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D-serine prevents cognitive deficits induced by acute stress

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ABSTRACT

Increasing evidence indicates that acute stress disrupts cognitive functions mediated by glutamate-NMDA receptors, although the mechanisms are not fully understood. Here we investigated whether D-serine and glycine, the endogenous co-agonists of the NMDA receptor, are regulated by acute stress. We studied the biochemical and behavioral effects of acute restraint stress in C57BL/6 mice. Acute restraint stress decreased D-serine levels in the prefrontal cortex and glycine levels in the hippocampus. Behaviorally, acute stress impaired memory consolidation in the object recognition task and prepulse inhibition of the startle response. Importantly, D-serine administration (1 g/kg, i.p.) prevented both stress-induced impairments. Taken together, our results show for the first time an interplay between stress and D-serine and warrant further research on the role of D-serine in stress-related disorders.

1. Introduction

Stressful events are inevitable in modern life. It is generally accepted that chronic stress promotes cognitive deficits, but the effects of acute stress on cognition are more complex, depending on the type, intensity and timing of stress (Popoli et al., 2012; Sandi, 2011). Many studies showed that acute stress enhances consolidation of stressful memories, enabling the individual to prepare for similar situations in the future (Barsegyan et al., 2010; Revest et al., 2005; Roozendal and McGaugh, 1996). However, acute stress can impair memory consolidation depending on the emotional arousal of the task. For instance, Li et al. (2012) found that acute stress inhibits memory consolidation in the object recognition task, a relatively stress-free memory paradigm.

Stress also affects the pre-attentive sensorimotor gating, a filtering mechanism that is thought to prevent sensory overload. Stress and glucocorticoids, a class of hormones released during stress, impair the pre-attentive prepulse inhibition of the acoustic startle response (PPI), a measure of sensorimotor gating (Richter et al., 2011; Sutherland and Conti, 2011).

The involvement of the N-methyl-D-aspartate subtype of glutamate receptor (NMDAR) activity in the effects of acute stress has been extensively investigated. Acute stress regulates glutamate release and NMDAR expression at the postsynaptic plasma membrane (Popoli et al., 2012). Furthermore, acute stress is a potent modulator of synaptic plasticity. For instance, acute stress impairs long-term potentiation (LTP) in the hippocampus-prefrontal cortex (PFC) pathway (Mailliet et al., 2008; Rocher et al., 2004), a phenomenon dependent on NMDAR (Nicoll and Roche, 2013). Acute stress can also cause deficits in LTP in the amygdala-PFC pathway (Maroun and Richter-Levin, 2003). Nevertheless, the mechanisms that mediate the effect of acute stress on cognition are not fully understood.

The NMDAR co-agonist site is endogenously activated by glycine or D-serine, and is critically involved in cognition (Collingridge et al., 2013). Glycine regulates NMDAR activity in the hippocampus (Bergeron et al., 1998; Martina et al., 2004) and inhibitors of glycine re-uptake enhance cognition in different animal models (Harada et al., 2012; Karasawa et al., 2008; Shimazaki et al., 2010). Importantly, evidence indicates that glycine might me modulated by stress (Elekes et al., 1986). On the other hand, D-serine is the

Abreviations: NMDAR, N-methyl-D-aspartate receptor; PPI, prepulse inhibition of the acoustic startle response; PFC, prefrontal cortex; LTP, long-term potentiation; SR, serine racemase.

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major endogenous co-agonist of the NMDAR in the forebrain (Mothet et al., 2000; Panatier et al., 2006) and is necessary for LTP in the hippocampus (Henneberger et al., 2010; Yang et al., 2003). Moreover, mice lacking serine racemase (SR) activity, the only enzyme that forms D-serine endogenously (Wolosker et al., 1999), present deficits in memory (Basu et al., 2009) and PPI response (Labrie et al., 2009), but the effect of acute stress on D-serine remains unknown. Hence, the question arises whether acute stress affects cognition through regulation of the D-serine pathway.

In this study, we investigated the effect of acute stress on levels of the co-agonists of NMDAR D-serine and glycine in the prefrontal cortex (PFC) and in the hippocampus of mice, two key regions in the stress response (Arnsten, 2009; Maras and Baram, 2012). Mice were subjected to acute restraint stress, a protocol that causes a strong stress response associated to cognitive deficits (Davies et al., 2013; Li et al., 2012). Then, we used the object recognition task to test whether D-serine administration could prevent the effect of stress on memory consolidation. Finally, we investigated if D-serine could prevent the stress-induced impairment in the PPI response.

2. Materials and methods

2.1. Animals

The Committee for the Use of Experimental Animals of our Institution approved all experimental protocols. We made all efforts to minimize animal suffering and the number of animals. We studied a total of 314 C57BL/6 male mice (2–5 months old). Mice were maintained in a temperature- and humidity-controlled room on a 12:12 h light/dark cycle. Food and water were supplied *ad libitum*. Experiments were performed between 9 am and 1 pm, during the light phase.

2.2. Acute restraint stress

Animals were restrained in a ventilated 50 mL Falcon tube for 90 min. Control mice were left in the home cage. The timing of the stress procedure is explained in each experiment.

