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MS-377, a novel selective σ_1 receptor ligand, reverses phencyclidine-induced release of dopamine and serotonin in rat brain

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Abstract

A novel selective σ_1 receptor ligand, (*R*)-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate (MS-377), inhibits phencyclidine (1-(1-phenylcyclohexyl)piperidine; PCP)-induced behaviors in animal models. In this study, we measured extracellular dopamine and serotonin levels in the rat brain after treatment with MS-377 alone, using in vivo microdialysis. We also examined the effects of MS-377 on extracellular dopamine and serotonin levels in the rat medial prefrontal cortex after treatment with PCP. MS-377 itself had no significant effects on dopamine release in the striatum (10 mg/kg, p.o.) nor on dopamine or serotonin release in the medial prefrontal cortex (1 and 10 mg/kg, p.o.). PCP (3 mg/kg, i.p.) markedly increased dopamine and serotonin release in the medial prefrontal cortex. MS-377 (1 mg/kg, p.o.), when administered 60 min prior to PCP, significantly attenuated this effect of PCP. These results suggest that the inhibitory effects of MS-377 on PCP-induced behaviors are partly mediated by inhibition of the increase in dopamine and serotonin release in the rat medial prefrontal cortex caused by PCP.

Keywords: σ_1 Receptor ligand; MS-377; Phencyclidine; Microdialysis, in vivo; Prefrontal cortex, medial

1. Introduction

Phencyclidine (1-(1-phenylcyclohexyl)piperidine; PCP) is a non-competitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, a type of glutamate receptor, and drug abuse with this agent causes the development of psychotic symptoms indistinguishable from those of schizophrenia (Javitt and Zukin, 1991). PCP causes various abnormal behaviors in animals and these are considered to be useful experimental models of schizophrenia. Behavioral and biochemical studies have suggested that these behaviors are closely related to dopamine and serotonin neural systems (Yamaguchi et al., 1986; Gleason and Shannon, 1997; Millan et al., 1999; Yamada et al., 1999). Both systemic and local administration of PCP increase the release of dopamine and serotonin in various brain regions such as the striatum, nucleus accumbens and medial prefrontal cortex (McCullough and Salamone, 1992; Hondo et al., 1994; Yonezawa et al., 1995; Millan et al., 1999), and this agent is preferentially effective in cortical rather than striatal regions. Therefore, the behavioral effects of PCP are partly mediated by activation of dopamine and serotonin transmission in these brain regions. In particular, the effects of PCP in the medial prefrontal cortex may play an important role in the pathophysiology of PCP-induced psychosis as well as schizophrenia, because the function of the prefrontal dopamine neuron systems has been suggested to be disturbed in schizophrenics (Weinberger, 1987; Deutch, 1992; Dolan et al., 1995). The mechanism of the effects of PCP on the release of dopamine and serotonin is not yet clearly understood because PCP has multiple sites of action. Although the most fundamental site of action is the NMDA receptor complex, PCP also acts as a noradrenaline and dopamine uptake inhibitor (Garey and Heath, 1976; Smith et al., 1977). Moreover, PCP has affinity for σ receptors (Su and Wu, 1990), and some

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behavioral effects of PCP may be mediated through σ receptors (Okuyama et al., 1993; Kitaichi et al., 1996; Yamada et al., 2000).

The existence of σ receptors was first postulated by Martin et al. (1976) to explain the psychotomimetic effects of benzomorphans such as *N*-allylnormetazocine and (+)-pentazocine. They were first defined as an opioid receptor subtype and were later suggested to be identical to PCP sites (Zukin and Zukin, 1981). However, σ receptors have been shown to be distinct from the PCP sites (Gundlach et al., 1985; Quirion et al., 1987) and to be widely distributed in various brain regions. It has been reported that several σ receptor ligands affect extracellular dopamine levels in the rat brain regions such as the striatum and medial prefrontal cortex (Gudelsky, 1995; Matsuno et al., 1995; Volonté et al., 1995), whereas other σ receptor ligands are not effective (Karbon et al., 1993; Poncelet et al., 1993). The reason why these σ receptor ligands show such multiple effects on extracellular dopamine levels are not clearly understood.

A novel selective σ_1 receptor ligand is (*R*)-(+)-1-(4-chlorophenyl)-3-[4-(2-methoxyethyl)piperazin-1-yl]methyl-2-pyrrolidinone L-tartrate (MS-377). MS-377 has selective affinity for σ_1 receptors ($K_i = 73$ nM), weak affinity for σ_2 receptors (6900 nM) and no affinity for any other receptors including dopamine, serotonin, PCP site, glutamate, γ-aminobutylic acid, adenosine, adrenergic receptors, etc. ($K_i > 10$ μM; Takahashi et al., 1999). In addition, MS-377 was shown to bind selectively to a single class of sites in the rat or guinea pig brain by Scatchard analysis using P2 membrane fractions, sites which were concluded to represent σ_1 receptors (Takahashi et al., 2000a; Karasawa et al., 2000). Therefore, the behavioral effects of this agent are considered to be mediated through σ_1 receptors. MS-377 inhibits PCP-induced behaviors such as head-weaving, rearing, hyperlocomotion and disruption of prepulse inhibition in animal models (Takahashi et al., 1999; Yamada et al., 2000). In addition, MS-377 inhibits 5-hydroxy-D,L-tryptophan-induced head twitches and prevents the development of methamphetamine-induced behavioral sensitization in rats, suggesting that this agent has inhibitory effects on dopamine and serotonin neurons (Takahashi et al., 1999, 2000b). Since the behavioral effects of PCP are closely related to activation of dopamine and serotonin transmission, MS-377 could affect dopamine and serotonin transmission.

In this study, we conducted two experiments using in vivo microdialysis. First, we examined the effects of MS-377 alone on extracellular dopamine and serotonin levels in the rat striatum and medial prefrontal cortex. For comparison, the effects of currently available typical and atypical antipsychotics, haloperidol and clozapine, were also tested. Second, we evaluated the effects of MS-377 on extracellular dopamine and serotonin levels in the rat medial prefrontal cortex after treatment with PCP. In addition, we measured locomotor activity after treatment with PCP in the same experiments and compared it with extracellular dopamine and serotonin levels.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 250–350 g, were housed in groups of three, under a 12/12-h light/dark cycle (lights on at 6:00 a.m.) with food and water ad libitum. Rats were adapted to laboratory conditions for 1 week prior to utilization. These experiments were performed in strict accordance with the guidelines for animal experiments of the Institute of Biological Science, Mitsui Pharmaceuticals, and were approved by the Animal Investigation Committee of the Institute.

