A Novel Azaindolizinone Derivative ZSET1446 (Spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one) Improves Methamphetamine-Induced Impairment of Recognition Memory in Mice by Activating Extracellular Signal-Regulated Kinase 1/2

Yukio Ito, Kazuhiro Takuma, Hiroyuki Mizoguchi, Taku Nagai, and Kiyofumi Yamada

Laboratory of Neuropsychopharmacology, Division of Life Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Japan

Received September 15, 2006; accepted November 6, 2006

ABSTRACT

The effect of ZSET1446 (spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one) on cognitive impairment in mice, previously treated with methamphetamine (METH) at a dose of 1 mg/kg for 7 days, was investigated. ZSET1446 showed a significant ameliorating effect on METH-induced impairment of recognition memory, although it had no effect on exploratory behavior. ZSET1446 (1 μg/kg) recovered the defect of the novelty-induced activation of extracellular signal-regulated kinase 1/2 (ERK1/2) in the prefrontal cortex (PFC) of METH-treated mice. The compound increased phosphorylated ERK1/2 levels in the hippocampus but not PFC of naive mice without affecting the total ERK1/2 levels. The ameliorating effect of ZSET1446 on recognition memory in METH-treated mice was negated by pretreatment with a mitogen-activated protein kinase/extracellular signal-regulated kinase kinase inhibitor, SL327 (α-[amino-(4-aminophenylthio)methylene]-2-(trifluoromethyl)phenylacetonitrile). Furthermore, the dopamine D1 receptor antagonist, SCH23390 [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine], and N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801 [5H-dibenzo[a,d]cyclohepten-5,10-imine (dizocilpine maleate)], blocked the ameliorating effect of ZSET1446 on METH-induced memory impairment, whereas the D2 receptor antagonist, raclopride, had no effect. These results suggest that the ameliorative effect of ZSET1446 on METH-induced memory impairment is associated with indirect activation of ERK1/2 following stimulation with dopamine D1 and NMDA receptors of the PFC. ZSET1446 would be a potential candidate for further preclinical study aimed at the treatment of cognitive deficits in Alzheimer’s disease and schizophrenia, as well as METH psychosis.

A novel azaindolizinone derivative ZSET1446 (spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one) has ameliorating effects on cognitive impairment induced by scopolamine and dizocilpine (Yamaguchi et al., 2002). ZSET1446 increases extracellular acetylcholine (ACh) levels in the cortex and hippocampus (Yamaguchi et al., 2002, 2003) and enhances nicotine-stimulated ACh release in the hippocampus in normal rats (Y. Yamaguchi et al., unpublished data). In addition, ZSET1446 shows ameliorating effects on the impairment of performance in a passive avoidance task caused by a single intracerebroventricular injection of amyloid β peptide (Aβ) fragment, Aβ25–35, or by lesions of the nucleus basalis magnocellularis by ibotenic acid (Yamaguchi et al., 2003). Furthermore, the compound ameliorates learning and memory impairment in rats with continuous intracerebroventricular infusion of Aβ1–40 in Y-maze, water maze, and passive avoidance tasks. The ameliorating effects of ZSET1446 are associated with reversal of the decrease in choline acetyltrans-
ferase activity in the medial septum and hippocampus, glutathione S-transferase-like immunoreactivity in the cortex and nicotine-stimulated ACh release in Δβ_{1-40}-infused rats to the levels of vehicle-infused control rats (Yamaguchi et al., 2006). Thus, ZSET1446 is a potential novel therapeutic agent for cognitive impairment associated with conditions such as Alzheimer’s disease, although the mechanism of action remains to be determined.

