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Anxiolytic-like activity of MGS0039, a selective group II mGlu receptor antagonist, is serotonin- and GABA-dependent

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Abstract:
In the present study, we examined the anxiolytic-like effects of (1R,2R,3R,5R,6R)-2-amino-3-(3,4-dichlorobenzyloxy)-6fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (MGS0039), a mGluR2/3 antagonist, in the Vogel conflict drinking test in rats. MGS0039 administered at the doses of 1 and 2 mg/kg *ip* (yet not at 3 mg/kg) produced anxiolytic-like effects in this test. Diazepam (2.5–10 mg/kg) was used as a reference drug. In the second part of our experiment, MGS0039 was tested at an effective dose of 2 mg/kg after a mixed injection with ritanserin (5-$HT_{2A/C}$ receptor antagonist) and WAY100635 (5-HT_{1A} receptor antagonist) or flumazenil (benzodiazepine receptor antagonist), and all of the compounds were found to attenuate the effect of MGS0039. The above results indicate that the mGluR2/3 antagonist MGS0039 may play a role in the therapy of anxiety and that its action may be mediated by serotonin and the GABAergic systems.

Key words:
MGS0039, anxiety, conflict drinking Vogel test, rats

Abbreviations: 5-HT – serotonin, GABA – γ-aminobutyric acid, mGluRs – metabotropic glutamate receptors, MGS0039 – (1R,2R,3R,5R,6R)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, WAY100635 – *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)-cyclohexane carboxamide trihydrochloride

Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Substantial data exist to indicate that glutamatergic neurotransmission is involved in a wide range of CNS disorders, including anxiety [for review see: 31, 39]. Metabotropic glutamate receptors are classified into three groups based on their amino acid sequence homology and intracellular coupling [33]. Group I mGlu receptors (mGluR1 and mGluR5) activate the phosphatidylinositol hydrolysis/Ca^{2+} signal transduction pathway, while group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7 and mGlu8) receptors inhibit adenylyl cyclase [6]. Group III mGlu receptors are located in the brain regions related to mood disorders [21].

The recent data on mGlu receptor ligands, which modulate the function of glutamatergic neurotransmission, show that substances that lead to a decrease in this transmission are potential anxiolytic and/or antidepressant drugs. Using a broad variety of tests, anxiolytic-like effects were observed after the administration of agonists of group II mGlu receptors [13, 22], and the selective group II receptor agonist compound LY354740 has progressed into phase III clinical trials for anxiety [9].

In light of the data supporting the pronounced anxiolytic effects of group II mGlu receptor agonists, it is puzzling that some experiments demonstrated that antagonists of group II mGlu receptors are also active in several models of anxiety. The mGlu2/3 antagonists (1R,2R,3R,5R,6R)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (MGS0039) and S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid (LY341495) reduced marble burying behavior in mice, suggesting the potential activity of group II mGlu receptor antagonists in the reduction of symptoms of obsessive-compulsive disorder (OCD) [37]. The same research group reported that MGS0039 reduced freezing behavior in the conditioned fear stress (CFS) model [42]. It was also reported that the group II mGlu receptor antagonists MGS0039 and LY341495, given *ip*, reduced stress-induced hyperthermia (SIH) in mice [17].

In the present study, we examined the anxiolytic-like effects of MGS0039 in the Vogel conflict drinking test (VCT), which is a widely employed model and is considered to be one of the most specific methods for the detection of potential anxiolytic activity [34]. MGS0039 produced anxiolytic-like effects in the VCT, and these effects were inhibited by the 5-HT_1 receptor antagonist WAY100635, the 5-$HT_{2A/C}$ receptor antagonist ritanserin and the benzodiazepine receptor antagonist flumazenil. The above results suggest the involvement of the serotoninergic and GABAergic systems in the anxiolytic-like effects mediated by the group II mGlu receptor antagonists.

