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Ameliorating effect of SA4503, a novel σ_1 receptor agonist, on memory impairments induced by cholinergic dysfunction in rats

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Abstract

We found a potent and selective σ_1 receptor agonist, SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride), with high affinity for the σ_1 receptor subtype (IC_{50} = 17 nM), but low affinity for the σ_2 receptor subtype (IC_{50} = 1800 nM). The binding activity and selectivity of SA4503 resembled those of (+)-pentazocine, a prototype σ_1 receptor agonist. We have previously shown that the σ_1 receptor agonist activated central cholinergic functions. Therefore, we examined the effects of SA4503 on the cholinergic dysfunction-induced memory impairments in a passive avoidance task. Scopolamine, a muscarinic acetylcholine receptor antagonist, produced memory impairment, when it was administered 30 min before the training session of the passive avoidance task in rats. Single administration of SA4503 significantly reduced the scopolamine-induced memory impairment. In addition, the lesioning by injection of α-amino-3-hydroxy-5-isoxazole acetic acid (ibotenic acid) into the basal forebrain area produced memory impairment in rats. Repeated administration of SA4503 after lesioning of the basal forebrain area ameliorated the basal forebrain lesion-induced memory impairment. Moreover, the ameliorating effect of SA4503 against the scopolamine-induced memory impairment was antagonized by both 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone (haloperidol), a σ receptor antagonist, and *N,N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride (NE-100), a putative σ_1 receptor antagonist. These results suggest that SA4503 has an anti-amnesic effect against cholinergic dysfunction-induced memory impairment, and that the effect of SA4503 is mediated by the σ_1 receptor subtype.

Keywords: σ_1 Receptor agonist; SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride); Cognitive enhancer; Scopolamine; Basal forebrain lesion; Passive avoidance task; (Rat)

1. Introduction

The central cholinergic system plays an important role in the complicated process of learning and memory (Deutsch, 1983; Smith, 1988). In clinical studies, it was shown that cholinergic activity decreased and that the neurons from the nucleus basalis of Meynert (corresponding to the basal forebrain area in rats), in which the cell bodies of acetylcholine neurons are abundant (Smith, 1988), were lost in the brains of patients with Alzheimer's disease (Bowen et al., 1983; Gottfries, 1985; Etienne et al., 1986). Therefore, these findings have provided a rationale for treating Alzheimer's disease patients with cholinomimetic drugs (Becker, 1991), and for the usefulness of animal models with central cholinergic dysfunction-induced memory impairment in testing therapeutic drugs for Alzheimer's disease (Flicker et al., 1983; Bartus et al., 1985; Yamazaki et al., 1995; Flood and Cherkin, 1986). Actually, 9-amino-1,2,3,4-tetrahydroacridine (tetrahydroaminoacridine), an acetylcholinesterase inhibitor, which has been used clinically for treating Alzheimer's disease (Summers et al., 1981, 1986), ameliorates the memory impairments caused by both scopolamine, a muscarinic acetylcholine receptor antagonist, and basal forebrain lesion in rodents (Chapin and Nielsen, 1988; Kwo-On-Yuen et al., 1990; Matsuno et al., 1993b; Yoshida and Suzuki, 1993). Therefore, drugs which activate the central cholinergic system and ameliorate the cholinergic dysfunction-induced memory impairments in rodents have been suggested to be effective in treating Alzheimer's disease.

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The central σ receptor was reported to be involved in neuronal transmission in the central nervous system (for reviews, see Walker et al., 1990; Ferris et al., 1991; Su, 1991). Particularly in the central cholinergic system, (2α,6α)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol ((±)-*N*-allylnormetazocine, (±)-SKF-10,047), a prototype σ receptor agonist (Itzhak, 1994), has been reported to enhance stimulation-evoked acetylcholine release in guinea-pig cerebral slices (Siniscalchi et al., 1987). In addition, (+)-SKF-10,047, a selective isomer of the σ_1 receptor subtype, also potentiated potassium chloride (KCl)-evoked acetylcholine release in rat hippocampal slices (Junien et al., 1991). Subsequently, we have shown that (+)-SKF-10,047 dose-dependently increased the extracellular acetylcholine level in the rat frontal cortex (Matsuno et al., 1992, 1993a) and hippocampus (Matsuno et al., 1995). Furthermore, (+)-SKF-10,047 was predominantly involved in the central cholinergic system, because it did not affect the cortical extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) level when it was administered in the same dose ranges (Matsuno et al., 1992). Thus, the selective σ_1 receptor agonist was expected to preferentially activate the central cholinergic system. In addition, we reported that (+)-SKF-10,047 ameliorated the impairment of passive avoidance tasks in mice and rats, and that these anti-amnesic effects of (+)-SKF-10,047 were mediated via the σ_1 receptor subtype and the central cholinergic system (Matsuno et al., 1994, 1995). These findings suggested that selective σ_1 receptor agonists may be useful as novel types of therapeutic drugs against Alzheimer's disease.

On the basis of this hypothesis, we found the novel σ_1 receptor agonist, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503) (Fig. 1). Our preliminary studies indicated that SA4503 bound to the σ_1 receptor subtype with high affinity but to the other 36 neurotransmitters' receptors, ion channel and second messenger systems with low affinity (Matsuno et al., 1996a), and that it increased the extracellular acetylcholine level in the rat frontal cortex (Kobayashi et al., 1996b; Matsuno et al., 1996b). In the present study, we examined the effect of SA4503 on the scopolamine- and basal forebrain lesion-induced memory impairment in rats. In addition, we examined whether the SA4503-induced anti-amnesic effect is mediated by the σ_1 receptor subtype.

