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REVERSAL OF NOVELTY-INDUCED HYPERLOCOMOTION AND HIPPOCAMPAL C-FOS EXPRESSION IN GLUA1 KNOCKOUT MALE MICE BY THE MGLUR2/3 AGONIST LY354740

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Abstract—Dysfunctional glutamatergic neurotransmission has been implicated in schizophrenia and mood disorders. As a putative model for these disorders, a mouse line lacking the GluA1 subunit (GluA1-KO) of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor displays a robust novelty-induced hyperlocomotion associated with excessive neuronal activation in the hippocampus. Agonists of metabotropic glutamate 2/3 receptors (mGluR2/3) inhibit glutamate release in various brain regions and they have been shown to inhibit neuronal activation in the hippocampus. Here, we tested a hypothesis that novelty-induced hyperlocomotion in the GluA1-KO mice is mediated via excessive hippocampal neuronal activation by analyzing whether an mGluR2/3 agonist inhibits this phenotypic feature. GluA1-KO mice and littermate wildtype (WT) controls were administered with (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) (15 mg/kg, i.p.) 30 min before a 2-h exposure to novel arenas after which c-Fos immunopositive cells were analyzed in the hippocampus. LY354740 (15 mg/kg) decreased hyperactivity in male GluA1-KO mice, with only a minimal effect in WT controls. This was observed in two cohorts of animals, one naïve to handling and injections, another pre-handled and accustomed to injections. LY354740 (15 mg/kg) also reduced the excessive c-Fos expression in the dorsal hippocampal CA1 pyramidal cell layer in maleGluA1-KO mice, while not affecting c-Fos levels in WT mice. In female mice, no significant effect for LY354740 (15 mg/kg) on hyperactive behavior or hippocampal c-Fos was observed in either genotype or treatment cohort. A higher dose of LY354740 (30 mg/kg) alleviated hyperlocomotion of GluA1-KO males, but not that of GluA1-KO females. In conclusion, the excessive behavioral hyperactivity of GluA1-KO mice can be partly prevented by reducing neuronal excitability in the hippocampus with the mGluR2/3 agonist suggesting that the hippocampal reactivity is strongly involved in the behavioral phenotype of GluA1-KO mice.

Key words: AMPA receptor, mGlu2/3 receptor, hyperlocomotion, hippocampus, immediate early genes, novelty.

INTRODUCTION

Disturbed glutamate signaling is implicated in several psychiatric disorders including schizophrenia and mood disorders (Inta et al., 2010; Field et al., 2011). Glutamate transmits fast excitatory actions via ionotropic receptors while its modulatory effects are mediated by metabotropic glutamate receptors (mGluR) (Conn and Pin, 1997; Traynelis et al., 2010). Ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype glutamatergic receptors are postsynaptic heteromeric transmembrane proteins formed by four different subunits (GluA1–4) (Traynelis et al., 2010). The GluA1 subunit is localized throughout the brain but especially highly expressed in the hippocampus (Keinanen et al., 1990), a region related to memory processing, novelty recognition, spatial learning and emotional regulation (Fanselow and Dong, 2010).

The functional role of GluA1 subunit is revealed by the GluA1 subunit-deficient (GluA1-knockout (KO)) mouse line (Zamanillo et al., 1999). In the GluA1-KO hippocampus, excitatory synaptic transmission is slightly reduced (Andrasfalvy et al., 2003; Jensen et al., 2003; Romberg et al., 2009) and long-term potentiation impaired at the CA3-CA1 synapses (Zamanillo et al., 1999; Hoffman et al., 2002; Jensen et al., 2003; Romberg et al., 2009). The GluA1-KO mice display a repertoire of behavioral alterations including defective spatial working memory (Reisel et al., 2002; Schmitt et al., 2003), enhanced spatial long-term memory (Sanderson et al., 2009), increased depressive behavior (Chourbaji et al., 2008), defective prepulse inhibition (Wiedholz et al., 2008), abnormalities linked to psychoaffective disorders (Barkus et al., 2012), reduced aggression (Vekovischeva et al., 2004) and addiction/dependence-related phenotypes (Vekovischeva et al., 2001; Aitta-aho et al., 2009, 2012). A robust behavioral feature of the GluA1-KO mice is strong hyperlocomotion in response to a novel environment (Zamanillo et al., 1999; Vekovischeva et al., 2001; Procaccini et al., 2011). This hyperlocomotion has been suggested to

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, analysis of variance; BSA, bovine serum albumin; DG, dentate gyrus; EAAT1/GLAST, excitatory amino acid transporter 1; KO, knockout; LY354740, (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; mGluR, metabotropic glutamate receptors; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo(f)quinoxaline-7-sulfonamide; TBS, Tris-buffered saline; TBST, TBS supplemented with 0.05% Tween 20; WT, wildtype.

