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A novel mGlu4 selective agonist LSP4-2022 increases behavioral despair in mouse models of antidepressant action

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ABSTRACT

Numerous data have indicated that metabotropic glutamate (mGlu) receptor ligands may be potentially useful as novel antidepressant drugs (ADs). The Group III mGlu receptor has not been explored much because of the limited access to selective ligands, but some behavioral studies have indicated that modulating group III mGlu receptors may result in benefits for the therapy of depression. Here, we investigated the potential antidepressant-like effects of a new mGlu4 selective orthosteric agonist, LSP4-2022. We found that the drug induced pro-depressant effects in the tail suspension test (TST) and the forced swim test (FST) in mice at doses that did not change the locomotor activity of the animals. Additional experiments that used knock-out (KO) mice and aimed to verify the selectivity of LSP4-2022 revealed that the drug induced strong pro-depressant-like effects in mGlu7 KO mice but did not affect the behavior of mGlu4 KO mice in the TST, suggesting that the activation of the mGlu4 receptor plays a role in producing the pro-depressant activity of the tested drug. The results of our study indicate that the inhibition rather than activation of mGlu4 receptors might induce antidepressant effects, but this hypothesis should be verified using a selective mGlu4 receptor antagonist, which is currently not available.

1. Introduction

One of the new leading hypotheses of depression is the glutamatergic hypothesis, which states that the main brain excitatory system plays a role in the pathophysiology of depression and suggests the possible antidepressant activity of the compounds that modulate glutamatergic neurotransmission via the activation or inhibition of glutamatergic receptors (Duman, 2014; Szewczyk et al., 2012). The most important evidence for this hypothesis is a set of clinical studies showing the rapid and sustained antidepressant effect of the noncompetitive NMDA receptor antagonist ketamine in patients suffering from major treatment-resistant depression (Serafini et al., 2014; Zarate et al., 2006). Although the effect of ketamine is spectacular, the drug itself has too many limitations to be used in therapy for depression, including the psychotic, dissociative and hypnotic effects of higher doses of the drug (Blier et al., 2012; Krystal et al., 2013).

New opportunities for using glutamatergic modulators as therapeutic agents emerged when the mGlu receptors were discovered (Nakanishi, 1992). Unlike the iGlu receptor ligands that correspond to fast excitation, mGlu ligands are responsible for the slower modulation of the excitatory currents (Conn and Pin, 1997). Thus, they are taken seriously as potentially effective drugs but are safer than ionotropic glutamate (iGlu) receptor ligands. A set of behavioral data and initial clinical trials have indicated that some mGlu receptor ligands, primarily mGlu5 and mGlu2/3 receptor antagonists, may be potentially useful as novel ADs (Chaki et al., 2013).

Group III is the most numerous and contains the most largely distributed mGlu receptor subtypes; however, the action of the ligands belonging to this group has been the least explored, primarily due to the limited amount of selective pharmacological tools (Mercier and Lodge, 2014). Nevertheless, the existing data suggest a role for the group III mGlu receptors in the pathophysiology of depression. Numerous studies using behavioral models of

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depression have shown changes in the expression of mGlu4, 7 and 8 receptors in mice and rats (O'Connor et al., 2013; Wierońska et al., 2008). Furthermore, it has been reported that the group III mGlu receptors of naive animals are sensitive to chronic treatment with ADs (O'Connor et al., 2013; Wierońska et al., 2007, 2008). Finally, a study using postmortem human brain samples of subjects with major depression indicated that the mGlu4 receptor gene was upregulated in depressed brains (Lopez et al., 2014).

It is worth noting that some behavioral studies have indicated the antidepressant action of group III mGlu receptor ligands in screening tests and behavioral models of depression. Preliminary experiments that were based solely on the application of agonists and antagonists of group III mGlu receptors to the lateral ventricles of a rat brain (i.c.v.) indicated that two substances, ACPT-I (a non-selective agonist of group III mGlu receptors, preferring the mGlu4 receptor) and RS-PPG (an mGlu8 receptor agonist), induced antidepressant effects in the FST in rats (Pałucha et al., 2004). Subsequent studies showed that a positive allosteric modulator (PAM) of the mGlu4 receptor, PHCCC, when administered i.c.v., intensified the antidepressant-like activity of ACPT-I in the FST in rats (Kłak et al., 2007); however, we could not confirm the antidepressant-like activity of ACPT-I after peripheral administrations in either the TST in mice or the FST in rats (Stachowicz et al., 2009). In contrast, promising results have been obtained using the first selective and bioavailable PAM of the mGlu7 receptor, AMN082, which induced a strong antidepressant-like effect in several animal models of depression in rodents (O'Connor and Cryan, 2013; Pałucha et al., 2007; Palucha-Poniewiera et al., 2010).

Using a considerable number of new selective pharmacological tools for the mGlu4 receptor, we received negative results on the screening tests used to evaluate antidepressant activity. Thus, Lu AF21934, which is a novel, selective, and brain-penetrant PAM of the mGlu4 receptor, did not induce any effect in the TST in mice (Sławińska et al., 2013). Another study showed a lack of an antidepressant-like effect of a novel orthosteric, a preferential agonist of the mGlu4 receptor, LSP1-2111, in the FST and TST in mice (Wierońska et al., 2010) at doses that induced distinct anxiolytic effects. In contrast, a recently published article by Kalinichev et al. (2014) showed that a novel mGlu4 receptor PAM, ADX88178, when given *per os*, induces an antidepressant-like effect in the FST in mice.

