Neuropsychopharmacology (2003) 28, 253–264

www.neuropsychopharmacology.org

Selective 5-HT_{1A} Antagonists WAY 100635 and NAD-299 Attenuate the Impairment of Passive Avoidance Caused by Scopolamine in the Rat

Ilga Misane[1,2] and Sven Ove Ögren*,[1]

[1]Division of Behavioral Neuroscience, Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; [2]Department of Molecular Neuroendocrinology, Max Planck Institute for Experimental Medicine, Goettingen, Germany

Systemic administration of the muscarinic-receptor antagonists atropine and scopolamine produces cognitive deficits in humans, nonhuman primates and rodents. In humans, these deficits resemble symptoms of dementia seen in Alzheimer's disease. The passive avoidance (PA) task has been one of the most frequently used animal models for studying cholinergic mechanisms in learning and memory. The present study examined the ability of two selective 5-HT_{1A} receptor antagonists WAY 100635 and NAD-299 (robalzotan) and two acetylcholinesterase (AChE) inhibitors tacrine and donepezil to attenuate the impairment of PA retention caused by the nonselective muscarinic receptor antagonist scopolamine in the rat. Although demonstrating differences in their temporal kinetics, both WAY 100635 and NAD-299 attenuated the impairment of PA caused by scopolamine (0.3 mg/kg s.c.). Donepezil did not block the PA deficit caused by the 0.3 mg/kg dose of scopolamine, but it prevented the inhibitory effects of the 0.2 mg/kg dose of scopolamine. In contrast, tacrine was effective *vs* both the 0.2 and 0.3 mg/kg doses of scopolamine. These results indicate that (1) a functional 5-HT_{1A} receptor antagonism can attenuate the anterograde amnesia produced by muscarinic-receptor blockade, and (2) the AChE inhibitors tacrine and donepezil differ in their ability to modify muscarinic-receptor-mediated function *in vivo*. These results suggest that 5-HT_{1A} receptor antagonists may have a potential in the treatment of cognitive symptoms in psychopathologies characterized by reduced ACh transmission such as Alzheimer's disease.
Neuropsychopharmacology (2003) **28,** 253–264. doi:10.1038/sj.npp.1300024

Keywords: passive avoidance; 5-HT_{1A} receptors; muscarinic receptors; acetylcholinesterase inhibitors

INTRODUCTION

The importance of forebrain cholinergic systems for cognitive functions has been known for decades. Systemic administration of the nonselective muscarinic receptor antagonists atropine and scopolamine causes cognitive deficits in humans (Christensen *et al*, 1992; Ebert and Kirch, 1998; Wesnes *et al*, 1991), nonhuman primates (Rupniak *et al*, 1989) and rodents (Patel and Tariot, 1991; Sunderland *et al*, 1986). In human volunteers, the pattern of cognitive impairment caused by scopolamine mimics in some aspects the cognitive symptomology seen in Alzheimer's disease (AD) (Ebert and Kirch, 1998) which is associated with the degeneration of cholinergic neurons in the basal forebrain resulting in a reduction of cholinergic neurotransmission in the forebrain (Araujo *et al*, 1988; DeKosky *et al*, 1996; Kuhl *et al*, 1999; Shinotoh *et al*, 2000; Shiozaki *et al*, 1999; Whitehouse *et al*, 1982). On the other hand, acetylcholinesterase (AChE) inhibitors, which increase synaptic acetylcholine (ACh) levels, are effective in the treatment of some cognitive symptoms of AD (Francis *et al*, 1999).

In addition to a deficit in cholinergic transmission, dementia is also related to degeneration of glutamatergic hippocampal and cortical pyramidal neurons (Aronica *et al*, 1998; Francis *et al*, 1999), which are targets for cholinergic and serotonergic innervation. Furthermore, the activity of glutamatergic neurons in the hippocampus and neocortex is modulated by cholinergic systems, which have facilitatory effects mediated via muscarinic receptors (Cole and Nicoll, 1984; Segal, 1982), and serotonergic systems, which have inhibitory effects partly mediated via 5-HT_{1A} receptors (Beck *et al*, 1992; Davies *et al*, 1987; Newberry *et al*, 1999; Pugliese *et al*, 1998). Thus, 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-*n*-propyloamino)tetralin hydrobromide (8-OH-DPAT) have been found to cause a hyperpolarization of limbic (hippocampus and entorhinal cortex) (Grunschlag *et al*, 1997; Sprouse and Aghajanian, 1988; Tada *et al*, 1999)

*Correspondence: Prof Sven Ove Ögren, Division of Behavioral Neuroscience, Department of Neuroscience, Karolinska Institutet, SE-171 77 Stockholm, Sweden, Tel: +46 8 728 7074, Fax: +46 8 302 875, E-mail: sven.ove.ogren@neuro.ki.se
Received 31 October 2001; revised 22 May 2002; accepted 30 June 2002

and neocortical pyramidal cells (Hajos *et al*, 1999). These effects are blocked by the 5-HT_{1A} receptor antagonists NAN-190 and WAY 100635 (Hajos *et al*, 1999; Schmitz *et al*, 1998).

Passive avoidance (PA) is one of the most frequently used animal models for studying the role of neurotransmitters in learning and memory processes (Bammer, 1982; Sarter *et al*, 1992a, b). This aversive learning task, which is based on classical (Pavlovian) fear conditioning, can be modified by alterations in both serotonergic and cholinergic transmission (Bammer, 1982; Misane and Ögren, 2000; Ögren, 1985). For instance, nonselective muscarinic receptor antagonists such as scopolamine injected prior to PA training cause a dose-dependent impairment of PA retention when tested 24 h after training (Cole and Jones, 1995; Meyers, 1965; Rush and Streit, 1992; Wilson and Cook, 1994). Conversely, pretraining administration of cholinomimetics such as AChE inhibitors (physostigmine, tacrine, donepezil) have been shown to antagonize the impairment of PA retention caused by scopolamine (Kojima *et al*, 1997; Rush and Streit, 1992; Yoshida and Suzuki, 1993). Unlike changes in cholinergic transmission, treatments that increase serotonergic (5-HT) activity in the brain, for example, the 5-HT-releasing compound *p*-chloroamphetamine, were shown to disrupt PA retention (Misane and Ögren, 2000; Ögren, 1985). The impairment of PA by *p*-chloroamphetamine was related to 5-HT release and subsequent activation of the postsynaptic 5-HT_{1A} receptors, but not the 5-HT_{2A} or 5-HT_{2C} receptors (Misane and Ögren, 2000).

Several studies using the selective 5-HT receptor agonists and antagonists have revealed the particular importance of 5-HT_{1A} receptor subtype in PA. The selective 5-HT_{1A} agonist 8-OH-DPAT given subcutaneously (s.c.) prior to PA training was found to produce a dose-dependent impairment of retention in the rat when examined 24 h later (Carli *et al*, 1992; Misane *et al*, 1998a; Misane and Ögren, 2000). The PA impairment caused by 8-OH-DPAT was related to stimulation of post- but not presynaptic 5-HT_{1A} receptors in the brain (Misane *et al*, 1998a; Misane and Ögren, 2000).

The role of muscarinic receptors and 5-HT_{1A} receptors themselves in PA has been extensively studied, but the potential functional interactions between these two receptor systems have received little attention. There is some evidence that 5-HT_{1A} and muscarinic receptors can interact in the regulation of PA performance. Coadministration of a subthreshold dose of 8-OH-DPAT (0.03 mg/kg s.c.) and scopolamine (0.1 mg/kg i.p.) before PA training resulted in an impairment of PA retention when tested 24 h later (Riekkinen, 1994). However, since this study was based on the use of the 5-HT_{1A} agonist 8-OH-DPAT and scopolamine and did not include a 5-HT_{1A} antagonist, the results do not allow conclusions as to the possible interaction between 5-HT_{1A}-receptor-mediated transmission and muscarinic-receptor-mediated transmission.

The present study examined whether a blockade of central 5-HT_{1A} receptors could modify the impairment of PA caused by muscarinic receptor blockade. For that purpose, the studies were performed under high-training conditions producing almost maximal (cutoff) retention latencies in control animals. This design allows a reliable detection of impairment of PA retention caused by muscarinic receptor blockade, but is not suitable for studying the facilitation of PA retention.

The effects of the highly selective 5-HT_{1A} receptor antagonists WAY 100635 and NAD-299 (robalzotan) on PA were examined alone or in combination with the nonspecific muscarinic receptor antagonist scopolamine in the rat. Robalzotan is a more selective 5-HT_{1A} receptor antagonist than WAY 100635, with a weak affinity for both NA and DA receptors (Johansson *et al*, 1997). Robalzotan is also a more short-acting drug in the rat compared with WAY 100635. For this reason, the temporal kinetics of WAY 100635 and robalzotan was compared in relation to scopolamine. In view of these data suggestive of the therapeutic potential of the 5-HT_{1A} antagonists, the two AChE inhibitors tacrine and donepezil (E2020) used in the treatment of AD were included as 'reference compounds'.

