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SA4503, a novel cognitive enhancer with σ_1 receptor agonist properties, facilitates NMDA receptor-dependent learning in mice

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Abstract

The selective σ_1 receptor agonist 1-(3,4-dimethoxyphenethyl)-4-(3-phenyl propyl)piperazine dihydrochloride (SA4503) was reported to reverse the amnesia induced by the muscarinic receptor antagonist scopolamine at sub-mg/kg doses. We examined its effect on the learning impairment induced in mice by the non-competitive NMDA receptor antagonist dizocilpine. Learning capacities were evaluated using spontaneous alternation in the Y-maze for spatial working memory, and step-down type passive avoidance. SA4503 (0.03–1 mg/kg s.c.) attenuated the dizocilpine (0.15 mg/kg i.p.)-induced memory deficits following a bell-shaped curve in both tests. These effects of SA4503 were blocked by haloperidol (0.05 mg/kg i.p.), implicating σ_1 receptors. SA4503 also reversed the alternation deficit induced by N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 mg/kg i.p.) at the same dosage, indicating that it acted on working memory through the nitric oxide (NO)-mediated signalling pathway. Furthermore, progesterone (2 mg/kg s.c.) blocked the SA4503 effects in the dizocilpine- and L-NAME-amnesia models, in accordance with the purported neurosteroids/σ_1 receptors interaction. These results demonstrate a promising neurobehavioural profile of SA4503, a ligand equally efficient to reverse the deficit in the glutamatergic as well as in the cholinergic amnesia model. Pertinent informations on the potential mechanism of the anti-amnesic effects of σ_1 receptor ligands were also obtained.

Keywords: σ_1 Receptor; Dizocilpine-induced amnesia; N^{ω}-Nitro-L-arginine methyl ester (L-NAME), induced amnesia; NMDA receptor; Learning

1. Introduction

We previously reported that high-affinity sigma (σ) receptor ligands, such as 1,3-di(2-tolyl)-guanidine (DTG), (+)-*N*-allyl-normetazocine ((+)-SKF-10,047), (+)-pentazocine, or 2-(4-morpholinoethyl-1)-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084), significantly attenuate, via the σ_1 receptor, the learning impairment induced in mice by the non-competitive NMDA receptor antagonist, dizocilpine (Maurice et al., 1994a,b,c). These effects were evidenced using spontaneous alternation, a behavior related to working memory, and the step-down and step-through type of passive avoidance tests, that examine long-term memory. Further evidence was recently provided by other authors, who used either the three-panel runaway task or the eight-radial-arm maze test with rats (Earley et al., 1995; Ohno and Watanabe, 1995). The exact mechanism by which σ_1 receptor ligands exert beneficial effects on the NMDA receptor-dependent learning impairment is still unknown. However, several authors have reported that selective σ_1 receptor ligands induce marked stimulation of several physiological responses to NMDA (*N*-methyl-D-aspartate), such as the NMDA-evoked [^{3}H]norepinephrine release from hippocampal slices (Monnet et al., 1991; Roman et al., 1991) and the NMDA-induced neuronal activation of CA_3 dorsal hippocampal neurons in vivo (Monnet et al., 1990, 1992). It has been proposed that σ_1 receptor ligands may exert their facilitating actions on the NMDA responses by modulation of the receptor-associated ion channel activity. Also, the ability of σ_1 receptor ligands to alleviate the amnesia induced by muscarinic acetylcholine receptor antagonists has been described (Earley et al., 1991; Matsuno et al., 1994, 1997). These results demonstrated the capacity of σ_1 receptor ligands to reduce the learning impairment in different pharmacological models, but questioned their selectivity for the neurotransmitter systems involved in learning processes.

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One of the most promising σ_1 receptor ligands is the highly selective agonist, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503). It has high affinity for the σ_1 sites ($IC_{50} = 17 \pm 1.9$ nM) and low affinity for the σ_2 sites ($IC_{50} = 1800 \pm 310$ nM; Matsuno et al., 1996). SA4503 induced an increase in cortical and hippocampal extracellular acetylcholine levels, without affecting the striatal acetylcholine level, as demonstrated by intracerebral in vivo microdialysis (Matsuno et al., 1997). In parallel, SA4503 attenuated the scopolamine-induced memory impairment in the passive avoidance task in rats, at sub-mg/kg dosages per os. This effect was blocked by the σ_1 receptor antagonists, haloperidol and *N,N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-ethylamine hydrochloride (NE-100) (Matsuno et al., 1997). The particularly promising profile of SA4503 led us to examine its ability to attenuate the learning impairments induced by dizocilpine, using the spontaneous alternation and passive avoidance tests in mice. The antagonist effect of haloperidol was also investigated. In addition, recent results provided new insights into the mechanism of the dizocilpine-induced learning impairment. Yamada et al. (1996a,b) reported that the spatial working memory deficits induced by NMDA receptor blockade involve nitric oxide (NO) formation, since a crossed pharmacology could be observed between the deficits in spontaneous alternation induced by dizocilpine or by the NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME). Moreover, Monnet et al. (1995) reported that neurosteroids, like σ_1 receptor ligands, modulate the NMDA-mediated physiological responses. In particular, dehydroepiandrosterone sulfate (DHEAS) potentiates, whereas pregnenolone sulfate inhibits, NMDA-induced [^{3}H]norepinephrine release. The σ receptor antagonists, haloperidol and 1[2-(3,4-dichlorophenyl)ethyl]-4-methyl piperazine (BD-1063), prevent these effects, while progesterone antagonizes the potentiating effect of DHEAS, and the inhibitory effects of DTG and pregnenolone sulfate (Monnet et al., 1995). Furthermore, DHEAS attenuated the dizocilpine-induced impairment of learning in mice in a haloperidol-sensitive manner (Maurice et al., 1997). We now have also examined the effect of SA4503 on the L-NAME-induced impairment of alternation and investigated the putative antagonist effect of progesterone on both amnesia models.

