Involvement of serotonergic system in the effect of a metabotropic glutamate 5 receptor antagonist in the novelty-suppressed feeding test

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**Abstract**

The blockade of metabotropic glutamate 5 (mGlu5) receptor has been reported to exert antidepressant effects in several animal models. We previously reported that both ketamine and an mGlu5 receptor antagonist exerted an effect in a novelty-suppressed feeding (NSF) test, and that the effect of ketamine may be mediated through an \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-dependent increase in serotonergic transmission. However, the involvement of the serotonergic system in the effect of mGlu5 receptor antagonists in the NSF test is not well understood. Therefore, we examined the roles of the serotonergic system in the effect of an mGlu5 receptor antagonist, 6-methyl-2-(phenylethynyl)pyridine hydrochloride (MPEP), in the NSF test in mice. The administration of MPEP significantly shortened the latency to feed, which was not attenuated by the AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX). The effect of MPEP was abolished by the tryptophan hydroxylase inhibitor, \( \text{para} \)-chlorophenylalanine (PCPA). Moreover, the effect of MPEP was blocked by a serotonin (5-HT)2A/2C receptor antagonist, ritanserin, but not by a 5-HT1A receptor antagonist, \( \text{N} \)-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-\( \text{N} \)-(2-pyridynyl) cyclohexane-carboxamide (WAY100635). These results suggest that the effect of an mGlu5 receptor antagonist may be mediated by the serotonergic system, including the stimulation of the 5-HT2A/2C receptor, in an AMPA receptor-independent manner in the NSF test.

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1. Introduction

Several lines of evidence have shown that modulation of the glutamatergic system may be an effective treatment for depressive symptoms, a hypothesis that has been supported by clinical observations using ketamine, a non-competitive \( N \)-methyl-\( l \)-aspartate (NMDA) receptor antagonist. Indeed, ketamine has been reported to exert rapid and sustained antidepressant effects in patients with major depressive disorder, even in patients with treatment-resistant depression (1–4), after a single injection as well as after repeated injections (1,2,5). In a search of alternatives for ketamine, which avoid undesirable side effects observed in ketamine therapy, investigations on neural mechanisms underlying the antidepressant effects of ketamine have been actively conducted. To date, ketamine has been proposed to exert antidepressant effects through the stimulation of brain-derived neurotrophic factor (BDNF)-mammalian target of rapamycin signaling and the blockade of eukaryotic elongation factor 2 kinase, both of which are mediated through the activation of the \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (6–8). In addition to these mechanisms, which may lead to an increase in synaptic protein synthesis and spine density (for a review, see Ref. (6)), the involvement of the serotonergic system in the actions of ketamine has been suggested. For example, a positron emission tomography study has revealed that treatment with high dose of ketamine increased serotonin (5-HT)1B receptor binding in the nucleus accumbens and the ventral pallidum in rhesus monkeys (9), and ketamine increased extracellular 5-HT levels in the prefrontal cortex in rats (10), with both mechanisms being mediated through AMPA receptor stimulation. Moreover, we recently reported that the depletion of 5-HT abolished the effect of ketamine in the novelty-suppressed feeding (NSF) test and that the stimulation of the

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postsynaptic 5-HT_{1A} receptor by 5-HT released via AMPA receptor stimulation may be involved in the effects of ketamine in this model (11). Thus, these findings indicate that the AMPA receptor-mediated activation of serotonergic systems may be involved in the antidepressant effect of ketamine.

Among the glutamate receptors, the metabotropic glutamate 5 (mGlu5) receptor has been reported to have roles in depression. Indeed, mGlu5 receptor levels are reportedly decreased in certain brain regions of depressed patients and rodent models of depression (12–14). In addition, mGlu5 receptor antagonists, such as 2-methyl-6-(phenylethynyl)-pyridine (MPEP), 3-[4-(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP), and [4-(difluoromethoxy)-3-(pyridine-2-ylethynyl)phenyl]5H-pyrrrolo[3,4-b]pyridine-6(7H)-yl methanone (GRN-529), reportedly exhibited antidepressant effects in several animal models of depression (15–18), raising the possibility that mGlu5 receptor blockade may be a useful approach for treating depression. The neural mechanisms underlying the antidepressant effects of mGlu5 receptor antagonists have not been fully elucidated, although interactions with NMDA receptor and BDNF signaling have been suggested (for a review, see Ref. (19)). Recently, the involvement of serotonergic systems in the antidepressant and anxiolytic effects of mGlu5 receptor antagonists has been reported. The antidepressant effect of MTEP was blocked by pretreatment with a tryptophan hydroxylase inhibitor, paroxetin, suggesting that activation of serotonergic systems may be involved in the antidepressant and anxiolytic effects of MTEP in mice (20). Additionally, MTEP increased the extracellular 5-HT levels in the prefrontal cortex in rats (21). Thus, the antidepressant effect of mGlu5 receptor antagonists may mediate an increase in serotonergic systems, as observed for ketamine.

