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Effect of rivastigmine on scopolamine-induced memory impairment in rats

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Abstract

The effect of rivastigmine on memory impairments induced in rats by scopolamine (0.5 mg/kg) was assessed in the Morris water maze and passive avoidance tests and compared with that of tacrine (2.5–17.7 mg/kg). Rivastigmine, (0.5–2.5 mg/kg) inhibited cholinesterase in the cortex and hippocampus by 21–60% and antagonised the deficits in working and reference memory. Tacrine (12.5 and 17.7 mg/kg) produced significantly less inhibition of cholinesterase in the hippocampus but more in the striatum than rivastigmine (0.75 and 1.5 mg/kg) and only antagonised the deficit in reference memory. Rivastigmine (1.5 and 2.5 mg/kg) or tacrine (12.5 mg/kg), injected immediately after completion of the acquisition trial in the passive avoidance test, antagonised the deficit induced by scopolamine (1 mg/kg) in memory retention. The inability of higher doses of the cholinesterase inhibitors to antagonise memory deficits induced by scopolamine may be related to excessive cholinergic stimulation in the central nervous system.

Keywords: Reference memory; Working memory; Passive avoidance; Cholinesterase inhibition, brain; Muscarinic receptor

1. Introduction

Degeneration of basal forebrain cortical cholinergic neurones occurs in the brains of subjects with Alzheimer's disease, and this correlates well with the degree of cognitive impairment (Bowen et al., 1976; Whitehouse et al., 1982). A number of cholinesterase inhibitors have been shown to improve cognitive function in such subjects (Stern et al., 1987; Knapp et al., 1994; Canal and Imbimbo 1996; Rogers et al., 1998). On the other hand, anticholinergic drugs, like scopolamine, can disrupt short-term or working memory in humans and animals (Stevens, 1981; Beatty et al., 1986; Kopelman and Corn, 1988). It was shown that cholinesterase inhibitors, including physostigmine, tacrine, donepezil and heptylphysostigmine antagonize the effect of scopolamine on spatial memory in the radial arm maze (Braida et al., 1996), Morris water maze and passive avoidance tests (Dawson et al., 1991; Yoshida and Suzuki, 1993). However, these drugs are effective over a very limited dose range, resulting in a typical inverted U-shaped dose-effect relationship (Braida et al., 1996). The reasons for this are not clear, but it has also been observed with physostigmine (Stern et al., 1987) and heptylphysostigmine (Canal and Imbimbo, 1996) in the treatment of memory deficits in patients with Alzheimer's disease. These drugs showed a decline in therapeutic efficacy when cholinesterase inhibition exceeded 40%.

Rivastigmine is a novel cholinesterase inhibitor with a relatively selective action on the enzyme in brain compared with that in the heart and skeletal muscle (Enz et al., 1993; Weinstock et al., 1994). In Alzheimer patients the drug produced a dose-related effect on cognitive function that was correlated with the degree of acetylcholinesterase inhibition in the cerebrospinal fluid (Cutler et al., 1998). The ability of the drug to antagonize memory impairments induced by scopolamine has not been evaluated. It was therefore of interest to see whether its lower propensity to cause peripheral cholinergic hyperactivity can increase the effective dose range compared to other cholinesterase inhibitors.

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There is a considerable discrepancy in the effective doses of the same drugs that have been evaluated in different studies for their ability to antagonize the scopolamine-induced memory impairments. This could have been related to the doses of scopolamine given, that ranged from 0.2–2 mg/kg (Dawson et al., 1991; Chopin and Briley, 1992; Wanibuchi et al., 1994; Braida et al., 1996, Riekkenen et al., 1996) and the particular test of memory. The present study assessed the effects of rivastigmine against memory impairments induced in rats by two doses of scopolamine in the Morris water maze and passive avoidance tests. Its effects were compared with those of tacrine and related to the degree of cholinesterase inhibition in the cortex and hippocampus. Any motor impairment induced by the drugs was compared to enzyme inhibition in the striatum and skeletal muscle.

2. Materials and methods

2.1. Animals

The study was performed on male Sprague–Dawley rats weighing 220–280 g, purchased from Harlan, Jerusalem, according to the guidelines of the University Committee for Institutional Animal Care, based on those of the National Institutes of Health, USA. The rats were housed 4 per cage for one week in the Animal House at an ambient temperature of 21 ± 1°C and a 12 h- diurnal light cycle, prior to testing. All behavioural experiments were carried out in a room adjacent to that in which the rats were housed under the same conditions of temperature and humidity and light cycle.

2.2. Behavioural experiments

2.2.1. Spatial memory test

The procedure used was a modification of that described by Morris (1984). A circular pool (140 cm in diameter, 50 cm high) was filled to depth of 30 cm with water at a temperature of 22 ± 1°C. The pool was divided into four quadrants of equal area, NE, NW, SE, SW. A glass platform (20 cm in diameter) was placed 1 cm below the surface, midway between the centre and rim of the pool in the NE quadrant. The rat was introduced into the pool in the SW quadrant, and an uninformed observer measured the time taken for it to find the escape platform. If it failed to do so within 180 s it was placed on the platform for 20 s and then removed from the pool. The rat was given two daily trials for 4 days with an inter-trial interval of 15 min. The point of entry of the rat into the pool and the location of the escape platform remained unchanged between trial 1 and 2 but was changed on each day. The decrease in escape latency from day to day in trial 1 represents reference or long-term memory while that from trial 1 to trial 2, is consistent with working, or short-term memory (Morris, 1983).