2. Materials and methods

2.1. Animals

Male Swiss mice (Breeding Centre of the Faculty of Pharmacy, Montpellier, France), aged 5–6 weeks and weighing 30–35 g at the beginning of the experiments, were used for the study. They were housed in plastic cages, with free access to laboratory chow and water, except during behavioural experiments, and kept in a controlled environment (23 ± 1°C, 40–60% humidity), with a 12-h light/dark cycle (light on at 8:00 a.m.). Experiments were carried out between 10:00 a.m. and 6:00 p.m., in a soundproof and air-conditioned experimental room, to which mice were allowed to adapt for at least 30 min before each experiment. Animal care and experimental procedures followed the protocols and guidelines approved by I.N.S.E.R.M.

2.2. Drugs and administration procedures

SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine) was supplied by Dr Kiyoshi Matsuno (Santen Pharmaceutical, Osaka, Japan); dizocilpine ((+)-MK-801 maleate) was from Research Biochemicals International (Natick, MA, USA); haloperidol (Haldol) was from Janssen (Boulogne-Billancourt, France); N^{ω}-Nitro-L-arginine methyl ester (L-NAME) and progesterone were from Sigma (Saint Quentin Fallavier, France). All ligands were dissolved in saline solution, except progesterone which was suspended in pure sesame oil (Sigma), and injected subcutaneously (s.c.) or intraperitoneally (i.p.) in a volume of 100 μl/20 g body weight. Ligands were administered simultaneously, 30 min before the Y-maze session or before the first passive avoidance training session. Injections were not repeated before the second training or retention session.

2.3. Spontaneous alternation performances

Spatial working memory performance was assessed by recording spontaneous alternation behavior in a Y-maze (Maurice et al., 1994a,b,c). The maze was made of black painted wood. Each arm was 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries, including possible returns into the same arm, were recorded using an Apple IIe computer. Alternation was defined as entries into all three arms on consecutive occasions. The number of maximum alternations was therefore the total number of arm entries minus two and the percentage of alternation was calculated as (actual alternations/maximum alternations) $\times$ 100.

2.4. Step-down type passive avoidance test

Long-term memory was examined using the step-down type of passive avoidance task (Maurice et al., 1994a,b,c). The apparatus consisted of a transparent acrylic cage (30 $\times$ 30 $\times$ 40 cm high) with a grid floor, inserted in a semi-soundproof outer box (35 $\times$ 35 $\times$ 90 cm high). The cage was illuminated with a 15-W lamp during the experimental period. A wooden platform (4 $\times$ 4 $\times$ 4 cm) was fixed at

the centre of the grid floor and electric shocks (1 Hz, 500 ms, 45 V DC) were delivered to the grid using an isolated pulse stimulator (Model 2100, AM Systems, Everett, WA, USA). The test consisted of two training sessions at 90-min intervals and of a retention session carried out 24 h after the first training session. During training sessions, each mouse was placed on the platform, and was submitted to shocks for 15 s after completely descending onto the grid floor. The step-down latency, and the numbers of vocalizations and flinching reactions were measured. Shock sensitivity was evaluated by summation of both these indices. Animals which failed to step down within 60 s during the second session, were considered to have remembered the task and were removed, without further shocks. The retention test was performed in the same way as training, except that no shocks were given. In this case, each mouse was once again placed on the platform, and latency was recorded, with an upper cut-off time of 300 s. Two parametric measures of retention were analyzed: the latency and the number of animals reaching the avoidance criterion defined as: latency during the retention greater than 3 times the latency during the second training session and greater than at least 60 s. Basically, median latency could be considered as a qualitative index of memory capacities, whereas the percentage of animals reaching criterion could be considered as a quantitative index.

2.5. Statistical analysis

Results were expressed as means ± S.E.M., with the exception of latencies, which were expressed in terms of medians and interquartile ranges. The data failed to show a normal distribution since cut-off times were set, and were analyzed with the Kruskal-Wallis non-parametric analysis of variance (KW value), followed by Dunn's non-parametric multiple comparisons test, or by the Mann-Whitney's non-parametric test, the levels for significance being $P < 0.01$ and $P < 0.05$.

3. Results

3.1. Effect of SA4503 on the dizocilpine-induced learning impairments in mice

The effect of SA4503 was first examined on spontaneous alternation behaviour in the Y-maze. SA4503 administered alone, in the 0.03–1 mg/kg s.c. dose range failed to affect the normal exploratory behavior in the Y-maze, as seen from the alternation percentage ($KW = 4.27$, $P > 0.05$, Fig. 1A), and the number of arms entered during the 8-min session ($KW = 4.18$, $P > 0.05$, Fig. 1B). Preadministration of dizocilpine (0.15 mg/kg i.p.) induced a marked decrease in spontaneous alternation (Fig. 1A) and an increase in the number of arm entries (Fig. 1B), which reflected the hyperactivity induced by the drug. The simultaneous administration of SA4503 led to a bell-shaped attenuation of the dizocilpine-induced deficit in alternation ($KW = 35.89$, $P < 0.01$), significant differences being observed at the 0.3 mg/kg dosage (Fig. 1A). No effect was observed on the increased number of arm entries ($KW = 24.42$, $P < 0.01$), non-significant attenuations appearing at the 0.1 and 1 mg/kg doses (Fig. 1B).

