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(S)-WAY 100135, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal scopolamine

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Abstract

Scopolamine, 3.75 $\mu g/\mu l$ infused bilaterally into the CA1 region of the dorsal hippocampus 10 min before each training session, impaired choice accuracy but had no effect on choice latency or errors of omission in rats trained in a two-platform spatial discrimination task. Administered subcutaneously at 3 and 10 mg/kg 30 min before each training session, *N*-tert-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride ((*S*)-WAY 100135), a 5-HT_{1A} receptor antagonist, prevented the impairment of choice accuracy induced by intrahippocampal scopolamine. No subcutaneous dose of (*S*)-WAY 100135 by itself modified the acquisition of spatial learning. Administered into the dorsal hippocampus 15 min before each training session, (*S*)-WAY 100135 at doses of 0.2, 1 and 5 $\mu g/\mu l$ did not affect the acquisition of spatial learning but dose dependently prevented the impairment of choice accuracy caused by scopolamine, 3.75 $\mu g/\mu l$ infused into the same area. These findings suggest that blockade of 5-HT_{1A} receptors can compensate the loss of cholinergic excitatory input on pyramidal cells, probably by favouring the action of other excitatory transmitters.

Keywords: Spatial learning; Hippocampus; Acetylcholine; Scopolamine; 5-HT_{1A} receptor; (*S*)-WAY 100135; Alzheimer disease; (Rat)

1. Introduction

Recent studies showed that subcutaneously administered 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a specific agonist at 5-HT receptors of the 5-HT_{1A} type, impaired rats' acquisition of a two-platform spatial discrimination task but did not modify their ability to learn visual discrimination (Carli and Samanin, 1992; Carli et al., 1995). Since the effect on spatial learning was actually potentiated in rats injected intracerebroventricularly with 5,7-dihydroxytryptamine to destroy 5-HT-containing neurons, it was suggested that postsynaptic 5-HT_{1A} receptors, possibly in the hippocampus, were involved (Carli and Samanin, 1992). This was supported by the finding that infusion of spiroxatrine and (*S*)-WAY 100135 (*N*-tert-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride), two potent 5-HT_{1A} receptor antagonists, in the CA1 region of the dorsal hippocampus antagonized the impairment of spatial learning caused by systemically administered 8-OH-DPAT (Carli et al., 1995). Moreover, 8-OH-DPAT administered directly into the hippocampus caused the same type of cognitive impairment and the effect was blocked by intrahippocampal administration of spiroxatrine (Carli et al., 1992). In these studies spiroxatrine and (*S*)-WAY 100135 by themselves did not change the rate of acquisition of spatial learning. The lack of effect may indicate that endogenous 5-HT does not exert a tonic influence on 5-HT_{1A} receptors in the hippocampus under normal conditions.

To get more information on the role of hippocampal 5-HT_{1A} receptors in spatial learning, we studied the effect of a selective 5-HT_{1A} receptor antagonist, (*S*)-WAY 100135. (Fletcher et al., 1993; Routledge et al., 1993), administered subcutaneously or into the hippocampus, on the impairment of spatial discrimination caused by an intrahippocampal dose of 3.75 $\mu g/\mu l$

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scopolamine. The latter dose was chosen on the basis of a previous study in which scopolamine (1.87–15 $\mu g/\mu l$) administered into the CA1 region of the dorsal hippocampus impaired learning in the two-platform spatial discrimination task in a dose-related manner (Carli et al., in preparation).

2. Materials and methods

2.1. Animals

Male albino rats (CD-COBS, Charles River, Italy) weighing 200–250 g were housed in pairs in a room at constant temperature (21 ± 1°C) and relative humidity (60%), with a regular light/dark schedule (07.00–19.00 h). Food (Altromin pellets for rats, Rieper, Italy) and water were available ad libitum.

2.2. Cannula implantation

The rats, anesthetized with Equithesin (9.7 mg/ml sodium pentobarbital in saline + 42.6 mg/ml chloral hydrate in propylenglycol + 21.2 mg/ml Mg_2SO_4 in ethanol; 3.0 ml/kg i.p.), were immobilized in a Kopf stereotaxic instrument. The skin was cut and the skull was cleaned for bilateral implantation of guide cannulae made of 23-gauge stainless steel tubing, 2 mm above the sites to be injected. The guide tubes were secured by acrylic dental cement anchored to three stainless steel screws fixed to the skull. To prevent clogging, stainless steel stylets, 30 gauge, were placed in the guide cannulae until the animals were given intracerebral injections 8 days later. The rats were accustomed to handling and on the days of acquisition training the stylets were withdrawn and replaced by bilateral injection units (30-gauge stainless steel tubing) terminating 2 mm below the tip of the guides.

