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Binding properties of [^{3}H]MS-377, a novel σ receptor ligand, to rat brain membranes

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Abstract

MS-377 ((R)-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate) is a novel selective σ receptor ligand, currently being developed for the treatment of schizophrenia. MS-377 showed anti-phencyclidine (PCP), anti-dopaminergic and anti-serotonergic activities, and we anticipated that the anti-psychotic activities of MS-377 were associated with σ_1 receptors. However, its pharmacological profile is partly distinct from those of selective σ_1 receptor ligands. Thus, one of the possible speculations is that MS-377 has another site of action. In the present study, we examined the binding properties of radiolabeled MS-377 ([^{3}H]MS-377) to rat brain membranes. [^{3}H]MS-377 showed saturable and reversible binding to rat brain membranes. Scatchard plot and Hill plot from saturation studies were linear, with K_d of 15.2 ± 6.6 nM, B_{max} of 599.4 ± 58.6 fmol/mg protein and Hill coefficient of 1.01 ± 0.01, indicating that [^{3}H]MS-377 bound to a single high-affinity site in rat brain membranes. Displacement studies revealed that the other σ reference compounds with different structures inhibited the specific binding of [^{3}H]MS-377 in a competitive manner. Stereoselectivity was observed for the inhibition of [^{3}H]MS-377 binding, (+)-isomers were more potent than (−)-isomers. Non-σ receptor ligand PCP showed weak inhibition of [^{3}H]MS-377 binding. The rank order of potency for the σ reference compounds to displace [^{3}H]MS-377 binding were as following: haloperidol > MS-377 = (+)-pentazocine > DTG (1,3-Ditolylguanidine) = (−)-pentazocine > BMY14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyramidinyl)-1-piperazine butanol) > (+)-SKF-10,047 > (−)-SKF-10,047 = PCP. These results suggested that the MS-377 selectively binds to σ binding site with high affinity in rat brain membranes. Therefore, the anti-psychotic activities of MS-377 are attributable to association with σ_1 receptors.

Keywords: MS-377; σ Binding site; Radiolabeled ligand binding; Brain membrane, rat

1. Introduction

σ Receptors were first postulated by Martin et al. (1976) to explain the psychotomimetic effects of benzomorphans such as *N*-allylnormetazocine ((+)-SKF-10,047) and (+)-pentazocine. They were first defined as an opioid receptor subtype and were later thought to be identical to phencyclidine (PCP) binding sites (Zukin and Zukin, 1981). However, σ receptors were distinct from opioid or PCP binding sites (Gundlach et al., 1986; Quirion et al., 1987), and then they could be classified into at least two subtypes, designated σ_1 and σ_2 (Walker et al., 1990; Quirion et al., 1992). These receptors were expressed in the central nervous system as well as several peripheral tissues of endocrine and immune system (Su, 1991; Liu et al., 1995; Hanner et al., 1996), and cDNAs of the σ_1 receptors have been cloned from several mammalian species in recent years (Hanner et al., 1996; Kekuda et al., 1996; Seth et al., 1997; Pan et al., 1998; Prasad et al., 1998; Seth et al., 1998). The amino acid sequences of σ_1 receptors were highly homologous among different mammalian species, but it shares no homology to known mammalian proteins. This evidence suggested that the σ_1 receptor is a distinct entity from any other known receptor, indicating its importance in cellular functions, although its physiological roles and endogenous ligands are not well established.

Some typical anti-psychotic agents such as haloperidol, perphenazine and chlorpromazine, which are dopamine D_2 antagonists, also have affinities for σ receptors (Tam and Cook, 1984; Snyder and Largent, 1989). Further, σ receptor binding was decreased in postmortem schizophrenic brain tissue compared to normal subjects (Helmeste et al., 1996), and this evidence suggests that σ receptors play a role in psychosis and are involved in the pathophysiology

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of schizophrenia. In addition, it has been demonstrated that NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]ethylamine monohydrochloride), a σ antagonist inhibited PCP-induced behaviors in animal models (Okuyama et al., 1993), and σ receptor ligands blocked the development of behavioral sensitization induced by methamphetamine and cocaine (Ujike et al., 1992, 1996). This evidence suggests that it is possible to create a new class of anti-psychotic drugs with selective high affinity for σ binding sites and without any affinity for dopamine receptors. Furthermore, σ receptor ligands are currently targeted as potential drugs in the pharmacotherapy of cocaine and amphetamine abuse (Witkin, 1994).