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats (2 months of age), weighing 300–350 g at the time of testing, were obtained from B&K UNIVERSAL AB (Sollentuna, Sweden). The animals were allowed at least a 5-day adaptation period at the animal maintenance facilities of the department before the start of the experiments. The animals were housed four per cage in standard plastic type IV Macrolon cages ($57 \times 35 \times 19$ cm, with 2 l wood-cuttings as bedding) and maintained at an ambient room temperature of $20 \pm 0.5^{\circ}$C with 40–50% relative humidity. A 12-h light/dark schedule (lights on at 06:00 h) was used throughout the experiment. The animals had free access to standard lab chow (Ewos R36, Ewos AB, Sweden) and tap water up to the time of the experiments. The cages were changed twice a week during the adaptation period. In order to decrease the influence of stress factors on performance, the cages were not cleaned during the days of PA training and retention. On the experimental days, the animals were brought to the experimental room and allowed to habituate to the environmental conditions for a period of approximately 60 min. Animal housing and all experimental procedures followed the provisions and general recommendations of the Swedish animal protection legislation. The experimental procedures were approved by the local Animal Ethics Committee (ethical N 80/96 and 116/00).

PA Procedure

PA was conducted as described earlier (Misane and Ögren, 2000; Misane *et al*, 1998b). A standard shuttle box (Ugo Basile, Comerio-Varese, Italy), with two communicating (7×7 cm sliding door built in the separating wall) compartments of equal size and a stainless-steel bar floor, was used. The right-hand compartment (shock compartment) was painted black to obtain a dark chamber. The left-hand compartment was illuminated by a bulb (24 V; 5 W) installed on the top Plexiglas cover.

PA training was conducted in a single session (day 1) during the light phase of a 12-h day/night cycle (09:00–16:00). In all the experiments, the animals ($n = 7$–16) were treated with the test compounds prior to training according

to the schedule described below. After the selected time interval following injection (day 1), the rat was placed in the light compartment with no access to the dark compartment and allowed to explore for 2 min. During the exploration phase in the PA apparatus, the behavior of the animals, including number of full rearings (move of the body through the vertical plane) and locomotor activity, was noted by the experimenter.

When 2 min expired, the sliding door was automatically opened by pressing a pedal and the rat was allowed to cross over into the dark compartment. Once the rat had entered the dark compartment, the sliding door was automatically closed and an inescapable, constant current, scrambled shock (5 s, 0.6 mA) was delivered through the grid floor. Latency to cross into the dark compartment (training latency) was recorded. If a rat failed to move into the dark compartment within 300 s (cutoff latency), the door was reopened and the rat was gently moved into the dark compartment by the experimenter, where it received footshock. Following training, the rat was immediately removed from the PA apparatus.

Retention performance was examined 24 h after training (day 2). The animal was placed in the light (safe) compartment, with access to the dark compartment (within 15 s) for a period of 300 s. The latency to enter the dark compartment with all four feet (retention latency) was automatically measured. If the rat failed to enter the dark compartment within 300 s, it was removed and assigned a maximum test latency score of 300 s.

Drugs

The following compounds were used in the present study: (−)-scopolamine hydrobromide (Sigma, St Louis, MO, USA); *N*-2-4-(2-methoxyphenyl)-1-piperazinylethyl-*N*-(2-pyridinyl)cyclohexane carboxamide trihydrochloride (WAY 100635) (Wyeth Research, Taplow, UK); (*R*)-3-*N*, *N*-dicyclobutylamino-8-fluoro-3,4-dihydro-3*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*)-tartrate monohydrate (NAD-299, robalzotan) (AstraZeneca, Södertälje, Sweden); tacrine hydrochloride (Sigma Chemical Co., St Louis, MO, USA); and 1-benzyl-4-(5,6-dimethoxy-1-indanon)-2-yl-methylpiperidine hydrochloride (donepezil, E2020) (kindly supplied by Anne-Lie Svensson, Karolinska Institutet, Huddinge University Hospital).

All drugs were dissolved in saline (NaCl 0.9%) and administered s.c. or i.p. (as indicated in the Results section and the figure legends) in volumes of 2 or 5 ml/kg, respectively.

The compounds were administered at the following doses and times before PA training: scopolamine (0.03–0.3 mg/kg) 40 min; WAY 100635 (0.03–1.0 mg/kg) 30 or 50 min; robalzotan (0.01–3.0 mg/kg) 15, 30 or 50 min; tacrine (0.3–3.0 mg/kg) 60 min; and donepezil (0.1–2.0 mg/kg) 30 or 90 min.

Statistical Analysis

The overall treatment effects in the PA studies were examined using one-way analysis of variance (ANOVA). For each significant F-ratio, Fisher's protected least significant difference test (Fisher's PLSD test) was used to analyze the statistical significance of appropriate multiple comparisons (Kirk, 1968). Subsequently, to analyze the possible dose-dependent effect of scopolamine itself and the dose dependency of 5-HT_{1A} antagonists and AChE inhibitors effects on the scopolamine effects, the analysis of polynomial regression was used and the coefficients of determination (R^2) were calculated to show the possible linearity between treatment and measured variables (training and retention latencies and number of rearings). In the drug combination studies with scopolamine, the respective scopolamine groups were used as control groups. Both when one-way ANOVA and regression ANOVA were applied, a probability level of $P<0.05$ was accepted as statistically significant. The *post hoc* tests were two tailed.

RESULTS

Effects of Scopolamine on PA

When examined 24 h after training, the retention latency in the saline-treated control group was close to 300 s with a small distribution in the responses (Figure 1), indicating that animals had acquired the task. When injected 40 min before PA training, scopolamine (0.03–0.3 mg/kg s.c.) caused an impairment of PA retention ($F_{3,28}=13.65$, $P<0.01$) with a significant effect at the 0.3 mg/kg dose ($P<0.01$ *vs* saline control group) (Figure 1). Polynomial regression ANOVA showed that the scopolamine effect was dose-dependent ($F_{2,29}=21.12$, $P<0.01$), although the treatment-latency relation was not strictly linear ($R^2=0.59$). Training latencies were not affected by scopolamine: $F_{3,28}=1.36$, $P>0.27$ (Table 1). Behavioral observations in the PA apparatus during the 2-min exploration period indicated that locomotor activity was increased while the number of full rearings was markedly decreased ($P<0.01$ *vs* saline control group) by the 0.3 mg/kg dose of scopolamine. Based on the dose–response experiments, the 0.3 mg/kg

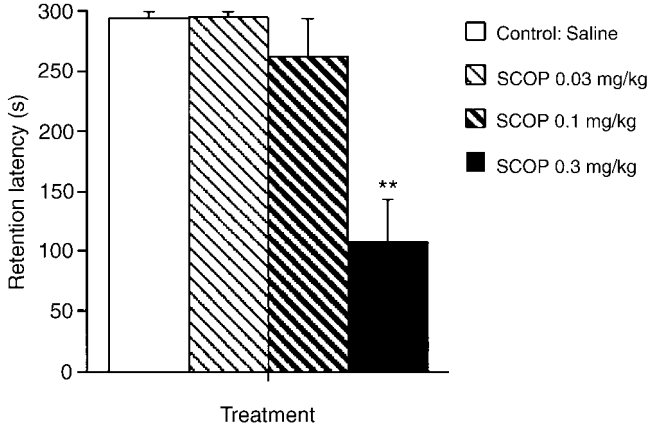

Figure 1 Dose-dependent effects of scopolamine on PA retention in the rat. Rats were injected with scopolamine (0.03–0.3 mg/kg s.c.) 40 min before the training session (exposure to inescapable foot shock). The saline (s.c. 2 ml/kg) control group was run concurrently with scopolamine-treated groups. The retention test was performed 24 h later. Vertical bars represent means (± SEM) of retention latencies. The maximal time of latency was set at 300 s (cutoff time). The statistical analysis was performed by one-way ANOVA followed by Fisher's PLSD test. $^{**}P<0.01$ *vs* saline control group, $n=8$. SCOP, scopolamine in all figures.

Table 1 Effects of Scopolamine, the 5-HT_{1A} Antagonists and AChE Inhibitors on PA Training in the Rat

Compound	Dose (mg/kg)/Training latency (s)				
Scopolamine	0/32.8 ± 12.7	0.03/20.1 ± 4.8	0.1/31.9 ± 5.7	0.3/15.2 ± 2.1	
WAY 100635	0/58.3 ± 23.9	0.03/49.6 ± 20.4	0.3/77.9 ± 34.2	1.0/57.4 ± 21.3	
NAD-299	0/50.6 ± 15.3	0.1/32.9 ± 10.0	0.3/34.7 ± 6.7	1.0/59.5 ± 34.8	3.0/106.1 ± 43.3
Tacrine	0/45.6 ± 16.5	0.3/72.4 ± 38.8	3.0/128.4 ± 31.8		
Donepezil	0/41.6 ± 10.9	0.1/60.7 ± 10.6	0.5/72.4 ± 21.0	1.0/116.6 ± 39.9*	2.0/170.8 ± 49.0**

The test compounds were administered before PA training at the times and by injection routes as follows: scopolamine (s.c.) 40 min, WAY 100635 (s.c.) 30 min, robalzotan (s.c.) 30 min, tacrine (i.p.) 60 min and donepezil (s.c.) 90 min. The values shown are mean durations (± SEM). The statistical analysis was performed by one-way ANOVA followed by Fisher's PLSD test (*$P<0.05$ and **$P<0.01$ *vs* corresponding saline control group, $n=7$–16); for details, see the Methods section.

