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An inverted U-shaped curve for heptylphysostigmine on radial maze performance in rats: comparison with other cholinesterase inhibitors

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Abstract

The potential of heptylphysostigmine tartrate (pyrrolo [2,3b] indol-5-ol, 3,3a,8,8a-hexahydro-1,3a,8-trimethylheptylcarbamate [ester, (3aS-cis)]) (MF201), a new second-generation cholinesterase inhibitor, to antagonize scopolamine-induced amnesia in rats was assessed in an 8-arm radial maze. Upon completing the training session, the rats were orally administered increasing doses of MF201 (2, 3, 4, 6 and 8 mg/kg) 60 min prior to a s.c. injection of scopolamine (0.25 mg/kg). 9-Amino-1,2,3,4-tetrahydroamino-acridine hydrochloride hydrate (tacrine) (0.25, 0.37, 0.5, 1 and 2 mg/kg), 1-benzil-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methyl piperidine (E2020) (0.125, 0.18, 0.25 and 0.5 mg/kg) and physostigmine (0.15, 0.25, 0.5 and 1 mg/kg) were orally administered and rats were tested in the same task. As previously described, scopolamine induced an impairment in radial maze performance, measured in terms of total number of errors, total time taken to complete the task and the percentage of amnesic animals. The reversal of scopolamine-induced impairment was characterized by the presence of an inverted U-shaped dose–response curve. A significant antagonistic effect was achieved with a dose (mg/kg) of 0.25 for E2020, 0.5 for tacrine and physostigmine and 3, 4 and 6 for MF201, the latter manifesting a broader spectrum of activity (3–6 mg/kg). While the maximal active doses restored the scopolamine-induced modified pattern of arm entry, they were ineffective in reducing hypermotility, suggesting the drugs have a specific effect on cognitive function.

Keywords: MF201; Tacrine; E2020; Physostigmine; 8-Arm radial maze; (Rat)

1. Introduction

The use of cholinesterase inhibitors to rectify the cholinergic deficit in Alzheimer's disease is seen as one of the most promising treatments following the important progress made in preclinical and clinical research on second-generation cholinesterase inhibitors (Giacobini, 1991).

The overall characteristics of these cholinesterase inhibitors (tacrine, E2020, ENA 713, etc.) are good penetration across the blood–brain barrier and potent and long-lasting inhibition of acetylcholinesterase with few peripheral cholinergic side-effects (Giacobini and Cuadra, 1994). Amongst these compounds, heptylphysostigmine (MF201) is a new carbamate derivative of physostigmine that causes a marked and long-lasting inhibition of cerebral cholinesterase as well as a prolonged elevation of acetylcholine levels in the rat cerebral cortex (Cuadra et al., 1994).

Until now, MF201 administered s.c. has been found to fully reverse, in rats and mice, scopolamine-induced amnesia in the passive avoidance test (Brufani et al., 1987; Dawson et al., 1991; Dawson and Iversen, 1993) and in the delay matching to position test in rats (Dawson et al., 1991). Moreover, s.c. injection of MF201 partially restored the scopolamine-induced deficit (Dawson et al., 1991) in the conditioned suppression of drinking test and in a 14 unit T-maze when administered i.p. (Iijima et al., 1992). MF201 (0.2–0.9 mg/kg/i.m.) was also able to antagonize the scopolamine-induced cognitive deficit of rhesus monkeys in a spatial delayed response task (Rupniak et al., 1992).

This study was performed in order to examine the effects of MF201 on scopolamine-induced deficit in a 8-arm radial maze test, using oral administration which is clinically more appropriate, and to test a wide range of

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doses in comparison with two second-generation cholinesterase inhibitors tacrine and E2020 and the classical physostigmine.

Particular attention was given to the possible interference of motor activity on cognitive performance.

2. Materials and methods

2.1. Animals

The study involved a total of 170 male Wistar albino rats (Charles River, Calco, Italy), aged ~ 3 months and weighing ~ 300 g upon receipt. The animals were individually caged with free access to water in an air-conditioned room (22 ± 2°C) illuminated between 08:00 and 20:00 h by overhead artificial lights.

2.2. Apparatus and procedure

2.2.1. Radial maze

The working memory was studied using a wooden 8-arm radial maze according to Olton and Samuelson (1976). 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 512 × 512 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 recording apparatus was located in a separate room.

After each trial, the maze was cleaned with a 2.5% cider vinegar solution. The animals, which had been previously deprived of food, were kept at 85% of their free-feeding body weight for the duration of the experiment. Water was available ad libitum.