As summarized in Table 1A, administration of SA4503 affected neither latency, nor shock sensitivity measured during the first passive avoidance training session as compared to those of control animals. Administration of dizocilpine did not affect latency, but instead showed a

Table 1
Lack of effect of SA4503 on sensitivity to electric shocks during the first passive avoidance training session

Treatments (mg/kg i.p.) +	(mg/kg s.c.)	*n*	Latency (s) Median [I.R.]	Shock sensitivity Mean ± S.E.M.
(A) SA4503 alone				
	Saline	18	4 [3–7]	17 ± 2
	SA4503 (0.03)	17	7 [4–12]	14 ± 2
	SA4503 (0.1)	17	5 [3–9]	15 ± 2
	SA4503 (0.3)	17	4 [3–8]	14 ± 2
	SA4503 (1)	18	9 [6–14]	19 ± 2
			$KW = 9.32$, $P > 0.05$	$KW = 6.54$, $P > 0.05$
(B) SA4503 + dizocilpine				
Dizocilpine (0.15)	Saline	12	3 [3–4]	32 ± 5 [a]
Dizocilpine (0.15)	SA4503 (0.03)	12	4 [3–7]	22 ± 4
Dizocilpine (0.15)	SA4503 (0.1)	12	5 [3–13]	21 ± 4
Dizocilpine (0.15)	SA4503 (0.3)	12	3 [3–4]	31 ± 3 [a]
Dizocilpine (0.15)	SA4503 (1)	12	3 [3–5]	27 ± 4
			$KW = 10.29$, $P < 0.05$	$KW = 5.63$, $P > 0.05$

The shock sensitivity scores represent the sum of the numbers of vocalizations plus flinching reactions in response to electric shocks (1 Hz, 500 ms, 45 V, for 15 s) received during the first training session (Kruskal-Wallis).
[a] $P < 0.01$ vs. saline-treated group.

general tendency to increase sensitivity (Table 1B). In addition, the administration of SA4503 with dizocilpine did not affect latency and tended to attenuate the dizocilpine-induced hypersensitivity, at the 0.03, 0.1, and 1 mg/kg doses, non-significantly as compared to the dizocilpine-treated group (Table 1B). During the retention test, the administration of SA4503 alone did not affect the performance of the mice, whether in terms of latency (Fig. 2A), or of percentage of animals reaching the avoidance criterion (Fig. 2B). The dizocilpine treatment markedly affected both parameters, and co-administration of SA4503 resulted in significant improvements in retention. Latency was significantly increased at 0.1 and 0.3 mg/kg (Fig. 2A), whereas the percentage of animals reaching criterion

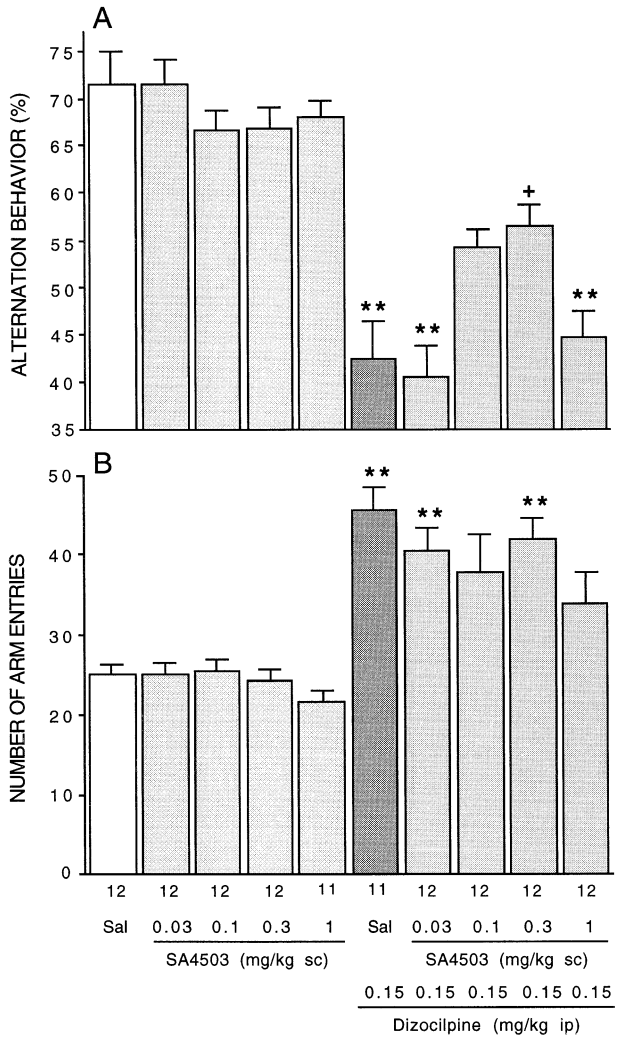

Fig. 1. Dose–response effect of SA4503 on the dizocilpine-induced decrease in spontaneous alternation in the Y-maze test. (A) Alternation behavior, and (B) total number of arm entries. SA4503 (0.03–1 mg/kg s.c.) was administered either 30 min before the session, or simultaneously with dizocilpine (0.15 mg/kg i.p.), which was given 30 min before the session. Results are expressed as means ± S.E.M., the number of animals per group being indicated within the columns in (A). Sal: saline solution. $^{**}P < 0.01$ vs. the control group, $^{+}P < 0.05$ vs. the dizocilpine-treated group (Dunn's test).

