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REVIEWS

SA4503: A Novel Sigma$_1$ Receptor Agonist

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INTRODUCTION

Sigma (σ) receptors, which were introduced by the pioneering studies of Martin et al. (90), have been postulated to account for the psychotomimetic effects or "canine delirium" observed in dogs after administration of (±)-*N*-allylnormetazocine ([±]-SKF-10,047), and classified as opioid receptor subtypes (90). Subsequent studies, based on the displacement of [^{3}H]phencyclidine (PCP) binding by (+)-SKF-10,047 (118,193), have shown that sigma receptors were identical to the PCP binding sites in the *N*-methyl-*D*-aspartate (NMDA) receptor channel complex. At present, although sigma receptors are distinct from opioid receptors and PCP binding sites in the NMDA receptor channel complex (15,79,88,140,159,161,171,172,179), they are still considered to be enigmatic molecular targets.

Based on biochemical studies, the existence of subtypes within the sigma receptor's family is accepted (6,10,49,57–59,66,141,148,166,182,188). Briefly, sigma receptors have been classified into at least two subtypes, termed sigma$_1$ and sigma$_2$. The sigma$_1$ receptor subtype, which is enriched in guinea pig brain, exhibits a high affinity for (+)-benzomorphans, and has been demonstrated to have an apparent molecular weight of about 25 kDa. The sigma$_2$ receptor subtype, first characterized in pheochromocytoma (PC 12) cells, displays a remarkable low affinity for (+)- benzomorphans as compared to the sigma$_1$ receptor subtype, and has an apparent molecular weight of 18 to 21 kDa. In the receptor displacement study, it was shown that some sigma receptor

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ligands, such as 1,3-di(2-tolyl)guanidine (DTG), (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ([+]-3-PPP), and dextromethorphan, have more than two binding sites in the guinea pig brain (192). From computer-assisted analysis of the same study, it has been proposed that the sigma receptors consist of four different subtypes, termed R_1, R_2, R_3, and R_4 receptor sites (192).

In parallel with these receptor binding studies, extensive studies to elucidate the physiological functions of sigma receptor subtypes in the central and peripheral nervous systems have been carried out (31,141,162,163,182). To date, the sigma$_1$ receptor subtype might be involved in some diseases in the central nervous system (CNS), such as schizophrenia (1,20,41,125), depression (41,94), dementia (1,98,109), and ischemia (109), and in the peripheral nervous system diseases, such as ulcers (135) and the neurogenic twitch contraction in the mouse vas deferens (104). On the other hand, the sigma$_2$ receptor subtype might be related to the regulation of motor function (15,92,181,182), ileal function (70), and the K^+ channel (63).

These sigma$_1$ and sigma$_2$ receptor subtypes were reported to be consistent with the aforementioned R_1 and R_3 receptor sites, respectively. The other two receptor sites, R_2 and R_4, were supposed to be consistent with the dextromethorphan-selective and low-affinity sites, respectively (192). The dextromethorphan-selective sites were reported to be associated with antitussive (176), anticonvulsant (72), and antiischemic activities (137), whereas the low-affinity sites were thought to be involved in the modulation of tonic K^+ channels in NCB-20 cells (188) and the regulation of twitch contraction induced by exogenous ATP in the mouse vas deferens (93). A more recent study has proposed that another subtype of sigma receptor (maybe sigma$_3$) might be linked to tyrosine hydroxylase and might regulate dopamine synthesis in the CNS (123).

Inspired by these findings, we have synthesized a number of compounds having high affinity and selectivity for the sigma$_1$ receptor subtype. As a result, we have found 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503; Fig. 1) as a candidate for the hypothesis that a sigma$_1$ receptor agonist may serve as a widely useful drug for CNS disorders.

CHEMISTRY

SA4503 has been synthesized by Santen Pharmaceutical Co., Ltd. This compound has a molecular weight of 441.44 and its empirical formula is $C_{23}H_{32}N_2O_2 \cdot 2HCl$. It has a melting point of 258 to 260°C (decomposition). It is odorless, colorless, and freely soluble in water. The crystal of SA4503 prepared in CH_3CN/H_2O is orthorhombic and shows space group of *Pna2$_1$*. The synthesis of SA4503 is summarized in Fig. 2 (40,68). Homoveratrilamine [1] was alkylated with 2-bromoethanol in the presence of K_2CO_3 in ethanol to give an oily compound [2]. After the chlorination of compound [2] by the addition of $SOCl_2$ in $CHCl_3$, compound [3] was coupled with 3-phenylpropylamine in dimethylformamide. The product was purified using silica-gel

Fig. 1. Chemical structure of SA4503.

column chromatography and converted to an HCl salt. Recrystallization of the salt in ethanol afforded SA4503 as a colorless crystal.

RECEPTOR BINDING STUDIES

Binding Affinity and Selectivity

Competitive binding experiments showed that SA4503 had a high affinity for [^{3}H](+)-pentazocine binding sites (the sigma$_1$ receptor subtype) in guinea pig brain membranes. The IC_{50} value of this compound for the sigma$_1$ receptor subtype was 17.4 ± 1.9 nM. On the other hand, SA4503 had a low affinity for [^{3}H]DTG binding sites in the presence of 200 nM (+)-pentazocine (the sigma$_2$ receptor subtype) in the

Fig. 2. Synthesis of SA4503.

same membranes, with an IC_{50} value of 1784.1 ± 314.4 nM (99,101,153). The inhibitory potency of SA4503 for the $sigma_1$ receptor subtype was about 100 times higher than that for the $sigma_2$ receptor subtype. This binding affinity was almost the same as that of (+)-pentazocine, a prototype $sigma_1$ receptor ligand. The IC_{50} values of carbetapentane, (+)-3-PPP, and (+)-SKF-10,047, the other prototype $sigma_1$ receptor ligands, for the $sigma_1$ receptor subtype were larger than that of SA4503 (99). In addition, 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. These binding affinities were about 100 times lower than that for the $sigma_1$ receptor subtype. Moreover, SA4503 had no affinity for 29 other receptors, ion channels, and second messenger systems examined (99). There are a few compounds that have high affinity and/or selectivity for the $sigma_1$ 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 $sigma_1$ receptor subtype over the $sigma_2$ receptor subtype (9,54,141,180). In addition, (+)-pentazocine and carbetapentane have high affinities for the $sigma_1$ receptor subtype (9,54,141,180). However, the binding affinity of SA4503 for the $sigma_1$ receptor subtype was superior to that of carbetapentane, (+)-SKF-10,047, or (+)-3-PPP. In addition, the selectivity of SA4503 for the $sigma_1$ receptor subtype over the $sigma_2$ receptor subtype was also superior to that of these three prototype $sigma_1$ receptor ligands (99). Moreover,

(+)-pentazocine and (+)-SKF-10,047 have been reported to bind to the NMDA receptor channel complex (61,79,157) and (+)-3-PPP to the dopamine autoreceptor (50,51,191). In contrast, SA4503 showed little binding to 36 other receptors, ion channels, and second messenger systems examined (99). Thus, we suggest that SA4503 is a novel, potent, and selective ligand for the sigma$_1$ receptor subtype.

Binding Profile for the Sigma$_1$ Receptor Subtype

Pertussis toxin (PTX), a blocker of the $G_{i/o}$ types of G-proteins (43), inhibits the binding of [^{3}H](+)-3-PPP in rat brain membranes (53), the (+)-3-PPP- and (+)-cinnamyl-1-phenyl-1-*N*-methyl-N-cyclopropylene (JO-1784) (147)-induced increase in NMDA-evoked [^{3}H]norepinephrine overflow in the rat hippocampus (119,121), and the JO-1784- and DTG-induced increase in NMDA-evoked neuronal activation of CA_3 dorsal hippocampus neurons (120). Similarly, PTX has been reported to affect the DTG-induced decrease in NMDA-evoked [^{3}H]norepinephrine overflow in the rat hippocampus (191,121). 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 in rat brain membranes (6,56,58). These lines of evidence suggest that the sigma$_1$ receptor subtype may be associated with G-proteins, particularly the $G_{i/o}$ types (14,141). In general, agonists for receptors coupled to G-proteins show a decrease of binding affinity in the presence of guanine nucleotides (25,42,80). For example, the affinity of dopamine D_2 or α_2-adrenergic receptor agonists for their respective receptors was lowered by guanine nucleotides (4,52,155). Similarly, (+)-SKF-10,047, (+)-3-PPP, and (±)-pentazocine, prototype sigma$_1$ receptor ligands, showed a decrease in their binding affinities in the presence of guanine nucleotides in the brain membranes (6,14,53), which suggest that these prototype ligands bind to the sigma$_1$ receptor subtype as agonists.

