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Binding properties of SA4503, a novel and selective σ_1 receptor agonist

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Abstract

The binding profiles of SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride), a novel σ receptor ligand, to σ_1 and σ_2 receptor subtypes in guinea pig and rat brain membranes were evaluated. SA4503 showed a high affinity for the σ_1 receptor subtype labeled by (+)-[^{3}H]pentazocine ($IC_{50} = 17.4 \pm 1.9$ nM), while it had about 100-fold less affinity for the σ_2 receptor subtype labeled by [^{3}H]1,3-di(2-tolyl)guanidine ([^{3}H]DTG) in the presence of 200 nM (+)-pentazocine. SA4503 showed little affinity for 36 other receptors, ion channels and second messenger systems. The inhibition curves of SA4503 for (+)-[^{3}H]pentazocine binding were shifted to the right in the presence of guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS), as similar to those of (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) and (+)-pentazocine, σ_1 receptor agonists. SA4503 significantly increased the K_D value, but did not affect the B_{max} value for specific (+)-[^{3}H]pentazocine binding. These results indicated that SA4503 is a potent and selective agonist for the σ_1 receptor subtype in the brain. In addition, SA4503 inhibited specific (+)-[^{3}H]pentazocine binding in a competitive manner.

Keywords: SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride); σ_1 Receptor subtype; Agonistic property; Competitive inhibition

1. Introduction

Since its classification was established (Hellewell and Bowen, 1990; Su et al., 1991; Quirion et al., 1992), intensive studies to clarify the physiological functions of the σ receptor subtypes have been carried out. Although the σ_2 receptor subtype has been reported to play a role in motor functions (Walker et al., 1993) and K^+ channels (Jeanjean et al., 1993), the physiological functions of the σ_1 receptor subtype are not well established. Recently, we found that (+)-*N*-allylnormetazocine ((+)-SKF-10,047), a selective σ_1 receptor agonist (Itzhak, 1994), increased acetylcholine release in the rat frontal cortex and hippocampus, and alleviated scopolamine- or *p*-chloroamphetamine-induced memory impairments in mice and rats (Matsuno et al., 1993a, 1994, 1995). In addition, these cholinergic facilitative effects of (+)-SKF-10,047 were superior to those of (−)-SKF-10,047, a stereoisomer with low affinity for the σ_1 receptor, and were completely antagonized by haloperidol (Matsuno et al., 1993a, 1994, 1995), a non-selective σ receptor antagonist. Thus, we suggested that the σ_1 receptor subtype plays an important role in the facilitation of central cholinergic function, and that the specific agonists for the σ_1 receptor subtype may be novel candidates as nootropic drugs.

A wide variety of structurally and pharmacologically diverse compounds have been reported to bind to the σ receptors. However, there have been few reports of potent and selective agonists or antagonists of the σ receptor subtypes. In our recent study, we found a novel σ receptor ligand, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)-piperazine dihydrochloride (SA4503) (Fig. 1). This compound increased acetylcholine release in the rat frontal cortex and hippocampus, and alleviated scopolamine-induced memory impairments in rats (Matsuno et al., 1996). In addition, these cholinergic facilitative effects were completely antagonized by haloperidol, a non-selective σ receptor antagonist, and NE-100, a selective σ_1 receptor antagonist (Chaki et al., 1994), (Matsuno et al., 1996). Thus, it is possible that SA4503 might be a novel agonist for the σ_1 receptor subtype.

In the present study, we characterized the binding properties of SA4503 to the σ_1 and σ_2 receptor subtypes, and compared these properties with those of prototype σ receptor ligands. Our present results indicated that SA4503 is a potent and selective agonist of the σ_1 receptor subtype in the brain.

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2. Materials and methods

The procedures involving animals and their care were conducted in accordance with our institutional guidelines which are in compliance 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) and male Wistar rats each weighing 200–300 g (Nihon SLC) were used. Animals were housed four 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 7:00 a.m. and 7:00 p.m.). Experiments were conducted following adaptation to laboratory conditions for at least 7 days.

2.2. Preparation of brain membranes

Membranes from guinea pig or rat whole brain were prepared as described previously (Matsuno et al., 1993a,b; Senda et al., 1995). Animals were killed by decapitation. Their brains were rapidly removed and homogenized with 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) with a Wheaton glass-glass homogenizer. The homogenates were centrifuged at 1000 × *g* for 10 min at 4°C, and the supernatant was collected and centrifuged at 47 000 × *g* for 20 min at 4°C. The resulting membrane pellets were resuspended with 20 vols. (w/v) of standard buffer. The suspension was centrifuged again, and the pelleted membranes were resuspended in fresh standard buffer for the binding assay. The protein content in the final suspensions was about 1.0 mg/ml (Lowry et al., 1951).