After 3 days of free exploration, performance in the maze was examined by placing each animal on the platform of the maze with all the doors closed. Following a 1-min acclimation period, all doors were simultaneously opened and the animals were trained to complete the maze as described elsewhere (Sala et al., 1991). 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. At the same time, the pattern of arm entry was examined according to McCann et al. (1987), who designed a method to analyse the angle chosen when a rat entered two consecutive arms. In the 8-arm radial maze, five possible angles exist: from 0° (which corresponds to a re-entry into the arm just visited) to 180° (corresponding to entry into the arm directly opposite the one just exited). Angles of 45, 90 and 135° may also be observed. The first eight arm entries of a session were used in our analysis but since the first arm entry originates from the center of the maze, only seven angles were actually measured for each session. The frequency of each choice was then determined (frequency = number of observations/7).

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 rats which failed to fulfil this criterion within 30 days were discarded.

After reaching the criterion performance, the animals were habituated to the treatment regimen for at least 1 week by means of a daily p.o. distilled water and s.c. saline injection, and then tested in the maze. Once criterion performance had been reached, groups of 6–10 rats each were randomly assigned to the following treatments: MF201 (2, 3, 4, 6 and 8 mg/kg); tacrine (0.25, 0.37, 0.5, 1 and 2 mg/kg); and E2020 (0.125, 0.18, 0.25 and 0.5 mg/kg). E2020 was synthetized by Mediolanum Farmaceutici. Due to its short half-life, physostigmine (0.15, 0.25, 0.5 and 1 mg/kg) was administered p.o. immediately before scopolamine, while the other compounds were administered 60 min beforehand. Scopolamine was administered 60 min prior to the test session. Drug tests were carried out at intervals of at least 7 days, and on the other days training was done without drug treatment until five consecutive criterion trials were again obtained. Each rat received no more than four treatments, all distinct, and the order of treatment was counterbalanced among subjects.

Scopolamine (0.25 mg/kg) was administered s.c. 60 min before the test to a further group of 10 animals (positive control). Different drug schedules were tested on single day and animals treated with scopolamine alone were always present.

2.2.2. Locomotor activity

Locomotor activity was tested in a Plexiglas square arena (43 × 43 × 12 cm) that was painted black and elevated 65 cm above the floor. The arena was placed in the center of a square room, measuring 6.25 m^2 in surface and lit by fluorescent lights.

All experiments were recorded through a video camera (Model CCD, Securit Alarmitalia) whose signals were digitized, interfaced by a PF6PLUS PAL 512 × 512 pixels (Imaging Technology), and sent to a video monitor (Tinitron KX-14CP1; Sony). 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 each cholinesterase inhibitor, the doses tested were those that maximally antagonized the scopolamine-induced cognitive impairment and the highest dose used in the radial maze.

Locomotor activity was recorded for 30 min starting 120 (or 60 min for physostigmine) after oral administration of the drug. Motor activity was evaluated in terms of total distance travelled (cm) over 30 min. At the end of the session, the animals were returned to their cages.

2.3. Drugs

Heptylphysostigmine tartrate (pyrrolo [2,3b] indol-5-ol, 3,3a,8,8a-hexahydro-1,3a, 8-trimethylheptylcarbamate [ester, (3aS-cis)]) (MF201), 1-benzil-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methyl piperidine hydrochloride (E2020) (kindly supplied by Mediolanum Farmaceutici, Milan, Italy), 9-amino-1,2,3,4-tetrahydroaminoacridine hydrochloride hydrate (tacrine) and physostigmine (Sigma, St Louis, MO) were dissolved in distilled water and administered p.o. 120 (or 60 min for physostigmine) 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 s.c. 60 min prior to the test, in a volume of 2 ml/kg.

2.4. Statistics

Performance on the day immediately preceding a pharmacological treatment was used as control for each drug dose.

All of the normally distributed data were expressed as mean ± S.E.M. and analysed by means of one-way ANOVA for a repeated measure design, followed by Tukey's t test where appropriate. Expressed against the log of the administered doses, the values for the cognitive parameters (the total number of errors and the time taken to complete the test) were calculated by the usual statistical analysis adapted to curvilinear regressions according to Steel and Torrie (1960). For amnesic animals, the χ^2 test was used to evaluate the significance of percentage scores.