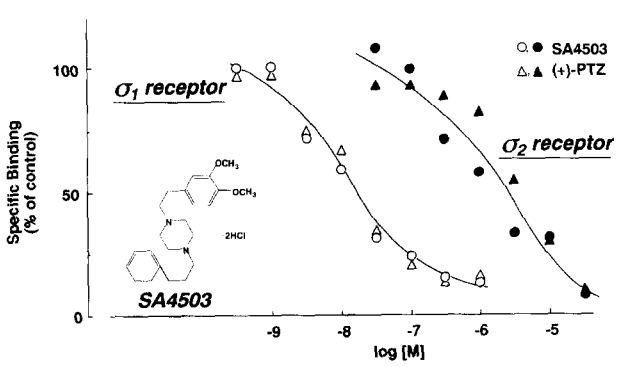

Fig. 1. Inhibition curves of SA4503 (○, ●) and (+)-pentazocine ((+)-PTZ, △, ▲) for the [^{3}H](+)-pentazocine (σ_1 receptor subtype: open symbol) and [^{3}H]DTG (with 200 nM (+)-pentazocine; σ_2 receptor subtype: closed symbol) binding in the guinea-pig brain membrane. Insert is the chemical structure of SA4503. The binding study was carried out with guinea-pig brain membranes which were incubated with each radioligand in the presence of several concentrations of SA4503 or (+)-pentazocine as described in the text. Values are expressed as in a typical experiment. Each experiment was repeated at least 3 times with similar results.

2. Materials and methods

The procedures involving animals and their care were conducted in conformity with the institutional guidelines which comply with the Guide for the Care and Use of Laboratory Animals (NIH Publication, No. 85-23, 1985).

2.1. Animals

Male Hartley guinea pigs, each weighing 300–400 g (Nihon SLC, Shizuoka, Japan), male Wistar rats, each weighing 200–300 g (Nihon SLC), and male F-344 rats, each weighing 250–350 g (Nihon Charles River, Kanagawa, Japan), were used. They were housed 4 per cage with free access to food and water in a controlled environment (23 ± 1°C, 55 ± 10% humidity), with a 12-h light-dark cycle (light on between 07:00 and 19:00 h). They were used following at least 7 days of adaptation to laboratory conditions.

2.2. σ Receptor-binding study

The membrane preparation from guinea-pig whole brain was obtained as described previously (Matsuno et al., 1993a,c; Senda et al., 1995b). The animals were decapitated. Their brains were removed and homogenized in 8 vols. (w/v) of ice-cold 0.32 M sucrose in 50 mM Tris-HCl buffer (pH 7.7 at 25°C, standard buffer) using a Wheaton glass-glass homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min at 4°C. The supernatant fraction was collected and centrifuged at 47 000 × *g* for 20 min at 4°C. The resulting membrane pellet was suspended in 20 vols. (w/v) of standard buffer. The suspension was centrifuged again, and the pellet was resuspended in fresh standard buffer for the binding assay. The protein content in the final suspension was about 0.6 mg/ml determined by the method of Lowry et al. (1951).

Binding of [^{3}H](+)-pentazocine and [^{3}H]1,3-di-(2-tolyl)guanidine ([^{3}H]DTG) (in the presence of 200 nM (+)-pentazocine) was used to assess the affinities of SA4503 and (+)-pentazocine for σ_1 and σ_2 receptor subtypes, respectively (Chaki et al., 1994; Leitner et al., 1994). The reaction was initiated by the addition of 0.2 ml of the membrane preparation to a mixture containing 5 nM [^{3}H](+)-pentazocine or 5 nM [^{3}H]DTG and unlabeled

SA4503 or (+)-pentazocine in a final volume of 1.0 ml. Incubations were carried out at 37°C for 150 min in the [^{3}H](+)-pentazocine-binding study and at 25°C for 90 min in the [^{3}H]DTG-binding study. The binding reaction was stopped by rapid vacuum filtration through Whatmann GF/B glass filters pre-soaked with 0.5% polyethyleneimine. The filters were washed 3 times with 4 ml of ice-cold standard buffer and counted in a liquid scintillation counter. Non-specific binding was determined in each case in the presence of 100 μM of each unlabeled ligand. Each compound was determined at least 3 times, each in triplicate.

2.3. Passive avoidance task

The apparatus for testing the step-through type passive avoidance learning tasks consisted of 2 compartments, 1 light compartment (25 cm long, 18 cm wide and 20 cm high) and 1 dark compartment (15 cm long, 20 cm wide and 20 cm high), equipped with a grid floor, and connected via a guillotine door (Matsuno et al., 1995).

Each rat was gently placed in the light compartment. After 10 s, the guillotine door was opened. When the rat entered the dark compartment, the guillotine door was closed. After 5 s, an electric shock (50 V for 3 s in the case of scopolamine-induced memory impairment and 70 V for 3 s in the case of basal forebrain lesion-induced memory impairment) was delivered to the animal via the grid floor. The time taken to do so was recorded in seconds. The retention test was carried out 24 h after the training session. The rat was put in the light compartment and the time taken to enter the dark compartment was recorded (step-through latency). The step-through latency was recorded up to a maximum cut-off time of 600 s. The training session and retention test were performed between 11:00 and 18:00 h.

