The novel nootropic compound DM232 (UNIFIRAM) ameliorates memory impairment in mice and rats

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Research Article

The Novel Nootropic Compound DM232 (UNIFIRAM) Ameliorates Memory Impairment in Mice and Rats

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The favorable pharmacological profile exhibited by piracetam stimulated the synthesis of related compounds potentially endowed with a higher nootropic potency. The antiamnesic and procognitive activity of DM232 (unifiram), a new compound structurally related to piracetam, was investigated. Mouse passive avoidance and rat Morris water maze and Social learning tests were employed. DM232 (0.001–1 mg kg^{-1} i.p. – 0.01–0.1 1 mg kg^{-1} p.o.) prevented amnesia induced by scopolamine (1.5 mg kg^{-1} i.p.), mecamylamine (20 mg kg^{-1} i.p.), baclofen (2 mg kg^{-1} i.p.), and clonidine (0.125 mg kg^{-1} i.p.). Furthermore, The antiamnesic effect of the investigated compound was comparable to that exerted by well-known nootropic drugs such as piracetam (30–100 mg kg^{-1} i.p.), aniracetam (100 mg kg^{-1} p.o.), rolipram (30 mg kg^{-1} p.o.), and nicotine (5 mg kg^{-1} i.p). DM232 (0.1 mg kg^{-1} i.p.) was also able to prevent amnesia induced by scopolamine (0.8 mg kg^{-1} i.p.) in the rat Morris watermaze test. In the rat social learning test, DM232 (0.1 mg kg^{-1} i.p.) injected in adults rats reduced the duration of active exploration of the familiar partner in the second session of the test. DM232, similarly to piracetam, reduced the duration of hypnosis induced by pentobarbital. At the highest effective doses, the investigated compound did not impair motor coordination (rota rod test), nor modified spontaneous (Animex). These results indicate DM232 (unifiram) as a novel cognition enhancer, strictly related to piracetam-like compounds, able to ameliorate memory impairment at doses about 1,000 times lower than the most active available nootropic compounds. Drug Dev. Res. 56:23–32, 2002.

Key words: nootropic drug; learning and memory; passive avoidance; Morris water maze; social learning

INTRODUCTION

Nootropics (noos = mind, tropein = towards) represent a heterogeneous compound group that includes the 2-pyrrolidinone derivatives piracetam, oxiracetam, pramiracetam, etiracetam, aniracetam, rolziracetam, and tenilsetam [Gouliaev and Senning, 1994]. The nootropic drugs facilitate learning and memory or overcome natural or induced cognitive impairments. The antiamnesic and memory-enhancing properties of these compounds have been demonstrated in various animal species and numerous experimental paradigms [Gouliaev and Senning, 1994]. Furthermore, these compounds facilitate the transcallosal, interhemispheric transfer of information [Okuyama and Aihara, 1988] and enhance long-term potentiation (LTP) in guinea-pig hippocampal slices [Satoh et al., 1986; Pugliese et al., 1989]. The members of this class present very low toxicity, no sedative or stimulatory effects, and lack of serious side effects of

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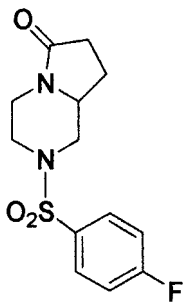

Fig. 1. The chemical structure of DM232 (unifiram): 4-(*para*-Fluorobenzensulfonyl)-1,4-diazabicyclo[4.3.0]nonan-9-one.

psychostimulants [Heise, 1987]. This favorable pharmacological profile stimulated the investigation of the potential antiamnesic activity of nootropics in human neurodegenerative pathologies. Nevertheless, results from controlled clinical trials have questioned the usefulness of nootropic compounds for treatment of cognitive disorder in humans [Sarter, 1991], several studies have demonstrated their usefulness for the treatment of cognitive impairment in the elderly [Vernon and Sorkin, 1991], in mild to moderate dementia [Chouinard et al., 1983; Nicholson, 1990], in Alzheimer's disease [Croisile et al., 1993; Parnetti et al., 1997], and for the treatment of cognitive deficit in early Parkinsonism [Oepen et al., 1985].