MS-377 ((R)-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate), a novel anti-psychotic agent with high affinity for σ receptors, is currently being developed for the treatment of schizophrenia. MS-377 had no or less affinities for other receptors including σ_2, dopamine, serotonin, PCP-site, and so on. MS-377 showed inhibitory effects on various drug-induced behaviors such as (+)-*N*-allylnormetazocine-induced head-weaving, 5-hydroxy-DL-tryptophan-induced head-twitching and apomorphine-induced climbing (Takahashi et al., 1999). Moreover, MS-377 inhibited PCP-induced head-weaving and rearing behaviors, which are recognized as comprehensive models of schizophrenia (Ellison, 1995; Javitt and Zukin, 1991). Especially, PCP-induced head-weaving was not affected by typical anti-psychotics, thus MS-377 was expected to be useful for treatment for schizophrenia against which typical anti-psychotics are ineffective. In addition, MS-377 did not induce catalepsy and did not prevent apomorphine-induced stereotyped behavior and PCP-induced ataxia in rats (Takahashi et al., 1999). In fact, several clinical trials of σ receptor ligands have been conducted; these agents were found to be effective against negative symptoms and to have no extrapyramidal side effects (Modell et al., 1996; Frieboes et al., 1997). Based on these data, the profile of MS-377 intimates the usefulness for the treatment of schizophrenia regardless of its response to typical anti-psychotic agents without any effect on the motor coordination. The anti-psychotic activities of MS-377 are attributable to the association with σ_1 receptors, because it had affinity for only σ_1 receptor. Since MS-377 has unique pharmacological profile distinct from those of σ_1 receptor ligands, it is also probable that MS-377 has another site of action. In this study, we characterized the binding properties of radiolabeled MS-377 ([^{3}H]MS-377) to rat brain membranes.

2. Materials and methods

2.1. Animals

Male Wistar rats (7 weeks old) (Nihon SLC, Japan) were used in this study. Animals were housed in groups of three, under a 12/12-h light/dark cycle (lights on at 0600 h) with food and water ad libitum. The present experiments were performed in strict accordance with the guidelines for animal experiments at the Institute of Biological Science, Mitsui Pharmaceuticals, and the protocol was approved by the Animal Investigation Committee of the Institute.

2.2. Drugs

MS-377, (+)-pentazocine, (−)-pentazocine and BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyramidinyl)-1-piperazine butanol) were synthesized by Mitsui Chemicals, and PCP was synthesized by Mitsui Pharmaceuticals. (+)-SKF-10,047 and (−)-SKF-10,047 was purchased from Research Biochemical. Haloperidol was purchased from Sigma, DTG (1,3-Ditolylguanidine) was purchased from Tokyo Chemical Industry, [^{3}H]MS-377 was obtained from Amersham International (specific activity 1.18 TBq/mmol). Drugs were dissolved in distilled water or dimethylformamide and then diluted with assay buffer.

2.3. Preparation of brain membranes

Receptor binding assays were carried out using the crude whole brain P2 membrane fraction, according to the σ_1 receptor binding assay (Bowen et al., 1992). Animals were decapitated and the whole brain (removed cerebellum) was rapidly excised and homogenized with 10 vols. (w/v) of ice-cold 0.32 M sucrose in 50 mM Tris–HCl buffer pH 7.4, with a teflon-glass homogenizer. The homogenate was centrifuged at $1000 \times g$ for 10 min at 4°C, and the supernatant was collected and centrifuged at $31{,}000 \times g$ for 15 min at 4°C. The precipitate was resuspended in 3 vols. (w/v) of the same buffer and incubated at 25°C for 15 min. After centrifugation at $31{,}000 \times g$ for 15 min at 4°C, the precipitate was resuspended in 1.5 vols. (w/v) of ice-cold 10 mM Tris–HCl buffer pH 7.6, at a protein concentration of 10–15 mg/ml, and stored at −80°C until use. Protein concentration was determined by the method of Bradford (1976), using the Bio-Rad protein assay reagent.

2.4. Receptor binding assay

For a typical experiment, each assay tube contained 50 μl of [^{3}H]MS-377, 250 μl of membranes suspension and 200 μl of assay buffer to a final assay volume of 0.5 ml, of which final protein concentration was approximately 0.4 mg/tube. Non-specific binding was determined in the presence of 10 μM unlabeled MS-377. All tubes were incubated for 180 min at 25°C, and the reaction was terminated by rapid filtration through a Whatman GF/B glass filter pre-soaked in 0.05% polyethyleneimine for at least 30 min. Filters were washed three times with 3 ml of ice-cold 50 mM Tris–HCl buffer pH 7.6, and filter-bound radioactivity was measured using a liquid scintillation counter. In the kinetic studies of association and dissocia-