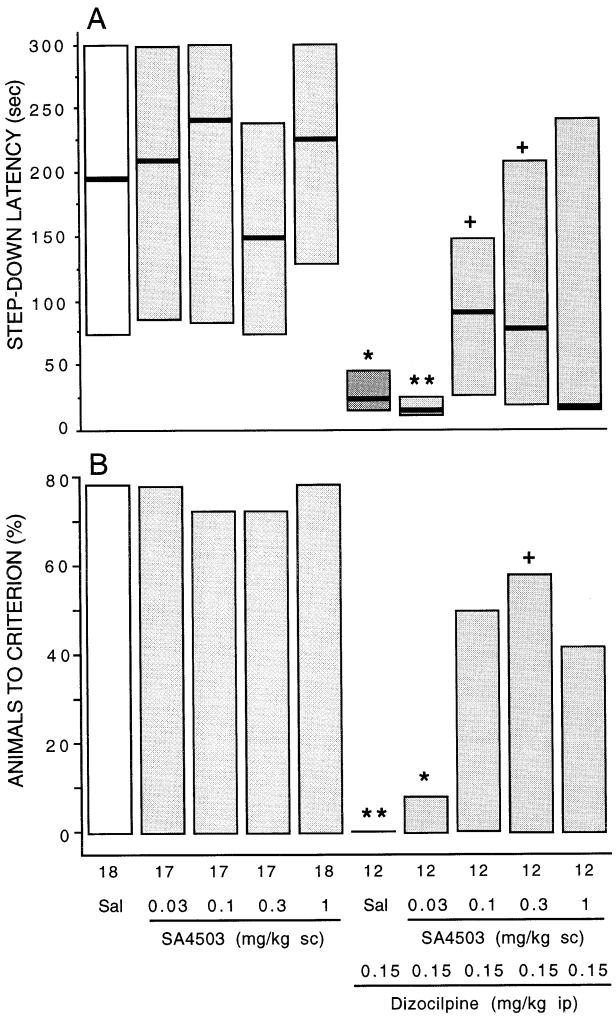

Fig. 2. Dose–response effect of SA4503 on the dizocilpine-induced learning impairment in the step-down type passive avoidance test. (A) Step-down latency and (B) percent of animals reaching criterion. SA4503 (0.03–1 mg/kg s.c.) was administered either 30 min before the first training session, or simultaneously with dizocilpine (0.15 mg/kg i.p.), which was given 30 min before the first training session. Results are expressed as median and interquartile range in (A) and percent in (B), the number of animals per group being indicated below the columns in (B). Sal, saline solution. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group, $^{+}P < 0.05$, $^{++}P < 0.01$ vs. the dizocilpine-treated group (Dunn's test).

was significantly increased at 0.3 mg/kg (Fig. 2B). Both the qualitative and quantitative parameters were improved by SA4503. These effects followed a bell-shaped dose-response profile, since the 1 mg/kg dose failed to show activity.

3.2. Effect of SA4503 on the L-NAME-induced spatial working memory deficit in mice

As previously reported by others (Yamada et al., 1996a,b), the administration of L-NAME (100 mg/kg i.p.) induced a marked decrease in spontaneous alternation (Fig. 3A), with no effect on the number of arm entries (Fig. 3B).

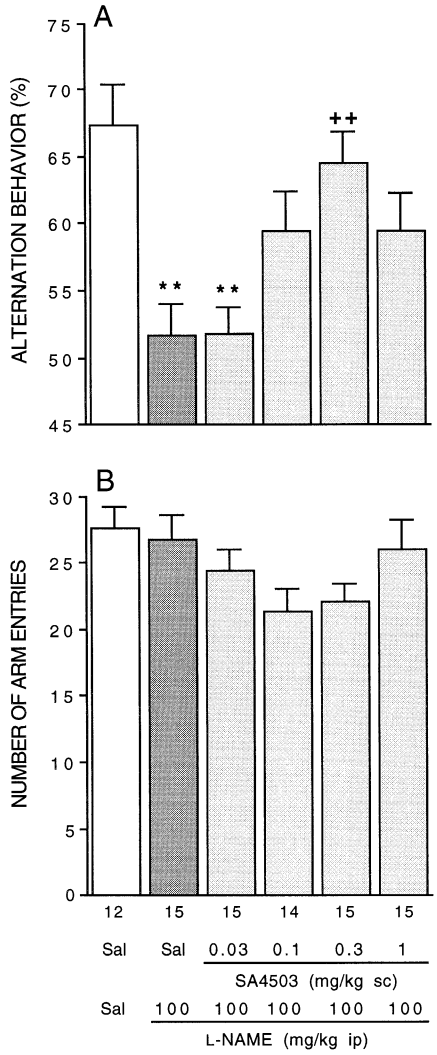

Fig. 3. Dose–response effect of SA4503 on the L-NAME-induced decrease in spontaneous alternation, in the Y-maze test. (A) Alternation behavior and (B) total number of arm entries. SA4503 (0.03–1 mg/kg s.c.) was administered simultaneously with L-NAME (100 mg/kg i.p.), which was given 30 min before the session. Results are expressed as means ± S.E.M., the number of animals per group being indicated within the columns in (A). Sal, saline solution. ** $P < 0.01$ vs. the control group, $^{++}$ $P < 0.01$ vs. the L-NAME-treated group (Dunn's test).

The simultaneous administration of SA4503, in the 0.03–1 mg/kg s.c. dose range, led to a bell-shaped attenuation of the L-NAME-induced deficit (KW = 25.61, $P < 0.01$; Fig. 3A), with no effect on the number of arm entries, whatever the dose (KW = 9.12, $P > 0.05$; Fig. 3B). The dose-response curves for the alternation behaviour in the dizocilpine model (Fig. 1A) and in the L-NAME model (Fig. 3A) showed excellent correlation, with significant attenuation at the 0.3 mg/kg dose.

We could also confirm the differential involvement of the NO signalling system in the NMDA receptor-dependent spatial working memory and long-term memory processes, since L-NAME administered using the same protocol did not affect step-down passive avoidance behaviour (Table 2). L-NAME, administered before the first training session, affected neither latencies, nor shock sensitivity measured during the first training session, compared to those of control animals. In addition, no difference in latencies was observed during the retention test, both groups showing marked learning of the task, confirming that NO is not involved in acquisition of the passive avoidance response.

3.3. Antagonism by haloperidol of the SA4503 effect on the dizocilpine-induced learning impairments

The effect of simultaneous administration of haloperidol (0.05 mg/kg i.p.) and SA4503 (0.3 mg/kg s.c.) on the dizocilpine-induced deficits was investigated using both tests, as summarized in Fig. 4. Haloperidol (0.05 mg/kg i.p.) did not affect the dizocilpine-induced impairment of alternation, but blocked the attenuating effect of the most effective dose (0.3 mg/kg s.c.) of SA4503 (KW = 37.55, $P < 0.01$; Fig. 4A). Haloperidol failed to affect the dizocilpine-induced deficits in passive avoidance, but blocked the anti-amnesic effect of SA4503, i.e. both latency (KW = 21.53, $P < 0.01$; Fig. 4B), and percentage of animals reaching criterion (KW = 27.73, $P < 0.01$; Fig. 4C), although the differences from the (SA4503 + dizocilpine)-treated group did not reach significance. Furthermore, haloperidol did not affect shock sensitivity during the first passive avoidance training session (KW = 7.60, $P > 0.05$; data not shown).

