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Research Paper

Deletion of serine racemase confers D-serine –dependent resilience to chronic social defeat stress

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ABSTRACT

The *N*-methyl-D-aspartate receptor (NMDAR) plays a key role in the pathophysiology of depression. Serine racemase (SRR, encoded by *Srr*) converts L-serine to D-serine, an endogenous co-agonist at the glycine site of the NMDAR. Knock-out (KO) of *Srr* did not alter behavioral signs of depression compared with wild-type (WT) mice as evaluated by locomotion, tail suspension, forced swimming, and 1% sucrose preference tests. However, chronic social defeat stress (CSDS: 10 days) caused a depression-like phenotype as measured by these same tests in WT mice but not in *Srr* KO mice, suggesting that decreased D-serine co-agonist activity confers resilience against CSDS. In WT mice, CSDS decreased brain-derived neurotrophic factor (BDNF) expression and phosphorylation/activation of its receptor TrkB in prefrontal cortex (PFC), dentate gyrus (DG), and the CA3 region of the hippocampus, but increased BDNF and phosphorylated TrkB in the nucleus accumbens (NAc). Conversely, CSDS did not alter BDNF or TrkB phosphorylation in any brain region of *Srr* KO mice. Administration of D-serine through drinking water (600 mg/L for 20 days) 10 days prior to and during CSDS restored the depression-like phenotype in *Srr* KO mice. These findings suggest that reducing brain D-serine may improve stress resilience, thereby reducing depression risk.

1. Introduction

Major depressive disorder (MDD) is among the most severe and debilitating of psychiatric illnesses. Although antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) are generally effective for the treatment of depression, it can take several weeks before patients experience the full therapeutic effects. Further, approximately one-third of depressed patients fail to respond fully to pharmacotherapy (Guidi et al., 2016). The precise molecular mechanisms underlying treatment-resistant depression are currently unknown.

Accumulating evidence suggests that the *N*-methyl-D-aspartate receptor (NMDAR) plays a central role in the pathophysiology of MDD (Sanacora et al., 2008; Hashimoto, 2009; Dang et al., 2014; Ohgi et al., 2015; Ghasemi et al., 2017; Haroon et al., 2017; Lener et al., 2017; Murrough et al., 2017). A study using postmortem brain samples showed increased levels of glutamate in the prefrontal cortex (PFC) of MDD patients compared with a control group (Hashimoto et al., 2007). Furthermore, the cerebrospinal fluid (CSF) glutamine-to-glutamate ratio was higher in elderly MDD patients than age-matched controls. Alternatively, absolute CSF levels of individual amino acids did not differ, suggesting that abnormalities in the glutamine-glutamate cycle may contribute to the pathophysiology of depression, at least in the elderly (Hashimoto et al., 2016; Hashimoto, 2018). Proton magnetic resonance spectroscopy

Abbreviation: BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CSDS, chronic social defeat stress; CSF, cerebrospinal fluid; DG, dentate gyrus; ECL, enhanced chemiluminescence; FST, forced swimming test; Glx, glutamine plus glutamate; HRP, horseradish peroxidase; KO, knock-out; LMT, locomotion test; LSD, least significant difference; MDD, major depressive disorder; MRS, magnetic resonance spectroscopy; NAc, nucleus accumbens; NMDAR, *N*-methyl-D-aspartate receptor; PFC, prefrontal cortex; p-TrkB, phosphorylated (activated) TrkB; PVDF, polyvinylidene difluoride; RT, room temperature; S.E.M., standard error of the mean; SIT, social interaction test; SNRIs, serotonin–norepinephrine reuptake inhibitors; SPT, sucrose preference test; SRR, Serine racemase; SSRIs, selective serotonin reuptake inhibitors; TBS, Tris buffered saline; TBST, Tris buffered saline + 0.1% Tween 20; TST, tail suspension test; WT, wild-type.

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(MRS) studies revealed a significant reduction in glutamine plus glutamate (Glx) levels, but not glutamate levels alone, in the brains of MDD patients compared with controls (Arnone et al., 2015). The NMDAR antagonist ketamine has rapid-acting and sustained antidepressant effects in treatment-resistant MDD patients (Newport et al., 2015; Kishimoto et al., 2016; Monteggia and Zarate, 2015; Hashimoto, 2016a, 2016b). Taken together, these findings suggest that an abnormality in NMDAR-mediate glutamatergic neurotransmission contributes to the pathophysiology of depression.

Serine racemase (SRR) converts L-serine to D-serine (Wolosker et al., 1999a; 1999b; Wolosker and Mori, 2012), the predominant endogenous co-agonist of the NMDAR in the mammalian brain (Mothet et al., 2000). In mammals, the SRR protein is encoded by the *SRR* gene. Studies of *Srr* knock-out (KO) mice demonstrated that SRR is the major enzyme responsible for D-serine production in the forebrain (Inoue et al., 2008; Basu et al., 2009; Horio et al., 2011, 2012; 2013). Dysregulation of D-serine and concomitant changes in NMDAR function are implicated in the pathophysiology of schizophrenia. For instance, the rs4523957 allelic variant of the *SRR* gene has been linked to schizophrenia (Schizophrenia Working Group on the Psychiatric Genomics Consortium, 2014). However, the role of SRR in the pathophysiology of depression has not been studied.