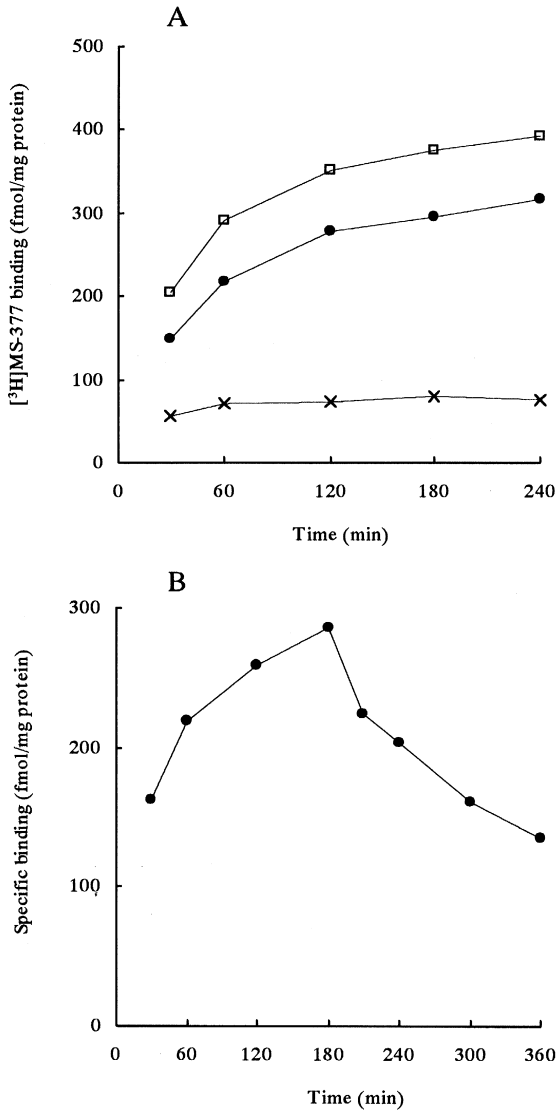

Fig. 1. Kinetics of [^{3}H]MS-377 binding to rat brain membranes. Rat brain membranes were incubated with 12.5 nM [^{3}H]MS-377 at 25°C. (A) Association experiment. Total binding, nonspecific binding and specific binding are indicated as □, × and ●, respectively. (B) Dissociation experiment. After equilibrium was reached (180 min), 10 μM of MS-377 was added and specific binding monitored for 180 min. The data are mean value of triplicate determinations.

tion, reaction was achieved at 25°C using 12.5 nM [^{3}H]MS-377. Association experiments were initiated by addition of membranes fraction, and incubated for various lengths of time. In dissociation experiments, after equilibrium was reached (180 min), dissociation was initiated by addition of an excess (10 μM) of unlabeled MS-377, and specific binding was monitored for 180 min. Saturation experiments were conducted over concentrations ranging from 0.78 to 50 nM of [^{3}H]MS-377. Saturation binding data were analyzed by Scatchard plot and Hill plot, and the dissociation constant (K_d), maximal number of binding sites (B_{max}) and Hill coefficient were calculated. In the competition binding assay, all drugs were tested at least at three to seven concentrations, and the percent inhibition of [^{3}H]MS-377 binding at each concentration tested was calculated. The IC_{50} value was determined by log–logit analysis, and K_i value was calculated using the K_d value obtained by Scatchard plot.

3. Results

3.1. Optimization of assay condition

Initially, optimization of assay condition was investigated. Specific binding of [^{3}H]MS-377 to rat brain membranes between pH 6 and 9 were not significantly differ-