Cognitive deficits are also a core feature in patients with schizophrenia. Furthermore, recent studies have demonstrated that chronic use of methamphetamine (METH) causes long-term cognitive deficits (Simon et al., 2000; Kamei et al., 2003; Nordahl et al., 2003), in addition to psychiatric signs such as hallucinations and delusions, which are indistinguishable from paranoid schizophrenia (Yui et al., 2002; Srisurapanont et al., 2003). In a previous study, we demonstrated that repeated METH treatment in mice impairs long-term recognition memory after withdrawal and that METH-induced cognitive impairment is reversed by an atypical antipsychotic, clozapine, but not haloperidol (Kamei et al., 2006). Thus, METH-induced cognitive impairment in mice may be a useful animal model for cognitive deficits in METH abusers and schizophrenic patients. In this study, to assess the effects of ZSET1446 on cognitive deficits in schizophrenia and paranoid schizophrenia, we investigated the effects of ZSET1446 on the impairment of recognition memory in METH-treated mice in a novel-object recognition test (NORT).

Materials and Methods

Animals. Male ICR mice (7–8 weeks old) were obtained from Japan SLC Inc. (Shizuoka, Japan). The animals were housed in plastic cages and kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity) with a 12-h light/dark cycle (lights on at 9:00 AM). Food (Labo MR Stock; Nihon Nosan Kogyo, Kanagawa, Japan) and tap water were available ad libitum. All animal care and use procedures were in accordance with the Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee of Kanazawa University.

Drugs. Methamphetamine hydrochloride (Dainippon Sumitomo Pharma Co Ltd, Osaka, Japan), S(-)-raclopride (+)-tartrate salt (Sigma-Aldrich, St. Louis, MO), R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-2-benazepine hydrochloride (SCH23390; Toeria Biosciences, Ellisville, MO) and dizocilpine hydrogen maleate (MK-801; Sigma-Aldrich) were dissolved in 100% dimethyl sulfoxide and injected at a volume of 0.02 ml/10 g. Fluoromethyl)phenylacetonitrile (SL327; Sigma) was dissolved in 1% carboxymethyl cellulose (CMC). α-[Amino-(4-aminophenylthio)methylene]-2-(trifluoromethyl)phenylacetonitrile (SL327; Sigma) was dissolved in saline. ZSET1446 was kindly provided by Zenyaku Kogyo Co. Ltd. (Tokyo, Japan) and was suspended in 1% carboxymethyl cellulose (CMC). α-[Amino-(4-aminophenylthio)methylene]-2-(trifluoromethyl)phenylacetonitrile (SL327; Sigma) was dissolved in 100% dimethyl sulfoxide and injected at a volume of 0.02 ml/10 g. All drugs except SL327 were administered at a volume of 0.1 ml/10 g b.w.t.

Novel-Object Recognition Test. The NORT was carried out as described previously (Nagai et al., 2003; Kamei et al., 2006). The experimental apparatus consisted of a Plexiglas open-field box (30 cm × 30 cm × 35 cm high), with a sawdust-covered floor. The apparatus was located in a sound-attenuated room and was illuminated with a 20-W bulb. The NORT procedure consisted of three sessions: habituation, training, and retention. Each mouse was individually habituated to the box, with 10 min of exploration in the absence of objects for 3 consecutive days (habituation session, days 1–3). During the training session, two novel objects were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore in the box for 10 min (day 4). The objects were constructed from a golf ball, wooden column, and wall socket, which were different in shape and color but similar in size. The animals were considered to be exploring the object when the head of the animal was facing the object or when the animal was touching or sniffing the object. The time spent exploring each object was recorded. After training, mice were immediately returned to their home cages. During the retention sessions, the animals were placed back into the same box 24 h (day 5) after the training session, in which one of the familiar objects used during training was replaced by a novel object. The animals were then allowed to explore freely for 5 min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index in the retention session, a ratio of the amount of time spent exploring the novel object over the total time spent exploring both objects, was used to measure cognitive function. In the training session, the preference index was calculated as a ratio of the time spent exploring the object that was replaced by the novel object in the retention session over the total exploring time.

Experimental Schedule. In typical experimental conditions, mice were given METH (1 mg/kg i.p.) once daily for 7 days. One day after the last treatment of METH, the NORT commenced, including habituation, training, and retention sessions. To examine the reversibility of METH-induced memory impairment, the NORT was also performed at 3 weeks after the last administration of METH. ZSET1446 or vehicle (1% CMC solution) was administered p.o. 1 h before the training session of the NORT. To test whether activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway was necessary for the ameliorating effects of ZSET1446 in the NORT, SL327 (50 mg/kg), a selective mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) inhibitor, was administered i.p. 1 h before the training session. SCH23390, raclopride, and MK-801 were administered i.p. 30 min before the training session.