2.2. Drugs

MS-377 was synthesized by Mitsui Chemicals, and PCP was synthesized by Mitsui Pharmaceuticals, Clozapine was purchased from Research Biochemicals, Haloperidol was purchased from Sigma, MS-377 and PCP were dissolved in saline. Haloperidol and clozapine were dissolved in 0.3% tartaric acid. All drugs were administered to rats in a volume of 1 ml/kg body weight.

2.3. Determination of extracellular dopamine levels in the rat striatum

Dialysis experiments were conducted with conscious, freely moving rats. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and guide cannulas (for CMA12, BAS) were implanted in the striatum according to the stereotaxic atlas of Paxinos and Watson (1986). The coordinates were A, +1.0 mm; L, 3.0 mm; V, 6.0 mm, from bregma and dura. Following a recovery period of at least 4 days, a dialysis probe (CMA12, membrane length 4 mm, BAS) was introduced and the striatum was perfused (1.5 μl/min) with Ringer's solution (140 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2$, 1 mM $MgCl_2$). Dialysates were collected every 20 min (30 μl) from 3 h after implantation of the dialysis probe. The first three samples were collected, and values were averaged and are referred to as baseline. Then, saline, MS-377 (10 mg/kg) and haloperidol (1 mg/kg) were administered orally to rats and dialysates were collected for 3 h. Dopamine levels in the dialysates were quantified by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Each sample was injected in a volume of 25 μl. The potential of the electrode of the electrochemical detector was set at +450 mV vs. an Ag/AgCl reference electrode. Dopamine was separated on a reverse-phase column (CA-5ODS, 2.1 × 150 mm, Eicom). The mobile phase was 0.1 M phosphate buffer (pH 6.0) containing 500

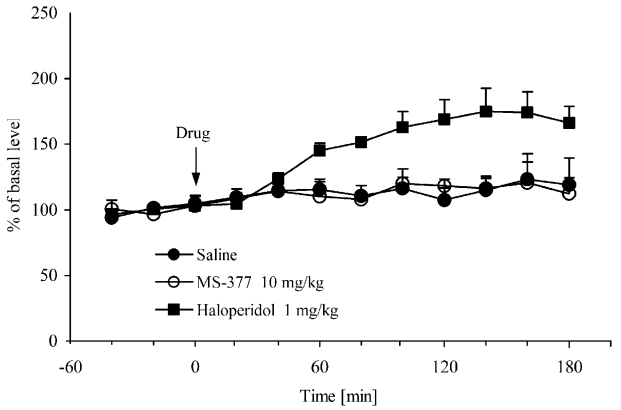

Fig. 1. Time course of changes in extracellular dopamine levels in the rat striatum after administration of saline, haloperidol (1 mg/kg) or MS-377 (10 mg/kg). Data represent means ± S.E.M. ($n = 3$). The arrow indicates p.o. administration of drugs or saline.

mg/l sodium octane sulfonate, 50 mg/l Na_2EDTA and 20% (v/v) methanol. The flow rate was 0.2 ml/min.

2.4. Determination of extracellular dopamine and serotonin levels in the rat medial prefrontal cortex

Dialysis experiments were conducted as described above with slight modifications. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and guide cannulas were implanted in the medial prefrontal cortex. The coordinates were A, +3.0 mm; L, 0.8 mm; V, 5.0 mm, from bregma and dura. Following a recovery period of at least 4 days, a dialysis probe was introduced and the medial prefrontal cortex was perfused (1.0 μl/min) with Ringer's solution. Dialysates were collected every 30 min (30 μl) from 4 h after implantation of the dialysis probe. The first three samples were collected, and values were averaged and are referred to as baseline. Then, saline, MS-377 (1, 10 mg/kg), haloperidol (1 mg/kg) and clozapine (20 mg/kg) were administered orally to rats and dialysates were collected for 3 h. In some experiments, PCP or saline was administered intraperitoneally 60 min after administration of saline and MS-377 (1 mg/kg). Dopamine and serotonin levels in dialysates were quantified by HPLC-ECD. Each sample was injected in a volume of 20 μl. The potential of the electrode of the electrochemical detector was set at +450 mV vs. an Ag/AgCl reference electrode. Dopamine and serotonin were separated on a reverse-phase column (CA-5ODS, 2.1 × 150 mm, Eicom). The mobile phase was 0.1 M phosphate buffer (pH 6.0) containing 500 mg/l sodium octane sulfonate, 50 mg/l Na_2EDTA and 20% (v/v) methanol. The flow rate was 0.23 ml/min.

2.5. Effects of MS-377 on PCP-induced increases in locomotor activity in rats

In the experiment with co-administration of PCP and MS-377, locomotor activity was measured simultaneously. Locomotor activity was measured automatically using an Animex auto locomotor counter (Muromachi Kikai). Rats were placed individually in plastic cages (38 × 25 × 30 cm) on the locomotor counter and dialysis experiments were started. After collection of basal samples, rats were administered drugs orally, and PCP was administered intraperitoneally at a dose of 3 mg/kg 60 min later. After PCP administration, locomotor activity was monitored for 2 h.

2.6. Statistics

The data from dialysis experiments are expressed as percentages of the baseline value prior to drug administration. All results are given as means ± S.E.M. and are expressed as the area under the curve (AUC), calculated from % of control for 180 min (drug alone) or 120 min (co-administration with PCP) after administration of drugs (p.o.) or PCP (i.p.), respectively. Statistical analysis of the different treatment groups was carried out by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer test for multiple comparisons using AUC values. The data from the behavioral experiments were analyzed by one-way ANOVA followed by the Tukey–Kramer test. In all comparisons, $P < 0.05$ was considered significant.

Table 1
Areas under the curve (AUC) of striatal dopamine and medial prefrontal cortical dopamine and serotonin levels for 180 min after administration of MS-377 and other antipsychotics in rats. Numbers in parentheses represent S.E.M.

