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C. Ghelardini · N. Galeotti · F. Gualtieri
M. N. Romanelli · C. Bucherelli · E. Baldi
A. Bartolini

DM235 (sunifiram): a novel nootropic with potential as a cognitive enhancer

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Abstract DM235 (sunifiram), a new compound structurally related to piracetam, prevented the amnesia induced by scopolamine (1.5 mg kg⁻¹ i.p.), after intraperitoneal (0.001–0.1 mg kg⁻¹) or oral (0.01–0.1 mg kg⁻¹) administration, as shown by a passive avoidance test in mice. The anti-amnesic effect of DM235 was comparable to that of well-known nootropic drugs such as piracetam (30–100 mg kg⁻¹ i.p.), aniracetam (100 mg kg⁻¹ p.o.) or rolipram (30 mg kg⁻¹ p.o.). DM235 also prevented mecamlamine (20 mg kg⁻¹ i.p.), baclofen (2 mg kg⁻¹ i.p.)- and clonidine (0.125 mg kg⁻¹ i.p.)-induced amnesia in the same test. In the Morris water maze test with rats, scopolamine (0.8 mg kg⁻¹ i.p.) inhibited the reduction of escape latency in both acquisition and retention/retraining tests. DM235 (0.1 mg kg⁻¹ i.p.), 20 min before each daily acquisition training, prevented the scopolamine-induced memory impairment. DM235 (1 mg kg⁻¹ i.p.) also reduced the duration of pentobarbitone-induced hypnosis in mice without modifying the induction time of hypnosis. At the highest effective doses, the investigated compound neither impaired motor coordination (rota-rod test), nor modified spontaneous motility and inspection activity (Animex and hole board tests).

These results indicate that DM235, a compound structurally related to piracetam, is a novel nootropic endowed with the capability to prevent cognitive deficits at very low doses. Indeed, its potency is about 1,000 times higher than that of the most active piracetam-like compounds.

Keywords DM235 · Sunifiram · Nootropic drugs · Piracetam · Learning and memory · Passive avoidance · Morris water maze

Introduction

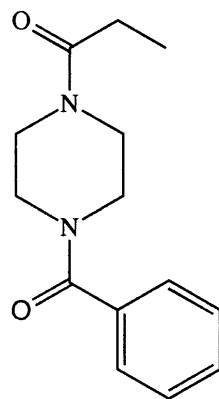
The so-called nootropic compounds are a group of pharmacologically active pyrrolidone derivatives that, in some respects, occupy a special position in the pharmacology of the central nervous system. The first pyrrolidone to come to the attention of clinicians was piracetam. This compound was developed in the late 1960s after pioneering research by Giurgea who also coined the term “nootropic”, meaning enhancement of learning and memory. Since then, there has been much pharmaceutical interest in a broad range of indications and in new compounds (aniracetam, oxiracetam, pramiracetam, nefiracetam, nebracetam, fasoracetam, levetiracetam, etc.). A wide range of animal models has been used to show improvements in cognitive function. These tests include maze and spatial learning, passive avoidance, matching-to-sample, active avoidance, choice reaction, conditional avoidance and motor tasks that show definitely positive effects on retention performance in laboratory animals (Verloes et al. 1988; Sarter 1991; Gouliarov and Senning 1994). Nootropic drugs also 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 show very low toxicity, have no sedative or stimulatory effects and lack the serious side effects of psychostimulants (Heise 1987). This favourable pharmacological profile has stimulated investigation of the potential anti-amnesic activity of nootropics in human neurodegenerative conditions. Clinical studies have focused on cognition enhancement and memory improvement by nootropic drugs. Some pyrrolidone derivatives, such as piracetam, aniracetam and oxiracetam, ameliorate the condition of elderly patients suffering from mild to moderate mental deterioration (Chouinard et al.