Western Blotting. Phosphorylation of ERK1/2 was examined by Western blotting as described previously (Mizoguchi et al., 2004; Kamei et al., 2006). The protein concentration was determined using a Protein Assay Rapid Kit (Wako Pure Chemicals, Osaka, Japan). Tissue samples from prefrontal cortex (PFC) were homogenized at 4°C in a lysis buffer composed of 20 mM Tris-HCl, 150 mM NaCl, 50 mM NaF, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM sodium orthovanadate, 0.1% SDS, 1% sodium deoxycholate, 0.5 mM dithiothreitol, 10 mM sodium pyrophosphate decahydrade, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin (pH 7.4). For the analysis of phosphorylated ERK, 20 μg of protein was boiled in a sample buffer [0.125 M Tris-HCl (pH 6.8), 2% SDS, 5% glyceral, 0.002% bromphenol blue, and 5% 2-mercaptoethanol], and, subsequently transferred to a polyvinylidene difluoride membrane (Millipore Corporation, Billerica, MA) or a nitrocellulose membrane (GE Healthcare Biosciences, Piscataway, NJ), and blocked with a Detector Block Kit (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Membranes were incubated with anti-phospho-ERK1/2 (phospho-p44/42 mitogen-activated protein kinase (Thr202/Tyr204) (E10) monoclonal antibody (1:2000 dilution; Cell Signaling Technology Inc., Beverly, MA) and washed with Tris-buffered saline-Tween 20 (10 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 1% Tween 20) three times for 10 min each. After incubation with a 1:5000 dilution of horseradish peroxidase-conjugated anti-mouse IgG for 1 h, membranes were washed with PBS-Tween 20 three times for 10 min each. The immune complex was detected using ECL Western blotting detection reagents (GE Healthcare Biosciences). The same membranes were stripped with a stripping buffer (137 mM NaCl, 2.7 mM KCl, 8.1 mM disodium hydrogen phosphate, 1.5 mM potassium dihydrogen phosphate, and 0.2% 2-mercaptoethanol) at 55°C for 30 min, incubated with anti-ERK1/2 (1:5000 dilution, anti-
mitogen-activated protein kinase 1/2; Upstate Biotechnology, Lake Placid, NY), and treated as described above.

As there was no change in the level of total ERK1/2, values of phosphorylated ERK1/2 were normalized to the values of total ERK1/2. All data from Western blotting are expressed as a percentage of the control.

Statistical Analysis. All data were expressed as means ± S.E. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test for multigroup comparisons. P values < 0.05 were taken to indicate statistically significant differences.

Results

Effect of ZSET1446 on METH-Induced Cognitive Impairment in Mice. We examined whether METH-induced cognitive impairment was reversed by ZSET1446 treatment. After the cessation of repeated METH (1 mg/kg s.c.) treatment for 7 days, mice were subjected to the NORT. ZSET1446 (0.1–100 μg/kg p.o.) was acutely administered 1 h before the training session. As shown in Fig. 1A, repeated METH treatment significantly reduced the exploratory preference for novel objects in the retention session [F(5,68) = 8.992, p < 0.05 by one-way ANOVA, p < 0.01 by post hoc test] (Fig. 1A). Treatment with ZSET1446 (1–100 μg/kg) significantly improved cognitive impairment in METH-treated mice (p < 0.05 and p < 0.01 by post hoc test) (Fig. 1A). The effect of ZSET1446 was bell-shaped, and the peak effect was observed at a dose of 1 μg/kg. Treatment with ZSET1446 affected neither the level of exploratory preference for novel objects in the training session [F(5,68) = 0.754, p = 0.5864 by one-way ANOVA] (Fig. 1A) nor the total exploration time in either the training [F(5,68) = 1.753, p = 0.1345 by one-way ANOVA] or retention sessions [F(5,68) = 1.753, p = 0.1345 by one-way ANOVA] in METH-treated mice (Fig. 1B). Notably, METH treatment did not affect the exploratory preference for the objects and total exploration time during the training session.