Drugs	Dose [mg/kg, p.o.]	$AUC_{0-180\ min}$		
		Striatum	Medial prefrontal cortex	
		Dopamine	Dopamine	Serotonin
Saline	–	20,484 (1652)	19,511 (1018)	14,887 (1091)
MS-377	1	–	19,016[a] (1878)	13,168 (1369)
	10	20,471[b] (1500)	20,667[a] (1247)	13,765 (618)
Clozapine	20	–	73,638[c] (5608)	21,822 (3732)
Haloperidol	1	26,783[d] (562)	25,152[a] (1743)	15,817 (1683)

[a] $P < 0.01$ vs. clozapine treatment group (Tukey–Kramer test).
[b] $P < 0.05$ vs. haloperidol treatment group (Tukey–Kramer test).
[c] $P < 0.01$ vs. saline treatment group (Tukey–Kramer test).
[d] $P < 0.05$ vs. saline treatment group (Tukey–Kramer test).

3. Results

3.1. Effects of MS-377 on extracellular dopamine levels in the rat striatum

Fig. 1 shows the changes in extracellular dopamine levels in the rat striatum after administration of saline, MS-377 (10 mg/kg, p.o.) and haloperidol (1 mg/kg, p.o.). Mean basal extracellular dopamine levels in the rat striatum for each group were 0.09–0.14 pmol/25 μl of dialysate. No change in extracellular dopamine levels was observed in the rat striatum after treatment with saline. MS-377 (10 mg/kg, p.o.) also had no effect on extracellular dopamine levels in the rat striatum. In contrast, haloperidol (1 mg/kg, p.o.) increased extracellular dopamine levels in the rat striatum, and a maximum increase in extracellular dopamine levels of 174.8 ± 7.7% was observed 140 min after administration of haloperidol. Analysis of AUC after administration of the drugs indicated significant differences between the haloperidol and saline treatment groups ($P < 0.05$, Table 1).

3.2. Effects of MS-377 on extracellular dopamine and serotonin levels in the rat medial prefrontal cortex

Figs. 2 and 3 show the changes in extracellular dopamine and serotonin levels after administration of saline, MS-377 (1, 10 mg/kg, p.o.) haloperidol (1 mg/kg, p.o.) and clozapine (20 mg/kg, p.o.) in the rat medial prefrontal cortex. Mean basal extracellular dopamine and serotonin levels in each group were 7.1–10.0 and 5.7–7.7 fmol/20 μl of dialysate, respectively. No changes were observed in extracellular dopamine or serotonin levels in the medial prefrontal cortex of rats treated with saline. MS-377 (1 and 10 mg/kg, p.o.) and haloperidol (1 mg/kg, p.o.) did not have a significant effect on extracellular dopamine or serotonin levels in the rat medial prefrontal cortex (Figs. 2 and 3). In contrast, clozapine (20 mg/kg, p.o.) increased

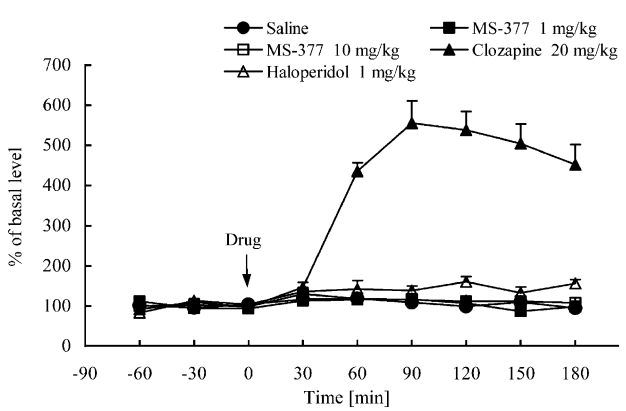

Fig. 2. Time course of changes in extracellular dopamine levels in the rat medial prefrontal cortex after administration of saline, MS-377 (1, 10 mg/kg), clozapine (20 mg/kg) or haloperidol (1 mg/kg). Values represent means ± S.E.M. ($n = 4–5$). The arrow indicates p.o. administration of drugs or saline.

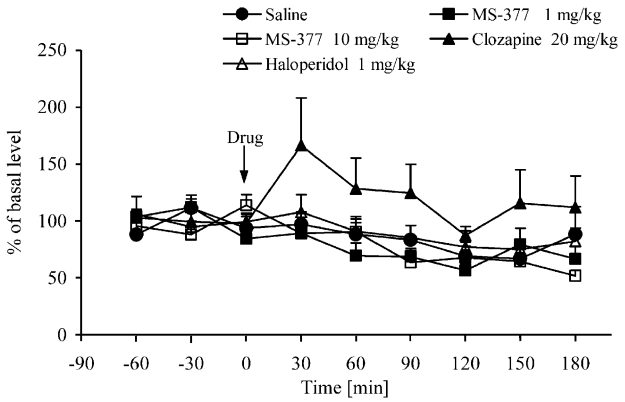

Fig. 3. Time course of changes in extracellular serotonin levels in the rat medial prefrontal cortex after administration of saline, MS-377 (1, 10 mg/kg, p.o.), clozapine (20 mg/kg, p.o.) or haloperidol (1 mg/kg, p.o.). Values represent means ± S.E.M. ($n = 4–5$). The arrow indicates administration of drugs or saline.

extracellular dopamine levels in the rat medial prefrontal cortex, and analysis of the AUC indicated significant differences between the clozapine and saline treatment