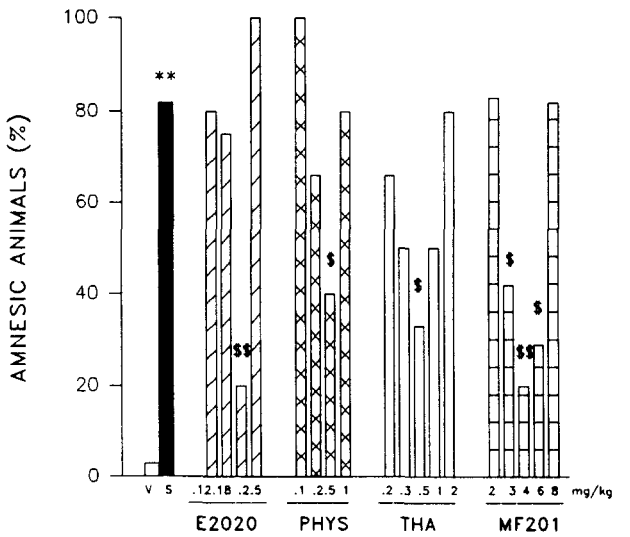

Fig. 2. Effect of increasing doses of E2020, physostigmine (PHYS), tacrine (THA) and MF201 on the percentage of amnesic animals. Scopolamine group (S); pooled value for rats treated with saline s.c. and distilled water p.o. the day before pharmacological treatment (V). ** $P < 0.01$ compared with vehicle; $ $P < 0.05$, $$ $P < 0.01$ compared with scopolamine (χ^2 test).

3. Results

3.1. Radial maze

In order to satisfy the criterion, training lasted 10–30 days, the pretreatment performance of each group of ani-

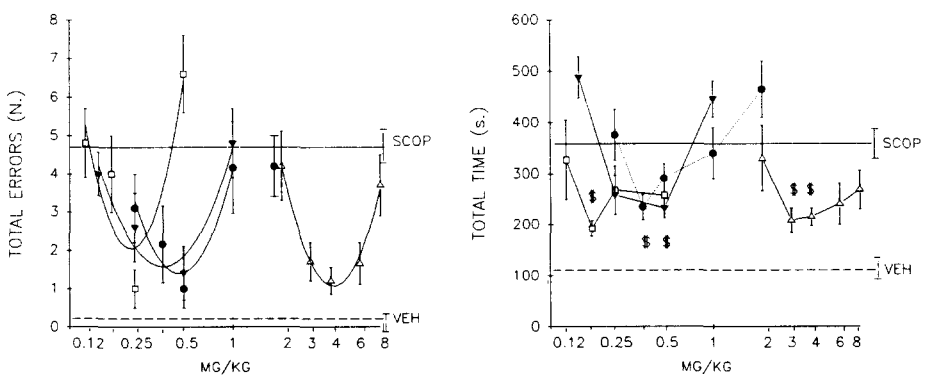

Fig. 1. Left: parabolic regression lines of the total number of errors (mean ± S.E.M.) plotted against log of administered doses of E2020 (□), physostigmine (▼), tacrine (●) and MF201 (△). Scopolamine group (SCOP) (mean ± S.E.M.); VEH represents the pooled mean ± S.E.M. value for rats treated with saline s.c. and distilled water p.o. the day before pharmacological treatment. Right: effect of increasing doses of E2020 (□), physostigmine (▼), tacrine (●) and MF201 (△) on the total time taken to complete the maze (mean ± S.E.M.). Scopolamine group (SCOP) (mean ± S.E.M.); VEH represents the pooled mean ± S.E.M. value for rats treated with saline s.c. and distilled water p.o. the day before pharmacological treatment. ** $P < 0.01$ compared with the vehicle group; $ $P < 0.05$ compared with the scopolamine group (ANOVA followed by Tukey's t test).

mals being similar. Under these training conditions, the pretest saline injections did not modify performance, which was ~ 90% of working memory (0.37 ± 0.09 total errors). Scopolamine significantly increased the mean total number of errors ($F(1,52) = 49.57$, $P < 0.0001$), the percentage of amnesic animals ($\chi^2 = 15.58$, $P < 0.001$) and the mean time taken to complete the maze ($F(1,52) = 47.11$, $P < 0.0001$) when compared to saline (Fig. 1, Fig. 2). The efficacy in reversing the increase in errors induced by scopolamine was best represented by an inverted U-shaped dose–response curve (Fig. 1). Only for the errors was a clear-cut U regression on the log of the administered doses found to be statistically symmetrical parabolas [$r(1,28) = 0.62$, $P < 0.01$ for E2020; $r(1,28) = 0.52$, $P < 0.01$ for physostigmine; $r(1,28) = 0.52$, $P < 0.01$ for tacrine; $r(1,39) = 0.52$, $P < 0.01$ for MF201]. In all the groups the mean time taken to complete the maze was only partially reversed. In fact, only 0.18 mg/kg of E2020, 0.37 mg/kg of tacrine, 0.5 mg/kg of physostigmine and 3–4 mg/kg of MF201 significantly reduced the increase in time induced by scopolamine [$F(1,33) = 4.38$, $P < 0.05$ for E2020; $F(1,33) = 4.21$, $P < 0.05$ for tacrine; $F(1,33) = 4.18$, $P < 0.05$ for physostigmine and $F(2,41) = 4.64$, $P < 0.01$ (Tukey's t test, $P < 0.05$) for MF201].