Table 2 Effects of the 5-HT_{1A} Antagonists and AChE Inhibitors on PA Retention in the Rat

Compound	Dose (mg/kg)/Retention latency (s)				
WAY 100635	0/293.1 ± 7.0	0.03/287.5 ± 12.5	0.3/300.0 ± 0.0	1.0/282.4 ± 17.6	
Robalzotan	0/292.8 ± 7.2	0.1/290.2 ± 9.8	0.3/291.1 ± 5.8	1.0/209.9 ± 45.0	3.0/177.9 ± 45.3**
Tacrine	0/300.0 ± 0.0	0.3/300.0 ± 0.0	3.0/291.6 ± 8.4		
Donepezil	0/246.1 ± 25.2	0.1/180.9 ± 44.2	0.5/296.5 ± 3.5	1.0/277.7 ± 22.3	2.0/220.1 ± 37.7

The test compounds were administered before PA training at the times and by injection routes as follows: WAY 100635 (s.c.) 30 min, robalzotan (s.c.) 30 min, tacrine (i.p.) 60 min and donepezil (s.c.) 90 min. The values shown are mean durations (± SEM). The statistical analysis was performed by one-way ANOVA followed by Fisher's PLSD test (**$P<0.01$ *vs* corresponding saline control group, $n=7$–16); for details, see the Methods section.

dose of scopolamine was used in most of the subsequent interaction studies with 5-HT_{1A} antagonists and AChE inhibitors.

Effects of the 5-HT_{1A} Antagonists and the AChE Inhibitors on PA

Both WAY 100635 (0.03–1.0 mg/kg s.c.) and robalzotan (0.01–1.0 mg/kg s.c.), and tacrine (0.3–3.0 mg/kg i.p.) and donepezil (0.1–2.0 mg/kg s.c.) failed to affect PA retention (Table 2). However, robalzotan caused an impairment of PA retention at the highest dose tested (3.0 mg/kg) ($P<0.01$ *vs* saline control group). Only donepezil caused a significant increase in PA training latency at the 1.0 and 2.0 mg/kg doses (Table 1).

WAY 100635 or robalzotan did not cause any apparent behavioral changes during the 2-min exploration phase in the PA apparatus, except for a decrease in rearing noted at the 3.0 mg/kg of robalzotan ($P<0.05$ *vs* saline control group).

At the highest doses tested, both tacrine (3.0 mg/kg IP) and donepezil (2.0 mg/kg s.c.) decreased rearing ($P<0.01$ and 0.05 *vs* saline control group, respectively). In addition, slight salivation, jaw and head movements resembling orofacial dyskinesias, and an increase in defecation (diarrhea) was observed in rats treated with the 2.0 mg/kg dose of donepezil and the 3.0 mg/kg dose of tacrine. In addition, at the 3.0 mg/kg dose, tacrine had an increased reactivity to sudden noise (such as opening the door in the PA apparatus). Importantly, no tremor was observed in the tacrine- or donepezil-treated rats.

Effects of WAY 100635 on Impairment of PA Caused by Scopolamine

The effects of WAY 100635 on scopolamine-induced impairment of PA retention were examined using two different injection schedules: WAY 100635 was given either 10 min after scopolamine or 10 min before scopolamine, that is 30 and 50 min before PA training, respectively.

When injected 10 min after scopolamine (0.3 mg/kg s.c.), WAY 100635 (0.03–1.0 mg/kg s.c.) failed to reverse the impairment of PA retention (Figure 2a). In contrast, when injected 10 min before scopolamine (0.3 mg/kg s.c.), WAY 100635 (0.3 and 1.0 mg/kg s.c.) almost completely blocked the impairment of PA retention ($P<0.01$ *vs* saline+scopolamine group and $P>0.15$ and 0.13 *vs* saline+saline control group for the 0.3 and 1.0 mg/kg doses of WAY 100635, respectively) (Figure 2b). Regression ANOVA revealed a highly significant treatment effect ($F_{2,21}=15.72$, $P<0.01$), although there was no strictly linear relation between the dose of WAY 100635 effect and retention latency ($R^2=0.60$). It is notable that regardless of the injection schedule, WAY 100635 failed to normalize rearing, which was nearly abolished because of the scopolamine treatment (data not shown).

Effects of Robalzotan on Impairment of PA Caused by Scopolamine

Owing to the relatively short half-life of robalzotan in the rat (Stenfors *et al*, 1998), the effects of robalzotan on scopolamine-induced impairment of PA retention were examined using four different injection schedules; the compound was given either 25 or 10 min after scopolamine and/or 10 min before scopolamine, that is 15, 30, and 50 min before PA training, respectively.

When injected 15 min before training, robalzotan (0.03–0.3 mg/kg s.c.) failed to attenuate the inhibitory effect of scopolamine (0.3 mg/kg s.c.) on PA retention (data not shown), and it did not alter training latencies ($F_{4,35}=0.69$, $P>0.60$).

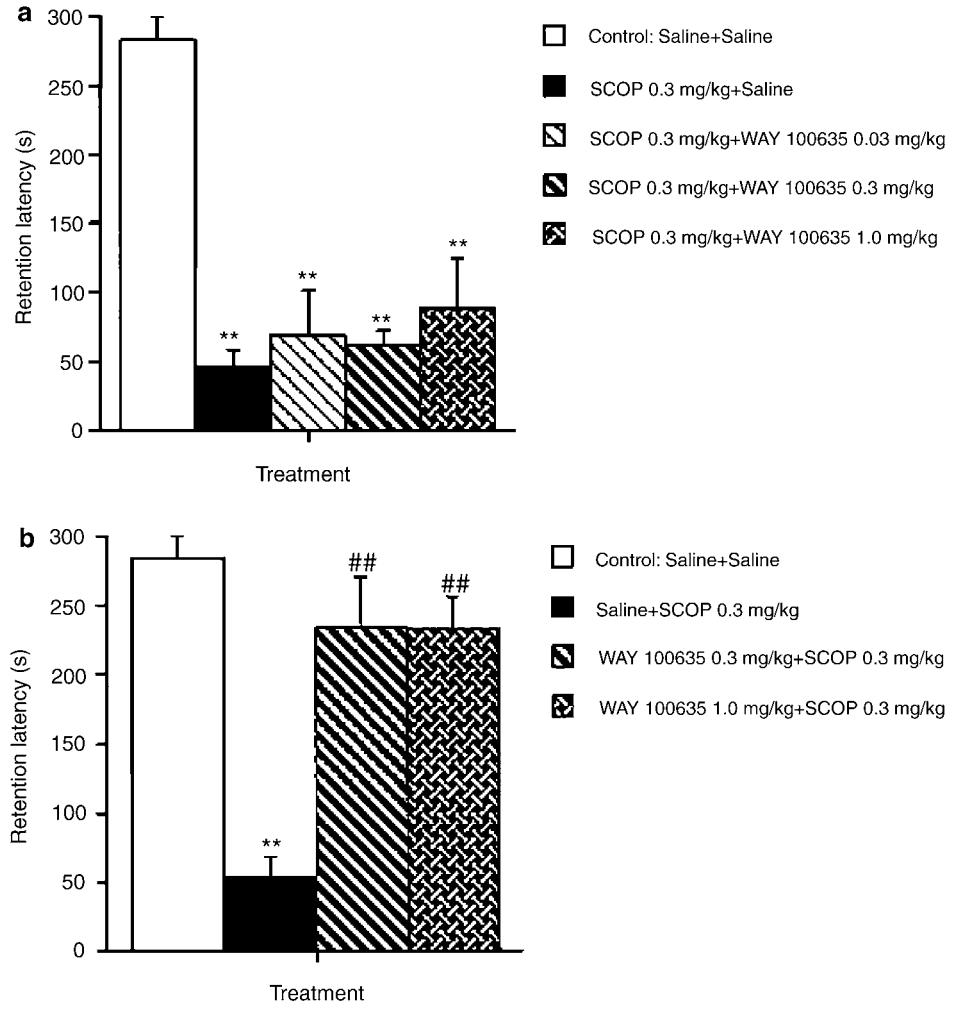

Figure 2 Combined effects of WAY 100635 and scopolamine on PA retention in the rat. (a) Rats were injected with scopolamine (0.3 mg/kg s.c.) and WAY 100635 (0.03–1.0 mg/kg s.c.) 40 and 30 min before the training session, respectively. (b) Rats were injected with WAY 100635 (0.3–1.0 mg/kg s.c.) and scopolamine (0.3 mg/kg s.c.) 50 and 40 min before the training session, respectively. The saline+saline (s.c. 2 ml/kg) control groups were run concurrently with scopolamine- and WAY 100635-treated groups. The retention test was performed 24 h later. Vertical bars represent means ($\pm$ SEM) of retention latencies. $^{**}P<0.01$ *vs* corresponding saline control group; $^{\#\#}P<0.01$ *vs* saline+scopolamine-treated group, $n=8$. For details of statistical analysis and general information, see the legend to Figure 1 and the Methods section.

A significant overall treatment effect in the retention test ($F_{6,65}=21.18$, $P<0.01$) was found when robalzotan (0.01–3.0 mg/kg s.c.) was injected 30 min before PA training (Figure 3a). Under these conditions, robalzotan displayed an inverse 'U-shape' type of activity. Regression ANOVA revealed a significant attenuation of scopolamine (0.3 mg/kg s.c.)-induced PA retention deficit by robalzotan at the 0.01–0.1 mg/kg dose range ($F_{2,37}=3.60$, $P<0.05$), although the dose–latency relation was not linear ($R^2=0.16$). It is notable that the drug effect was not significant at the highest 0.3–3.0 mg/kg dose ranges of robalzotan tested. A similar 'U-shape' pattern was also found for training latencies. While scopolamine itself did not affect the training latency ($P>0.95$ *vs* saline+saline control group), a profound ($P<0.01$ *vs* saline+saline control and scopolamine+saline groups) increase in training latencies was seen when scopolamine was combined with robalzotan at the 0.03–0.3 mg/kg dose range. The maximum effect was found at the 0.1 mg/kg dose.

