Stimulation of Metabotropic Glutamate (mGlu) 2 Receptor and Blockade of mGlu1 Receptor Improve Social Memory Impairment Elicited by MK-801 in Rats

Hirohiko Hikichi1,*, Ayaka Kaku1, Jun-ichi Karasawa1, and Shigeyuki Chaki1
1Discovery Pharmacology I, Molecular Function and Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan

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Abstract. Glutamatergic dysfunction has been implicated in psychiatric disorders such as schizophrenia. Both the stimulation of the metabotropic glutamate (mGlu) 2/3 receptor and the blockade of the mGlu1 receptor have been shown to be effective in a number of animal models of schizophrenia. However, the efficacy for social cognition, which is poorly managed by current medication, has not been fully addressed. The present study evaluated the effects of an mGlu2/3-receptor agonist and an mGlu1-receptor antagonist on social memory impairment in rats. Pretreatment with an mGlu2/3-receptor agonist, (-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268), or an mGlu1-receptor antagonist, (3,4-dihydro-2H-pyrano[2,3-b]quinolin-7-yl)-(cis-4-methoxycyclohexyl)-methanone (JNJ16259685), improved social memory impairment induced by 5R,10S- (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801) without affecting the social interactions. In addition, the intraperitoneal administration of an mGlu2-receptor potentiator, 3’-[(2-cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1H-inden-5-yl)oxy]methyl]-[1,1’-biphenyl]-4-carboxylic acid (BINA), also improved the MK-801–induced impairment of social memory, which was blocked by pretreatment with an mGlu2/3-receptor antagonist, (2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495). These findings indicate that both the stimulation of the mGlu2 receptor and the inhibition of an mGlu1 receptor improve social memory impairment elicited by MK-801, and both manipulations could be effective approaches for the treatment of certain cognitive dysfunctions observed in schizophrenic patients.

Keywords: social memory, mGlu2/3-receptor agonist, mGlu2-receptor potentiator, mGlu1-receptor antagonist

Introduction

In addition to the dopamine hypothesis of schizophrenia, abnormalities in glutamatergic transmissions have been suggested to be involved in the pathophysiology of schizophrenia (1 – 3). Among glutamate receptors, metabotropic glutamate (mGlu) receptors, which consist of 8 subtypes (mGlu1 – 8), have emerged as attractive therapeutic targets for the development of novel interventions for psychiatric disorders. Of these, group I (mGlu1 and mGlu5) and group II (mGlu2 and mGlu3) receptors have been proposed to have important roles in the pathophysiology of schizophrenia. Indeed, a growing body of evidence has shown that mGlu2/3 receptor agonists/potentiators, mGlu5 receptor potentiators, and mGlu1 receptor antagonists exhibit antipsychotic activity in numerous experimental animal models of schizophrenia (4). For example, mGlu2/3-receptor agonists as well as mGlu2-receptor potentiators have been reported to improve behavioral abnormalities induced by pharmacological and environmental manipulations, which represent both positive and negative symptoms of schizophrenia (5 – 7). Likewise, mGlu1-receptor antagonists [4-[1-(2-fluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-
4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide] (FTIDC) and 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-furo[2,3-d]pyridine-4-carboxylic acid, 4-[(2S)-2-amino-4-(methylthio)-1-oxobutyl]amino-, 2,2-dioxide monohydrate (LY2140023) ameliorated both positive and negative symptoms of schizophrenia (10), although the efficacy of LY2140023 is still controversial.

In contrast, the efficacy of mGlu2/3-receptor agonists and mGlu1-receptor antagonists for cognitive dysfunctions has not been fully investigated. An mGlu2/3-receptor agonist has been reported to improve working memory deficit in both rodents (11) and healthy volunteers (12), while its efficacy in relation to other cognitive domains remains to be investigated. Moreover, previous studies did not address whether mGlu1-receptor antagonists were effective in animal models of the cognitive deficits that are observed in schizophrenia.