The scopolamine-induced memory impairment was produced by the administration of scopolamine (0.75 mg/kg i.p.) to Wistar rats 30 min prior to the training session (Matsuno et al., 1995, 1996b). SA4503 and (+)-pentazocine were administered orally or s.c. 30 min before the training session, respectively. Tetrahydroaminoacridine was administered orally 60 min prior to the training session. To examine the antagonistic effects of 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone (haloperidol) or *N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride (NE-100) (Okuyama et al., 1993), they were administered i.p. 60 min or orally 15 min before the administration of SA4503 or (+)-pentazocine, respectively.

Lesions of the bilateral basal forebrain areas were made by the method of Nabeshima et al. (1991) with minor modifications. Briefly, F-344 rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and fixed on a stereotaxic apparatus. Bilateral neurotoxic lesions of the basal forebrain areas were produced by injecting α-amino-3-hydroxy-5-isoxazole acetic acid (ibotenic acid). An injection needle connected to a 5-μl microsyringe was inserted into the basal forebrain area, according to the Paxinos and Watson (1986) atlas of the rat brain (1.5 mm posterior, 2.8 mm bilateral to the bregma, 7.2 mm below the dura). Ibotenic acid dissolved in 50 mM phosphate buffer (pH 7.4), at a concentration of 20 μg/μl, 0.5 μl (10 μg/side) was infused for 5 min. The injection needle was left in place for 10 min more to ensure that the drug had diffused away from the needle tip. Sham-operated rats received 50 mM phosphate buffer instead of ibotenic acid. 10 days after the basal forebrain lesion, administration of SA4503 started. Each rat received SA4503 or vehicle orally for 15 days: initially, the drugs were administered once a day for 13 days before the passive avoidance task, and then 30 min prior to the training session and retention test. The day after the retention test of the passive avoidance task, cortical choline acetyltransferase and acetylcholinesterase activity in the sham-operated and basal forebrain-lesioned control groups assays were done (Matsuno et al., 1993a). The rats were decapitated, and the frontal cortex was rapidly dissected (Glowinski and Iversen, 1966). The enzyme solution for the measurement of choline acetyltransferase activity was prepared from dissected frontal cortex by homogenization in 12.5 ml of 25 mM sodium phosphate buffer (pH 7.4) per g of wet weight, using a Teflon homogenizer, followed by centrifugation at 20000 × *g* for 60 min at 4°C. The supernatant was diluted 10-fold in the above buffer and used as an enzyme solution in which the final concentration of protein was about 0.2 mg/ml. The standard incubation mixture consisted of the following components in a total volume of 200 μl (final concentrations in parentheses): 100 μl of 0.1 M sodium phosphate buffer, pH 7.4 (0.05 M) containing 0.4 mM acetyl-CoA (0.2 mM), 10 mM choline chloride (5 mM), 0.2 mM physostigmine (0.1 mM), 0.3 M sodium chloride (0.15 M) and 20 mM (ethylenedinitrilo)tetraacetic acid (EDTA)-2Na (10 mM), and 100 μl of enzyme solution. Incubation was carried out at 37°C for 20 min, and the reaction was stopped with 50 μl of 1 M perchloric acid in an ice-bath; 10 min later 6 μl of 1 mM isopropylhomocholine was added as an internal standard, and the reaction mixture was centrifuged at 1600 × *g* for 10 min at 4°C. A 100-μl aliquot of the supernatant was taken, of which a 10-μl aliquot was injected into the high-performance liquid chromatography and electrochemical detector (HPLC-ECD) system. The resultant acetylcholine was detected with the same system.

The enzyme solution for the measurement of acetylcholinesterase activity was prepared from dissected frontal cortex by homogenization in 12 ml of 25 mM potassium phosphate buffer (pH 7.0) per g of wet weight, using a Teflon homogenizer at 4°C. The homogenate was diluted 70 times in the above buffer and used as an enzyme solution in which the final concentration of protein was about 0.1 mg/ml. The standard incubation mixture con-

sisted of the following components in a total volume of 1.5 ml (final concentrations in parentheses): 1 ml of 0.075 M potassium phosphate buffer, pH 7.0 (0.05 M) containing 0.15 M sodium chloride (0.1 M) and 0.03 M magnesium chloride (0.02 M), 3 mM acetylcholine in water (2 mM), and 500 μl of enzyme solution. Incubation was carried out at 37°C for 15 min, and then stopped with 400 μl of 5% metaphosphoric acid in an ice-bath; 10 min after stop of the reaction, 200 μl of 1 mM ethylhomocholine in water was added as an internal standard, and the reaction mixture was centrifuged at 1600 × *g* for 10 min at 4°C. A 100-μl aliquot of the supernatant was taken, of which a 10-μl aliquot was injected into the HPLC-ECD system. The resultant choline was detected with the same system. For the control experiments, the enzyme solution was boiled at 95°C for 5 min. The protein content was determined by the method of Lowry et al. (1951).