The coordinates, calculated from the interaural line according to Paxinos and Watson (1982), were: A = +5.2 mm L = ±2.0 and H = +7.3 so as to have access to the CA1 region of the dorsal hippocampus.

On completion of the experiment the location of the infusion was verified visually. The rats were killed and their brains were removed and quickly frozen on dry ice. To check the position of the cannulae tracks, the frozen brains were cut in the coronal plane in a Cryocut. For each experiment we only included in the results data from rats in which the bilateral tracks of the injection tips were in the CA1 pyramidal layer and in the stratum radiatum. Their correct lateral position was within 2.0 ± 0.5 mm of the bregma. The correct fronto-caudal position of the injection sites was between the coronal section −3.30 and −3.80 mm from bregma. The vertical location of the target was between −2.4 and −2.8 mm relative to the skull surface, according to the atlas of Paxinos and Watson (1982). Only 10% of cannulated rats were discarded, mostly because of infection at the injection site.

2.3. Apparatus

A circular 'swimming pool' was used, 1.5 m in diameter, 0.5 m high. The pool was filled to a depth of 0.29 m with water (26 ± 1°C) which was rendered opaque by the addition of a food dye (coffee color, Bayo, Italy). The water was changed daily. The pool was located centrally in a large room and was surrounded by various visual cues: a blackened window with a big white cross, a white wall with a big black cross, a long table, a door and a picture-covered wall with a rack for cages. The objects could be covered, when required, by black curtains around the maze. When open, the curtains were collected together at one corner of the room, forming another prominent visual cue. The room was lit by a 100-W light bulb in the center of the ceiling at 2.4 m above the water surface. The light intensity at the water surface was 80 lux (measured by a Illuminometer, Mod 5200, Kyoritsu, Japan).

Two visible grey platforms were used. The fixed one protruded 1.5–2.0 cm above the water surface. Its top was square (11 × 11 cm) and made of Perspex. The second platform also protruded 1.5–2.0 cm above the water and was made of the same material but was filled with expanded polystyrene. It was 'anchored' by thread to a solid movable base on the bottom of the pool. Thus one platform was rigid and provided support, the other sank when the rats tried to climb on it.

2.4. Training procedure

The black curtains were drawn back together to allow a full view of extra-maze cues. The rats were trained to swim to the rigid grey escape platform while avoiding the floating grey platform. For all rats, the fixed escape platform (correct) was always in the same place at the centre of one of the eight equal sectors. The floating platform (incorrect) was positioned over successive trials in a quasi-random sequence of eight locations around the pool, subject to the constraint that the spatial relationship between the platform and the starting position did not consistently reward either right- or left-turning tendencies.

The rats were trained with ten trials a day for 5 days. A trial began with the rat being placed in the pool while held at, and facing, the side wall. Eight possible starting locations were used in quasi-random sequence across trials. A trial ended when the rat escaped onto the rigid platform, where it was allowed to sit for 15 s before being returned to a holding cage before the next trial. The rats were trained in squads of four. Inter-trial intervals were approximately 2–4 min.

Each rat's daily testing lasted approximately 30 min. A correct trial was that in which the rats climbed onto the rigid platform without touching the floating platform with their forepaws or snout. The occasional incident of brushing the floating platform in passing was not considered an error. If the rat did not choose to escape onto either platform (correct or incorrect) in 60 s, it was taken out of the pool and an omission error was scored. We measured (1) the first choice in each trial (correct/incorrect), (2) the latency to escape (s), and (3) the number of omissions.

2.5. Treatment schedules

2.5.1. Subcutaneous (S)-WAY 100135

On each acquisition training day, 20 min before intrahippocampal injection of scopolamine or saline, the rats were injected subcutaneously with (*S*)-WAY 100135 (Wyeth Research, UK) dissolved in saline (3 or 10 mg/kg) or with saline (2 ml/kg). Scopolamine hydrobromide (Sigma, USA), 3.75 μg/μl dissolved in saline, was infused bilaterally into the hippocampus. Control rats received 1 μl of saline in the hippocampus. Saline or scopolamine solution was delivered at a rate of 0.5 μl/min by a Hamilton syringe, mounted in a CMA/100 infusion pump (CMA Microdialysis, Stockholm, Sweden), connected by PP10 tubing to a 30-gauge stainless steel cannula. The solution was administered over a 2-min period. The injection cannulae were left in place for another minute before withdrawal to allow diffusion from the tip and to prevent reflux of the solution. Ten minutes after intrahippocampal injection of scopolamine or saline, the rats started their daily acquisition training. A total of 50 rats (7–9 per group) were run in this experiment.