2.3. σ_1 and σ_2 receptor binding

The σ_1 receptor subtype was labeled using (+)-[^{3}H]pentazocine, while the σ_2 receptor subtype was labeled using 1,3-[^{3}H]di(2-tolyl)guanidine ([^{3}H]DTG) in the presence of 200 nM (+)-pentazocine (Chaki et al., 1994; Leitner et al., 1994). The reaction was initiated by addition of 0.2 ml of the membrane preparation to the mixture containing ^{3}H-ligands, and unlabeled σ receptor ligands 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. Competition studies were carried out with 5 nM (+)-[^{3}H]pentazocine or 5 nM [^{3}H]DTG. Saturation studies were conducted over a concentration range of 0.1–50.0 nM (+)-[^{3}H]pentazocine. GTP shift studies were performed with 5 nM (+)-[^{3}H]pentazocine and at least eight concentrations of SA4503 or σ receptor ligands in the absence and presence of 500 μM guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS). Non-specific binding was determined in the presence of 100 μM of each unlabeled ligand. Each experiment was repeated at least three times, and each assay was conducted in triplicate. The binding reaction was stopped by rapid vacuum filtration through Whatmann GF/B glass filters presoaked with 0.5% polyethyleneimine. The filters were washed three times with 4 ml of ice-cold standard buffer and counted in a liquid scintillation counter.

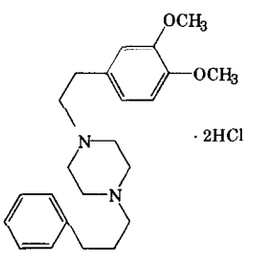

Fig. 1. Chemical structure of SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperizine dihydrochloride).

2.4. Drugs

The following drugs were used: SA4503 and (+)-pentazocine (synthesized by Santen Pharmaceutical Co.); (+)-SKF-10,047, (−)-SKF-10,047, carbetapentane, DTG and (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) (Research Biochemical); haloperidol (Sumitomo Pharmaceutical Co.); GTPγS (Sigma); (+)-[^{3}H]pentazocine (specific activity 1.4 TBq/mmol, New England Nuclear (NEN), Boston, MA) and [^{3}H]DTG (specific activity 1.3 TBq/mmol, NEN). Other chemicals and reagents of an analytical grade were obtained from commercial suppliers. SA4503 or σ receptor ligands were dissolved in dimethyl sulfoxide (DMSO) containing equimolar HCl. The final concentration of DMSO in each preparation was less than 1%.

2.5. Statistical analysis

The results are expressed as means ± S.E.M. The concentration of test drug causing 50% inhibition of radioligand binding (IC_{50}) was determined by Hill's analysis using computer-assisted linear least-squares regression analysis. The values of apparent dissociation constants (K_D) and maximal number of binding sites (B_{max}) were also calculated by linear least-squares regression analysis. The statistical significance of differences between treatment groups and controls was determined by analysis of variance (ANOVA) followed by Dunnet's multiple range comparison test, and difference between two groups was analyzed using Student's unpaired *t* test.

3. Results

3.1. Inhibitory potencies of SA4503 and σ receptor ligands for σ_1 and σ_2 receptor subtypes

Competition binding experiments showed that SA4503 had a high affinity for (+)-[^{3}H]pentazocine binding sites in guinea pig brain membranes (Fig. 2). The IC_{50} value of this compound for the σ_1 receptor subtype was 17.4 ± 1.9 nM (Table 1). On the other hand, this compound had a low affinity for the σ_2 receptor subtype, with an IC_{50} value of 1784.1 ± 314.4 nM (Fig. 2 and Table 1). The inhibitory potency of SA4503 for the σ_1 receptor subtype was about 100 times higher than that for the σ_2 receptor subtype. This binding affinity was almost the same as that of (+)-pentazocine, a prototype σ_1 receptor agonist (Fig. 2 and Table 1). The IC_{50} values of carbetapentane, DTG, (+)-3-PPP, (+)-SKF-10,047 and (−)-SKF-10,047 for the σ_1 receptor subtype were higher than that of SA4503 (Fig. 2 and Table 1).

3.2. Selectivity of SA4503 for σ receptors

SA4503 had weak binding affinities for α_1 adrenergic, dopamine D_2, serotonin $(5\text{-HT})_{1A}$, 5-HT_2, histamine H_1, muscarinic M_1 and muscarinic M_2 receptors at a concentration of 10 μM. However, these binding affinities were about 100 times lower than that for the σ_1 receptor subtype (Table 2). SA4503 had no affinity for 29 other receptors, ion channels and second messenger systems examined (Table 2).