Social cognition is an aspect of learning and memory that is particularly impaired in schizophrenia (13, 14). Although clozapine but not typical antipsychotics might improve a particular domain of social cognition (15), social cognitive dysfunction is not improved adequately by currently available antipsychotics and is one of the serious unmet medical needs in the treatment of schizophrenia. Therefore, the development of novel drugs that can improve social cognitive dysfunction in schizophrenia is desired. We previously demonstrated that social memory was impaired by a non-competitive NMDA-receptor antagonist, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801), and that impaired social cognition was improved by treatment with the atypical antipsychotic clozapine, but not the typical antipsychotic haloperidol (16). Therefore, MK-801–induced social memory impairment may be useful as an animal model for evaluating the improvement of cognitive impairment, although social memory in rodents is a far simpler construct than in humans.

In the present study, we investigated the effects of both an mGlu2/3-receptor agonist and an mGlu1-receptor antagonist on social memory impairment using a social recognition test in rats. In addition, given that mGlu2 receptor stimulation has been involved in the antipsychotic actions of mGlu2/3 receptor agonists in some animal models, the role of the mGlu2 receptor in the social recognition test was also investigated using a selective mGlu2-receptor potentiator.

Materials and Methods

Animals
Male Sprague-Dawley rats (purchased from Charles River, Yokohama) were used for the social recognition test. The adult rats weighed 280 – 400 g, while the juveniles weighed 60 – 125 g at the time of the test. The rats were housed in groups of four and were maintained under a 12-h light/dark cycle (lights on at 7:00 A.M.) at a constant temperature of 23°C and a relative humidity of 50%. Food and water were available ad libitum. All the studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987).

Social recognition test
The social recognition test was performed as described previously (16). The behavior tests were performed in an open-topped box (28 × 47 × 30 cm, length × width × height) containing sawdust. An adult rat was placed in the test cage and was allowed to habituate for 30 min. Then, an unfamiliar juvenile rat was placed in the test cage with the adult rat for 5 min (the first test session). The length of time during which the adult rats showed exploratory behavior (sniffing, grooming, and close following) toward the juvenile rat during the test session was recorded and was defined as the first investigation duration. The juvenile was then removed from the test cage and returned to its home cage, while the adult was left in the test cage. Thirty minutes later, the same juvenile (familiar) was then placed in the test cage once again for a 5-min test session (the second test session); the length of time spent by the adult in exploring the juvenile during this test session was defined as the second investigation duration. Observers who kept unaware of the treatment group scored the investigation duration (the first and the second test sessions). The social memory for each adult rat was defined by determining the ratio of the second investigation duration to the first investigation duration (RID). MK-801 was administered intraperitoneally to the adult rats 30 min prior to the first exposure to the juvenile. (−)-2-Oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268) and 3′-[[(2-cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1H-inden-5-yl)oxy]methyl]-[1′-biphenyl]-4-carboxylic acid (BINA) were respectively administered subcutaneously and intraperitoneally to the rats at 60 min prior to the first exposure to the juvenile. (2S)-2-amino-2-[(1S,2S)-2-carboxy-
cycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495) was administered intraperitoneally to the rats at 90 min prior to the first exposure to the juvenile. (3,4-Dihydro-2H-pyrano[2,3-b]quinolin-7-yl)-(cis-4-methoxycyclohexyl)methanone (JNJ16259685) was administered intraperitoneally to the rats at 60 min prior to the first exposure to the juvenile.

Drugs
LY379268 was purchased from Abcam (Cambridge, UK). LY341495 and JNJ16259685 were purchased from Tocris Bioscience (Bristol, UK). BINA was synthesized at Taisho Medicinal Research Laboratories. MK-801 was purchased from Sigma-Aldrich (St. Louis, MO, USA). LY379268 was dissolved in saline for subcutaneous administration. JNJ16259685 and BINA were suspended in 0.5% methylcellulose for intraperitoneal administration. LY341495 was dissolved in 66.6 mM phosphate buffer (pH 8.0) for intraperitoneal administration. MK-801 was dissolved in saline for intraperitoneal administration. The volume of administration was 2 mL/kg. The dose selection for all the drugs were based on previous reports (16 – 19) and our preliminary studies; none of the doses caused overt behavioral changes when administered by themselves.

Data analyses
All the statistical analyses were performed using SAS software (SAS Institute Japan, Tokyo). Data from the behavioral experiments were analyzed using the Student’s t-test or a one-way analysis of variance followed by the Dunnett’s post hoc test or Tukey-Kramer post hoc test. A value of $P < 0.05$ was regarded as significant.