Robalzotan (0.1–3.0 mg/kg s.c.) injected 50 min before training failed to attenuate the inhibitory effect of scopolamine (0.3 mg/kg s.c.) on PA retention (Figure 3b). In addition, no significant overall treatment effect was found for training latencies ($F_{4,35}=0.78$, $P>0.51$). However, when robalzotan (0.03–0.3 mg/kg s.c.) was injected twice both 50 and 30 min before PA training (Figure 3c), ANOVA revealed a significant overall treatment effect on the retention test ($F_{4,35}=5.56$, $P<0.01$). In this experiment, robalzotan attenuated the PA retention deficit caused by scopolamine (0.3 mg/kg s.c.) at the 0.3 mg/kg dose ($P<0.05$ *vs* control group and saline+scopolamine+saline-treated group). However, regression ANOVA showed that the robalzotan effect was not dose dependent ($F_{2,29}=2.48$, $P>0.10$, $R^2=0.15$). It is notable that regardless of the injection schedule (before and/or after scopolamine), robalzotan failed to normalize rearing, which was nearly abolished because of the scopolamine treatment (data not shown).

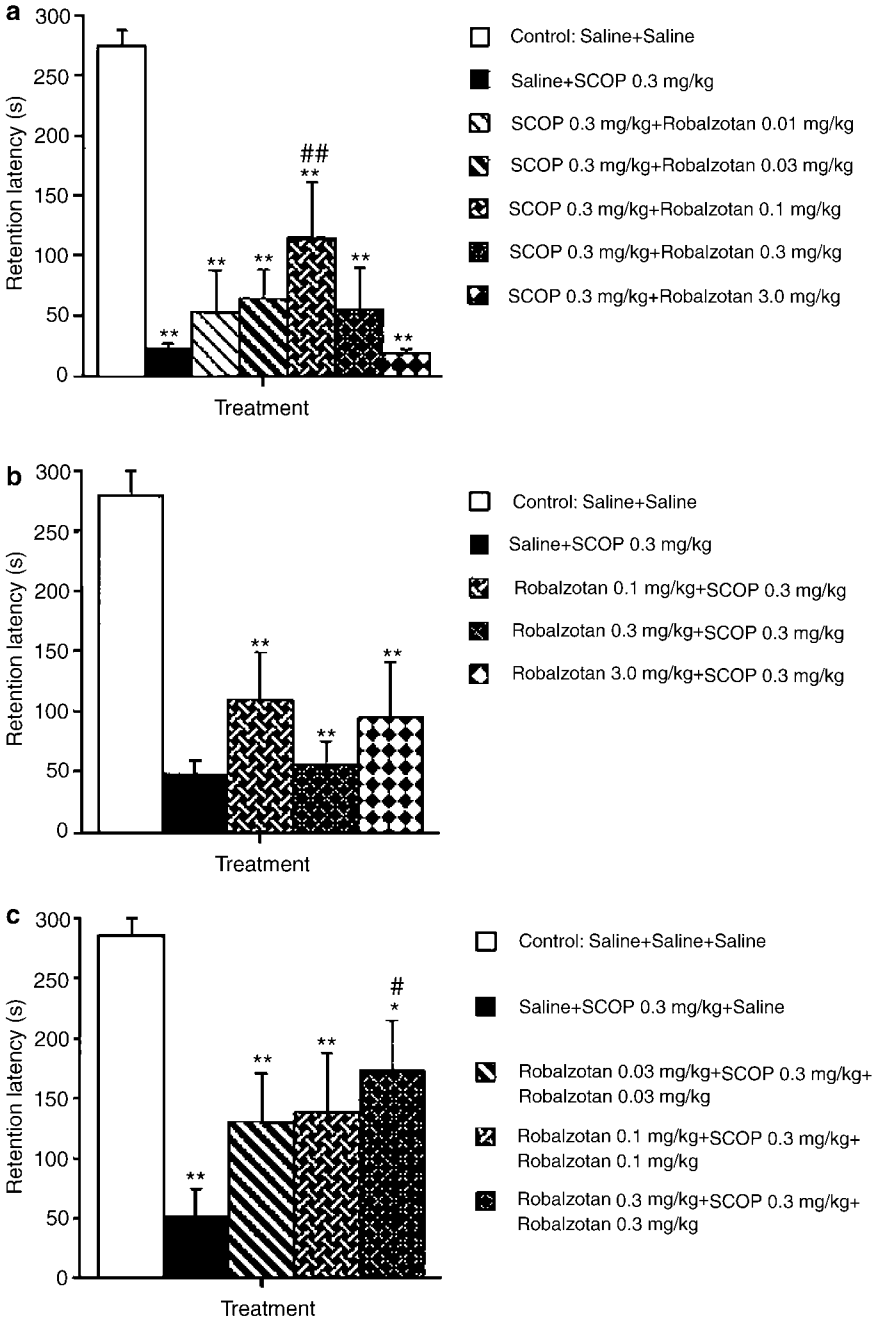

Figure 3 Combined effects of robalzotan and scopolamine on PA retention in the rat. (a) Rats were injected with scopolamine (0.3 mg/kg s.c.) and robalzotan (0.01–3.0 mg/kg s.c.) 40 and 30 min before the training session, respectively. (b) Rats were injected with robalzotan (0.1–3.0 mg/kg s.c.) and scopolamine (0.3 mg/kg s.c.) 50 and 40 min before the training session, respectively. (c) Rats were injected with robalzotan (0.03–0.3 mg/kg s.c.) 50 and 30 min before the training session. Scopolamine (0.3 mg/kg s.c.) was given 40 min before training. The saline (double or triple injections, s.c. 2 ml/kg) control groups were run concurrently with scopolamine- and robalzotan-treated groups. The retention test was performed 24 h later. Vertical bars represent means ($\pm$ SEM) of retention latencies. $^{*}P<0.05$ and $^{**}P<0.01$ *vs* corresponding saline control group; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ *vs* corresponding scopolamine control group, $n=8$–16. For details of statistical analysis and general information, see the legend to Figure 1 and the Methods section.

Effects of Tacrine on Impairment of PA Caused by Scopolamine

The activity of tacrine was tested *vs* two doses of scopolamine (0.2 and 0.3 mg/kg s.c.).

Figure 4a shows that tacrine (3.0 mg/kg i.p.) attenuated the deficit of PA caused by the 0.3 mg/kg dose of scopolamine ($P<0.05$ *vs* saline+saline control and $P<0.05$ *vs* saline+scopolamine-treated group), while the 0.3 mg/kg dose of tacrine did not have any significant effect. Regression ANOVA revealed that the tacrine effect was dose dependent ($F_{2,18}=4.28$, $P<0.05$) without the linear treatment–latency relation ($R^2=0.32$). No overall treatment effect on training was found ($F_{3,24}=0.71$, $P>0.55$).

Tacrine (3.0 mg/kg i.p.) also attenuated the impairment of PA retention caused by the lower 0.2 mg/kg dose of