We have previously demonstrated that repeated METH treatment caused an enduring memory deficit, so that METH-treated mice showed recognition memory impairment even 3 weeks after the withdrawal of METH (Kamei et al., 2006). Figure 2 shows the effect of ZSET1446 on METH-induced impairment of recognition memory after the withdrawal of METH for 3 weeks. ZSET1446 (1 μg/kg) significantly improved the cognitive impairment in METH-treated mice [F(2,21) = 12.905, p < 0.05 by one-way ANOVA and p < 0.01 by post hoc test] (Fig. 2A). Treatment with ZSET1446 affected neither the level of exploratory preference for the novel object in the training session [F(2,21) = 2.199, p = 0.1358 by one-way ANOVA] (Fig. 2A) nor the total exploration time in either the training [F(2,21) = 0.520, p = 0.6018 by one-way ANOVA] or retention sessions [F(2,21) = 0.304, p = 0.7409 by one-way ANOVA] in METH-treated mice (Fig. 2B).

Effect of ZSET1446 on the Defect of Novelty-Induced Activation of ERK1/2 in the Prefrontal Cortex of METH-Treated Mice. In a previous study, we demonstrated novelty-induced ERK1/2 activation in the PFC when mice were exposed to novel objects, leading to the formation of long-lasting object recognition memory. Furthermore, memory impairment in METH-treated mice was associated with the dysfunction of ERK1/2 signaling in the PFC (Kamei et al., 2006). To examine the mechanism by which ZSET1446 ameliorates the impairment of recognition memory in METH-treated mice, we examined the effect of ZSET1446 on ERK1/2 phosphorylation in the PFC of METH-treated mice when they were exposed to novel objects. Accordingly, the animals were killed immediately after the exposure to novel objects in the training session, and phosphorylated ERK1/2 levels in the PFC were determined by Western blotting. In agreement with the previous study, a significant increase in the phosphorylation of ERK1/2 levels was observed in the PFC of saline-treated mice immediately after a 10-min exposure to novel objects, and repeated METH treatment abolished novelty-induced ERK1/2 activation in the PFC [F(5,22) = 6.842, p < 0.01 by one-way ANOVA and p < 0.05 by post hoc test] (Fig. 3). ZSET1446 (1 μg/kg) significantly recovered the defect of novelty-induced activation of ERK1/2 in the PFC of METH-treated mice (p < 0.05 by post hoc test) (Fig. 3). The levels of total ERK1/2 did not differ among the groups examined.

We also examined the effect of ZSET1446 on ERK1/2 phos-
Effect on ERK1/2 phosphorylation in the PFC by post hoc test (Fig. 4B); however, the compound had no variance: \( F(3,20) \). In the hippocampus in a dose- and time-dependent manner \( p < 0.01 \), compared with saline (S) + vehicle (veh) group that was not exposed to novel objects (baseline). \#p < 0.05, compared with saline + vehicle group that was exposed to novel objects (exposure). †, \( p < 0.05 \) compared with METH + vehicle group (exposure).

Fig. 3. Effect of ZSET1446 on the defect of novelty-induced ERK1/2 phosphorylation in the PFC of METH-treated mice. ZSET1446 (1 \( \mu \)g/kg p.o.) or vehicle (1% CMC) was administered in mice that had been previously treated with either saline or METH (1 mg/kg) for 7 days, 1 h before exposure to novel objects. Values indicate the mean \( \pm \) SE (n = 4–6). Analysis of variance: \( F(2,21) = 2.119, p = 0.1358 \) for Fig. 2A training; \( F(2,21) = 12.905, p < 0.05 \) for Fig. 2A retention; \( F(2,21) = 0.526, p = 0.6018 \) for Fig. 2B training; \( F(2,21) = 0.304, p = 0.7409 \) for Fig. 2B retention. ••, \( p < 0.01 \), compared with saline + vehicle group. #†, \( p < 0.05 \), compared with METH + vehicle group.