Results
Effects of the mGlu2/3-receptor agonist and the mGlu2-receptor potentiator
The intraperitoneal administration of MK-801 (0.1 mg/kg) significantly increased the RID (Figs. 1A, 2A, and 3), indicating that MK-801 impaired short-term social memory, as we previously reported (16). The administration of the mGlu2/3-receptor agonist LY379268 and the mGlu2-receptor potentiator BINA significantly improved the social memory impairment elicited by MK-801 in rats {[$F(2,32) = 5.51, P < 0.01$] and [$F(2,43) = 4.75, P < 0.05$] (Figs. 1A and 2A)}. When an unfamiliar juvenile rat was exposed to an adult rat during the second test session, LY379268 and BINA did not influence the RID to the unfamiliar juvenile rats (Figs. 1B and 2B), suggesting that LY379268 and BINA did not affect social memory.

Fig. 1. Effect of LY379268 in the social recognition test in rats. Effect of LY379268 on MK-801–induced social memory impairment (A) and effect of LY379268 on social interaction to unfamiliar juvenile (B) were investigated. Data represent the mean ± S.E.M., $n = 11 – 12$ animals per each group. *$P < 0.05$, compared with response to vehicle + saline (Student’s $t$-test). **$P < 0.01$, compared with the response to vehicle + MK-801 (Dunnett’s test). No significant difference was observed (Student’s $t$-test) (B).

Fig. 2. Effect of BINA in the social recognition test in rats. Effect of BINA on MK-801–induced social memory impairment (A) and effect of BINA on social interaction to unfamiliar juvenile (B) were investigated. Data represent the mean ± S.E.M., $n = 11 – 16$ animals per each group. *$P < 0.05$, compared with the response to vehicle + saline (Student’s $t$-test). *$P < 0.05$, compared with the response to vehicle + MK-801 (Dunnett’s test). No significant difference was observed (Student’s $t$-test) (B).
investigation itself. In addition, pretreatment with the mGlu2/3-receptor antagonist LY341495 at a dose of 3 mg/kg significantly blocked the effect of BINA on the MK-801–induced impairment of social memory {[F(2,36) = 14.79, P < 0.01] (Fig. 3)}, indicating that the stimulation of the mGlu2 receptor mediates the effect of BINA on social memory.

**Effects of the mGlu1-receptor antagonist**

The intraperitoneal administration of MK-801 (0.1 mg/kg) significantly increased the RID (Fig. 4A), indicating that MK-801 impaired short-term social memory. The mGlu1-receptor antagonist JNJ16259685 significantly improved social memory impairment elicited by MK-801 [F(2,34) = 10.94, P < 0.01] (Fig. 4A). When an unfamiliar juvenile rat was exposed to an adult rat during the second test session, JNJ16259685 did not influence the RID to unfamiliar rats (Fig. 4B), suggesting that JNJ16259685 did not affect social investigation itself.

**Discussion**

We demonstrated, for the first time, that the activation of mGlu2/3 receptors and the blockade of mGlu1 receptors improved social memory impairment in rats treated with an NMDA-receptor antagonist and that the effects of an mGlu2/3-receptor agonist may be mediated via at least the mGlu2 receptor. In the present study, the mGlu2/3-receptor agonist LY379268, mGlu2-receptor potentiator BINA, and mGlu1-receptor antagonist JNJ16259685 improved the social memory impairment elicited by MK-801 in rats, while all the compounds did not affect social interactions with unfamiliar juvenile rats. Therefore, the improvements induced by LY379268, BINA, and JNJ16259685 on social memory impairment are unlikely to reflect nonspecific effects. Although we used a different animal model, the improvement of social memory impairment by the stimulation of mGlu2/3 receptors is consistent with previous findings that rats treated neonatally with phencyclidine (PCP) exhibited deficits in social discrimination and that an mGlu2/3-receptor agonist improved the deficits in social discrimination (20).