Fig. 2 highlights the percentage of amnesic animals. All the compounds reduced this percentage but only at the following dosages: 0.25 for E2020 ($P < 0.01$, χ^2 test), 0.5 for physostigmine and tacrine ($P < 0.02$, χ^2 test), while 3, 6 ($P < 0.02$) and 4 mg/kg ($P < 0.01$, χ^2 test) for MF201.

Fig. 3 shows the effects of cholinesterase inhibitors on the mean frequency of each of the five possible angles between consecutive arm entry. After training, 80% of the animals demostrated a tendency, which was not affected

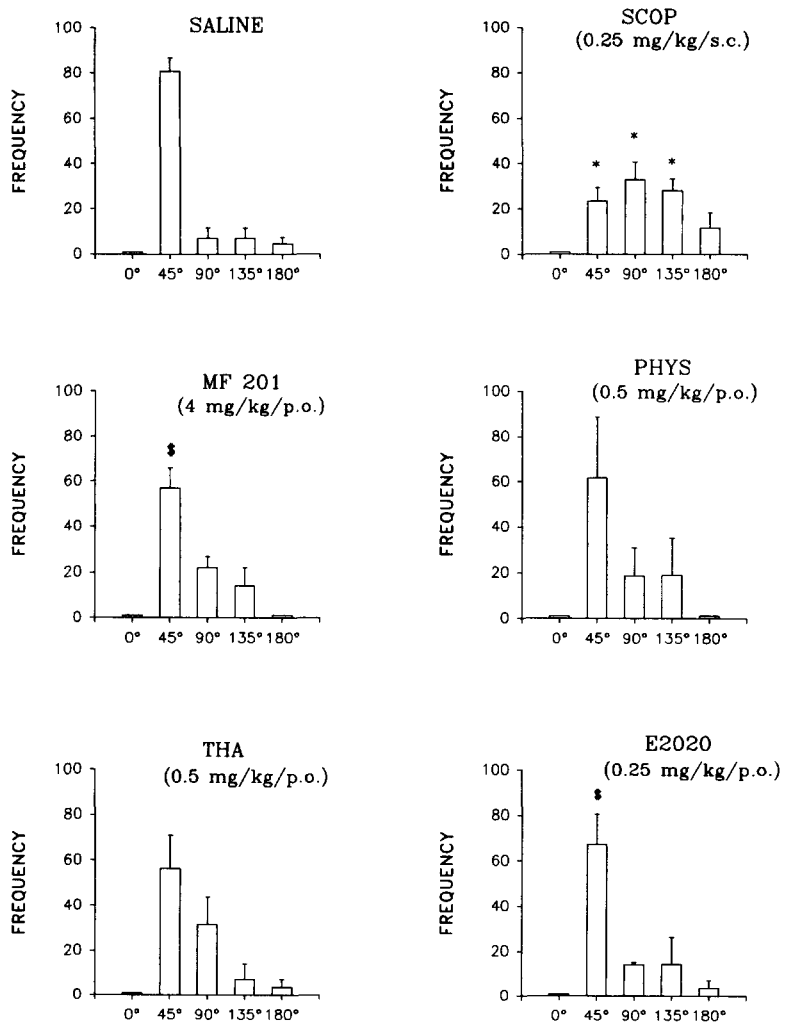

Fig. 3. Effect of different cholinesterase inhibitors on the pattern of arm entry (mean ± S.E.M.). Abscissa: the 5 possible angles between consecutive arm entries. Ordinates: frequency of occurrence during the first 8 entries in any one session (mean ± S.E.M.). * $P < 0.05$ compared with vehicle group; $ $P < 0.05$ compared with scopolamine group (ANOVA followed by Tukey's t test).

by saline, to enter an arm adjacent (45°) to the one which was being exited. A shift to the right in the distribution of angles was clearly apparent after scopolamine treatment compared with controls ($F(1,40) = 45.51$, $P < 0.0001$). All the compounds, at the maximally active dose (0.25 mg/kg for E2020; 0.5 mg/kg for physostigmine and tacrine; 4 mg/kg for MF201), showed a tendency to restore the pattern of arm entry modified by scopolamine, but this was significant for only MF201 and E2020 [$F(1,27) = 8.48$, $P < 0.01$ and $F(1,27) = 11.46$, $P < 0.01$, respectively].