We recently reported that an mGlu5 receptor antagonist exhibited both acute and sustained effects in the NSF test (22), a model which measures latency to feed in an aversive environment and is sensitive to chronic but not acute treatment with antidepressants, and acute and sustained effects were also observed with ketamine (23). Using this model, we investigated the roles of the serotonergic system in the action of ketamine, as described above. Therefore, the NSF test is likely to be a useful model for comparing the neural mechanisms of an mGlu5 receptor antagonist, particularly the roles of the serotonergic system, with those of ketamine. However, the involvement of the serotonergic system in the action of an mGlu5 receptor antagonist in the NSF test has not been investigated.

In the present study, we first investigated the involvement of the serotonergic system in the effect of an mGlu5 receptor antagonist, MPEP, in the NSF test by depleting 5-HT with PCPA. Then, we investigated the roles of 5-HT receptor subtypes using the respective antagonists. Moreover, we investigated the involvement of AMPA receptor stimulation in the action of an mGlu5 receptor antagonist, since AMPA receptor stimulation reportedly mediates the enhancement of the serotonergic system by ketamine.

2. Materials and methods

2.1. Animals and housing

Nine-week-old male C57BL/6J mice (Charles River Laboratories, Yokohama) were used for all the experiments. The animals were maintained under a controlled temperature (23 ± 3 °C) and humidity (50 ± 20%) with a 12-h light/dark cycle (lights on at 7:00 a.m.). Food and water were provided ad libitum, except for the deprivation of food for 24 h prior to the NSF test. All the studies were performed according to 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).

2.2. Drug administration

MPEP (Sigma–Aldrich Co., St. Louis, MO, USA) was dissolved in 0.5% methylcellulose (0.5% MC). 2,3-Dixo-6-nitro-1,2,3,4-tetrahydrobenzof[7]quinolxalone-7-Sulfonamide (NBQX) (Tocris Cookson Ltd., Bristol, UK) was suspended in saline. PCPA (Wako Pure Chemical Industries, Ltd, Osaka) and ritanserin (Sigma–Aldrich Co., St. Louis, MO, USA) were suspended in 0.5% MC. N-[2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-N-(2-pyridyl)cyclohexane-carboxamide (WAY100635) (Sigma–Aldrich Co., St. Louis, MO, USA) was dissolved in saline. MPEP (3 mg/kg) was administered intraperitoneally (i.p.) 60 min prior to the test. NBQX (1, 3, and 10 mg/kg) and WAY100635 (0.3, 1, and 3 mg/kg) were administered subcutaneously (s.c.) at 65 min and 90 min prior to the test, respectively. Ritanserin (0.125, 0.25, and 0.5 mg/kg) was administered i.p. 90 min prior to the test. PCPA (300 mg/kg) was administered i.p. twice daily (at 7:00–11:00 and 16:00–19:00) for 3 consecutive days, and the tests were conducted 18 h after the final administration. All the drugs were injected at a volume of 10 mL/kg body weight. The doses for the systemic administration of MPEP, NBQX, PCPA, WAY100635, and ritanserin were selected based on previous studies (11,22).

2.3. Novelty-suppressed feeding test in mice

The NSF test was performed during a 5-min period, as described previously (11). Of note, we previously reported that fluvoxamine exerted an effect following treatment for 28 days in the NSF test, while MPEP exerted an effect after single treatment under the same condition (22). The mice were weighed, and all food was removed from their cages. Water continued to be provided ad libitum. Approximately 24 h after the removal of the food, the mice were transferred to the testing room, placed in a clean holding cage, and allowed to habituate for 30 min. The testing apparatus consisted of a Plexiglas box (45 × 45 × 20 cm) in an illuminated (approximately 1000 lux), soundproofed box. The floor of the box was covered with 1 cm of wooden bedding. A small piece of mouse chow was placed in the center of the box on a white circular filter paper (11 cm in diameter). Each subject was placed in the corner of the testing arena, and the time until the first feeding episode was recorded. Immediately after the mouse began to eat the chow, the tested animal was placed alone in its home cage with a weighed piece of chow for 5 min. At the end of this period, the amount of food consumed was determined by weighing the piece of chow. After all the mice from a single cage had been tested, the mice were returned to their home cage with food and water provided ad libitum. NBQX, PCPA, WAY100635, and ritanserin did not affect the latency to feed in the NSF test at the doses used in the present study (11). None of the treatments affected the amount of food consumed at doses used in the test (data not shown).