In rat brain membranes, SA4503 also inhibited [^{3}H](+)-pentazocine binding. The IC_{50} value of this compound for the sigma$_1$ receptor subtype in rat brain membranes preparations was 6.8 ± 3.6 nM. It was smaller than that in guinea pig brain membranes as described above (99). Interestingly, the inhibition curve of SA4503 for [^{3}H](+)-pentazocine binding in rat brain membranes was shifted to the right in the presence of 500 μM guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS) (99). 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, prototype sigma$_1$ receptor agonists, were also shifted to the right in the presence of GTPγS (99). These findings indicate that SA4503 may act as an agonist for the sigma$_1$ receptor subtype.

In guinea pig brain membranes, inclusion of SA4503, at concentrations nearly equivalent to and twice 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} values (99). Similarly, inclusion of haloperidol, (+)-pentazocine, or DTG also increased the K_D values of [^{3}H](+)-pentazocine binding, but not the B_{max} values (99). DTG and haloperidol have been reported to competitively inhibit the binding of [^{3}H](+)-3-PPP in rat brain membranes (10). In addition, haloperidol also competitively

inhibited the [^{3}H](+)-SKF-10,047 binding in guinea pig brain membranes (117). Moreover, inclusion of haloperidol, (+)-pentazocine, or (+)-3-PPP inhibited the [^{3}H](+)-pentazocine binding in guinea pig brain membranes in a competitive manner (23,73). In agreement with these reports, our results indicate that SA4503 binds to the sigma$_1$ receptor subtype competitively (99).

PHARMACOLOGICAL STUDIES

Efficacy in Amnesia Models

Prototype sigma receptor agonists, such as (+)-SKF-10,047, (±)-pentazocine, DTG, and (+)-3-PPP, alleviated scopolamine- and *p*-chloroamphetamine (PCA)-induced memory impairments in mice and rats (100,102,103,150,153). Similarly, JO-1784 alleviated scopolamine-induced amnesia in rats (28). Moreover, (+)-SKF-10,047, (+)-pentazocine, DTG, and 2-(4-morpholinoethyl-1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084)(165), attenuated dizocilpine ([+]-MK-801)-, nimodipine-, and carbon monoxide (CO)-induced impairment of learning in mice (105–107,114,115) and impairment of working memory in rats (129). It is particularly interesting that PRE-084 and JO-1784 alleviated the age-related learning impairment in the senescence-accelerated mouse (SAM) (113). Moreover, the anti-amnesic effects of (+)-SKF-10,047 were superior to those of (–)-SKF-10,047 (100,103,105,107,129,150), and were completely antagonized by haloperidol, a non-specific sigma receptor antagonist and *N*,*N*-dipropyl-2-(4-methoxy-3-[2-phenylethoxy]phenyl)ethylamine monohydrochloride (NE-100), a putative sigma$_1$ receptor antagonist (100,131,150,153). These results indicate that the facilitation of the cognitive function elicited by these agonists are mediated by the sigma receptor, particularly the sigma$_1$ receptor subtype. Noteworthy, these sigma$_1$ receptor agonist-induced facilitations were observed at all stages of learning and memory processes, such as acquisition, consolidation, and retrieval stages (103,150).

In the rat step-through test, the disruption of the retention performance elicited by scopolamine was alleviated by orally (p.o.) administered 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 p.o. (101,153). Simultaneous intraperitoneal (i.p.) injection of haloperidol at doses of 0.05 and 0.1 mg/kg significantly reduced the anti-amnesic effect by SA4503 (0.1 mg/kg p.o.) (153). This anti-amnesic effect of SA4503 was also significantly antagonized by pretreatment with NE-100 at doses of 0.5 and 1.0 mg/kg p.o. (153). In addition, the disruption of the retention performance elicited by basal forebrain (BF) lesion was also alleviated by repeated oral administration of SA4503 at doses ranging from 0.25 to 1.0 mg/kg/d for 15 d. A significant anti-amnesic effect was observed at a dose of 0.5 mg/kg p.o. (153). In the rat Morris water maze (MWM) test, the prolonged escape latency of BF-lesioned rats in the training period was significantly shortened by repeated administration of SA4503 at oral doses ranging from 0.25 to 1.0 mg/kg/d (151,152). In addition, although the BF le-

sion reduced the number of times each rat crossed the goal area in the probe trial, repeated oral administration of SA4503 0.25 mg/kg/d significantly ameliorated this reduction (152).

In the mouse step-down test, subcutaneously (s.c.) administered SA4503 at a dose of 0.3 mg/kg alleviated β_{25-35} amyloid peptide-induced disruption of the retention performance (110). SA4503 at doses of 0.1 and 0.3 mg/kg s.c. also alleviated dizocilpine- and N^{ω}-nitro-L-arginine methyl ester (L-NAME)–induced memory impairment (111). These anti-amnesic effects of SA4503 were significantly antagonized by simultaneous administration of haloperidol at doses of 0.05 and 0.1 mg/kg i.p. (110,111).

Efficacy in Depression Models

Sigma receptors were reported to be involved in central noradrenergic and/or glutamatergic transmission (30,33,71,146). For example, (+)-pentazocine, haloperidol, (+)-3-PPP, (+)-SKF-10,047, and DTG inhibited [^{3}H]norepinephrine uptake in rat brain synaptosomes (71,146). In addition, (+)-pentazocine increased [^{3}H]norepinephrine release from rat cortical slices (71). In contrast, haloperidol, rimcazole (32), and ifenprodil (67) inhibited glutamate release from rat striatal slices (30). Similarly, DTG blocked NMDA receptor-mediated responses in both rat and mouse cultured neurons (33). The central noradrenergic and/or glutamatergic systems play important roles in the pathophysiology of depression. The hypothesis that the activation of norepinephrine transmission is involved in the relief of behavioral despair has been well documented (11). In addition, competitive and non-competitive antagonists for the NMDA receptor channel complex reduced the immobility time and mimicked the effects of clinically effective antidepressant drugs in behavioral depression models (177). Thus, it is possible that sigma receptors play an important role in the pathophysiology of depression. Moreover, binding studies have demonstrated the interesting phenomenon that norepinephrine or serotonin uptake inhibitors potently blocked [^{3}H](+)-3-PPP binding to sigma receptors in the rat brain (149). In addition, monoamine oxidase inhibitors also displaced [^{3}H](+)-3-PPP binding to sigma receptors in the C57BL/6 mouse brain (55). A tricyclic antidepressant without norepinephrine and serotonin uptake inhibitory activity, opipramol, also potently inhibited [^{3}H](+)-3-PPP binding to sigma receptors in the guinea pig brain (122).

A prototype sigma$_1$ receptor agonist, (+)-pentazocine, reduced immobility time in the mouse forced swimming test in a dose-dependent manner (94). On the other hand, DTG and JO-1784, both non-selective sigma receptor agonists, also reduced the immobility time in the same test (94). Similarly, (+)-pentazocine and DTG reduced the immobility time in the mouse tail suspension test (87,178). Also, 3-phenyl-1-(1-propyl-1,2, 5,6-tetrahydro-pyridin-3-yl)-propan-1-one oxime (PD 128298), which has a high affinity for the sigma$_1$ receptor subtype, reduced the duration of immobility in rat behavioral despair test (139). Although NE-100 alone did not affect the immobility time, pretreatment with NE-100 completely antagonized the (+)-pentazocine- and DTG-induced reduction in immobility responses (94,178). These results indicate that sigma$_1$ receptor agonists alleviate the behavioral despair in animals.