On each day, the rats were injected subcutaneously with saline (1 ml), or rivastigmine (0.5, 0.75, 1.5, 2.5, 3.5 mg) or tacrine (2.5, 5, 8.85, 10, 12.5 or 17.7 mg/kg), 8–11 rats per treatment, followed 10 min later by scopolamine (0.5 mg/kg). In one experiment scopolamine (1 mg/kg) was substituted for 0.5 mg/kg and given with rivastigmine (1.5 mg/kg). Other rats were injected with saline (1 ml/kg), rivastigmine (1.5, 2.5 or 3.5 mg/kg) or tacrine (12.5 or 17.7 mg/kg) alone. Atropine methyl nitrate (0.5 mg/kg) was given instead of scopolamine, to block peripheral muscarinic receptors only, preceded by saline, rivastigmine (3.5 mg/kg) or tacrine (17.7 mg/kg). All rats were tested for spatial memory 20 min after the injection of scopolamine, methyl atropine or saline.

2.2.2. Passive avoidance test

The step-through passive avoidance apparatus consisted of one illuminated and one dark chamber, separated by a guillotine door. The test consisted of an acquisition and a retention trial, which were carried out on groups of 9–11 rats per treatment. In the acquisition trial, each rat was injected with saline or rivastigmine, followed 10 min later by scopolamine (0.5 or 1 mg/kg) or saline (1 ml/kg). It was then placed in the illuminated compartment and allowed to explore for 10 s. A guillotine door separating the chambers was opened and the latency to enter the dark compartment assessed. As soon as the rat entered the dark compartment the door was closed and an inescapable footshock (1.0 mA for 5 s) was delivered through the grid floor. In other rats, rivastigmine or tacrine was injected immediately after they had been removed from the apparatus and had entered the dark chamber. A retention test was performed 24 h later in which the rats were put into the illuminated compartment and the time to enter the dark compartment was recorded as step-through latency. The rats were allowed to remain in the illuminated chamber for 3 min and a maximum latency of 180 s was recorded for rats that did not pass into the dark compartment.

2.3. Evaluation of cholinergic side effects

Rivastigmine (2.5 and 3.5 mg/kg) or tacrine (17.7 mg/kg), was injected subcutaneously alone, or 10 min before scopolamine (0.5 mg/kg). The presence or absence of signs of cholinergic hyperactivity, splayed hindlimbs and diarrhoea was noted 15, 30 and 45 min, respectively, after injection of the cholinesterase inhibitor. The intensity of whole body tremor was also scored at these times (0–3), (0 = absent; 1 = mild; 2 = moderate; 3 = severe).

2.4. Cholinesterase assay

Rats were injected subcutaneously with saline (1 ml/kg), rivastigmine (0.5, 0.75, 1.5, 2.5, 3.5 mg/kg) or

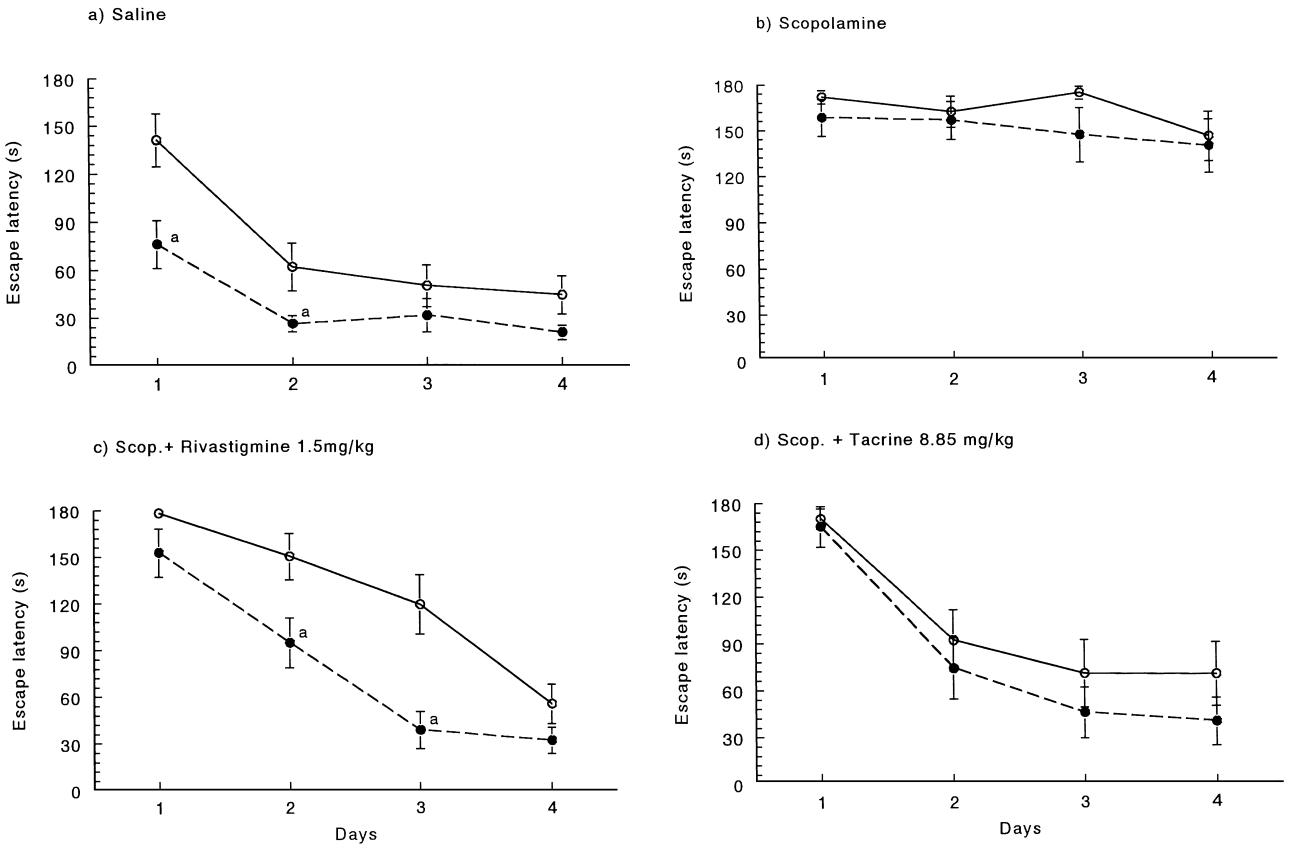

Fig. 1. The effect of saline, scopolamine, with and without rivastigmine or tacrine in trials 1 and 2 in the Morris water maze. Values represent mean ± S.E.M. Trial 1; ○–○: trial 2; ●–●. [a]Significantly different from value in trial 1, $P < 0.05$.