3.2. Locomotor activity

Fig. 4 shows the mean distance travelled in 30 min for each treated group ($n = 6$). The mean distance covered by the control animals was 3553.98 ± 413.19 cm and was unmodified following p.o. administration of cholinesterase inhibitors alone. Scopolamine significantly increased locomotor activity ($F(1,10) = 8.82$, $P < 0.01$). All the drugs tested were unable to reverse this anticholinergic hyperstimulation even at the highest doses tested.

4. Discussion

As described in the literature (Eckerman et al., 1980; Okaichi and Jarrard, 1982; Peele and Baron, 1988; Okaichi et al., 1989), scopolamine induced a significant increase in the total number of errors, percentage of amnesic animals, total time taken to complete the task and modified the pattern of arm entry. Performance in a radial arm maze by hippocampectomized subjects yields a pattern of disruption similar to that found in scopolamine-treated subjects (Anisman and Kokkinidis, 1975).

Following oral administration all the compounds reversed the scopolamine-induced working memory deficit, with an inverted U-shaped dose–response curve. Our results agree with those findings where cholinergic agonists, including cholinesterase inhibitors, in different tasks and species, at low doses improve retention but at higher doses

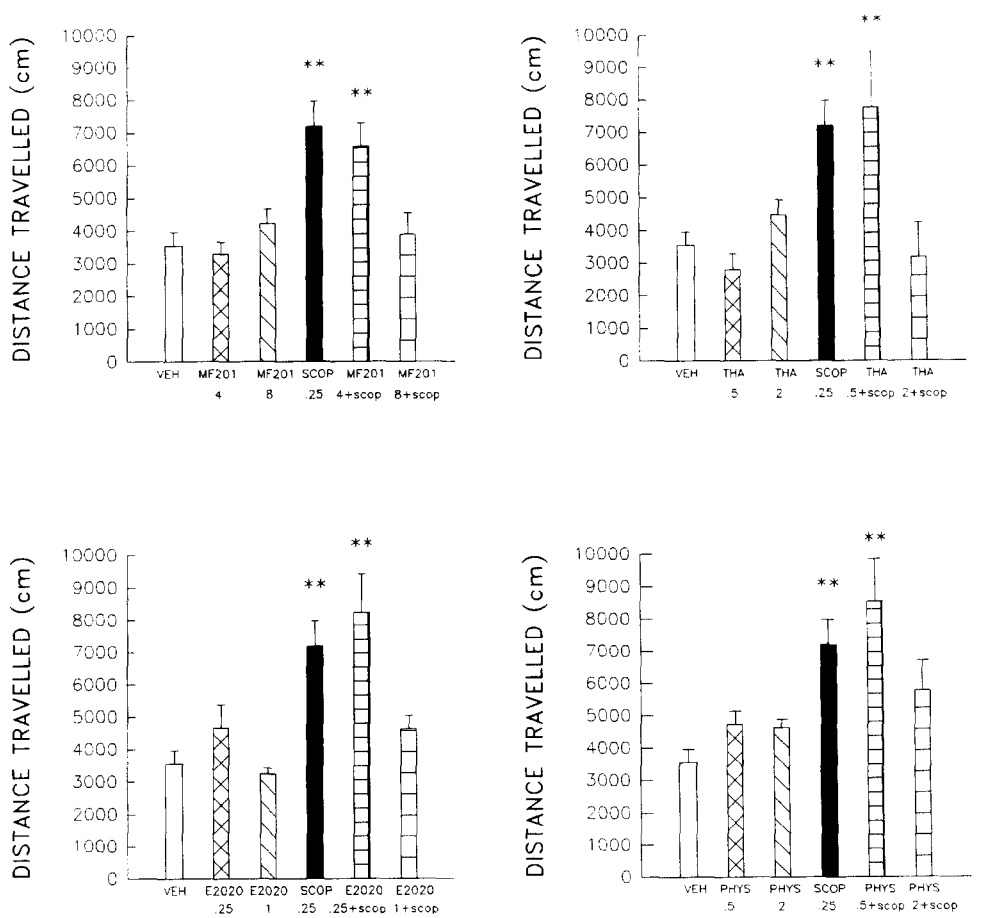

Fig. 4. Effect of MF201, E2020, tacrine (THA), physostigmine (PHYS) and scopolamine (SCOP) on locomotor activity evaluated in terms of total distance travelled (cm) in 30 min. The results are shown as the mean ± S.E.M. VEH represents the control group. ** $P < 0.01$ compared with vehicle (ANOVA test).

impair it (Flood et al., 1981; Waite and Thal, 1995; Wanibuchi et al., 1994).