SA4503 shortened the immobility time in the mouse forced swimming test at doses ranging from 0.1 to 1.0 mg/kg p.o. Significant reduction in immobility time by SA4503 was observed at doses of 0.1 and 1.0 mg/kg p.o. (94). Pretreatment with NE-100 0.5 mg/kg p.o. completely antagonized the reduction in immobility responses induced by SA4503 1.0 mg/kg p.o. (94). In the mouse tail suspension test, a single administration of SA4503, 1.0 mg/kg p.o., significantly reduced the duration of immobility (87,178). In this model, the repeated administration of SA4503 at doses of 1.0 and 3.0 mg/kg p.o. for 7 and 14 d also reduced the immobility time (87,178). It is interesting that the effects of SA4503 on immobility seem to be augmented by its repeated administration. The effect of a single administration of SA4503 1.0 mg/kg p.o. was significantly antagonized by pretreatment with NE-100 0.5 mg/kg p.o. (87,178). Additionally, repeated administration of SA4503 producing antidepressant effects did not affect the increase in body weight or behavioral response, whereas repeated administrations of desipramine and fluoxetine producing antidepressant effects significantly inhibited the increase in body weight (178).

Efficacy in Ischemia Models

Several sigma receptor agonists and antagonists are reported to have neuroprotective actions in both *in vitro* and *in vivo* ischemic models. For example, (+)-SKF-10,047 and (±)-pentazocine attenuated hypoxia-induced neuronal injury in mouse neocortical neuron culture (44) and glutamate-induced excitotoxicity in rat cerebellar granule cells in the absence of glucose (85). In addition, DTG, J0 1784, and α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol (BMY-14802) had neuroprotective effects against hypoxia/hypoglycemia-mediated neurotoxicity in rat primary neuronal cultures (84). With regard to the *in vivo* model, (+)-SKF-10,047, BMY-14802, U-50,488H analogs, and JO-compounds reduced CA_1 neuronal loss in the gerbil model of global brain ischemia (16,18,29,85,86,132,133). In addition, BMY-14802 protected against nitrogen anoxia- and hypoxia-induced lethality in rats and mice (138,175). Moreover, in the cat transient focal ischemia model, 4-phenyl-1-(4-phenylbutyl)piperidine (PPBP) (48) protected against acute injury in both cortex and caudate nucleus (170). Although the mechanisms of the neuroprotective action elicited by sigma receptor agonists and antagonists remain controversial, some groups have suggested that sigma receptors play an important role in these neuroprotective effects (16,18,132,138,170,175). Moreover, the neuroprotective effects mediated by sigma receptors are reported to be involved in the inhibition of the the ischemia-induced glutamate release at presynaptic sites (83,84), buffering the glutamate-induced Ca^{2+} influx at postsynaptic sites (22,74), and in the prevention of the increase in nitric oxide (NO) synthase activity (81,132).

SA4503 at concentrations ranging from 1 nM to 10 μM suppressed hypoxia/hypoglycemia-induced neurotoxicity in rat primary neuronal cultures. Significant inhibition was observed at concentrations ranging from 10 nM to 10 μM (126). The protective effect induced by SA4503 at 100 nM was significantly antagonized by NE-100 at 100 nM (126). Contrary to this observation, SA4503 showed no effect on NMDA-in-

duced neurotoxicity in rat primary neuronal cultures (126). Similarly, (+)-pentazocine suppressed hypoxia/hypoglycemia-induced neurotoxicity in a dose-dependent manner, while NMDA-induced neurotoxicity was unaffected (126). Since significant suppression by (+)-pentazocine was observed only at 1 μM, SA4503 was more potent than (+)-pentazocine. This neuroprotective effect of (+)-pentazocine at 1 μM was also blocked by NE-100 at 100 nM (126). On the other hand, (+)-3-PPP had no effect on the hypoxia/hypoglycemia-induced neurotoxicity at concentrations ranging from 1 nM to 10 μM (126). Under normal conditions, SA4503 *per se* had no cytotoxic effects on primary neuronal cultures at concentrations of 1 and 10 μM (126).

In cultured rat retinal neurons, SA4503 and (+)-pentazocine reduced glutamate-induced neurotoxicity in a dose-dependent manner at concentrations ranging from 100 nM to 100 μM. These neuroprotective effects were antagonized by NE-100 at 10 and 100 μM (154).

NEUROCHEMICAL STUDIES

Stimulation of Acetylcholine Release in Rat Brain

Central cholinergic neurons have an important role in the function of learning and memory (24,158). In particular, dysfunction of central cholinergic neurotransmission was reported in patients with dementias, such as Alzheimer's disease and senile dementia. For example, it has been shown that marked degeneration of cholinergic neurons in the nucleus basalis of Meynert and decrease of choline acetyltransferase (ChAT) activity in cerebral cortex took place in the postmortem brain of patients with Alzheimer's disease (19,26,136,185). Drugs activating the central cholinergic neurotransmission may be effective, therefore, in the treatment of dementias. Tetrahydroaminoacridine (THA), an acetylcholinesterase (AChE) inhibitor, is thought to augment cholinergic neurotransmission and to prevent the hydrolysis of the extracellular acetylcholine (128). This compound has been reported to improve cognitive dysfunction in patients with Alzheimer's disease (167,168) and animal models of amnesia (103,128).

In the central cholinergic systems, (+)-SKF-10,047 potentiated electrical stimulation- and KCl-evoked acetylcholine release in guinea pig (156) and rat (65) cerebral slices. Using an *in vivo* microdialysis method, we have found that (+)-SKF-10,047, (±)-pentazocine, DTG, and (+)-3-PPP increased in a dose-dependent manner extracellular acetylcholine levels in rat frontal cortex (95,97) and hippocampus (100). In addition, the regulation of acetylcholine transmission by sigma receptor agonists is different depending upon the brain region. Namely, the sigma receptor agonists (+)-SKF-10,047 and DTG scarcely affected extracellular acetylcholine level in the striatum (75). Those cerebral areas, such as frontal cortex and hippocampus, are considered to play an important role in the function of learning and memory (89,144,169). Moreover, the increase in extracellular acetylcholine levels elicited by (+)-SKF-10,047 was greater than that by (–)-SKF-10,047 (97,100). In addition, the

(+)-SKF-10,047- and DTG-induced increases in extracellular acetylcholine levels of the frontal cortex and hippocampus were significantly reduced by simultaneous administration of haloperidol (97,100). These results indicate that sigma$_1$ receptor agonists may be effective in the treatment of dementias such as Alzheimer's disease and senile dementia.

The extracellular acetylcholine levels in rat frontal cortex were increased in a dose-dependent manner by the administration of SA4503 at doses ranging from 5 to 20 mg/kg p.o. Maximal effects were observed at 30 and 45 min after administration of SA4503 at doses of 10 and 20 mg/kg p.o., respectively. Significant increases at doses of 10 and 20 mg/kg p.o. were observed from 30 to 60 min after administration (78,101). Similarly, SA4503 at a dose of 20 mg/kg p.o. significantly increased extracellular acetylcholine levels in the rat hippocampus from 30 to 60 min up to 105 min after administration. Contrary to these observations, SA4503, at doses of 10 and 20 mg/kg p.o., failed to affect extracellular acetylcholine levels in rat striatum (78,101).