Influence of a MEK Inhibitor on the Ameliorating Effect of ZSET1446 against METH-Induced Cognitive Impairment. We have already found that treatment with SL327 (30 and 50 mg/kg i.p.), a selective MEK inhibitor, 1 h before the training session dose dependently impairs long-term recognition memory in naive mice (unpublished data). Then, we examined the effect of SL327 (50 mg/kg) administered before the training session on the improvement of METH-induced impairment of recognition memory by ZSET1446 to determine the involvement of ERK1/2 activation in the effect of ZSET1446. In the training session, treatment with SL327 alone did not affect the exploratory preference for the objects \( F(4,26) = 0.432, p = 0.7840 \) by one-way ANOVA (Fig. 5A). In the retention test, SL327 completely blocked the ameliorating effect of ZSET1446 on the impairment of exploratory preference for a novel object in METH-treated mice \( F(4,26) = 18.738, p < 0.05 \) by one-way ANOVA and \( p < 0.01 \) by post hoc test (Fig. 5A), although it had no effect on METH-induced impairment of memory retention. The antagonistic effect of SL327 on ZSET1446-induced improvement of exploratory preference in METH-treated mice was not associated with changes in the total exploration time [training: \( F(4,26) = 0.375, p = 0.8244 \); retention: \( F(4,26) = 1.642, p = 0.1938 \) by one-way ANOVA] (Fig. 5B).

Effects of Dopamine Receptor Antagonists on Ameliorative Effect of ZSET1446 against METH-Induced Cognitive Impairment. As ZSET1446 had no effect on ERK1/2 phosphorylation in the PFC of naive mice, it is unlikely that the compound directly activates ERK1/2 in the PFC of METH-treated mice. Rather, it is possible that ZSET1446 may indirectly activate ERK1/2 signaling in the PFC. We have previously shown that the ERK1/2 signaling pathway linked to dopamine D1 receptors (Valjent et al., 2000; Zanassi et al., 2001) is involved in METH-associated contextual memory in rats (Mizoguchi et al., 2004). Moreover, we have demonstrated that repeated METH treatment in mice induces cognitive impairment in the NORT, which is accompanied by dysfunction of the dopamine D1 receptor-ERK1/2 pathway in the PFC (Kamei et al., 2006). Therefore, we investigated whether activation of dopamine receptors was involved in the ameliorating effect of ZSET1446 on mem-
ory impairment in METH-treated mice. We have already found that the dopamine D1 receptor antagonist SCH23390 (0.03 and 0.05 mg/kg i.p.) impaired long-term recognition memory, whereas the D2 receptor antagonist raclopride (0.1 and 0.3 mg/kg i.p.) had no effect in naive mice without affecting locomotor activity or total exploration time of novel objects in the training session (unpublished data). Accordingly, SCH23390 (0.05 mg/kg i.p.) or raclopride (0.3 mg/kg i.p.) was administered before the training session. In the training session, treatment with SCH23390 alone did not affect the exploratory preference for the objects \[ F(6,49) = 0.459, p = 0.8352 \] by one-way ANOVA (Fig. 6A). In the retention test, SCH23390 significantly blocked the ameliorating effect of ZSET1446 on the impairment of exploratory preference for a novel object in METH-treated mice \[ F(6,49) = 18.539, p < 0.05 \] by post hoc test (Fig. 6A), although it had no effect on METH-induced impairment of memory retention. In contrast, treatment with raclopride had no effect on exploratory preference in the training and retention sessions (Fig. 6A). Treatment with antagonists did not affect the total exploration time in either the training \[ F(6,49) = 1.007, p = 0.4319 \] by one-way ANOVA) (Fig. 6B) or retention sessions \[ F(6,49) = 0.735, p = 0.6218 \] by one-way ANOVA) (Fig. 6B).