tacrine (12.5 or 17.7 mg/kg). The rats were decapitated, 30 or 45 min later and the brain and skeletal muscle removed. The hippocampus, frontal cortex and striatum were dissected out. The brain regions and muscle were rapidly weighed and homogenised in 0.1 M phosphate buffer (90 mg/ml for cortex and hippocampus, 20 mg/ml for striatum and 250 mg/ml for tibialis muscle), pH 8.0 containing 1% Triton. Cholinesterase activity was measured by the method of Ellman et al, (1961) on 25 μl aliquots of enzyme homogenates. The inhibition of cholinesterase (%) induced by rivastigmine and tacrine was calculated by comparison with enzyme activity obtained from rats injected with saline under the same conditions.

2.5. Statistical analyses

Morris water maze latencies were analysed by two-way ANOVA with days as one variable and treatment as another, followed by a post-hoc Student's *t* test with a Bonferroni correction. Step-through passive avoidance latencies for acquisition and retention trials and mean values for % inhibition of cholinesterase in different tissues were analysed by one-way analysis of variance followed by a post-hoc Student's *t* test with a Bonferroni correction. Mean scores for tremor were analysed by the non-parametric Mann–Whitney test and the incidence of the other cholinergic symptoms, by Chi-square test. All data are

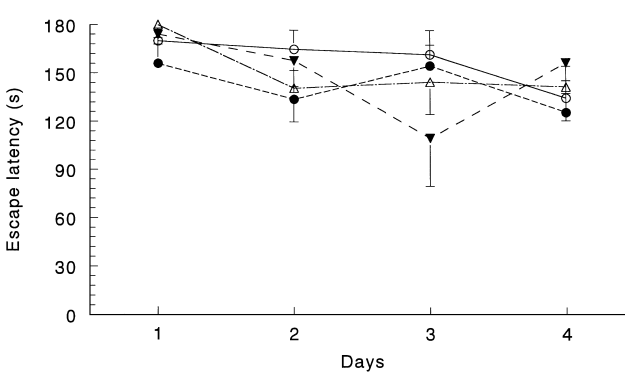

Fig. 2. Antagonism of scopolamine-induced memory impairment by different doses of rivastigmine in the Morris water maze. Values represent mean ± S.E.M of the total escape latencies for 4 daily trials. Trial 1; open columns: trial 2; cross-hatched columns. Scop = scopolamine (0.5 mg/kg) R = rivastigmine. [a]Significantly different from value in trial 1, $P < 0.05$; [b]significantly different from respective values in rats given scopolamine in trials 1 and 2, $P < 0.05$.

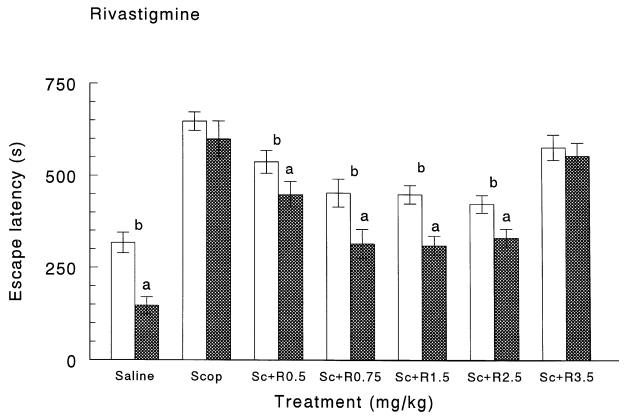

Fig. 3. Failure of rivastigmine to antagonize impairment in spatial memory induced by scopolamine (1 mg/kg). Values represent mean ± S.E.M. Scopolamine (1 mg/kg); ○–○: trial 1; ●–●: trial 2; scopolamine (1 mg/kg) plus rivastigmine (1.5 mg/kg) △–△: trial 1; ▼–▼: trial 2.

expressed as the mean value for the group ± S.E.M. A $P < 0.05$ was considered statistically significant.

2.6. Drugs

Rivastigmine bitartrate, (ENA713) Novartis Basle, Switzerland; atropine methyl nitrate, (−)-scopolamine HCl and Tacrine HCl, Sigma Holon, Israel. All doses are expressed in mg/kg of the respective salt.

3. Results

3.1. Morris water maze

Saline-treated rats showed a marked reduction in escape latencies from the first to second trials on days 1 and 2 ($P < 0.001$) in the Morris water maze and reached stable latencies after the 4th trial (Fig. 1a). The escape latencies in trials 1 and 2 remained essentially unchanged throughout the 4-day testing period in the rats given scopolamine (0.5 mg/kg) (Fig. 1b). The escape latencies in rats given saline, scopolamine with and without rivastigmine showed a highly significant effect of day [trial 1]; $F(3, 331) = 46.63$, $P < 0.0001$: [trial 2]; $F(3, 331) = 55.46$, $P < 0.0001$: treatment [trial 1]; $F(6, 331) = 15.60$, $P < 0.0001$: [trial 2]; $F(6, 331) = 29.70$, $P < 0.0001$: and a day by treatment interaction [trial 1]; $F(18, 331) = 2.38$, $P <$