On the other hand, THA increased extracellular acetylcholine levels in rat frontal cortex in a dose-dependent manner at doses of 5 and 10 mg/kg p.o. Maximal significant effects were observed at 45 and 60 min after administration of THA at doses of 5 and 10 mg/kg p.o., respectively (78), THA at a dose of 20 mg/kg p.o. increased extracellular acetylcholine levels by approximately 200% in rat hippocampus. This increase was, however, not significant (78). Interestingly, THA at doses of 5 and 10 mg/kg p.o. increased extracellular acetylcholine level in the rat striatum in a dose-dependent manner. Moreover, the significant increases of striatal extracellular acetylcholine level elicited by THA at a dose of 10 mg/kg p.o. were observed at 30 min after administration, and did not decline even after 150 min (78,101).

The increase in extracellular acetylcholine levels in rat frontal cortex induced by SA4503 10 mg/kg p.o. was significantly antagonized by simultaneous administration of haloperidol 0.1 mg/kg i.p. (78). Oral administration of NE-100 0.5 mg/kg 15 min before SA4503 also significantly antagonized the increase in extracellular acetylcholine levels of rat frontal cortex induced by SA4503 10.0 mg/kg p.o. (78). In addition, intracortical perfusion of tetrodotoxin (1 μM) significantly antagonized the increase in the extracellular acetylcholine level of rat frontal cortex induced by SA4503 10.0 mg/kg p.o. (78).

SA4503 at concentrations ranging from 1 nM to 10 μM did not have any significant effects on AChE, ChAT, or sodium-dependent high affinity choline uptake (SDHACU) activities in the rat frontal cortex and hippocampus (78). On the other hand, THA, 4-(1-naphthylvinyl)pyridine (NVP), and hemicholinium-3 (HCh-3), inhibited in a concentration-dependent manner the activities of AChE, ChAT, and SDHACU in both regions (78). Although AChE, ChAT, and SDHACU are closely involved in the regulation of extracellular acetylcholine levels, the mechanism by which SA4503 increased extracellular acetylcholine level did not involve the regulation of either acetylcholine-related enzymes or SDHACU activities.

Stimulation of Dopamine Neurotransmission

Sigma receptors have been reported to interact with midbrain dopamine neurons. For example, it has been reported that (+)-SKF-10,047 increased the firing rate of the substantia nigra pars compacta (A9) and the ventral tegmental area (A10) dopamine neurons (13,37,39,160), but non-benzomorphan sigma receptor agonists, such as DTG and (+)-3-PPP, decreased the activity of these midbrain dopamine neurons (39,51,160). In contrast, (+)-pentazocine increased the activity of A10 dopamine neurons and decreased the activity of A9 dopamine neurons (39,160). In addition, behavioral studies in animals have suggested a functional interaction between the sigma receptors and dopamine neurons (38,60). Moreover, the interaction between the sigma receptors and dopamine neurons in the CNS has also been supported by anatomical and autoradiographic evidence (17,45,46). In addition to these findings, we further showed that the prototype sigma$_1$ receptor agonists, (+)-SKF-10,047 and (±)-pentazocine, activated mesocortical dopamine neurons, whereas they did not affect nigrostriatal dopamine neurons (96). In addition, these activations were antagonized by rimcazole, a prototype sigma receptor antagonist (96). On the other hand, DTG, a non-specific sigma receptor agonist, also activated the mesocortical dopamine neurons, whereas it suppressed the activity of nigrostriatal dopamine neurons. Interestingly, the DTG-activated transmission in the mesocortical dopamine neurons was not antagonized by rimcazole (96).

SA4503 at a dose of 1 mg/kg p.o. significantly increased dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) contents in the rat frontal cortex (77). On the other hand, SA4503 at doses ranging from 0.1 to 10 mg/kg p.o. did not affect dopamine or its metabolites levels in other regions including hippocampus, striatum, midbrain, cerebellum, medulla/pons, and hypothalamus (77). These increases of cortical dopamine and DOPAC levels induced by SA4503, 1.0 mg/kg p.o., were completely reversed by NE-100 0.25 mg/kg p.o. which did not affect the levels when administered alone (77). In addition, cortical L-3,4-dihydroxyphenylalanine (L-DOPA) levels in *m*-hydrobenzylhydrazine (NSD-1015)-treated rats were significantly increased in comparison with those in control rats. SA4503, at a dose of 1.0 mg/kg p.o., increased L-DOPA accumulation by about 125% of the control level in the rat frontal cortex while inhibiting dopa decarboxylase activity (77). It has been reported that L-DOPA accumulation following the inhibition of dopa decarboxylase is an index of the dopamine synthesis rate, possibly by activation of tyrosine hydroxylase (12,139). Therefore, SA4503 might enhance the dopamine synthesis rate resulting in the activation of tyrosine hydroxylase. Additionally, SA4503, at doses ranging from 0.1 to 10 mg/kg p.o., did not have any effects on norepinephrine, 5-HT, or their metabolites levels in all regions.

Dopamine neurons have been reported to be involved in depressive disorders (64,186). In addition, various kinds of effective antidepressants, such as tricyclic antidepressant 5-HT reuptake inhibitors, and 5-HT$_{1A}$ receptor agonists, increased extracellular dopamine levels in the rat prefrontal cortex (173,174). The prefrontal cortex is thought to play an important role in the pathophysiology of depressive disorders, because positron emission tomography (PET) studies showed that metabolic abnormali-

Fig. 3. Hypothetical mechanism of antidepressive effect of sigma $_1$ receptor agonist.

ties in this region were correlated with the state and severity of the depressive disorder (5,27). Therefore, the antidepressive effect of SA4503 may be associated with the facilitation of cortical dopamine transmission mediated through the sigma $_1$ receptor subtype (Fig. 3). Thus, the sigma $_1$ receptor subtype plays an important role in the activation of mesocortical dopamine neurons and might be involved in the pathophysiology of depression.

BEHAVIORAL STUDIES

Cholinomimetic Side Effects

Augmentation of cholinergic transmission has been reported to induce some cholinomimetic side effects, such as miosis, lacrimation, and hypothermia in humans (36,134) and animals (128,190). In particular, it is well known that the activation of striatal cholinergic function may produce tremor, gait dysfunction, and catalepsy syndrome in rats (3,134). Thus, it is possible that SA4503 produces these cholinomimetic side effects, because this compound augments the cholinergic transmission.

SA4503 at doses of 10 and 20 mg/kg p.o. did not affect body temperature in rats. Moreover, SA4503 did not cause other cholinomimetic side effects, such as tremors,

miosis, and lacrimation in rats (78). On the contrary, THA at a dose of 10 mg/kg p.o. produced these cholinomimetic side effects (78). Similarly, THA at a dose of 20 mg/kg p.o. induced catalepsy at 60 and 90 min after administration. Haloperidol also induced catalepsy at doses of 0.5 and 1.0 mg/kg i.p. However, SA4503 at doses of 10 and 20 mg/kg p.o. had no cataleptic activity in rats (101).

Psychotomimetic Effects

It has been reported that the sigma$_1$ receptor subtype may be involved in the induction of psychotomimetic behavior. For example, (+)-SKF-10,047 and related benzomorphans induced psychotomimetic reactions in humans (7,47,69) and animals (90,124,131). Similarly, stereotyped behaviors were observed in rats that received the sigma receptor ligands (+)-3-PPP and DTG (15).

SA4503 at doses ranging from 0.1 to 20.0 mg/kg p.o. did not produce PCP-like stereotyped behaviors, such as head weaving, turning, and backpedaling in rats (76). On the contrary, (+)-SKF-10,047 at doses ranging from 5.0 to 20.0 mg/kg s.c. markedly produced PCP-like stereotyped behavior in rats (76). Additionally, the PCP (5 mg/kg i.p.)-induced stereotyped behavior was significantly augmented by (+)-SKF-10,047 5.0 mg/kg s.c., but not by SA4503 at doses ranging from 0.1 to 20 mg/kg p.o. (76).