2.5.2. Intrahippocampal (S)-WAY 100135

(*S*)-WAY 100135 was dissolved in saline and infused bilaterally into the dorsal hippocampus on each acquisition training day in a volume of 1 μl each side 5 min before scopolamine. Saline or various doses of (S)-WAY 100135 (0.2, 1.0 and 5.0 μg/μl) were delivered at a rate of 0.5 μl/min by a Hamilton syringe, mounted in a CMA/100 infusion pump (CMA Microdialysis, Stockholm, Sweden), connected by PP10 tubing to a 30-gauge stainless steel cannula. The solution was administered over a 2-min period. The injection cannulae were left in place for another min before withdrawal to allow diffusion from the tip and prevent reflux of the solution. The infusion protocol for 3.75 μg/μl of scopolamine administered in the hippocampus was the same as described above. Control rats received two 1-μl infusions of saline in the hippocampus. Ten minutes after intrahippocampal injection of scopolamine or saline, the rats started their daily acquisition training. A total of 79 rats (8–16 per group) were run in this experiment.

2.6. Statistical analysis and measures

Choice accuracy of spatial discrimination was measured as the proportion of correct choices (total correct choices/total correct choices + total incorrect choices). The data were expressed as percentages and, after arc-sine transformation, were analysed by analysis of variance (ANOVA).

Choice latency was defined as the time in seconds taken by the rat to swim from the starting location to either the correct or incorrect platform. For each training day the mean latency to escape was calculated for each rat (total latency/total number of trials) and, after logarithmic transformation, analysed by ANOVA. Angular and log transformations were done to normalize the distributions in accordance with the ANOVA model (Winer, 1971).

Errors of omission were measured as the number of failures to choose in 60 s. Trials in which the animals made errors of omission were not counted for the measurement of choice accuracy and latency.

The effects of subcutaneous or intrahippocampal (*S*)-WAY 100135 on the scopolamine-induced deficit in the two-platform spatial discrimination learning task were analysed by a three-factor ANOVA (two between, one within subjects). The first between-subjects factor was (*S*)-WAY 100135 (either three or four levels), the second was two levels of scopolamine. There were five levels of the within-subjects factor time (i.e. repeated measures over the 5 training days). Significant interactions between time, scopolamine and (*S*)-WAY 100135 were further analysed by comparing scopolamine- and (*S*)-WAY 100135-treated and non-treated rats at each level separately by a SPLIT-PLOT ANOVA (treatment × day). Significant two-way interactions between (*S*)-WAY100135 and scopolamine were further analysed by comparing treatment group means of the five training sessions, using Tukey's test. The number of omissions was analysed by a between-subjects two-way ANOVA.

Statistical analysis was done using the SAS Institute (USA) statistical software run on a Micro VAX 3500 computer (Digital, USA).

3. Results

Multiple treatments seemed practicable since signs of tissue loss were very limited and the rats appeared healthy and alert.

3.1. Subcutaneous (S)-WAY 100135

The results on choice accuracy are shown in Fig. 1A. All rats started with a similar performance (about 50% correct choices). The accuracy of controls and rats