2.4. Response to electric footshock

The flinch, vocalization and jump thresholds with electric footshock were measured as described previously (Matsuno et al., 1994). The apparatus for testing responses to electric footshock consisted of a 25 × 25 × 30-cm Plexiglas rectangular box featuring a grid floor, 2.1-mm stainless steel rods set 7.5 mm apart. Each rat (Wistar) was placed on the grid floor and a scrambled electric footshock was delivered. The shock intensity was raised stepwise manually from 0.1 mA to 2.0 mA (every 0.1 mA; shock duration: 0.5 s; inter-shock interval: 5 s) until a flinch, vocalization and jump were observed. SA4503 and (+)-pentazocine were administered orally and s.c. 30 min prior to testing, respectively.

2.5. Statistical analysis

In the binding study, the concentrations of test drugs causing 50% inhibition of radioligand binding (IC_{50}) were determined by Hill's analysis using a computer-assisted linear least-squares regression analysis, and the results were expressed as the means ± S.E.M. In the behavioral study, the results were expressed as medians and interquartile ranges. Statistical comparisons were made with the Mann-Whitney *U*-test for 2 groups and with the Kruskal-Wallis test, a non-parametric one-way analysis of variance, followed by a Dunn-type test (the number of animals is not equal) or a Tukey-type test (the number of animals is equal) for multiple comparisons. Choline acetyltransferase and acetylcholinesterase activity in the basal forebrain-lesioned control groups was expressed as the mean ± S.E.M. of the percentages for the average of the sham-operated group. Statistical comparisons between the sham-operated and basal forebrain-lesioned control groups were made using the Mann-Whitney *U*-test. Differences with *P* values of less than 0.05 were considered to be statistically significant.

2.6. Drugs

The following drugs were used: SA4503, (+)-pentazocine and NE-100 (synthesized in our laboratory); DTG (Research Biochemicals, Wayland, MA, USA); scopolamine hydrobromide (Tokyo Kasei, Tokyo, Japan); tetrahydroaminoacridine (Aldrich Chemical, Milwaukee, WI, USA); haloperidol (Sumitomo Pharmaceutical, Osaka, Japan); ibotenic acid (Sigma, St. Louis, MO, USA); [^{3}H](+)-pentazocine (specific activity 1169.2 GBq/mmol, New England Nuclear (NEN), Boston, MA, USA) and [^{3}H]DTG (specific activity 1457.8 GBq/mmol, NEN). Other chemicals and reagents of analytical grade were obtained from commercial suppliers.

In the in vitro study, SA4503 and (+)-pentazocine were dissolved in dimethyl sulfoxide (DMSO) containing equimolar hydrochloric acid (HCl). The final concentration of DMSO was less than 1%.

In the in vivo study, scopolamine and haloperidol were dissolved in saline. SA4503, tetrahydroaminoacridine and NE-100 were suspended in 1% methylcellulose. (+)-Pentazocine was initially dissolved in 1 M HCl and neutralized with 1 M NaOH. All drugs were given at a dose of 0.5 ml/100 g body weight. Doses are expressed in terms of the salts.

3. Results

3.1. σ Receptor-binding study

Competition binding experiments showed that SA4503 had high affinity for the σ_1 receptor subtype in guinea-pig brain membrane (Fig. 1). The IC_{50} value of SA4503 was 17 ± 1.9 nM, compared to 14 ± 3.0 nM for (+)-pentazocine, a selective σ_1 receptor agonist. However, both SA4503 and (+)-pentazocine had low affinity for the σ_2 receptor subtype (SA4503: IC_{50} = 1800 ± 310 nM; (+)-pentazocine: IC_{50} = 2900 ± 580 nM). The affinity of SA4503 for the σ_1 receptor subtype was about 100-times greater than that for the σ_2 receptor subtype.

3.2. Effects of single administration of SA4503, (+)-pentazocine and tetrahydroaminoacridine on the scopolamine-induced memory impairment

The vehicle-treated rats that received an electric shock during the training session showed a prolonged step-through latency in the retention test. Scopolamine administered 30 min before the training session significantly shortened the step-through latency in the retention test ($H(4) = 28.60$, $P < 0.01$, Fig. 2A; $H(4) = 27.16$, $P < 0.01$, Fig. 2B; $H(4) = 27.56$, $P < 0.01$, Fig. 2C; $H(6) = 46.43$, $P < 0.01$, Fig. 4A; $H(6) = 57.93$, $P < 0.01$, Fig. 4B; $H(7) = 45.34$, $P < 0.01$, Fig. 5A; $H(7) = 73.01$, $P < 0.01$, Fig. 5B), which indicated disruption of the retention performance in the passive avoidance response.