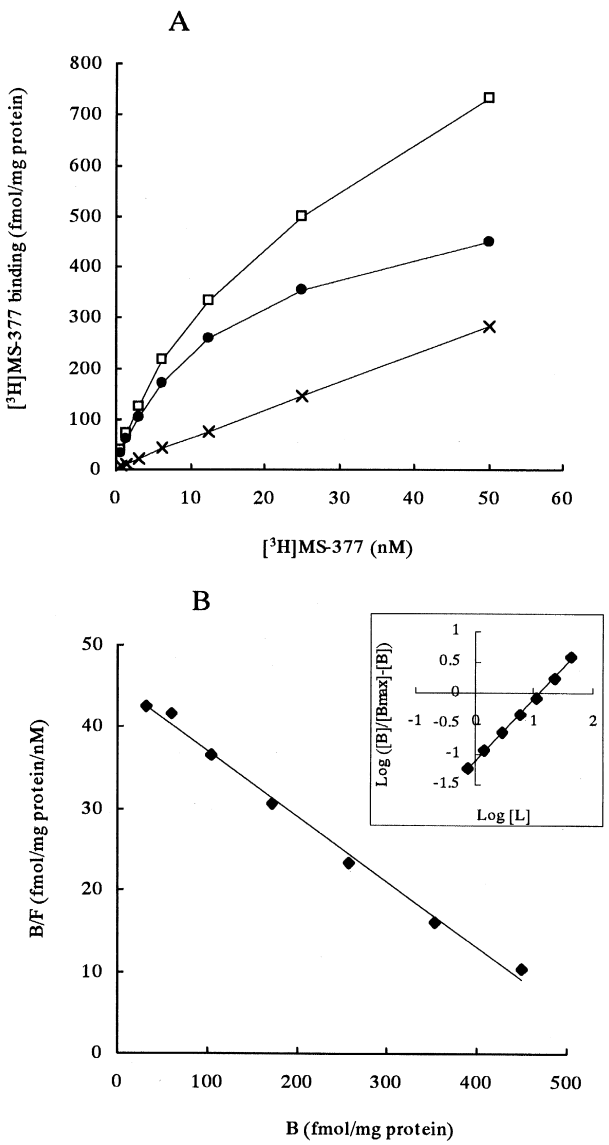

Fig. 2. Saturation isotherm of [^{3}H]MS-377 binding to rat brain membranes. Ligand concentrations ranged from 0.78 to 50 nM. (A) Saturation curves of the binding data. Total binding, nonspecific binding and specific binding are indicated as □, × and ●, respectively. (B) Scatchard plot of the same data as in (A). Inset: Hill plot of the same data. The data are mean value of four independent experiments done in triplicate. Mean values of K_d, B_{max} and Hill coefficient are 15.2±6.6 nM, 599.4±58.6 fmol/mg protein and 1.01±0.01, respectively.

ent; however, it appeared maximal between pH 7 and 8 (data not shown). The specific binding was linear at final protein concentrations of 0.125 to 0.5 mg (data not shown). At 25°C, the specific binding increased in a time-dependent manner, and in excess of 120 min, the binding gradually increased and reached equilibrium at 180 min (Fig.1). At 37°C, the binding increased precipitously, but gradually decreased in a time-dependent manner. At 0°C, the binding decreased by 1/3, with a similar time course of those at 25°C. As determined, following conditions were employed: buffer pH 7.6; final protein concentration; 0.4 mg/tube, incubation temperature; 25°C, incubation time; 180 min.

3.2. Kinetic studies

Binding of [^{3}H]MS-377 to rat brain membranes is saturable (Fig. 1A). After equilibrium was reached (180 min), an excess (10 μM) of unlabeled MS-377 was added. Then, the displacement of [^{3}H]MS-377 at binding sites occurred, and complete displacement was achieved 180 min after the addition of unlabeled MS-377 (Fig. 1B). These kinetic studies revealed that the binding of [^{3}H]MS-377 to rat brain membranes was reversible.

3.3. Saturation studies

Saturation studies of the binding of [^{3}H]MS-377 with ligand concentrations ranging from 0.78 to 50 nM revealed that the specific binding was saturable (Fig. 2A). Scatchard plot and Hill plot of these data were linear and indicated that [^{3}H]MS-377 bound to a single site in rat brain membranes (Fig. 2B). The K_d, B_{max} and Hill coefficient were calculated to be 15.2 ± 6.6 nM, 599.4 ± 58.6 fmol/mg protein, and 1.01 ± 0.01, respectively.

3.4. Displacement studies

Several σ reference compounds were examined for their competition potencies with the specific binding of [^{3}H]MS-377 to rat brain membranes. Table 1 summarizes K_i values of test compounds at [^{3}H]MS-377 binding sites

Table 1
Affinity constants of various σ reference compounds that displace [^{3}H]MS-377 binding to rat brain membranes

Compounds	K_i (nM)	
Haloperidol	10.2 ± 5.2	(3)
MS-377	26.4 ± 5.9	(5)
(+)-Pentazocine	39.4 ± 10.5	(5)
DTG	206 ± 63	(5)
(−)-Pentazocine	207 ± 59	(3)
BMY14802	306 ± 99	(3)
(+)-SKF-10,047	713 ± 205	(3)
(−)-SKF-10,047	5133 ± 1626	(3)
PCP	6667 ± 1882	(3)

Values are expressed as means ± S.D. of three to five independent experiments shown in parentheses.