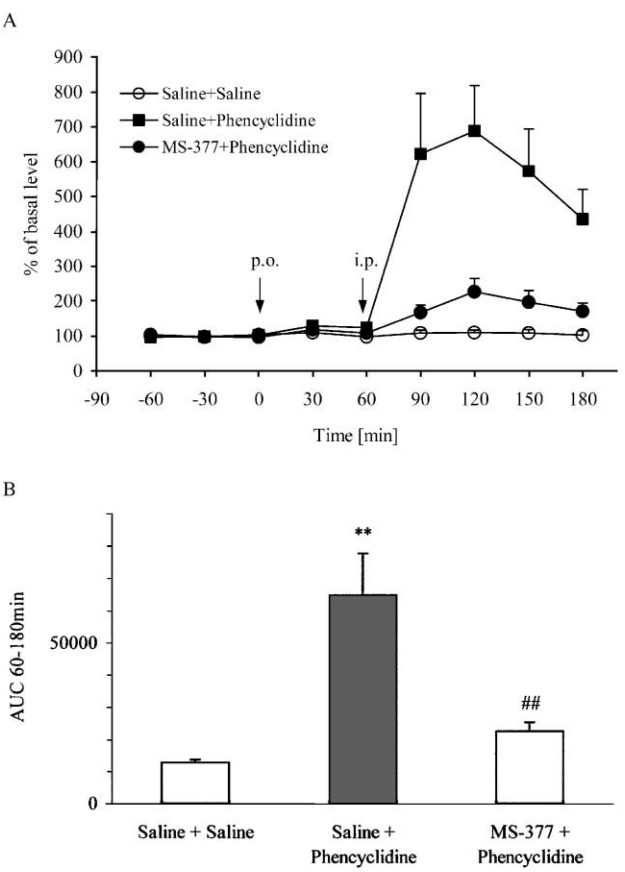

Fig. 4. (A) Time course of the effect of MS-377 on the phencyclidine-induced increase in extracellular dopamine levels in the rat medial prefrontal cortex. Values represent means ± S.E.M. ($n = 5$). The left arrow indicates p.o. administration of saline or MS-377 (1 mg/kg) and the right arrow indicates i.p. administration of saline or phencyclidine (3 mg/kg). (B) AUC of extracellular dopamine levels after i.p. administration of phencyclidine or saline. **: $P < 0.01$ vs. saline + saline group, ##: $P < 0.01$ vs. saline + phencyclidine group (Tukey–Kramer test).

groups ($P < 0.01$, Fig. 2, Table 1). Clozapine induced a 166.4 ± 41.7% increase in extracellular serotonin levels in the rat medial prefrontal cortex 30 min after administration, but analysis of AUC indicated no significant difference between the saline and clozapine treatment groups (Fig. 3, Table 1).

3.3. Effects of MS-377 on the PCP-induced increase in extracellular dopamine and serotonin levels in the rat medial prefrontal cortex

Figs. 4 and 5 show the changes in extracellular dopamine and serotonin levels in the rat medial prefrontal cortex after administration of saline–saline, saline–PCP and MS-377–PCP. No changes in extracellular dopamine and serotonin levels were observed in the saline–saline group. PCP (3 mg/kg, i.p.) increased extracellular dopamine levels in the rat medial prefrontal cortex (Fig. 4a) from 30 min after injection, and a maximum increase in extracellular dopamine levels of 704.5 ± 131.7% was observed 60 min after injection of PCP in the saline–PCP group. When MS-377 (1 mg/kg, p.o.) was administered 60 min before injection of PCP, the increase in extracellular dopamine levels was significantly suppressed (Fig. 4a), while MS-377 alone showed no significant effect. The maximum increase in extracellular dopamine level of 230.6 ± 36.3% was observed 60 min after injection of PCP in the MS-377–PCP group. Analysis of the AUC indicated a significant difference between saline–PCP and MS-377–PCP groups ($P < 0.01$). PCP (3 mg/kg, i.p.) also increased extracellular serotonin levels in the rat medial prefrontal cortex (Fig. 5a, b). The maximum increase in extracellular serotonin level of 306.8 ± 59.3% was observed 30 min after injection of PCP in the saline–PCP group. MS-377 administered 60 min before injection of PCP completely suppressed the effect of PCP on extracellular serotonin levels ($P < 0.05$, Fig. 5b).

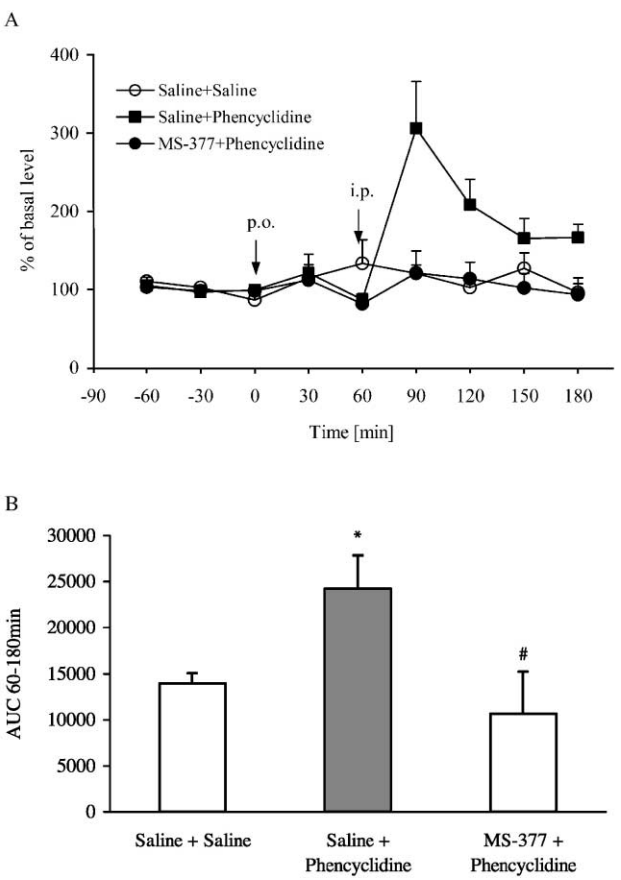

Fig. 5. (A) Time course of the effect of MS-377 on the phencyclidine-induced increase in extracellular serotonin levels in the rat medial prefrontal cortex. Values represent means ± S.E.M. ($n = 5$). The left arrow indicates p.o. administration of saline or MS-377 (1 mg/kg) and the right arrow indicates i.p. administration of saline or phencyclidine (3 mg/kg). (B) AUC of extracellular serotonin levels after i.p. administration of phencyclidine or saline. *: $P < 0.05$ vs. saline + saline group, #: $P < 0.05$ vs. saline + phencyclidine group (Tukey–Kramer test).