Dystonia

Several sigma receptor ligands were reported to be involved in the regulation of movement and posture in both behavioral and biochemical studies. For example, sigma receptors are concentrated in brain structures that control movement, such as red nucleus and substantia nigra (46,79). In addition, unilateral microinjection of sigma receptor ligands, such as DTG, haloperidol, (+)-SKF-10,047, and (+)-3-PPP, into the red nucleus induced neck dystonia in rats (92,130,182,183). The potency of these sigma receptor ligands in inducing neck dystonia correlated significantly with their binding affinities for the sigma receptor labeled with [^{3}H]DTG (92,182). Similarly, other ligands with a weak affinity for sigma receptors failed to induce neck dystonia in rats (92,182,183).

The unilateral injection of SA4503 into the red nucleus at doses ranging from 1 to 10 nmol/0.5 µl induced little postural changes in the head angle (127), whereas microinjection of DTG produced apparent neck dystonia. This neck dystonia was significant at 5 and 10 nmol/0.5 µl (127).

PHARMACOKINETICS AND TOXICOLOGICAL STUDIES

SA4503, administered orally, was rapidly absorbed in rats and cynomolgus monkeys. Plasma concentrations of SA4503 reached C_{max} at 0.5 h and at 1.0 to 1.8 h after oral administration to rats and cynomolgus monkeys, respectively. The $t_{1/2}$ in the ter-

minal phase in rats and cynomolgus monkeys were 1.6 to 3.5 h and 2.4 to 4.3 h, respectively. After oral administration of SA4503 to male fasting rats, the concentration of SA4503 in cerebral tissue reached C_{max} at 0.5 to 1.0 h and then declined with a $t_{1/2}$ of 1.2 to 2.6 h. The ratio of cerebral to plasma concentration was 6.7 at 5 mg/kg p.o. SA4503 concentrations was distributed equally to various paths of the brain.

The lethal single oral doses of SA4503 were about 100 times higher than the doses effective in the amnesia model. The toxic repeated oral doses of SA4503 in a one-month toxicity study in rats were about 25 times higher than the doses effective in the amnesia model. SA4503 was not mutagenic in the Ames test.

SUMMARY AND FUTURE VISTAS

Based on the classification of sigma receptor subtypes, we concluded that the $sigma_1$ receptor subtype plays an important role in the facilitation of central cholinergic and dopaminergic functions, and suggest that specific agonists for the $sigma_1$ receptor subtype might have nootropic, antiischemic, and/or antidepressant properties. Thus, a novel and selective $sigma_1$ receptor agonist, SA4503, is expected to be useful and effective in patients with cognitive disorders, such as Alzheimer's disease, cerebrovascular dementia, or depression (Table 1). Our conclusion is supported by the clinical study which reported that sigma binding sites in CA1 area of the anterior hippocampus were reduced in the postmortem brains of Alzheimer's disease (62).

TABLE 1. Summary of pharmacological effects of SA4503 in various models

Model	Treatment	Effective Doses
Amnesia model (*in vivo*)		
Rat/PA	Scopolamine	0.1, 0.25 mg/kg (p.o.)
Rat/PA	BF-lesion	0.5 mg/kg (p.o.) × 15 d
Rat/MWM	BF-lesion	0.25 mg/kg (p.o.) × 15 d
Mouse/PA, Y-maze	β_{25-35} Amyloid	0.3 mg/kg (s.c.)
Mouse/PA, Y-maze	Dizocilpine	0.1, 0.3mg/kg(s.c.)
Mouse/PA, Y-maze	L-NAME	0.3 mg/kg (s.c.)
Depression model (*in vivo*)		
Mouse/Forced swim		0.1, 1.0 mg/kg (p.o.)
Mouse/Tail suspension		1.0, 3.0 mg/kg (p.o.)
		1.0, 3.0 mg/kg (p.o.) × 7, 14 d
Hypoxia model (*in vitro*)		
Rat brain primary culture		1 nM – 1 μM
Rat retinal cultured neuron		100 nM – 100 μM

Abbreviations: PA, Passive avoidance; MWM, Morris water maze; BF, basal forebrain; L-NAME, N^{ω}-nitro-L-arginine methyl ester.

Some recent reports have shown a close relationship between sigma$_1$ receptor subtype and steroids, such as androgens and neurosteroids. For example, the steroids, such as progesterone, testosterone, pregnenolone sulfate, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS), have all been reported to bind to the sigma receptors in the brain (112,164), liver (189), and placenta (142). In addition, these steroids have been reported to improve memory impairment in mice and rats (34,35,82,91,108,110,116,145) and augment hippocampal acetylcholine release in rats (143).

The amelioration of memory impairment elicited by DHEAS and pregnenolone sulfate was antagonized by nonspecific sigma receptor antagonists, haloperidol and BMY-14802 (108,110). On the other hand, the ameliorating effects elicited by SA4503 and (+)-pentazocine were antagonized by progesterone, similar to DHEAS and pregnenolone (110,111). In addition, both sigma$_1$ receptor agonists and neurosteroids modulate the [^{3}H]norepinephrine release evoked by NMDA in rat hippocampal slices. These modulations were antagonized by haloperidol and progesterone (121). Similarly, DHEA potentiated the excitatory response of pyramidal neurons to NMDA in the CA3 regions of the dorsal hippocampus in the rat via the sigma receptor, because this effect of DHEA was reversed by haloperidol and NE-100 (8,21). Moreover, progesterone blocked not only the DHEAS-, but also (+)-pentazocine-, DTG-, and JO-1784-induced augmentation of NMDA-evoked neuronal activation of CA3 dorsal hippocampal neurons (8,21). On the other hand, DHEA and DHEAS are reported to be the most abundant circulating steroid hormones in humans. In addition, because the plasma concentrations of DHEA and DHEAS steadily decrease with aging, these steroids are likely to be involved in numerous age-related diseases, such as cognitive impairment, (2,184).

Thus, we suggest that the sigma$_1$ receptor agonists are useful for hormone replacement therapy and can be used in the treatment of cognitive disorders, such as Alzheimer's disease, cerebrovascular dementia, and depression. In fact, a recent study has reported that DHEAS showed antidepressive and memory enhancing effects in humans (187).

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REFERENCES

1. Amano M, Yamada K, Matsuno K, Nabeshima T. Neuropharmacological effects of sigma recepto r ligands: Anxiolytic, anti-amnesic and neuroprotective effects (abstract in English). *Jpn J Psychopharmacol* 1996;16:73–84.
2. Barrou Z, Charry P, Lidy C. Dehydroepiandrosterone (DHEA) and aging. *Arch Gerontol Geriatr* 1997;24:233–241.
3. Bartholini G, Stadler H, Lloyd KG. Cholinergic-dopaminergic interregulations within the e xtrapyramidal systems. In: Waser PG, ed. *Cholinergic Mechanisms*. New York: Raven Press, 1975:411–418.