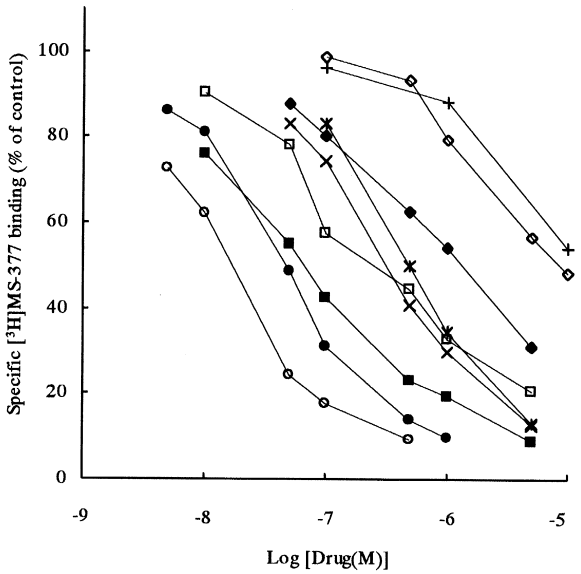

Fig. 3. Effects of several representative σ receptor ligands on [^{3}H]MS-377 binding to rat brain membranes. The data are mean values of three to five independent experiments. Key: MS-377 (●), haloperidol (○), (+)-pentazocine (■), (−)-pentazocine (□), DTG (×), BMY14802 (*), (+)-SKF-10,047 (◆), (−)-SKF-10,047 (◇), and PCP (+).

calculated from competition data, and competition curves are illustrated in Fig. 3. [^{3}H]MS-377 binding was inhibited by unlabeled MS-377, with K_i value of 26 nM, and (+)-pentazocine showed similar inhibition to that of unlabeled MS-377, with K_i value of 39 nM. Haloperidol was the most potent inhibitor, with K_i value of 10 nM. DTG, BMY14802 and (+)-SKF-10,047 showed moderate inhibition, with K_i values of 206, 306 and 713 nM, respectively. Non-σ receptor ligand PCP showed weak inhibition only at a concentration of 10^{-5} M. Stereoselectivity was observed for (+)-pentazocine, which was five-fold more potent than (−)-isomer, and for (+)-SKF-10,047, which was seven-fold more potent than (−)-isomer. The rank order of potency for the σ reference compounds to displace [^{3}H]MS-377 binding were as following: haloperidol > MS-377 = (+)-pentazocine > DTG = (−)-pentazocine > BMY14802 > (+)-SKF-10,047 > (−)-SKF-10,047 = PCP.

4. Discussion

Studies of σ receptor ligands, especially receptor binding and autoradiographic studies, have been carried out exclusively with the use of guinea pig brain, because σ receptors appear to be more abundant in the guinea pig brain (Bowen and Hellewell, 1988; Walker et al., 1990). Several studies have shown differences in σ receptors between rat and guinea pig (Bowen et al., 1989; Bowen and Hellewell, 1988). On the other hand, almost its behavioral studies have been carried out in rats and mice. Therefore, we considered not only in vivo studies but also in vitro studies should be carried out using same species,

in spite that the primary structure of σ_1 receptors is highly conserved among a variety of mammalian species (Pan et al., 1998). In the present study, we examined the binding properties of the novel σ receptor ligand MS-377 to rat brain membranes. Results from kinetic studies revealed that the binding of [^{3}H]MS-377 was time dependent and reached equilibrium at 180 min, and displacement of [^{3}H]MS-377 binding was occurred when an excess of unlabeled MS-377 was added, indicating that binding of [^{3}H]MS-377 was reversible. Saturation studies revealed that the [^{3}H]MS-377 binding was saturable, and Scatchard plot analysis indicated [^{3}H]MS-377 bound to a single high-affinity site in rat brain membranes. In order to verify that the [^{3}H]MS-377 binding site in rat brain membranes is a σ binding sites, inhibitory effects of σ reference compounds with different structure on [^{3}H]MS-377 binding were studied. Haloperidol, commonly used as a σ receptor ligand (Quirion et al., 1992), was the most potent inhibitor to the [^{3}H]MS-377 binding site. (+)-Pentazocine, which have been established as selective σ_1 receptor ligand (De Costa et al., 1989; Hellewell and Bowen, 1990), and unlabeled MS-377 showed almost the same effect against [^{3}H]MS-377 binding to rat brain membranes. DTG, which is putative $\sigma_{1/2}$ ligand (Rothman et al., 1990), showed moderate inhibition of [^{3}H]MS-377 binding. Non-σ receptor ligand PCP (Largent et al., 1985) had negligible affinities for [^{3}H]MS-377 binding site. Furthermore, differences in the binding affinities of stereoisomers of pentazocine and SKF-10,047 for the [^{3}H]MS-377 binding site were studied, since stereoselective profile was observed for σ_1 binding site, but not for σ_2 binding site (Walker et al., 1990; Quirion et al., 1992). And marked differences in the binding affinity of these stereoisomers were observed in this study. Overall, effects of those σ reference compounds with different structure to the [^{3}H]MS-377 binding site was similar to the binding profile, which has been reported previously for other σ receptor ligands, such as [^{3}H](+)-SKF-10,047, [^{3}H](+)-3-PPP ((+)3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine), [^{3}H](+)-pentazocine, [^{3}H]DuP-734 (1-(cyclopropylmethyl)-4-(2′-(4″-fluorophenyl)-2′-oxoethyl)piperidine) and [^{3}H]NE-100 (Largent et al., 1984; Steinfels et al., 1989; Heroux et al., 1992; DeHaven-Hudkins et al., 1992; Tanaka et al., 1995), even though these experiment had been done using guinea pig brain membranes. These results suggested that the MS-377 selectively binds to σ binding site in rat brain membranes. In addition, the marked difference in affinity between (+) and (−) stereoisomers suggested that MS-377 is relatively selective for σ_1 receptors.