3.3. Effects of GTPγS on the SA4503-induced inhibition of (+)-[^{3}H]pentazocine binding

In rat brain membranes, SA4503 also inhibited (+)-[^{3}H]pentazocine binding (Fig. 3). The IC_{50} value of this compound for the σ_1 receptor subtype in these membrane preparations was smaller than that in the guinea pig brain membranes (Table 3). SA4503 was a more potent ligand than (+)-pentazocine in rat brain membrane preparations

Table 1
Inhibitory effects of SA4503 and σ receptor ligands against σ_1 and σ_2 receptor subtypes in guinea pig brain membranes

Compounds	IC_{50}(nM)		Ratio
	σ_1	σ_2	(σ_2/σ_1)
SA4503	17.4 ± 1.9	1784.1 ± 314.4	102.5
(+)-Pentazocine	13.7 ± 3.0	2874.6 ± 575.0	209.8
Carbetapentane	128.0 ± 26.2	1953.7 ± 286.9	15.3
DTG	246.4 ± 56.6	362.2 ± 125.2	1.5
(+)-3-PPP	397.0 ± 27.5	5545.0 ± 3331.0	14.0
(+)-SKF-10,047	605.1 ± 128.6	19857.4 ± 4925.5	32.8
(−)-SKF-10,047	17947.1 ± 1871.9	15565.8 ± 3165.1	0.9

The results are expressed as means ± S.E.M. of 3–4 experiments, each conducted in triplicate.

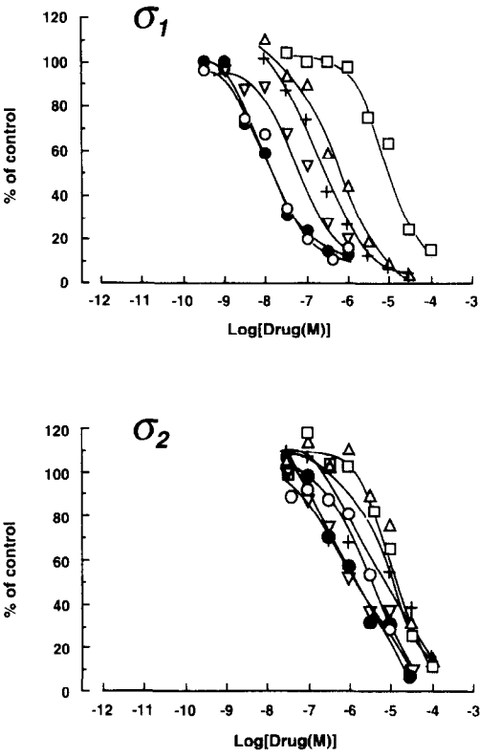

Fig. 2. Displacement curves of SA4503 and σ receptor ligands against σ_1 or σ_2 receptors in guinea pig brain membranes. Binding affinities to σ_1 and σ_2 receptor subtypes were assessed with 5 nM (+)-[^{3}H]pentazocine or 5 nM [^{3}H]DTG with 200 nM (+)-pentazocine, respectively. Incubations in the (+)-[^{3}H]pentazocine and [^{3}H]DTG binding studies were carried out at 37°C for 150 min and at 25°C for 90 min, respectively. (●) SA4503; (○) (+)-pentazocine; (▽) carbetapentane; (+) (+)-3-PPP; (△) (+)-SKF-10,047; (□) (−)-SKF-10,047. The data shown are from typical experiments representing the means of triplicate determinations.

(Table 3). Interestingly, the inhibition curve of SA4503 for (+)-[^{3}H]pentazocine binding was shifted to the right in the presence of GTPγS (Fig. 3). The inhibitory potency of SA4503 in the presence of GTPγS was about ten times weaker than that in the absence of GTPγS. Inhibition curves of (+)-3-PPP and (+)-pentazocine, σ_1 receptor agonists, were also shifted to the right in the presence of GTPγS (Fig. 3 and Table 3).

3.4. Characteristics of competitive effects of SA4503 and σ receptor ligands on specific (+)-[^{3}H]pentazocine binding

Inclusion of SA4503, at concentrations nearly equivalent to and twice to its IC_{50} value, increased K_D values of (+)-[^{3}H]pentazocine binding by 2.2-fold and 2.6-fold, respectively. However, this compound did not affect the B_{max} value (Fig. 4 and Table 4). Similarly, inclusion of haloperidol, (+)-pentazocine and DTG also increased the K_D values of (+)-[^{3}H]pentazocine binding, but not the B_{max} values (Fig. 4 and Table 4).