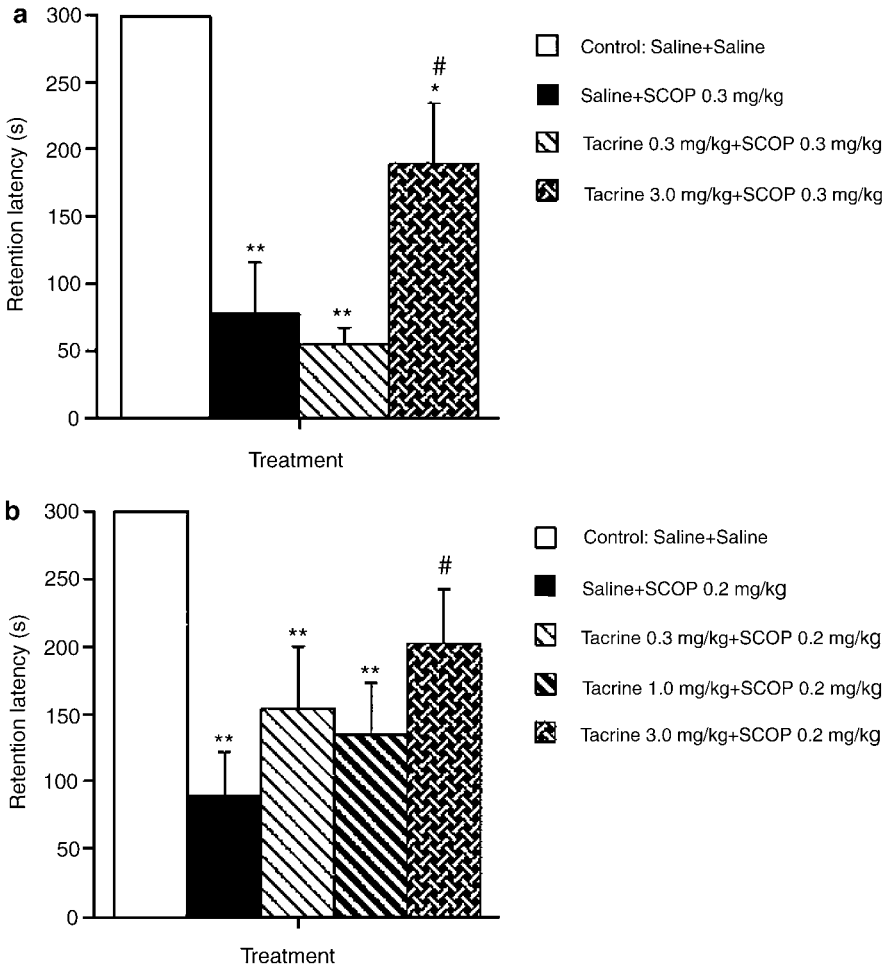

Figure 4 Combined effects of tacrine and scopolamine on PA retention in the rat. (a) Rats were injected with tacrine (0.3–3.0 mg/kg i.p,) and scopolamine (0.3 mg/kg s.c.) 60 and 40 min before the training session, respectively. (b) Rats were injected with tacrine (0.3–3.0 mg/kg i.p.) and scopolamine (0.2 mg/kg s.c.) 60 and 40 min before the training session, respectively. The saline (i.p. 5 ml/kg)+saline (s.c. 2 ml/kg) control groups were run concurrently with tacrine- and scopolamine-treated groups. The retention test was performed 24 h later. Vertical bars represent means (± SEM) of retention latencies. $^{*}P<0.05$ and $^{**}P<0.01$ *vs* corresponding saline+saline control group; $^{\#}P<0.05$ *vs* corresponding saline+scopolamine-treated group, $n=7$–8. For details of statistical analysis and general information, see the legend to Figure 1 and the Methods section.

scopolamine ($P>0.06$ *vs* saline+saline control and $P<0.05$ *vs* saline+scopolamine-treated group) (Figure 4b); however, the tacrine effect was not dose dependent ($F_{2,29}=1.65$, $P=0.21$, $R^2=0.10$). In this experiment, ANOVA revealed a significant effect on training latencies ($F_{4,35}=2.70$, $P<0.05$).

Tacrine failed to normalize rearing, which was nearly abolished following treatment with either the 0.2 or 0.3 mg/kg dose of scopolamine (data not shown).

Effects of Donepezil on Impairment of PA Caused by Scopolamine

The effects of donepezil on the scopolamine-induced impairment of PA retention were examined using two different injection schedules. Donepezil was given either before or after scopolamine, that is 90 and 30 min before PA.

When injected 30 or 90 min before training, donepezil (0.1–2.0 mg/kg s.c.) failed to block the inhibitory effect of scopolamine (0.3 mg/kg s.c.) on PA retention (Figures 5a and b) and it did not alter training latencies ($F_{5,42}=0.96$, $P>0.44$ and $F_{5,58}=0.94$, $P>0.45$, respectively).

In view of the inability of donepezil to counteract the impairment of PA retention caused by the 0.3 mg/kg dose of scopolamine, donepezil was also examined using the 0.2 mg/kg dose of scopolamine. Under this condition, when given 90 min before PA training, donepezil (0.5–1.0 mg/kg s.c.) almost completely blocked the inhibitory effects of scopolamine (Figure 5c), although this effect was not dose dependent ($F_{2,21}=2.57$, $P>0.10$, $R^2=0.20$). No overall treatment-effect was found for training latencies ($F_{3,28}=0.75$, $P>0.53$).

Regardless of the injection time (90 or 30 min before training), donepezil (0.1–2.0 mg/kg s.c.) failed to normalize rearing in the PA apparatus, which was nearly abolished following the 0.2 and 0.3 mg/kg doses of scopolamine (data not shown).

DISCUSSION

In agreement with previous studies (Bammer, 1982), systemic (s.c.) scopolamine administration before training

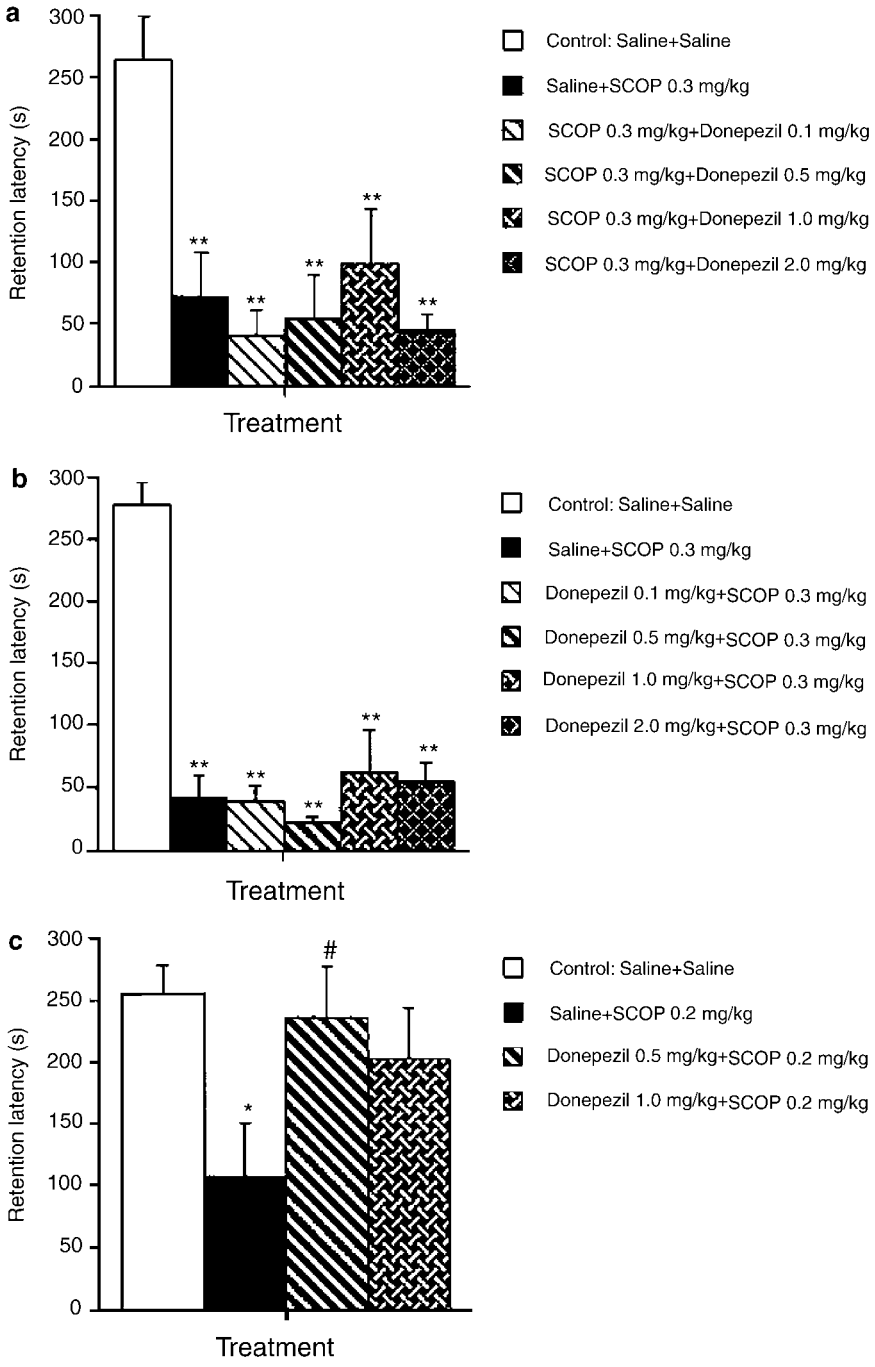

Figure 5 Combined effects of donepezil and scopolamine on PA retention in the rat. (a) Rats were injected with scopolamine (0.3 mg/kg s.c.) and donepezil (0.1–2.0 mg/kg s.c.) 40 min and 30 min before the training session, respectively. (b) Rats were injected with donepezil (0.1–2.0 mg/kg s.c.) and scopolamine (0.3 mg/kg s.c.) 90 and 40 min before the training session, respectively. (c) Rats were injected with donepezil (0.5–1.0 mg/kg s.c.) and scopolamine (0.2 mg/kg s.c.) 90 and 40 min before the training session, respectively. The saline+saline (s.c. 2 ml/kg) control groups were run concurrently with donepezil- and scopolamine-treated groups. The retention test was performed 24 h later. Vertical bars represent means (± SEM) of retention latencies. $^{*}P<0.05$ and $^{**}P<0.01$ *vs* corresponding saline+saline control group; $^{\#}P<0.05$ *vs* saline+scopolamine-treated group, $n=8$–16. For details of statistical analysis and general information, see the legend to Figure 1 and the Methods section.

produced a dose-related impairment of step-through PA retention when examined 24 h later. The interval between the s.c. administration of scopolamine and training in this study was 40 min, which is longer than in most previous PA studies (varying usually between 5 and 30 min) (Bammer, 1982). The choice of this time interval was based on *in vivo* microdialysis studies, in which s.c. scopolamine produced an increase in the ACh levels in the ventral hippocampus with a peak effect after 40–60 min (Antoniou *et al*, 1997; Ögren *et al*, 1996; Toide and Arima, 1989).

