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European Journal of Pharmacology 501 (2004) 121–125

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Short communication

Anxiolytic-like activity of MGS0039, a potent group II metabotropic glutamate receptor antagonist, in a marble-burying behavior test

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Received 2 August 2004; accepted 9 August 2004
Available online 12 September 2004

Abstract

Glutamatergic abnormalities are involved in several psychiatric disorders. Clinical evidence demonstrates altered glutamatergic neurotransmission in patients suffering from obsessive-compulsive disorder. MGS0039, (1*R*,2*R*,3*R*,5*R*,6*R*)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, is a novel group II metabotropic glutamate (mGlu) receptor antagonist. We examined MGS0039's potential anti-obsessive-compulsive disorder activity, using the marble-burying behavior test as a model of obsessive-compulsive disorder. MGS0039 as well as LY341495 ((2*S*,1′*S*,2′*S*)-2-(9-xanthylmethyl)-2-(2′-carboxycycloprolyl)glycine), another group II mGlu receptor antagonist, inhibited marble-burying behavior. We also demonstrated that this effect was significantly attenuated by a group II mGlu receptor agonist. This data indicates that group II mGlu receptor antagonists may exert anti-obsessive-compulsive disorder effects in clinical use.

Keywords: MGS0039; Glutamate; Obsessive-compulsive disorder; Marble-burying behavior test

1. Introduction

It is now well recognized that glutamate is the principal excitatory neurotransmitter in the central nervous system. Animal models and human clinical data clearly show that glutamatergic neurotransmission is potentially involved in a very wide range of central nervous system disorders, including epilepsy, neurodegenerative diseases, stroke, traumatic brain injury, pain, psychiatric diseases (Danysz et al., 1995; Meldrum, 2000). At present, abnormalities of glutamatergic neurotransmission have been observed in several psychiatric disorders such as schizophrenia, attention deficit hyperactivity disorder, bipolar disorder, major depression and obsessive-compulsive disorder (Clinton et al., 2003; Carlsson, 2001; McCullumsmith and Meador-Woodruff, 2002; Rosenberg et al., 2000).

Obsessive-compulsive disorder is characterized by repeated, persistent and dysphoric thoughts (obsessions), which are ego-dystonic, repetitive, seemingly purposeful behaviors (compulsions) (Rasmussen and Eisen, 1992). Although obsessive-compulsive disorder is grouped as an anxiety disorder, benzodiazepines, clinically effective anxiolytic drugs, are generally ineffective in relieving obsessive-compulsive disorder symptoms. To date, the most efficacious treatments for obsessive-compulsive disorder are anti-depressants, including clomipramine and selective serotonin reuptake inhibitors (SSRIs) (Boyer et al., 1991).

Some clinical evidence indicates that glutamatergic abnormalities are associated with obsessive-compulsive disorder symptoms. Brain imaging studies of obsessive-compulsive disorder patients have demonstrated a reversible glutamatergically mediated thalamo-cortical-striatal dysfunction (Rosenberg et al., 2001). Moreover, using proton magnetic resonance spectroscopy, it is clear that abnormal glutamatergic neurotransmissions were observed in the

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0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.08.016

caudate of obsessive-compulsive disorder patients (Rosenberg et al., 2000). Therefore, regulating glutamatergic neurotransmission may result in effective therapy for obsessive-compulsive disorder patients.

Glutamate mediates its effects via two types of receptors: ionotropic receptors and metabotropic receptors (Monaghan et al., 1989; Conn and Pin, 1997). At present, eight subtypes of mGlu receptors have been cloned (Conn and Pin, 1997). Based on the expression pattern of distinct mGlu receptors in the central nerve system, agonists or antagonists of mGlu receptors are believed to offer the potential for new therapeutic agents for a number of neurological and psychiatric disorders (Knopfel et al., 1995; Conn and Pin, 1997; Nicoletti et al., 1997). MGlu receptors are classified into three subgroups (groups I–III). Group II mGlu receptors are mainly localized in presynapses and serve an important role in regulating glutamatergic neurotransmission (Cartmell and Schoepp, 2000).