Preliminary pharmacological studies reported that 1,4-diazabicyclo[4.3.0]nonan-9-ones, structurally related to piracetam, could represent a new class of nootropic agents [Manetti et al., 2000a]. Among them, the compound labeled DM232 (unifiram; Fig. 1) appeared to be endowed with highest potency and the best pharmacological profile. The aim of the present study was to further investigate the capability of DM232 to ameliorate impaired or unimpaired memory functions in mice and rats.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (23–30 g), 4-month (400–450 g), and 4-week (90–110 g) -old male Wistar rats and 70-day-old male hooded Long-Evans (average body weight 270 g) from Morini (San Polo d'Enza, Italy) were used. The mice were housed fifteen per cage whereas the rats were individually housed in stainless-steel cages. The cages were placed in the experimental room 24 h before the test for adaptation. The animals were fed a standard laboratory diet and tap water ad libitum and kept at $23 \pm 1^{\circ}C$ with a 12-h light/dark cycle, light on at 7 a.m. The animals always had free access to food and water. All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Passive-Avoidance Test

The test was performed according to the step-through method described by Jarvik and Kopp [1967]. The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. Mice, as soon as they entered the dark compartment, received a punishing electrical shock (0.3 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. The maximum entry latency allowed in the training and retention sessions was, respectively, 60 and 180 s.

Spatial Reference Memory in the Morris Watermaze

Spatial learning was assessed in an open field watermaze [Morris, 1984] consisting of a large circular tank (diameter 1.5 m; depth 0.6 m) containing water at $24 \pm 1^{\circ}C$ to a depth of 0.3 m. The rats' task was to escape from the water by locating a hidden escape platform (diameter 14 cm) submerged 1.5 cm below the surface of the water. The water was made opaque by the addition of 3 liters of semi-skimmed milk, which prevents the animals from seeing the platform. The pool was located on the floor in the center of an acoustically insulated room (4×4) kept at a constant temperature ($24 \pm 1^{\circ}C$). Illumination inside the room, containing various prominent cues, was 60 lux. The swim paths taken by the animals in the pool were monitored by a video camera mounted in the ceiling. The resulting video signal was relayed to a video recorder.

All rats were trained to find a hidden escape platform, in a fixed location. They received 5 days of training with 10 trial block per day. The platform was located in the center of a chosen quadrant of the pool. The rats were placed into the pool facing the side wall at a position chosen randomly across trials and allowed to swim until they found the platform, or for a maximum of 60 sec. Any rat that failed to find the platform in time was guided to its location by the experimenter. The rats were then allowed to remain on the platform for 20 sec. Rats were gently removed from the platform and placed for 20 sec in a cage on the floor of the same room before commencing the next trial. On completion of behavioral testing, the rats were returned to their home cages where they were briefly warmed under a heating lamp. Ninety-six hours after the last acquisition training, rats received again the same behavioral procedure (retention/retraining test).

The latencies to reach the platform were recorded blindly by means of a stopwatch. Data reported for each day training were the means of ten trials.

Social Learning Test

The social learning test was performed according to Mondadori et al. [1992]. Male adult Wistar rats were used throughout the experiments and juvenile males 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 the same day of the experiment. Experimental sessions were always conducted between 10 a.m. and 2 p.m. 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. Data were reported as the difference between exploration activity in the two sessions of the test.

Pentobarbital-Induced Hypnosis

After mice were treated with pentobarbital, their loss of righting reflex was measured. The duration of hypnosis was as the time to regain the righting reflex.

Hole Board Test

The hole board test utilizes a 40-cm-square plane with 16 flush-mounted cylindrical holes (diameter 3 cm) distributed 4 by 4 in an equidistant, grid-like manner. The mice were placed in the center of the board one by one and left to move about freely for a period of 5 min each. Two photoelectric beams, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signaled the movement of the animals on the surface of the plane. Miniature photoelectric cells, in each of the 16 holes, recorded the exploration of the holes (head plunging activity) by the mice.

Rota Rod Test

The apparatus consists of a base platform and a rotating rod of 3-cm diameter with a non-skid surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus, up to 5 mice were tested simultaneously on the apparatus, with a rod-rotation speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s, according to Vaught et al. [1985]. Performance time was measured before and 15, 30, and 45 min after subcutaneous administration of the drugs.

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. Then signals were automatically converted to numbers. On the day of the experiment, the rats were treated and then the cage, containing 3 rats, was put on the measuring platform. Activity counts were made for 5 min at 15-min intervals for 45 min (total of 3 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.

Binding Studies

The binding profile of DM232 has been evaluated under the auspices of NIMH Psychoactive Drug Screening Program by Dr. B. Roth, CWR University, Cleveland, OH. For more details on the investigated receptors see: http://rothlab.cwru.edu/rothlabhome-pag/default.htm .