Scopolamine markedly elevates extrasynaptic ACh at the time of PA training mainly because of the blockade of presynaptic M_2 muscarinic receptors (Ögren *et al*, 1996; Stillman *et al*, 1996). At the same time scopolamine blocks

several postsynaptic muscarinic (M_1–M_5) receptors (Bymaster *et al*, 1993), which explains its inhibitory effects on cognitive processing. Thus, the action of scopolamine on pre-/postsynaptic muscarinic receptors is of critical importance for its effects on learning and memory and the ability of AChE inhibitors to attenuate these effects.

The most important finding of this study is the observation that muscarinic and 5-HT_{1A} receptors can interact in the regulation of aversive learning. The 'prototypic' selective 5-HT_{1A} receptor antagonist WAY 100635 and the selective 5-HT_{1A} receptor antagonist robalzotan both attenuated the inhibitory effects of scopolamine. However, WAY 100635 was active *vs* scopolamine when injected 50 but not 30 min before PA training. In contrast, robalzotan was found to attenuate the PA impairment by scopolamine when injected 30 but not 50 min before PA training. This attenuating effect was even more pronounced when robalzotan was injected twice, that is, both 30 and 50 min before PA training, which is consistent with the kinetic concentrations of robalzotan in the mouse brain (Stenfors *et al*, 1998). In contrast to robalzotan, WAY 100635 failed to reverse the deficit of PA retention caused by scopolamine. This suggests potential differences in the action of the two 5-HT_{1A} receptor antagonists, and it is possible that the temporal kinetics of the 5-HT_{1A} receptor antagonists in relationship to muscarinic receptor blockade by scopolamine is of importance. In addition, unlike WAY 100635, robalzotan itself at the highest dose tested (3.0 mg/kg) impaired retention performance (Table 2) and this dose failed to attenuate scopolamine effects (Figure 3a). This finding suggests that at higher doses, robalzotan can increase 5-HT_{1A}-mediated transmission probably via increased 5-HT release or partial agonistic 5-HT_{1A} receptor activity.

The role of 5-HT in learning and memory seems to be mainly related to the acquisition or the encoding phase (Ögren, 1985). Thus, the activation of 5-HT_{1A} receptors before but not immediately after training has been shown to impair retention in associative learning tasks such as step-through passive avoidance and fear conditioning (Misane *et al*, 1998a; Misane and Ögren, 2000; Stiedl *et al*, 2000). It is notable that inhibitory effects of pretraining administration of 8-OH-DPAT on PA retention could not be explained by state-dependent factors (Misane *et al*, 1998a). Our recent studies show that scopolamine injected s.c. immediately after training at the doses used in this study failed to impair PA retention tested 24 h later.

Thus, both alterations in 5-HT_{1A}- and muscarinic-receptor-mediated transmission appear to interfere with the formation of contextual short-term representation and, thereby, possibly produce a deficit in the long-term memory representation of the context. This suggests that the modulatory action of 5-HT and 5-HT_{1A} receptors on muscarinic-receptor-mediated transmission is mainly related to encoding mechanisms important for acquisition. The exact neurobiological mechanisms underlying the anterograde amnestic effect of scopolamine are still not well understood, but probably involve multiple forebrain areas important for learning and memory.

The mechanisms and the sites of action mediating the functional interaction between muscarinic and 5-HT_{1A} receptors are presently unknown. The use of the double immunocytochemical technique has shown that the 5-HT_{1A} receptors are colocalized with choline acetyltransferase (ChAT) in the medial septum and diagonal band of Broca (MS-dBB) in the rat (Kia *et al*, 1996). About 25% of the cholinergic neurons in the septal complex were found to express 5-HT_{1A} receptor immunoreactivity. In the dorsal and ventral cholinergic cell groups of the MS–dBB, the number of neurons coexpressing both ChAT and 5-HT_{1A} receptors was in the range of 40–44% (Kia *et al*, 1996). Triple labeling immunofluorescent techniques have also identified 5-HT-immunoreceptive neurons in close proximity to the soma and dendrites of cholinergic neurons in the nucleus basalis (NB) of the guinea pig (Fort *et al*, 1998; Khateb *et al*, 1993). Interestingly, 5-HT and the 5-HT_{1A} agonist 8-OH-DPAT were also found to hyperpolarize the ChAT-immunoreactive cells *in vitro*, suggesting an ability to inhibit tonic firing and also to modulate the low threshold bursting of the cholinergic NB neurons (Khateb *et al*, 1993). Because the NB neurons provide the main cholinergic innervation of the cerebral cortex (Lehmann *et al*, 1980; Lewis and Shute, 1967; Rye *et al*, 1984), changes in the activity of these neurons will influence cortical functioning, for example, the sleep–waking cycle (Stewart *et al*, 1984). It seems likely that a blockade of endogenous 5-HT_{1A}-receptor-mediated transmission in the basal forebrain could result in a disinhibition of cholinergic MS–dBB and/or NB neurons. These findings support the view that the postsynaptic 5-HT_{1A} receptors in the basal forebrain can modulate cholinergic transmission and, thus, influence learning and memory. Direct support for this view has recently been provided by *in vivo* microdialysis studies in awake rats performed in our laboratory. Both robalzotan and WAY 100635 have been found to increase basal ACh release in the hippocampus and cortex of the rat (J Kehr, personal communications). Robalzotan produced a two-fold increase in cortical and hippocampal ACh in the dose range that attenuated the PA deficit caused by scopolamine in this study. Also WAY 100635 increased ACh release in the same dose range as that blocking the action of scopolamine in the PA test. However, quantitatively robalzotan was more effective than WAY 100635 in its elevations of extracellular ACh. In view of the anatomical localization of brain 5-HT_{1A} receptors, this finding suggests that the 5-HT_{1A} antagonists can enhance cholinergic transmission in the forebrain by blocking 5-HT_{1A} receptors located on the ascending cholinergic neuronal systems.

Muscarinic and 5-HT_{1A} receptors are localized on the pyramidal hippocampal cells (Azmitia *et al*, 1996; van der Zee and Luiten, 1999; van der Zee *et al*, 1989), and electrophysiological studies (Grunschlag *et al*, 1997; Kobayashi *et al*, 1997; Sprouse and Aghajanian, 1988; Stewart *et al*, 1992; Tada *et al*, 1999; Wang and Tang, 1998) have shown that muscarinic and 5-HT_{1A} receptors are involved in the regulation of pyramidal cell activity. These effects might explain the marked inhibitory effects of the 5-HT_{1A} agonists in various types of learning in rodents ranging from aversive conditioning (Carli *et al*, 1993; Misane *et al*, 1998a; Misane and Ögren, 2000) to spatial learning (Bertrand *et al*, 2000; Carli *et al*, 1992) (see Meneses, 1999). Therefore, it is likely that another site for the 5-HT_{1A}–muscarinic receptor interactions might involve populations of cortico-limbic pyramidal cells. In support of

this hypothesis, subcutaneous WAY 100635 was found to prevent the impairment of spatial learning caused by intrahippocampal scopolamine or 7-chloro-kynurenic acid (an NMDA receptor antagonist), suggesting that blockade of 5-HT_{1A} receptors can compensate for the loss of cholinergic or NMDA-receptor-mediated excitatory input to pyramidal cells in the hippocampus (Carli *et al*, 1997). This assumption receives support from other learning tasks in rodents. Thus, the selective 5-HT_{1A} antagonist WAY 100635 when injected both s.c. and intrahippocampally reversed the impairment of the two-platform discrimination task caused by intrahippocampal scopolamine (Carli *et al*, 1997). However, detailed hypotheses on the potential interactive sites of muscarinic and 5-HT_{1A} receptors and their therapeutical relevance must await a more detailed experimental analysis.

It is generally believed that AChE inhibitors such as physostigmine act by increasing synaptic ACh (Kawashima *et al*, 1994; Scali *et al*, 1997), resulting in competition with scopolamine at the postsynaptic muscarinic receptors. In agreement with this view, tacrine and donepezil attenuated or completely blocked the inhibitory effects of scopolamine depending on the dose of the muscarinic receptor antagonist. It is clear, however, that noncompetitive AChE inhibitors including physostigmine and tacrine in general can only attenuate but not fully block the cognitive impairments caused by scopolamine in different learning and memory tasks (Chopin and Briley, 1992; Kojima *et al*, 1997; Rush and Streit, 1992; Yoshida and Suzuki, 1993). The more potent effect of tacrine compared to donepezil seen in this study might be because of differences in the mechanism of action of these two AChE inhibitors. Donepezil is a piperidine-based mixed (competitive/noncompetitive)-type AChE inhibitor that has higher selectivity for AChE *vs* butyrylcholinesterase in the CNS than tacrine (Rogers, 1998). In addition, tacrine acts directly on both muscarinic and nicotinic receptors and potassium channels (Flynn and Mash, 1989; Halliwell and Grove, 1989; Hirai *et al*, 1997; Nilsson *et al*, 1987).

The relatively low potency of tacrine and particularly donepezil may seem paradoxical because the enzyme inhibitors will essentially increase cholinergic transmission at all muscarinic synapses. However, such a mechanism might be counterproductive since AChE inhibitors can also affect 'noncholinergic' neurons via the mode of extrasynaptic transmission (Mrzljak *et al*, 1998, Umbriaco *et al*, 1994, 1995). In addition, at higher doses AChE inhibitors might 'overstimulate' ACh receptor systems in the brain. Thus, adequate performance of PA retention seems to depend on an optimal level of cholinergic receptor stimulation (Deutsch, 1971). This means that a delicate balance between pre- and postsynaptic ACh receptor mechanisms is probably required to compete with the functional muscarinic receptor blockade caused by the high-affinity receptor antagonist scopolamine.

