Pharmacology Biochemistry and Behavior, Vol. 59, No. 4, pp. 897–901, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00526-1

Long-Lasting Antiamnesic Effect of a Novel Anticholinesterase Inhibitor (MF268)

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Received 29 April 1997; Revised 11 July 1997; Accepted 11 July 1997

BRAIDA, D., E. PALADINI, P. GRIFFINI, M. LAMPERTI, L. COLIBRETTI AND M. SALA. *Long-lasting antiamnesic effect of a novel anticholinesterase inhibitor (MF268).* PHARMACOL BIOCHEM BEHAV **59**(4) 897–901, 1998.— In the present study a short (120 min) and long-lasting (360 min) antagonism of scopolamine-induced amnesia in rats was investigated in an eight-arm radial maze, by (3a *S*, 8a *R*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol[8-(cis2,6-dimethyl-morpholin-4-yl)octyl]-carbamate *L*-bitartrate hydrate (MF268), a new cholinesterase inhibitor. Upon completing the training session, the rats were orally administered increasing doses of MF268 (2, 3, 6, 7, and 8 mg/kg) 60 min prior to SC injection of scopolamine (0.25 mg/kg). Following a further 60 min the rat was placed in the maze. The reversal of scopolamine-induced impairment was characterized by an inverted U-shaped dose–response curve. A significant reduction in the number of errors, and time taken to complete the maze was observed with a dose of 6 mg/kg. The compound improved memory retention without affecting scopolamine-induced hypermotility. When the same dose was administered 360 min prior to the test a significant reduction in the number of amnesic animals was observed, whereas no cognitive improvement was detected when either 1-Benzil-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methyl piperidine hydrochloride (E2020) (0.25 mg/kg) or tacrine (0.5 mg/kg) were administered 360 min prior to the test. The kinetics of whole-brain cholinesterase confirmed the long-lasting activity for MF268. A clinical relevance for the use of MF268 in AD treatment is suggested. © 1998 Elsevier Science Inc.

MF268 E2020 Tacrine Radial maze Brain cholinesterase Inhibition Locomotor activity Rat

THERE is sufficient clinical and experimental evidence to indicate that hippocampal and cortical cholinergic innervation is dramatically reduced in Alzheimer's disease (AD) patients. It has, therefore, been postulated that compounds that are able to potentiate cholinergic transmission in these regions, might be used to alleviate the symptoms associated to AD. Preclinical and clinical research on new AChE inhibitors has strongly advanced during the last few years, particularly for second generation AChE inhibitors, which are characterized by high BBB penetration, potent, long-lasting and selective AChE inhibition.

Recently, heptylphysostigmine, a second-generation cholinesterase inhibitor that induces a marked and long-lasting inhibition of brain cholinesterase as well as a prolonged elevation of ACh levels in the rat cerebral cortex (4) has been found to fully reverse scopolamine-induced amnesia in rats using an eight-arm radial maze (3).

MF268, a new eseroline derivative, which interacts with both the catalytic and regulatory anionic sites of AChE, is undergoing development due to its extra long-lasting AChE inhibition. It is a tight-binding inhibitor with an in vitro IC_{50} (inhibitor concentration 50%) on whole rat brain AChE equal to 9×10^{-9} M, following a 120-min incubation period. Oral (2–5 mg/kg) as well as subcutaneous (0.5–2 mg/kg) administration of MF268 has been shown to induce a marked and substained increase in rat cortical levels of extracellular ACh (460 and 1200% after oral; 360 and 2500% after subcutaneous administration, respectively), without significantly altering any other neurotransmitters. T_{max} was observed after 5–6 h (16).

On the basis of these biochemical data, we conducted the present study to verify whether MF268, administered orally, was able to reverse scopolamine-induced cognitive deficit in an eight-arm radial maze.

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As standard control compounds of the second generation, tacrine and E2020 were used in the same task. Tacrine is a centrally active noncompetitive reversible AChE inhibitor that has been reported as the first drug approved in the United States for the treatment of AD (8). E2020 is a new synthesized indanone-piperidine derivative that was shown to potently and selectively inhibit AChE activity (5).

METHOD

Animals

Male Wistar albino rats (Charles River, Calco, Italy), weighing about 300 g, were individually caged with free access to water in an air-conditioned (22 ± 2°C) room. A 12-h light cycle (0800 to 2000 h) was used.