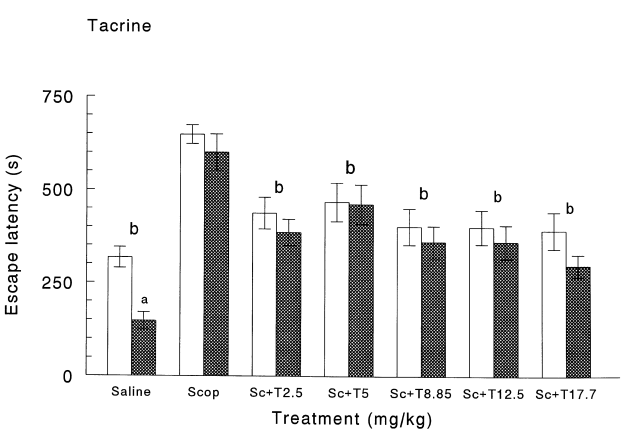

Fig. 4. Antagonism of scopolamine-induced memory impairment by different doses of tacrine in the Morris water maze. Values represent mean ± S.E.M of the total latencies for 4 daily trials. Open columns; trial 1: cross-hatched columns; trial 2. Scop = scopolamine (0.5 mg/kg). T = tacrine. [b]Significantly different from respective values in rats given scopolamine plus saline in trial 1 and 2, $P < 0.05$.

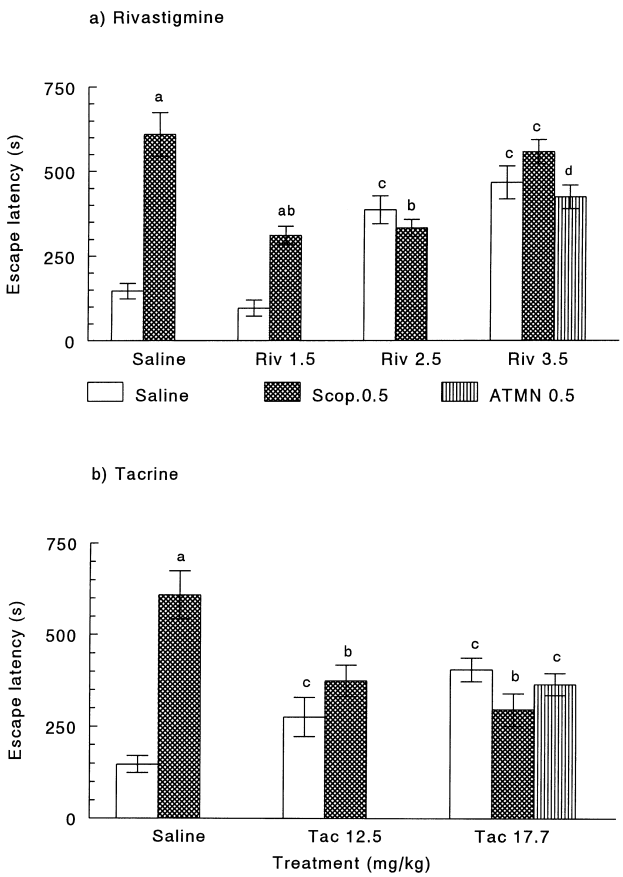

Fig. 5. Effect of rivastigmine and tacrine alone, or with scopolamine or atropine methyl nitrate on escape latencies in trial 2. Values represent mean ± S.E.M of the total latencies for 4 daily trials. (a) Scop = scopolamine, ATMN = atropine methyl nitrate. Open columns: saline or rivastigmine. Cross-hatched columns: scopolamine + saline or rivastigmine; vertical line columns: atropine methyl nitrate (0.5 mg/kg) plus saline or rivastigmine. [a]Significantly different from rats given saline, or rivastigmine alone, $P < 0.05$; [b]significantly different from rats given scopolamine alone, $P < 0.05$; [c]significantly different from saline control, $P < 0.05$; [d]significantly different from scopolamine (0.5 mg/kg) plus rivastigmine (3.5 mg/kg), $P < 0.05$. (b) Legend as for (a) but tacrine is substituted for rivastigmine. [a]Significantly different from rats given saline, or tacrine alone, $P < 0.05$; [b]significantly different from rats given scopolamine alone, $P < 0.05$; [c]significantly different from saline control, $P < 0.05$.

Table 1
Antagonism of scopolamine-induced amnesia in the passive avoidance test

Drug (mg/kg)	Saline (1 ml/kg)		Scopolamine (0.5 mg/kg)		Scopolamine (1 mg/kg)	
	Acquisition	Retention	Acquisition	Retention	Acquisition	Retention
Saline	10.7 ± 1.5	150 ± 13	10.6 ± 1.5	59.9 ± 15.9[a]	13.3 ± 2.9	36.7 ± 14.6[a]
Rivastigmine 0.75	–	–	7.4 ± 1.1	86.4 ± 28.2	–	–
Rivastigmine 1.5	19.1 ± 4.6	157 ± 21	14.4 ± 3.1	117.8 ± 17.8[b]	8.3 ± 1.5[c]	33.6 ± 12.6
Rivastigmine 2.5	43.7 ± 10.4[a]	110 ± 28	7.3 ± 1.5[c]	75.6 ± 28.1	27.0 ± 4.0	55.4 ± 24.2
Rivastigmine 3.5			–	–	25.5 ± 2.6	29.8 ± 5.0
Tacrine 12.5	119 ± 21[a]	88.6 ± 21.4[a]	–	–	–	–

[a]Significantly different from saline, $P < 0.05$.
[b]Significantly different from scopolamine, $P < 0.05$.
[c]Significantly different from rivastigmine alone, $P < 0.05$.