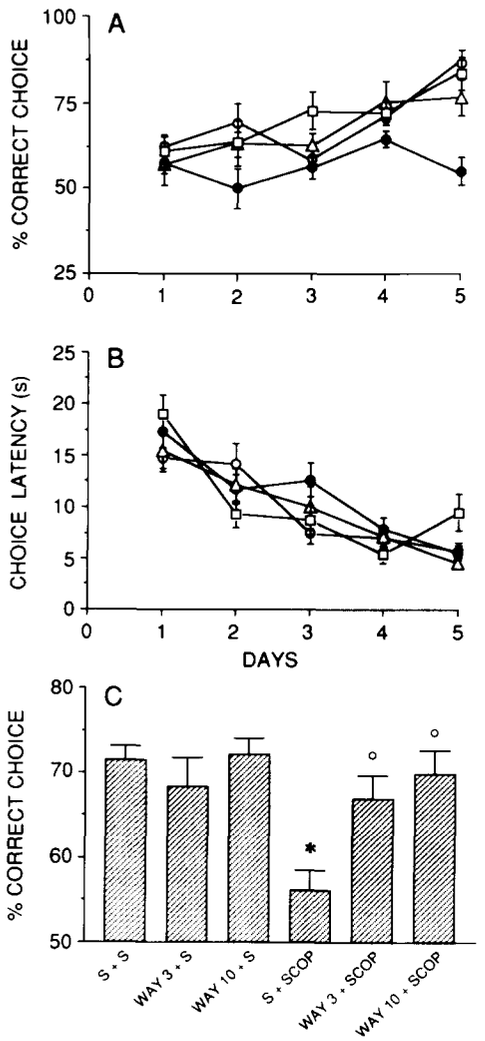

Fig. 1. Effects of subcutaneous doses of (*S*)-WAY 100135 (3 and 10 mg/kg) on the percentage of correct choices (A), mean ± S.E.M. of correct choices in the five training sessions (C) and choice latency (B) of rats given saline or scopolamine (3.75 μg/μl) intrahippocampally. On each acquisition day, (*S*)-WAY 100135 was injected 20 min before scopolamine, which was given 10 min before the training session. For descriptive convenience the acquisition curves of (*S*)-WAY 100135, 3 and 10 mg/kg + saline are not shown (means ± S.E.M. for 7–9 rats per group for each training day for (*S*)-WAY 100135 3 mg/kg + saline were: 60 ± 5.3; 68.5 ± 5.3; 64.2 ± 6.1; 68.5 ± 6.3; 80 ± 4.3; for (*S*)-WAY 100135 10 mg/kg + saline were: 66 ± 7.4; 56.4 ± 5.8; 72.8 ± 3.5; 78.5 ± 2.6; 85.7 ± 3.6). Symbols: saline + saline (○), saline + scopolamine (●), (*S*)-WAY 100135 3 mg/kg + scopolamine (△), (*S*)-WAY 100135 10 mg/kg + scopolamine (□). Abbreviations: S = saline; WAY 3 or WAY 10 = (*S*)-WAY 100135 3 or 10 mg/kg; Scop = scopolamine. $^{*}P < 0.05$ vs. saline + saline; $^{\circ}P < 0.05$ vs. saline + scopolamine.

treated with 3 and 10 mg/kg (*S*)-WAY 100135 plus 1 μl of saline in the dorsal hippocampus improved similarly over days (time: $F(4,176) = 17.55$, $P < 0.001$; time × (*S*)-WAY 100135: $F(8,176) = 1.20$, $P > 0.05$). Rats injected before each acquisition session with 3.75 μg/μl of scopolamine in the same hippocampal area still showed about 50% of correct choices at the end of training. Although the differences from controls were not significant on any particular day of training (time × scopolamine: $F(4,176) = 1.12$, $P > 0.05$; main effect of scopolamine $F(1,44) = 4.07$, $P < 0.05$), rats injected with scopolamine were on the whole significantly impaired in their choice accuracy (comparison of treatment group means for the 5 training days; scopolamine; $F(1,16) = 24.02$, $P < 0.0005$).

The histograms in Fig. 1C present the results as means ± S.E.M. of the five training sessions. Overall ANOVA showed a non-significant three-way interaction time × (*S*)-WAY 100135 × scopolamine ($F(8,176) = 1.83$, $P > 0.05$) but a statistically significant two-way interaction between (*S*)-WAY 100135 and scopolamine ($F(2,44) = 3.62$, $P < 0.05$), suggesting that, independently of the training day, systemically injected (*S*)-WAY 100135 prevented scopolamine's effect on accuracy. The post-hoc Tukey's test indicated that saline + scopolamine-treated rats had a significantly worse discriminative accuracy than those treated with saline + saline (Tukey's test $P < 0.05$), while rats treated with 3 or 10 mg/kg (*S*)-WAY 100135 + scopolamine had a significantly better choice accuracy than those treated with saline + scopolamine (Tukey's test $P < 0.05$). The choice accuracy of rats treated with 3 mg/kg (*S*)-WAY 100135 + scopolamine was not significantly different from that of rats receiving 10 mg/kg (*S*)-WAY 100135 + scopolamine (Tukey's test $P > 0.05$). Neither 3 nor 10 mg/kg (*S*)-WAY 100135 by itself modified swim maze acquisition (Tukey's test $P > 0.05$).

