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S 15535, a benzodioxopiperazine acting as presynaptic agonist and postsynaptic 5-HT_{1A} receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal scopolamine

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1 The effect of S 15535 (4-benzodioxan-5-yl)1-(indan-2-yl)piperazine), an agonist at presynaptic and antagonist at postsynaptic 5-HT_{1A} receptors, on the impairment of spatial learning caused by intrahippocampal scopolamine in a two-platform spatial discrimination task was studied.

2 Scopolamine (4.0 $\mu g\ \mu l^{-1}$), injected bilaterally into the CA1 region of the dorsal hippocampus 10 min before each training session, impaired choice accuracy with no effect on choice latency and errors of omission.

3 Administered subcutaneously 30 min before each training session, S 15535 1.0 (but not 0.3) mg kg^{-1} did not modify choice accuracy but prevented its impairment by intrahippocampal scopolamine.

4 WAY 100635, a 5-HT_{1A} receptor antagonist, injected into the dorsal raphe at 1.0 μg 0.5 μl^{-1} 5 min before scopolamine, had no effect on choice accuracy and latency or errors of omission and did not modify the effect of scopolamine but completely antagonized the effect of S 15535 (1.0 mg kg^{-1}) on scopolamine-induced impairment of choice accuracy.

5 The results confirm a previous report (Carli *et al*., 1998) that stimulation of presynaptic 5-HT_{1A} receptors in the dorsal raphe counteracts the deficit caused by intrahippocampal scopolamine, probably by facilitating the transfer of facilitatory information from the entorhinal cortex to the hippocampus.

6 Drugs that stimulate action on presynaptic 5-HT_{1A} receptors, such as S 15535 and other partial 5-HT_{1A} receptors agonists, may be useful in the symptomatic treatment of human memory disturbances associated with loss of cholinergic innervation to the hippocampus.

Keywords: Spatial learning; hippocampus; dorsal raphe; presynaptic 5-HT_{1A} receptors; S 15535 {4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine)}; rat; memory disturbances

Abbreviations: ANOVA, analysis of variance; DR, dorsal raphe; 5-HT_{1A}, serotonin$_{1A}$ receptors; S 15535, {4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine)}; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazynil]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide

Introduction

The density of serotonin 1A (5-HT_{1A}) and muscarinic receptors is high in the CA1 region of the dorsal hippocampus (Pazos *et al*., 1988; Yamamura & Snyder, 1974), an area traditionally linked with cognitive function, particularly spatial memory, at least in rodents (Morris *et al*., 1982; O'Keefe & Nadel, 1978). Electrophysiological studies have shown that stimulation of 5-HT_{1A} and muscarinic receptors causes respectively hyperpolarization and excitation of hippocampal pyramidal cells (Andrade & Nicoll, 1987; Bernardo & Prince, 1981; Colino & Halliwell, 1987; Segal, 1982). These findings suggest that 5-HT_{1A} and muscarinic cholinergic receptors in the dorsal hippocampus have opposite functions in regulating spatial learning in rats.

A series of studies in our laboratory have in fact shown that stimulation of 5-HT_{1A} receptors and blockade of muscarinic receptors in the CA1 region of the dorsal hippocampus impair spatial learning in a two-platform spatial discrimination task, with no effect on motor/motivational performance or non-spatial visual learning (Carli & Samanin, 1992; Carli *et al*., 1992; 1995a; 1997b). The impairment of spatial learning by intrahippocampal scopolamine was prevented by blockade of hippocampal 5-HT_{1A} receptors (Carli *et al*., 1995b; 1997a), so the loss of cholinergic excitatory input on pyramidal cells may be compensated by blockade of the inhibitory 5-HT_{1A} receptors.

A similar conclusion was reached by Harder *et al*. (1996) in a study in which blockade of 5-HT_{1A} receptors by WAY 100635, a potent 5-HT_{1A} receptor antagonist (Forster *et al*., 1995), improved the cognitive deficit caused by transection of the fornix in the marmoset.

The 5-HT_{1A} receptors are heterogeneously distributed in the central nervous system and their density is high in the nucleus raphe dorsalis (Pazos *et al*., 1988) where they act as presynaptic somatodendritic 5-HT receptors (Sprouse & Aghajanian, 1986). Stimulation of presynaptic 5-HT_{1A} receptors in the dorsal raphe reversed the deficit of spatial learning caused by intrahippocampal scopolamine (Carli *et al*., 1998), probably by facilitating the transfer of facilitatory information to the hippocampus. On the basis of these and previous findings it was suggested that partial agonists such as buspirone, gepirone, ipsapirone and others which behave as

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full agonists and antagonists respectively at pre and postsynaptic 5-HT_{1A} receptors (Sprouse & Aghajanian, 1986) may be useful in the symptomatic treatment of memory disturbances associated with loss of hippocampal cholinergic transmission.