0.001: [trial 2]; $F(18, 331) = 2.43$, $P < 0.001$. There was also a highly significant effect of day, [trial 1]; $F(3, 315) = 43.6$, $P < 0.0001$: [trial 2]; $F(3, 315) = 41.1$, $P < 0.0001$: and of treatment, [trial 1]; $F(3, 315) = 13.68$, $P < 0.0001$: [trial 2]; $F(3, 315) = 20.83$, $P < 0.0001$: and a day-by-treatment interaction [trial 1]; $F(18, 315) = 1.83$, $P < 0.025$: [trial 2]; $F(18, 315) = 2.38$, $P < 0.025$: for escape latency when tacrine was substituted for rivastigmine. Rivastigmine (0.5–2.5 mg/kg), and tacrine (2.5–17.7 mg/kg) significantly antagonized the effect of scopolamine on escape latency in both trials. The effect of one dose of each of these drugs given 10 min before scopolamine, on escape latencies in trials 1 and 2 is shown in Fig. 1c and d, respectively. The cholinesterase inhibitors significantly shortened escape latencies in each daily trial compared with those in rats given scopolamine alone, ($P < 0.05$). There were no significant differences in escape latencies after 0.5 or 1 mg/kg of scopolamine and rivastigmine (1.5 mg/kg) failed to antagonize the deficit in spatial memory induced by the higher dose of scopolamine (Fig. 2). The effect of other doses of rivastigmine and tacrine on escape latencies summed for the 4 test days are shown for trials 1 and 2 in Figs. 3 and 4. The latencies for trial 2 were significantly lower than in trial 1 for rats given saline alone, or scopolamine (0.5 mg/kg) plus rivastigmine ($P < 0.05$) at all doses except 3.5 mg/kg (Figs. 1a,c and 2). On the other hand, tacrine did not cause a significant difference in escape latency between trial 1 and trial 2 at any dose (Figs. 1d and 4).

The effect of rivastigmine (1.5, 2.5 and 3.5 mg/kg) and tacrine (12.5 and 17.7 mg/kg) alone, or with scopolamine on escape latencies in trial 2 is shown in Fig. 5a and b. Rivastigmine (1.5 mg/kg) tended to decrease escape latency ($P = 0.08$) compared to saline, but significantly increased escape latency at 2.5 and 3.5 mg/kg ($P < 0.005$). Rats given a combination of scopolamine and rivastigmine (3.5 mg/kg) did not show a better performance in working or reference memory in the Morris water maze than those given scopolamine alone, despite the fact that scopolamine virtually abolished the tremor and muscle weakness (see Section 3.3).

In order to determine whether the high escape latencies induced by a combination of rivastigmine and scopolamine were due to excess cholinergic stimulation in the brain, atropine methyl nitrate (0.5 mg/kg) was substituted for scopolamine in another group of rats. This dose was effective in antagonising the peripheral cholinergic effects, salivation and diarrhoea, produced by rivastigmine. The summed escape latencies in trials 1 and 2 of rats given atropine methyl nitrate alone were 301 ± 42 s and 154 ± 35 s, respectively, and did not differ from saline controls. Atropine methyl nitrate did not influence the degree of tremor or hind-limb abduction (data not shown), nor did it alter the escape latencies of rats given rivastigmine, which remained significantly shorter than those induced by rivastigmine plus scopolamine ($P < 0.005$) (Fig. 5a).

Tacrine also increased escape latency when given alone at doses of 12.5 and 17.7 mg/kg in each daily trial ($P < 0.01$), but unlike rivastigmine, it significantly antagonized the effect of scopolamine on escape latency ($P < 0.001$). Moreover, the combination of tacrine (17.7 mg/kg) and either scopolamine or atropine methyl nitrate did not result in longer escape latencies than those of tacrine alone (Fig. 5b).

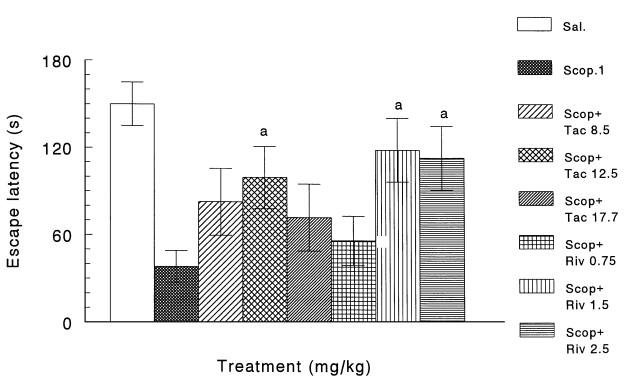

Fig. 6. Effect of rivastigmine and tacrine on memory retention in the passive avoidance test. Values represent mean ± S.E.M. [a]Significantly different from scopolamine (1 mg/kg), $P < 0.05$.

3.2. Passive avoidance test

Scopolamine (0.5 and 1 mg/kg) significantly reduced step-through latency in the retention trial ($P < 0.025$ and $P < 0.001$, respectively) (Table 1). Rivastigmine (1.5 mg/kg) given alone produced step-through latencies in both the acquisition and retention trials which were similar to those in saline controls. This dose antagonized the memory impairment caused by scopolamine (0.5 mg/kg), but not by (1 mg/kg). Higher and lower doses of rivastigmine were ineffective against scopolamine (0.5 mg/kg). Although rivastigmine (2.5 mg/kg) alone, significantly increased step-through latency in the acquisition trial, it did not impair memory retention. Scopolamine (0.5 mg/kg), but not (1 mg/kg) prevented the elevation in step-through latency induced by rivastigmine (2.5 mg/kg). On the other hand, administration of rivastigmine (1.5 and 2.5 mg/kg) immediately after the acquisition trial, antagonized the amnesia induced by scopolamine (1 mg/kg) (Fig. 6).