On the first day of the acquisition training all rats had the same choice latency (Fig. 1B). With training all rats improved their performance similarly. The analysis of choice latency data showed that only the effect of training was statisticaly significant (time: $F(4,176) = 67.7$, $P < 0.0001$). Treating the rats with scopolamine, (*S*)-WAY 100135 or both had no effect on choice latency (time × (*S*)-WAY 100135 × scopolamine $F(8,176) = 1.11$; time × (*S*)-WAY 100135: $F(8,176) = 1.55$; time × scopolamine: $F(4,176) = 1.82$; (*S*)-WAY 100135 × scopolamine $F(2,44) = 0.26$; all $P > 0.05$, main effect of (*S*)-WAY 100135: $F(2,44) = 1.21$; scopolamine: $F(1,4) = 0.30$, both $P > 0.05$).

The number of omissions was not significantly affected by scopolamine, (*S*)-WAY 100135, or the two together in any session during training (data not shown).

3.2. Intrahippocampal (S)-WAY 100135

The results on choice accuracy are shown in Fig. 2A. The accuracy of controls improved each day and reached about 85% correct choices on day 5 of training (time: $F(4,284) = 36.54$, $P < 0.001$). Rats treated with scopolamine before each daily session made significantly fewer correct choices at the end of the 5 days of

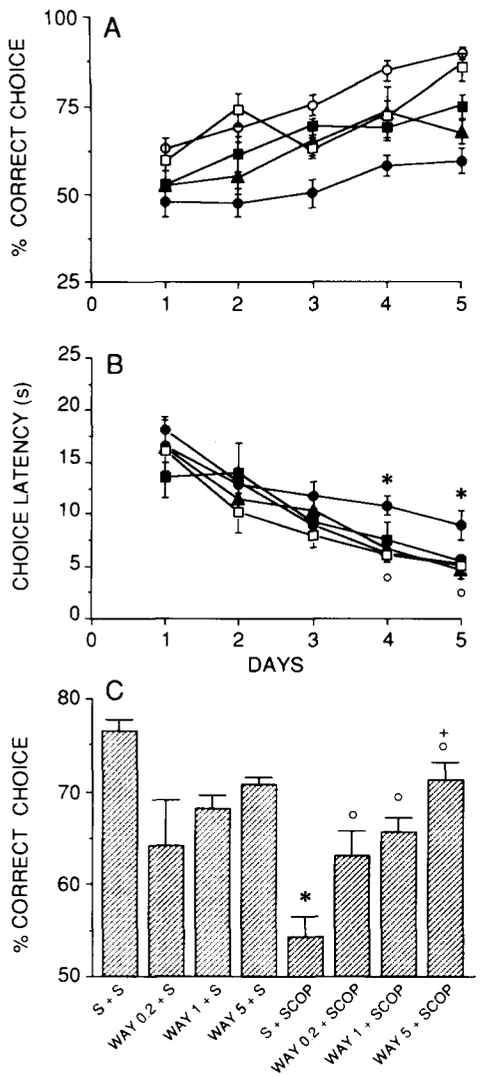

Fig. 2. Effect of intrahippocampal (*S*)-WAY 100135 (0.2, 1 and 5 $\mu g/\mu l$) on the percentage of correct choices (A), means ± S.E.M. of correct choices in the five training sessions (C) and choice latency (B) of rats given saline or scopolamine (3.75 $\mu g/\mu l$) intrahippocampally. On each acquisition day, (*S*)-WAY 100135 was infused 5 min before scopolamine, which was given 10 min before the training session. For descriptive convenience the acquisition curves of (*S*)-WAY 100135 + saline are not shown (mean ± S.E.M. for 8–16 rats per group for each training day were: (*S*)-WAY 100135 0.2 $\mu g/\mu l$ + saline 46.6 ± 6.0; 59.8 ± 9.5; 64.5 ± 5.3; 70 ± 6.3; 80 ± 5.3; (*S*)-WAY 100135 1 $\mu g/\mu l$ + saline 52.2 ± 4.5; 60.5 ± 5.9; 68.5 ± 4.4; 75.6 ± 5.3; 84.2 ± 1.7; (*S*)-WAY 100135 5 $\mu g/\mu l$ + saline 58.1 ± 7.1; 62.5 ± 3.5; 72.7 ± 3.4; 77.5 ± 2.4; 82.5 ± 3.6). Symbols: saline + saline (○), saline + scopolamine (●), (*S*)-WAY 100135 0.2 $\mu g/\mu l$ + scopolamine (▲), (*S*)-WAY 100135 1 $\mu g/\mu l$ + scopolamine (■), (*S*)-WAY 100135 5 $\mu g/\mu l$ + scopolamine (□). Abbreviations: S = saline; WAY 0.2, WAY 1 and WAY 5 = (*S*)-WAY 100135 0.2, 1 and 5 $\mu g/\mu l$ respectively. Scop = scopolamine. $^{*}P < 0.05$ vs. saline + saline; $^{\circ}P < 0.05$ vs. saline + scopolamine; $^{+}P < 0.05$ vs. WAY 0.2 + scopolamine.