4. Battaglia G, Titeler M. [^{3}H]N-Propylapomorphine and [^{3}H]spiperone binding in brain indicate two states of the D_2-dopamine receptor. *Eur J Pharmacol* 1982;81:493–498.
5. Baxter LR, Schwartz JM, Phelps ME, et al. Reduction of prefrontal cortex glucose metabol ism common to three types of depression. *Arch Gen Psychiatry* 1989;46:243.
6. Beart PM, O'Shea RD, Manallack DT. Regulation of σ receptors: High- and low-affinity agonist states, GTP shifts, and up-regulation by rimcazole and 1,3-di(2-tolyl)guanidine. *J Neurochem* 1989;53:779–788.
7. Bellville JW, Forrest WH Jr. Respiratory and subjective effect of d- and l-pentazocine. *Clin Pharmacol Ther* 1968;9:142–151.
8. Bergeron R, De Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced b y dehydroepiandrosterone and its suppression by progesterone: Effects mediated via sigma recept ors. *J Neurosci* 1996;16:1193–1202.
9. Bonhaus DW, Loury DN, Jakeman LB, et al. [^{3}H]BIMU-1, a 5-hydroxytryptamine$_3$ receptor ligand in NG-108 cells, selectively labels *sigma*-2 binding sites in guinea pig hippocampus. *J Pharmacol Exp Ther* 1993;267:961–970.
10. Bowen WD, Hellewell SB, McGarry KA. Evidence for a multi-site model of the rat brain σ receptor. *Eur J Pharmacol* 1989;163:309–318.
11. Bunny WE, Davies JM. Norepinephrine in depressive reactions. *Arch Gen Psychiatry* 1965;13:483–494.
12. Carlsson A, Kehr W, Lindqvist M, Magnusson T, Atack CV. Regulation of monoamine metabolis m in the central nervous system. *Pharmacol Rev* 1972;24:371–384.
13. Ceci A, Smith M, French ED. Activation of the A_{10} mesolimbic system by the σ receptor agonist (+)SKF 10,047 can be blocked by rimcazole, a novel putative antipsychotic. *Eur J Pharmacol* 1988;154:53–57.
14. Connick JH, Hanlon G, Roberts J, France L, Fox PK, Nicholson CD. Multiple G binding sites in guinea pig and rat brain membranes: G-protein interactions. *Br J Pharmacol* l992;107:726–731.
15. Contreras PC, Contreras ML, Compton RP, O'Donohue TL. Biochemical and behavioral charac - terization of PCP and sigma receptors. In: Domino EF and Kamenka J-M, eds. *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology*. Ann Arbor: NPP Books, 1988:327–334.
16. Contreras PC, Gray NM, Ragan DM, Lanthorn TH. BMY-14802 protects against ischemia-induced neuronal damage in the gerbil. *Life Sci* 1992;51:1145–1149.
17. Contreras PC, Quirion R, Gehlert DR, Contreras ML, O'Donohue TL. Autoradiographic distrib ution of non-dopaminergic binding sites labeled by [^{3}H]haloperidol in rat brain. *Neurosci Lett* 1987;75:133–140.
18. Contreras PC, Ragan DM, Bremer ME, et al. Evaluation of U-50,488H analogs for neuroprotec tive activity in the gerbil. *Brain Res* 1991;546:79–82.
19. Davies P, Maloney AJF. Selective loss of central cholinergic neurons in Alzheimer's dise ase. *Lancet* 1976;2:1403.
20. Debonnel G. Current hypotheses on sigma receptors and their physiological role: Possib le implications in psychiatry. *J Psychiatr Neurosci* 1993;18:157–172.
21. Debonnel G, Bergeron R, De Montigny C. Potentiation by dehydroepiandrosterone of the neuronal response to *N*-methyl-*D*-aspartate in the CA_3 region of the rat dorsal hippocampus: An effect mediated via sigma receptors. *J Endocrinol* 1996;150:S33–S42.
22. DeCoster MA, Klette KL, Knight ES, Tortella FC. σ Receptor-mediated neuroprotection against gluta - mate toxicity in primary rat neuronal cultures. *Brain Res* 1995;671:45–53.
23. DeHaven-Hudkins DL, Fleissner LC, Ford-Rice FY. Characterization of the binding of [^{3}H](+)-pentazocine to σ recognition sites in guinea pig brain. *Eur J Pharmacol Mol Pharmacol* 1992;227:371–378.
24. Deutsch JA. The cholinergic synapse and the site of memory. In: Deutsch JA, ed. *The Physiological Basis of Memory*. New York: Academic Press, 1983;367–386.
25. Dolphin AC. Nucleotide binding proteins in signal transduction and disease. *Trends Neurosci* 1987;10:53–57.
26. Drachman DA. Memory and cognitive function in man: Does the cholinergic system have a sp ecific role? *Neurology* 1977;27:783–790.
27. Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME. A functional anatom ical study of unipolar depression. *J Neurosci* 1992;12:3628–3641.

28. Earley B, Burke M, Leonard BE, Gouret C-J, Junien J-L. Evidence for an anti-amnesic effect of JO-1784 in the rat: A potent and selective ligand for the sigma receptor. *Brain Res* 1991;546:282–286.
29. Earley B, Canney M, O'Neill M, Leonard BE, Junien J-L. Experimental cerebral ischaemia in mongolian gerbils: Effects of JO-1784 on locomotor activity and ischaemia-induced hippocampal damage. *Med Sci Res* 1995;23:555–559.
30. Ellis Y, Davies JA. The effects of sigma ligands on the release of glutamate from rat striatal slices. *Naunyn-Schmied Arch Pharmacol* 1994;350:143–148.
31. Ferris CD, Hirsch DJ, Brooks BP, Snyder SH. σ Receptors: From molecule to man. *J Neurochem* 1991;57:729–737.
32. Ferris RM, Tang FLM, Chang K-J, Russell A. Evidence that the potential antipsychotic agent rimcazole (BW 234U) is a specific, competitive antagonist of sigma sites in brain. *Life Sci* 1986;38:2329–2337.
33. Fletcher EJ, Church J, Adbel-Hamid K, MacDonald JF. Selective reduction of *N*-methyl-*D*-aspartate-evoked responses by 1,3-di(2-tolyl)guanidine in mouse and rat cultured hippocampal pyramidal neurons. *Br J Pharmacol* 1993;109:1196–1205.
34. Flood JF, Roberts E. Dehydroepiandrosterone sulfate improves memory in aging mice. *Brain Res* 1988;448:178–181.
35. Flood JF, Morley JE, Roberts E. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc Natl Acad Sci USA* 1992;89:1567–1571.
36. Forsyth DR, Surmon DJ, Morgan RA, Wilcock GK. Clinical experience with and side effects of tacrine hydrochloride in Alzheimer's disease: A pilot study. *Age Ageing* 1989;18:223–229.
37. Freeman AS, Bunney BS. The effects of phencyclidine and *N*-allylnormetazocine on midbrain dopamine neuronal activity. *Eur J Pharmacol* 1984;104:287–293.
38. French ED. Effects of *N*-allylnormetazocine (SKF 10,047), phencyclidine, and other psychomotor stimulants in the rat following 6-hydroxydopamine lesion of the ventral tegmental area. *Neuropharmacology* 1986;25:447–450.
39. French ED, Ceci A. Noncompetitive *N*-methyl-*D*-aspartate antagonists are potent activators of ventral tegmental A_{10} dopamine neurons. *Neurosci Lett* 1990;119:159–162.
40. Fujimura K, Matsumoto J, Niwa M, et al. Synthesis, structure, and quantitative structure-activity relationships of σ receptor ligands, 1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazines. *Bioorg Med Chem* 1997;5:1675–1683.
41. Gilligan PJ, Tam SW. Sigma receptor ligands: Potential drugs for the treatment of CNS disorders? *Drug News Persp* 1994;7:13–18.
42. Gilman AG. Receptor-regulated G proteins. *Trends Neurosci* 1986;9:460–463.
43. Gilman AG. G proteins: Transducers of receptor-generated signals. *Annu Rev Biochem* 1987;56:615–649.
44. Goldberg MP, Viseskul V, Choi DW. Phencyclidine receptor ligands attenuate cortical neuronal injury after *N*-methyl-*D*-aspartate exposure or hypoxia. *J Pharmacol Exp Ther* 1988;245:1081–1087.
45. Graybiel AM, Besson M-J, Weber E. Neuroleptic-sensitive binding sites in the nigrostriatal system: Evidence for differential distribution of sigma sites in the substantia nigra, pars compacta of the cat. *J Neurosci* 1989;9:326–338.
46. Gundlach AL, Largent BL, Snyder SH. Autoradiographic localization of sigma receptor binding sites in guinea pig and rat central nervous system with (+)^{3}H-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine. *J Neurosci* 1986;6:1757–1770.
47. Haertzen CA. Subjective effects of narcotic antagonists cyclazocine and nalorphine on the addiction research center inventory (ARCI). *Psychopharmacology* 1970;18:366–377.
48. Hashimoto K, London ED. Further characterization of [^{3}H]ifenprodil binding to σ receptors in rat brain. *Eur J Pharmacol* 1993;236:159–163.
49. Hellewell SB, Bowen WD. A sigma-like binding site in rat pheochromocytoma (PC12) cells: Decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor form from that of guinea pig brain. *Brain Res* 1990;527:244–253.
50. Hjorth S, Carlsson A, Wikström H, et al. 3-PPP, a new centrally acting DA receptor agonist with selectivity for autoreceptors. *Life Sci* 1981;28:1225–1238.