The purpose of this study was to examine whether SRR contributes to the pathophysiology of depression by comparing behavioral endophenotypes between wild-type (WT) and *Srr* KO mice in the locomotion test (LMT), tail suspension test (TST), forced swimming test (FST), and 1% sucrose preference test (SPT). These tests were performed in control mice and in mice exposed to chronic social defeat stress (CSDS). We then examined the expression levels of brain-derived neurotrophic factor (BDNF) and its receptor TrkB in selected brain regions as multiple studies have implicated BDNF–TrkB signaling in the pathophysiology of depression (Nestler et al., 2002; Hashimoto et al., 2004; Duman and Monteggia, 2006; Hashimoto, 2010, 2013; Castrén, 2014; Björkholm and Monteggia, 2016; Zhang et al., 2016). Finally, we examined whether supplementation of D-serine through drinking water can affect stress resilience following CSDS.

2. Materials and methods

2.1. Animals

Adult male WT and *Srr* KO C57BL/6 mice (8 weeks old, body weight 20–25 g) and adult male CD1 mice (ICR, 13–15 weeks old, body weight >40 g) were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The *Srr* KO mice and WT mice were maintained and tested as reported previously (Inoue et al., 2008; Horio et al., 2011, 2012; 2013; Miya et al., 2008). Animals were housed at a controlled temperature under a 12 h/12 h light/dark cycle (lights on between 07:00–19:00 h) with ad libitum food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The study was approved by the Chiba University Institutional Animal Care and Use Committee and conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA.

2.2. Behavioral tests

2.2.1. Locomotion

Locomotor activity was measured by the SCANETMV-40 animal movement analysis system (MELQUEST Co., Ltd., Toyama, Japan). Mice were placed in experimental cages (length × width × height: 560 × 560 × 330 mm) and free ambulation was recorded for 60 min. Cages were cleaned between tests.

2.2.2. TST

The tip of the mouse tail was placed by a small piece of adhesive tape about 2 cm, and mice were hung individually on a hook in a single hole. The immobility time was recorded for 10 min. When the mice hung passively and completely motionless, they were considered the immobility.

2.2.3. FST

The FST was performed by an automated forced-swim apparatus (SCANETMV-40, MELQUEST Co., Ltd., Toyama, Japan). Mice were independently put into a plastic cylinder (diameter: 23 cm; height: 31 cm) containing water at a depth of 15 cm and kept the water temperature at 23 ± 1 °C. The immobility time over 6 min was measured as total time – active time by the apparatus analysis software.

2.2.4. SPT

Mice were offered water and 1% sucrose solution (free choice) for 48 h, then deprived water and food for 4 h. Next, mice were exposed to two bottles (water and 1% sucrose solution) which were pre-weighed for 1 h. The bottles were weighed again at end of this period. Sucrose preference was measured as a proportion of sucrose solution consumed relative to the total liquid (water + 1% sucrose solution) consumed.

2.3. Chronic social defeat stress model

The procedure for CSDS was performed as previously reported (Zhang et al., 2015b; Yang et al., 2015b; Yao et al., 2016; Yang et al., 2016b; Ren et al., 2016; Ma et al., 2016; Dong et al., 2017). CD1 male mice with consistent attack latencies were housed in cages fitted with perforated Plexiglas separators, which allow sensory contact without physical contact, and used to stress/defeat the experimental WT or *Srr* KO mouse ("intruder mouse"). Every day mice were exposed to a 10 min long defeat episode, and then housed for the remainder of the day in the compartment next to the aggressor. This procedure was repeated for 10 consecutive days. At 24 h after the last session, all mice were housed individually. On day 11, a social interaction test (SIT) was performed to identify subgroups of mice that were susceptible and unsusceptible (resilient) to social defeat stress. This was accomplished by placing a test mouse in an interaction test box (42 × 42 cm) with an empty wire-mesh cage (10 × 4.5 cm) located at one end. The movement of the mouse was tracked for 2.5 min. Then, an unfamiliar CD1 aggressor mouse was placed in the wire-mesh cage and the behavior of the test mouse monitored for an additional 2.5 min. The duration that the test mouse spent in the "interaction zone" (defined as the 8 cm wide area surrounding the wire-mesh cage) was recorded by a stopwatch. The interaction ratio was calculated as time spent in the interaction zone with an aggressor to the time spent in the interaction zone without an aggressor (empty wire-mesh cage). An interaction ratio of 1 was set as the cutoff: mice with scores <1 were defined as "susceptible" to social defeat stress and those with scores ≥1 were defined as "resilient." Approximately 70%–80% of mice were susceptible after CSDS. Susceptible mice were randomly divided for subsequent experiments. Control C57BL/6 mice not exposed to CSDS (WT-no CSDS and KO-no CSDS groups) were housed in the home cage before the behavioral tests.