The presence of an inverted U-shaped dose–response relation has already been observed with the clinical use of cholinesterase inhibitors (Mohs et al., 1985; Becker et al., 1991; Imbimbo and Lucchelli, 1994). Due to the nature of these experiments (acute dosing), it is difficult to explain this observation by simpling saying that it is caused by 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. All four compounds have been shown to increase cortical acetylcholine through the preservation of otherwise hydrolyzed acetylcholine in the cortical extracellular space, even though the percent increase in acetylcholine does not seem to be correlated to cholinesterase inhibition (Giacobini and Cuadra, 1994). However, large increases in cortical acetylcholine will activate presynaptic M_2/M_4 receptors and reduce further acetylcholine release.

Maximum antagonistic effects were attained at a dose (mg/kg) of 0.25 for E2020, 0.5 for physostigmine and tacrine and 4 for MF201. However, MF201 was seen to be active over a wider dose range (3–6 mg/kg), while for the others only a single dose was found to be significantly active. Due to the fact that in humans the beneficial mnemonic effect of the present day drugs is typically small, with a narrow therapeutic window (Heise, 1987), the broader window seen with MF201 may be an advantage. In addition, the fact that the animals that developed a pattern of selecting adjacent arms and which were treated with the different cholinesterase inhibitors were less disrupted by scopolamine suggests that the increased cholinergic activity can ameliorate performance in spatial tasks.

Concerning the time taken to complete the maze, no treatment was able to restore this parameter back to control values. It must be stressed that s.c. methylscopolamine does not modify the efficiency (number of errors), but significantly increases the running time in the radial maze (Dennes and Barnes, 1993; Magnani et al., 1992). This would tend to suggest that the observed increase in the time taken to complete the maze is probably influenced by peripheral anticholinergic side-effects.

Indeed, MF201 (4–8 mg/kg) was unable to significantly block scopolamine-induced peripheral side-effects, such as mydriasis, drying of the mucosa and reduced intestinal motility, when tested 2h after administration (data not shown). However, when these effects were assessed 30 min after MF201 administration, a complete inhibition of the scopolamine-induced peripheral side-effects was observed (unpubl. data). This would suggest that MF201 and maybe the other cholinesterase inhibitors are unable to counteract the peripheral effects of scopolamine at the time used for the radial maze.

The time chosen for the radial maze test (120 min after treatment) was optimal for MF201 to maximally increase the levels of acetylcholine in the brain (De Sarno et al., 1989). Due to the lipophilic characteristics of MF201, there is not a uniform distribution of the drug. This probably results in a different degree of cholinesterase inhibition in various tissues. This could explain why some peripheral side-effects induced by scopolamine were not antagonized with the working doses of MF201.

For all the compounds, the doses that significantly improved memory performance were unable to antagonize scopolamine-induced hypermotility, a phenomenon already observed for MF201 (Dawson et al., 1991). This suggests that the transient amnesia did not result from a sensory or motor or motivational incapacity, but rather from a difficulty to use the visual spatial information relevant to each stage of the test. Therefore, these compounds might act, with a certain degree of selectivity, on memory function.

The improvement in cognitive function observed with tacrine, physostigmine and E2020 in the radial maze is in accordance with previous studies using the same task (Ogura et al., 1989; Magnani et al., 1992; M'Harzi et al., 1995).

Furthermore, similar results for tacrine and physostigmine have been obtained in a variety of experimental manipulations such as pharmacological blockade (Yamamoto et al., 1993), cholinergic lesions (Murray and Fibiger, 1986; Aaltonen et al., 1991; M'Harzi et al., 1995; Waite et al., 1995), chronic alcohol (Hodges et al., 1990) or barbital treatment (Mohammed et al., 1990) and ischemic lesion (Yamamoto et al., 1993).

There is considerable clinical evidence which supports our findings. In fact, with metrifonate (Becker et al., 1990) and physostigmine (Thal et al., 1986), a U-shaped dose–response relationship between cholinesterase inhibition levels and cognitive improvement in Alzheimer's disease was found. On this basis, it is proposed that the maximum benefit of cholinesterase inhibition in Alzheimer's disease occurs when there is 30–60% inhibition. In our experiments, MF201 (4 mg/kg) inhibited red blood cell and whole brain cholinesterase by 60 and 45%, respectively. At the highest dose tested (8 mg/kg) the inhibition observed was 80 and 65%, respectively. In the case of physostigmine, the 0.5 mg/kg dose induced a 35 and 14% inhibition of red blood cell and brain acetylcholinesterase 20 min after administration (unpubl. data). However, as mentioned above, Giacobini and Cuadra (1994) have shown that physostigmine can induce a large increase in cortical acetylcholine with moderate levels of cholinesterase inhibition compared to other inhibitors. Since tacrine and E2020 follow simple mixed competitive inhibition kinetics, no effects ex vivo can be measured.