MGS0039, (1*R*,2*R*,3*R*,5*R*,6*R*)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, is a novel and potent antagonist of group II mGlu receptor and exhibits antidepressant-like activity (Chaki et al., 2004). In the present study, we examined whether glutamatergic change induced by MGS0039 resulted in anti-obsessive-compulsive disorder activity in the marble-burying behavior test. The marble-burying behavior test has been suggested as a useful model for evaluating anti-obsessive-compulsive disorder drugs, because no change in the intensity of the marble-burying behavior occurred during repeated testing (this is considered as compulsive behavior) and because SSRIs, which have been found effective in treating symptoms of clinical obsessive-compulsive disorder, antagonize this burying behavior (Njung'e and Handley, 1991a,b).

2. Materials and methods

Male ICR mice (25–35 g, Charles River, Yokohama, Japan) were used. Mice were housed in groups of 10 until the experiments begun. Living cages for group housing were measured 24×35×15 cm. Mice were maintained under a 12 h light/dark cycle (lights on 7:00 AM). Temperature was held constant at 23 °C with a relative humidity of 50%. Food and water were made available ad libitum.

MGS0039, LY341495 ((2*S*,1′*S*,2′*S*)-2-(9-xanthylmethyl)-2-(2′-carboxycycloprolyl)glycine), LY354740 ((1*S*,2*S*,5*R*,6*S*)-2-amino-bicyclo[3.1.0]hexane-2,6-di carboxylic acid) and fluvoxamine were synthesized at Taisho Medical Research Laboratories. For the behavioral study, MGS0039 and LY341495 were dissolved in a 1/15 M phosphate buffer (pH 8.0). LY354740 and Fluvoxamine were dissolved in a 0.3% Tween80/saline solution.

Marble-burying behavior was determined according to the method previously reported (Chaki et al., 2003). Mice were individually placed in transparent, polycarbonate cages (22×32×13.5 cm) containing a 5-cm layer of sawdust and 24 glass marbles (1.5 cm in diameter) were evenly distributed on the sawdust in the cages. Thirty minutes later, the animals were removed from the cages and the number of marbles at least two-thirds buried in the sawdust was recorded. MGS0039 or LY341495 was administered intraperitoneally 1 h prior to the test. LY354740 and fluvoxamine was dosed 30 min prior to the test intraperitoneally and subcutaneously, respectively.

Spontaneous locomotor activity was determined as reported (Chaki et al., 2003). Mice were housed individually in transparent acrylic cages (30 cm diameter, 30 cm height), and spontaneous locomotor activity was recorded for 30 min, using a SCANET apparatus (Neuroscience, Tokyo, Japan) placed in a sound-proof box. Locomotor activity was defined as the number of photobeam breaks recorded during a 30-min test period. MGS0039 was administered intraperitoneally 1 h before the start of measurement.

All statistical analysis was carried out using the PC application SAS (SAS institute Japan, Tokyo, Japan). Data from in vivo experiments were analyzed by one-way analysis of variance (ANOVA). Significant differences between groups were determined, using Dunnett's test.

All studies were reviewed by the Taisho Pharmaceutical Animal Care Committee and met Japanese Experimental Animal Research Association standards, as defined in the *Guidelines for Animal Experiments* (1987).