We previously reported that MS-377 showed anti-PCP, anti-dopaminergic and anti-serotonergic activities in pharmacological studies (Takahashi et al., 1999). MS-377 had affinity for only σ_1 receptor and not for any other receptors, ion channels and second messenger systems (unpublished data). Thus, we suggested that the anti-psychotic activities of MS-377 were not directly associated with dopamine D_2 receptors, 5-hydroxytryptamine (5-HT) receptors and PCP-*N*-methyl-D-aspartate (NMDA) site, and were at least partly attributable to σ_1 receptors. However, MS-377 also has unique pharmacological profile distinct from those of more potent and selective σ_1 ligands such as NE-100. For example, MS-377 attenuated the development of methamphetamine-induced behavioral sensitization (unpublished data). It also enhanced the efficacy of dopamine D_2 antagonists without deterioration of extrapyramidal side effects, though MS-377 has no anti-dopaminergic activity at doses employed in this experiments (unpublished data). Kamei et al. (1997) reported that (+)-SKF-10,047 acts on a subpopulation of σ_1 sites, which are closely associated with dopaminergic neurons, and the possible existence of a σ_3 subtype has also been reported (Wyrick and Booth, 1995). Therefore, MS-377 may have another site of action different from the well-known subtypes of σ receptors, and the anti-dopaminergic activity of MS-377 may be associated with such receptors. However, the results of these reported studies suggested that the MS-377 selectively binds, with high affinity, to σ_1 binding site in rat brain membranes. Therefore, the anti-psychotic activities of MS-377 are attributable to association with σ_1 receptor. And these indicated that σ receptor ligands indirectly interact with dopamine and serotonin neurons, PCP-NMDA site.

Differences in the pharmacological profile between MS-377 and any other σ reference compounds remain unexplained from binding properties. (+)-Pentazocine and NE-100, both of which are σ_1 receptor ligands, have similar binding properties and distribution in guinea pig brain (DeHaven-Hudkins et al., 1992; Walker et al., 1992; Tanaka et al., 1995; Okuyama et al., 1995). Interestingly, Yamamoto et al. (1999) reported that the differences between [^{3}H](+)-pentazocine and [^{3}H]NE-100 in site-directed mutagenesis of σ_1 receptor binding studies implies the presence of different recognition sites in σ_1 receptor for each compound. In the view of this finding, MS-377 may have specific recognition sites in σ_1 receptor. To clarify the mechanism for distinctive activities of MS-377, further studies on the binding sites in the brain and intracellular signal transduction of σ receptors are necessary.

In conclusion, MS-377 binds saturably and reversibly to a single σ_1 binding site in rat brain membranes with high affinity. Therefore, the anti-psychotic activities of MS-377 are attributable to association with σ_1 receptors. We considered that the MS-377 could be a new and unique σ receptor ligand and this will be a nice tool for investigating the relationship between σ binding sites and schizophrenia. And we also expect that MS-377 shows favorable efficacy against schizophrenia clinically.

References

Bowen, W.D., Hellewell, S.B., 1988. Characterization of sigma receptors on PC12 cells: pharmacological differences from rat and guinea pig

rain indicate sigma receptor heterogeneity. Soc. Neurosci. Abstr. 14, 703.

Bowen, W.D., Hellewell, S.B., McGarry, K.A., 1989. Evidence for a multi-site model of the rat brain σ receptor. Eur. J. Pharmacol. 163, 309–318.

Bowen, W.D., Walker, J.M., De Costa, R.B., Wu, R., Tolentino, P.J., Finn, D., Rothman, R.B., Rice, K.C., 1992. Characterization of the enantiomers of *cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl) cyclohexylamine (BD737 and BD738): novel compounds with high affinity, selectivity and biological efficacy at sigma receptors. J. Pharmacol. Exp. Ther. 262, 32–40.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

De Costa, B.R., Bowen, W.D., Hellewell, S.B., Walker, J.M., Thurkauf, A., Jacobson, A.E., Rice, K.C., 1989. Syntheses and evaluation of optically pure [^{3}H]-(+)-pentazocine, a highly potent and selective radioligand for σ receptors. FEBS Lett. 251, 53–58.