Reagents and Compounds

The following drugs were used: DM232 (unifiram) prepared in the Department of Pharmaceutical Sciences of University of the Florence according to the method described by Manetti et al. [2000a]; scopolamine hydrobromide, piracetam, (±)baclofen (Sigma); mecamylamine hydrochloride, clonidine hydrochloride, rolipram (RBI); nicotine hydrogentartrate (Fluka); aniracetam (A. Menarini Industrie Farmaceutiche Riunite); pentobarbital (Sagatal).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution, for i.p. injection, or dispersed in sodium carboxymethyl cellulose 1%, for p.o. administration, immediately before use. Drug concentrations were

prepared so that the necessary dose could be administered in a volume of 10 ml kg^{-1} by i.p. or p.o. injection for mice and 3 ml kg^{-1} by i.p. administration for rats.

Pharmacological Treatments

For memory disruption in the passive avoidance test, mice were i.p. injected with amnesic drugs (scopolamine, baclofen, mecamylamine, clonidine) immediately after termination of the training session. DM232, piracetam, aniracetam, rolipram, and nicotine were injected 20 (i.p.) or 30 (p.o.) min before the training session.

In the watermaze experiments, rats were i.p. injected with DM232 and/or scopolamine 20 min before each daily acquisition training. The day of the retention/retraining rats were all i.p. injected with saline solution 20 min before the test.

In the rat social learning test, DM232 and piracetam were i.p. injected 20 min before the first session of the experiment.

Statistical Analysis

All experimental results are given as the means $\pm$s.e.m. Analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, was used to verify the significance between two means in mouse behavioral data. Mixed ANOVAs with pharmacological treatments as a between-subjects variable and the training days as a within-subjects variable and Newman-Keuls multiple comparisons test were used for rat behavioral experiments. Data were analysed with the StatView software for the Macintosh (1992). P values <0.05 were considered significant.

RESULTS

Effect of DM232 in the Mouse Passive Avoidance Test

Pretreatment with DM232 prevented the amnesia induced by the administration of the antimuscarinic drug scopolamine (1.5 mg kg^{-1} i.p.) after i.p. (0.001–1 mg kg^{-1}, Fig. 2A) and p.o. (0.01–0.1 mg kg^{-1}, Fig. 2B) injection in the mouse passive avoidance test. The maximal antiamnesic effect of DM232 was obtained with the dose of 0.01 mg kg^{-1} and maintained up to 1 mg kg^{-1}. The DM232-induced antiamnesic effect was of the same intensity of that exerted by the well-known nootropic drugs piracetam (30 mg kg^{-1} i.p., Fig. 2A), aniracetam (100 mg kg^{-1} p.o., Fig. 2B) and rolipram (30 mg kg^{-1} p.o., Fig. 2B). Lower doses of DM232 (0.0001 mg kg^{-1} i.p., data not shown), piracetam (10 mg kg^{-1} i.p., Fig. 2A), aniracetam (50 mg kg^{-1} p.o., Fig. 2B), and rolipram (10 mg kg^{-1} p.o., Fig. 2B) were devoid of any ameliorative effect on scopolamine-induced amnesia. At 1 mg kg^{-1} i.p., the entrance latency value, in the retention session, of the DM232-treated group was comparable to that produced by control mice.

The administration of DM232 (0.01–0.1 mg kg^{-1} i.p.) antagonized the memory disruption produced by mecamylamine (20 mg kg^{-1} i.p.), similarly to the antiamnesic effect produced by nicotine (5 mg kg^{-1} i.p.) and piracetam (30 mg kg^{-1} i.p.). A dose 10-times lower of DM232 was completely ineffective (Fig. 3).

DM232, at the doses of 0.01–0.1 mg kg^{-1} i.p., was also able to prevent baclofen (2 mg kg^{-1} i.p.) induced amnesia, whereas at the dose of 0.001 mg kg^{-1} i.p. was devoid of any effect (Fig. 4). The DM232 prevention of memory impairment was comparable to the effect produced by piracetam (30 mg kg^{-1} i.p., Fig. 4).

Clonidine, at the dose of 0.125 mg kg^{-1} i.p., induced amnesia in the mouse passive avoidance test, which was prevented by DM232 (0.01–0.1 mg kg^{-1} i.p.) and piracetam (30 mg kg^{-1} i.p.), used as reference drug (Fig. 5).

At active doses DM232 did not enhance the entrance latency in unamnesic mice in comparison with the control group (Fig. 2). Furthermore, there were no differences observed in the various entrance latencies of every group in the training session of the passive avoidance test (Figs. 2–5).