C. Ghelardini (✉) · N. Galeotti · A. Bartolini
Department of Preclinical and Clinical Pharmacology,
University of Florence,
Viale G. Pieraccini 6, 50139 Florence, Italy
e-mail: ghelard@pharm.unifi.it,
Tel.: +39-55-4271312, Fax: +39-55-4271280

F. Gualtieri · M.N. Romanelli
Department of Pharmaceutical Sciences, University of Florence,
Via G. Capponi 6, 50121 Florence, Italy

C. Bucherelli · E. Baldi
Department of Physiological Sciences, University of Florence,
Viale G.B. Morgagni 63, 50134 Florence, Italy

Fig. 1 DM235 (sunifiram): 1-benzoyl-4-propionylpiperazine



1983; Maina et al. 1989; Nicholson 1990; Vernon and Sorkin 1991; Lee and Benfield 1994), of geriatric patients with cerebrovascular insufficiency (Foltyn et al. 1983), in Alzheimer's disease (Senin et al. 1991; Croisile et al. 1993; Parnetti et al. 1997) and are useful in the treatment of cognitive deficits in early Parkinsonism (Oepen et al. 1985).

Preliminary pharmacological studies have shown that 1,4-diazabicyclo[4.3.0]nonan-9-ones, structurally related to piracetam, could represent a class of nootropic agents (Manetti et al. 2000a, 2000b). Among them, the compound DM235 (sunifiram; Fig. 1) appears to be endowed with the best pharmacological profile. The aim of the present study was to investigate further the ability of DM235 to ameliorate impaired or unimpaired memory functions in mice and rats.

Materials and methods

Animals. Male Swiss albino mice (23–30 g) and 70-day-old male hooded Long-Evans (average body weight 270 g) from Morini (San Polo d'Enza, Italy) were used. Mice were housed 15 per cage; the rats were housed individually in stainless-steel cages. For adaptation, the cages were placed in the experimental room 24 h prior to tests. Animals always had free access to a standard laboratory diet (TRM, Harlan, Padua, Italy) and tap water and were kept at $23 \pm 1^\circ\text{C}$ with a 12-h light/dark cycle (light on at 7 a.m.). All experiments were carried out according to the guidelines of the European Community Council for experimental animal care. All experiments were performed blind.

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 s). The latency times for entering the dark compartment were measured in the training test and 24 h later in the retention test. The maximum entry latencies allowed in the training and retention sessions were 60 and 180 s respectively.

Spatial reference memory in the Morris water maze. Spatial learning was assessed in an open-field water maze (Morris 1984), consisting of a large, circular, transparent tank (diameter 1.5 m; depth 0.6 m) containing water at $24 \pm 1^\circ\text{C}$ at a depth of 0.3 m. The rats' task was to escape from the water by locating a hidden, transparent escape platform (diameter 14 cm) submerged 1.5 cm below the surface of the water. The water was made partially opaque by the

addition of 3 l semi-skimmed milk that prevented the animals from seeing the platform. The pool was located on the floor in the centre of an acoustically insulated room (4×4 m) kept at a constant temperature ($24 \pm 1^\circ\text{C}$). 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 a ten-trial block per day. The platform was located in the centre of a chosen quadrant of the pool. The rats were placed into the pool facing the side wall at a position chosen randomly (the start points were chosen randomly, always starting from the external edge) across trials and allowed to swim until they found the platform, or for a maximum of 60 s. 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 s. They were then removed gently from the platform and placed for 20 s in a cage on the floor of the same room before commencing the next trial. On completion of behavioural testing the rats were returned to their home cages where they were warmed briefly under a heating lamp. Then, 96 h after the last acquisition training, the rats were again subjected to the same behavioural procedure (retention/retraining test). The latencies for reaching the platform were recorded blindly using a stopwatch. Data reported for each day's training were the means of ten trials.

Pentobarbitone-induced hypnosis. After mice had been given pentobarbitone sodium (60 mg kg^{-1} i.p.), the loss of the righting reflex was measured. The duration of hypnosis was taken as the time required to regain the righting reflex. Mice were pretreated with DM235 (0.1–1 mg kg^{-1} i.p.), or piracetam (30 mg kg^{-1} i.p.) 20 or 30 min respectively before the injection of pentobarbitone.

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 plane of the hole board is made of black metal; the separation of the holes from each other is 5.5 cm; the distance of the outermost holes from the edge of the board is 5 cm. The mice were placed in the centre 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 four equal quadrants, automatically signalled 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 made of black plastic. The 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 allowing up to five mice to be tested simultaneously, with a rod rotation speed of 16 rpm. The integrity of motor coordination was assessed as 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 intraperitoneal 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. Signals were converted automatically to numbers. On the day of the experiment the rats were treated and the cage, containing three rats, then 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.