Animal care was in accordance with the State regulations governing the care and treatment of laboratory animals.

Cholinesterase Inhibition

Animals were orally administered (3a *S*, 8a *R*)-1,2,3,3a 8,8a-hexahydro-1,3a,8-trimethylpyrrolo [2,3-b] indol-5-ol[8-(cis2,6-dimethyl-morpholin-4-yl)octyl]-carbamate L-bitartrate hydrate (MF268) (2 and 5 mg/kg). After 30 min, 120 min, 360 min, 8 h, and 15 h the rats were decapitated. The whole brain was removed, washed to remove external blood, and then dissected into two. Only one hemisphere was homogenized in distilled water (1 g/5 ml wt/vol). Aliquots (25 μl) of brain homogenate were placed into the differential pH meter to determine the amount of free enzyme present (2).

Radial Maze

The working memory was studied using a computerized wooden eight-arm radial maze (as previously described) (12). Briefly, the 75 × 7.5 cm arms, each with 10-cm high white plastic side walls, radiated from a central 30-cm wide octagonal platform that served as a starting base. Small plastic cups mounted at the end of each arm served as receptacles for food reinforcers. Access to the arms, in which a 45-mg food pellet was placed, was controlled by eight pneumatically operated sheet metal guillotine doors. The entire maze was painted black, elevated 50 cm from the floor, and placed in the center of a small room (2.5 × 2.5 m) lit by fluorescent lights and provided with several extramaze cues. Animal behavior was monitored by a video camera (Model CCD, Securit Alarmitalia) whose signals were digitized, interfaced by a PF6PLUS PAL 512X512 pixels (Imaging Technology, Woburn, MA), and sent to a video monitor (Tinitron KX-14CP1, Sony, Japan). Image analysis and pattern recognition were elaborated by a Delta System computer (Addonics) using software provided by Biomedica Mangoni (Pisa, Italy).

The animals were kept at 85% of their free feeding body weight for the duration of the experiment. After 3 days of free exploration, the animals were trained to complete the maze as described elsewhere (12). During each session working memory was scored on the basis of the total number of errors, the percentage of amnesic animals (number of animals making more than one error), and the total time taken to complete the test. Training, at the rate of one session/day, continued until the rats had fulfilled the criterion, i.e., entering seven different arms out of the first eight choices on 5 successive days. The animals that failed to fulfill this criterion within 30 days were discarded.

After reaching criterion, the animals were habituated to handling and injections prior to actual drug treatments by means of a daily administration of distilled water (PO) and saline (SC injection) and then tested in the maze. When a stable baseline responding was ensured (a week at the most), groups of 8–10 rats each were randomly assigned to the following treatments: MF268 (2, 3, 5, 6, 7, and 8 mg/kg), tacrine (0.5 mg/kg), and E2020 (0.25 mg/kg). E2020 was synthetized by Mediolanum Farmaceutici. MF268 was administered PO 120 and 360 min before the test, while the other compounds were administered PO 360 min before the test. Scopolamine was administered SC 60 min prior to the test session. The tests with the compound under investigation were carried out at 7-day intervals, and on the other days training was carried out without any treatment, until criterion was once again reached. Five percent of the animals did not reattain predrug performance level, so they were discarded. Each rat received no more than four different treatments, and the order of treatment was counterbalanced among subjects.

Scopolamine (0.25 mg/kg) was administered SC 60 min before the test to a further group of eight animals (positive control). Different drug schedules were tested on a single day and scopolamine treated group was always present.

Locomotor Activity

Locomotor activity was recorded as previously described (3). Briefly, all experiments were recorded through a video camera for 30 min, 120 min after oral administration of the drug, in a Plexiglas black square arena (43 × 43 × 12 cm) and expressed in terms of total distance travelled (cm). Image analysis and pattern recognition were elaborated by a Delta System computer (Addonics) using software provided by Biomedica Mangoni (Pisa, Italy). The recording apparatus was located in a separate room. For MF268, the doses tested were: the one that maximally antagonized scopolamine-induced cognitive impairment (6 mg/kg), and two higher ones (8 and 12 mg/kg).