Effect of DM232 in the Rat Morris Watermaze Test

All rats swam well in the pool and showed no evidence of any sensorimotor impairment. As training proceeded, the rats spent progressively less time at, or near, the side walls and learned to use the platform as a means of escape from the water. Consequently, all animals showed a reduction in escape latencies and path lengths with training. A significant reduction of the average escape latencies of daily blocks during the 5 days of watermaze acquisition and the retention/retraining was revealed (Fig. 6A). There was also a significant main effect of treatments. The scopolamine group rats were traveling significantly further before finding the platform than the other three groups of rats on day 5 (Fig. 6A) and on retention/retraining day (Fig. 6B). DM232 (0.1 mg kg^{-1} i.p.) was able to revert the memory impairment induced by scopolamine on both acquisition and retention/retraining days, whereas, when administered alone, it was unable to ameliorate unimpaired memory processes (Fig. 6).

No difference between the escape latencies of the last day of acquisition and the retention/retraining test was observed (Fig. 6).

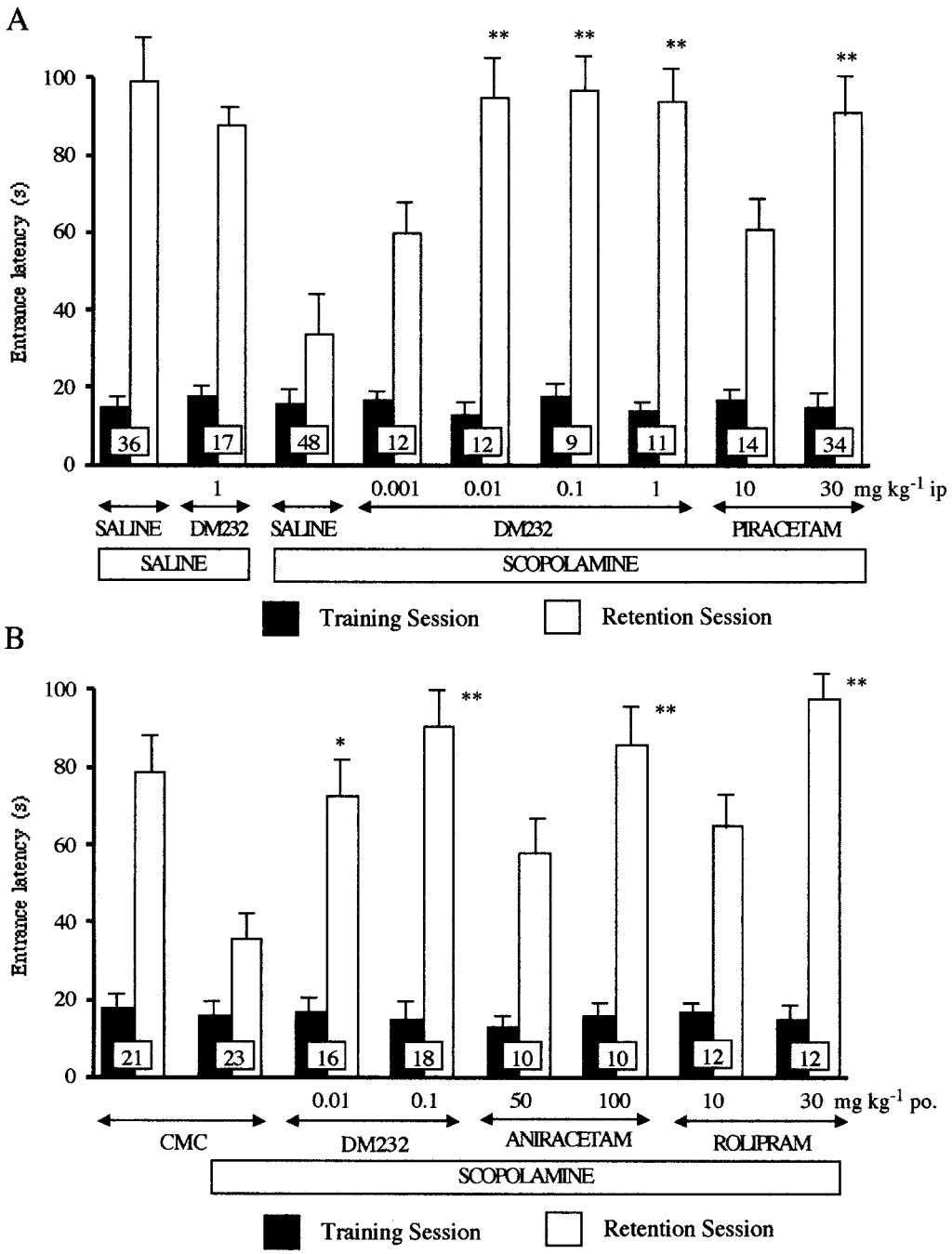

Fig. 2. Dose-response curves of DM232 (ip) in comparison with piracetam (**A**) and DM-232 (po) in comparison with aniracetam and rolipram (**B**) on amnesia induced by scopolamine (1.5 mg kg^{-1} ip) in mouse passive avoidance test. DM232, piracetam, aniracetam, and rolipram were administered 20 min (ip) and 30 min (po) before training session while scopolamine was injected immediately after. The number of mice is inside the column. $^{*}P<0.05$ $^{**}P<0.01$ in comparison with scopolamine-treated mice.