Materials and Methods

Animals and housing

The experiments were carried out on male Wistar rats weighing 250–300 g. The animals were grouped in cages (60 × 38 × 20 cm) and were exposed to a natural day-night cycle at a room temperature of 19–21°C, with free access to food and tap water prior to the experiment. All experiments were performed in the light phase of the natural light-dark cycle (from May till December) between 9 a.m. and 2 p.m. All of the experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Conflict drinking test (Vogel test)

A modification of the method of Vogel et al. [40] was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. After the adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for 10 min with free access to the drinking bottle. Afterwards, they were allowed a 30 min free-drinking session in their home cage. After another 24 h of water deprivation, the rats were again placed in the test chamber and were allowed to drink for 30 s. Immediately afterwards, any drinking attempts were punished with an electric shock (0.5 mA). The impulses were delivered every 2 s (timed from the moment when the preceding shock was delivered) between the grid floor and the spout of the drinking bottle. Each shock lasted 1 s, and if the rat was drinking when an impulse was released, it received a shock. The number of shocks received throughout a 5 min experimental session was recorded by an experimenter who observed the behavioral reaction (e.g., body jerks) of the rats to the electric shock.

Shock threshold and free-drinking tests

To check for the possibility of drug-induced changes in the perception of the stimulus or in the thirst drive, which might have contributed to activity in the conflict-drinking test, the stimulus threshold was measured, and a free drinking experiment was carried out. In both cases, the rats were treated in a manner similar to that described in the conflict-drinking test, including the two 24 h water deprivation periods, separated by 30 min of water availability. In the shock threshold test, the rats were placed individually in the box, and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise by manually increasing the current (0.1, 0.2, 0.3, 0.4, 0.5 mA) delivered through the grid floor until

a rat showed an avoidance reaction (e.g., jumps, jerks, recorded by an observer oblivious to the treatment) to the electric stimulus. There was a 15 s shock-free interval between the steps.

In the free-drinking test, each animal was allowed to drink from the water spout. Licking was not punished. The total amount of water (ml) consumed during 5 min was recorded for each rat. In both of these tests and the Vogel test, the animals were used only once.

Drug treatment

(1R,2R,3R,5R,6R)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (MGS0039, Taisho Pharmaceutical Co. Ltd. Saitama, Japan) and diazepam (Polfa, Poznań, Poland) were suspended in a 1% aqueous solution of Tween 80. Both substances were administered intraperitoneally (*ip*) 60 min before the test. Flumazenil (Hoffman-La Roche, Basel) and ritanserin (Research Biochemicals Inc., Natick MA) were suspended in a 1% aqueous solution of Tween 80 and administered intraperitoneally (*ip*) 60 and 30 min, respectively, before the tests. *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)-cyclohexane carboxamide trihydrochloride (WAY100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) was used as an aqueous solution and administered (*sc*) 45 min before the test. Control rats received the vehicle according to the same schedule.

Statistical analysis

The obtained data are presented as the mean ± SEM and were evaluated by a one-way analysis of variance followed by Dunnett's test, with $p < 0.05$ considered significant, using the GraphPad Prism 4.0 program.

Results

MGS0039, an mGluR2/3 receptor antagonist, administered at doses of 1 and 2 mg/kg exerted an anxiolytic-like effect, significantly ($p < 0.05$) increasing the number of shocks accepted during the experimental session in the conflict drinking test (Fig. 1), ($F(3,28) = 3,352$; $p < 0.05$).

When tested in the shock threshold and free-drinking tests at the effective dose from the conflict drinking test, MGS0039 did not change the threshold current but did decrease the water intake (Tab. 1), ($F(3,20) = 4.336$; $p < 0.05$). Diazepam (used as a positive standard), administered at doses of 2.5, 5 and 10 mg/kg, significantly increased the number of the accepted shocks (Fig. 2), ($F(3,20) = 40.81$; $p < 0.001$) in the Vogel test in rats. Moreover, in control experiments, neither the threshold current nor water intake were changed by diazepam administered at doses that were effective in the conflict drinking test, unlike the effects observed with the vehicle treatment (data not shown).