Drugs. The following drugs were used: DM235 (sunifiram) 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, ($\pm$)-baclofen (Sigma); mecamlamine hydrochloride, clonidine hydrochloride, ($\pm$)-rolipram (RBI); nicotine hydrogentartrate (Fluka); aniracetam (A. Menarini Industrie Farmaceutiche Riunite); pentobarbitone (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 such that the necessary dose could be administered in volumes of 10 ml kg⁻¹ (i.p. or p.o.) for mice and 3 ml kg⁻¹ (i.p.) for rats.

Pharmacological treatments. For memory disruption in the passive avoidance test, mice were injected i.p. with amnesic drugs immediately after termination of the training trial (scopolamine, baclofen, mecamlamine) or 60 min before the training trial (clonidine). DM235, piracetam, aniracetam, rolipram and nicotine were injected 20 (i.p.) or 30 (p.o.) min before the training trial. In the water maze experiments, rats were injected i.p. with DM235 and/or scopolamine 20 min before each daily acquisition training. The day of the retention/retraining rats were all injected i.p. with saline solution 20 min before the test.

Statistical analysis. All experimental results are given as means $\pm$ SEM. Analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD) procedure for post-hoc comparison, was used to verify significance of differences between means in mouse behavioural 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 behavioural experiments. Data were analysed using StatView software on a Macintosh computer. $P < 0.05$ (2-tailed) was considered significant.

Results

In the passive avoidance test in mice, pretreatment with DM235 prevented the amnesia induced by the administration of the antimuscarinic drug scopolamine (1.5 mg kg⁻¹ i.p.) after i.p. (0.001–0.1 mg kg⁻¹, Fig. 2A) or p.o. (0.01–0.1 mg kg⁻¹, Fig. 2B) injection. The maximal anti-amnesic effect of DM235 was obtained with the dose of 0.001 mg kg⁻¹ i.p. and maintained up to 0.1 mg kg⁻¹ i.p. The DM235-induced anti-amnesic effect was of the same intensity as that exerted by the well-known nootropic drugs piracetam (30 mg kg⁻¹ i.p.), aniracetam (100 mg kg⁻¹ p.o.) or rolipram (30 mg kg⁻¹ p.o.) (Fig. 2A and B). Lower doses of DM235 (0.0001 mg kg⁻¹ i.p.), piracetam (10 mg kg⁻¹ i.p.) aniracetam (50 mg kg⁻¹ p.o.) or rolipram (10 mg kg⁻¹ p.o.) (Fig. 2A and B) were devoid of any ameliorative effect on scopolamine-induced amnesia. At 0.1 mg kg⁻¹ i.p., the entrance latency value of the DM235-treated group in the retention session was comparable to that produced by control mice.

The administration of DM235 (0.01–0.1 mg kg⁻¹ i.p.) antagonized the memory disruption produced by mecamlamine (20 mg kg⁻¹ i.p.), similarly to the anti-amnesic effect produced by nicotine (5 mg kg⁻¹ i.p.) and piracetam (30 mg kg⁻¹ i.p.). DM235 at 0.001 mg kg⁻¹ i.p. was completely ineffective (Fig. 3).

DM235 at (0.01 and 0.1 mg kg⁻¹ i.p.) also prevented the amnesia induced by baclofen (2 mg kg⁻¹ i.p.), but at