Tacrine (12.5 mg/kg) greatly prolonged step-through latency when given alone in the acquisition trial. It was not tested as pretreatment before scopolamine. When administered immediately after the trial to rats that had received scopolamine (1 mg/kg) the memory impairment was reduced significantly. A higher or lower dose of tacrine was less effective (Fig. 6).

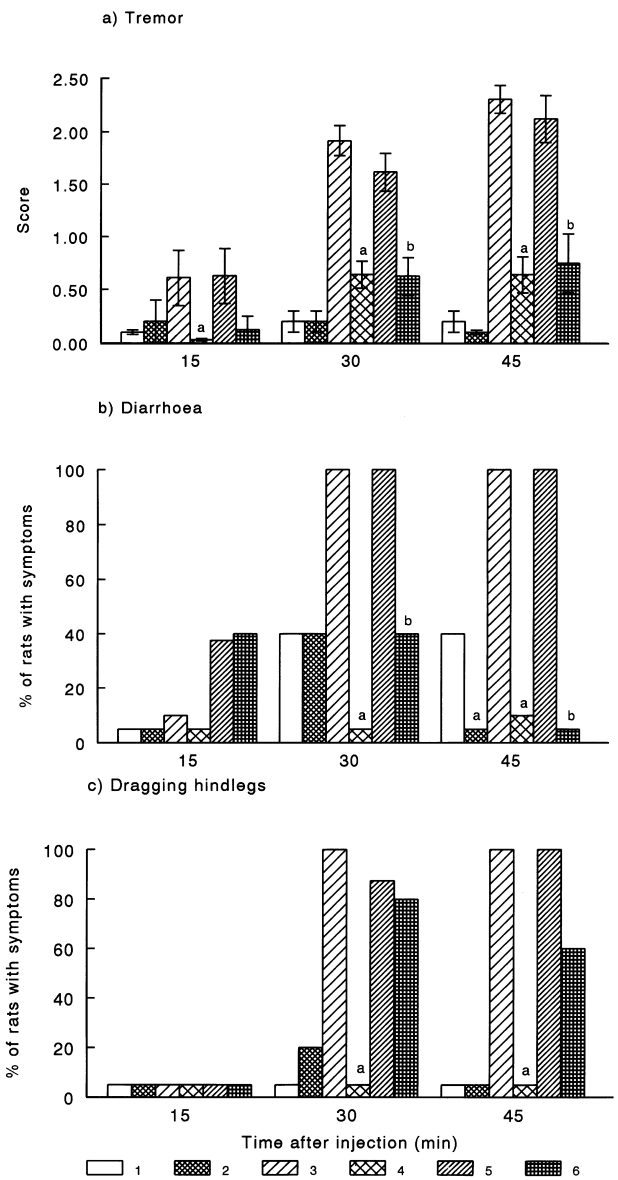

Fig. 7. Effect of scopolamine on tremor, diarrhoea and hindlimb abduction induced rivastigmine and tacrine. (1) Rivastigmine (2.5 mg/kg); (2) rivastigmine (2.5 mg/kg) plus scopolamine (0.5 mg/kg); (3) rivastigmine (3.5 mg/kg); (4) rivastigmine (3.5 mg/kg) plus scopolamine (0.5 mg/kg); (5) tacrine (17.7 mg/kg); (6) tacrine (17.7 mg/kg) plus scopolamine (0.5 mg/kg). [a]Significantly different from rivastigmine or tacrine alone, $P < 0.05$.

3.3. Cholinergic side effects

Rivastigmine (2.5 mg/kg) caused only a very mild degree of tremor and diarrhoea in less than 30% of the animals. All the rats given either tacrine (17.7 mg/kg) or rivastigmine (3.5 mg/kg) had moderate to severe tremor (Fig. 7a), diarrhoea (Fig. 7b), and hindlimb abduction, 30 and 45 min after injection (Fig. 7c). Scopolamine markedly reduced the diarrhoea and tremor induced by each drug to a similar extent, and the hindlimb abduction induced by rivastigmine, but not by tacrine.

3.4. Cholinesterase inhibition

Table 2 shows the cholinesterase activity in 3 brain regions, skeletal muscle and heart of rats injected with saline and the effect of different doses of rivastigmine and tacrine on this activity. Rivastigmine induced a dose-dependent inhibition of the enzyme in all brain areas and periphery, 30 min after injection. The degree of inhibition was similar in the cortex and hippocampus. At doses below 2.5 mg/kg, the drug had a significantly greater effect in the cortex than in the striatum and skeletal muscle (Table 2). Tacrine (12.5 and 17.7 mg/kg) produced similar inhibition in the cortex to that induced by rivastigmine (0.75 and 1.5 mg/kg), respectively, but was significantly less effective in blocking the enzyme in the hippocampus and more effective in the striatum and skeletal muscle.

4. Discussion

It is generally agreed that the cholinergic system in the basal forebrain plays an important role in learning and memory. Scopolamine interferes with memory and cognitive function in humans (Beatty et al., 1986) and experi-

Table 2
Inhibition of cholinesterase (% of control) induced by rivastigmine and tacrine 30 min after injection
Values represent mean ± S.E.M.