51. Hjorth S, Clark D, Carlsson A. Lack of functional evidence for the involvement of sigma opia te receptors in the actions of the 3-PPP enantiomers on central dopaminergic systems: Discrepanci es between *in vitro* and *in vivo* observations. *Life Sci* 1985;37:673–684.
52. Hoffman BB, Mullikin-Kilpatrick D, Lefkowitz RJ. Heterogeneity of radioligand bindin g to α-adrenergic receptors. *J Biol Chem* 1980;255:4645–4652.
53. Itzhak Y. Multiple affinity binding states of the σ receptor: Effect of GTP-binding protein-modifying agents. *Mol Pharmacol* 1989;36:512–517.
54. Itzhak Y. Multiple sigma binding sites in the brain. In: Itzhak Y, ed. *Sigma Receptors*. San Diego: Academic Press Inc., 1994:113–137.
55. Itzhak Y, Kassim CO. Clorgyline displays high affinity for σ binding sites in C57BL/6 mouse brain. *Eur J Pharmacol* 1990;176:107–108.
56. Itzhak Y, Khouri M. Regulation of the binding of σ- and phencyclidine (PCP) receptor ligands in rat brain membranes by guanine nucleotides and ions. *Neurosci Lett* 1988;85:147–152.
57. Itzhak Y, Stein I. Sigma binding sites in the brain: An emerging concept for multiple sites and their relevance for psychiatric disorders. *Life Sci* 1990;47:1073–1081.
58. Itzhak Y, Stein I. Regulation of σ receptors and responsiveness to guanine nucleotides following re peated exposure of rats to haloperidol: Further evidence for multiple σ binding sites. *Brain Res* 1991;566:166–172.
59. Itzhak Y, Stein I, Zhang S-H, Kassim CO, Cristante D. Binding of σ-ligands to C57BL/6 mouse brain membranes: Effects of monoamine oxidase inhibitors and subcellular distribution studies suggest the existence of σ receptor subtypes. *J Pharmacol Exp Ther* 1991;257:141–148.
60. Iwamoto ET. Locomotor activity and antinociception after putative *mu*, *kappa* and *sigma* opioid receptor agonists in the rat: Influence of dopaminergic agonists and antagonists. *J Pharmacol Exp Ther* 1981;217:451–460.
61. Iyengar S, Dilworth VM, Mick SJ, et al. Sigma receptors modulate both A9 and A10 dopaminer gic neurons in the rat brain: Functional interaction with NMDA receptors. *Brain Res* 1990;524:322–326.
62. Jansen KLR, Faull RLM, Storey P, Leslie RA. Loss of sigma binding sites in the CA1 area of th e anterior hippocampus in Alzheimer's disease correlates with CA1 pyramidal cell loss. *Brain Res* 1993;623:299–302.
63. Jeanjean AP, Mestre M, Maloteaux J-M, Laduron PM. Is the σ_2 receptor in rat brain related to the K^+ channel of class III antiarrhythmic drug? *Eur J Pharmacol* 1993;241:111–116.
64. Jimerson DC. Role of dopamine mechanisms in the affective disorders. In: Meltzer HY, ed. *Psychopharmacology*: *The Third Generation of Progress*. New York: Raven Press, 1987:505–511.
65. Junien JL, Roman FJ, Brunelle G, Pascaud X. JO 1784, a novel σ ligand, potentiates [^{3}H]acetylcholine release from rat hippocampal slices. *Eur J Pharmacol* 1991;200:343–345.
66. Karbon EW, Naper K, Pontecorvo MJ. [^{3}H]DTG and [^{3}H](+)-3-PPP label pharmacologically distinct σ binding sites in guinea pig membranes. *Eur J Pharmacol* 1991;193:21–27.
67. Karbon EW, Patch RJ, Pontecorvo MJ, Ferkany JW. Ifenprodil potently interacts with [^{3}H](+)-3-PPP-labeled σ binding sites in guinea pig brain membranes. *Eur J Pharmacol* 1990;176:247–248.
68. Kawashima Y, Matsumoto J, Matsuno K, Senda T, Hirano K. Novel 1,4-di(phenylalkyl)piperazin e. WO 95/04050.
69. Keats AS, Telford J. Narcotic antagonists as analgesics. Clinical aspects. In: Gould RF , ed. *Molecular Modification in Drug Design*; *Advances in Chemistry Series 45*. Washington: American Chemical Society, 1964:170–176.
70. Kinney GG, Harris EW, Ray R, Hudzik TJ. σ_2 Site-mediated inhibition of electrically evoked guinea pig ileum longitudinal muscle/myenteric plexus contractions. *Eur J Pharmacol* 1995;294:547–553.
71. Kinouchi K, Maeda S, Saito K, Inoki R, Fukumitsu K, Yoshiya I. Effects of D- and L-pentazoc ine on the release and uptake of norepinephrine in rat brain cortex. *Res Commun Chem Path Pharmacol* 1989;63:201–213.
72. Klein M, Musacchio JM. High affinity dextromethorphan binding sites in guinea pig brain ef fect of sigma ligands and other agents. *J Pharmacol Exp Ther* 1989;251:207–215.