Drugs

(3a *S*, 8a *R*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol[8-(cis2,6-dimethyl-morpholin-4-yl)octyl]-carbamate L-bitartrate hydrate (MF268), 1-Benzil-4-[5,6-dimethoxy-1-indanon)-2-yl]-methyl piperidine hydrochloride (E2020) (kindly supplied by Mediolanum Farmaceutici), 9-amino-1,2,3,4-tetrahydroaminoacridine hydrochloride hydrate (tacrine) (Sigma, St. Louis, MO) were dissolved in distilled water and administered either 120 and/or 360 min before the test session. The drugs were prepared daily and administered in a volume of 5 ml/kg.

Scopolamine hydrobromide (Sigma) was dissolved in saline and administered SC 60 min prior to test, in a volume of 2 ml/kg.

Data Analysis

Performance on the day immediately preceeding a drug administration of all groups of animals was used as its own control.

All of the normally distributed data were expressed as mean ± SEM and analyzed by means of one-way ANOVA for a repeated-measure design, followed by Tukey's test, where appropriate.

The cognitive parameters (total number of errors and time taken to complete the test) were plotted against log administered dose and calculated using statistical analysis adapted to curvilinear regressions (13). The χ^2 test was used to statistically analyse the number of amnesic animals.

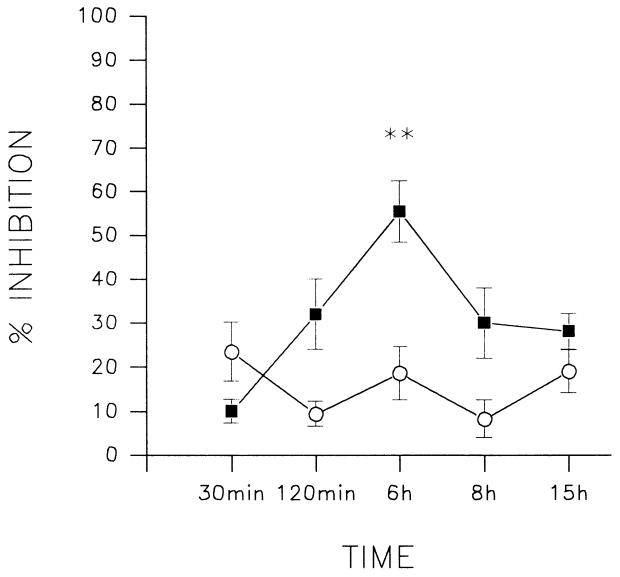


FIG. 1. Time course of whole rat brain AChE inhibition after a single oral dose of MF268, 2.0 (○) and 5.0 (■) mg/kg. $^{**}p < 0.002$ compared with 30 min (ANOVA test).

RESULTS

At the oral dose of 5 mg/kg MF268 significantly inhibited rat whole brain cholinesterase by 55% after 360 min ($F = 16.95$, $p < 0.002$) (Fig. 1). Its long duration of action was evident by the fact that after 15 h a 30% inhibiton was still present. At this dose no cholinergic side effects were seen throughout the experiment.

To satisfy the criterion in the radial maze, training lasted 10–30 days, the pretreatment performance between each group of animals being similar. Pretest saline injection did not modify performance, which was about 90% of working memory (0.12 ± 0.13 total errors). Scopolamine significantly increased the mean total number of errors, $F(1, 16) = 45.63$, $p < 0.0001$, the percentage of amnesic animals ($p < 0.0001$, χ^2 test), and the mean time taken to complete the test, $F(1, 16) = 25.08$, $p < 0.0001$, when compared to the control group (Fig. 2). The reduction in scopolamine-induced amnesia observed with MF268, administered 120 min before the test, resulted in statistically symmetrical parabolas, log dose vs. number of errors ($r = 0.87$, $p < 0.01$) and log dose vs. time taken ($r = 0.59$, $p < 0.05$). The 6 mg/kg dose was maximally active in reversing scopolamine-increased errors and time, $F(7, 50) = 4.28$, $p < 0.001$, and $F(7, 50) = 3.54$, $p < 0.05$ for errors and time, respectively; Tukey's test: $p < 0.01$.

Eighty-six percent of the animals treated with scopolamine were amnesic. With MF268, a U-shaped dose-dependent reduction was observed, even though it never reached the statistically significant level.

