $*P < 0.01$ in comparison with dicyclomine-treated mice.

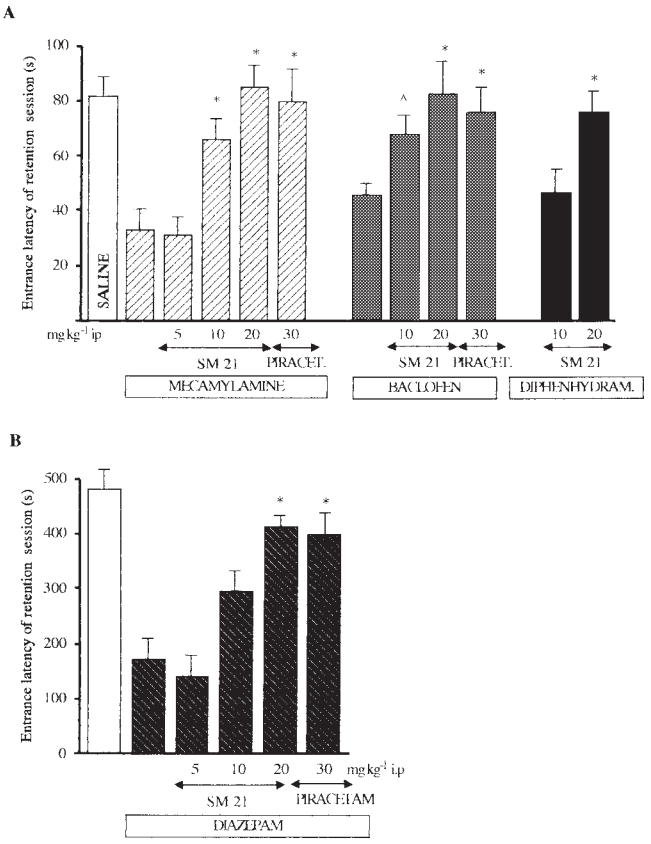

Fig. 4. Dose–response curves of SM 21 in comparison with piracetam on amnesia induced in mouse by mecamylamine, baclofen, and diphenhydramine (**A**) and in rat by diazepam (**B**) in passive avoidance test. SM 21 and piracetam were administered 20 min before training session, while the other drugs were injected immediately after. Each column represents the mean of at least 18 mice or 8 rats. $^P < 0.05$; $*P < 0.01$ in comparison with amnesic drug-treated animals.

the nootropic drug piracetam, regardless of the amnesic drug used (Fig. 4A,B).

Prevention by SM 21 of the Amnesia Induced by Hypoxia

A strong reduction in memory was obtained in mice exposed for 8 min to 5% O_2 in water-saturated nitrogen in the passive avoidance test (Fig. 5). The intensity of amnesia was comparable to that produced by all the amnesic drugs used in this study. At doses ranging between 10–30 mg kg^{-1} i.p., SM 21 was able to prevent the amnesic effect of this low oxygen concentration with an efficacy comparable to that produced by piracetam, used as a nootropic reference drug (Fig. 5). On the other hand, SM 21 was inactive in preventing hypoxia-induced amnesia at lower doses (Fig. 5). At the highest active dose SM 21 did not increase the entrance latency in nonhypoxic mice in comparison with the saline group (Fig. 5).

Effect of SM 21 on the rat social learning test

Adult rats were treated with SM 21 (5–10 mg kg^{-1} i.p.) and piracetam (30 mg kg^{-1} i.p.), or saline 20 min before the first session of the test. In the adult animals treated with SM 21 (10 mg kg^{-1} i.p.), the duration of active exploration of the familiar partner at the second pairing at 24 h was shortened in comparison with saline-treated rats (Fig. 6). No curtailment was observed if an unknown partner was presented (Fig. 6). At the dose of 5 mg kg^{-1} i.p., SM 21 was ineffective. In the same experimental conditions, a nootropic drug such as

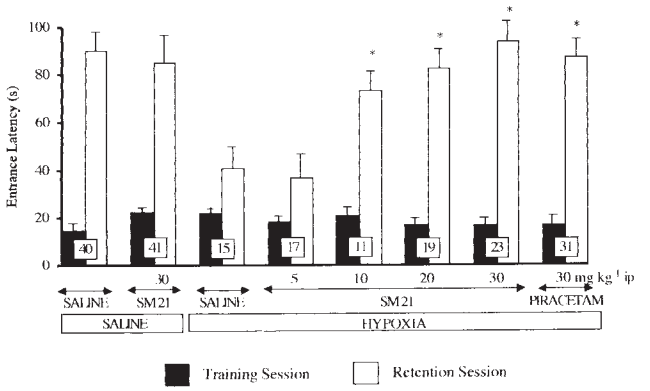

Fig. 5. Comparison of prevention by SM 21 and by piracetam of hypoxia-induced amnesia in the mouse passive avoidance test. Mice were exposed to hypoxic environment (5% O_2, 95% N_2) for 8 min. SM 21 and piracetam were injected 20 min before the training test. The number of mice is inside the column. $*P < 0.01$ in comparison with hypoxic-treated mice.