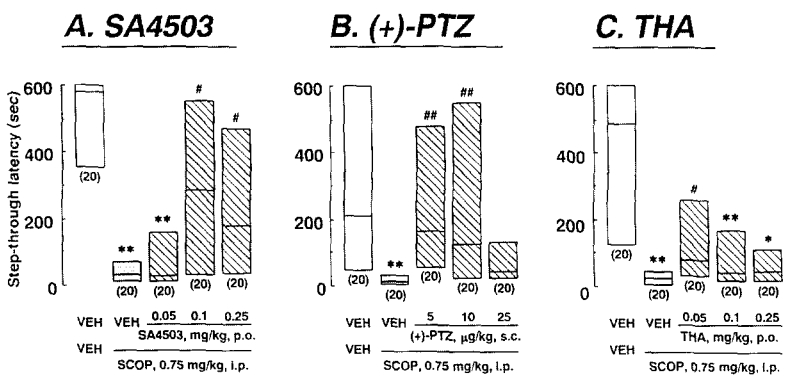

Fig. 2. Effects of single administration of SA4503 (A), (+)-pentazocine ((+)-PTZ, B) and tetrahydroaminoacridine (THA, C) on scopolamine (SCOP)-induced memory impairment in the passive avoidance task in rats. SA4503 and (+)-pentazocine were administered orally and s.c., respectively, 30 min prior to the training session. Tetrahydroaminoacridine was administered orally 60 min prior to the training session. Scopolamine was administered i.p. 30 min before the training session. The retention test was performed 24 h after the training session. Values are expressed as medians and interquartile ranges. The number of rats in each group is indicated in parentheses. * $P < 0.05$, ** $P < 0.01$ as compared with the vehicle (VEH) + VEH group. # $P < 0.05$, ## $P < 0.01$ as compared with the SCOP + VEH group.

This disruption of the retention performance elicited by scopolamine was alleviated by SA4503 at doses ranging from 0.05 to 0.25 mg/kg. Significant anti-amnesic effects were observed at doses of 0.1 and 0.25 mg/kg ($H(3) = 14.09$, $P < 0.01$, Fig. 2A). (+)-Pentazocine also significantly reduced this memory impairment at doses of 5 and 10 μg/kg ($H(3) = 22.75$, $P < 0.01$, Fig. 2B). Furthermore, tetrahydroaminoacridine (0.05 mg/kg), an acetylcholinesterase inhibitor, ameliorated scopolamine-induced memory impairment ($H(3) = 8.72$, $P < 0.05$, Fig. 2C). The step-through latencies in the training session were left unchanged by treatment with these drugs (data not shown).

3.3. Effect of repeated administrations of SA4503 on the basal forebrain lesion-induced memory impairment

The basal forebrain lesion produced aphagia and ataxia for 3–5 days after surgery. These behavioral changes were ameliorated when water and a small amount of food were provided in the home cage, and did not persist beyond 7 days after surgery.

The sham-operated rats that received an electric shock during the training session showed a prolonged step-through latency in the retention test. However, the basal forebrain-lesioned rats had a shortened step-through latency in the retention test ($H(4) = 19.53$, $P < 0.01$, Fig. 3), which indicated disruption of the retention performance in the passive avoidance response.

This disruption of the retention performance elicited by basal forebrain lesion was alleviated by repeated p.o. administration of SA4503 at doses ranging from 0.25 to 1.0 mg/kg per day. A significant anti-amnesic effect was observed at the 0.5 mg/kg per day dose ($H(3) = 11.67$, $P < 0.01$, Fig. 3). The step-through latency of each group in the training session was unchanged (data not shown).

Cortical choline acetyltransferase activity and acetylcholinesterase activity in the basal forebrain-lesioned control rats were significantly reduced to 79 ± 4 and 76 ± 7%, respectively, in comparison with those in the sham-oper-

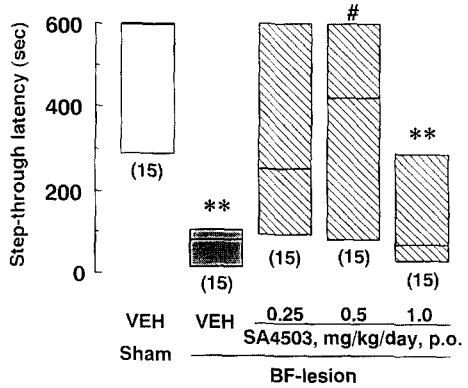

Fig. 3. Effect of repeated administration of SA4503 on basal forebrain (BF) lesion-induced memory impairment in the passive avoidance task in rats. SA4503 was administered orally once a day for 13 days before the passive avoidance task, and 30 min prior to the training session and retention test of the passive avoidance task. For further details, see legend to Fig. 2 and Section 2. ** $P < 0.01$ as compared with the sham + vehicle (VEH) group. # $P < 0.05$ as compared with the BF lesion + VEH group.

Table 1
Effects of SA4503 and (+)-pentazocine on the flinch, vocalization and jump thresholds with electric footshock in rats

Compounds	n	Threshold (mA)		
		Flinch	Vocalization	Jump
SA4503 (mg/kg p.o.)				
VEH	10	0.2 (0.2–0.3)	0.6 (0.5–0.7)	0.7 (0.6–0.8)
0.1	10	0.3 (0.2–0.3)	0.6 (0.6–0.6)	0.8 (0.8–0.9)
0.25	10	0.3 (0.2–0.3)	0.6 (0.5–0.7)	0.8 (0.7–0.9)
(+)-Pentazocine (μg/kg s.c.)				
VEH	10	0.3 (0.2–0.3)	0.6 (0.5–0.7)	0.7 (0.6–0.7)
5	10	0.3 (0.2–0.3)	0.7 (0.5–0.8)	0.7 (0.6–1.0)
10	10	0.3 (0.2–0.3)	0.7 (0.5–0.7)	0.7 (0.7–0.8)

Each drug was given 30 min before the start of measurements. The VEH groups were given the vehicle corresponding to each drug, respectively. Values are expressed as medians and interquartile ranges.