Here, we decided to investigate the potential antidepressant-like effects of a new mGlu4 selective orthosteric agonist, LSP4-2022, a compound that is 100 times more potent on the mGlu4 receptor than on the other group III mGlu receptors in the brain and is inactive on any other mGlu receptor (Goudet et al., 2012). This new selective compound has been shown to be active on native receptors and to display antiparkinsonian activity (Goudet et al., 2012) and analgesic effects (Vilar et al., 2013), thus demonstrating its ability to cross the blood–brain barrier. Importantly, it was reported that although it is much more potent at mGlu4 (EC_{50} = 0.1 μM), this ligand is the most potent mGlu7 orthosteric agonist ever described, with an EC_{50} of 10 μM (Flor and Acher, 2012). Therefore, it was interesting to test this compound as a potential antidepressant drug. Two screening tests (the FST and the TST) were used in the study, and the selectivity of LSP4-2022 was verified using mGlu4 and mGlu7 KO mice and their wild-type (WT) littermates.

2. Materials and methods

2.1. Animals and housing

Male C57BL/6J mice (Charles River, Germany), weighing 23–25 g at the beginning of the experiments, were used in the study. Heterozygous mGlu4 C57BL/6J mice were obtained from Abbot Laboratories (a gift from Dr. Bespalov), and heterozygous mGlu7 mice were obtained from Novartis Pharma AG. All animals (mGlu4 KO and WT mice, as well as mGlu7 KO and WT mice) were bred in our institute, essentially as described by Mitsukawa et al. (2006). The phenotypes of newborn mice were analyzed according to Sansig et al. (2001) using polymerase chain reaction (PCR). The animals were kept under standard laboratory conditions of lighting (light phase: 7:00–19:00) and temperature (19–21 °C). Food and water were freely available. The experiments were performed during the light period (9:00–14:00) by an observer who was unaware of the treatment. All procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee and were approved by the Ethics Committee of the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

2.2. Drugs and treatment

LSP4–2022 was synthesized in the laboratory of F.A. following a previously described procedure (Selvam et al., 2010). LSP4-2022 was dissolved in sterile water, and the pH was adjusted to 7.0. The drug was administered intraperitoneally (i.p.) 45 min before the behavioral test. Imipramine hydrochloride (Sigma–Aldrich, St. Louis, USA) was dissolved in 0.9% NaCl and administered i.p. 30 min before the behavioral test. NaCl (0.9%) was used as a vehicle. All solutions were prepared immediately prior to the experiments and were administered at a constant volume of 10 ml/kg.

2.3. Tail suspension test

The tail suspension test was performed according to the procedure of Steru et al. (1985). C57BL/6J mice were individually suspended by their tails by a plastic string that was positioned horizontally 75 cm above the tabletop using adhesive tape placed approximately 1 cm from the tip of the tail. The immobility duration was recorded for 6 min. The mice were considered immobile only when they hung down passively and were completely motionless.

2.4. Forced swim test

C57BL/6J mice were placed individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm of water and maintained at 23 °C. The animals were left in the cylinder for 6 min. After the first 2 min, the total duration of immobility was measured during a 4-min test. The mouse was judged to be immobile when it remained floating passively.

2.5. Locomotor activity

The spontaneous locomotor activity of the mice was measured in Plexiglas locomotor activity chambers (40 × 20 × 15 cm) in a 20-station photobeam activity system (Opto-M3 Activity Meter, Columbus Instruments, USA), where the animals were placed individually 45 min after the drug injections. The distance traveled (cm) was recorded for 15–30 min.

2.6. qRT-PCR (real-time PCR)

Total RNA was extracted from the hippocampus and frontal cortex of mGlu7 KO and WT mice with the TRIzol® (Ambion) method. The RNA quality and quantity was checked by spectrophotometry (NanoDrop1000) and electrophoresis in an agarose gel. One microgram of an RNA sample was digested with gDNA Wipeout Buffer to remove the genomic DNA contamination. cDNA was synthetized from RNA using the Reverse Transcription method (Qiagen – QuantiTect Reverse Transcription Kit) with oligo-dT random primers. *Grm4* gene expression was determined by real-time PCR (CFX96 Touch, Bio-Rad) performed with TaqMan probe assays (Applied Biosystems). The *Actb* gene was selected as a reference gene. The TaqMan Expression Assay IDs were as follows: Mm01306128_m1 for *Grm4* and Mm00607939_s1 for *Actb*. All PCR reactions were conducted in triplicate. For each reaction, 50 ng of cDNA was used. Data analysis was based on the ΔΔCt method.

2.7. Statistics

The results obtained in the TST and FST are presented as the means ± SEM and were evaluated by one-way ANOVA followed by Dunnett's post-hoc test or two-way ANOVA (when KO and WT mice were used in the experiment) followed by Bonferroni post-hoc test. A repeated-measures ANOVA was used to analyze the locomotor activity results. For each analysis, the difference between two curves was analyzed considering two parameters: the time and the drug treatment. An analysis of data obtained using real-time PCR was based on the ΔΔCt method, and Student's t-test was used to compare ΔΔCt in KO mice compared with their respective WT controls (the means ± SD). GraphPad Prism version 5.00 for Windows, version 5.04, 2010 (GraphPad Software Inc., San Diego, CA) was used to analyze the data.