In view of its nonselectivity for muscarinic receptors, the effects of systemic scopolamine administration on PA are probably resulting from impairments of a range of information processes such as attention, learning and memory. In addition, results obtained with the local administration of scopolamine suggest that its effects on aversive learning are mediated by limbic forebrain circuits involving entorhinal and parietal cortices, hippocampus, amygdala and septum (Izquierdo *et al*, 1992, 1998; Nomura *et al*, 1994; Riekkinen *et al*, 1995). In this context, the ability of the 5-HT_{1A} receptor antagonists to attenuate the impairment of PA caused by systemic scopolamine administration in a manner similar to tacrine is both unexpected and intriguing.

Taken together, these data support the hypothesis that cholinergic transmission in cortico-limbic brain regions can be enhanced by 5-HT_{1A} receptor blockade. The 5-HT_{1A} receptor antagonists might, therefore, be of value for the treatment of human psychopathologies associated with a reduction in ACh transmission such as AD. The lack of peripheral cholinergic side effects (salivation, tremor, etc) by this type of compounds may be of particular importance in human therapy.

ACKNOWLEDGEMENTS

We thank Dr Olof Thorberg (R&D Local Discovery, AstraZeneca, Södertälje, Sweden) for his constructive comments on the manuscript. This work was supported in part by a grant from the Swedish Medical Research Council (MFR; project No K98-14X-11588-03A) and The Research Funds from Karolinska Institutet (SOÖ).

REFERENCES

Antoniou K, Kehr J, Snitt K, Ögren SO (1997). Differential effects of the neuropeptide galanin on striatal acetylcholine release in anaesthetized and awake rats. *Br J Pharmacol* **121**: 1180–1186.

Araujo DM, Lapchak PA, Robitaille Y, Gauthier S, Quirion R (1988). Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. *J Neurochem* **50**: 1914–1923.

Aronica E, Dickson DW, Kress Y, Morrison JH, Zukin RS (1998). Non-plaque dystrophic dendrites in Alzheimer hippocampus: a new pathological structure revealed by glutamate receptor immunocytochemistry. *Neuroscience* **82**: 979–991.

Azmitia EC, Gannon PJ, Kheck NM, Whitaker-Azmitia PM (1996). Cellular localization of the 5-HT_{1A} receptor in primate brain neurons and glial cells. *Neuropsychopharmacology* **14**: 35–46.

Bammer G (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: a review and some new results. *Neurosci Biobehav Rev* **6**: 247–296.

Beck SG, Choi KC, List TJ (1992). Comparison of 5-hydroxytryptamine1A-mediated hyperpolarization in CA1 and CA3 hippocampal pyramidal cells. *J Pharmacol Exp Ther* **263**: 350–359.

Bertrand F, Lehmann O, Lazarus C, Jeltsch H, Cassel JC (2000). Intraseptal infusions of 8-OH-DPAT in the rat impairs water-maze performances: effects on memory or anxiety? *Neurosci Lett* **279**: 45–48.

Bymaster FP, Heath I, Hendrix JC, Shannon HE (1993). Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. *J Pharmacol Exp Ther* **267**: 16–24.

Carli M, Bonalumi P, Samanin R (1997). WAY 100635, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal administration of scopolamine or 7-chloro-kynurenic acid. *Brain Res* **774**: 167–174.

Carli M, Tatarczynska E, Cervo L, Samanin R (1993). Stimulation of hippocampal 5-HT_{1A} receptors causes amnesia and anxioly-

tic-like but not antidepressant-like effects in the rat. *Eur J Pharmacol* **234**: 215–221.

Carli M, Tranchina S, Samanin R (1992). 8-Hydroxy-2-(di-*n*-propyloamino)tetralin, a 5-HT_{1A} receptor agonist, impairs performance in a passive avoidance task. *Eur J Pharmacol* **211**: 227–234.

Chopin P, Briley M (1992). Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. *Psychopharmacology* **106**: 26–30.

Christensen H, Maltby N, Jorm AF, Creasey H, Broe GA (1992). Cholinergic 'blockade' as a model of the cognitive deficits in Alzheimer's disease. *Brain* **115**: 1681–1699.

Cole BJ, Jones GH (1995). Double dissociation between the effects of muscarinic antagonists and benzodiazepine receptor agonists on the acquisition and retention of passive avoidance. *Psychopharmacology* **118**: 37–41.

Cole AE, Nicoll RA (1984). The pharmacology of cholinergic excitatory responses in hippocampal pyramidal cells. *Brain Res* **305**: 283–290.

Davies MF, Deisz RA, Prince DA, Peroutka SJ (1987). Two distinct effects of 5-hydroxytryptamine on single cortical neurons. *Brain Res* **423**: 347–352.

DeKosky ST, Scheff SW, Styren SD (1996). Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration* **5**: 417–421.

Deutsch JA (1971). The cholinergic synapse and the site of memory. *Science* **174**: 788–794.

Ebert U, Kirch W (1998). Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur J Clin Invest* **28**: 944–949.

Flynn DD, Mash DC (1989). Multiple *in vitro* interactions with and differential *in vivo* regulation of muscarinic receptor subtypes by tetrahydroaminoacridine. *J Pharmacol Exp Ther* **250**: 573–581.

Fort P, Khateb A, Serafin M, Muhlethaler M, Jones BE (1998). Pharmacological characterization and differentiation of non-cholinergic nucleus basalis neurons *in vitro*. *Neuroreport* **9**: 61–65.

Francis PT, Palmer AM, Snape M, Wilcock GK (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurol Neurosurg Psychiatry* **66**: 137–147.

Grunschlag CR, Haas HL, Stevens DR (1997). 5-HT inhibits lateral entorhinal cortical neurons of the rat *in vitro* by activation of potassium channel-coupled 5-HT_{1A} receptors. *Brain Res* **770**: 10–17.

Hajos M, Hajos-Korcsok E, Sharp T (1999). Role of the medial prefrontal cortex in 5-HT_{1A} receptor-induced inhibition of 5-HT neuronal activity in the rat. *Br J Pharmacol* **126**: 1741–1750.

Halliwell JV, Grove EA (1989). 9-Amino-1,2,3,4-tetrahydroacridine (THA) blocks agonist-induced potassium conductance in rat hippocampal neurones. *Eur J Pharmacol* **163**: 369–372.

Hirai K, Kato K, Nakayama T, Hayako H, Ishihara Y, Goto G *et al* (1997). Neurochemical effects of 3-[1-(phenylmethyl)-4-piperidinyl]-1-(2,3,4,5,-tetrahydro-1*H*-1-benzazepin-8-yl)-1-propanone fumarate (TAK-147), a novel acetylcholinesterase inhibitor, in rats. *J Pharmacol Exp Ther* **280**: 1261–1269.

Izquierdo I, da Cunha C, Rosat R, Jerusalinsky D, Ferreira MB, Medina JH (1992). Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behav Neural Biol* **58**: 16–26.

Izquierdo I, Izquierdo LA, Barros DM, Mello e Souza T, de Souza MM, Quevedo J *et al* (1998). Differential involvement of cortical receptor mechanisms in working, short-term and long-term memory. *Behav Pharmacol* **9**: 421–427.

Johansson L, Sohn D, Thorberg SO, Jackson DM, Kelder D, Larsson LG *et al* (1997). The pharmacological characterization of a novel selective 5-hydroxytryptamine1A receptor antagonist, NAD-299. *J Pharmacol Exp Ther* **283**: 216–225.

Kawashima K, Sato A, Yoshizawa M, Fujii T, Fujimoto K, Suzuki T (1994). Effects of the centrally acting cholinesterase inhibitors tetrahydroaminoacridine and E2020 on the basal concentration of extracellular acetylcholine in the hippocampus of freely moving rats. *Naunyn Schmiedebergs Arch Pharmacol* **350**: 523–528.

Khateb A, Fort P, Alonso A, Jones BE, Muhlethaler M (1993). Pharmacological and immunohistochemical evidence for serotonergic modulation of cholinergic nucleus basalis neurons. *Eur J Neurosci* **5**: 541–547.

Kia HK, Brisorgueil MJ, Daval G, Langlois X, Hamon M, Verge D (1996). Serotonin1A receptors are expressed by a subpopulation of cholinergic neurons in the rat medial septum and diagonal band of Broca—a double immunocytochemical study. *Neuroscience* **74**: 143–154.

Kirk RE (1968). *Experimental Design: Procedures for the Behavioural Sciences*. Brooks/Cole: Belmont, CA.

Kobayashi M, Ohno M, Shibata S, Yamamoto T, Watanabe S (1997). Concurrent blockade of beta-adrenergic and muscarinic receptors suppresses synergistically long-term potentiation of population spikes in the rat hippocampal CA1 region. *Brain Res* **777**: 242–246.

Kojima J, Nakajima K, Ochiai M, Nakayama K (1997). Effects of NIK-247 on cholinesterase and scopolamine-induced amnesia. *Methods Find Exp Clin Pharmacol* **19**: 245–251.

Kuhl DE, Koeppe RA, Minoshima S, Snyder SE, Ficaro EP, Foster NL *et al* (1999). *In vivo* mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. *Neurology* **52**: 691–699.