The involvement of the mGlu2 receptor in the antipsychotic effects of mGlu2/3-receptor agonists has been suggested since the antipsychotic effects of (−)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039) (21) or LY379268 (5) were no longer observed in mice lacking the mGlu2 receptor but not the mGlu3 receptor and selective mGlu2-receptor potentiators exert an antipsychotic effect (6, 18). The present results that BINA improved the MK-801–induced impairment of social memory and that the improvement induced by BINA on social memory impairment was blocked by pretreatment with the mGlu2/3 receptor agonist LY341495 strongly support the hypothesis that the effects of mGlu2/3-receptor agonists on social memory impairment are mediated via the mGlu2 receptor. The present finding
is consistent with the previous report that an mGlu2 receptor potentiator, [N-(4-(2-methoxyphenoxy)phenyl)-N-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine] (LY487379), reversed social discrimination deficits in rats treated neonatally with PCP (20). Of note, because LY341495 exerts an antagonistic activity at the mGlu8 receptor with a 10 times lower potency than at the mGlu2 receptor (22) and the potency of BINA at the mGlu8 receptor has not been determined (18), a possible role of the mGlu8 receptor in social memory could not be ruled out.

The neural mechanisms underlying the activation of mGlu2 receptors and the blockade of the mGlu1 receptor have not been fully elucidated. The administration of NMDA-receptor antagonists, such as PCP and ketamine, reportedly increase glutamate release in the prefrontal cortex (11, 23), presumably as a result of the disinhibition of γ-aminobutyric acid (GABA) interneurons (24). The injection of an NMDA-receptor antagonist into the medial prefrontal cortex increased glutamate release and impaired attentional performance (25). Thus, the dysfunction of NMDA receptors within the medial prefrontal cortex may increase glutamate release, leading to cognitive impairments. mGlu2/3-receptor agonists have been reported to attenuate NMDA-receptor antagonists–induced increases in glutamate release in the prefrontal cortex (11, 23). The attenuation of NMDA-receptor antagonists–evoked hyperactivity in the prefrontal cortex by mGlu2/3-receptor agonists or mGlu2-receptor potentiators was also supported by studies using 2-deoxyglucose uptake (26) and pharmacological magnetic resonance imaging (27–29). Therefore, the inhibition of the increase in glutamate release through the stimulation of presynaptic mGlu2 receptors might ameliorate the hyperactivity of pyramidal neurons in the prefrontal cortex, and this mechanism might be involved in the action of mGlu2/3-receptor agonists / mGlu2-receptor potentiators on social memory impairment.

Moreover, the mGlu1 receptor in the prefrontal cortex has been postulated to regulate GABA release negatively through retrograde endogenous cannabinoid signaling (30–33), which results in the regulation of pyramidal neuron activity. Because enhanced mGlu1 receptor–mediated signaling, resulting from increased mGlu1 receptor expression and decreased regulator of G-protein signaling 4 expression, has been observed in the prefrontal cortex of schizophrenic patients (34–36), increased glutamate release may occur as a result of the disinhibition of GABA interneurons through the hyperactivation of mGlu1 receptor–mediated signaling. This assumption is supported by the report that the injection of a group I mGlu-receptor agonist into the prefrontal cortex increased glutamate release, which was blocked by the co-applica-

References

5 Woolley ML, Pemberton DJ, Bate S, Corti C, Jones DN. The mGlu2 but not the mGlu3 receptor mediates the actions of the

6 Galici R, Echemendia NG, Rodriguez AL, Conn PJ. A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. J Pharmacol Exp Ther. 2005;315:1181–1187.


8 Satow A, Maehara S, Ise S, Hikichi H, Fukushima M, Suzuki G, et al. Pharmacological effects of the metabotropic glutamate receptor 1 antagonist compared with those of the metabotropic glutamate receptor 5 antagonist and metabotropic glutamate receptor 2/3 agonist in rodents: detailed investigations with a selective allosteric metabotropic glutamate receptor 1 antagonist, FTIDC [4-[1-(2-fluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazole-4-yl]-bispropyl-N-methyl-3,6-dihydroprydidine-1(2H)-carboxamide]. J Pharmacol Exp Ther. 2008;326: 577–586.


15 Savina I, Beninger RJ. Schizophrenic patients treated with clozapine or olanzapine perform better on theory of mind tasks than those treated with risperidone or typical antipsychotic medications. Schizophr Res. 2007;94:128–138.


31 Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. Pre-