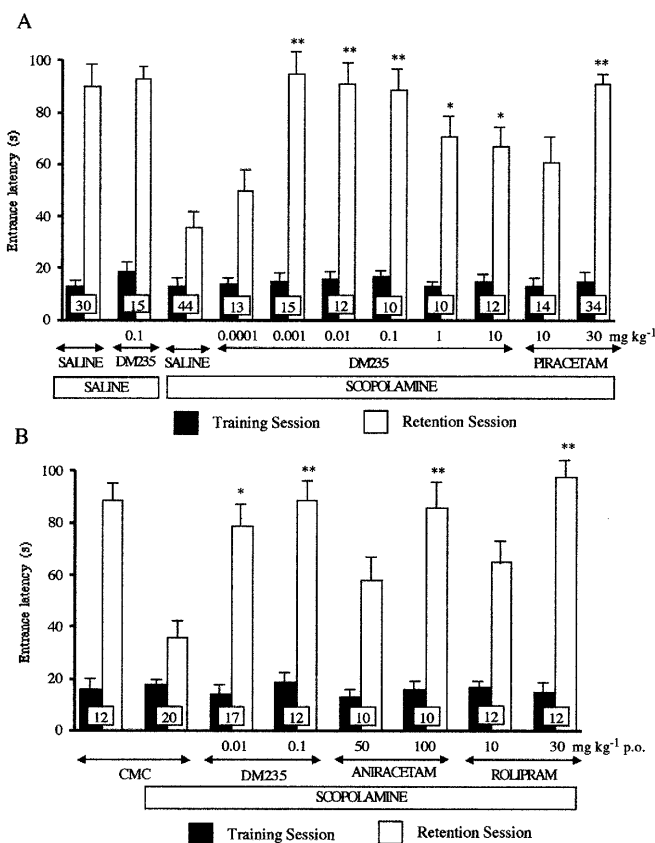


Fig. 2 Dose/response curve for DM 235 administered either i.p. in comparison with piracetam (A) or orally in comparison with aniracetam and rolipram (B) on amnesia induced by scopolamine (1.5 mg kg⁻¹ i.p.) in a passive avoidance test in mice. DM235, piracetam, aniracetam and rolipram were administered 20 min (i.p.) or 30 min (p.o.) before the training session while scopolamine was injected immediately after. The numbers in the columns indicate the numbers of mice tested. ** $P < 0.01$, * $P < 0.05$ vs. scopolamine-treated mice

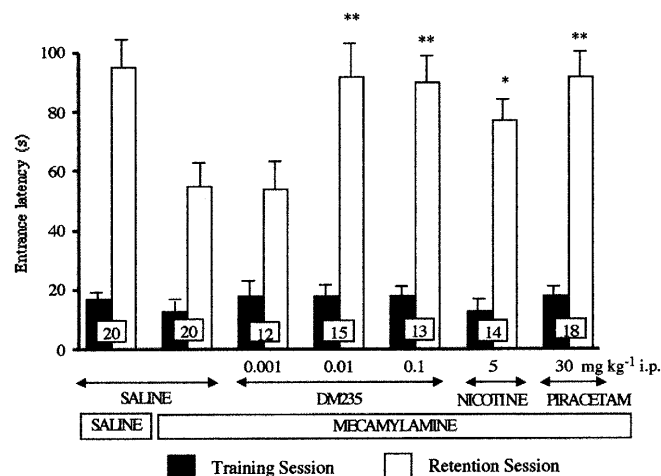


Fig. 3 Dose/response curve for DM235 (administered i.p.) in comparison with nicotine and piracetam on amnesia induced by mecamlamine (20 mg kg⁻¹ i.p.) in a passive avoidance test in mice. DM235, nicotine and piracetam were administered 20 min before training session while mecamlamine was injected immediately after. ** $P < 0.01$; * $P < 0.05$ vs. mecamlamine-treated mice

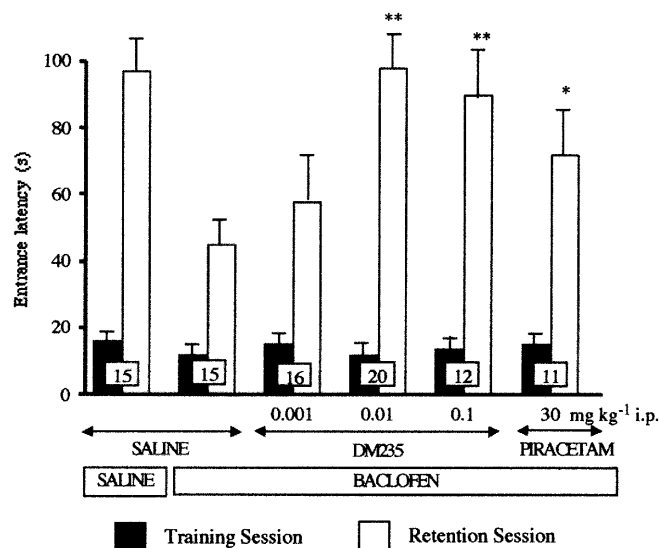


Fig. 4 Dose/response curve for DM235 (administered i.p.) in comparison with piracetam on amnesia induced by baclofen in a passive avoidance test in mice. DM235 and piracetam were administered 20 min before training session while baclofen ($2 \text{ mg kg}^{-1} \text{ i.p.}$) was injected immediately after. $**P < 0.01$; $*P < 0.05$ vs. baclofen-treated mice