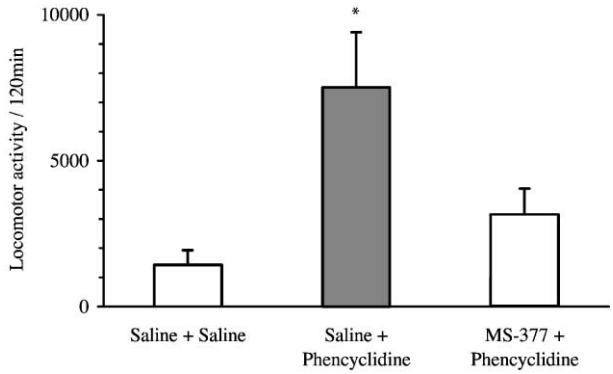

Fig. 6. Effect of MS-377 on phencyclidine-induced hyperlocomotion. Values represent means ± S.E.M. of locomotor activity for 120 min from i.p. administration of saline or phencyclidine. *: $P < 0.05$ vs. saline + saline group (Tukey–Kramer test).

3.4. Effects of MS-377 on the PCP-induced increase in locomotor activity in rats

Locomotor activity in rats after treatment with saline or PCP was measured in the same experiments as the determination of extracellular dopamine and serotonin levels. PCP-treated rats showed a significantly higher locomotor activity than saline-treated controls ($P < 0.05$, Fig. 6). MS-377 showed a tendency to suppress this effect of PCP, but this suppression was not significant ($P = 0.065$, Fig. 6).

4. Discussion

In this study, MS-377 did not have a significant effect on extracellular dopamine levels in either the medial prefrontal cortex or the striatum. The prototype σ_1 receptor agonist (+)-*N*-allylnormetazocine was reported previously to increase extracellular dopamine levels in the rat striatum (Volonté et al., 1995). (+)-*N*-allylnormetazocine and

(±)-pentazocine increased extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the rat frontal cortex (Matsuno et al., 1995). Kobayashi et al. (1997) reported that a selective σ_1 receptor agonist, 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503), increased dopamine and DOPAC levels in the rat frontal cortex, and these effects were fully antagonized by the selective σ_1 receptor antagonist *N,N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)ethylamine (NE-100). Several non-selective σ receptor ligands, α-(4-fluorophenyl)-4-(5-fluoro-2-pyramidinyl)-1-piperazine butanol (BMY14802) and (1-(cyclopropylmethyl)-4-2′4″-fluorophenyl)-(2′-oxoethyl)piperidine hydroborate (Dup 734), have been reported to increase extracellular dopamine levels in the striatum (Gudelsky and Nash, 1992; Gudelsky, 1995), but (*Z*)-3-(hexahydroazepin-1-yl)-1-(3-chloro 4-cyclohexylphenyl)propene-1, hydrochloride (SR 31742A) and 6-[6-(4-hydroxypiperidinyl)hexyloxy]-3-methylflavone hydrochloride (NPC 16377), more selective σ receptor ligands, have no detectable effects on dopamine metabolism (Karbon et al., 1993; Poncelet et al., 1993). In addition, Gudelsky (1999) reported that (+)-pentazocine initially increased dopamine levels, but prolonged treatment decreased dopamine levels in the rat striatum. These findings suggested that the activation of σ receptors modulates extracellular dopamine levels in multiple ways. Interestingly, Yamamoto et al. (1999) reported that σ_1 receptors have different recognition sites for ligands. Moreover, [^{3}H]MS-377 was taken up into primary cultured neuronal cells through σ_1 receptors, and the order of potency of σ_1 receptor ligands for inhibition of this uptake was not the same as the order of affinity for their binding to membrane σ_1 receptors (Yamamoto et al., 2000). Therefore, there appear to be more than two pathways for σ_1 receptor ligand transport into neuronal cells, which are involved in their multiple pharmacological effects. It remains unclear whether σ_1 receptors are taken up into cells with σ_1 receptor ligands; however, σ_1 receptor ligands are considered to show various pharmacological effects after being taken up into cells. This uptake of σ_1 receptor ligands may play an important role in their pharmacological effects because σ_1 receptors are predominantly present in the microsomal fraction (Yamamoto et al., 1999; Hayashi et al., 2000). Taken together, the present results indicated that one group of selective σ_1 receptor ligands, including MS-377, have no acute effect on the basal levels of extracellular dopamine in the rat medial prefrontal cortex and striatum by themselves. Single administration of MS-377 alone had no significant behavioral effect at doses up to 100 mg/kg, p.o. (unpublished data). The present results are consistent with the behavioral profile of this agent alone.

It has been reported that various atypical antipsychotics increase extracellular dopamine levels in the rat medial frontal cortex (Moghaddam and Bunney, 1990; Nomikos et al., 1994; Gray and Connick, 1998; Kuroki et al., 1999b). In this study, 20 mg/kg of clozapine significantly increased extracellular dopamine levels in the rat medial prefrontal cortex. Clozapine is the prototype atypical antipsychotic (Kane et al., 1988) and has a greater therapeutic efficacy than other antipsychotics, especially against the negative symptoms of schizophrenia. Reduced dopamine activity in mesocortical projections is involved in these negative symptoms (Weinberger and Lipska, 1995), and the efficacy of clozapine against these symptoms may be related to its ability to increase extracellular dopamine levels in the prefrontal cortex. The mechanism of the effect of clozapine on extracellular dopamine levels in the prefrontal cortex is not understood; however, blockade of dopamine D_4 or 5-HT_{2A} receptors or activation of 5-HT_{1A} receptors may be involved (Schmidt and Fadayel, 1995; Rollema et al., 1997; Broderick and Piercey, 1998).

In contrast, 1 mg/kg of haloperidol induced no significant changes in extracellular dopamine levels in the rat prefrontal cortex, whereas it induced significant increases in the striatum. Haloperidol appears to have less effect on extracellular dopamine levels in the prefrontal cortex than in the striatum or nucleus accumbens (Moghaddam and Bunney, 1990; Nomikos et al., 1994; Kuroki et al., 1999b). This is considered to be due to the low density of dopamine D_2 receptors in the prefrontal cortex (Bouthenet et al., 1987).

In this study, MS-377 and haloperidol had no effect on extracellular serotonin levels in the rat prefrontal cortex. Only clozapine showed a tendency to increase the serotonin level, but this effect was not significant. Ichikawa et al. (1998) reported that clozapine increased extracellular serotonin levels in the rat nucleus accumbens, but not in the medial prefrontal cortex. In contrast, it has been reported that clozapine decreases extracellular serotonin levels in the rat nucleus accumbens (Ferre and Artigas, 1995) and ventral hippocampus (Bengtsson et al., 1998). Further studies are needed to clarify the effects of antipsychotics on extracellular serotonin levels in various brain regions.