A particularly interesting compound is S 15535 (4-benzodioxan-5-yl)1-(indan-2-yl)piperazine) which behaves as a highly selective, potent antagonist at postsynaptic 5-HT_{1A} receptors and as an agonist at 5-HT_{1A} autoreceptors (Millan *et al*., 1993; 1994). In the present study we examined whether systemically administered S 15535 prevented the scopolamine-induced deficit in spatial learning. To clarify the role of presynaptic 5-HT_{1A} receptors, in one experiment we studied the ability of systemically administered S 15535 to prevent the scopolamine-induced deficit in rats that had received WAY 100635 in the nucleus raphe dorsalis.

As in previous studies (Carli & Samanin, 1992; Carli *et al*., 1995a,b; 1997a,b; 1998), we used a two-platform discrimination task in a water maze in which changes in motor or motivational performance are minimized by measuring choice accuracy and latency separately, and motivational changes can be deduced from the number of omissions. Correct performance of the task obliges the rats to use spatial mapping abilities, as shown by the fact that drawing a black curtain around the pool to hide all the extra-maze cues completely blocks acquisition of the task (Morris *et al*., 1986; our unpublished results).

Methods

Animals

Male Crl:CD(SD)BR rats (Charles River, Italy) were housed in groups of ten in standard laboratory conditions (temperature $20 \pm 1^{\circ}C$ and 60% relative humidity) in a room illuminated from 07.00 to 19.00 h. Food and water were freely available.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the national (D.L. n. 116, G.U., suppl., 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Cannula implantation and histology

The rats, anaesthetized with Equithesin (9.7 mg ml^{-1} sodium pentobarbital in saline +42.6 mg ml^{-1} chloral hydrate in propylenglycol +21.2 mg ml^{-1} Mg_2SO_4 in ethanol; 3.0 ml kg^{-1} i.p.), were immobilized in a Kopf stereotaxic instrument. The skin was cut and the skull cleaned for 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, 30-gauge stainless steel stylets were placed in the guide cannulae until the animals were given intracerebral injections.

A single guide cannula was implanted to give access to the dorsal raphe (DR) nucleus. It was positioned at an angle of 20° relative to the saggital plan to avoid damage to the sinus. The coordinates calculated from the interaural line were A, +1.1 mm; L, −1.4 mm; H, +3.2 (Paxinos & Watson, 1982). To gain access to the CA1 region of the dorsal hippocampus bilateral guide cannulae were implanted at coordinates calculated from the interaural line: A = +5.2 mm L = ±2.0 and H = +7.3 (Paxinos & Watson, 1982). In one experiment rats were simultaneously implanted with three guide cannulae (bilaterally into the CA1 region of the hippocampus and singly into the DR).

After the guide cannulae were implanted the rats were housed singly and were allowed 7 days of recovery. During the last 3 days rats were adapted to the injection procedure by removing each one from its home cage and transporting it to the place where the injection was made. The dummy cannulae were removed and the rat was held firmly for 3 min (approximately the time needed for the injection procedure) after which the dummy cannulae were put back. No solutions were infused during these sham injections. On the days of acquisition training the stylets were withdrawn and replaced by injection units (30-gauge stainless steel tubing) terminating 2 mm below the tip of the guides.

On completion of each experiment rats were killed and their brain removed and frozen on dry ice. To check the position of the cannulae tracks, brain sections 40 μm thick were cut in the coronal plane in a Cryo-cut, and the location of the infusion was verified visually. For each experiment only data from rats in which the cannulae were located in the appropriate structures were included in the results.

Some rats were killed and their brains were removed, stored initially in formalin (10%) and then in a sucrose solution (30%). Coronal sections 30 μm thick were cut in the coronal plane in a Cryo-cut, mounted on treated slides and stained with cresyl violet. Representative photographs of the histological sections from animals which received a single injection into the DR and bilateral injections into the CA1 region of dorsal hippocampus on each of the five training days are shown in Figure 1.

Apparatus

A circular 'swimming pool' was used, 1.5 m in diameter and 0.5 m high. The pool was filled to a depth of 0.29 m with water ($26 \pm 1^{\circ}C$) rendered opaque by the addition of a food dye (coffee colour, Bayo, Italy). The water was changed daily. The pool was placed in the middle of 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 centre of the ceiling, 2.4 m above the water surface. The light intensity at the water surface was 80 lux (measured by an Illuminometer, Mod 5200, Kyoritsu, Japan).

Two visible platforms were used. The fixed one protruded 1.5–2.0 cm above the water. 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, and the other sank when the rats tried to climb onto it.