Effect of NMDA Receptor Antagonist on the Ameliorative Effect of ZSET1446 against METH-Induced Cognitive Impairment. Several lines of evidence have demonstrated the interaction between NMDA and dopamine D1 receptors (Wang and O'Donnell, 2001; Lee et al., 2002). The dopamine D1 receptor plays a key role in NMDA receptor-dependent long-term potentiation (LTP) in hippocampal-PFC circuits (Gurden et al., 2000). Repeated METH administration impairs LTP in hippocampal-PFC (Ishikawa et al., 2005). Moreover, both protein synthesis and NMDA receptors are required for the consolidation of recognition memory in the PFC (Akirav and Maroun, 2006). Therefore, we examined whether NMDA receptors were involved in the ameliorative effect of ZSET1446 on memory impairment in METH-treated mice. A NMDA receptor blocker MK-801 was
reported to impair object recognition memory in naive rats (de Lima et al., 2005). We also found that MK-801 (0.1 mg/kg i.p.) impaired long-term recognition memory 24 h after the training session in naive mice (data not shown). Therefore, MK-801 (0.1 mg/kg i.p.) was administered before the training session. In the training session, treatment with MK-801 alone did not affect the exploratory preference for the objects \( F(4,39) = 0.582, p = 0.6775 \) by one-way ANOVA (Fig. 7A). In the retention test, MK-801 blocked the ameliorating effect of ZSET1446 on the impairment of exploratory preference for a novel object in METH-treated mice \( F(4,39) = 9.940, p < 0.05 \) by one-way ANOVA and \( p < 0.01 \) by post hoc test (Fig. 7A), although it had no effect on METH-induced impairment of memory retention. The antagonistic effect of MK-801 on ZSET1446-induced improvement of exploratory preference in METH-treated mice was not associated with changes in total exploration time [training: \( F(4,39) = 0.622, p = 0.6497 \); retention: \( F(4,39) = 0.537, p = 0.7092 \) by one-way ANOVA] (Fig. 7B).

**Discussion**

We have recently demonstrated that repeated administration of METH in mice induces object recognition impairment, which is associated with dysfunction of the dopamine D1 receptor-ERK1/2 pathway in the PFC. Furthermore, cognitive impairment as well as behavioral sensitization induced by repeated METH treatment persisted for at least 28 days after withdrawal from METH (Kamei et al., 2006). These observations in METH-treated mice are consistent with recent studies in human subjects demonstrating that long-term METH use is associated with cognitive deficits (Simon et al., 2000; Volkow et al., 2001). Alternatively, the fact that METH...
significantly reduced the exploratory preference for novel objects in the retention session could be interpreted as a neophobia. However, a possible involvement of neophobia can be excluded because METH treatment had no effect on total exploration time of novel objects during the training session. Furthermore, it is unlikely that the deficit of recognition memory in repeated METH-treated mice is due to the changes in attention, curiosity, or locomotor activity because repeated METH treatment had no effect on total exploratory time or locomotor activity during training and retention sessions (Kamei et al., 2006).

Accumulating evidence suggests that the dopaminergic system in the PFC is involved in cognitive function. For instance, disruption of dopamine transmission in the PFC by infusions of dopamine D1 receptor antagonists or by excitotoxic lesions impairs the performance of object retrieval-detour tasks, as well as delayed response tasks in nonhuman primates (Sawaguchi and Goldman-Rakic 1991; Dias et al., 1996a,b). A recent study with functional magnetic resonance imaging showed that dysfunction in the PFC of METH abusers is related to cognitive impairment (Paulus et al., 2002). Accordingly, cognitive impairment in METH abusers may be associated with deficits in dopamine transmission in the PFC.

Clozapine, but not haloperidol, completely restored the cognitive impairment induced by METH treatment when repeatedly administered for 7 days after withdrawal from METH, although acute treatment with these antipsychotics had no effect (Kamei et al., 2006). The data are consistent with clinical evidence that clozapine is superior to typical neuroleptics in improving cognitive deficits in schizophrenic patients (Lee et al., 1999). Thus, we propose that cognitive impairment induced by repeated METH treatment in mice is a useful model to study cognitive deficits in schizophrenia and METH psychosis.