3.4. Antagonism by progesterone of the SA4503 effect on the dizocilpine-induced learning impairments

The simultaneous administration of the neurosteroid, progesterone with SA4503 (0.3 mg/kg s.c.), on the di-

Table 2
Lack of effect of L-NAME on passive avoidance behaviour in mice

Treatment (mg/kg i.p.)	*n*	First training session		Retention	
		Latency (s) Median [I.R.]	Shock sensitivity Mean ± S.E.M.	Latency (s) Median [I.R.]	Mice to criterion (%)
Saline	14	8 [6–13]	16 ± 2	132 [78–300]	71.4
L-NAME (100)	14	9 [4–15]	13 ± 1	124 [45–247]	57.1

Saline solution or L-NAME, 100 mg/kg i.p., was administered 20 min before the first training session. Shock sensitivity scores represent the sum of the numbers of vocalizations plus flinching reactions in response to shock. Differences were not significant (Mann-Whitney's test).

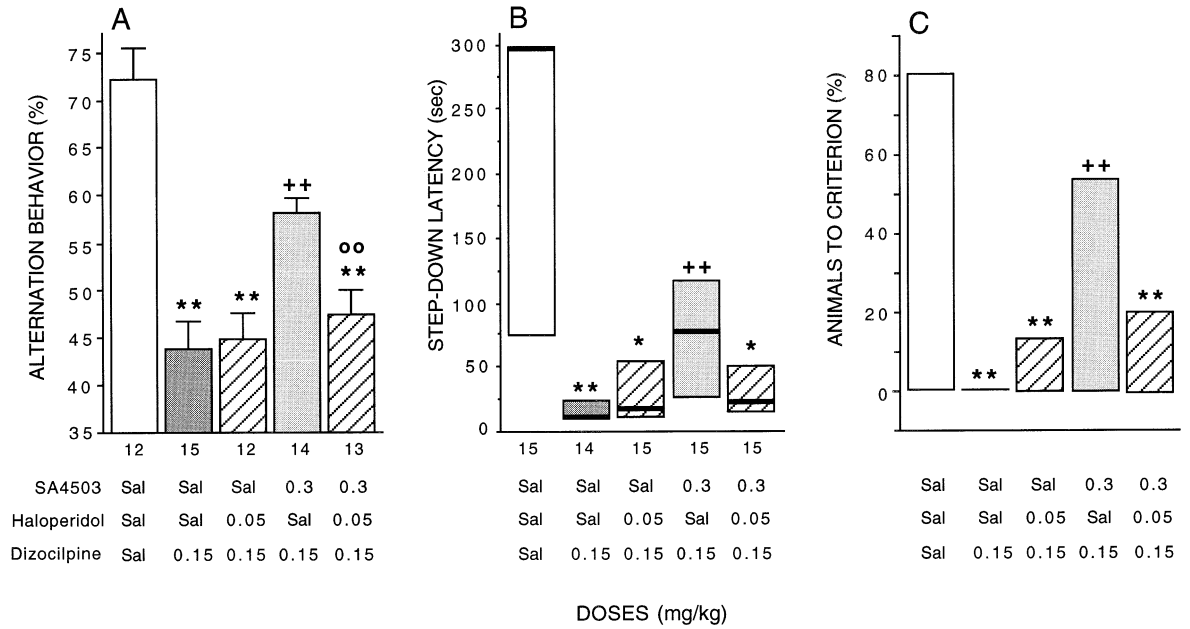

Fig. 4. Antagonism by haloperidol of the SA4503 effect on dizocilpine-induced learning impairments: (A) alternation behavior in the Y-maze test, (B) step-down latency and (C) percentage of animals reaching criterion in the step-down type passive avoidance test. Haloperidol (0.05 mg/kg i.p.) was administered 10 min before SA4503 (0.3 mg/kg s.c.), administered simultaneously with dizocilpine (0.15 mg/kg i.p.), which was given 30 min before the Y-maze session or the first passive avoidance training. The number of animals per group is indicated below the columns in (A) and (C). Sal,: saline solution. * $P < 0.05$, ** $P < 0.01$ vs. the control group, $^{++}$ $P < 0.05$ vs. the (dizocilpine + Sal)-treated group, ^{oo} $P < 0.01$ vs. the (dizocilpine + SA4503)-treated group (Dunn's test).

zocilpine-induced deficits was investigated using both tests (Fig. 5). The dose of progesterone was selected based on the previous finding that progesterone, 2–20 mg/kg, did not itself affect memory processes, but that 2 and 20 mg/kg allowed a significant block of the anti-amnesic effects of other neurosteroids, such as pregnenolone or