4. Discussion

There are a few compounds which have high affinity and/or selectivity for the σ_1 receptor subtype. For example, (+)-pentazocine, carbetapentane, (+)-SKF-10,047 and (+)-3-PPP have been reported to show a high degree of selectivity for binding to the σ_1 receptor subtype over the σ_2 receptor subtype (Quirion et al., 1992; Vilner and Bowen, 1992; Bonhaus et al., 1993; Itzhak, 1994). In addition, (+)-pentazocine and carbetapentane have high affinities for the σ_1 receptor subtype (Quirion et al., 1992; Vilner and Bowen, 1992; Bonhaus et al., 1993; Itzhak, 1994). In the present study, however, the binding affinity of SA4503 for the σ_1 receptor subtype was higher than

Table 2
SA4503 is either inactive or has low binding affinities for the following receptors, ion channels or second messenger systems

Receptor	Radioligand	% inhibition (average, $n = 2$)			Assay reference
		1 nM	100 nM	10 μM	
Adenosines					
Adenosine A_1	[^{3}H]CPX	−7.2	1.8	−3.2	Bruns et al., 1987
Adenosine A_2	[^{3}H]CGS 21680	10.3	15.6	49.0	Jarvis et al., 1989
Adrenergics					
α_1	[^{3}H]Prazosin	0.7	0.1	83.1	Timmermans et al., 1981
α_2	[^{3}H]RX 781094	0.7	0.0	27.1	Doxey et al., 1983
β	[^{3}H]DHA	−2.1	−3.0	18.5	Riva and Creese, 1989
Excitatory amino acid					
Glutamate	[^{3}H]Glutamate	4.7	0.3	7.8	Slevin et al., 1982
NMDA	[^{3}H]CGS 19755	−5.8	−4.7	−0.3	Lehmann et al., 1988
Kainate	[^{3}H]Kainic acid	0.8	−2.4	4.7	London and Coyle, 1979
Quisqualate	[^{3}H]AMPA	−4.3	0.6	8.8	Murphy et al., 1987
Glycine	[^{3}H]Glycine	18.1	−0.3	−2.6	Snell et al., 1988
PCP	[^{3}H]TCP	−7.4	11.8	30.9	Vignon et al., 1983
MK-801	[^{3}H]MK-801	0.3	−0.6	1.6	Javitt and Zukin, 1989
Inhibitory amino acid					
Glycine	[^{3}H]Strychnine	−7.0	18.8	0.7	Young and Snyder, 1974
$GABA_A$	[^{3}H]GABA	−3.7	−3.5	5.7	Enna et al., 1977
$GABA_B$	[^{3}H]GABA + isoguvacine	−2.8	−3.5	5.6	Scherer et al., 1988
Benzodiazepine	[^{3}H]Flunitrazepam	−3.0	−5.1	−1.9	Sweetnam and Tallman, 1986
Cholinergics					
Muscarinic M_1	[^{3}H]Pirenzepine	−4.6	−1.1	52.3	Watson et al., 1983
Muscarinic M_2	[^{3}H]AF-DX384	−3.6	−5.3	69.6	Eberlein et al., 1989
Muscarinic M_3	[^{3}H]NMeSCOP	3.0	9.9	33.1	Hanack and Pfeiffer, 1990
Nicotinic	[^{3}H]NMCI	2.4	7.2	0.7	Boksa and Quirion, 1987
Biogenic amines					
Dopamine D_1	[^{3}H]SCH23390	1.7	2.6	12.8	Billard et al., 1984
Dopamine D_2	[^{3}H]Sulpiride	−21.4	−5.9	80.9	Imafuku, 1987
5-HT_{1A}	[^{3}H]8-OH-DPAT	−2.5	−1.0	62.0	Hoyer et al., 1985
5-HT_2	[^{3}H]Ketanserin	−25.0	−10.8	87.9	Leysen et al., 1982
5-HT_3	[^{3}H]GR 65630	0.1	11.9	12.1	Lummis et al., 1990
Histamine H_1	[^{3}H]Pyrilamine	−10.3	−4.7	75.9	Chang et al., 1979
Opioids					
μ opioid	[^{3}H]DAGO	−5.5	−5.8	19.0	Gillan and Kosterlitz, 1982
δ opioid	[^{3}H]DPDPEN	9.2	13.1	39.0	Akiyama et al., 1985
κ opioid	[^{3}H]U69593	−2.0	−22.4	14.9	Lahti et al., 1985
Channel proteins					
Ca^{2+} channel (L-type)	[^{3}H]Nitrendipine	4.1	1.1	22.3	Gould et al., 1984
Ca^{2+} channel (N-type)	ω-[^{125}I]Conotoxin	−1.3	−8.6	−2.2	Wagner et al., 1988
K^+ channel	[^{125}I]Apamin	−1.2	5.3	8.3	Seager et al., 1987
Cl^- channel	[^{3}H]TBOB	2.8	1.1	4.8	Lawrence et al., 1985
Second messenger systems					
Protein kinase C	[^{3}H]PDBU	−0.7	2.5	7.6	Dunphy et al., 1980
Adenylate cyclase	[^{3}H]Forskolin	−3.4	−10.1	−19.1	Seamon et al., 1984
Inositol triphosphate	[^{3}H]IP_3	−2.0	−0.4	8.8	Worley et al., 1987