In this study, acute treatment with ZSET1446 significantly improved cognitive impairment without affecting motor function in METH-treated mice. These results suggest that ZSET1446 is effective for the treatment of cognitive deficits in patients with schizophrenia and METH psychosis. Furthermore, the effect of ZSET1446 may be superior to clozapine and other antipsychotics because acute treatment with ZSET1446 showed an improving effect whereas repeated treatment was necessary for the effect of clozapine.

Recent studies have demonstrated that the ERK1/2 signaling pathway linked to dopamine D1 receptors (Valjent et al., 2000; Zanassi et al., 2001) is involved in the rewarding effects induced by METH (Mizoguchi et al., 2004) and the behavioral sensitization and rewarding effects induced by cocaine (Valjent et al., 2000). Regarding the mechanism underlying the repeated METH-induced memory impairment, we have already demonstrated dysfunction of the ERK1/2 pathway in the PFC. Hyperphosphorylation of ERK1/2 was found in the PFC when control mice were exposed to novel objects whereas this activation was abolished in repeated METH-treated mice. Inhibition of ERK1/2 by the microinjection of PD98059, a selective MEK inhibitor, into the PFC resulted in cognitive impairment (Kamei et al., 2006). We also found that SL327 (30 and 50 mg/kg i.p.) significantly impaired long-term recognition memory 24 h after the training session in naive mice (unpublished data). In this study, ZSET1446 ameliorated the METH-induced defect of ERK1/2 hyperphosphorylation in the PFC of mice exposed to novel objects. In addition, the ameliorating effect of ZSET1446 on METH-induced object-recognition impairment was completely blocked by pretreatment with the MEK inhibitor SL327. Accordingly, these results suggest that the ameliorating effect of ZSET1446 on METH-induced cognitive impairment is related to the activation of ERK1/2 in the PFC.

In this study, we demonstrated that ZSET1446 increased phosphorylated ERK1/2 levels in the hippocampus, but not in the PFC, of naive mice. It is not clear whether this phenomenon is related to the ameliorating effect of ZSET1446 on METH-induced memory impairment, but ZSET1446 significantly increased the exploratory preference for novel objects in normal mice in the NORT (data not shown). As ZSET1446 (10⁻⁵ M) has no effect on kinase activity, including ERK1/2 (unpublished data), the data suggest that ZSET1446 indirectly activates ERK1/2 in the brain. Accordingly, it is likely that ERK1/2 in the PFC of repeated METH-treated mice may be activated indirectly by acute ZSET1446 treatment.

It is known that ERK1/2 is critically linked to dopamine D1 receptors coupled with Gs protein (Valjent et al., 2000; Zanassi et al., 2001). Several second messengers could be responsible for the link between dopamine D1 receptors and ERK1/2. Namely, cAMP, the first second messenger to be related to dopamine D1 receptor antagonists show no effect. Moreover, it has been demonstrated that dopaminergic dysfunction in the PFC is related to the cognitive dysfunction of schizophrenia (Okubo et al., 1997) and that the improving effect of clozapine is related to the enhancement of dopamine transmission in the PFC (Kuroki et al., 1999; Youngren et al., 1999). In this study, we demonstrated that coadministration of a dopamine D1 receptor antagonist SCH23390 or SCH39166 into the PFC of monkeys (Sawaguchi and Goldman-Rakic, 1991, 1994) or rats (Seamans et al., 1995) produces delay-related impairment in spatial working memory performance, whereas comparable infusions of dopamine D2 receptor antagonists do not affect performance. Moreover, it has been proposed that dopaminergic hypofunction in the PFC is related to the cognitive dysfunction of schizophrenia (Okubo et al., 1997) and that the improving effect of clozapine is related to the enhancement of dopamine transmission in the PFC (Kuroki et al., 1999; Youngren et al., 1999).

In this study, we demonstrated that coadministration of a dopamine D1 receptor antagonist blocked the ameliorating effect of ZSET1446 on METH-induced memory impairment, whereas a D2 receptor antagonist had no effect. Therefore, it is suggested that activation of dopamine D1 receptors in the PFC is necessary for the ameliorating effect of ZSET1446.