73. Klein M, Cooper TB, Musacchio JM. Effects of haloperidol and reduced haloperidol on bind ing to σ sites. *Eur J Pharmacol* 1994;254:239–248.
74. Klette KL, DeCoster MA, Moreton JE, Tortella FC. Role of calcium in sigma-mediated neurop rotection in rat primary cortical neurons. *Brain Res* 1995;704:31–41.
75. Kobayashi T, Matsuno K, Mita S. Regional differences of the effect of σ receptor ligands on the acetylcholine release in the rat brain. *J Neural Transm* 1996;103:661–669.
76. Kobayashi T, Matsuno K, Mita S. Sigma$_1$ receptor subtype does not interact with stereotyped behaviors in rats. *Pharmacol Biochem Behav* 1998 (in press).
77. Kobayashi T, Matsuno K, Murai M, Mita S. σ_1 Receptor subtype is involved in the facilitation of cortical dopaminergic transmission in the rat brain. *Neurochem Res* 1997;22:1105–1109.
78. Kobayashi T, Matsuno K, Nakata K, Mita S. Enhancement of acetylcholine release by SA4503, a novel σ_1 receptor agonist, in the rat brain. *J Pharmacol Exp Ther* 1996;279:106–113.
79. Largent BL, Gundlach AL, Snyder SH. Pharmacological and autoradiographic discriminati on of *sigma* and phencyclidine receptor binding sites in brain with (+)-[^{3}H]SKF 10,047, (+)-[^{3}H]-3-[3-hydroxyphenyl]-N-(1-propyl)piperidine and [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine. *J Pharmacol Exp Ther* 1986;238:739–748.
80. Lefkowitz RJ, Caron MG. Stiles GL Mechanisms of membrane receptor regulation. *N Engl J Med* 1984;310:1570–1579.
81. Lesage AS, DeLoore KL, Peeters L, Leysen JE. Neuroprotective sigma ligands interfere wit h the glutamate-activated NOS pathway in hippocampal cell culture. *Synapse* 1995;20:156–164.
82. Li P-K, Rhodes ME, Jagannathan S, Johnson DA. Reversal of scopolamine-induced amnesia i n rats by the steroid sulfatase inhibitor estrone-3-O-sulfamate. *Cog Brain Res* 1995;2:251–254.
83. Lobner D, Lipton P. σ-Ligands and non-competitive NMDA antagonists inhibit glutamate release during cerebral ischemia. *Neurosci Lett* 1990;117:169–174.
84. Lockhart BP, Soulard P, Benicourt C, Privat A, Junien J-L. Distinct neuroprotective pro files for σ ligands against *N*-methyl-*D*-aspartate (NMDA), and hypoxia-mediated neurotoxicity in neuronal culture toxicity studies. *Brain Res* 1995;675:110–120.
85. Lysko PG, Yue T-L, Gu J-L, Feuerstein G. Neuroprotective mechanism of (+)SKF 10,047 *in vitro* and in gerbil global brain ischemia. *Stroke* 1992;23:1319–1324.
86. Lysko PG, Gagnon RC, Yue T-L, Gu J-L, Feuerstein G. Neuroprotective effects of SKF 10,047 in cultured rat cerebellar neurons and in gerbil global brain ischemia. *Stroke* 1992;23;414–419.
87. Maeda H, Ukai M, Nanya Y, Kameyama T, Matsuno K. Psychotropic effects of the novel σ_1 receptor agonist SA4503: Evaluations using tail-suspension and conditioned suppression tests. *Jpn J Psychopharmacol* 1996;16:251.
88. Manallack DT, Beart PM, Gundlach AL. Psychotomimetic σ-opiates and PCP. *Trends Pharmacol Sci* 1986;7:448–451.
89. Mandel RJ, Gage FH, Thal LJ. Spatial learning in rats: Correlation with cortical choline acetyltransferase and improvement with NGF following NBM damage. *Exp Neurology* 1989;104:208–217.
90. Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE. The effects of morphine- and nalo rphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther* 1976;197:517–532.
91. Mathis C, Vogel E, Cagniard B, Criscuolo F, Ungerer A. The neurosteroid pregnenolone sulfat e blocks deficits induced by a competitive NMDA antagonist in active avoidance and lever-press learni ng tasks in mice. *Neuropharmacology* 1996;35:1057–1064.
92. Matsumoto RR, Hemstreet MK, Lai NL, et al. Drug specificity of pharmacological dystonia. *Pharmacol Biochem Behav* 1990;36:151–155.
93. Matsuno K, Kobayashi T, Mita S. Involvement of σ receptors in the increase in contraction of mouse vas deferens induced by exogenous ATP. *J Pharm Pharmacol* 1996;48:96–99.
94. Matsuno K, Kobayashi T, Tanaka MK, Mita S. σ_1 Receptor subtype is involved in the relief of behavioral despair in the mouse forced swimming test. *Eur J Pharmacol* 1996;312:267–271.
95. Matsuno K, Matsunaga K, Mita S. Increase of extracellular acetylcholine level in rat fr ontal cortex induced by (+)*N*-allylnormetazocine as measured by brain microdialysis. *Brain Res* 1992;575:315–319.

96. Matsuno K, Matsunaga KH, Mita S. Acute effects of σ ligands on the extracellular DOPAC level in rat frontal cortex and striatum. *Neurochem Res* 1995;20:233–238.
97. Matsuno K, Matsunaga K, Senda T, Mita S. Increase in extracellular acetylcholine level by σ ligands in rat frontal cortex. *J Pharmacol Exp Ther* 1993;265:851–859.
98. Matsuno K, Mita S. Sigma receptor ligands. *Farmashia* 1995;31:505–508.
99. Matsuno K, Nakazawa M, Okamoto K, Kawashima Y, Mita S. Binding properties of SA4503, a novel and selective σ_1 receptor agonist. *Eur J Pharmacol* 1996;306:271–279.
100. Matsuno K, Senda T, Kobayashi T, Mita S. Involvement of σ_1 receptor in (+)-*N*-allylnormetazocine-stimulated hippocampal cholinergic functions in rats. *Brain Res* 1995;690:200–206.
101. Matsuno K, Senda T, Kobayashi T, Okamoto K, Nakata K. Mita S. SA4503, a novel cognitive enhancer, with σ_1 receptor agonistic properties. *Behav Brain Res* 1997;83:221–224.
102. Matsuno K, Senda T, Matsunaga K, Mita S, Kaneto H. Similar ameliorating effects of benz omorphans and 5-HT_2 antagonists on drug-induced impairment of passive avoidance response in mice: Comparison with acetylcholinesterase inhibitors. *Psychopharmacology* 1993;112:134–141.
103. Matsuno K, Senda T, Matsunaga K, Mita S. Ameliorating effects of σ receptor ligands on the impairment of passive avoidance tasks in mice: Involvement in the central acetylcholinergic syst em. *Eur J Pharmacol* 1994;261:43–51.
104. Matsuno K, Senda T, Mita S. Correlation between potentiation of neurogenic twitch cont raction and benzomorphan σ receptor binding potency in the mouse vas deferens. *Eur J Pharmacol* 1993;231:451–457.
105. Maurice T, Hiramatsu M, Itoh J, Kameyama T, Hasegawa T, Nabeshima T. Behavioral evidence f or a modulating role of a ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-i nduced amnesia. *Brain Res* 1994;647:44–56.
106. Maurice T, Hiramatsu M, Itoh J, Kameyama T, Hasegawa T, Nabeshima T. Low dose of 1,3-di(2-to lyl)guanidine (DTG) attenuates MK-801-induced spatial working memory impairment in mice. *Psychopharmacology* 1994;114:520–522.
107. Maurice T, Hiramatsu M, Kameyama T, Hasegawa T, Nabeshima T. Behavioral evidence for a mod ulating role of σ ligands in memory processes. II. Reversion of carbon monoxide-induced amnesia. *Brain Res* 1994;647:57–64.
108. Maurice T, Junien J-L, Privat A. Dehydroepiandrosterone sulfate attenuates dizocilpi ne-induced learning impairment in mice via σ_1 receptors. *Behav Brain Res* 1997;83:159–164.
109. Maurice T, Lockhart BP. Neuroprotective and anti-amnesic potentials of sigma (σ) receptor ligands. *Prog Neuro-Psychopharmacol Biol Psychiat* 1997;21:69–102.
110. Maurice T, Privat A. Selective σ_1 receptor agonists and neurosteroids attenuate β[25-35]-amyloid peptide-induced amnesia in mice through a common mechanism. *Soc Neurosci Abstr* 1996;22:2006.
111. Maurice T, Privat A. SA4503, a novel cognitive enhancer with σ_1 receptor agonist properties, facilitates NMDA receptor-dependent learning in mice. *Eur J Pharmacol* 1997;328:9–18.
112. Maurice T, Roman FJ, Privat A. Modulation by neurosteroids of the *in vivo* (+)-[^{3}H]SKF-10,047 binding to σ_1 receptors in the mouse forebrain. *J Neurosci Res* 1996;46:734–743.
113. Maurice T, Roman FJ, Su T-P, Privat A. Beneficial effects of sigma agonists on the age-rel ated learning impairment in the senescence-accelerated mouse (SAM). *Brain Res* 1996;733:219–230.
114. Maurice T, Su T-P, Parish DW, Nabeshima T, Privat A. PRE-084, a σ selective PCP derivative, attenuates MK-801-induced impairment of learning in mice. *Pharmacol Biochem Behav* 1994;49:859–869.
115. Maurice T, Su T-P, Parish DW, Privat A. Prevention of nimodipine-induced impairment of learning by the selective σ ligand PRE-084. *J Neural Transm* 1995;102:1–18.
116. Mayo W, Dellu F, Robel P, et al. Infusion of neurosteroids into the nucleus basalis magnoc ellularis affects cognitive processes in the rat. *Brain Res* 1993;607:324–328.
117. McCann DJ, Su T-P. Haloperidol competitively inhibits the binding of (+)-[^{3}H]SKF-10,047 to σ sites. *Eur J Pharmacol* 1990;180:361–364.
118. Mendelsohn DJ, Rabin RA, Johnson BG, Kerchner GA. Sigma opioid receptor: Characterization and co-identity with the phencyclidine receptor. *J Pharmacol Exp Ther* 1985;233:597–602.