Training procedure

The black curtains were drawn together to allow a full view of extra-maze cues. Rats were trained to swim to the rigid

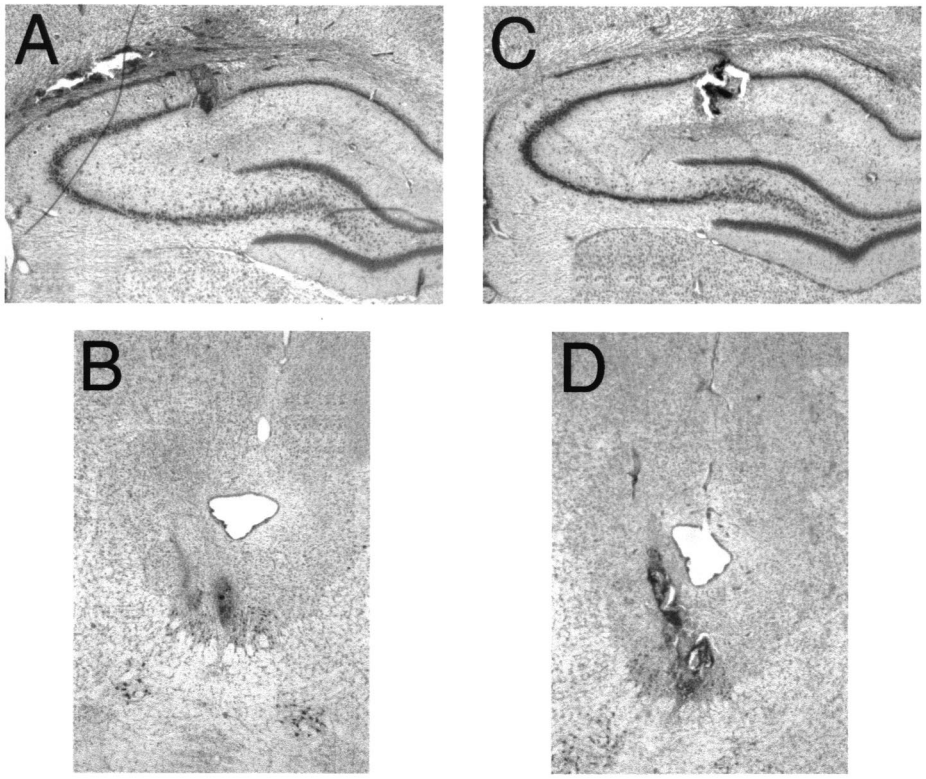

Figure 1 Representative photographs of the histological section showing the cannulae tracks and the infusion sites into the CA1 region of the dorsal hippocampus (A and C) and into the corresponding dorsal raphe nucleus of the mesencephalon (B and D).

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 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 until the next trial. The rats were trained in squads of four. Inter-trial intervals were approximately 2–4 min so each daily testing lasted approximately 30 min for each rat. 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 past 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.

The parameters measured were (1) the first choice in each trial (correct/incorrect), (2) the latency to escape (s), and (3) the number of omissions.

Treatment schedules

After recovery from surgery and adaptation to the injection procedure rats were allocated to different treatment groups. Scopolamine HBr (Sigma, U.S.A.), WAY 100635 (Pharmacia-Upjohn, Italy) or saline solutions were delivered at a rate of 0.5 μl min^{-1} by a Hamilton syringe mounted on a CMA/100 infusion pump (CMA Microdialysis, Stockholm, Sweden), connected by PP10 tubing to a 30-gauge stainless steel cannula (injection unit) terminating 2 mm below the tip of the guides. A total of 0.5 μl of WAY 100635 dissolved in saline, or saline alone, was administered by a single injection into the DR over a 1-min period; bilateral injections of 2 μl scopolamine (dissolved in saline), or saline alone, into the hippocampus were made over 2 min. The injection cannulae were left in place for another minute before withdrawal to allow diffusion from the tip and prevent reflux of the solution.

Each animal received only one drug regimen given over all testing days.

The number of animals in each group is given here in brackets. In the first experiment, on each acquisition training day, the rats were injected subcutaneously (s.c.) with vehicle 2 ml kg^{-1} ($n=9$) or S 15535 (Servier, France) 0.3 mg kg^{-1} ($n=8$) or 1.0 mg kg^{-1} ($n=10$) dissolved in vehicle (distilled water) and 20 min later they received a bilateral injection 1.0 μl of vehicle into the dorsal hippocampus (stratum radiatum of area CA1). Other rats were injected subcutaneously with 2 ml kg^{-1} vehicle ($n=9$), 0.3 mg kg^{-1} ($n=8$) or 1.0 mg kg^{-1} S 15535 ($n=9$) and bilaterally infused with 4.0 μg μl^{-1} scopolamine into the hippocampus.

In the second experiment rats were injected subcutaneously on each training day with 2 ml kg^{-1} vehicle and after 15 min injected with either 0.5 μl saline ($n=10$) or 1.0 μg 0.5 μl^{-1} WAY 100635 ($n=8$) into the DR. Other rats were injected subcutaneously with 1.0 mg kg^{-1} S 15535 followed, 15 min later, by 0.5 μl saline ($n=8$) or 1.0 μg 0.5 μl^{-1} WAY 100635 ($n=8$) into the DR. Five minutes later all animals received a

bilateral injection of 4.0 μg μl^{-1} scopolamine into the dorsal hippocampus.