Tab. 1 Effects of MGS0039 on the shock threshold of and the amount of water consumed by water-deprived rats. MGS0039 and ritanserin were administered *ip* 60 min before the test, and WAY100635 45 min and flumazenil were administered 30 min before the test. The values are expressed as the mean ± SEM, with n as the number of rats per group. The symbols indicate differences in Newman-Keuls Multiple Comparison Test: * $p < 0.05$ compared to control, # $p < 0.05$ compared to the MGS-group, ns – not significant

Compound and dose (mg/kg)	n	Shock threshold (mA)	Water consumption (ml)
Vehicle	6	0.39 ± 0.04	8.78 ± 0.54
MGS0039 (2)	6	0.40 ± 0.04	6.96 ± 0.45*
		ns	F(3,20) = 4.336 p < 0.05
Vehicle	6	0.5 ± 0.01	9.3 ± 0.5
MGS0039 (2)	6	0.45 ± 0.05	7.4 ± 0.5*
Ritanserin (0.5)	6	0.5 ± 0.04	9.5 ± 0.5
Ritanserin (0.5) + MGS0039 (2)	6	0.5 ± 0.02	8.2 ± 0.6
		ns	F(3,20) = 9.23 p = 0.0065
Vehicle	6	0.5 ± 0.01	9.3 ± 0.5
MGS0039 (2)	6	0.45 ± 0.05	7.4 ± 0.5*
WAY100635 (0.1)	6	0.5 ± 0.02	8.5 ± 0.4
WAY100635 (0.1) + MGS0039 (2)	6	0.5 ± 0.02	8.7 ± 0.5#
		ns	F(3,20) = 3.18 p = 0.0899
Vehicle	6	0.5 ± 0.01	9.3 ± 0.5
MGS0039 (2)	6	0.45 ± 0.05	7.4 ± 0.5*
Flumazenil (10)	6	0.5 ± 0.04	9.4 ± 0.5
Flumazenil (10) + MGS0039 (2)	6	0.5 ± 0.02	8.8 ± 0.6
		ns	F(3,20) = 5.63 p = 0.0278

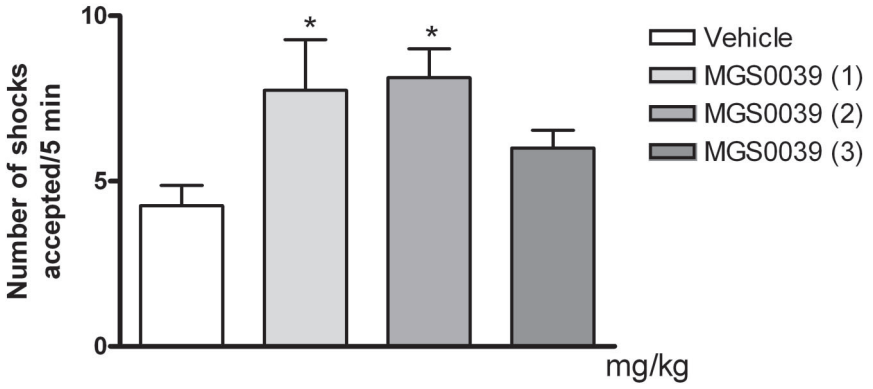

Fig. 1. Effect of MGS0039 in the Vogel conflict drinking test in rats. MGS0039 was administered *ip* 60 min before the test. The values are expressed as the mean ± SEM of the number of shocks accepted during a 5 min experimental session; n = 8 rats per group. The symbols indicate a significant difference found using Dunnett's test, * $p < 0.05$ *vs.* control

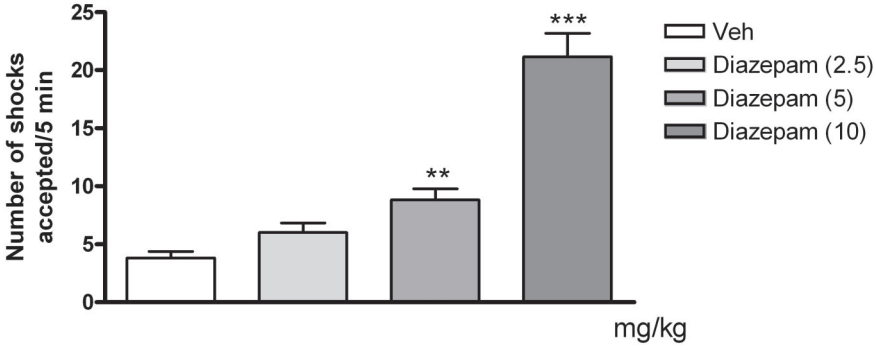

Fig. 2. Effect of diazepam in the Vogel conflict drinking test in rats. Diazepam was administered *ip* 60 min before the test. The values are expressed as the mean ± SEM of the number of shocks accepted during a 5-min experimental session; n = 8 rats per group. The symbols indicate a significant difference found using Dunnett's test, ** $p < 0.01$ *** $p < 0.001$ *vs.* control