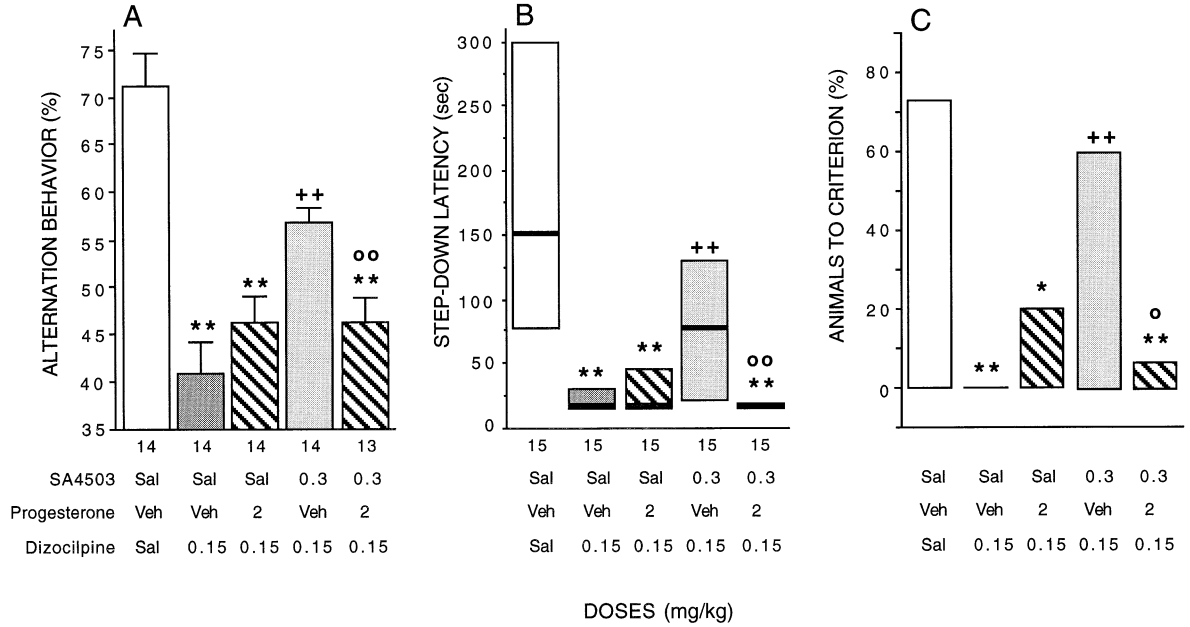

Fig. 5. Antagonism by progesterone of the SA4503 effect on the dizocilpine-induced learning impairments: (A) alternation behavior in the Y-maze test, (B) step-down latency and (C) percentage of animals reaching criterion in the step-down type passive avoidance test. Progesterone (2 mg/kg s.c.) was administered 10 min before SA4503 (0.3 mg/kg s.c.), administered simultaneously with dizocilpine (0.15 mg/kg i.p.), which was given 30 min before the Y-maze session or the first passive avoidance training. The number of animals per group is indicated below the columns in (A) and (C). Sal, saline solution; Veh, vehicle for progesterone was sesame oil. * $P < 0.05$, ** $P < 0.01$ vs. the control group, $^{++}$ $P < 0.05$ vs. the (dizocilpine + Sal)-treated group, $^{\circ}$ $P < 0.05$, ^{oo} $P < 0.01$ vs. the (dizocilpine + SA4503)-treated group (Dunn's test).

dehydroepiandrosterone sulfate (manuscript in preparation). Progesterone, 2 mg/kg s.c., did not affect the dizocilpine-induced deficits in alternation, and blocked the attenuating effect of SA4503 (KW = 36.11, $P < 0.01$; Fig. 5A). In the passive avoidance task, progesterone did not alter the dizocilpine-induced retention deficits, but prevented the anti-amnesic effect of SA4503, in terms of latency (KW = 29.28, $P < 0.01$; Fig. 5B) and percentage of animals reaching criterion (KW = 29.26, $P < 0.01$; Fig. 5C). Furthermore, progesterone did not modify shock sensitivity during the first passive avoidance training session (KW = 4.00, $P > 0.05$; data not shown).

3.5. Antagonism by haloperidol and progesterone of the SA4503 effect on the L-NAME-induced spatial working memory deficit

The antagonist effects of haloperidol and progesterone were also examined on the anti-amnesic effect of SA4503 in the L-NAME-induced deficits in spontaneous alternation, as shown in Fig. 6. Haloperidol (0.05 mg/kg i.p.) did not itself attenuate the L-NAME-induced deficit, but significantly blocked the beneficial effect of SA4503 (KW = 18.33, $P < 0.01$; Fig. 6A). Similarly, progesterone (2 mg/kg s.c.) failed to alter the L-NAME-induced deficit in alternation, but prevented the SA4503 effect (KW = 25.76, $P < 0.01$; Fig. 6B).

4. Discussion

The present study demonstrated the anti-amnesic effects of SA4503, a novel cognitive enhancer with selective σ_1 receptor agonist properties (Matsuno et al., 1996), on learning impairments related to the blockade of NMDA receptor activation. SA4503 attenuated the deficits in working and in long-term memory induced by dizocilpine, the non-competitive NMDA receptor antagonist. The drug was efficient at low doses, in the 100–300 μg/kg s.c. range, with a bell-shaped curve for the effect, as previously observed for other σ_1 receptor agonists (Maurice et al., 1994a,b,c). These effects involved σ_1 receptors, since they could be blocked by the σ_1 receptor antagonist, haloperidol. These results confirmed the promising neurobehavioural profile of the drug, which was previously shown to attenuate scopolamine-induced amnesia in rodents at a similar dose (Matsuno et al., 1997). The present report provides new data on the potential mechanism of the σ_1 receptor-mediated anti-amnesic effects.

SA4503 is a ligand with high affinity and selectivity for the σ_1 receptor subtype (Matsuno et al., 1996). It exerts significant effects on cholinergic neurotransmission, increasing extracellular acetylcholine levels as measured by in vivo microdialysis in the rat hippocampus and cortex, in a haloperidol (0.1 mg/kg)-sensitive manner (Matsuno et al., 1997). It was previously reported that other prototypical σ_1 receptor ligands, such as (+)-SKF-10,047, increase acetylcholine release from guinea-pig cortical slices in