PCP at 3 mg/kg significantly increased extracellular dopamine and serotonin levels in the rat medial prefrontal cortex. This result is consistent with previous reports (Hondo et al., 1994; Etou et al., 1998; Martin et al., 1998; Kuroki et al., 1999a; Millan et al., 1999), and the effect of PCP on extracellular dopamine levels appears to be mediated by antagonism of the NMDA receptor complex (Umino et al., 1998; Yonezawa et al., 1998). However, it has also been reported that PCP stimulates dopamine release via σ_1 receptors (Ault and Werling, 1999). Moreover, Yamamoto et al. (1995) reported that selective σ_1 receptor ligands without affinity for the NMDA receptor complex inhibited [^{3}H]TCP binding to primary cultured neuronal cells, suggesting that σ_1 receptor ligands could indirectly modulate the effect of PCP. In this study, MS-377 at 1 mg/kg significantly suppressed the PCP-induced increase in extracellular dopamine and serotonin levels in the rat medial prefrontal cortex. The pharmacological effects

of MS-377 are mainly mediated through σ_1 receptors because this agent is highly selective for σ_1 receptors and it has less or no affinity for other receptors, including the NMDA receptor complex (Takahashi et al., 1999; Karasawa et al., 2000). Therefore, MS-377 probably attenuated the PCP-induced increase in dopamine and serotonin levels via σ_1 receptors. The present results suggested that the PCP-induced increase in extracellular dopamine and serotonin levels in several brain regions not only is attributable to an interaction with the NMDA receptor complex but is also directly and/or indirectly mediated by σ_1 receptors.

The result of the present study showing that acute administration of PCP increased extracellular dopamine levels in the rat medial prefrontal cortex seems to be inconsistent with the hypothesis of prefrontal dopaminergic hypofunction proposed by Weinberger (1987). However, Verma and Moghaddam (1996) reported that NMDA receptor antagonists produced cognitive dysfunction in spite of an increase in dopamine release in the prefrontal cortex. In addition, Murphy et al. (1996) has suggested that excessive dopaminergic activity in the prefrontal cortex impairs cognitive function. Interestingly, a similar cognitive dysfunction was observed after subchronic administration of PCP in rats and monkeys, and these animals showed reduced dopamine utilization in the prefrontal cortex (Jentsch et al., 1998,1999). These results suggest that both an excess and a deficiency in prefrontal dopamine output caused by PCP may be related to impaired function of the medial prefrontal cortex in the PCP model of psychosis. MS-377 itself caused neither an excess nor a deficiency in dopamine output from the medial prefrontal cortex, and it reversed the dopamine overflow induced by PCP. Therefore, MS-377 has a beneficial effect in the PCP model of psychosis.

PCP causes various abnormal behaviors in animal models such as head-weaving, rearing, sniffing and hyperlocomotion. These behaviors are considered to be related to dopamine and serotonin neural activation (Yamaguchi et al., 1986; Gleason and Shannon, 1997; Millan et al., 1999). In particular, PCP-induced hyperlocomotion has been shown to be caused by both dopamine and serotonin activation (Millan et al., 1999). In this study, locomotor activity was measured in the same experiments, and PCP was shown to significantly increase locomotor activity. These results suggest that dopamine and serotonin neural activation in the medial prefrontal cortex is involved in PCP-induced hyperlocomotion. MS-377 suppressed the PCP-induced increase in extracellular dopamine and serotonin levels, and tended to suppress locomotor activity (not significantly). In addition, MS-377 inhibited PCP-induced head-weaving (Takahashi et al., 1999), rearing (Takahashi et al., 2001) and disruption of prepulse inhibition (Yamada et al., 2000). PCP-induced head-weaving and rearing are closely related to dopamine and serotonin neural activation and/or σ receptors (Yamaguchi et al., 1986; Kitaichi et al., 1996). In addition, Yamada et al. (1999) reported that the potency of drugs to restore the PCP-induced disruption of prepulse inhibition was significantly correlated to their K_i 5-HT_{2A} affinity. These findings suggest that the inhibitory effects of MS-377 on PCP-induced behaviors are at least partly attributable to suppression of dopamine and serotonin release activated by PCP. These effects of MS-377 may be mediated directly through σ_1 receptors or indirectly through modification of the NMDA receptor complex via σ_1 receptors. Further studies are needed to clarify this point.

In summary, MS-377, a novel selective σ_1 receptor ligand, did not have any effect on extracellular dopamine or serotonin levels by itself. However, it suppressed the PCP-induced increase in extracellular dopamine and serotonin levels in the rat medial prefrontal cortex. These results suggest that the inhibitory effects of this agent on various PCP-induced behaviors in animal models are attributable to the inhibition of the increase in dopamine and serotonin release caused by PCP without affecting their basal levels. The precise mechanism of the inhibitory effects of MS-377 is unclear, but σ_1 receptors may be partly involved in the PCP-induced increase in dopamine and serotonin levels and this agent suppressed the effects of PCP via σ_1 receptors. Since the PCP model is recognized as the good model of schizophrenia, MS-377 may be useful for the treatment of schizophrenia.

References

Ault, D.T., Werling, L.L., 1999. Phencyclidine and dizocilpine modulate dopamine release from rat nucleus accumbens via σ receptors. Eur. J. Pharmacol. 386, 145–153.

Bengtsson, H.J., Kullberg, A., Millan, M.J., Hjorth, S., 1998. The role of 5-HT_{1A} autoreceptors and α_1-adrenoceptors in the modulation of 5-HT release—III. Clozapine and the novel putative antipsychotic S 16924. Neuropharmacology 37, 349–356.

Bouthenet, M.-L., Martres, M.-P., Sales, N., Schwartz, J.-C., 1987. A detailed mapping of dopamine D_2 receptors in rat central nervous system by autoradiography with [^{125}I]iodosulpiride. Neuroscience 20, 117–155.

Broderick, P.A., Piercey, M.F., 1998. Clozapine, haloperidol, and the D_4 antagonist PNU-101387G: in vivo effects on mesocortical, mesolimbic and nigrostriatal dopamine and serotonin release. J. Neural Transm. 105, 749–767.