those of carbetapentane, (+)-SKF-10,047 and (+)-3-PPP. In addition, the selectivity of SA4503 for the σ_1 receptor subtype over the σ_2 receptor subtype was also superior to these three σ receptor ligands. Moreover, (+)-pentazocine and (+)-SKF-10,047 have been reported to bind to the *N*-methyl-D-aspartate (NMDA) receptor channel complex (Largent et al., 1986; Sircar et al., 1986; Iyengar et al., 1990). Similarly, (+)-3-PPP has also been reported to bind the dopamine autoreceptor (Hjorth et al., 1981, 1985; Zhang et al., 1992). In contrast, SA4503 showed little affinity for 36 other receptors, ion channels and second messenger systems examined. Thus, we suggest that SA4503 is a novel, potent and selective ligand for the σ_1 receptor subtype.

Pertussis toxin (PTX), a blocker of the $G_{i/o}$ types of G-proteins (Gilman, 1987), has been shown to inhibit the binding of (+)-[^{3}H]3-PPP (Itzhak, 1989) and the (+)-3-PPP-induced increase in NMDA-evoked [^{3}H]norepinephrine overflow (Monnet et al., 1992). In addition, GTP and its stable analog, guanyl-5′-imidodiphosphate (Gpp(NH)p), have been reported to decrease the binding of (+)-[^{3}H]SKF-10,047 and (+)-[^{3}H]3-PPP, but not [^{3}H]DTG (Itzhak and Khouri, 1988; Beart et al., 1989; Itzhak and Stein, 1991). These lines of evidence suggested that σ receptors, particularly the σ_1 receptor subtype, may be associated with G-proteins, particularly the $G_{i/o}$ types (Connick et al., 1992; Quirion et al., 1992). In general, agonists for receptors coupled to G-proteins show a decrease of binding affinity in the presence of guanine nucleotides (Lefkowitz et al., 1984; Gilman, 1986; Dolphin, 1987). For example, the affinity of dopamine D_2 or α_2-adrenergic receptor agonists for their respective receptors were lowered in the presence of guanine nucleotides (Hoffman et al., 1980; Battaglia and Titeler, 1982; Sibley et al., 1982). Similarly, (+)-SKF-10,047 and (+)-3-PPP, σ_1 receptor agonists, have been reported to show a decrease of binding affinity in the presence of guanine nucleotides (Beart et al., 1989; Connick et al., 1992). In the present study, we demonstrated that the inhibition curve of SA4503 for (+)-[^{3}H]pentazocine binding was shifted to the right in the presence of GTPγS. This finding indicates that SA4503 may act as an agonist for the σ_1 receptor subtype.

DTG and haloperidol have been reported to inhibit the

Note to Table 2:

These assays were performed as a contract study by Nova Pharmaceuticals (Baltimore, MD, USA). The following incubation conditions were used to perform the binding assay: adenosine A_1, 8-[^{3}H]cyclopentyl-1,3-dipropylxanthine (CPX) in 50 mM Tris-HCl buffer (pH 7.7) at 25°C for 60 min with rat cortical membranes; adenosine A_2, [^{3}H]CGS 21680 in 50 mM Tris-HCl buffer (pH 7.7) at 25°C for 90 min with bovine striatal membranes; α_1-adrenergic, [^{3}H]prazosin in 50 mM Tris-HCl buffer (pH 7.7) at 25°C for 60 min with rat forebrain membranes; α_2-adrenergic, [^{3}H]RX 781094 in 50 mM Tris-HCl buffer (pH 7.4) at 0°C for 90 min with rat cortical membranes; β-adrenergic, [^{3}H]dihydroalprenolol (DHA) in 50 mM Tris-HCl buffer (pH 7.4) at 37°C for 30 min with rat cortical membranes; exitatory amino acid (EAA)-glutamate, [^{3}H]glutamate in 50 mM Tris-HCl buffer (pH 7.4) at 37°C for 60 min with rat cerebellar membranes; EAA-*N*-methyl-D-aspartate (NMDA), [^{3}H]CGS 19755 in 50 mM Tris-acetate buffer (pH 7.4) at 0–4°C for 45 min with rat forebrain membranes; EAA-kainate, [^{3}H]kainic acid in 50 mM Tris-HCl buffer (pH 7.1) at 2°C for 60 min with rat forebrain membranes; EAA-quisqualate, [^{3}H]amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) in 10 mM K_2HPO_4 buffer containing 100 mM potassium thiocyanate (pH 7.5) at 0–4°C for 30 min with rat forebrain membranes; EAA-glycine, [^{3}H]glycine in 50 mM Hepes buffer (pH 7.1) at 4°C for 30 min with rat cortical membranes; EAA-phencyclidine (PCP), *N*-[^{3}H](1(2-thienyl)cyclohexyl)-3,4-piperidine (TCP) in 5.0 mM Tris-HCl buffer (pH 7.7) at 4°C for 60 min with rat forebrain membranes; EAA-MK-801, [^{3}H]MK-801 in 20 mM Hepes buffer (pH 7.5) at 25°C for 90 min with rat forebrain membranes; inhibitory amino acid (IAA)-glycine, [^{3}H]strychnine in 50 mM Na-KPO_4 buffer containing 200 mM NaCl (pH 7.1) at 4°C for 10 min with rat spinal cord membranes; IAA-γ-aminobutyric acid $(GABA)_A$, [^{3}H]GABA in 50 mM Tris-HCl buffer (pH 7.4) at 0–4°C for 15 min with bovine cerebellar membranes; IAA-$GABA_B$, [^{3}H]GABA (in the presence of 100 μM isoguavacine) in 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$ (pH 7.4) at 25°C for 15 min with rat cortical membranes; IAA-benzodiazepine, [^{3}H]flunitrazepam in 10 mM Na-KPO_4 buffer (pH 7.7) at 0–4°C for 45 min with bovine cortical membranes; muscarinic M_1, [^{3}H]pirenzepine in 10 mM Na-KPO_4 buffer (pH 7.4) at 25°C for 60 min with bovine striatal membranes; muscarinic M_2, [^{3}H]AF-DX 384 in 10 mM Na-KPO_4 buffer (pH 7.4) at 25°C for 30 min with rat cardiac membranes; muscarinic M_3, *N*-[^{3}H]methylscopolamine (NMeSCOP) in 30 mM Hepes buffer containing 142 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 3.6 mM $NaHCO_3$, 1.0 mM $MgCl_2$ and 5.6 mM D-glucose (pH 7.4) at 37°C for 120 min with guinea pig ileum membranes; nicotinic, *N*-[^{3}H]methylcarbamylcholine iodide (NMCI) in 50 mM Tris-HCl buffer containing 120 mM NaCl, 5.0 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 3.0 μM atropine sulfate (pH 7.4) at 4°C for 60 min with rat cortical membranes; dopamine D_1, [^{3}H]SCH 23390 in 50 mM Hepes buffer containing 1.0 mM EDTA, 4.0 mM $MgSO_4$ and 10 μM ketanserin (pH 7.4) at 37°C for 60 min with rat striatal membranes; dopamine D_2, [^{3}H]sulpiride in 50 mM Tris-HCl buffer containing 100 mM NaCl (pH 7.5) at 25°C for 60 min with rat striatal membranes; serotonin $(5\text{-HT})_{1A}$, 2-[^{3}H]dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) in 50 mM Tris-HCl buffer (pH 7.4) at 37°C for 10 min with bovine hippocampal membranes; 5-HT_2, [^{3}H]ketanserin in 50 mM Tris-HCl buffer (pH 7.6) at 37°C for 15 min with rat cortical membranes; 5-HT_3, [^{3}H]GR 65630 in 20 mM Hepes buffer containing 150 mM NaCl (pH 7.4) at 25°C for 30 min with N1E-115 cells; histamine H_1, [^{3}H]pyrilamine in 50 mM Na-KPO_4 buffer (pH 7.5) at 25°C for 30 min with bovine cerebellar membranes; μ-opioids, [^{3}H]Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol (DAGO) in 50 mM Tris-HCl buffer (pH 7.4) at 25°C for 90 min with rat forebrain membranes; δ-opioids, [^{3}H](2-D-penicillamine, 5-D-penicillamine)enkephalin (DPDPEN) in 50 mM Tris-HCl buffer (pH 7.4) at 25°C for 90 min with rat forebrain membranes; κ-opioids, [^{3}H]U69593 in 50 mM Hepes buffer (pH 7.4) at 30°C for 120 min with guinea pig cerebellar membranes; Ca^{2+} channel (L-type), [^{3}H]nitrendipine in 50 mM Tris-HCl buffer (pH 7.7) at 25°C for 60 min with rat cortical membranes; Ca^{2+} channel (N-type), ω-[^{3}H]conotoxin in 50 mM Hepes buffer containing 0.2% BSA (pH 7.4) at 25°C for 30 min with rat cortical membranes; K^+ channel, [^{3}H]apamin in 50 mM Tris-HCl buffer containing 0.1% BSA and 5 mM KCl (pH 7.4) at 4°C for 60 min with rat forebrain membranes; Cl^- channel, 1-[^{3}H]phenyl-4-*t*-butyl-2,6,7-trioxabicyclo(2.2.2)octane (TBOB) in 20 mM Na-KPO_4 buffer containing 500 mM NaCl (pH 7.5) at 25°C for 75 min with rat cortical membranes; protein kinase C, [^{3}H]phorbol ester dibutyrate (PDBU) in 50 mM Tris-HCl buffer containing 1.0% BSA and 0.5 mM $CaCl_2$ (pH 7.4) at 37°C for 30 min with mouse brain membranes; adenylate cyclase, [^{3}H]forskolin in 50 mM Tris-HCl buffer containing 5.0 mM $MgCl_2$ (pH 7.4) at 25°C for 60 min with rat forebrain membranes; inositol triphosphate (IP_3), [^{3}H]IP_3 in 50 mM Tris-HCl buffer containing 1 mM EDTA (pH 8.3) at 0°C for 10 min with rat cerebellar membranes.