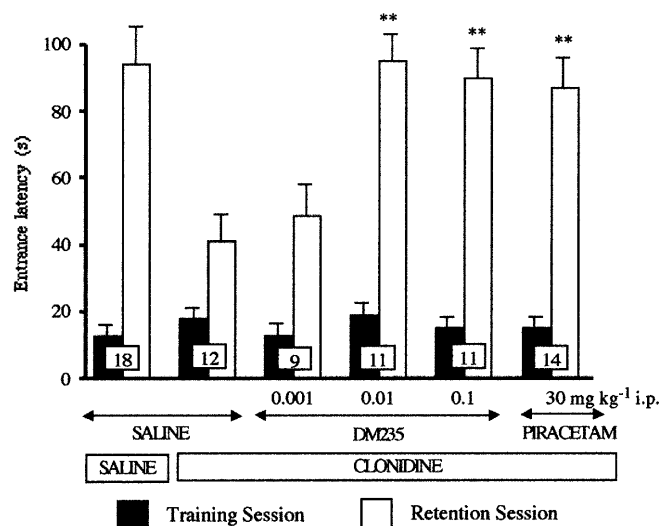


Fig. 5 Dose/response curve of DM235 (i.p.) in comparison with piracetam on amnesia induced by clonidine in a passive avoidance test in mice. DM235 and piracetam were administered 20 min before training session while clonidine ($0.125 \text{ mg kg}^{-1} \text{ i.p.}$) was injected 60 min before test. $**P < 0.01$ vs. clonidine-treated mice

$0.001 \text{ mg kg}^{-1} \text{ i.p.}$ DM235 was devoid of any effect (Fig. 4). The prevention of memory impairment by DM235 was comparable to the effect of piracetam ($30 \text{ mg kg}^{-1} \text{ i.p.}$, Fig. 4).

The memory impairment induced by clonidine ($0.125 \text{ mg kg}^{-1} \text{ i.p.}$) in the mouse passive avoidance test was prevented by DM235 ($0.01\text{--}0.1 \text{ mg kg}^{-1} \text{ i.p.}$) and piracetam ($30 \text{ mg kg}^{-1} \text{ i.p.}$), used as reference drug

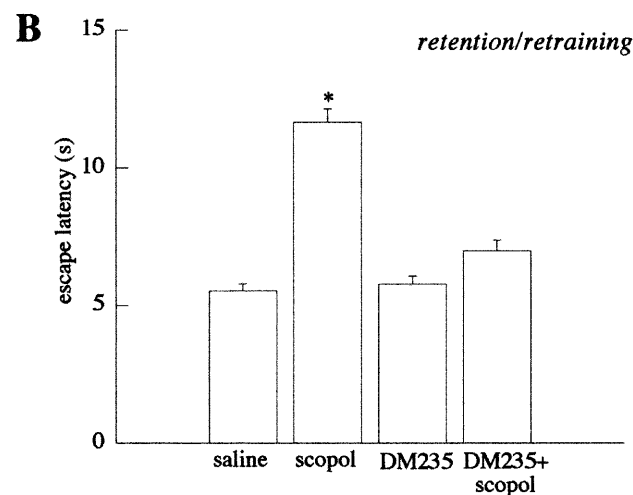
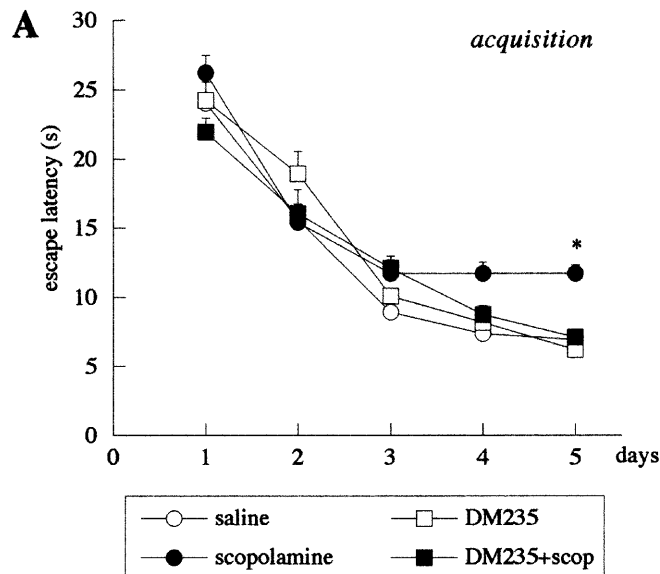