Treatment	*N*	Cortex	Hippocampus	Striatum	Muscle
Control activity[a]	5	18.2 ± 0.7	19.6 ± 0.3	116.6 ± 1.9	2.40 ± 0.13
% inhibition by drug					
Rivastigmine, 0.5 mg/kg	7	30.1 ± 5.5	21.0 ± 6.7	11.6 ± 4.9[b]	15.4 ± 4.9[b]
Rivastigmine, 0.75 mg/kg	4	41.1 ± 5.2	39.9 ± 1.9	12.7 ± 4.0[b]	26.2 ± 8.7
Rivastigmine, 1.5 mg/kg	4	49.2 ± 1.8	46.0 ± 3.7	36.2 ± 4.6[b]	36.7 ± 3.3[b]
Rivastigmine, 2.5 mg/kg	3	64.5 ± 2.2	62.3 ± 5.9	57.1 ± 3.3	63.2 ± 4.0
Rivastigmine, 3.5 mg/kg	3	71.4 ± 1.0	63.4 ± 2.5	71.5 ± 4.5	70.3 ± 5.6
Tacrine, 12.5 mg/kg	4	39.8 ± 4.4	10.0 ± 4.7[b,c]	42.8 ± 5.1[c]	47.7 ± 8.3[c]
Tacrine, 17.7 mg/kg	4	45.6 ± 6.1	12.2 ± 7.6[b,d]	44.0 ± 7.9	51.9 ± 6.9[d]

[a]Activity of cholinesterase in μmol of acetylthiocholine hydrolysed per g/min.
[b]Significantly different from value in cortex, $P < 0.05$.
[c]Significantly different from value in same area for rivastigmine (0.75 mg/kg) $P < 0.05$.
[d]Significantly different from value in same area for rivastigmine (1.5 mg/kg), $P < 0.05$.

mental animals (Stevens, 1981; Sutherland et al., 1982) by blocking muscarinic receptors in these brain regions. The present study compared the effects of a novel cholinesterase inhibitor, rivastigmine with that of tacrine against scopolamine-induced memory impairments in the Morris water maze and passive avoidance tests. In the former, a paradigm was used that enabled us to differentiate an effect of the cholinesterase inhibitors on working and reference memory (Morris, 1983). It was found that the anti-amnesic effects of these drugs depended on the dose of scopolamine used and the particular test.

Scopolamine (0.5 and 1 mg/kg) caused similar degrees of impairment in both reference and working memory in the Morris water maze. Rivastigmine (0.75 and 1.5 mg/kg) were effective in antagonizing the memory deficit induced by scopolamine (0.5 mg/kg), but not against that induced by scopolamine (1 mg/kg). This suggests that 0.5 mg/kg is the optimal dose of scopolamine to block spatial memory and that a dose of rivastigmine (1.5 mg/kg) that inhibits brain cholinesterase by more than 45% is unable to cause sufficient accumulation of acetylcholine to displace the antagonist from muscarinic receptors. Alternatively, scopolamine may have other actions at higher doses, which are not affected by cholinesterase inhibition.

Rivastigmine (0.5–2.5 mg/kg), inhibited cholinesterase in the cortex and hippocampus by 20–60%, and significantly reduced the effects of scopolamine on reference and working memory. A dose of 3.5 mg/kg, which inhibited cholinesterase in all brain areas and skeletal muscle by more than 70%, failed to prevent the memory impairment induced by scopolamine.

Antagonism by rivastigmine of scopolamine-induced amnesia in the passive avoidance test also depended on the dose of scopolamine and whether the cholinesterase inhibitor was administered before or after the acquisition trial. When injected before scopolamine (0.5 mg), rivastigmine showed the typical inverted U-shaped relationship for antagonism reported for other cholinesterase inhibitors (Yoshida and Suzuki, 1993), but this was narrower than that seen in the Morris water maze. Significant antagonism was achieved with a dose of 1.5 mg/kg but not 0.75 or 2.5 mg/kg. None of these doses were effective against scopolamine (1 mg/kg). When given alone, rivastigmine (2.5 mg/kg) induced alert immobile behaviour (Enz et al., 1993) and significantly prolonged step-through latency during the acquisition phase. Although the immobile behaviour was antagonized by scopolamine (0.5 mg/kg) and the step-through latency restored to that of saline control rats, the amnesia in the retention trial was unaffected by rivastigmine. On the other hand, scopolamine (1 mg/kg) did not affect the prolongation of step-through latency induced by rivastigmine (2.5 mg/kg) in the acquisition trial. This showed that when the rats were under the influence of a combination of rivastigmine (2.5 mg/kg) and scopolamine (0.5 mg/kg), or rivastigmine (1.5 mg/kg) and scopolamine (1 mg/kg) during the acquisition trial, their memory in the retention test was impaired. However, rivastigmine (1.5 or 2.5 mg/kg) was able to antagonize the amnesia produced by scopolamine (1 mg/kg), when it was injected immediately after the completion of the acquisition trial. The efficacy of post-test administration was demonstrated in other studies in mice with physostigmine (Matsuno et al., 1993). It has the advantage that the performance in the retention trial is not confounded by a possible effect of the drug on pain threshold or motor activity during the acquisition phase, thereby enabling one to detect an effect on memory consolidation (McGaugh, 1989).

The inverted U-shaped dose response curve for antagonism by rivastigmine of scopolamine-induced memory impairments, is reminiscent of that seen with other cholinesterase inhibitors, tacrine (Yoshida and Suzuki, 1993), donepezil, physostigmine, and heptyl physostigmine (Braida et al., 1996). However, in antagonising the effect

of scopolamine in the Morris water maze, rivastigmine appears to be effective over a wider range of doses than those reported for the above drugs. The lack of effect of higher doses of these drugs was attributed by Braida et al. (1996) to activation of presynaptic muscarinic autoreceptors by the excess acetylcholine formed, thereby reducing its release. While presynaptic inhibition could explain such a phenomenon in the absence of scopolamine, it is unlikely to occur in its presence, as scopolamine also blocks autoreceptors and increases acetylcholine release (Messamore et al., 1993).