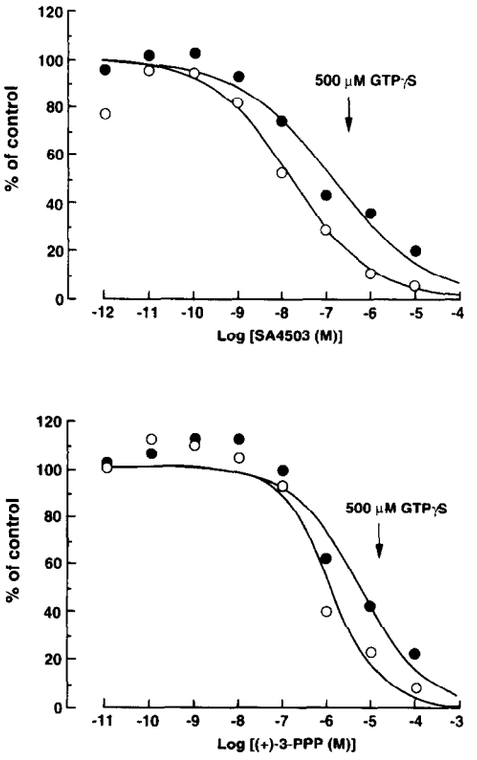

Fig. 3. Displacement curves of SA4503 (upper) and (+)-3-PPP (lower) against (+)-[^{3}H]pentazocine binding in rat brain membranes in the absence (○) and presence (●) of GTPγS. Membranes were incubated with 5 nM (+)-[^{3}H]pentazocine and at least eight concentrations of SA4503 and (+)-3-PPP at 25°C for 150 min in the absence or presence of 500 μM GTPγS. The data shown are from typical experiments representing the means of triplicate determinations.