training (time × scopolamine: $F(4,284) = 2.62$, $P < 0.05$). Further analysis of choice accuracy by a split-plot ANOVA confirmed that differences between saline- and scopolamine-treated rats in the acquisition of the task were significant (scopolamine × day: $F(4,112) = 3.58$, $P < 0.01$; scopolamine: $F(1,28) = 74.25$, $P < 0.001$). Infusion of various doses of (*S*)-WAY 100135 by itself into the CA1 field of the dorsal hippocampus did not modify swim maze acquisition (time × (*S*)-WAY 100135: $F(12,284) = 0.63$, $P > 0.05$; (*S*)-WAY 1001345: $F(3,71) = 1.78$, $P > 0.05$).

Fig. 2C presents the results as means ± S.E.M. of the five training sessions. Since the overall three-way ANOVA showed a non-significant interaction between time, scopolamine and (*S*)-WAY 100135 ($F(12,284) = 1.26$, $P > 0.05$) but a highly significant two-way interaction between scopolamine and (*S*)-WAY 100135 ($F(3,71) = 11.28, P < 0.0001$), the choice accuracy data were further analysed by comparing treatment group means for the five training sessions, using Tukey's test. This showed that doses of 0.2, 1 and 5 $\mu g/\mu l$ of (*S*)-WAY 100135 (Tukey's test $P < 0.05$) infused into the CA1 field of the dorsal hippocampus prevented the impairment of choice accuracy caused by infusion of 3.75 $\mu g/\mu l$ scopolamine in the same area. The post-hoc Tukey's test indicated also that rats receiving 5 $\mu g/\mu l$ (*S*)-WAY 100135 + scopolamine had a significantly better choice accuracy than those injected with 0.2 $\mu g/\mu l$ (*S*)-WAY 100135 + scopolamine ($P < 0.5$).

All rats started with a similar latency and improved their performance over the trials, as shown by the progressive reduction in latency (time: $F(4,284) = 101.28$, $P < 0.0001$) (Fig. 2B). The overall ANOVA showed significant three-way (time × (*S*)-WAY 100135 × scopolamine: $F(12,284) = 3.27$, $P < 0.001$) and two-way ((*S*)-WAY 100135 × scopolamine: $F(3,71) = 3.29$, $P < 0.5$) interactions. Further statistical analysis separately for each training day showed significant two-way interactions between (*S*)-WAY 100135 and scopolamine starting from day 3 (day 3: $F(3,71) = 3.54$, $P < 0.05$; day 4: $F(3,71) = 6.39$, $P < 0.0005$; day 5: $F(3,71) = 4.14$, $P < 0.01$).

These results suggest that rats treated with scopolamine had a significantly longer choice latency than control rats on days 4 and 5 (Tukey's test, $P < 0.05$) and that (*S*)-WAY 100135 by itself had no effect on choice latency (Tukey's test, $P > 0.05$). The lengthening of the choice latency induced by scopolamine was antagonized on day 4 by 0.2 and 5 $\mu g/\mu l$ (*S*)-WAY 100135 (Tukey's test, $P < 0.05$) but on day 5 only 0.2 $\mu g/\mu l$ had a significant effect (Tukey's test, $P < 0.05$).

Scopolamine and each dose of (*S*)-WAY 100135, or the two combined, had no significant effect on the errors of omission (data not shown).

4. Discussion

Administered subcutaneously at doses of 3 and 10 mg/kg, (*S*)-WAY 100135 by itself did not affect spatial

discrimination but it prevented the deficit induced by intrahippocampal scopolamine. (*S*)-WAY 100135 is a selective 5-HT_{1A} receptor antagonist (Fletcher et al., 1993; Routledge et al., 1993) and the doses used in the present study completely blocked the effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on hippocampal 5-HT release (Routledge et al., 1993) and motor behaviour (Fletcher et al., 1993) in the rat. These findings suggest that these doses do effectively block 5-HT_{1A} receptors in the rat brain.