Effect of DM232 in the Rat Social Learning Test

Adults rats were treated with DM232 (0.01–0.1 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 DM232 (0.1 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. 7). No curtailment was observed if an unknown partner was presented (Fig. 7). At the dose of 0.01 mg kg^{-1} i.p., DM232 was ineffective. In the same experimental conditions, a nootropic drug, such as piracetam, reduced the time spent on exploratory behavior

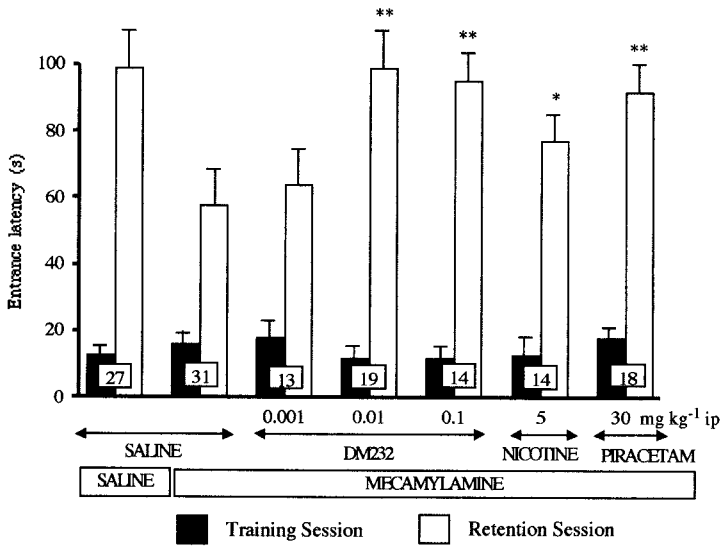

Fig. 3. Dose-response curve of DM232 (ip) in comparison with nicotine and piracetam on amnesia induced by mecamylamine (20 mg kg^{-1} ip) in mouse passive avoidance test. DM232, nicotine, and piracetam were administered 20 min before training session while mecamylamine was injected immediately after. The number of mice is inside the column. $^{*}P<0.05$ $^{**}P<0.01$ in comparison with mecamylamine-treated mice.

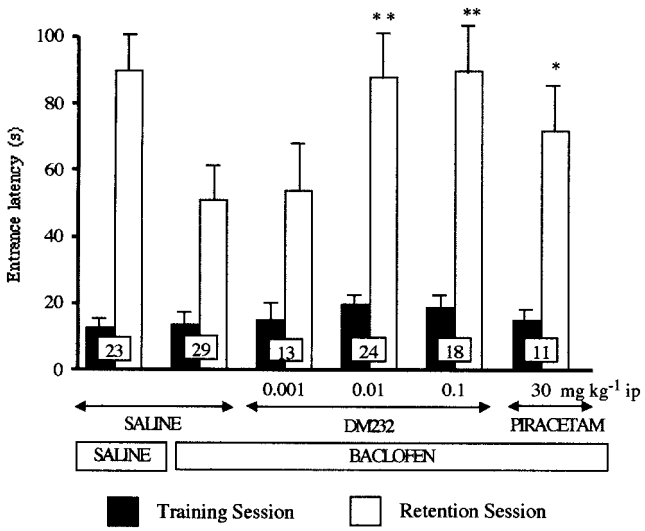

Fig. 4. Dose response curve of DM232 (ip) in comparison with piracetam on amnesia induced by baclofen in mouse passive-avoidance test. DM232 and piracetam were administered 20 min before training session while baclofen (2.0 mg kg^{-1} ip) was injected immediately after. The number of mice is inside the column. $^{*}P<0.05$ $^{**}P<0.01$, in comparison with baclofen-treated mice.