Interest in the role of NMDA receptors in learning and memory originates primarily from extensive evidence that these receptors are essential for the induction of LTP. Several lines of evidence have demonstrated an interaction between NMDA and dopamine D1 receptors (Wang and O'Donnell, 2001; Lee et al., 2002). D1 receptor-stimulated activation of protein kinase A induces phosphorylation of the NR1 subunit of the NMDA receptor, which is involved in D1 receptor-mediated phosphorylation of cAMP response element-binding protein (Dudman et al., 2003). These signaling pathways implicated in the PFC are involved in the enhancement of dopamine transmission in the PFC (Kuroki et al., 1999; Youngren et al., 1999). In this study, we demonstrated that coadministration of a dopamine D1 receptor antagonist blocked the ameliorating effect of ZSET1446 on METH-induced memory impairment, whereas a D2 receptor antagonist had no effect. Therefore, it is suggested that activation of dopamine D1 receptors in the PFC is necessary for the ameliorating effect of ZSET1446.
pathways play a key role in NMDA-dependent LTP in hippocampal-PFC circuits (Gurden et al., 2000). Considering the interaction between D1 and NMDA receptors, it is possible that the ameliorating effect of ZSET1446 is also associated with the activation of NMDA receptors. In fact, we found that MK-801 significantly attenuated the effect of ZSET1446 on METH-induced memory impairment. As discussed above, our findings suggest that dopamine D1-ERK1/2 and NMDA receptor systems are required for the effects of ZSET1446. However, because dopamine D1-ERK1/2 and NMDA receptor systems are critical in recognition memory, one might expect that the effects of ZSET1446 would be reversed by these antagonists regardless of the mechanisms of ZSET1446 to attenuate the cognitive impairment in METH-treated mice. However, if the action site of ZSET1446 is downstream of dopamine D1-ERK1/2 and NMDA receptor systems, the receptor antagonists or the MEK inhibitor would fail to reverse the effect of ZSET1446. Accordingly, our data suggest that ZSET1446 acts upstream of dopamine D1-ERK1/2 and NMDA receptor systems.

It has been reported that ZSET1446 increases extracellular ACh levels in the cortex and hippocampus (Yamaguchi et al., 2002, 2003) and extracellular dopamine levels in the hippocampus in normal rats (Y. Yamaguchi et al., unpublished data) although ZSET1446 (10−5 M) does not act on any receptor system (Y. Yamaguchi et al., unpublished data). These findings suggest that the cholinergic system, in addition to the dopaminergic system, may be involved in the pharmacological effect of ZSET1446. As shown in this study, the dopamine D1 receptor antagonist and NMDA receptor antagonist completely blocked the ameliorating effect of ZSET1446 in METH-treated mice. Therefore, it is likely that ZSET1446 attenuates impairment of recognition memory in METH-treated mice by stimulating neurotransmitter release such as dopamine and glutamate, which results in indirect activation of the ERK1/2 pathway in the PFC via dopamine D1 and NMDA receptors. Further studies are necessary to clarify the molecular mechanism of action of this compound.

In conclusion, the ameliorating effect of ZSET1446 on METH-induced memory impairment is associated with indirect activation of the ERK1/2 pathway after stimulation of dopamine D1 and NMDA receptors in the PFC. ZSET1446 is a potential candidate for further preclinical study aimed at the treatment of cognitive deficits in Alzheimer’s disease and schizophrenia as well as METH psychosis.

Acknowledgments
We thank Zenyaku Kogyo Co. Ltd. for providing ZSET1446.

References
Youngren KD, Ingles FM, Pirvoto JF, Jedema HP, Bradberry CW, Goldman-Rakic PS, Roth RH, and Moghaddam B (1999) Clozapine preferentially increases dopa...
mine release in the rhesus monkey prefrontal cortex compared with the caudate nucleus. Neuropsychopharmacology 20:403–412.

Address correspondence to: Dr. Kiyofumi Yamada, Laboratory of Neuropsychopharmacology, Division of Life Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa 920-1192, Japan. E-mail: kyamada@p.kanazawa-u.ac.jp