Fig. 6A,B The effect of DM235 (sunifiram) ($0.1 \text{ mg kg}^{-1} \text{ i.p.}$) on spatial reference memory in rats in the Morris water maze test. **A** Effect of DM235 on scopolamine ($0.8 \text{ mg kg}^{-1} \text{ i.p.}$)-induced impairment of acquisition. Task acquisition is reflected as a decrease in escape latency. Means $\pm$ SEM, $*P < 0.05$ vs. saline-treated rats. **B** Effect of DM235 on scopolamine-induced impairment on the retention-retraining day. Means $\pm$ SEM, $*P < 0.05$ vs. saline-treated rats

(Fig. 5). A tenfold lower dose of DM235 was ineffective (Fig. 5).

At active doses DM235 did not enhance the entrance latency in normal 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, 3, 4, 5).

In the Morris water maze test, all rats swam well in the pool and showed no evidence of sensorimotor impairment. As training proceeded, the rats spent progressively less time at, or near, the side walls and learned to use the

Table 1 Lack of effect of DM-235 in comparison with piracetam in the mouse rota rod test. Means $\pm$ SEM; $n=10$ mice

		Dose	Number of falls (30 s)			
			Before treatment	After treatment		
				15 min	30 min	45 min
Saline		10 ml kg ⁻¹ i.p.	3.4 $\pm$ 0.4	2.2 $\pm$ 0.3	1.7 $\pm$ 0.2	1.1 $\pm$ 0.2
DM235		0.1 mg kg ⁻¹ i.p.	3.7 $\pm$ 0.5	2.5 $\pm$ 0.4	1.5 $\pm$ 0.4	1.3 $\pm$ 0.3
DM235		1.0 mg kg ⁻¹ i.p.	3.5 $\pm$ 0.3	1.9 $\pm$ 0.4	1.3 $\pm$ 0.3	1.2 $\pm$ 0.3
DM235		10 mg kg ⁻¹ i.p.	3.8 $\pm$ 0.5	2.3 $\pm$ 0.4	1.5 $\pm$ 0.3	0.8 $\pm$ 0.2
Piracetam		30 mg kg ⁻¹ i.p.	3.6 $\pm$ 0.4	2.6 $\pm$ 0.4	1.0 $\pm$ 0.2	1.0 $\pm$ 0.2

Table 2 Lack of effect of DM-235 in the rat Animex test. Means $\pm$ SEM; $n=10$ rats

		Dose	Number of counts		
			After treatment		
			15 min	30 min	45 min
Saline		10 ml kg ⁻¹ i.p.	688 $\pm$ 45	112 $\pm$ 19	37 $\pm$ 13
DM235		0.1 mg kg ⁻¹ i.p.	691 $\pm$ 50	125 $\pm$ 21	45 $\pm$ 10

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 in the daily blocks during the 5 days of water maze acquisition and the retention/retraining was revealed (Fig. 6A). There was also a significant main effect of treatment. The scopolamine-treated rats showed longer latencies and path lengths before finding the platform than the other three groups of rats on day 5 (Fig. 6A) and on retention/retraining day (Fig. 6B). DM235 (0.1 mg kg⁻¹ i.p.) reversed the memory impairment induced by scopolamine on both acquisition and retention/retraining days, whereas, when administered alone, it did not ameliorate unimpaired memory processes (Fig. 6). There were no differences between the escape latencies of the last day of acquisition and the retention/retraining test (Fig. 6).