MF268 (6, 8, and 12 mg/kg), did not modify the locomotor activity in rats, whereas scopolamine significantly increased it, $F(7, 56) = 3.31$, $p < 0.01$ (Fig. 3). MF268 was unable to reverse this anticholinergic hyperstimulation except at the highest dose employed (12 mg/kg).

When different cholinesterase inhibitors were administered 360 min before the test (Fig. 4), ANOVA evidenced a significant effect for drug treatment, $F(4, 35) = 6.66$, $p < 0.0005$. A post hoc test showed a significant difference only between

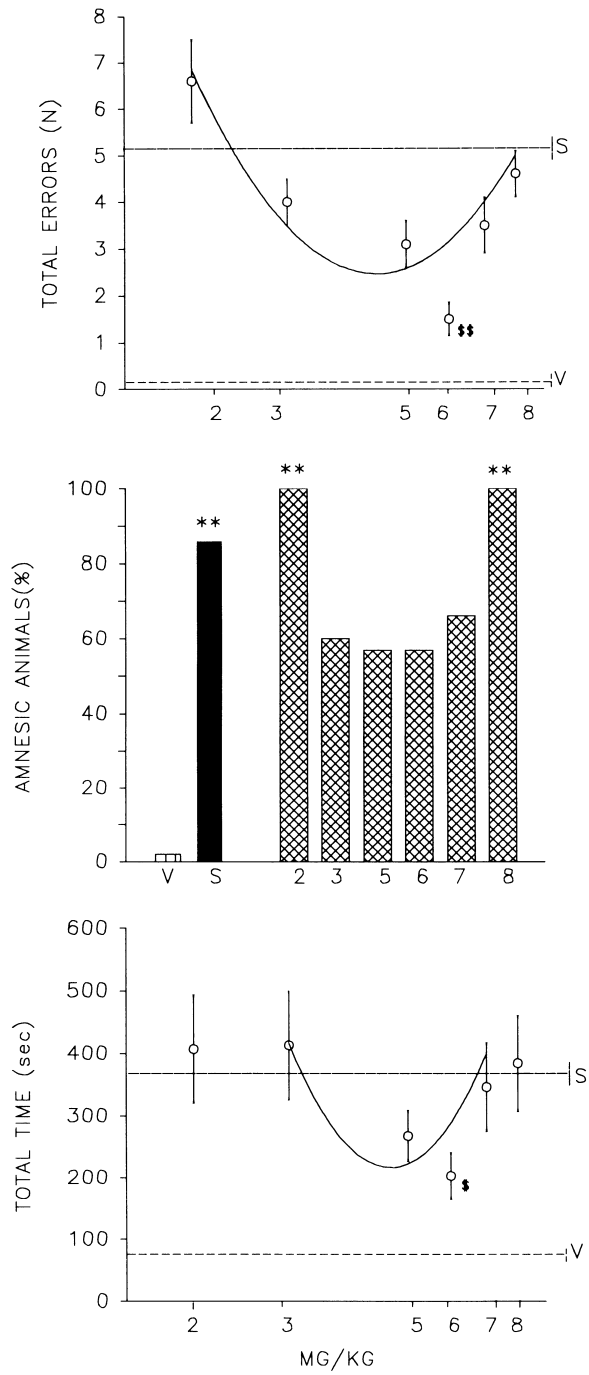


FIG. 2. (Top) Parabolic regression line of the total number of errors (mean ± SEM) plotted against log/dose of MF268 PO administered 120 min before the test. (Middle) Effect of increasing doses of MF268 on the percentage of amnesic animals. (Bottom) Parabolic regression line of the total time (mean ± SEM) taken to complete the maze. Scopolamine group (S); pooled value for rats treated with saline SC and distilled water PO the day before pharmacological treatment (V). $^{**}p < 0.01$ compared with vehicle (χ^2 test); $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ compared with the scopolamine group (ANOVA followed by Tukey's test).