Control rats ($n=9$) received 2 ml kg^{-1} vehicle s.c. followed by a single injection of 0.5 μl saline into the DR and a bilateral injection of 1.0 μl saline into the dorsal hippocampus.

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). 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). 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 s.c. S 15535 on the scopolamine-induced deficit in accuracy (per cent correct choices) in the two-platform spatial discrimination task were analysed by three-way ANOVA for repeated measures. The first between-subjects factor was S 15535 (three levels), and the second scopolamine (two levels). There were five levels of the repeated within-subjects factor time. Significant three-way interactions between factor S 15535, scopolamine and time were further analysed by two-way ANOVA, examining the effects of factors S 15535 in saline- or scopolamine-treated rats. The interaction between time and factors S 15535 or scopolamine were further analysed by comparing the effects of factors S 15535 or scopolamine for each day separately.

Significant two-way interactions between factor S 15535 and scopolamine were further analysed by comparing treatment group means of the five training sessions, using Tukey's test. The same statistical analysis was applied to choice latencies.

The effects of WAY 100635 injected into the DR on the effect of S 15535 on the scopolamine-induced deficit in accuracy (per cent correct choices) was examined by three-way ANOVA for repeated measures. The between-subjects factors were S 15535 and WAY 100635. There were five levels of the repeated within subjects factor time. In this experiment one-way ANOVA was used to compare control rats and rats injected with vehicle s.c., saline into the DR and scopolamine into the hippocampus. *Post-hoc* comparisons between the treatment groups were made for each day separately by Tukey's test. The same statistical analysis was applied to choice latency data.

When required, angular and log10 transformations were done to normalize the distributions in accordance with the ANOVA model (Winer, 1971).

The analyses of variance for unbalanced data were performed by the general linear model procedure (GLM) using the SAS Institute Inc. (U.S.A.) statistical software run on a Micro VAX 3500 computer (Digital, U.S.A.). The degrees of freedom associated with the F values for the univariate tests of within-subjects effects were adjusted by the Greenhouse-Geisser (G-G) epsilon (ε). In the result section we have reported only the adjusted $Pr > F$ (G-G) without showing the degrees of freedom of the F values of the repeated factor time. The degrees of freedom of the F values are reported only for the between-subjects factors S 15535, WAY 100635 and their interaction.

The numbers of omissions during the acquisition training were analysed by between-subjects two-way ANOVA.

Results

Histology

Examination of the stained coronal sections showed that multiple injections into the hippocampus and dorsal raphe caused very limited tissue damage in the majority of rats. The degree of gliosis around the injection needle track was similar in all experimental groups. Figure 1 shows some representative photographs of the histological sections of rat brains from the experiment in which the effect of s.c. S 15535 in combination with WAY 100635 injected into the DR was examined in rats injected with scopolamine into the CA1 area of the dorsal hippocampus.

Effects of S 15535 on intrahippocampal scopolamine-induced deficit

The results on choice accuracy are shown in Figure 2A. The accuracy of rats on the first day of acquisition training was not appreciably different, their performance ranging from 50 to 60% of correct choices. The accuracy of the control rats improved each day, reaching about 90% of correct choices on day 5 of training. Rats injected with 4.0 μg μl^{-1} scopolamine in the CA1 area of the dorsal hippocampus before each acquisition session still showed about 60% of correct choices on the last day of training (time $\times$ scopolamine, $F=5.03$ $P=0.001$). Injecting the animals with S 15535 had no real effect on any particular day of training (time $\times$ S 15535, $F=1.04$ $P=0.40$).

ANOVA showed a non-significant three-way interaction time $\times$ scopolamine $\times$ S 15535 ($F=1.25$ $P=0.27$), but a significant two-way interaction between scopolamine and S 15535 ($F_{2,45}=10.4$ $P=0.0002$), suggesting that, independently of the training day, S 15535 prevented scopolamine-induced impairment of accuracy. The choice accuracy data were further analysed by comparing treatment group means of the five training sessions.

The histograms in Figure 2B present the results as means $\pm$ s.e.mean of the five training sessions. The *post-hoc* Tukey's test indicated that saline + scopolamine treated rats had significantly worse discriminative accuracy than controls (saline + saline) ($P<0.05$) and that rats given 1.0 mg kg^{-1} S 15535 + scopolamine had significantly better choice accuracy than those treated with saline + scopolamine ($P<0.05$). S 15535 0.3 mg kg^{-1} s.c. did not reverse the scopolamine-induced choice accuracy impairment ($P>0.05$). Tukey's test showed that S 15535 by itself had no effect on choice accuracy at any dose tested.