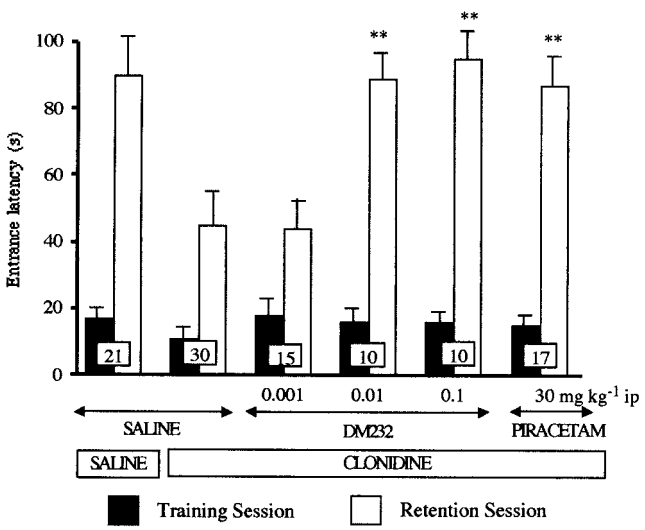

Fig. 5. Dose-response curves of DM232 (ip) in comparison with piracetam on amnesia induced by clonidine in mouse passive avoidance test. DM232 and piracetam were administered 20 min before training session while clonidine (0.125 mg kg^{-1} ip) was injected immediately after. The number of mice is inside the column. $^{**}P<0.01$ in comparison with clonidine-treated mice.

(Fig. 7). All drugs used did not modify the duration of active exploration in the first session of the test in comparison with saline-treated rats (data not shown).

Effect of DM232 on Pentobarbital-Induced Hypnosis

DM232 (0.1 mg kg^{-1} i.p.) significantly reduced the total sleeping time ($18.3\pm3.1^{*}$ min) induced by 60 mg kg^{-1} i.p. pentobarbital (27.5 ± 6.3 min), similarly to the effect induced by 30 mg kg^{-1} i.p. piracetam ($12.5\pm5.3^{*}$ min). At the same doses, both drugs did not modify the induction time of hypnosis (control: 3.8 ± 1.6; DM232: 4.1 ± 1.9; piracetam: 4.5 ± 2.1 min) ($^{*}P<0.05$ vs. pentobarbital).

Effect of DM232 on Motor Coordination and Spontaneous Motility

It should be noted that DM232 elicited its ameliorating effect on cognitive processes without

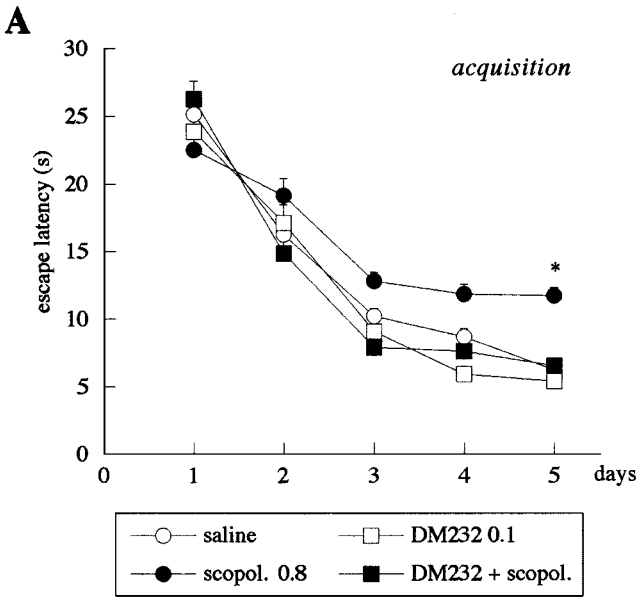

Fig. 6. The effect of DM232 (0.1 mg kg^{-1} ip) on spatial reference memory in the Morris water maze test. **A**: Effect of DM232 on scopolamine (0.8 mg kg^{-1} ip)-induced impairment of rat acquisition. Task acquisition is reflected as a decrease in escape latency. *$P<0.05$ in comparison with saline-treated rats. Vertical lines represent s.e.m. **B**: Effect of DM232 on scopolamine-induced impairment in the retention/retaining day. *$P<0.05$ in comparison with saline-treated rats. Vertical lines represent s.e.m.