Previous studies have shown that the impairment of spatial learning caused by systemically administered 8-OH-DPAT is mediated by stimulation of 5-HT_{1A} receptors in the dorsal hippocampus (Carli et al., 1995). The inhibitory role of hippocampal 5-HT_{1A} receptors in spatial learning was confirmed by the finding that administration of 8-OH-DPAT in the CA1 region of the dorsal hippocampus impaired acquisition of the spatial discrimination task, and the effect was blocked by a 5-HT_{1A} receptor antagonist (Carli et al., 1992). That the effect of systemically administered (*S*)-WAY 100135 was due to its ability to block 5-HT_{1A} receptors in the dorsal hippocampus is borne out by the fact that when this drug was administered into the CA1 region of the dorsal hippocampus, it dose dependently (0.2–5 $\mu g/\mu l$) antagonized the deficit caused by intrahippocampal scopolamine. The inability of (*S*)-WAY 100135 by itself to affect spatial learning may indicate that hippocampal 5-HT_{1A} receptors are not under tonic influence by endogenous 5-HT under normal conditions.

It is unlikely that (*S*)-WAY 100135 prevented the effect of scopolamine by increasing acetylcholine release since inhibitory 5-HT receptors on cholinergic terminals in the hippocampus are of the 5-HT_{1B} type (Maura and Raiteri, 1986) and strangely enough, acetylcholine release was enhanced on infusing the hippocampus with 8-OH-DPAT, a 5-HT_{1A} receptor agonist (Izumi et al., 1994).

High densities of muscarinic and 5-HT_{1A} receptors are found in the CA1 region of the dorsal hippocampus (Yamamura and Snyder, 1974; Pazos et al., 1988). Stimulation of muscarinic and 5-HT_{1A} receptors causes excitation and hyperpolarization of pyramidal cells respectively (Benardo and Prince, 1981; Segal, 1982; Andrade and Nicoll, 1987; Colino and Halliwell, 1987). It is likely therefore that the behavioural interaction between (*S*)-WAY 100135 and scopolamine results from their opposite effects on the activity of pyramidal neurons. By blocking the hyperpolarizing action of endogenous 5-HT, (*S*)-WAY 100135 may compensate the loss of cholinergic excitatory input by favouring the action of other excitatory transmitters on pyramidal cells. Recently, blockade of 5-HT_{1A} receptors was found to enhance glutamate release from hippocampal slices of guinea pigs (Matsuyama et al., 1994). 5-HT receptors other than 5-HT_{1A} can also contribute to the facilitatory effect since after blockade of 5-HT_{1A} receptors exogenous 5-HT increases the excitability of CA1 pyramidal neurons (Chaput et al., 1990). The 5-HT_4 type may be one of the receptors mediating the depolarizing response since it was recently reported that 5-HT_{1A} and 5-HT_4 receptors exert inhibitory and excitatory effects respectively on the same pyramidal neurons of the hippocampus (Roychowdhury et al., 1994).

What is the importance of the muscarinic/5-HT_{1A} interaction in the hippocampus for human cognitive disorders?

Although other transmitter systems such as those using noradrenaline and serotonin are deficient in the brain of patients with Alzheimer disease (Perry, 1987), a high correlation was found between the behavioural deficits and a reduction of choline acetyltransferase activity in the hippocampus of these patients (Katzman et al., 1986). The hippocampus appears to be a critical region for controlling learning and memory processes. Behavioural deficits after excitotoxic lesion of cholinergic projections to the cerebral cortex have been interpreted as changes in sensorimotor behaviour and attention rather than changes in cognition per se (Dunnett et al., 1991). A marked depletion of cortical choline acetyltransferase activity in patients with dominantly inherited olivopontocerebellar atrophy was not associated with disabling dementia, as it is in Alzheimer disease (Kish et al., 1989). Finally, recent studies have shown that lesions of specific parts of the hippocampus, particularly the CA1 pyramidal cells, are sufficient to cause learning and memory disturbances (Zola-Morgan et al., 1986). Should direct injection of scopolamine in the hippocampus model some important aspects of dementing disorders, 5-HT_{1A} receptor antagonists may offer a novel tool for the symptomatic treatment of memory disturbances in Alzheimer disease.

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References

Andrade, R. and R.A. Nicoll, 1987, Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus, Naunyn-Schmied. Arch. Pharmacol. 336, 5.

Benardo, L.S. and D.A. Prince, 1981, Acetylcholine induced modulation of hippocampal pyramidal neurons, Brain Res. 211, 227.

Carli, M. and R. Samanin, 1992, 8-Hydroxy-2-(di-*n*-propylamino)tetralin impairs spatial learning in a water maze: role of postsynaptic 5-HT_{1A} receptors, Br. J. Pharmacol. 105, 720.