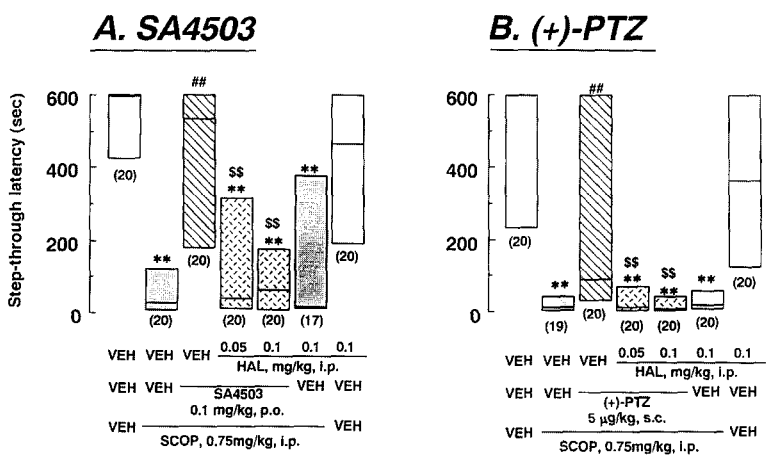

Fig. 4. Antagonism by haloperidol (HAL) of the ameliorating effects of SA4503 (A)- and (+)-pentazocine ((+)-PTZ, B) against scopolamine (SCOP)-induced memory impairment in the passive avoidance task in rats. SA4503 and (+)-pentazocine were administered orally and s.c 30 min prior to the training session, respectively. Haloperidol was administered i.p. 90 min before the training session. For further details, see legend to Fig. 2. ** $P < 0.01$ as compared with the vehicle (VEH) + VEH + VEH group. $^{\#\#}$ $P < 0.01$ as compared with the SCOP + VEH + VEH group. $^{\$\$}$ $P < 0.01$ as compared with the SCOP + SA4503 + VEH or SCOP + (+)-PTZ + VEH group.

ated rats ($P < 0.01$ for both enzymes activities-the averages of enzymes activities in sham-operated rats were: choline acetyltransferase; 1865 ± 168 pmol/min per mg protein, acetylcholinesterase; 50 ± 4 nmol/min per mg protein).

3.4. Antagonism with haloperidol and NE-100 against the ameliorating effects of SA4503 and (+)-pentazocine on the scopolamine-induced memory impairment

To clarify whether the SA4503- and (+)-pentazocine-induced anti-amnesic actions were mediated through the σ receptor, the effects of the σ receptor antagonists were studied. Pre-treatment with haloperidol (0.05 and 0.1 mg/kg), a prototype σ receptor antagonist, reduced the SA4503 (0.1 mg/kg)- and (+)-pentazocine (5 μg/kg)-induced anti-amnesic actions in the rats, respectively (SA4503 vs. haloperidol: $H(2) = 14.82$, $P < 0.01$; (+)-pentazocine vs. haloperidol: $H(2) = 15.89$, $P < 0.01$, Fig. 4). In addition, the ameliorating effects of SA4503 and (+)-pentazocine on the scopolamine-induced memory impairment in rats were antagonized by pre-treatment with NE-100 (0.25, 0.5 and 1.0 mg/kg), a putative σ_1 receptor antagonist, respectively (SA4503 vs. NE-100: $H(3) = 13.46$, $P < 0.01$; (+)-pentazocine vs. NE-100: $H(3) = 16.36$, $P < 0.01$, Fig. 5). The retention performance of normal and scopolamine-treated rats was not affected by either haloperidol (0.1 mg/kg) or NE-100 (1.0 mg/kg) alone at the highest dose used, respectively (Figs. 4 and 5).

3.5. Effects of SA4503 and (+)-pentazocine on the response to electric footshock

As changes in the response to electric footshock in the training session could influence passive avoidance learning, we examined the effects of SA4503 and (+)-penta-

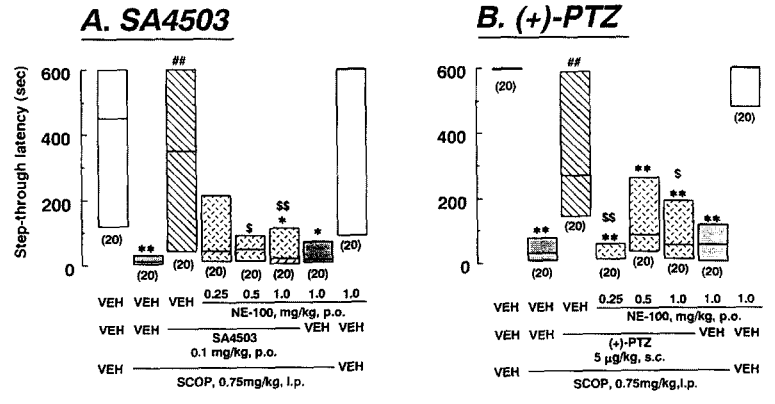

Fig. 5. Antagonism by NE-100 of the ameliorating effects of SA4503 (A)- and (+)-pentazocine ((+)-PTZ, B) against scopolamine (SCOP)-induced memory impairment in the passive avoidance task in rats. SA4503 and (+)-pentazocine were administered orally and s.c. 30 min prior to the training session, respectively. NE-100 was administered orally 45 min before the training session. For further details, see legend to Fig. 2. * $P < 0.05$, ** $P < 0.01$ as compared with the vehicle (VEH) + VEH + VEH group. $^{\#\#}$ $P < 0.01$ as compared with the SCOP + VEH + VEH group. $^{\$}$ $P < 0.05$, $^{\$\$}$ $P < 0.01$ as compared with the SCOP + SA4503 + VEH or SCOP + (+)-PTZ + VEH group.