Lehmann J, Nagy JI, Atmadia S, Fibiger HC (1980). The nucleus basalis magnocellularis: the origin of a cholinergic projection to the neocortex of the rat. *Neuroscience* **5**: 1161–1174.

Lewis PR, Shute CC (1967). The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. *Brain* **90**: 521–540.

Meneses A (1999). 5-HT system and cognition. *Neurosci Biobehav Rev* **23**: 1111–1125.

Meyers B (1965). Some effects of scopolamine on a passive avoidance response in rats. *Psychopharmacologia* **8**: 111–119.

Misane I, Johansson C, Ögren SO (1998a). Analysis of the 5-HT_{1A} receptor involvement in passive avoidance in the rat. *Br J Pharmacol* **125**: 499–509.

Misane I, Ögren SO (2000). Multiple 5-HT receptors in passive avoidance: comparative studies of *p*-chloroamphetamine and 8-OH-DPAT. *Neuropsychopharmacology* **22**: 168–190.

Misane I, Razani H, Wang FH, Jansson A, Fuxe K, Ögren SO (1998b). *In vivo* modulation of 5-HT_{1A} receptor-mediated response by the neuropeptide galanin in the rat. *Eur J Neurosci* **10**: 1230–1240.

Mrzljak L, Levey AI, Belcher S, Goldman-Rakic PS (1998). Localization of the m2 muscarinic acetylcholine receptor protein and mRNA in cortical neurons of the normal and cholinergically deafferented rhesus monkey. *J Comp Neurol* **390**: 122–132.

Newberry NR, Footitt DR, Papanastassiou V, Reynolds DJ (1999). Action of 5-HT on human neocortical neurones *in vitro*. *Brain Res* **833**: 93–1000.

Nilsson L, Adem A, Hardy J, Winblad B, Nordberg A (1987). Do tetrahydroaminoacridine (THA) and physostigmine restore acetylcholine release in Alzheimer brains via nicotinic receptors? *J Neural Transm* **70**: 357–368.

Nomura Y, Nishiyama N, Saito H, Matsuki N (1994). Role of cholinergic neurotransmission in the amygdala on performances of passive avoidance learning in mice. *Biol Pharm Bull* **17**: 490–494.

Ögren SO (1985). Evidence for a role of brain serotonergic neurotransmission in avoidance learning. *Acta Physiol Scand Suppl* **544**: 1–71.

Ögren SO, Kehr J, Schött PA (1996). Effects of ventral hippocampal galanin on spatial learning and on *in vivo* acetylcholine release in the rat. *Neuroscience* **75**: 1127–1140.

Patel S, Tariot PN (1991). Pharmacologic models of Alzheimer's disease. *Psychiatr Clin North Am* **14**: 287–308.

Pugliese AM, Passani MB, Corradetti R (1998). Effect of the selective 5-HT_{1A} receptor antagonist WAY 100635 on the inhibition of e.p.s.ps produced by 5-HT in the CA1 region of rat hippocampal slices. *Br J Pharmacol* **124**: 93–100.

Riekkinen PJ (1994). 5-HT_{1A} and muscarinic acetylcholine receptors jointly regulate passive avoidance behavior. *Eur J Pharmacol* **262**: 77–90.

Riekkinen Jr P, Kuitunen J, Riekkinen M (1995). Effects of scopolamine infusions into the anterior and posterior cingulate on passive avoidance and water maze navigation. *Brain Res* **685**: 46–54.

Rogers SL (1998). Perspectives in the management of Alzheimer's disease: clinical profile of donepezil. *Dement Geriatr Cogn Disord* **9**: 29–42.

Rupniak NM, Steventon MJ, Field MJ, Jennings CA, Iversen SD (1989). Comparison of the effects of four cholinomimetic agents on cognition in primates following disruption by scopolamine or by lists of objects. *Psychopharmacology* **99**: 189–195.

Rush DK, Streit K (1992). Memory modulation with peripherally acting cholinergic drugs. *Psychopharmacology* **106**: 375–382.

Rye DB, Wainer BH, Mesulam MM, Mufson EJ, Saper CB (1984). Cortical projections arising from the basal forebrain: a study of cholinergic and noncholinergic components employing combined retrograde tracing and immunohistochemical localization of choline acetyltransferase. *Neuroscience* **13**: 627–643.

Sarter M, Hagan J, Dudchenko P (1992a). Behavioral screening for cognition enhancers: from indiscriminate to valid testing: Part I. *Psychopharmacology* **107**: 144–159.

Sarter M, Hagan J, Dudchenko P (1992b). Behavioral screening for cognition enhancers: from indiscriminate to valid testing: Part II. *Psychopharmacology* **107**: 461–473.

Scali C, Giovannini MG, Prosperi C, Bartolini L, Pepeu G (1997). Tacrine administration enhances extracellular acetylcholine *in vivo* and restores the cognitive impairment in aged rats. *Pharmacol Res* **36**: 463–469.

Schmitz D, Gloveli T, Empson RM, Heinemann U (1998). Serotonin reduces polysynaptic inhibition via 5-HT_{1A} receptors in the superdicial entorhinal cortex. *J Neurophysiol* **80**: 1116–1121.

Segal M (1982). Multiple actions of acetylcholine at a muscarinic receptor studied in the rat hippocampal slice. *Brain Res* **246**: 77–87.

Shinotoh H, Namba H, Fukushi K, Nagatsuka S, Tanaka N, Aotsuka A *et al* (2000). Progressive loss of cortical acetylcholinesterase activity in association with cognitive decline in Alzheimer's disease: a positron emission tomography study. *Ann Neurol* **48**: 194–200.

Shiozaki K, Iseki E, Uchiyama H, Watanabe Y, Haga T, Kameyama K *et al* (1999). Alterations of muscarinic acetylcholine receptor subtypes in diffuse Lewy body disease: relation to Alzheimer's disease. *J Neurol Neurosurg Psychiatry* **67**: 209–213.

Sprouse JS, Aghajanian GK (1988). Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology* **27**: 707–715.

Stenfors C, Werner T, Ross SB (1998). *In vivo* labelling of the mouse brain 5-hydroxytryptamine receptor with the novel selective antagonist [^{3}H]NAD-299. *Arch Pharmacol* **357**: 500–507.

Stewart M, Luo Y, Fox SE (1992). Effects of atropine on hippocampal theta cells and complex-spike cells. *Brain Res* **591**: 122–128.

Stewart DJ, MacFabe DF, Vanderwolf CH (1984). Cholinergic activation of the electrocorticogram: role of the substantia innominata and effects of atropine and quinuclidinyl benzilate. *Brain Res* **322**: 219–232.

Stiedl O, Misane I, Spiess J, Ögren SO (2000). Involvement of the 5-HT_{1A} receptors in classical fear conditioning in C57BL/6J mice. *J Neurosci* **20**: 8515–8527.

Stillman MJ, Shukitt-Hale B, Galli RL, Levy A, Lieberman HR (1996). Effects of M2 antagonists on *in vivo* hippocampal acetylcholine levels. *Brain Res Bull* **41**: 221–226.

Sunderland T, Tariot PN, Weingartner H, Murphy DL, Newhouse PA, Mueller EA, Cohen RM (1986). Pharmacologic modelling of Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* **10**: 599–610.

Tada K, Kasamo K, Ueda N, Suzuki T, Kojima T, Ishikawa K (1999). Anxiolytic 5-hydroxytryptamine1A agonists suppress firing activity of dorsal hippocampus CA1 pyramidal neurons through a postsynaptic mechanism: single-unit study in unanesthetized, unrestrained rats. *J Pharmacol Exp Ther* **288**: 843–848.

Toide K, Arima T (1989). Effects of cholinergic drugs on extracellular levels of acetylcholine and choline in rat cortex, hippocampus and striatum studied by brain dialysis. *Eur J Pharmacol* **173**: 133–141.

Umbriaco D, Garcia S, Beaulieu C, Descarries L (1995). Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum rediatum of adult rat hippocampus (CA1). *Hippocampus* **5**: 605–620.

Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK (1994). Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J Comp Neurol* **348**: 351–373.

van der Zee EA, Luiten PG (1999). Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. *Prog Neurobiol* **58**: 409–471.

van der Zee EA, Matsuyama T, Strosberg AD, Traber J, Luiten PG (1989). Demonstration of muscarinic acetylcholine receptor-like immunoreactivity in the rat forebrain and upper brainstem. *Histochemistry* **92**: 475–485.

Wang T, Tang XC (1998). Reversal of scopolamine-induced deficits in radial maze performance by (−)-huperzine A: comparison with E2020 and tacrine. *Eur J Pharmacol* **349**: 137–142.

Wesnes KA, Simpson PM, White L, Pinker S, Jertz G, Murphy M *et al* (1991). Cholinesterase inhibition in the scopolamine model of dementia. *Ann NY Acad Sci* **640**: 268–271.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**: 1237–1239.

Wilson WJ, Cook JA (1994). Cholinergic manipulations and passive avoidance in the rat: effects on acquisition and recall. *Acta Neurobiol Exp* **54**: 377–391.

Yoshida S, Suzuki N (1993). Antiamnesic and cholinomimetic side-effects of the cholinesterase inhibitors, physostigmine, tacrine and NIK-247 in rats. *Eur J Pharmacol* **250**: 117–124.