2.4. Statistical analysis

The results were expressed as the mean ± S.E.M. Statistical significance was determined using a one-way analysis of variance (ANOVA) or a two-way ANOVA, followed by the Student’s t-test and the Dunnett’s test or the LSD post-hoc test for comparing the treated group with a control group and multi-group comparisons, respectively. Statistical differences between the two sets of groups were determined using the Student’s t-test. A value of P <0.05 was considered statistically significant.
3. Results

3.1. Effect of 5-HT depletion on the action of MPEP in the NSF test

MPEP significantly reduced the latency period until feeding in the NSF test \(F(3,40) = 4.46, P < 0.01\) (Fig. 1). The decrease in the latency to feed induced by MPEP (3 mg/kg i.p.) was blocked by pretreatment with PCPA (300 mg/kg i.p., twice daily for 3 days) \(F(1,40) = 5.46, P < 0.05\); PCPA, \(F(1,40) = 3.07, P = 0.09\); interaction, \(F(1,40) = 4.87, P < 0.05\) (Fig. 1). Pretreatment with PCPA itself did not affect the latency to feed (Fig. 1).

3.2. Effect of a 5-HT1A receptor antagonist on the action of MPEP in the NSF test

MPEP significantly reduced the latency period until feeding in the NSF test \(F(1,22) = 8.25, P < 0.01\) (Fig. 2). The decrease in the latency to feed induced by MPEP (3 mg/kg i.p.) was not blocked by pretreatment with a 5-HT1A receptor antagonist, WAY100635 (0.3, 1, and 3 mg/kg s.c.) \(F(3,43) = 0.06, P = 0.98\) (Fig. 2).

3.3. Effect of a 5-HT2A/2C receptor antagonist on the action of MPEP in the NSF test

MPEP significantly reduced the latency period until feeding in the NSF test \(F(1,22) = 12.36, P < 0.01\) (Fig. 3). The decrease in the latency to feed induced by MPEP (3 mg/kg i.p.) was blocked by pretreatment with a 5-HT2A/2C receptor antagonist, ritanserin (0.5 mg/kg i.p.) \(F(3,44) = 3.86, P < 0.05\) (Fig. 3).

3.4. Effect of an AMPA receptor antagonist on the action of MPEP in the NSF test

MPEP significantly reduced the latency period until feeding in the NSF test \(F(1,21) = 14.54, P < 0.01\) (Fig. 4). The decrease in the latency to feed induced by MPEP (3 mg/kg i.p.) was not blocked by pretreatment with an AMPA receptor antagonist, NBQX (1, 3 and 10 mg/kg s.c.) \(F(3,44) = 0.59, P = 0.63\) (Fig. 4).

4. Discussion

In the present study, we demonstrated that, similar to ketamine, an mGlu5 receptor antagonist exerted its effect through the serotonergic system in the NSF test, although the mechanisms of the involvement of the serotonergic system were different. The main purpose of the present study was to investigate the involvement of the serotonergic system in the effect of MPEP in the NSF test. In the present study, the effect of MPEP was blocked by...
pretreatment with a tryptophan hydroxylase inhibitor, PCPA, suggesting that serotonergic transmission plays a role in the effect of the mGlu5 receptor antagonist in the NSF test. It should be noted that this is the first report to demonstrate the involvement of serotonergic transmission in the effect of an mGlu5 receptor antagonist in the NSF test. Previously, we demonstrated that treatment with PCPA (300 mg/kg twice daily for 3 days) caused a 74.8% reduction in the 5-HT content in the frontal cortex in mice, compared with a vehicle-treated group, and abolished the head-twitch response induced by a 5-HT release-promoting agent, PCA (11). Therefore, the treatment condition with PCPA used in this study is sufficient for the pharmacological depletion of 5-HT in mouse brain. This finding is consistent with previous reports that the antidepressant-like effect of MTEP was attenuated by PCPA treatment in the TST (20), indicating that serotonergic transmission may play a key role in the actions of mGlu5 receptor antagonists across animal models.