zocine on the response to electric footshock. As shown in Table 1, SA4503 (0.1 and 0.25 mg/kg) and (+)-pentazocine (5 and 10 μg/kg) did not alter the flinch, vocalization and jump thresholds significantly (SA4503: $H(2) = 0.38$, $P > 0.05$ (flinch), $H(2) = 0.02$, $P > 0.05$ (vocalization), $H(2) = 2.63$, $P > 0.05$ (jump); (+)-pentazocine: $H(2) = 0.10$, $P > 0.05$ (flinch), $H(2) = 3.02$, $P > 0.05$ (vocalization), $H(2) = 1.24$, $P > 0.05$ (jump)).

4. Discussion

The present study showed that SA4503 significantly attenuated the cholinergic dysfunction-induced memory impairments, and that its anti-amnesic effect was mediated by the σ_1 receptor subtype.

Recently, selective σ_1 receptor agonists have been reported to reduce the experimental memory impairment in animals. For example, Maurice et al. (1994a,b) showed that (+)-pentazocine and (+)-SKF-10,047, selective σ_1 receptor agonists (Itzhak, 1994), attenuated the (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*b*]cyclohepten-5,10-imine hydrogen maleate ((+)-MK-801)- and carbon monoxide-induced memory impairment in mice. In addition, we reported that (+)-SKF-10,047 reduced the *p*-chloroamphetamine- and scopolamine-induced memory impairment in mice (Matsuno et al., 1993b,1994; Senda et al., 1995a) and rats (Matsuno et al., 1995) when it was administered before or immediately after the training session or before the retention test of the passive avoidance task. Moreover, we showed that the (+)-SKF-10,047-induced anti-amnesic effect was involved in the central cholinergic system (Matsuno et al., 1994), because its ameliorating effect was antagonized by either scopolamine or 2,2′-(4,4′-biphenylene)bis(2-hydroxy-4,4-dimethyl-morpholinium bromide) (hemicholinium-3), an inhibitor of the Na^+-dependent high-affinity choline uptake. This was supported by our previous finding that (+)-SKF-10,047 dose-dependently increased the extracellular acetylcholine level in the rat frontal cortex and hippocampus as measured by microdialysis (Matsuno et al., 1992, 1993a,1995) and also by the finding that (+)-SKF-10,047 potentiated KCl-evoked acetylcholine release in rat hippocampal slices (Junien et al., 1991). It was similarly reported that SA4503, a selective σ_1 receptor agonist, increased the extracellular acetylcholine level in the rat frontal cortex, as does (+)-SKF-10,047 (Matsuno et al., 1996b). These findings suggest that the ameliorating effect of SA4503 on cholinergic dysfunction-induced memory impairment may be mediated by the activation of central cholinergic functions.

As described in Section 1, the testing of the efficacy of therapeutic drugs against dementing disorders, such as Alzheimer's disease, has involved animal models with memory impairment induced by central cholinergic dysfunction (Flicker et al., 1983; Bartus et al., 1985; Flood and Cherkin, 1986). Therefore, in the present study, we investigated the effect of SA4503 on scopolamine- and basal forebrain lesion-induced memory impairments in rats. Scopolamine, a muscarinic acetylcholine receptor antagonist, is well known to produce memory impairment as a result of cholinergic dysfunction via the blockade of acetylcholine transmission (Kameyama et al., 1986; Yoshida and Suzuki, 1993). The present results showed that tetrahydroaminoacridine, an acetylcholinesterase inhibitor which activates the cholinergic system, reduces the scopolamine-induced memory impairment in rats. In addition, it was reported that lesioning of the basal forebrain area produces destruction of the cell bodies of acetylcholine neurons and reduction of the activity of both cortical choline acetyltransferase and acetylcholinesterase in rats (Miyamoto et al., 1987; Mandel and Thal, 1988; Nabeshima et al., 1991; Matsuoka et al., 1992), because it is known that the basal forebrain area has abundant cell bodies of acetylcholine neurons which project to the cortex (Collerton, 1986; Etienne et al., 1986). In agreement with these reports, the present results show that, in basal forebrain-lesioned rats, cortical choline acetyltransferase and acetylcholinesterase both had their activity reduced. These findings suggest that the scopolamine- and the basal forebrain lesion-induced memory impairment observed in the present study were due to dysfunction of the central cholinergic system. Because SA4503 ameliorated these memory impairments caused by the cholinergic dysfunction, it was suggested that SA4503 may be used as a therapeutic drug for the treatment of dementing disorders, such as Alzheimer's disease.