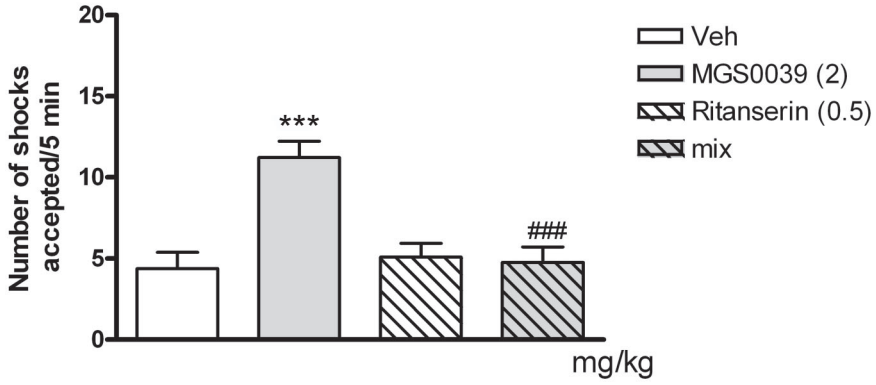

Fig. 3. Effect of ritanserin on the anxiolytic-like effects of MGS0039 in the Vogel conflict drinking test in rats. MGS0039 and ritanserin were administered *ip* 60 min before the test. The values are expressed as the mean ± SEM of the number of shocks accepted during a 5-min experimental session; n = 8 rats per group. *** $p < 0.001$ compared to control, ### $p < 0.001$ *vs.* MGS0039 (2 mg/kg)-treated rats

In the second part of our experiments, the influence of benzodiazepine and serotoninergic antagonists was examined. The anxiolytic-like effect of MGS0039 (2 mg/kg) was significantly attenuated by ritanserin (0.5 mg/kg). Two-way ANOVA revealed that the effects of MGS0039 was statistically significant ($F(1,26) = 26.21$, $p < 0.0001$) and that there was a significant interaction between the effects of MGS0039 and ritanserin [$F(1,31) = 14.67$, $p < 0.0001$] (Fig. 3). Furthermore, the anxiolytic-like effect of MGS0039 (2 mg/kg) was significantly attenuated by WAY100635 (0.1 mg/kg) ($F(1,26) = 21.26$, $p < 0.0001$) (Fig. 4). Flumazenil also attenuated the effect of MGS0039 ($F(1,26) = 7.29$, $p = 0.012$) (Fig. 5).

When MGS0039 was combined with the administration of ritanserin, WAY100635 or flumazenil in the shock threshold and free-drinking tests in doses effective in the conflict drinking test, there was no change of the threshold current, yet the water intake did decrease (Tab. 1).

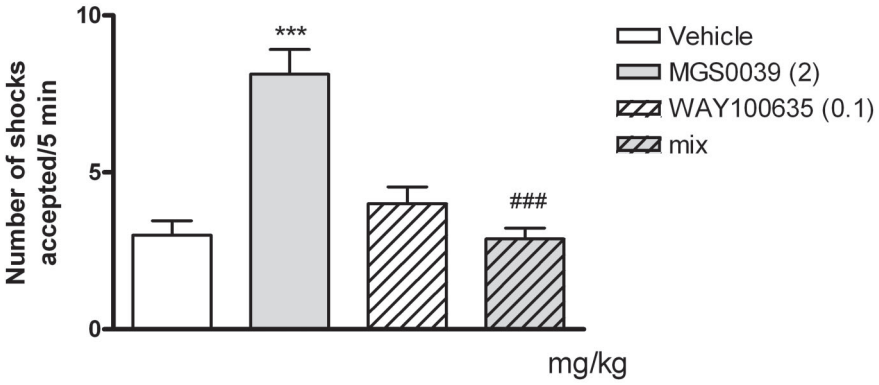

Fig. 4. Effect of WAY100635 on the anxiolytic-like effects of MGS0039 in the Vogel conflict drinking test in rats. MGS0039 was administered *ip* 60 min before the test, and WAY100635 was administered *sc* 45 min before the test. The values are expressed as the mean ± SEM of the number of shocks accepted during a 5-min experimental session; n = 8 rats per group. *** $p < 0.001$ compared to control, ### $p < 0.001$ *vs.* MGS0039 (2 mg/kg)-treated rats

