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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

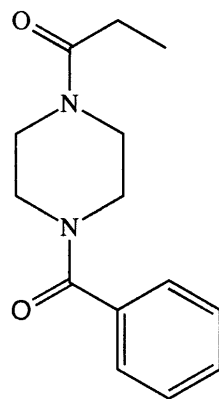
The so-called nootropic compounds are a group of pharmacologically active pyrrolidone derivatives that, in some respects, occupy a special position in the pharmacology of the central nervous system. The first pyrrolidone to come to the attention of clinicians was piracetam. This compound was developed 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; Gouliavov 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.

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Fig. 1 DM235 (sunifiram):
1-benzoyl-4-propionylpiper-
azine



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.