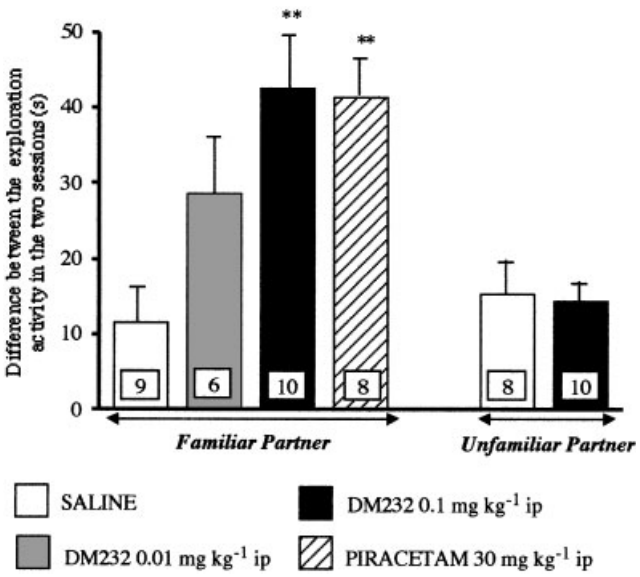

Fig. 7. Effect of D232 in comparison with piracetam in the rat social learning test. DM232 and piracetam were administered 20 min before the first session. The number of rats is inside the column. **$P<0.01$ in comparison with saline-treated rats.

TABLE 1. Lack of Effect of DM-232 in Comparison With Piracetam in the Mouse Rota Rod Test*

		Endurance time on rota rod (s)			
			After treatment (min)		
	Dose	Before treatment	15	30	45
Saline	10 ml kg^{-1} ip	3.3±0.3	1.9±0.3	1.4±0.2	0.8±0.2
DM232	0.1 ml kg^{-1} ip	3.2±0.5	2.2±0.4	1.7±0.4	1.2±0.2
DM232	1.0 mg kg^{-1} ip	3.6±0.3	2.2±0.4	1.5±0.2	1.1±0.2
DM232	10 mg kg^{-1} ip	3.5±0.4	2.6±0.3	1.6± 0.3	1.2±0.3
Piracetam	30 mg kg^{-1} ip	3.2±0.4	2.5±0.5	1.4±0.3	1.3±0.4

*Each value represents the mean of at least 10 mice.

TABLE 2. Lack of Effect of DM-232 in the Rat Animex Test*

		Number of counts after treatment		
	Dose	15 min	30 min	45 min
Saline	10 ml kg^{-1} ip	691±47	123±21	44±16
DM232	1 mg kg^{-1} ip	715±56	115±28	51±11

*Each value represents the mean of at least 10 mice.

changing animals' gross behavior. No modification of motor coordination was revealed by the mouse rota rod test. DM232 (1–10 mg kg^{-1} i.p.) did not modify the number of falls from the rotating rod in comparison with saline-treated mice (Table 1). Furthermore, mouse and rat spontaneous motility, as revealed, respectively, by the rat Animex apparatus (Table 2) and the mouse hole board test (data not shown) was unmodified by DM232 administration (1 mg kg^{-1} i.p.) in comparison with saline-treated animals.

Binding Profile of DM232

DM232, at 10^{-6} M, did not reveal any affinity towards the most important central receptors, such as

serotoninergic, dopaminergic, muscarinic, nicotinic adrenergic, glutamatergic, histaminergic, opiod, and GABAergic (data not shown).

DISCUSSION

The present results describe the effects observed with DM232 on experimentally impaired and unimpaired memory processes in mice in rats. DM232 ameliorates cognitive processes not only by preventing amnesia induced by pharmacological treatments in the mouse passive avoidance and rat Morris watermaze tests, but also by producing a procognitive activity in a rat social learning task.

DM232 prevented amnesia induced by the antimuscarinic drug scopolamine after i.p. and p.o. administration and by the nicotinic antagonist mecamylamine in the mouse passive avoidance test. Furthermore, DM232 was able to overcome the impairment of the acquisition and retention/retraining produced by scopolamine in the rat Morris watermaze test. The lack of difference in the escape latencies between the last day of acquisition and the retention/retraining test led us to exclude the induction of state-dependent effects [McGaugh, 1989; Overton, 1991].

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 [Levin and Bowman 1986]. Animals treated with the M_1 selective antagonist pirenzepine and dicyclomine had impaired memory processes in various paradigms in both mice and rats [Caufield et al., 1983; Sala et al., 1991; Ghelardini et al., 1997]. 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, 1981].