3. Results

Fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), significantly blocked marble-burying activity at doses of 10 and 30 mg/kg [$F(3,36)=8.40$, $P<0.01$] (Fig. 1). Mice treated with MGS0039, a potent and selective antagonist of group II mGlu receptor, reduced marble-burying in a significant and dose-dependent manner at doses of 3 and 10 mg/kg [$F(3,36)=5.32$, $P<0.01$] (Fig. 1). No significant change was observed in spontaneous locomotor activity in MGS0039 treated mice (counts/30 min vehicle, 10191±1069; 1 mg/kg, 9082±889; 3 mg/kg, 9528±1169; 10 mg/kg, 8179±442). LY341495, another potent antagonist of group II mGlu receptor, also reduced marble-burying behavior at doses of 1, 3, and 10 mg/kg [$F(3,36)=7.94$, $P<0.01$] (Fig. 1). This reduction of marble-burying in MGS0039 treated mice was significantly attenuated by administration of mGlu receptor agonist, LY354740 [$F(1,18)=6.18$, $P<0.05$ and $F(3,36)=4.82$, $P<0.01$] (Fig. 2). Moreover, LY354740 itself had no

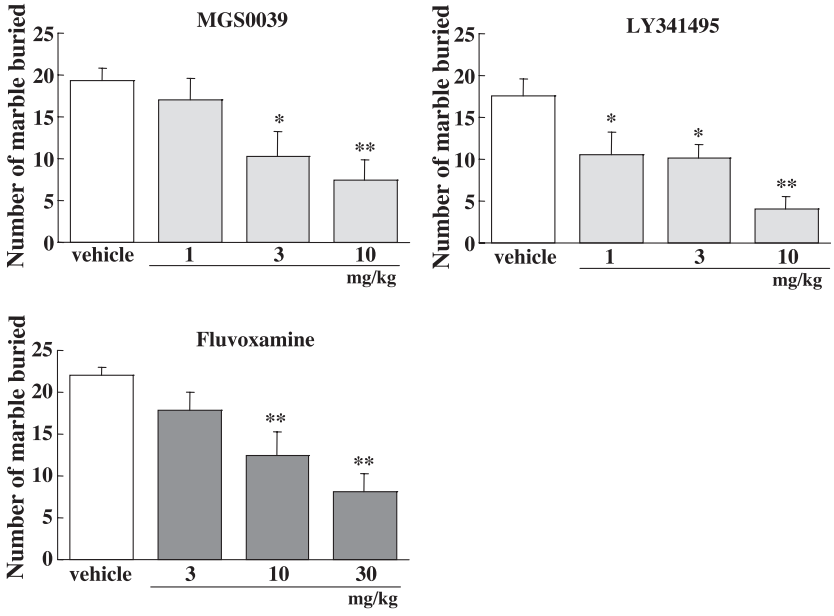

Fig. 1. Effects of fluvoxamine, MGS0039 and LY341495 on marble-burying behavior test in mice. Data represent mean±SEM (n=10). *P<0.05, **P<0.01 versus vehicle-treated group (Dunnett's test).

effect on marble-burying behavior [$F(3,36)=0.18$, $P=0.907$] (Fig. 2).

4. Discussion

In the previous study, we demonstrated that MGS0039 showed high affinity and selectivity for group II mGlu receptor and no affinity for serotonergic and noradrenergic receptors and transporters even at 10 μM (Chaki et al., 2004).

In the present study, we obtained the results that MGS0039 as well as fluvoxamine showed anxiolytic-like effect in the marble-burying behavioral test, which is considered a model for obsessive-compulsive disorder (Njung'e and Handley, 1991a), without affecting spontaneous locomotor activity. LY341495, another potent antagonist of group II mGlu receptor, significantly reduced marble-burying behavior, as did MGS0039.

It has been reported that group II mGlu receptor agonists have anxiolytic-like activity (Grillon et al., 2003; Collado et al., 2002; Helton et al., 1998). In this study, we showed that a group II mGlu receptor agonist itself has no effect on marble-burying behavior. Moreover, a group II mGlu receptor agonist, LY354740 attenuated this reduction of marble-burying behavior. Thus, the anxiolytic-like effect in marble-burying behavior may be attributable to the blockade of group II mGlu receptor.