Next, we investigated the involvement of the 5-HT receptor subtype in the effect of MPEP in the NSF test. 5-HT1A and 5-HT2A/2C receptors were investigated in the present study because these receptors play important roles in the antidepressant and anxiolytic-like effects of agents (24,25). We found that the effect of MPEP was blocked by a 5-HT2A/2C receptor antagonist, ritanserin, but not by a 5-HT1A receptor antagonist, WAY100635, in the NSF test. These results suggest that the stimulation of the 5-HT2A/2C receptor, but not the 5-HT1A receptor, mediates the effect of MPEP in the NSF test. These findings are consistent with previous reports that the antidepressant and anxiolytic effects of MTEP were attenuated by ritanserin but not WAY100635 in the TST and Vogel conflict drinking test (20,21). Given that both MPEP and MTEP do not have activities at 5-HT receptors and mGlu5 receptor antagonists have been reported to increase 5-HT release in the prefrontal cortex and hippocampus (21,26,27), the blockade of mGlu5 receptors may indirectly stimulate the 5-HT2A/2C receptor through an increase in 5-HT release, leading to the antidepressant/anxiolytic effects in animal models, including the NSF test.

Although the effects of both an mGlu5 receptor antagonist and ketamine in the NSF test are mediated through serotonergic transmission, the mechanism of the mGlu5 receptor antagonist differs from that of ketamine, since we previously reported that the 5-HT1A receptor, but not the 5-HT2A/2C receptor, is involved in the effect of ketamine (11). Ketamine reportedly increases 5-HT release via the stimulation of the AMPA receptor (10) in the prefrontal cortex, which may lead to the stimulation of the postsynaptic 5-HT1A receptor and its subsequent effects. Interestingly, a clear distinction existed between the mGlu5 receptor antagonist and ketamine with regard to the role of the effects of the AMPA receptor in the NSF test. While the effect of MPEP in the NSF was not attenuated by NBQX in the present study, we reported that the effect of ketamine was blocked by NBQX in the same paradigm. Therefore, the mGlu5 receptor antagonist may increase 5-HT release via a different neural mechanism from that of ketamine, i.e., an AMPA receptor-independent mechanism, which may explain the involvement of distinct 5-HT receptor subtypes in the effects in the NSF test.

The neural mechanism of 5-HT release and the activation of the 5-HT2A/2C receptor induced by an mGlu5 receptor antagonist in the NSF test remain to be elucidated. Treatment with MTEP reportedly increases 5-HT release without elevating 5-HT1A in the prefrontal cortex in rats, indicating that the blockade of the mGlu5 receptor may inhibit the 5-HT transporter to increase 5-HT release (21). However, Heidbreder et al. (2003) reported that MPEP had a moderate affinity for the norepinephrine (NE) transporter, but not for the 5-HT transporter, as evaluated using radioligand binding assays (26). Moreover, 5-HT transporter inhibitors reportedly do not exert an effect after acute treatment in the NSF test (28), which is in accord with our previous finding (22). Therefore, it is unlikely that an mGlu5 receptor antagonist increases 5-HT release by inhibiting the 5-HT transporter. Of note, a previous study showed that gene deletion of the mGlu5 receptor in mice increased the behavioral response to a 5-HT2A receptor agonist, suggesting that blockade of the mGlu5 receptor may enhance the sensitivity to the 5-HT2A receptor (29). Moreover, 5-HT2 receptors are positioned on GABAergic neurons (30), and the stimulation of 5-HT2 receptors increases GABA release in the prefrontal cortex (31). Given that the GABAergic system is known to be disrupted in depressed patients (for a review, see Ref. (32)), it is intriguing to speculate that regulation of the GABAergic system via the 5-HT2 receptor may be involved in the antidepressive effect of mGlu5 receptor antagonists.

The present study has a notable limitation. The specificity of the mGlu5 receptor antagonist, MPEP, was not optimal, as it also inhibits the NMDA receptor and NE transporter (26,33) as well as acting as a positive allosteric modulator of the mGlu4 receptor (34). However, MPEP acts on the above-mentioned receptors and transporter at a concentration more than 1000 times higher than that blocks the mGlu5 receptor (an IC50 value of 36 nM) (35), and MPEP did not exhibit an antidepressant-like effect in mGlu5 receptor-knockout mice in the forced swimming test (36). Thus, the effect of MPEP at a dose 3 mg/kg can most likely be attributed to the blockade of the mGlu5 receptor.

In conclusion, we have provided the first evidence that the effect of an mGlu5 receptor antagonist may be mediated through serotonergic transmission, presumably an increase in 5-HT release and the subsequent stimulation of 5-HT2A/2C receptor in the NSF test, and that the neural mechanism differs from that of ketamine. Further investigation of the neural mechanisms of mGlu5 receptor antagonists and comparisons of the mechanisms with those of ketamine may warrant the clinical efficacy of mGlu5 receptor antagonists for the treatment of depression and anxiety disorders.

Conflicts of interest

All authors declare no conflict of interest.

References