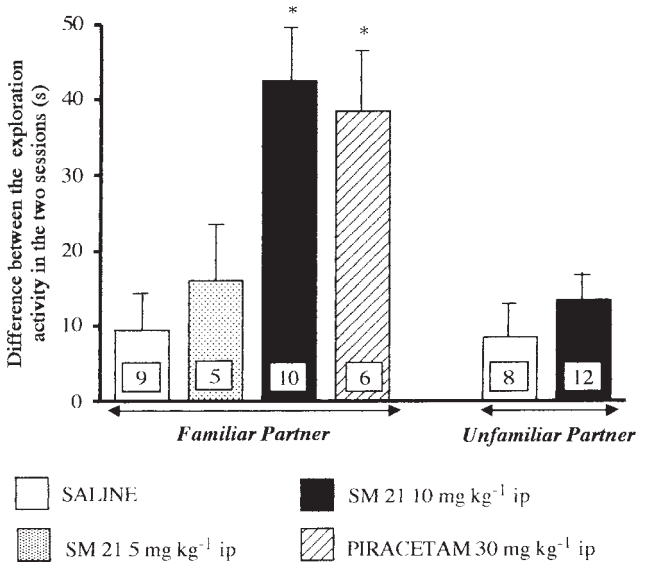

Fig. 6. Comparison of the effect of SM 21 and of piracetam in the rat social learning test. SM 21 and piracetam were administered 20 min before the first session. The number of rats is inside the column. *$P < 0.01$ in comparison with saline-treated rats.

piracetam reduced the time spent on exploratory behavior (Fig. 6). All drugs used did not modify the duration of active exploration in comparison with saline-treated rats (data not shown).

Effect of Subacute Treatment With SM 21

SM 21 induced tolerance after repeated administration. SM 21, injected twice daily for 2 weeks at a dose at which it demonstrates full antiamnesic activity (10 mg kg^{-1} i.p.), lost the ability to prevent amnesia induced by dicyclomine (Fig. 7). By contrast, in the same experimental conditions piracetam (30 mg kg^{-1} i.p.), used as reference drug, still exerted antiamnesic activity (Fig. 7). Subacute treatment with SM 21 (10 mg kg^{-1} i.p.) did not evidence any loss of body weight nor the typical symptomatology of withdrawal syndrome.

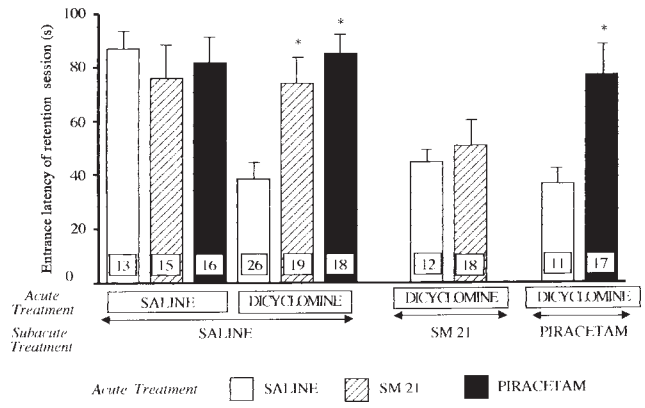

Fig. 7. Comparison of the effect of subacute treatment with SM 21 and piracetam on dicyclomine-induced amnesia in mouse passive-avoidance test. SM 21 (10 mg kg^{-1} i.p.) and piracetam (30 mg kg^{-1} i.p.) were injected twice daily for 14 days; on the day of the experiment, both were administered 20 min before training. The number of mice is inside the column. *$P < 0.01$ in comparison with dicyclomine-treated mice.