Obsessive-compulsive disorder is a neuropsychiatric disorder characterized by recurrent thoughts and/or repetitive behavior that causes marked distress and interferes with normal functioning (Rasmussen and Eisen, 1992). Although obsessive-compulsive disorder is classified as an anxiety disorder, widely prescribed anxiolytic agents such as benzodiazepines are generally ineffective in relieving obsessive-compulsive disorder symptoms. Clinical reports indicate that SSRIs and clomipramine

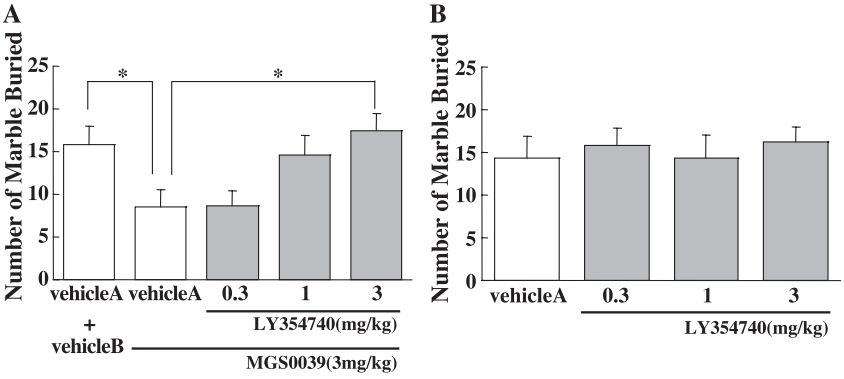

Fig. 2. Effects of co-administration of MGS0039 (3 mg/kg) and LY354740 on marble-burying behavior test in mice (A). Effect of LY354740 on marble-burying behavior test in mice (B). Data represent mean±SEM (n=10). vehicle A: 0.3% tween 80 saline, vehicle B: 1/15M PBS (pH=8), *P<0.05 versus vehicle A or vehicle B-treated group (Dunnett's test).

are effective in reducing obsessive-compulsive disorder symptoms (Gorman and Kent, 1999). The marble-burying behavior test has been suggested as a useful model for evaluating anti-obsessive-compulsive disorder drugs because no change in the intensity of the marble-burying behavior occurred during repeated testing (this is considered as compulsive behavior) and because antidepressants such as tricyclics and SSRIs suppress marble-burying behavior (Millan et al., 2001; Njung'e and Handley, 1991b). In our previous report on MGS0039, we performed several kinds of animal behavioral tests and showed that the effective profile of MGS0039 in animal behavioral tests resembled that of SSRIs (Chaki et al., 2004). For example, in a forced swimming test of rats, MGS0039 increased swimming behavior without affecting climbing behavior, as did fluvoxamine (Chaki et al., 2004). These results appear to indicate that the activity of group II mGlu receptor antagonists mimics that of SSRIs. The interaction of the blockade of group II mGlu receptor with serotonergic transmission remains unknown and needs to be clarified in future studies.

Caudate glutamatergic dysfunctions have been observed in obsessive-compulsive disorder patients in clinical studies. After 12 weeks of treatment with paroxetin, an SSRI, these glutamatergic changes were normalized and were observed to be associated with reduced obsessive-compulsive disorder symptoms (Rosenberg et al., 2000). Such data indicates that obsessive-compulsive disorder symptoms may be improved by the alteration of glutamatergic neurotransmission. It was reported that group II mGlu receptor is localized mainly on presynaptic endings to regulate glutamate release (Cartmell and Schoepp, 2000). Based on the anxiolytic-like effects of a group II mGlu receptor antagonist in marble-burying behavior, it appears that blockade of the presynaptic inhibition of glutamate release by group II mGlu receptor antagonists may represent an effective treatment method for relieving obsessive-compulsive disorder symptoms.

In conclusion, MGS0039 significantly inhibits marble-burying behavior, which is a model for evaluating clinical potential in obsessive-compulsive disorder treatment. Although the underlying anxiolytic-like mechanisms of group II mGlu receptor antagonists remain unknown, this result suggests that MGS0039 may exhibit anti-obsessive-compulsive disorder effect, in addition to its antidepressive effects.

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