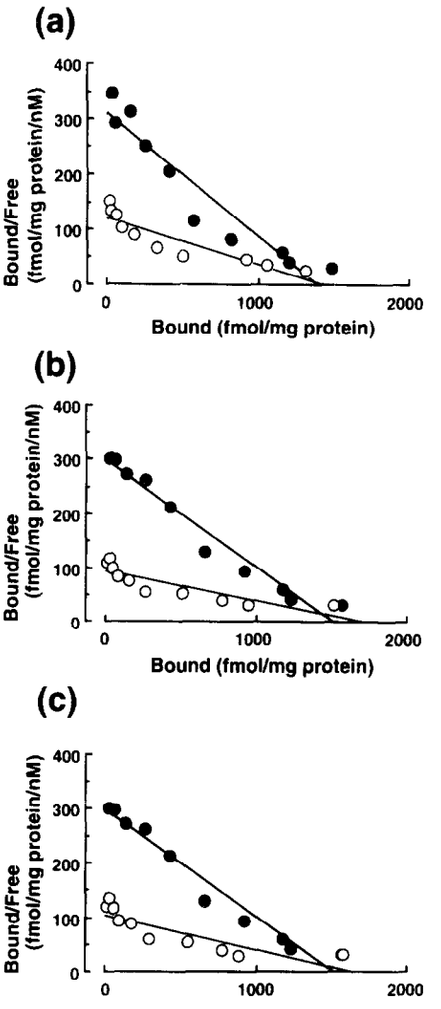

Fig. 4. Representative Scatchard plots of (+)-[^{3}H]pentazocine binding to guinea pig brain membranes with 15 nM SA4503 (a), 15 nM (+)-pentazocine (b) and 3 nM haloperidol (c). Membranes were incubated at 25°C for 150 min with the ligand over a concentration range from 0.1 to 50.0 nM (+)-[^{3}H]pentazocine, and these σ receptor ligands at the IC_{50} concentration for displacement of (+)-[^{3}H]pentazocine binding. The data shown are from typical experiments representing the means of triplicate determinations.

binding of (+)-[^{3}H]3-PPP in a competitive manner (Bowen et al., 1989). In addition, haloperidol competitively inhibited the (+)-[^{3}H]SKF-10,047 binding (McCann and Su, 1990). Similarly, inclusion of haloperidol, (+)-pentazocine and (+)-3-PPP inhibited the (+)-[^{3}H]pentazocine binding in a competitive manner (DeHaven-Hudkins et al., 1992; Klein et al., 1994). These results were obtained from data which each non-radiolabeled σ receptor ligand significantly increased the K_D values of these ^{3}H-ligand bindings, but did not affect the B_{max} values. Similar to these reports, we found that SA4503 significantly caused an increase of the K_D value and no change in the B_{max} value of the (+)-[^{3}H]pentazocine binding. Thus, SA4503 bound to the σ_1 receptor subtype in a competitive manner.

Table 3
Inhibitory effects of SA4503, (+)-3-PPP and (+)-pentazocine for σ_1 receptor subtype labeled with (+)-[^{3}H]pentazocine in rat brain in the absence and presence of GTPγS

Compounds	IC_{50}(nM)		Ratio
	None	+500 μM GTPγS	
SA4503	6.78±3.55	65.75±20.26	9.7
(+)-3-PPP	529.67±203.96	2639.77±1575.46	5.0
(+)-Pentazocine	19.61±2.85	44.41±18.85	2.3

The results are expressed as means±S.E.M. of 3–4 experiments, each conducted in triplicate.

Finally, as described in the Introduction, we suggested that the σ_1 receptor subtype plays an important role in the facilitation of central cholinergic function, and that specific agonists for the σ_1 receptor subtype may be good candidates as novel nootropic drugs. Similar to our suggestions, the σ_1 receptor subtype has been reported to interact with the central cholinergic systems. For example, σ_1 receptor agonists, such as (+)-SKF-10,047 and (+)-pentazocine, inhibited carbachol-induced inositol phosphate accumulation in the rat brain (Bowen et al., 1988; Brog and Beinfeld, 1990; Candura et al., 1990). In addition, (+)-

Table 4
Effects of SA4503 and σ receptor ligands on kinetic parameters of specific (+)-[^{3}H]pentazocine binding to guinea pig brain membranes

Compounds	K_D (nM)	B_{max} (fmol/mg protein)
Control	6.9 ± 1.1	1811.0 ± 153.7
SA4503 (15 nM)	15.0 ± 2.2 [a]	1856.7 ± 296.1
SA4503 (30 nM)	18.1 ± 1.7 [a]	1713.3 ± 204.8
Haloperidol (3 nM)	14.0 ± 1.0 [c]	1480.6 ± 104.1
(+)-Pentazocine (15 nM)	17.6 ± 0.6 [c]	1665.5 ± 26.1
DTG (250 nM)	11.8 ± 1.4 [b]	1358.0 ± 88.4

The results are expressed as means ± S.E.M. of 3–7 experiments, each conducted in triplicate. [a] $P < 0.01$ as compared with control (Dunnett's multiple-range test). [b] $P < 0.05$ and [c] $P < 0.01$ as compared with control (Student's unpaired t test).

SKF-10,047 enhanced stimulation-evoked and KCl-evoked acetylcholine release in guinea pig cerebral slices (Siniscalchi et al., 1987) and rat hippocampal slices (Junien et al., 1991), respectively. Moreover, (+)-SKF-10,047 and (+)-pentazocine attenuated the dizocilpine- and carbon monoxide-induced amnesia in mice (Maurice et al., 1994a,b). Recently, dehydroepiandrosterone sulfate attenuated the dizocilpine-induced learning impairment in mice, which was mediated through an interaction with σ_1 receptor subtype (Maurice et al., 1996). Therefore, SA4503 may be useful for treating disorders involving cholinergic dysfunction such as Alzheimer's disease and senile dementia.

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