It has been demonstrated, by microdialysis studies, that DM232 is able to weakly increase ACh release from rat parietal cortex [Manetti et al., 2000b], a cerebral structure involved in the modulation of cognitive processes [Bartus et al., 1982]. Therefore, the potentiation of the cholinergic system induced by DM232 could make it able to prevent amnesia induced by an antimuscarinic drug as well as by the administration of a nicotinic antagonist. The modulation of the cholinergic system was also postulated as the mechanism of action of the piracetam-like nootropic drugs [Pepeu and Spignoli, 1989]. In particular, it has been reported that piracetam might alter presynaptic cholinergic functions, possibly by enhancing high-affinity neuronal uptake of choline [Pedata et al., 1984], but these data are still a matter of controversy [Franklin et al., 1986]. Pilch and Müller [1988] showed that piracetam elevated muscarinic receptor density in the frontal cortex of aged but not of young mice. Aniracetam was found to be able to increase the acetylcholine content in the hippocampus and cerebral cortex [Toide, 1989].

However, in binding studies, DM232 at 10^{-6} M did not reveal any affinity towards not only muscarinic and nicotinic receptors, but also for the most important central receptors (data not shown) that might explain the potentiation of the endogenous cholinergic system induced by the investigated compound. The lack of receptorial affinity is also a characteristic feature of the nootropic compounds. These drugs, with the only exception of nefiracetam, which shows high affinity for the $GABA_A$ receptors, do not seem to act at any well-characterized receptor system [Gouliaev and Senning, 1994]. Indeed, as already postulated for nootropic compounds [Mondadori et al., 1991; Muller et al., 1999], the DM232 effects on the cholinergic system might be of secondary origin.

Amnesia can also be obtained by modulating neurotransmitter systems different than the cholinergic. GABA is the main inhibitory neurotransmitter in the brain and it plays an important role in learning and memory. The activation of $GABA_A$ as well as $GABA_B$ receptors has been reported to impair memory performances [Jerusalinsky et al., 1994; Swartzwelder et al., 1987]. The α_2-agonists are known to exert a variety of effects on the central nervous system. Central depression usually accompanies therapeutic doses of α_2-agonists and impairment of cognitive functions is also observed [Voronina et al., 1991; Genzoka-Papazova et al., 1997].

DM232 was able to prevent amnesia induced by the administration of baclofen and clonidine. Thus, DM232 counteracts amnesia not only induced by anticholinergic drugs, but also that obtained independently from a cholinergic system blockade. DM232 ameliorates cognitive processes not only by preventing amnesia induced by pharmacological treatments, but also by producing a procognitive activity in a rat social learning task in which adults rats with unimpaired memory were used. DM232, as well as piracetam, improved cognitive performance by prolonging the time physiologically spent by rats to delete mnemonic information.

DM232 did not show any procognitive activity in the passive avoidance and Morris watermaze tests when given alone. However, an improvement in cognition of young animals that have no memory

impairment is difficult to demonstrate. As a matter of fact, not only DM232 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].

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 the exploratory activity in the social learning test and the escape latency in the Morris watermaze test, were not modified by the administration of DM232.

DM232 was also able to reduce the total sleeping time induced by pentobarbital without modifying the induction time of hypnosis. This effect was comparable to that exerted by piracetam. The ability of nootropics to reduce sleeping time was already observed in humans. Hollister [1985] reported that piracetam increases waking hours in elderly. This waking effect would be beneficial for patients in which senile dementia is associated with persistent sleepiness.

The amelioration of memory process induced by DM232 is obtained without any induction of side effects. DM232, at the highest effective doses, did not impair motor coordination, as revealed by the rota rod test, nor modify spontaneous motility, as indicated by the Animex apparatus and the hole board test. Furthermore, DM232, at a dose 1,000 times higher than the minimal effective dose, is still devoid of any alteration of behavioral parameters.

The present results provide evidence that DM232 is a new antiamnesic and procognitive compound for which the belonging to the class of nootropic drugs can be supposed. As a matter of fact, DM232 shows, not only a chemical structural similarity with piracetam [Manetti et al., 2000a], but also exhibits a pharmacological profile comparable to that of nootropics. DM232 is, in fact, endowed with the main pharmacological properties of piracetam-like compounds: facilitation of memory processes, lack of toxicity and side effects, lack of affinity towards the most important central receptors. However, DM232 differs from nootropics for its potency. Even if it exerts the same pharmacological effects, DM232 is at least 1,000 times more potent than the most active nootropic drugs, such as oxiracetam, nefiracetam, etiracetam, and aniracetam [Gouliaev and Senning, 1994].

In conclusion, these results indicate DM232 as a novel cognition enhancer, strictly related to piracetam-like compounds, but endowed with higher potency. These observations, together with the lack of side effects at a dose 1,000 times higher than the minimal active one, permits considering DM232 a promising compound for the treatment of human cognitive deficits.

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