Effect of SM 21 on Mouse Rota-Rod Test and Rat Animex Apparatus

It should be noted that SM 21 elicited its modulatory effect on cognitive processes without changing either gross behavior or motor coordination and spontaneous motility as revealed, respectively, by the mouse rota-rod test (Table 1) and the rat Animex apparatus (Fig. 8). SM 21, administered at the highest active doses, did not reduce the endurance time on the rotating rod in comparison with saline-treated mice (Table 1). By contrast, in the same experimental conditions physostigmine (0.2 mg kg^{-1} i.p.) reduced time spent by the animals on the rotating rod (Table 1).

The spontaneous motility of rats was unmodified by SM 21 administration (30 mg kg^{-1} i.p.) as revealed by the Animex apparatus in comparison with saline-treated rats (Fig. 8).

DISCUSSION

The present results describe acute and subacute effects observed with SM 21 on experimentally impaired memory in mice and unimpaired learning in rats. SM 21 has been demonstrated to ameliorate cognitive processes not only by preventing amnesia induced by pharmacological treatments or exposure to hypoxic environment in the passive avoidance test, but also by producing a procognitive activity in a social learning task.

That stimulation of the cholinergic system improves cognitive processes has long been observed [Coyle, 1995]. On the other hand, a blockade of the cholinergic system produces a disruption of memory functions. The administration of scopolamine, an unselective muscarinic ACh receptor antagonist, results in impaired learning and memory in humans [Frumier et al., 1976] and animals [Dilts and Berry, 1967; Levin and Bowman, 1986]. Animals treated with the M_1 selective antagonist pirenzepine [Hammer et al., 1980] had impaired passive avoidance learning in mice [Caufield, 1993] and impaired spatial learning [Hagan et al., 1987; Hunter and Roberts, 1988], radial arm maze performance [Sala et al., 1991], and active avoidance acquisition [Sen and Bhattacharya, 1991] in rats. Moreover, the M_1 selective antagonists dicyclomine [Nilvebrant and Sparf, 1986] and S-(–)-ET-126 [Ghelardini et al., 1996] were able to induce amnesia in a mouse passive avoidance task [Ghelardini et al., 1997b; Matucci et al., 1997]. Disruption of the cholinergic system can also be obtained by the use of cholinotoxins. The

TABLE I. Effect of SM 21 in Comparison With Physostigmine in the Rota-Rod Test

	Endurance time on rota-rod(s)			
		After treatment		
	Before treatment	15 min	30 min	45 min
Saline ip	103.5 ± 5.6 (11)	99.7 ± 7.2 (11)	102.9 ± 5.9 (11)	96.3 ± 4.9 (11)
SM 21 30 mg kg^{-1} ip	98.5 ± 6.9 (10)	103.6 ± 7.7 (10)	96.2 ± 8.3 (10)	101.2 ± 8.6 (10)
Physostigmine 0.2 mg kg^{-1} ip	102.4 ± 4.5 (10)	65.3 ± 8.1* (10)	65.7 ± 7.3* (10)	87.4 ± 8.4* (10)

*$P < 0.05$ in comparison with the respective pretest value. The number of mice is shown in parentheses.

i.c.v. injection of the ethylcholine aziridinium ion (AF-64A), a selective cholinotoxin, produced a deficient performance in a rat passive avoidance task and a delayed alteration task in the T-maze test [Nakahara et al., 1988]. Furthermore, the administration of nicotinic ACh receptor antagonists, such as mecamylamine, produces a dose-dependent impairment of performance in the passive avoidance test [Elrod and Buccafusco, 1991].

It has been demonstrated by microdialysis studies that SM 21 is able to increase ACh release from rat cerebral cortex [Ghelardini et al., 1997a] and hippocampus (data not shown), two cerebral structures highly involved in the modulation of cognitive processes [Bartus et al., 1982]. Therefore, it is not unexpected that SM 21 is able to prevent amnesia induced by selective and unselective antimuscarinic drugs, by disruption of the cholinergic neurones by the use of the antimuscarinic toxin AF-64A, as well as by the administration of a nicotinic antagonist.

Amnesia can also be obtained by modulating neurotransmitter systems different from the cholinergic. GABA is the main inhibitory neurotransmitter in the brain and it plays an important role in learning and memory. The activation of $GABA_A$ receptors impairs memory performance [Jerusalinsky et al., 1994] and the stimulation of $GABA_B$ receptors by baclofen disrupts memory after systemic, intraamygdala or intraseptal administration [Swartzwelder et al., 1987; Castellano et al., 1989; Stackman and Walsh, 1994]. Furthermore, benzodiazepines have long been known to impair many forms of learning in many species, including humans [Izquierdo and Medina, 1991]. The antihistaminics are known to exert a variety of effects on the central nervous system. Central depression usually accompanies therapeutic doses of the H_1 antagonists, which appears to be related to occupancy of cerebral H_1 receptors; impairment of cognitive functions is a common manifestation [Simons and Simons, 1994]. Furthermore, the administration of the cerebral H_1 antagonist diphenhydramine induces amnesia in animals also [Kamei et al., 1990; Galeotti et al., 1998].