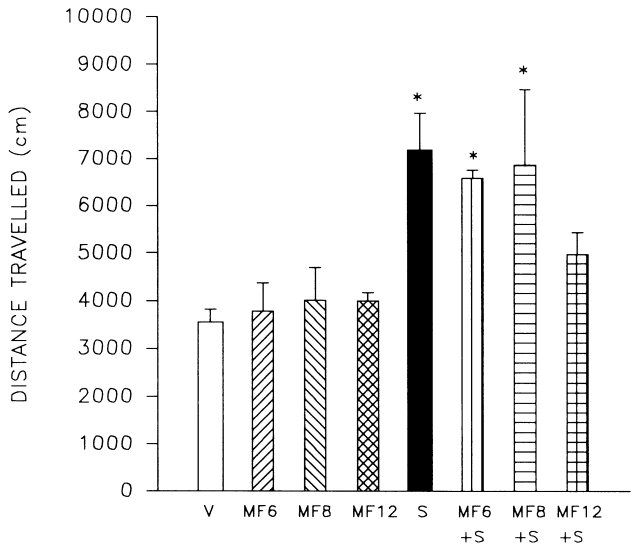


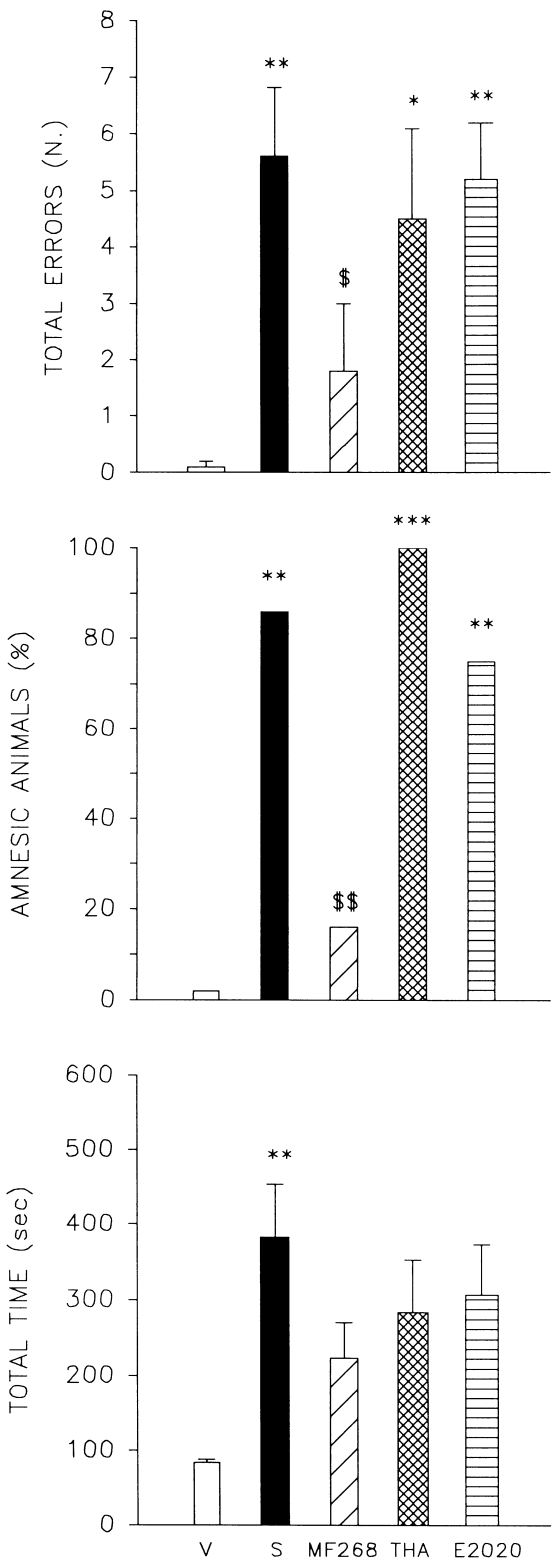
FIG. 3. Effect of MF268 (6, 8, and 12 mg/kg/PO) alone and in association with scopolamine (S) on locomotor activity evaluated in terms of total distance travelled (cm) in 30 min. The results are shown as mean ± SEM. V represents the control group. $^{*}p < 0.05$, compared with the control group (ANOVA followed by Tukey's test).

MF268 treatment and scopolamine ($p < 0.01$, Tukey's test). Moreover, MF268 significantly reduced the percentage of amnesic animals (16%) compared to tacrine (100%) and E2020 (75%) ($p < 0.01$, χ^2 test). Regarding the time taken to complete the task, no difference was detected between any of the treatments and the scopolamine group.