Deutch, A.Y., 1992. The regulation of subcortical dopamine systems by the prefrontal cortex: interactions of central dopamine systems and the pathogenesis of schizophrenia. J. Neural Transm. 36, 61–89.

Dolan, R.J., Fletcher, P., Frith, C.D., Friston, K.J., Frackowiak, R.S., Grasby, P.M., 1995. Dopaminergic modulation of impaired cognitive activation in the anterior cingulate cortex in schizophrenia. Nature 378, 180–182.

Etou, K., Kuroki, T., Kawahara, T., Yonezawa, Y., Tashiro, N., Uchimura, H., 1998. Ceruletide inhibits phencyclidine-induced dopamine and serotonin release in rat prefrontal cortex. Pharmacol. Biochem. Behav. 61, 427–434.

Ferre, S., Artigas, F., 1995. Clozapine decreases serotonin extracellular levels in the nucleus accumbens by a dopamine receptor-independent mechanism. Neurosci. Lett. 187, 61–64.

Garey, R.E., Heath, R.G., 1976. The effects of phencyclidine on the uptake of ^{3}H-catecholamines by rat striatal and hypothalamic synaptosomes. Life Sci. 18, 1105–1110.

Gleason, S.D., Shannon, H.E., 1997. Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin subtype selective antagonists in mice. Psychopharmacology 129, 79–84.

Gray, A.M., Connick, J.H., 1998. Clozapine-induced dopamine levels in the rat striatum and nucleus accumbens are not affected by muscarinic antagonism. Eur. J. Pharmacol. 362, 127–136.

Gudelsky, G.A., 1995. Effects of sigma receptor ligands on the extracellular concentration of dopamine in the striatum and prefrontal cortex of the rat. Eur. J. Pharmacol. 286, 223–228.

Gudelsky, G.A., 1999. Biphasic effect of sigma receptor ligands on the extracellular concentration of dopamine in the striatum of the rat. J. Neural Transm. 106, 849–856.

Gudelsky, G.A., Nash, J.F., 1992. Neuroendocrinological and neurochemical effects of sigma ligands. Neuropharmacology 31, 157–162.

Gundlach, A.L., Largent, B.L., Snyder, S.H., 1985. Phencyclidine and σ opiate receptors in brain: biochemical and autoradiographical differentiation. Eur. J. Pharmacol. 113, 465–466.

Hayashi, T., Maurice, T., Su, T., 2000. Ca^{2+} signaling via σ_1-receptors: novel regulatory mechanism affecting intracellular Ca^{2+} concentration. J. Pharmacol. Exp. Ther. 293, 788–798.

Hondo, H., Yonezawa, Y., Nakahara, T., Nakamura, K., Hirano, M., Uchimura, H., Tashiro, N., 1994. Effect of phencyclidine on dopamine release in the rat prefrontal cortex: an in vivo microdialysis study. Brain Res. 633, 337–342.

Ichikawa, J., Kuroki, T., Meltzler, H.Y., 1998. Effects of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. Eur. J. Pharmacol. 351, 163–171.

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

Jentsch, J.D., Dazzi, L., Chhatwal, J.P., Verrico, C.D., Roth, R.H., 1998. Reduced prefrontal cortical dopamine, but not acetylcholine, release in vivo after repeated, intermittent phencyclidine administration to rats. Neurosci. Lett. 258, 175–178.

Jentsch, J.D., Taylor, J.R., Elsworth, J.D., Redmond Jr., D.E., Roth, R.H., 1999. Altered frontal cortical dopaminergic transmission in monkeys after subchronic phencyclidine exposure: involvement in frontostriatal cognitive deficits. Neuroscience 90, 823–832.

The Clozaril Collaborative Group, Kane, J., Honigfeld, G., Singer, J., Meltzler, H., 1988. Clozapine for treatment-resistant schizophrenic. Arch. Gen. Psychiatry 45, 789–796.

Karasawa, J., Takahashi, S., Horikomi, K., 2000. Binding properties of [^{3}H]MS-377, a novel σ receptor ligand, to rat brain membranes. Eur. J. Pharmacol. 400, 51–57.

Karbon, E.W., Abreu, M.E., Erickson, R.H., Kaiser, C., Natalie, K.J., Clissold, D.B., Borosky, S., Bailey, M., Martin, L.A., Pontecorvo, M.J., Enna, S.J., Ferkany, J.W., 1993. NPC 16377, a potent and selective σ-ligand: I. Receptor binding, neurochemical and neuroendocrine profile. J. Pharmacol. Exp. Ther. 265, 866–875.

Kitaichi, K., Noda, Y., Hasegawa, T., Furukawa, H., Nabeshima, T., 1996. In vivo functional interaction between phencyclidine binding sites and σ receptors to produce head-weaving behavior in rats. Eur. J. Pharmacol. 318, 205–211.

Kobayashi, T., Matsuno, K., Murai, M., Mita, S., 1997. Sigma 1 receptor subtype is involved in the facilitation of cortical dopaminergic transmission in the rat brain. Neurochem. Res. 22, 1105–1109.

Kuroki, T., Kawahara, T., Yonezawa, Y., Tashiro, N., 1999a. Effects of the serotonin2A/2C receptor agonist and antagonist on phencyclidine-induced dopamine release in rat medial prefrontal cortex. Prog. Neuropsychopharmacol. Biol. Psychiatry 23, 1259–1275.

Kuroki, T., Meltzler, H.Y., Ichikawa, J., 1999b. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J. Pharmacol. Exp. Ther. 288, 774–781.

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 nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532.

Martin, P., Carlsson, M.L., Hjorth, S., 1998. Systemic PCP treatment elevates brain extracellular 5-HT: a microdialysis study in awake rats. NeuroReport 9, 2985–2988.

Matsuno, K., Matsunaga, K.H., Mita, S., 1995. Acute effects of sigma ligands on the extracellular DOPAC level in rat frontal cortex and striatum. Neurochem. Res. 20, 233–238.

McCullough, L.D., Salamone, J.D., 1992. Increases in extracellular dopamine levels and locomotor activity after direct infusion of phencyclidine into the nucleus accumbens. Brain Res. 577, 1–9.