DM235 (1 mg kg⁻¹ i.p.) significantly reduced the total sleeping time (22.1 $\pm$ 3.4 min) induced by 60 mg kg⁻¹ i.p. pentobarbital (27.7 $\pm$ 3.3 min), similar to the effect induced by 30 mg kg⁻¹ i.p. piracetam (12.5 $\pm$ 5.3 min), whereas the dose of 0.1 mg kg⁻¹ i.p. was ineffective. At the same doses both drugs did not modify the induction time of hypnosis (control 3.9 $\pm$ 1.8; DM235: 4.3 $\pm$ 2.0; piracetam: 4.5 $\pm$ 2.1 min).

It should be noted that DM235 elicited its anti-amnesic effect without changing the animals' gross behaviour. No modification of motor coordination was revealed by the rota-rod test in the mouse. DM235 (0.1–10 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, the spontaneous mobility of mice and rats, as revealed by the hole board test (data not shown) and the Animex apparatus (Table 2) respectively was unmodified by DM235 (0.1 mg kg⁻¹ i.p.) in comparison with saline-treated animals.

Discussion

The present results describe the effects observed with DM235 on experimentally impaired memory processes in mice and rats. DM235 ameliorates cognitive processes by preventing pharmacologically-induced amnesia in the mouse passive avoidance and rat Morris water maze tests. Previous results have also demonstrated a procognitive activity of that DM235 in a social learning task in rats (Manetti et al. 2000a).

DM235 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, DM235 reversed the impairment of acquisition and retention/retraining produced by scopolamine in the Morris water maze test in rats. The lack of difference in the escape latencies between the last day of acquisition and the retention/retraining test excluded the possibility of induction of state-dependent effects (McGaugh 1989; Overton 1991). These results are in agreement with data obtained after administration of nootropic drugs. Pyrrolidone derivatives not only reverse the amnesia induced by impairment of the cholinergic system in a passive avoidance task (Verloes et al. 1988), but nootropic compounds, such as ne-firacetam and oxiracetam, also counteract cognitive deficits in a Morris water maze paradigm (Pitsikas and Algeri 1992; Fordyce et al. 1995; DeFord et al. 2001).

That stimulation of the cholinergic system improves cognitive processes has long been observed (Deutsch 1971; Bartus et al. 1982; Coyle 1995). Conversely, blockade of the cholinergic system disrupts memory functions. The administration of scopolamine, an unselective muscarinic ACh receptor antagonist, impairs learning and memory in humans (Frumier et al. 1976) and animals (Levin and Bowman 1986). Mice or rats treated with the M₁ selective antagonists pirenzepine and dicyclomine show impaired memory processes in various paradigms (Caulfield 1993; Sala et al. 1991; Ghelardini et al. 1997). Furthermore, the administration of a nicotinic ACh receptor antagonist, such as mecamylamine, dose-dependently impairs performance in passive avoidance tests (Elrod and Buccafusco 1991).

Microdialysis studies have shown that DM235 increases ACh release from the rat parietal cortex (Manetti et al. 2000b), a cerebral structure involved in the modula-

tion of cognitive processes (Bartus et al. 1982). Therefore, the potentiation of the cholinergic system by DM235 could make it able to prevent amnesia induced by an antimuscarinic drug as well as by the administration of a nicotinic antagonist. Extensive study of the modes of action of the pyrrolidone derivatives has revealed various pharmacological effects, with striking differences between drugs suggesting that no single predominant mode of action is shared by the whole class of drugs. Most, however, influence cholinergic function. Acetylcholine production and turnover are stimulated by most pyrrolidone derivatives but with varying actions at both muscarinic and nicotinic receptors. In particular, piracetam might alter presynaptic cholinergic function, possibly by enhancing high-affinity neuronal uptake of choline (Pedata et al. 1984), but these data are controversial (Franklin et al. 1986). Pilch and Müller (1988) have shown that piracetam elevates muscarinic receptor density in the frontal cortex of aged but not of young mice. Increased acetylcholine release has been demonstrated for piracetam (Hitzenberger et al. 1998) and aniracetam increases acetylcholine content in the hippocampus and cerebral cortex (Toide 1989).