Moreover, the present findings suggest that SA4503, like (+)-pentazocine, acted as agonist for σ_1 receptor subtype, because the anti-amnesic effect of SA4503 was antagonized by haloperidol or NE-100. Reportedly, haloperidol antagonizes the (+)-SKF-10,047- or DTG-induced colonic motor response to eating (Junien et al., 1990) and duodenal bicarbonate output in rats (Pascaud et al., 1990). Haloperidol also antagonized the (+)-SKF-10,047 or (+)-pentazocine-induced increase in latency of the N126 peak of visual evoked potential recordings in rabbits (Steinfels et al., 1988; Tam et al., 1988). In addition, the head-weaving behavior induced by (+)-SKF-10,047 was antagonized by haloperidol and NE-100 (Okuyama et al., 1993). Monnet et al. (1992) also showed that (+)-pentazocine and (+)-cinnamyl-1-phenyl-1-*N*-methyl-*N*-cyclopropylene (JO-1784), a selective σ receptor ligand (Roman et al., 1990), potentiated *N*-methyl-D-aspartate (NMDA)-induced excitation of pyramidal neurons in the CA_3 region of rats dorsal hippocampus, and that the JO-1784-induced potentiation was antagonized by haloperidol, which did not affect the NMDA-induced excitation. Moreover, binding studies showed that NE-100 had high affinity for the σ receptor and negligible affinity for other receptors, such as dopaminergic, serotonergic and phencyclidine receptors (Okuyama et al., 1993), that the affinities of (+)-pentazocine and NE-100 for the σ_1 re-

ceptor subtype were about 100 and 55 times greater than that for the σ_2 receptor subtype, respectively (Chaki et al., 1994), and that haloperidol bound to both σ_1 and σ_2 receptor subtypes with the same potency (Hellewell and Bowen, 1990; Chaki et al., 1994). The present study also showed that (+)-pentazocine was more selective for the σ_1 receptor subtype than for the σ_2 receptor subtype. Thus, it is assumed that (+)-pentazocine is a selective agonist for the σ_1 receptor subtype, that haloperidol is an antagonist for the σ_1 and/or σ_2 receptor subtype and that NE-100 is a selective antagonist for the σ_1 receptor subtype (Tam et al., 1988; Su, 1991; Monnet et al., 1992; Okuyama et al., 1993). Therefore, we considered that SA4503 was an agonist for the σ_1 receptor subtype. This possibility was supported by our previous finding that the inhibitory potency of SA4503 for [^{3}H](+)-pentazocine binding was weakened in the presence of guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS) (Matsuno et al., 1996a).

The present study showed that the ameliorating effects of SA4503 and (+)-pentazocine were antagonized by haloperidol, which has been reported to interact not only with the σ_1 and/or σ_2 receptor subtype (Hellewell and Bowen, 1990; Chaki et al., 1994), but also with the dopamine D_2 receptor (Burt et al., 1976; Okuyama et al., 1993). Therefore, there is a possibility that the anti-amnesic effect of SA4503 is involved in the D_2 dopaminergic system. However, *R*(−)-10,11-dihydroxyaporphine ((−)-apomorphine), a dopamine receptor agonist, and *N*-phenethyl-*N*-propyl-2-(3-hydroxyphenyl)ethylamine (RU-24213), a dopamine D_2 receptor agonist, reportedly induce memory impairment via the dopamine D_2 receptor (Ichihara et al., 1988, 1992). In addition, *S*(−)-5-(aminosulfonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-2-methoxybenzamide ((−)-sulpiride), a dopamine D_2 receptor antagonist, enhances passive avoidance learning (Ichihara et al., 1988). These findings suggested that the activation of the dopamine D_2 receptor results in memory dysfunction. Therefore, the SA4503 and (+)-pentazocine-induced anti-amnesic effects did not result from activation of the dopamine D_2 receptor.

Finally, it was suggested that SA4503 was more suitable as a therapeutic drug for the treatment of dementing disorders than acetylcholinesterase inhibitors, such as tetrahydroaminoacridine, and other σ_1 receptor agonists, such as (+)-SKF-10,047 and (+)-pentazocine, because it could be expected that the SA4503-induced aversive effects would be less than those elicited by tetrahydroaminoacridine, (+)-SKF-10,047 and (+)-pentazocine. Previously, we reported that the activation of the cholinergic system induced by σ_1 receptor agonists showed regional differences in rat brain. Namely, (+)-SKF-10,047 increased the extracellular acetylcholine level in the frontal cortex, while it did not change it in the striatum (Kobayashi et al., 1996a). Like (+)-SKF-10,047, SA4503 also increased the extracellular acetylcholine level in the frontal cortex, while it did not change it in the striatum (Kobayashi et al., 1996b; Matsuno et al., 1996b). On the contrary, tetrahydroaminoacridine increased the extracellular acetylcholine level in both regions (Matsuno et al., 1996b). These findings indicate that, while tetrahydroaminoacridine produces striatal acetylcholine-mediated aversive effects, such as gait dysfunction, SA4503 does not produce these aversive effects. This speculation was supported by our previous finding that tetrahydroaminoacridine, but not SA4503, induces catalepsy in rats (Matsuno et al., 1996b). In addition, the selectivity of SA4503 for the σ_1 receptor subtype was higher than those of (+)-SKF-10,047 and (+)-pentazocine. Namely, (+)-SKF-10,047 and (+)-pentazocine bound not only to the σ_1 receptor subtype but also to the phencyclidine receptor (Carroll et al., 1992) which is well known to be involved in hallucination in the human (Contreras et al., 1988). However, our previous finding indicated that the binding ability of SA4503 for the phencyclidine receptor was negligible (Matsuno et al., 1996a). Therefore, it was suggested that the SA4503-induced aversive effects which are mediated by the phencyclidine receptor were less than those with (+)-SKF-10,047 and (+)-pentazocine.

In conclusion, SA4503 ameliorates the cholinergic dysfunction-induced memory impairment, and this effect of SA4503 is mediated by the σ_1 receptor subtype.

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