Millan, M.J., Brocco, M., Gobert, A., Joly, F., Bervoets, K., Rivet, J., Newman-Tancredi, A., Audinot, V., Maurel, S., 1999. Contrasting mechanisms of action and sensitivity to antipsychotics of phencyclidine versus amphetamine: importance of nucleus accumbens 5-HT_{2A} sites for PCP-induced locomotion in the rat. Eur. J. Neurosci. 11, 4419–4432.

Moghaddam, B., Bunney, B.S., 1990. Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens and striatum of the rat: an in vivo microdialysis study. J. Neurochem. 541, 1755–1760.

Murphy, B.L., Arnsten, A.F., Goldman-Rakic, P.S., Roth, R.H., 1996. Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. Proc. Natl. Acad. Sci. U. S. A. 93, 1325–1329.

Nomikos, G.G., Iurlo, M., Andersson, J.L., Kimura, K., Svensson, T.H., 1994. Systemic administration of amperozide, a new atypical antipsychotic drug, preferentially increases dopamine release in the rat medial prefrontal cortex. Psychopharmacology (Berlin) 115, 147–156.

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, PL285–PL290.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. 2nd edn. Academic Press, Sydney.

Poncelet, M., Santucci, V., Paul, R., Gueudet, C., Lavastre, S., Guitard, J., Steinberg, R., Terranova, J.P., Breliere, J.C., Soubrie, P., Fur Le, G., 1993. Neuropharmacological profile of a novel and selective ligand of the sigma site: SR 31742A. Neuropharmacology 32, 605–615.

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.

Rollema, H., Lu, Y., Schmidt, A.W., Zorn, S.H., 1997. Clozapine increases dopamine release in prefrontal cortex by 5-HT_{1A} receptor activation. Eur. J. Pharmacol. 338, R3–R5.

Schmidt, C.J., Fadayel, G.M., 1995. The selective 5-HT_{2A} receptor antagonist MDL 100,907, increases dopamine efflux in the prefrontal cortex of the rat. Eur. J. Pharmacol. 273, 273–279.

Smith, R.C., Meltzer, H.Y., Arora, R.C., Davis, J.M., 1977. Effects of phencyclidine on [^{3}H]catecholamine and [^{3}H]serotonin uptake in synaptosomal preparations from rat brain. Biochem. Pharmacol. 26, 1435–1439.

Su, T.P., Wu, X.Z., 1990. Guinea pig vas deferens contains sigma but not phencyclidine receptors. Neurosci. Lett. 108, 341–345.

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 antipsychotic agent with selective affinity for σ receptors. Psychopharmacology 145, 295–302.

Takahashi, S., Karasawa, J., Horikomi, K., 2000a. Membrane binding property of a novel antipsychotic agent MS-377 in guinea pig brain. In: Kato, T. (Ed.), Frontier of the Mechanisms of Memory and Dementia. Elsevier, pp. 203–204.

Takahashi, S., Miwa, T., Horikomi, K., 2000b. Involvement of σ_1 receptors in methamphetamine-induced behavioral sensitization in rats. Neurosci. Lett. 289, 21–24.

Takahashi, S., Takagi, K., Horikomi, K., 2001. Effects of a novel, selective, σ_1-ligand, MS-377, on phencyclidine-induced behaviour. Naunyn-Schmiedeberg's Arch. Pharmacol. 364, 81–86.

Umino, A., Takahashi, K., Nishikawa, T., 1998. Characterization of the phencyclidine-induced increase in prefrontal cortical dopamine metabolism in the rat. Br. J. Pharmacol. 124, 377–385.

Verma, A., Moghaddam, B., 1996. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alteration performance in rats: modulation by dopamine. J. Neurosci. 16, 373–379.

Volonté, M., Ceci, A., Borsini, F., 1995. Effect of the 5-hydroxytryptamine3 receptor antagonist itasetron (DAU 6215) on (+)-*N*-allylnormetazocine-induced dopamine release in the nucleus accumbens and in the corpus striatum of the rat: an in vivo microdialysis study. J. Pharmacol. Exp. Ther. 275, 358–367.

Weinberger, D.R., 1987. Implications of normal brain development for the pathogenesis of schizophrenia. Arch. Gen. Psychiatry 44, 660–669.

Weinberger, D.R., Lipska, B.K., 1995. Cortical maldevelopment, antipsychotic drugs and schizophrenia: a search for a common ground. Schizophr. Res. 16, 87–110.

Yamada, S., Harano, M., Annoh, N., Nakamura, K., Tanaka, M., 1999. Involvement of serotonin 2A receptors in phencyclidine-induced disruption of prepulse inhibition of the acoustic startle in rats. Biol. Psychiatry 46, 832–838.

Yamada, S., Yamauchi, K., Hisadomi, S., Annoh, N., Tanaka, M., 2000. Effect of σ-1 ligand, MS-377 on apomorphine or phencyclidine-induced disruption of prepulse inhibition of acoustic startle in rat. Eur. J. Pharmacol. 402, 251–254.

Yamaguchi, K., Nabeshima, T., Kameyama, T., 1986. Role of dopaminergic and GABAergic mechanisms in discrete brain areas in phencyclidine-induced locomotor stimulation and turning behavior. J. Pharmacobio-Dyn. 9, 975–986.

Yamamoto, H., Yamamoto, T., Sagi, N., Okuyama, S., Kawai, N., Baba, A., Moroji, T., 1995. NE-100, a novel sigma ligand: effects on [^{3}H]TCP binding to intact primary cultured neuronal cells. Life Sci. 56, PL39–PL43.

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.

Yamamoto, H., Yamamoto, T., Nukada, T., Sagi, N., Shinohara, K., Takahashi, S., Karasawa, J., Okuyama, S., Sora, I., 2000. Sigma-1 receptor ligands are preferentially uptaken into the neuronal cells compared to glial cells. Soc. Neurosci. Abstr. 26, 1160.

Yonezawa, Y., Kuroki, T., Tashiro, N., Hondo, H., Uchimura, H., 1995. Potentiation of phencyclidine-induced dopamine release in the rat striatum by the blockade of dopamine D_2 receptor. Eur. J. Pharmacol. 285, 305–308.

Yonezawa, Y., Kuroki, T., Kawahara, T., Tashiro, N., Uchimura, H., 1998. Involvement of gamma-aminobutyric acid neurotransmission in phencyclidine-induced dopamine release in the medial prefrontal cortex. Eur. J. Pharmacol. 341, 45–56.

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