In the various types of mazes used to test for memory impairment, correct performance does not only depend on intact cholinergic transmission in the basal forebrain, but also on normal motor function that enables the animal to reach the appropriate goals. Inhibition of cholinesterase in the basal ganglia and skeletal muscle results in an excess of acetylcholine, which can interfere with motor activity. The selectivity of rivastigmine (0.5–1.5 mg/kg) for the enzyme in the cortex and hippocampus, demonstrated in the current and previous studies (Enz et al., 1993; Weinstock et al., 1994), explains why there was no impairment of swimming ability. Higher doses of rivastigmine (2.5 and 3.5 mg/kg) inhibited cholinesterase in the striatum and muscle by more than 50% and significantly increased escape latencies above those of saline controls. Clear signs of motor impairment, associated with tremor and retraction of the hindlimbs were seen with a dose of 3.5 mg/kg. This resulted from excess activity of acetylcholine on muscarinic receptors in the basal ganglia rather than on nicotinic receptors in the neuromuscular junction, since both symptoms were considerably reduced by scopolamine. Rats that had received a combination of rivastigmine (3.5 mg/kg) and scopolamine (0.5 mg/kg) were able to swim without apparent difficulty. However, the pattern changed from that seen with lower doses, in which rats either swam across the pool or in concentric circles, to one of more pronounced thigmotaxis. Several of the rats even swam close to, or over the escape platform without attempting to step onto it. These findings support those in the passive avoidance test, in which the higher step-through latency obtained with rivastigmine was also antagonized by scopolamine (0.5 mg/kg), but the memory impairment induced by the latter drug was not prevented. They also suggest that the altered behaviour of rats given the higher doses of rivastigmine in the water maze and passive avoidance tests did not result from excessive muscle weakness.

Flood et al. (1983) obtained an inverted U-shaped dose response curve for antagonism by cholinesterase inhibitors of scopolamine-induced memory impairment in mice, even when the drugs were injected into the cerebral ventricles, indicating a locus of action in the central nervous system. Rats given a combination of atropine methyl nitrate and rivastigmine (3.5 mg/kg) showed lower escape latencies that those given the anticholinesterase with scopolamine. This suggests that the impaired performance of the latter was associated with blockade of muscarinic receptors in the central nervous system. This could have resulted in increased levels of acetylcholine, which caused excessive stimulation followed by blockade of brain nicotinic receptors, and supports the finding cited above in mice.

Tacrine has been shown to cause some improvement in cognitive function in subjects with Alzheimer's disease (Knapp et al., 1994). However, it is not clear if this results from inhibition of cholinesterase (Kaul, 1962), activation of nicotinic or muscarinic receptors (Nordberg et al., 1989), stimulation of acetylcholine release (Svensson et al., 1996), or from a combination of all of these actions. We found that tacrine caused relatively more inhibition than rivastigmine in the striatum and less in the hippocampus in doses that induced similar enzyme inhibition in the cortex. This agrees with the finding of Nielsen et al. (1989) that tacrine increased acetylcholine in the striatum at a dose of 5 mg/kg, but only at 10 mg/kg, in the hippocampus. Tacrine (2.5–17.7 mg/kg) decreased the impairment by scopolamine in reference memory in the Morris water maze test. Higher doses caused death from respiratory failure. We have no explanation for the finding that tacrine did not show the inverted U-shaped dose response curve in this test. It clearly did so in the passive avoidance test in our study and in that of Wanibuchi et al., (1994) and in the radial arm maze (Braida et al., 1996).

In contrast to rivastigmine, tacrine did not lower escape latency significantly more in trial 2 than trial 1 on any day of testing, indicating that it did not affect the interference by scopolamine of working memory. One could argue that its apparent failure to do so, at least at a dose of 8.5 mg/kg, may have been due to the fact that escape latencies in trial 1 were already lower than those obtained with rivastigmine (1.5 mg/kg) on the second and third days. Thus, the rats may not have been able to shorten them even more in trial 2. This does not appear to be justifiable, since rats given saline had even lower latencies on day 2 in trial 1, but were still able to reduce them further in trial 2. Moreover, tacrine (12.5 mg/kg) produced the same mean value for escape latency in trial 1 to that seen after rivastigmine (0.75 mg/kg), but only the latter drug significantly shortened the latencies in trial 2. These findings support the conclusion of Riekkenen et al. (1991) that tacrine is relatively ineffective in antagonizing working memory in rats. They suggested that the lack of effect of tacrine could have been due to deficits in motor function. However, this is unlikely because they did not see any interference with swimming ability or reference memory at the dose they used.

The data in the present study suggested an alternative explanation for the low efficacy of tacrine against deficits in working memory. Working but not reference memory in rats can be disrupted by dorsal hippocampal lesions (Kitajima et al., 1992), indicating that it is dependent on

intact hippocampal function. Tacrine inhibited cholinesterase in the hippocampus by only 20%, even at higher doses, compared to 40% inhibition of the enzyme by rivastigmine at a dose of 0.75 mg/kg. Although the actual inhibition by tacrine in vivo may have been greater than that measured because of a dilution effect (Hallak and Giacobini, 1989), we obtained more than 40% inhibition of the enzyme in the cortex and striatum. This showed that the drug was less effective in the hippocampus than in other brain regions and may have produced insufficient amounts of acetylcholine (Nielsen et al., 1989) to displace scopolamine from receptors involved in the mediation of working memory.

In conclusion, the data show that rivastigmine (0.5–2.5 mg/kg) is an effective antagonist of the scopolamine-induced deficits in both reference and working memory. These doses of rivastigmine inhibited cholinesterase in the cortex and hippocampus from 20–60%, while causing significantly less enzyme inhibition in the striatum and skeletal muscle. Tacrine is less effective against the impairment of working than reference memory, does not show the brain selectivity, and causes less inhibition in the hippocampus than in the striatum. Rivastigmine acts over a wider dose range than tacrine in counteracting the effect of scopolamine on memory retention in the passive avoidance test. These findings concur with the dose-related inhibition of cholinesterase in the cerebral spinal fluid of subjects with Alzheimer disease that was correlated with the improvement in cognitive function (Cutler et al., 1998).

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