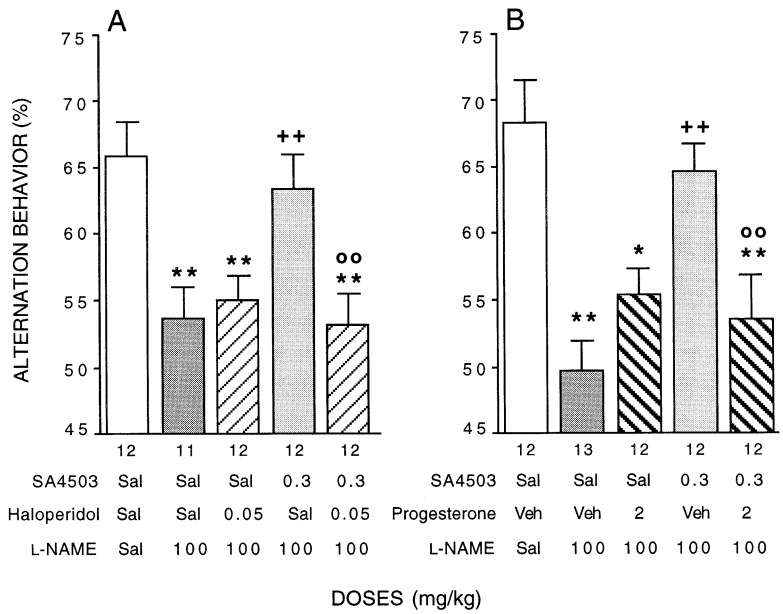

Fig. 6. Antagonism by (A) haloperidol or (B) progesterone of the SA4503 effect on the L-NAME-induced deficit in spontaneous alternation behavior in the Y-maze test. Haloperidol (0.05 mg/kg i.p.) or progesterone (2 mg/kg s.c.) was administered 10 min before SA4503 (0.3 mg/kg s.c.), administered simultaneously with L-NAME (100 mg/kg i.p.), which was given 30 min before the session. Sal, saline solution; Veh, vehicle for progesterone was sesame oil. * $P < 0.05$, ** $P < 0.01$ vs. the control group, $^{++}$ $P < 0.05$ vs. the (L-NAME + Sal)-treated group, ^{oo} $P < 0.01$ vs. the (L-NAME + SA4503)-treated group (Dunn's test).

vitro and in the rat frontal cortex in vivo (Siniscalchi et al., 1987; Matsuno et al., 1992, 1993a, 1995). (+)-SKF-10,047 also potentiates the KCl-evoked [^{3}H]acetylcholine release from rat hippocampal slices in vitro and the high-affinity [^{3}H]choline uptake from mouse hippocampal synaptosomes ex vivo, these latter effects being stereoselective and reversed by haloperidol (Junien et al., 1991; Roman et al., 1992). Interestingly, the drug did not affect the extracellular acetylcholine levels in the striatum, suggesting that it may not cause striatal cholinomimetic side-effects, in contrast to tacrine (Matsuno et al., 1997). Consequently, these ligands more likely attenuated the amnesia induced by blockade of the muscarinic acetylcholine receptors, by, e.g., scopolamine. Earley et al. (1991) first reported that the σ receptor ligands, DTG, (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) and (+)-*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-1-ethyl-but-3-en-1-yl-amine hydrochloride (JO-1784), at doses in the mg/kg range, significantly prevented the scopolamine-induced step-through passive avoidance deficits. Matsuno et al. (1993b, 1994, 1995) examined the effects of the σ_1 receptor agonists, (+)-SKF-10,047, (±)-pentazocine, DTG, or (+)-3-PPP, on the memory impairment induced by scopolamine and by the serotonin depleter, *p*-chloroamphetamine, using the step-down passive avoidance task in mice. The ligands demonstrated anti-amnesic abilities when they were administered before or after training, or before retention, indicating that they could improve memory during either the acquisition, consolidation or retention phase (Matsuno et al., 1994). Similar results were recently obtained with SA4503, the drug being effective at low doses, in the 0.1–0.25 mg/kg p.o. range (Matsuno et al., 1997).

It appears that SA4503 has a similar efficiency against the learning impairment induced by either blockade of the cholinergic systems or antagonism of the activation of the NMDA receptor, although the models may involve different mechanisms. Indeed, selective σ_1 receptor ligands attenuated the dizocilpine-induced learning impairments. We have previously reported that DTG, (+)-SKF-10,047, (+)-pentazocine and PRE-084 reverse the dizocilpine-induced learning impairments in a variety of behavioral tests in mice (Maurice et al., 1994a,b,c). Results of recent studies (Earley et al., 1995; Ohno and Watanabe, 1995), and the present study further support our conclusions, indicating that σ_1 receptor agonists are able to reverse the behavioural effects induced by blockade of the NMDA receptor-mediated neurotransmission. A functional interaction between σ_1 receptors and NMDA receptor complex was established in several previous studies, showing that σ_1 receptor agonists allowed a marked potentiation of several NMDA-evoked responses (Monnet et al., 1990, 1991, 1992; Roman et al., 1991). Low doses of σ_1 receptor agonists, including DTG, JO-1784, (+)-pentazocine and BD-737, enhanced the NMDA-induced electrophysiological activation of CA_3 pyramidal neurons in the rat hippocampus. This effect was antagonized by haloperidol, BMY-14802 and (+)-3-PPP (Monnet et al., 1990, 1992). In vitro, low nanomolar doses of σ_1 receptor ligands modulated the NMDA-evoked [^{3}H]norepinephrine release from rat hippocampal slices (Monnet et al., 1991; Roman et al., 1991). Such physiological actions are likely to underlie behavioural involvement in learning and memory processes, since both hippocampal NMDA and norepinephrine systems are involved in memory. The effect of SA4503 on these NMDA-evoked responses has not yet been characterized, but it may be similar to that of other σ_1 receptor agonists as shown by its efficiency to attenuate the dizocilpine-induced learning impairment. Interestingly, the efficient dose range for the attenuating effect of SA4503 on learning impairment appeared to be bell-shaped, as usually observed for cognitive enhancers. It is proposed that the cognitive enhancing effect involves primarily facilitation of attention, and thus, at low doses, the improvement of attention will concern attention to specific cues necessary for learning, while at higher doses, there will also be attention to non-specific cues that, in turn, impede the quality of learning. An alternative explanation could thus be that the bell-shaped curve for behavioural responses reflect directly the bell-shaped dose-response effects observed in electrophysiological or superfusion studies (Monnet et al., 1990, 1991, 1992; Roman et al., 1991). The mechanism of the σ_1 receptor-mediated potentiation of the NMDA-evoked responses could explain the bell-shaped curve of behavioural responses, although this remains to be clarified.