On the first day of training, all rats had the same choice latency (Figure 2C) and improved similarly over the 5 days of training. Overall statistical analysis showed no significant three-way (time $\times$ scopolamine $\times$ S 15535, $F=1.65$ $P=0.13$) or two-way interactions between scopolamine and S 15535 ($F_{1,45}=0.26$ $P=0.77$) or any significant main effect of scopolamine ($F_{1,45}=0.23$ $P=0.86$) or S 15535 ($F_{2,45}=0.15$ $P=0.86$) but there was a highly significant main effect of time ($F=66.58$ $P=0.0001$).

For the sake of descriptive convenience the acquisition curves of S 15535 0.3 and 1.0 mg kg^{-1} + saline are not shown in Figure 2A (correct choices) or Figure 2C (choice latencies). Mean $\pm$ s.e.mean percentage correct choices for each training day for S 15535 0.3 mg kg^{-1} + saline were: 55.6 $\pm$ 2.9, 72.8 $\pm$ 5.9, 77.2 $\pm$ 2.4, 77.5 $\pm$ 4.1 and 86.2 $\pm$ 4.6; for S 15535 1.0 mg kg^{-1} + saline: 47.2 $\pm$ 4.5, 60.0 $\pm$ 3.9, 62.2 $\pm$ 3.2, 76.6 $\pm$ 3.7 and 77.8 $\pm$ 3.6. Mean $\pm$ s.e.mean of choice latencies

in seconds for each training day for S 15535 0.3 mg kg^{-1} + saline were: 15.2±1.8, 13.5±1.6, 9.6±1.2, 6.3±0.6 and 4.4±0.3; for S 15535 1.0 mg kg^{-1} + saline: 15.6±2.1, 12.1±1.2, 9.4±1.2, 8.5±1.1 and 6.5±0.7. Scopolamine and each dose of S 15535, or the two combined, had no significant effect on errors of omission (data not shown).

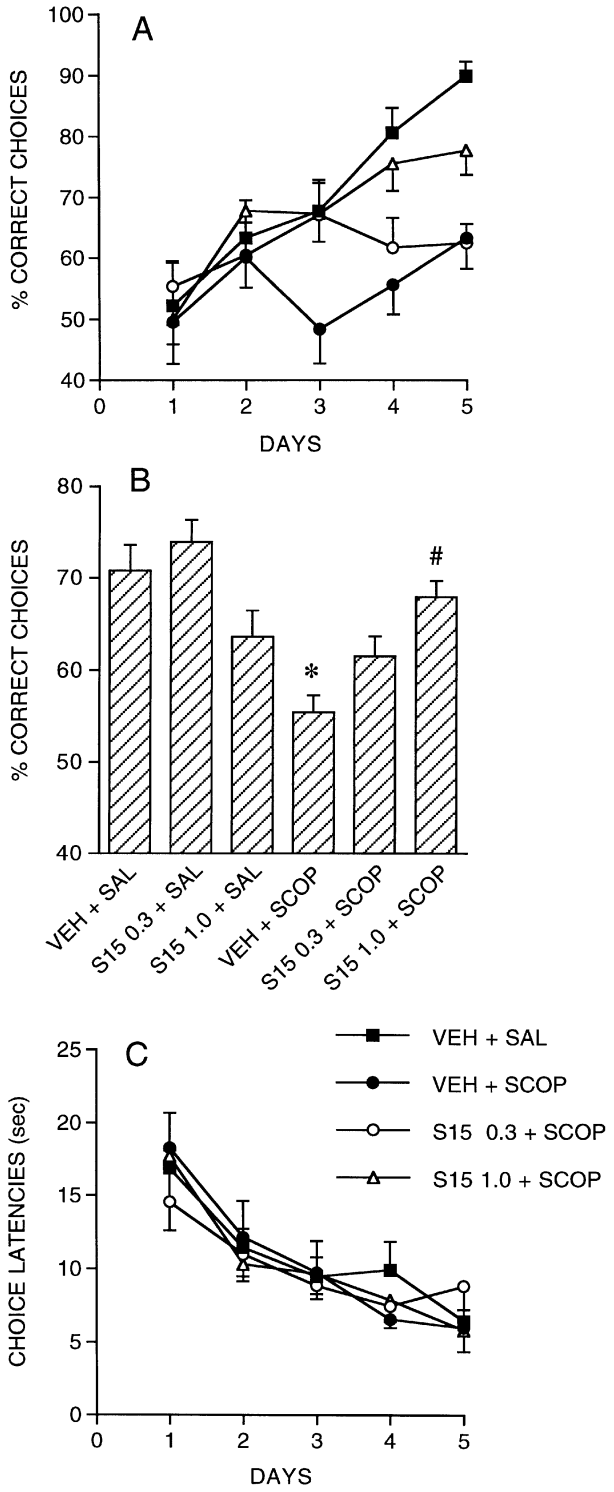

Figure 2 Effects of vehicle (VEH), 0.3 or 1.0 mg kg^{-1} of S 15535 ((S 15 0.3) or (S 15 1.0) respectively) injected subcutaneously on the percentage of correct choices (A), means±s.e.mean of correct choices in the five training sessions (B) and choice latencies (C) of rats given saline (SAL) or scopolamine (SCOP) (4.0 μg μl^{-1}) intrahippocampally. On each acquisition day, S 15535 (S 15) was injected 20 min before scopolamine which was given 10 min before the training session. *$P<0.05$ vs VEH+SAL (Tukey's test); #$P<0.05$ vs VEH+SCOP (Tukey's test).