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model schizoaffective disorders as it was reduced by the antipsychotic drug haloperidol (Wiedholz et al., 2008) and partially also by the mood stabilizer lithium (Fitzgerald et al., 2010). Recently, we reported that the novelty-induced hyperlocomotion of GluA1-KO mice was accompanied with excessive neuronal activation in the hippocampus as detected by the increased number of c-Fos-positive cells. This is in line with the findings of Fumagalli et al. (2011), who showed that immobilization stress increased mRNA expression of another immediate early gene, Arc (activity-regulated cytoskeletal associated protein) in the GluA1-KO hippocampus. Thus, novelty-induced excessive hippocampal neuronal activation in the GluA1-KO mice is probably not due to increased spatial exploration, which itself is known to induce immediately early gene expression in hippocampal place cells, but rather reflects the excessive reactivity of hippocampal neurons to different types of environmental challenges/stressors including immobilization stress (Fumagalli et al., 2011) and novelty (Procaccini et al., 2011). Our finding, that the systemic administration of the AMPA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo(f) quinoxaline-7-sulfonamide (NBQX) alleviated locomotor hyperactivity of the GluA1-KO mice (Procaccini et al., 2011) indicates that increased glutamatergic signaling might underlie at least partially behavioral responses of KO mice to novelty. Interestingly, Chourbaji et al. (2008) reported increased levels of glutamate in the KO hippocampus. Based on these findings we decided to analyze here whether the modulation of glutamate signaling through group II mGluRs would inhibit the behavioral and hippocampal hyperactivity of GluA1-KO mice.

Group II mGluRs, mGluR2 and mGluR3, located in presynaptic terminals regulate predominantly the release of glutamate; in postsynaptic and glial locations they mediate various cellular metabolic actions (Anwyl, 1999; Cartmell and Schoepp, 2000). mGluR2/3 agonists reduce presynaptic glutamate release in cortical and subcortical slice preparations (reviewed in Marek, 2010). This is also observed in the hippocampus, at the entorhinal cortex-dentate gyrus (DG) or -CA1synapses (Macek et al., 1996; Kilbride et al., 1998; Kew et al., 2001, 2002; Capogna, 2004; Higgins et al., 2004; Price et al., 2005; Giocomo and Hasselmo, 2006; Ceolin et al., 2011), and at the DG-CA3 synapses (Kamiya et al., 1996; Yokoi et al., 1996; Scanziani et al., 1997). In microdialysis studies, mGluR2/3 agonists reduce extracellular glutamate and monoamine levels increased by psychotomimetic and stimulant drugs, the majority of the results having been obtained from the prefrontal cortex or nucleus accumbens (see Fell et al., 2012), but a few also from the ventral hippocampus (Lorrain et al., 2003; Fell et al., 2010). Pretreatment with the mGluR2/3 agonists have been shown to reduce the number of c-Fos-positive cells in the dorsal hippocampus after mild stress or other challenges (Linden et al., 2004; Zhao et al., 2006; Wischhof and Koch, 2012). In agreement with these neurochemical effects, mGluR2/3 agonists have shown antipsychotic and antianxiety properties in clinical trials (Patil et al., 2007; Dunayevich et al., 2008). In preclinical animal models of psychiatric disorders, mGluR2/3 agonists inhibit hyperactivity produced by the stimulant amphetamine and psychotomimetic phencyclidine (reviewed in Fell et al., 2012) and display anxiolytic effects (reviewed in Swanson et al., 2005).

In this study, we tested a potent and selective mGluR2/3 agonist (1S,2S,5R,6S)-2-aminobicyclo[3.1.0] hexane-2,6-dicarboxylic acid (LY354740) (Monn et al., 1997) for its efficacy in reversing novelty-induced responses of GluA1-KOmice.

EXPERIMENTAL PROCEDURES

Animals

All experimental animal procedures had the ethical approval from the Southern Finland Provincial Government. All efforts were made to minimize the number and suffering of animals. GluA1-KO mice were obtained by genetic inactivation of the *Gria1* gene and genotyped as previously described (Zamanillo et al., 1999). Both GluA1-KO mice and their wildtype (WT) littermate controls derived from the breeding of heterozygous parents after at least 10 backcrosses to C57BL/6J mice (Aitta-aho et al., 2012). We used two cohorts of mice. Cohort 1 of naïve mice contained a total of 118 mice: 29 WT males (26.3 ± 2.1 weeks; 31.4 ± 0.8 g), 27 WT females (28.8 ± 2.6 weeks; 25.3 ± 0.6 g), 27 KO males (26.2 ± 2.0 weeks; 30.7 ± 0.9 g), and 35 KO females (24.2 ± 1.9 weeks; 24.1 ± 0.5 g). Cohort 2 contained 82 pre-handled mice: 20 WT males (18.9 ± 0.2 weeks; 31.1 ± 0.6 g), 22 WT females (18.6 ± 0.3 weeks; 23.6 ± 0.4 g), 20 KO males (18.6 ± 0.2 weeks; 28.4 ± 0.4 g), and 20 KO females (18.8 ± 0.2 weeks; 22.2 ± 0.4 g). After weaning, the mice were maintained with their littermates in same-sex groups of either 1–2 (small plastic cages, 20 × 27 × 13 cm; Tecniplast, Buguggiate, Italy) or 4–6 mice (bigger cages, 40 × 30 × 15 cm), with food pellets (Harlan BV., Horst, Netherlands) and tap water available *ad libitum* at standard housing conditions (12-h light–dark cycle, lights on at 06:00 a.m.; temperature, 20–23 °C; relative humidity, 50–60%; aspen chip beddings; a wooden toy and shelter were located in each home cage).

Locomotor activity in novel cages

In the first experiment (Cohort 1), all mice were naïve to both injections and locomotor activity cages. In the second experiment (Cohort 2) mice were shortly handled and injected with saline (i.p.) daily for 1 week but naïve to locomotor activity cages. On the trial day, mice were acclimated to the test room in their home cages for at least 1 h, then they were injected (i.p.) with LY354740 or saline and immediately returned to home cages. After 30 min, they were transferred to novel testing cages (40 × 30 × 20 cm, covered with transparent lids with ventilation holes) for 2 h. Locomotor activity was detected as previously described (Procaccini et al., 2011). Horizontal movements (m) of