Carli, M., M. Lazarova, E. Tatarczynska and R. Samanin, 1992, Stimulation of 5-HT_{1A} receptors in the dorsal hippocampus impairs acquisition and performance of a spatial task in a water maze, Brain Res. 595, 50.

Carli, M., R. Luschi, P. Garofalo and R. Samanin, 1995, 8-OH-DPAT impairs spatial but not visual learning in a water maze by stimulating 5-HT_{1A} receptors in the hippocampus, Behav. Brain Res. 67, 67.

Chaput, Y., R.C. Araneda and R. Andrade, 1990, Pharmacological and functional analysis of a novel serotonin receptor in the rat hippocampus, Eur. J. Pharmacol. 182, 441.

Colino, A. and J.V. Halliwell, 1987, Differential modulation of three separate k-conductances in hippocampal CA1 neurons by serotonin, Nature 328, 73.

Dunnett, S.B., B.J. Everitt and T.W. Robbins, 1991, The basal forebrain-cortical cholinergic system: interpreting the functional consequences of excitotoxic lesions, Trends Neurosci. 14, 494.

Fletcher, A., D.J. Bill, S.J. Bill, I.A. Cliffe, G.M. Dover, E.A. Forster, J.T. Haskins, D. Jones, H.L. Mansell and Y. Reilly, 1993, WAY100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors, Eur. J. Pharmacol. 237, 283.

Izumi, J., M. Washizuka, N. Miura, Y. Hiraga and Y. Ikeda, 1994, Hippocampal serotonin 5-HT_{1A} receptor enhances acetylcholine release in conscious rats, J. Neurochem. 62, 1804.

Katzman, R., T. Brown and P. Fuld, 1986, Significance of neurotransmitter abnormalities in Alzheimer's disease, in: Neuropeptides in Neurologic and Psychiatric Disease, 2nd edn., eds. J.B. Martin and J.D. Barchas (Raven Press, New York) p. 279.

Kish, S.J., Y. Robitaille, M. El-Awar, J.H.N. Deck, J. Simmons, L. Schut, L.-J. Chang, L. DiStefano and M. Freedman, 1989, Non-Alzheimer-type pattern of brain cholineacetyltransferase reduction in dominantly inherited olivopontocerebellar atrophy, Ann. Neurol. 26, 362.

Matsuyama, S., K. Nei, H. Shuntoh and C. Tanaka, 1994, Inhibitory modulation of NMDA receptor-mediated glutamate release via 5-HT_{1A} receptor in hippocampal dentate gyrus, Soc. Neurosci. Abstr. 20, 731.

Maura, G. and M. Raiteri, 1986, Cholinergic terminals in rat hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release, Eur. J. Pharmacol. 129, 333.

Paxinos, G. and C. Watson, 1982, The Rat Brain in Stereotaxis Coordinates (Academic Press, Sydney).

Paxos, A., D. Hoyer, M.M. Dietl and J.M. Palacios, 1988, Autoradiography of serotonin receptors, in: Neuronal Serotonin, eds. N.N. Osborne and M. Hamon (Wiley, Chichester) p. 507.

Perry, E.K., 1987, Cortical neurotransmitter chemistry in Alzheimer's disease, in: Psychopharmacology. The Third Generation of Progress, ed. H.Y. Meltzer (Raven Press, New York) p. 887.

Routledge, C., J. Gurling, I.K. Wright and C.T. Dourish, 1993, Neurochemical profile of the selective and silent 5-HT_{1A} receptor antagonist WAY100135: an in vivo microdialysis study, Eur. J. Pharmacol. 239, 195.

Roychowdhury, S., H. Haas and E.G. Anderson, 1994, 5-HT_{1A} and 5-HT_4 receptor colocalization on hippocampal pyramidal cells, Neuropharmacology 33, 551.

Segal, M., 1982, Multiple action of acetylcholine at a muscarinic receptor studied in the rat hippocampal slice, Brain Res. 246, 77.

Winer, B.J., 1971, Statistical Principles in Experimental Design, 2nd edn. (McGraw-Hill, Tokyo).

Yamamura, H.I. and S.H. Snyder, 1974, Postsynaptic localization of muscarinic cholinergic receptor binding in rat hippocampus, Brain Res. 78, 320.

Zola-Morgan, S., L.R. Squire and D.G. Amaral, 1986, Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus, J. Neurosci. 6, 2950.