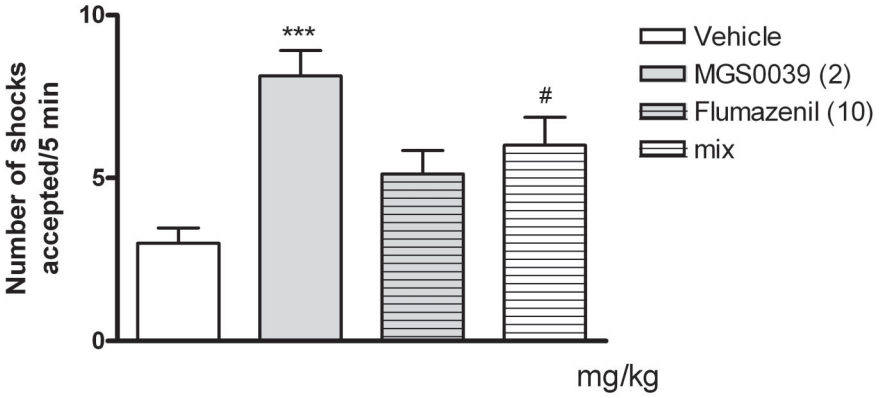

Fig. 5. Effect of flumazenil on the anxiolytic-like effects of MGS0039 in the Vogel conflict drinking test in rats. MGS0039 and flumazenil were administered *ip* 60 and 30 min, respectively, before the test. The values are expressed as the mean ± SEM of the number of shocks accepted during a 5-min experimental session; n = 8 rats per group. *** $p < 0.001$ compared to control, # $p < 0.05$ *vs.* MGS0039 (2 mg/kg)-treated rats

Discussion

Previous studies have demonstrated that MGS0039, a potent and selective antagonist of mGlu2/3 with a low affinity for the mGlu7 receptor [29], produced anxiolytic effects in a conditioned non-conflict test designed to reveal anxiolysis because it reduced freezing behavior in the conditioned fear stress model [42]. MGS0039 was also active in unconditioned models of anxiety. It reduced SIH in singly-housed mice [17], and it reduced the marble burying behavior in mice [37]. Although the spectrum of tests in which the anxiolytic-like effects of MGS0039 were demonstrated is quite broad, the compound was not effective in the elevated plus maze and in the social interaction tests [4].

The VCT shows high predictivity, constructive value and validity as a tool to discover new anxiolytics [27] and belongs to the conditioned, conflicting experimental models of anxiety. Here, we demonstrated that the mGlu2/3 receptor antagonist MGS0039 significantly increased the number of shocks accepted in the VCT in rats. The action of MGS0039 appears to be specific because this compound, in doses effective in the Vogel test, did not affect the response to the threshold current and increased water intake. However, we observed an inverse U-shaped profile in its anxiolytic efficacy, which means that the window of its anxiolytic efficacy may be narrow. Additionally, our data show that its efficacy is lower when compared to benzodiazepines, and the same result was shown in the stress-induced hyperthermia test [17], which may put its practical use as an anxiolytic in question.

Substantial data indicate that stress increases the level of glutamate in the brain; therefore, stress-related disorders, including anxiety, may be characterized by an increased glutamate level [18, 24, 25, 28]. Moreover, blocking glutamatergic transmission by influencing the ionotropic or metabotropic systems causes anxiolytic-like effects in preclinical studies [for review see: 5, 31].

The anxiolytic-like activity of mGlu2/3 receptor agonists is a well-established phenomenon found in a number of preclinical tests [for review see: 31, 39]. The presynaptic localization of mGlu2/3 receptors results in the attenuation of glutamate release after their stimulation [for review see: 36], which may form the basis of anxiolysis. Therefore, the anxiolytic-like efficacy of an mGlu2/3 antagonist that enhances the release of glutamate is puzzling. However, group II mGlu receptors also function as presynaptic heteroreceptors on GABAergic neurons [30], and stimulation of these receptors leads to a decreased release of GABA, which plays a role in central disinhibition. Anxiolysis *via* increased GABAergic transmission forms the basis of the anxiolytic effect of benzodiazepines, which are positive allosteric modulators at the $GABA_A$ receptors [12]. As such, blocking the mGlu2/3 receptors located on GABAergic nerve terminals should lead to an increase in the central inhibition and to anxiolytic responses. The inhibition of the anxiolytic action of MGS0039 by flumazenil administration implicates the participation of GABA (benzodiazepine sites) in the anxiolytic effect of MGS0039 and may support this line of thinking.