Effects of WAY 100635 injected into the DR on the reversal of scopolamine-induced deficit by S 15535

Since intrahippocampal saline-treated rats given subcutaneous S 15535 (Figure 2) or WAY 100635 (1.0 μg 0.5 μl^{-1}) injected into the DR (Carli *et al.*, 1998) showed no effect on choice

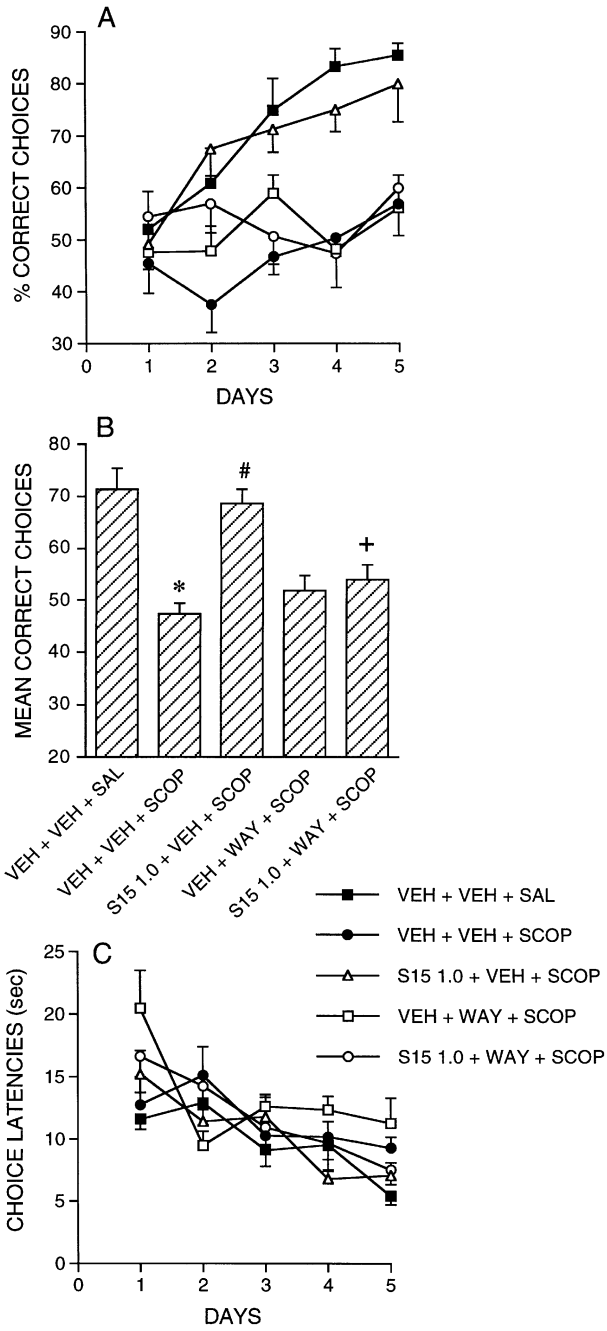

Figure 3 Effects of 1.0 μg 0.5 μl^{-1} WAY 100635 (WAY) or 0.5 μl vehicle (VEH) injected into the dorsal raphe (DR) of rats injected subcutaneously with 1.0 mg kg^{-1} of S 15535 (S 15 1.0) or 2 ml kg^{-1} vehicle (VEH) on the percentage of correct choices (A), means±s.e.mean of correct choices in the five training sessions (B) and choice latencies (C) of rats given scopolamine (SCOP) (4.0 μg μl^{-1}) intrahippocampally. Control rats received vehicle (VEH) in the DR plus vehicle s.c. (VEH) plus saline in the hippocampus (SAL). On each acquisition day, S 15535 was given 15 min before WAY 100635 or vehicle which were injected into the DR 5 min before scopolamine. The rats started their daily training session 10 min later. *$P<0.05$ vs VEH+VEH+SAL (Tukey's test); #$P<0.05$ vs VEH+VEH+SCOP (Tukey's test); +$P<0.05$ vs S 15 1.0+VEH+SCOP (Tukey's test).

accuracy or latency, we examined how WAY 100635 injected into the DR modified the effect of S 15535 on the scopolamine-induced deficit in accuracy (per cent correct choices) only in the intrahippocampal scopolamine-treated rats. One group of control rats which received saline injected into the hippocampus was used to examine the effects of scopolamine injected into the hippocampus on correct choices and choice latencies.