A major consequence of these results is that σ_1 receptor may allow a significant improvement in amnesia models related to normal or pathological aging, since both cholinergic and glutamatergic systems are affected during aging. We recently reported that the selective σ_1 receptor ligands, JO-1784 and PRE-084, had beneficial effects in a model of age-related cognitive deficits, the senescence-accelerated (SAM) mice (Maurice et al., 1996a). SA4503, since it appears to be one of the most efficient σ_1 receptor ligands, is currently being tested against the age-related learning deficits of SAM.

One point that remains to be elucidated is whether the effect of SA4503 on cholinergic systems is related to modulation of NMDA receptor activation. First, NMDA receptors regulate acetylcholine release in several brain structures, including the cortex and hippocampus (Lodge and Johnston, 1985; Snell and Johnson, 1986; Jones et al., 1987; Nishimura and Boegman, 1990). Nicotine and other nicotinic acetylcholine receptor agonists potentiate the activity of glutamatergic synapses in the neocortex through the involvement of presynaptic receptors (Vidal, 1994). A functional cooperation between cholinergic and glutamatergic systems, in the cortex and presumably in the hippocampus, may underlie the mechanism of their involvement in learning and memory processes (Vidal, 1994). In turn, selective σ_1 receptor agonists, such as SA4503, facilitate acetylcholine receptor functions as efficiently as

NMDA receptor activation, yielding cognitive enhancers of particular interest. It has been shown that σ_1 receptor ligands improve the cholinergic-dependent memory processes during the three different memory phases (Matsuno et al., 1994). However, NMDA receptor activation is known to be involved mainly in learning processes, the NMDA receptor antagonists being ineffective if they are administered during consolidation or just before retention (Danysz and Wroblewski, 1989; Venable and Kelly, 1990). It thus seems likely that direct and independent interactions between σ_1 receptor ligands and either the cholinergic or glutamatergic systems are involved in their effects on memory. Further studies are in progress to establish if the effects of SA4503 on the cholinergic system (Matsuno et al., 1997) and on the glutamatergic system (the present study) involve distinct mechanisms.

Previous studies by Yamada et al. (1996a,b) demonstrated that although activation of the NMDA receptors is involved in both the spatial working memory and long-term memory processes, different neuronal mechanisms are involved. The NO/cyclic GMP signalling system may play a role in spatial working memory, whereas either cerebral protein synthesis or interaction with the metabolism of neurotransmitters, such as serotonin or dopamine, may be involved in the passive avoidance response (Yamada et al., 1996b). Indeed, similar pharmacology could be observed between the dizocilpine- and L-NAME-induced impairments of alternation, in particular the NO synthase substrate, L-arginine, the generator of NO *S*-nitroso-*N*-acetylpenicillamine, or the cGMP analog, bromo-cGMP, inhibiting the dizocilpine- and L-NAME-induced deficits (Yamada et al., 1996a). The authors concluded that the spatial working memory impairment induced by blockade of NMDA receptors may be due primarily to the reduction of NO/cGMP signalling pathways, as proposed for the development of long-term potentiation in the hippocampus (Yamada et al., 1996b). We now confirmed that L-NAME induced deficits in alternation, but did not affect the passive avoidance response. We also observed that SA4503 attenuated L-NAME-induced deficits and the dizocilpine-induced deficits similarly, i.e., over a similar dose-range, with a bell-shaped dose-response profile, and in a haloperidol- and progesterone-sensitive manner. These observations confirmed the crossed pharmacology between the two models, and demonstrated that σ_1 receptor ligands exert their beneficial effect on the learning impairments induced by blockade of the NMDA receptor activation through an effect on NO signalling pathways, i.e., by facilitating the NMDA receptor-dependent second messenger system. It remains to be found if SA4503 modulates, through an effect on NO formation, the release or uptake of neurotransmitters potentially involved in learning and memory processes.

Finally, it was important to show that progesterone, which did not affect the behaviour of control or dizocilpine-treated animals, blocked the SA4503 effects on both amnesia models. The interaction between neurosteroids and σ_1 receptors was first suggested by Su et al. (1988), who used in vitro [^{3}H](+)-SKF-10,047 binding in rat brain homogenates and [^{3}H](+)-haloperidol binding in spleen homogenates. Progesterone was a potent inhibitor of the binding to σ_1 receptors, with a K_i value of about 268 nM. Several studies have extended these initial observations, in particular by using in vivo binding of [^{3}H](+)-SKF-10,047 to σ_1 sites in the mouse forebrain (Maurice et al., 1996b). Furthermore, results of functional studies have also suggested that progesterone acts as a potent σ_1 receptor antagonist. Monnet et al. (1995) reported that progesterone mimics the antagonist effect of haloperidol by blocking the effects of DHEAS and pregnenolone sulfate and the effect of the non-steroidal σ_1 receptor agonists, on the NMDA-evoked release of [^{3}H]norepinephrine from rat hippocampal slices. Bergeron et al. (1996) reported that dehydroepiandrosterone potentiates the NMDA-evoked electric neuronal activity in the CA_3 area of the rat hippocampus, an effect blocked by the σ_1 receptor antagonists, haloperidol and NE-100, and by progesterone. The present results extend these functional observations by demonstrating the behavioral relevance of the neurosteroids/σ_1 systems interaction and the clear antagonist effect of progesterone. The neuromodulatory effects induced by the σ_1 systems must thus be examined, keeping in mind the major role of the physiologic modulations of progesterone levels.

In summary, we now reported on the beneficial effects of the selective σ_1 receptor agonist, SA4503, on the learning processes involved in short-term as well as long-term memory, depending on the activation of the NMDA receptor. New insights into the mechanism of the anti-amnesic effect of σ_1 receptor ligands were presented. Finally, the ligand appears as a particularly promising therapeutic tool that may improve age-related cognitive deficits, because of its equal efficiency against cholinergic as well as glutamatergic amnesia models.

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