DeHaven-Hudkins, D.L., Fleissner, L.C., Ford-Rice, F.Y., 1992. Characterization of the binding of [^{3}H](+)-pentazocine to σ recognition sites in guinea pig brain. Eur. J. Pharmacol. 227, 371–378.

Ellison, G., 1995. The *N*-methyl-D-asparate antagonists phencyclidine, ketamine and dizocilpine as both behavioral and anatomical models of the dementias. Brain Res. Rev. 20, 250–267.

Frieboes, R.M., Murck, H., Wiedemann, K., Holsboer, F., Steiger, A., 1997. Open clinical trial on the sigma ligand panamesine in patients with schizophrenia. Psychopharmacology 132, 82–88.

Gundlach, A.L., Langent, B.L., Snyder, S.H., 1986. Phencyclidine and σ opiate receptors in brain: biological and autoradiographical differentiation. Eur. J. Pharmacol. 113, 465–466.

Hanner, M., Moebius, F.F., Flandorfer, A., Knaus, H.G., Striessing, J., Kempner, E., Glossmann, H., 1996. Purification, molecular cloning and expression of the mammalian σ_1-binding site. Proc. Natl. Acad. Sci. U. S. A. 93, 8072–8077.

Hellewell, S.B., Bowen, W.D., 1990. A sigma-like binding site in rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor from that of guinea pig brain. Brain Res. 527, 244–253.

Helmeste, D.M., Tang, S.W., Bunney, W.E. Jr., Potkin, S.G., Jones, E.G., 1996. Decrease in σ but no increase in striatal dopamine D4 sites in schizophrenic brains. Eur. J. Pharmacol. 314, R3–R5.

Heroux, J.A., Tam, S.W., De Souza, E.B., 1992. Autoradiographic identification and characterization of σ receptors in guinea pig brain using [^{3}H]1(cyclopropylmethyl)-4-(2′-(4″-fluorophenyl)-2′-oxoethyl)piperidine ([^{3}H]Dup734), a novel σ receptor ligand. Brain Res. 598, 76–86.

Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148, 1301–1308.

Kamei, H., Noda, Y., Kameyama, T., Nabeshima, T., 1997. Role of (+)-SKF-10,047 sensitive sub-population of receptors in amelioration of conditioned fear stress in rat: association with mesolimbic dopaminergic systems. Eur. J. Pharmacol. 319, 165–172.

Kekuda, R., Prasad, P.D., Fei, Y.J., Leibach, F.H., Ganapathy, V., 1996. Cloning and functional expression of the human type 1 sigma receptor (hSigmaR1). Biochem. Biophys. Res. Commun. 229, 553–558.

Largent, B.L., Gundlach, A.L., Snyder, S.H., 1984. Psychotomimetic opiate receptors labeled and visualized with (+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine. Proc. Natl. Acad. Sci. U. S. A. 81, 4983–4987.

Largent, B.L., Gundlach, A.L., Snyder, S.H., 1985. Pharmacological and autoradiographic discrimination of sigma and phencyclidine receptor binding sites in brain with [^{3}H](+)SKF10047, [^{3}H](+)3-[3-hydroxyphenyl]-*N*-(1-propyl)piperidine and [^{3}H]1-[1-(2-thyenyl)cyclohexyl] piperidine. J. Pharmacol. Exp. Ther. 238, 739–748.

Liu, Y., Whitlock, B.B., Pultz, J.A., Wolf, S.A., 1995. Sigma-1 receptors modulate functional activity of rat splenocytes. J. Neuroimmunol. 59, 143–154.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., Gilbert, P.E., 1976. The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532.

Modell, S., Naber, D., Holzbach, R., 1996. Efficacy and safety of an opiate sigma-receptor antagonist (SL 82.0715) in schizophrenic patients with negative symptoms: an open dose-range study. Pharmacopsychiatry 29, 63–66.

Okuyama, S., Imagawa, Y., Ogawa, S., Araki, H., Ajima, A., Tanaka, M., Muramatsu, M., Nakazato, A., Yamaguchi, K., Yoshida, M., Otomo, S., 1993. NE-100, a novel sigma receptor ligand: in vivo tests. Life Sci. 53, 285–290.

Okuyama, S., Chaki, S., Yae, T., Nakazato, A., Muramatsu, M., 1995. Autoradiographic characterization of binding sites for [^{3}H]NE-100 in guinea pig brain. Life Sci. 57, 333–337.

Pan, Y.-X., Mey, J., Xu, J., Wan, B.-L., Zucherman, A., Pasternac, G.W., 1998. Cloning and characterization of a mouse σ_1 receptor. J. Neurochem. 70, 2279–2285.