The results on choice accuracy are shown in Figure 3A. All rats started with 50% correct choices. Rats given saline in the hippocampus improved steadily throughout the training, reaching 85% of correct choices, whereas rats injected with scopolamine into the hippocampus achieved only 55% correct choices at the end of training. The difference between the mean per cent correct choices of the five training days for the two treatment groups was higly significant (main effect of scopolamine $F_{1,17}=23.27$ $P=0.0002$). The effect of scopolamine was indipendent of the training day (time × scopolamine $F=2.09$ $P=0.11$).

Overall ANOVA examining the effects of the combination of 1.0 mg kg^{-1} S 15535 or 2 ml kg^{-1} saline s.c. and 1.0 μg 0.5 μl^{-1} WAY 100635 or 0.5 μl saline injected into the DR in intrahippocampal scopolamine-treated rats showed a non-significant three-way interaction (time × S 15535 × WAY 100635, $F=1.5$ $P=0.23$) but a significant two-way interaction between S 15535 and WAY 100635 ($F_{1,30}=12.41$ $P=0.0014$).

The histograms in Figure 3B present the results as means ± s.e.mean of the five training sessions. The *post-hoc* Tukey's test indicated that control rats treated with saline in the hippocampus had significantly better discriminative accuracy than those given scopolamine in the hippocampus ($P<0.05$). Scopolamine-treated rats injected with S 15535 made significantly more correct choices than those injected with saline ($P<0.05$; Tukey's test) and rats treated with S 15535 + WAY 100635 in the DR had significantly worse choice accuracy than those injected with S 15535 + saline in DR ($P<0.05$; Tukey's test). Injection of WAY 100635 into the DR by itself did not modify choice accuracy of scopolamine-treated rats ($P>0.05$; Tukey's test).

The results on choice latency are shown in Figure 3C. One-way ANOVA comparing control rats given saline or scopolamine in the hippocampus showed the drugs had no significant effect on choice latency (main effect scopolamine, $F_{1,17}=1.98$ $P=0.17$; time × scopolamine, $F=1.45$ $P=0.24$) (Figure 3C). In both groups choice latencies significantly declined with training (time, $F=5.55$ $P=0.003$).

Overall statistical analysis on scopolamine-treated rats showed no significant three-way (time × WAY 100635 × S 15535, $F=2,13$ $P=0.10$) or two-way interactions between WAY 100635 and S 15535 ($F_{1,30}=0.20$ $P=0.65$) or any significant main effect of WAY 100635 ($F_{1,30}=3.04$ $P=0.09$) or S 15535 ($F_{1,30}=2,27$ $P=0.14$) but there was a significant main effect of time ($F=10.2$ $P=0.0001$) (Figure 3C). WAY 100635 or S 15535, or the two combined, had no effect on errors of omission in intrahippocampal scopolamine-treated rats (data not shown).

Discussion

As previously found with the other ligands of 5-HT$_{1A}$ receptors WAY 100135 (Carli *et al*., 1995b) and WAY 100635 (Carli *et al*., 1997a), in the present study S 15535 0.3 and 1 mg kg^{-1} s.c. had no effect on the performance of the rat in the two-platform spatial discrimination task, but 1 mg kg^{-1} significantly antagonized the impairment of choice accuracy caused by scopolamine 4 μg μl^{-1} infused into the dorsal hippocampus. At 0.3 mg kg^{-1} S 15535 showed a non significant tendency to antagonize the effect of scopolamine.

Previous studies in our laboratory suggest that blockade of postsynaptic 5-HT$_{1A}$ receptors prevents the deficit of spatial learning caused by intrahippocampal scopolamine in the two-platform discrimination task. This is clearly shown by the fact that direct blockade of 5-HT$_{1A}$ receptors in the dorsal hippocampus prevented the deficit of spatial learning caused by infusing scopolamine in that brain region (Carli *et al*., 1995b), whereas blockade of 5-HT$_{1A}$ autoreceptos in the DR had no effect on the performance of the rat or on the impairment caused by intrahippocampal scopolamine (Carli *et al*., 1998).

Although systemically administered S 15535 may prevent the scopolamine-induced deficit in spatial learning by blocking postsynaptic 5-HT$_{1A}$ receptors, this compound is a highly active agonist in tests of 5-HT$_{1A}$ autoreceptor-mediated activity (Millan *et al*., 1994). Thus, in view of our recent finding that stimulation of presynaptic 5-HT$_{1A}$ receptors in the dorsal raphe did not affect the performance of the rat but antagonized the impairment of choice accuracy caused by intrahippocampal scopolamine (Carli *et al*., 1998), we examined whether 5-HT$_{1A}$ receptors in the DR were involved in the effect of S 15535.

In a previous study (Carli *et al*., 1998) WAY 100635 injected into the DR at 1.0 μg 0.5 μl^{-1} had no effect on rats' performance or on the impairment of choice accuracy caused by intrahippocampal scopolamine but completely prevented 1.0 μg 0.5 μl^{-1} 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist, antagonizing the scopolamine-induced deficit when administered in the DR. We therefore used the same condition to clarify the role of presynaptic 5-HT$_{1A}$ receptors in the effect of S 15535.