The importance of the 5-HT system in anxiolysis is a generally accepted phenomenon [for review see: 27]. For example, selective serotonin reuptake inhibitors (SSRI) were shown to be effective in a broad spectrum of anxiety disorders [20]. WAY100635, a selective 5-HT_{1A} receptor antagonist [10] that was inactive on its own, inhibited the anxiolytic-like action of MGS0039 in the Vogel test in rats. This finding corresponds with that of Iijima et al. [17], who observed that WAY100635 reversed the anti-hyperthermia effects of MGS0039 in the SIH test in mice, suggesting that this effect is mediated by stimulation of the 5-HT_{1A} receptors. 5HT-$_{1A}$ receptors are localized presynaptically on serotonergic neurons (mainly in the raphe nucleus), where they play the role of autoreceptors regulating the release of serotonin [35]. The postsynaptic localization was reported in non-serotonergic neurons, including glutamatergic neurons [7, 35]. Postsynaptic 5-HT_{1A} receptors exist mainly in limbic structures, including the amygdala [14], the brain structure supposedly responsible for playing a major role in anxiety [8]. Serotonin released from 5-HT terminals in the central nucleus of the amygdala through the action of 5-HT_{1A} receptors localized on the glutamatergic postsynaptic element contributes to the inhibition of the increased firing of these neurons [2, 3] and may contribute to anxiolysis, hence the effectiveness of WAY100635 in blocking the anxiolytic effects of MGS0039.

Ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist [15], also blocked the anxiolytic-like effects of MGS0039, demonstrating the involvement of this serotonin receptor subtype in the therapeutic process. The specificity of the effect of ritanserin is supported by the fact that it had no influence either on the water intake or the shock threshold, either by itself or in combination with MGS0039. It must also be noted that the anxiolytic-like action of the group III mGlu receptor antagonist, (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG), was also blocked by ritanserin [38]. Because group II and group III mGlu receptors share the presynaptic localization and because blocking the activity of both groups enhances the release of glutamate [36], it is possible that these two receptor types may enter into similar interactions with 5-$HT_{2A/2C}$ receptors.

In an electrophysiological study, MGS0039 dose-dependently significantly increased the firing rate of the dorsal raphe nucleus (DRN) serotonergic neurons and significantly increased the extracellular level of serotonin in the rat medial prefrontal cortex in a microdialysis study [19]. Another mGlu2/3 antagonist, LY341495, increased the frequency and amplitude of 5-HT-induced EPSCs in pyramidal cells in the medial prefrontal cortex [26]. Therefore, an increase in serotonergic transmission may be a result of a group II mGlu receptor blockage, indicating a functional interaction between mGlu2 and 5HT_{2A} receptors. 5-HT_2 receptors are candidates for mediating the anxiolytic action of SSRI-s. The data indicate that 5-HT_2 receptor agonists may be beneficial in the treatment of some anxiety disorders [1, 16, 41]. It can be speculated that the stimulation of presynaptically-localized group II mGlu receptors on serotonergic nerve terminals leads to a decrease in the release of serotonin. As such, MGS0039, *via* the blockage of the presynaptic receptors, may lead to 5-HT overflow and to anxiolysis. This effect may account for ritanserin's ability to block the action of MGS0039. The functional and structural interaction between mGlu2 and 5HT_{2A} receptors is well-documented, and the interaction has been implicated in psychosis [11, 23, 26]. Such interaction may also be involved in the mechanisms related to anxiety.

The results of our studies show that the anxiolytic action of MGS0039, which depends on the serotoner-

gic system, differs from its antidepressant-like action, which is serotonin-independent [32]. This may reflect a fundamental difference in the anxiety *versus* depression circuitry in the brain in the context of group II mGlu receptor functioning.

When considered en bloc, our data strongly indicate the existence of complex interactions between the serotonergic, GABAergic and glutamatergic systems on the extent of the anxiolytic-like action of group II mGlu receptor antagonists.

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