In conclusion, MF201 appears to be efficacious in selectively antagonizing cognitive deficit in a 8-arm radial maze over a relatively wide range of doses when compared with other cholinesterase inhibitors. Even if it is most unlikely that scopolamine can provide an useful model of the memory failure in Alzheimer's disease, these findings together with other reports of an improvement of memory

in different cognitive tasks, show some promise for the treatment of Alzheimer's disease. Whether or not this compound has a role will become apparent when the results of current clinical trials are published.

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References

Aaltonen, M., P. Riekkinen, J. Sirvio and P. Riekkinen, Jr., 1991, Effects of THA on passive avoidance and spatial performance in quisqualic acid nucleus basalis-lesioned rats, Pharmacol. Biochem. Behav. 39, 563.

Anisman, H. and L. Kokkinidis, 1975, Effects of scopolamine, *d*-amphetamine and other drugs affecting catecholamines on spontaneous alternation and locomotor activity in mice, Psychopharmacology 45, 55.

Becker, R.E., J. Colliver, R. Elble, E. Feldmann, E. Giacobini, V. Kumar, S. Markwell, P. Moriearty, R. Parks, S.D. Shillcutt, L. Unni, S. Vicari, C. Womack and R.F. Zec, 1990, Effects of metrifonate, a long-acting cholinesterase inhibitor, in Alzheimer disease: report of an open trial, Drug Dev. Res. 19, 425.

Becker, R.E., P. Moriearty and L. Unni, 1991, The second generation of cholinesterase inhibitors: clinical and pharmacological effects, in: Cholinergic Basis for Alzheimer Therapy, eds. R. Becker and E. Giacobini (Birkhauser, Boston, MA), p. 211.

Brufani, M., C. Castellano, M. Marta, A. Oliverio, P.G. Pagella, M. Pavone and P.L. Rugarli, 1987, A long-lasting cholinesterase inhibitor affecting neural and behavioral processes, Pharmacol. Biochem. Behav. 26, 625.

Cuadra, G., K. Summers and E. Giacobini, 1994, Cholinesterase inhibitor effects on neurotransmitters in rat cortex in vivo, J. Pharm. Exp. Ther. 270, 1.

Dawson, G.R. and S.D. Iversen, 1993, The effects of novel cholinesterase inhibitors and selective muscarinic receptor agonists in tests of reference and working memory, Behav. Brain Res. 57, 143.

Dawson, G.R., G. Bentley, F. Draper, W. Rycroft, S.D. Iversen and P.G. Pagella, 1991, The behavioral effects of heptyl physostigmine, a new cholinesterase inhibitor, in tests of long-term and working memory in rodents, Pharmacol. Biochem. Behav. 39, 865.

Dennes, R.P. and J.C. Barnes, 1993, Attenuation of scopolamine-induced spatial memory deficits in the rat by cholinimimetic and non-cholinomimetic drugs using a novel task in the 12-arm radial maze, Psychopharmacology 111, 435.

De Sarno, P., M. Pomponi, E. Giacobini, X.C. Tang and E. Williams, 1989, The effect of heptyl-physostigmine, a new cholinesterase inhibitor, on the central cholinergic system of the rat, Neurochem. Res. 14, 971.

Eckerman, D.A., W.A. Gordon, J.D. Edwards, R. McPhail and M.I. Gage, 1980, Effects of scopolamine, pentobarbital, and amphetamine on radial arm maze performance in the rat, Pharmacol. Biochem. Behav. 12, 595.

Flood, J.F., D.W. Landry and M.E. Jarvik, 1981, Cholinergic receptor interaction and their effects on long-term memory processing, Brain Res. 215, 177.

Giacobini, E., 1991, The second generation of cholinesterase inhibitors: pharmacological aspects, in: Cholinergic Basis for Alzheimer Therapy, eds. R.E. Becker and E. Giacobini (Birkhauser, Boston, MA), p. 247.

Giacobini, E. and G. Cuadra, 1994, Second and third generation cholinesterase inhibitors: from preclinical studies to clinical efficacy, in: Alzheimer Disease: Therapeutic Strategies, eds. E. Giacobini and R.E. Becker (Birkhauser, Boston, MA), p. 1.

Heise, G.A., 1987, Facilitation of memory and cognition by drugs, TIPS 8, 65.

Hodges, H., A.M. Ribeiro, J.A. Gray and R.M. Marchbanks, 1990, Low dose tetrahydroaminoacridine (THA) improves cognitive function but does not affect brain acetylcholine in rats, Pharmacol. Biochem. Behav. 36, 291.