Prasad, P.L., Li, H.W., Fei, Y.J., Ganapathy, M.E., Fujita, T., Plumley, L.H., Yang-Feng, T.L., Leibach, F.H., Ganapathy, V., 1998. Exon–intron structure, analysis of promoter region, and chromosomal localization of the human type 1 sigma receptor gene. J. Neurochem. 70, 443–451.

Quirion, R., Chicheportiche, R., Contreras, P.C., Johnson, K.M., Lodge, D., Tam, S.W., Woods, J.H., Zukin, S.R., 1987. Classification and nomenclature of phencyclidine and sigma receptor sites. Trends Neurosci. 10, 444–446.

Quirion, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.-P., Tam, S.W., Taylor, D.P., 1992. A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13, 85–86.

Rothman, R.B., Reid, A., Mahboubu, A., Kim, C., De Costa, B.R., Jacobson, A.E., Rice, K.C., 1990. Labeling by [^{3}H]1,3-Di(2-tolyl)guanidine of two high affinity binding sites in guinea pig brain: evidence for allosteric regulation by calcium channel antagonists and pseudoallosteric modulation by σ ligands. Mol. Pharmacol. 39, 222–232.

Seth, P., Leibach, F.H., Ganapathy, V., 1997. Cloning and structural analysis of the cDNA and the gene encoding the murine type 1 sigma receptor. Biochem. Biophys. Res. Commun. 241, 535–540.

Seth, P., Fei, Y.J., Li, H.W., Huang, W., Leibach, F.H., Ganapathy, V., 1998. Cloning and functional characterization of a σ receptor from rat brain. J. Neurochem. 70, 922–931.

Snyder, S.H., Largent, B.L., 1989. Receptor mechanisms in anti-psychotic drug action: focus on sigma receptors. J. Neuropsychiatry 1, 7–15.

Steinfels, G.F., Tam, S.W., Cook, L., 1989. Electrophysiological effects of selective σ-receptor agonists, antagonists, and the selective phencyclidine receptor agonist MK-801 on midbrain dopamine neurons. Neuropsychopharmacology 2, 201–208.

Su, T.P., 1991. Putative links between nervous, endocrine and immune systems. Eur. J. Biochem. 200, 633–642.

Takahashi, S., Sonehara, K., Takagi, K., Miwa, T., Horikomi, K., Mita, N., Nagase, H., Iizuka, K., Sakai, K., 1999. Pharmacological profile of MS-377, a novel anti-psychotic agent with selective affinity for σ receptors. Psychopharmacology 145, 295–302.

Tanaka, M., Shirasaki, T., Kaku, S., Muramatsu, M., Otomo, S., 1995. Characterization of binding of [^{3}H]NE-100, a novel sigma-receptor ligand, to guinea pig brain membranes. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 244–251.

Tam, S.W., Cook, L., 1984. σ opiates and certain anti-psychotic drugs mutually inhibit (+)-[^{3}H]SKF 10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes. Proc. Natl. Acad. Sci. U. S. A. 81, 5618–5621.

Ujike, H., Kanzaki, A., Okumuma, K., Akiyama, K., Otsuki, S., 1992. Sigma (σ) antagonist BMY 14802 prevents methamphetamine-induced sensitization. Life Sci. 50, PL129–PL134.

Ujike, H., Kuroda, S., Otsuki, S., 1996. σ Receptor antagonists block the development of sensitization to cocaine. Eur. J. Pharmacol. 296, 123–128.

Walker, J.M., Bowen, W.D., Walker, F.O., Matsumoto, R.R., De Costa, B., Rice, K.C., 1990. Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402.

Walker, J.M., Bowen, W.D., Goldstein, S.R., Robert, A.H., Patrick, S.L., Hohmann, A.G., De Costa, B., 1992. Autoradiographic distribution of [^{3}H] (+)-pentazocine and [^{3}H]1,3-di-*o*-tolylguanidine (DTG) binding sites in guinea pig brain: a comparative study. Brain Res. 581, 33–38.

Witkin, J.M., 1994. Pharmacotherapy of cocaine abuse: preclinical development. Neurosci. Behav. Rev. 18, 121–142.

Wyrick, S.D., Booth, S.R., 1995. Progress in sigma receptor research. Drugs Future 20, 1033–1044.

Yamamoto, H., Miura, R., Yamamoto, T., Shinohara, K., Watanabe, M., Okuyama, S., Nakazato, A., Nukada, T., 1999. Amino acid residues in the transmembrane domain of the type 1 sigma receptor critical for ligand binding. FEBS Lett. 445, 19–22.

Zukin, R.S., Zukin, S.R., 1981. Demonstration of [^{3}H]cyclazocine binding to multiple receptor sites. Mol. Pharmacol. 20, 246–254.