DISCUSSION

Our results clearly show that MF268 is able to antagonize the deleterious effects of scopolamine on memory, confirming the ability of the anticholinergic drug to disrupt performance in the radial maze as in hippocampectomized subjects (1). On all the cognitive parameters measure, MF268 exhibited an inverted-U dose–response curve, when administered 120 min before the test. Maximum antagonistic effect was attained at a dose of 6 mg/kg, when considering total number of errors and time taken, while only a slight reduction in the total number of amnesic animals was observed. Our previous data about tacrine and E2020, administered orally 120 min prior to the same test, indicated a presence of an inverted U-shaped dose–response curve. A significant antagonistic effect was achieved with a dose of 0.25 mg/kg for E2020 and 0.5 mg/kg for tacrine (3). Our findings are also in accordance with others (6, 14, 15), in which cholinergic agonists, including cholinesterase inhibitors, improve performance at low doses but are inefficacious at higher ones. It is difficult to explain this observation by simply suggesting downregulation or tolerance. We are inclined to believe that the activation of presynaptic autoreceptors may play a role in reducing the efficacy of the cholinesterase inhibitors.

MF268 did not completely restore the time taken to complete the maze, to control values, nor was it able to block sco-



of the amnesic animals (middle), and the total time taken to complete the maze (bottom). The results are shown as mean ± SEM. V represents the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ compared with scopolamine (ANOVA followed by Tukey's test or χ^2 test where appropriate).

FIG. 4. Effect of MF268 (6 mg/kg), tacrine (THA) (0.5 mg/kg), E2020 (0.25 mg/kg) (administered PO 360 min before the test), and scopolamine (S) on the total number of errors (top), the percentage

polamine-induced peripheral side effects (i.e., mydriasis and drying of the mucosa; data not shown). This would suggest that the observed increase in the time taken to complete the maze is probably influenced by these side effects, as previously reported (10). At the dose that significantly improved memory performance, MF268 was unable to antagonize scopolamine-induced hypermotility. This would suggest that the transient amnesia did not result from a sensory or motor or motivational disability, but from a difficulty to use the visual spatial information relevant to each stage of the test. Therefore, the compound seems to exhibit a certain degree of selectivity on memory function.

When the compound was tested 360 min after treatment, at the dose achieving maximal activity after 120 min, a significant reduction was detected in the number of amnesic animals as well as the total number of errors, as was a slight decrease in the time taken. This would suggest that MF268 possesses a long-lasting effect on memory. This finding is supported by ex vivo biochemical data in which MF268 (5 mg/kg) manifests maximum inhibition on whole-brain cholinesterase 360 min after treatment. What's more, a 30% inhibition is still present after 15 h.

The present data are consistent with microdialysis studies in which MF268 (5 mg/kg/PO) has been shown to induce a significant increase in extracellular ACh in rat cerebral cortex (+1200%) for 360 min but disagree with the level of AChE inhibition found (9.7%) (16). At this regard, the low level of AChE inhibition observed in this report could be ascribed to methodological differences, because AChE activity was assayed in whole-brain homogenates at 0°C, while our experiments were carried out at 37°C. A similar duration of action has also been reported for MF268 (2 mg/kg) after SC administration, maximum effect on ACh being +2000%. Compared to MF268, our data demonstrate a lack of ability of tacrine and E2020 to restore memory when administered 360 min before the test, at a dose previously found maximally active on memory function (3). In fact, AChE inhibition for both compounds has been found to be maximal after 60 min with a progressive loss of activity (7, 11).

In conclusion MF268 appears to have a long-lasting activity in selectively antagonizing cognitive deficit in an eight-radial maze, exhibiting an inverted-U shaped dose–response curve. Moreover, the fact that the maximally active dose shows a moderate degree (55%) of whole-brain AChE inhibition is in agreement with the findings obtained in AD patients where moderate (30–60%) steady-state AChE inhibition is associated to a maximal cognitive and clinical improvement (9). Even if it is most unlikely that scopolamine-induced amnesia can provide a useful model of AD, the long duration of action together with the slow onset of its activity make MF268 a possible candidate for the treatment of AD.

ACKNOWLEDGEMENTS

This work was supported by a grant from MEDIOLANUM Farmaceutici (Milan, Italy) and a grant (60%, 1994) from the Italian Ministry for University and Scientific and Technological Research (MURST) to M. Sala.

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