Iijima, S., N.H. Greig, P. Garofalo, E.L. Spangler, B. Heller, A. Brossi and D.K. Ingram, 1992, The long-lasting cholinesterase inhibitor heptyl-physostigmine attenuates the scopolamine-induced learning impairment of rats in a 14-unit T-maze, Neurosci. Lett. 144, 79.

Imbimbo, B.P. and P.E. Lucchelli, 1994, A pharmacodynamic strategy to optimize the clinical response to eptastigmine (MF-201), in: Alzheimer Disease: Therapeutic Strategies, eds. E. Giacobini and R.E. Becker (Birkhauser, Boston, MA), p. 103.

Magnani, M., O. Pozzi, R. Biagetti, S. Banfi and L. Dorigotti, 1992, Oxiracetam antagonizes the disruptive effects of scopolamine on memory in the radial maze, Psychopharmacology 106, 175.

McCann, D., R.A. Rabin and J.C. Winter, 1987, Use of the radial maze in studies of phencyclidine and other drugs of abuse, Physiol. Behav. 40, 805.

M'Harzi, M., A.M. Palou, C. Oberlander and F. Barzaghi, 1995, Antagonism of scopolamine induced memory impairments in rats by the muscarinic agonist RU 35926 (CI-979), Pharmacol. Biochem. Behav. 51, 119.

Mohammed, A., G. Wahlstrom, T. Archer and A. Norberg, 1990, Learning deficits in aged rats pretreated chronically with barbital and tested late in abstinence: alleviation by tetrahydroaminoacridine, J Neural. Transm. 2, 285.

Mohs, R.C., B.M. Davis, C.A. Johns, A.A. Mathe, B.S. Greenwald, T.B. Honath and K. Davis, 1985, Oral physostigmine treatment of patients with Alzheimer's disease, Am. J. Psychiatry 142, 28.

Murray, C.L. and H.C. Fibiger, 1986, The effect of pramiracetam (CI-879) on the acquisition of a radial arm maze task, Psychopharmacology 89, 378.

Ogura, H., T. Kosasa, S. Araki, Y. Yamanishi and K. Yamatsu, 1989, E2020, a novel centrally acting acetylcholinesterase inhibitor (3): behavioral study of cholinergic action in rats, Jpn. J. Pharmacol. 49 (Suppl.), Abstr. P 0–300.

Okaichi, H. and L.E. Jarrard, 1982, Scopolamine impairs performance of a place and cue task in rats, Behav. Neural Biol. 35, 319.

Okaichi, H., Y. Oshima and L.E. Jarrard, 1989, Scopolamine impairs both working and reference memory in rats: a replication and extension, Pharmacol. Biochem. Behav. 34, 599.

Olton, D.S. and R.J. Samuelson, 1976, Remembrance of places passed: spatial memory in rats, J. Exp. Psychol. (Anim. Behav. Process.) 2, 97.

Peele, D.B. and S.P. Baron, 1988, Effects of selection delays on radial maze peformance: acquisition and effects of scopolamine, Pharmacol. Biochem. Behav. 29, 143.

Rupniak, N.M.J., S.J. Tye, C. Brazell, A. Heald, S.D. Iversen and P.G. Pagella, 1992, Reversal of cognitive impairment by heptyl physostigmine, a long-lasting cholinesterase inhibitor, in primates, J. Neurol. Sci. 107, 246.

Sala, M., D. Braida, P. Calcaterra, M.P. Leone, F.A. Comotti, S. Gianola and E. Gori, 1991, Effect of centrally administered atropine and pirenzepine on radial arm maze performance in the rat, Eur. J. Pharmacol. 194, 45.

Steel, R.G. and J.H. Torrie, 1960, Principles and Procedures of Statistics (McGraw-Hill Book Company, New York, NY).

Thal, L.J., D.M. Masur, N.S. Sharpless, P.A. Fuld and P. Davies, 1986, Acute and chronic effects of oral physostigmine and lecitin in Alzheimer's disease, Progr. Neuropsychopharmacol. Biol. Psychiatry 10, 627.

Waite, J.J. and L.J. Thal, 1995, The behavioral effects of heptylphysostigmine on rats lesioned in the nucleus basalis, Neurosci. Res. 21, 251.

Wanibuchi, F., T. Nishida, H. Yamashita, K. Hidaka, K. Koshiya, S. Tsukamoto and S. Usuda, 1994, Characterization of a novel muscarinic receptor agonist, YM796: comparison with cholinesterase inhibitors in vivo pharmacological studies, Eur. J. Pharmacol. 265, 151.

Yamamoto, T., M. Ohno, I. Kitajima, S. Yatsugi and S. Ueki, 1993, Ameliorative effects of the centrally active cholinesterase inhibitor, NIK-247, on impairment of working memory in rats, Physiol. Behav. 53, 5.