Affinity for central receptors might explain the potentiation of the endogenous cholinergic system by DM235. In binding studies, however, DM235 (10^{-6} M) showed affinity for neither muscarinic and nicotinic receptors, nor for the other most important central receptors (data not shown). A lack of receptor affinity is also a characteristic feature of the nootropic compounds. These drugs, with the 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 effects of DM235 on the cholinergic system might be secondary.

Amnesia can also be obtained by modulating neurotransmitter systems other than the cholinergic system. GABA is the main inhibitory neurotransmitter in the brain and plays an important role in learning and memory. The activation of GABA_A and GABA_B receptors reportedly impairs memory performance (Jerusalinsky et al. 1994; Swartzwelder et al. 1987). Cognitive processes, including learning and memory, can be ameliorated by GABA_B receptor antagonists (Mondadori et al. 1992). α_2 -Agonists also 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).

DM235, even in the absence of any interaction with GABA_B or α_2 -adrenoceptors, prevented the amnesia induced by baclofen and clonidine. Thus, DM235 counteracts amnesia not only induced by anticholinergic drugs, but also that induced by blockade of the cholinergic system. The capability for reversing the amnesia induced by activation of GABA_B receptors is also observed with fasoracetam (Ogasawara et al. 1999). The involvement of the GABAergic system in the mechanism of action of

pyrrolidone derivatives has also been postulated. The chemical similarity between GABA and pyrrolidones has prompted many studies on the role of the GABAergic system in the amelioration of memory processes by nootropic drugs. However, with the sole exception of fasoracetam, which up-regulates GABA_B receptors (Ogasawara et al. 1999), few of these drugs have any substantial action at GABA receptor sites (Gouliaev and Senning 1994).

DM235 did not show any procognitive activity in the passive avoidance and Morris water maze 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 DM235 but also well-known nootropic drugs such as piracetam, aniracetam, nefiracetam and oxiracetam, or cholinomimetics such as physostigmine and oxotremorine, do not facilitate memory in unamnesic animals (Pitsikas and Algeri 1992; Gouliaev and Senning 1994; Coyle 1995; Fordyce et al. 1995; DeFord et al. 2001). In the first session, neither the latency for entering the dark compartment of the light-dark box in the passive avoidance test nor the escape latency in the Morris water maze test were modified by the administration of DM235.

The ability of nootropics to reduce sleeping time has been observed in humans. Hollister (1985) has reported that piracetam increases waking hours in elderly. This waking effect would be beneficial for patients in whom senile dementia is associated with persistent sleepiness. DM235 reduced the total sleeping time induced by pentobarbitone without modifying the induction time of hypnosis. This effect was comparable to that exerted by piracetam, even though weaker than that produced by the nootropic compound and present only at high doses.

The amelioration of memory process induced by DM235 occurred without any side effects. DM235, at the highest effective doses, neither impaired motor coordination, as revealed by the rota-rod test, nor modified spontaneous motility, as indicated by the Animex apparatus and the hole board test. Furthermore, DM235 at a dose 1,000 times higher than the minimal effective dose, is still devoid of any alteration of behavioural parameters.

The present results provide evidence that DM235 is a new anti-amnesic compound for which membership of the class of nootropic drugs can be supposed. Indeed, DM235, even though not a pyrrolidone derivative, shows a high degree of structural similarity with piracetam (Manetti et al. 2000a) and also exhibits a pharmacological profile comparable to that of nootropics. DM235 is, in fact, endowed with the main pharmacological properties of piracetam-like compounds: facilitation of memory processes, lack of toxicity and side effects and a lack of affinity towards the most important central receptors. DM235 differs from pyrrolidone derivatives in its potency. Even though it exerts the same pharmacological effects, DM235 is at least 100 times more potent than the most active nootropic drugs such as oxiracetam, nefiracetam, etiracetam, aniracetam (Gouliaev and Senning 1994).

In conclusion, these results indicate that DM235 is a novel cognition enhancer, chemically and pharmacologi-

cally closely related to the 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 minimally effective dose, suggest that DM235 may be a promising compound for the treatment of human cognitive deficits.

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