In agreement with our previous findings (Carli *et al*., 1998), 1.0 μg 0.5 μl^{-1} WAY 100635 injected into the DR did not affect choice accuracy and latency or errors of omission, or modify the effect of intrahippocampal scopolamine. This is in line with previous findings that WAY 100635 by itself had no effect on extracellular 5-HT (Gurling *et al*., 1994; Invernizzi *et al*., 1997), confirming that somatodendritic 5-HT$_{1A}$ receptors are not tonically activated by endogeneous levels of 5-HT (Gartside *et al*., 1995; Invernizzi *et al*., 1996). Nevertheless, WAY 100635 completely antagonized the effect of systemically administered S 15535 on scopolamine-induced impairment of choice accuracy. Thus presynaptic 5-HT$_{1A}$ receptors clearly appear to be involved in the ability of S 15535 to counteract the impairment of spatial learning caused by scopolamine.

Although blockade of postsynaptic 5-HT$_{1A}$ receptors may contribute to this effect, at the dose used in the present study the drug very likely modified the effect of scopolamine mainly by acting on presynaptic 5-HT$_{1A}$ receptors. In line with this are findings by Millan *et al*. (1997) that at doses as low as 0.3 mg kg^{-1} s.c. S 15535 reduced extracellular 5-HT levels in the rat hippocampus, a result reflecting its agonist properties at 5-HT$_{1A}$ autoreceptors, whereas higher doses of S 15535 were needed to block postsynaptic 5-HT$_{1A}$ receptor-mediated responses (Millan *et al*., 1994).

At the same doses as in the present study S 15535 showed anxiolytic-like activity in various rat models (Millan *et al*., 1997; Cervo *et al*., 1997). It is unlikely that this activity interfered with rats' performance since S 15535 had no effect on motivational indices such as choice latency and errors of omission that one would expect to be affected by an anxiolytic effect in an aversively motivated task. Moreover, anxiolytic drugs such as benzodiazepines cause an impairment of spatial learning in a water maze (McNaughton & Morris, 1987). In

view of recent suggestions that enhanced anxiety may contribute to learning deficit following cholinergic blockade (Smythe *et al*., 1996; 1998; Leri & Franklin, 1998) S 15535 might perhaps reduce the deficit caused by scopolamine, in part, through its anxiolytic activity. Stimulation of 5-HT_{1A} receptors in the DR caused anxiolytic-like effects in various models (Higgins *et al*., 1988; Handley, 1995). Although further studies are necessary to clarify this point, the present findings and our own results with 8-OH-DPAT injected into the DR (Carli *et al*., 1998) add new information on the functional output of stimulating 5-HT_{1A} receptors in this brain region. Reductions of 5-HT transmission in terminal regions are presumably involved in the anxiolytic and memory-enhancing effects of compounds stimulating 5-HT_{1A} autoreceptors.

The fact that S 15535, as previously found with 8-OH-DPAT administered into the DR had no effect on spatial performance (Carli *et al*., 1998) but reduced 5-HT transmission in terminal regions (Invernizzi *et al*., 1991) confirms that 5-HT neurons originating from the DR have no direct effect on the acquisition of spatial memory but modulate the effect of other systems more directly involved.

It is unlikely that 5-HT neurones originating in the nucleus raphe medianus that innervate the hippocampal formation were indirectly involved since 8-OH-DPAT injections in this nucleus, unlike those in the DR, did not attenuate the learning deficit caused by intrahippocampal scopolamine and actually caused an impairment of choice accuracy in the two-platform spatial discrimination task (unpublished results).

The entorhinal cortex provides a major cortical input to the hippocampus through a pathway using glutamate as neurotransmitter (Iijima *et al*., 1996; see Francis *et al*., 1993) and selective lesions in this area cause a selective deficit in an allocentric memory task (Holscher & Schmidt, 1994). Superficial cells of the entorhinal cortex are inhibited by serotonin (Schmitz *et al*., 1995) and are preferentially innervated by serotonergic neurons in the dorsal raphe (Jacobs & Azmitia, 1992). S 15535 by stimulating 5-HT_{1A} receptors in the DR may reduce 5-HT transmission in this region. The reduction of a serotonergic inhibitory influence to the entorhinal cortical cells, may facilitate the transfer of excitatory information from the entorhinal cortex to the hippocampus and thus compensate the loss of cholinergic input to the hippocampal pyramidal cells. This hypothesis is currently under investigation in our laboratory.

Whatever the neuronal mechanism and brain region involved, S 15535 and probably other partial agonists at central 5-HT_{1A} receptors may offer a novel approach for the symptomatic treatment of memory disturbances in man, including Alzheimer's disease where a close correlation has been found between the reduction in acetylcholine markers in the hippocampus and behavioural deficit (Katzman *et al*., 1986).

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