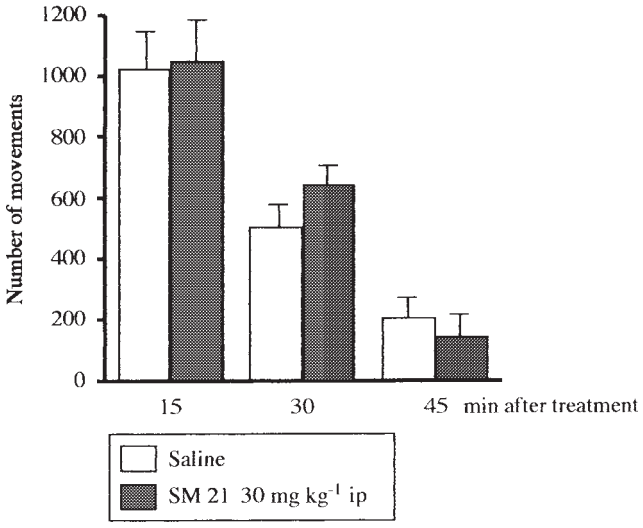

Fig. 8. Lack of effect of SM 21 on rat spontaneous motility. Vertical lines show SEM. Each column represents the mean of eight rats.

Cerebral hypoxia is associated with a series of molecular events which can culminate in neuronal cell death. Exposure of cerebral structure to an environment with a low concentration of oxygen is well known to produce amnesia. The administration of cholinomimetics or nootropic drugs can prevent the amnesic effect produced by hypoxia in both laboratory animals [DeNoble et al., 1986; Coyle, 1995] and humans [Saletu and Grünberger, 1984].

SM 21 was able to prevent amnesia induced by the administration of baclofen, diazepam, diphenhydramine, and hypoxia. Thus, SM 21 counteracts amnesia not only induced by antimuscarinic drugs, but also that obtained independently from a cholinergic blockade.

SM 21 is also endowed with antinociceptive properties [Ghelardini et al., 1997a], but the analgesia and antiamnesia seem to be unrelated, since the first antiamnesic dose was lower than that able to enhance the pain threshold. A complete prevention of amnesia was, in fact, obtained at a dose (10 mg kg^{-1}) that was weakly analgesic only in the hot-plate test [Ghelardini et al., 1997a]. The time-course of the antiamnesic activity of SM 21 was equal to that observed for its antinociceptive action, reaching its maximum between 15–30 min after injection (data not shown). Therefore, in the learning and memory experiments SM 21 was administered 20 min before the training session.

In the passive avoidance test, an improvement of cognition in animals which have no memory impairment

is difficult to demonstrate. As a matter of fact, not only SM 21 but also well-known nootropic drugs, such as piracetam and aniracetam, or cholinomimetics, such as physostigmine and oxotremorine, do not show any memory facilitation in unamnesic animals [Gouliaev and Senning, 1994; Coyle, 1995]. However, a procognitive activity of SM 21 was unmasked by using a social learning test in which adult rats with unimpaired memory were used. SM 21, as well as piracetam, improved cognitive performance by prolonging the time spent by rats deleting mnemonic information.

In the first session, the latency to enter the dark compartment of the light–dark box in the passive avoidance test, as well as the duration of exploratory activity in the social learning test, were not modified by the administration of SM 21. This observation was confirmed by evaluation of the motor coordination in mice and spontaneous motility in rats. SM 21, at the highest doses used, did not impair motor coordination as revealed by the rotarod test or modify spontaneous motility as indicated by the Animex apparatus. Furthermore, SM 21 did not elicit the typical cholinergic symptoms (tremors, sialorrhea, diarrhea, rhinorrhea, lacrimation, etc.) produced by injection of direct postsynaptic muscarinic agonists. In other words, SM 21 is able to counteract amnesia and to exert a procognitive activity in a more physiological manner than the cholinergic activators, such as physostigmine or AF-102B, used as reference drugs.

In conclusion, these results indicate the ability of SM 21 to modulate memory processes. On these bases, SM 21 could be considered a new potential antiamnesic drug useful in the treatment of cognitive disorders.

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