2.3. Corticosterone measurement

Mice were euthanized immediately after acute restraint stress by lethal injection of ketamine (300 mg/kg i.p.) and blood was immediately collected by cardiac puncture using heparin (40 U/ml) for corticosterone quantification. Plasma was obtained after sample centrifugation for 10 min at 1000 × g and stored at −20 °C until use. Corticosterone levels were determined by means of a radioimmunoassay following manufacturer's guidelines (MP Biomedicals, Solon, OH, USA).

2.4. Drug administration

D-serine (Sigma, MO, USA) was dissolved in 0.9% saline. We injected D-serine (1 g/kg or 100 mg/kg) or saline i.p. in the time points explained in each experiment.

2.5. D-Serine Levels and serine racemase (SR) phosphorylation

We performed euthanasia immediately after the stress protocol or after removal of controls from the home cage. The PFC and hippocampus of each animal were rapidly dissected and immediately stored in liquid nitrogen. Tissue fragments were homogenized at 4 °C in RIPA buffer (Sigma, St. Louis, USA). Total protein concentrations were determined using the Bicinchoninic Acid (BCA) Protein Assay kit (Pierce, Rockfors, USA). We analyzed D-serine and glycine levels by high-performance liquid chromatography (HPLC) as described (Hashimoto et al., 1992; Panizzutti et al., 2001). D-serine and glycine levels were normalized by protein content. We immunoprecipitated SR from the brain homogenates and evaluate phosphorylation in serine residues using immunoblotting. First, we incubated the homogenates (0.5–1 mg of protein/mL) overnight at 4 °C with goat polyclonal anti-SR antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by incubation with protein A/G-agarose beads (1:20; Santa Cruz Biotechnology) for 3 h at 4 °C. The beads were washed 6 times with PBS supplemented with 1% Triton X-100 (PBS-T) and 300 mM NaCl, followed by 3 times with PBS. Immunoprecipitated samples were resolved in 5–15% polyacrylamide gel (BioRad, CA, USA) and transferred to nitrocellulose membranes (Millipore, MA, USA). Membranes were blocked in room temperature with blocking solution (LI-COR Biosciences) followed by overnight incubation at 4 °C with the primary antibodies: mouse monoclonal anti-phosphoserine antibody (1:5000; Sigma) and rabbit polyclonal anti-serine racemase (1:750; Santa Cruz Biotechnology, CA, USA). After incubation and subsequent extensive washing with PBS-T, membranes were incubated with appropriate secondary antibody conjugated with infrared dyes (1:10000–15000; LI-COR Biosciences) for 1 h, and extensively washed with PBS-T. We visualized and quantified the immunoreactive bands using an Odyssey InfraredImaging System (LI-COR Biosciences). Results are presented as the ratio between phosphoserine and serine racemase immunoreactivities, as a percentage of the control group.

2.6. NMDAR Quantitative immunoblotting

To study NMDAR levels, we prepared synaptoneurosomes as described (Hollingsworth et al., 1985). The primary antibodies used were anti-NR2A (1:400, Upstate Cell Signalling Solutions, New York, USA), anti-NR2B (1:1000, Millipore, California, USA), and anti-actin (1:1000, Millipore, California, USA). Immunoreactive bands were visualized and quantified using Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, USA). The relative levels of NR2A and NR2B were calculated as a ratio against actin.

2.7. Microdialysis

We implanted a microdialysis guide cannula (CMA Microdialysis AB, Stockholm, Sweden) in the PFC of adult male mice (20–30 g of body weight). Animals were pretreated with atropine sulfate (0.2 mg/kg i.p.) and anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). Rectal temperature was maintained at 37 °C by keeping the animal on a heating pad (Insight, Ribeirao Preto, Brazil). Nociceptive reflexes, as well as cardiac and respiratory rhythms, were monitored throughout the surgical procedure and additional doses of ketamine and xylazine were administered as needed. In the case of respiratory distress, another atropine sulfate (0.2 mg/kg) injection was given. After a deep level of anesthesia was reached, the animal was placed in a stereotaxic frame (David Kopf Instruments, California, USA) with a topical local anesthetic (xilocaine) applied on the ear bars. We performed a craniotomy unilaterally in the right hemisphere over the PFC (AP: 2.5 mm; ML: 0.6 mm, relative to Bregma; DV: −1.0 mm). Animals received penicillin (22 000 UI/kg, i.m.) and ketoprofen (2 mg/kg, i.m.) immediately after surgery and daily in the following 3 days, and were left undisturbed in the home cages for recovery for 5–10 days. In the day of the experiment we replaced the dummy probe with a 2 mm CMA/7 microdialysis probe (CMA Microdialysis AB) and equilibrated with perfusion fluid CNS (CMA Microdialysis AB) for 2 h at a rate of 1 ml/min. Dialysate samples were collected at 20 min intervals over 60 min (baseline) and then D-serine (1 g/kg, i.p.) was given. After that, dialysate samples were collected at 20 min intervals for a 240 min period. Each dialysate sample was immediately stored at 4 °C and then analyzed using HPLC as described before.

2.8. Prepulse inhibition of the startle response (PPI)

We measured the acoustic startle response using the LE 118-8 Startle and Fear Interface (Panlab, Barcelona, Spain). For acclimatization, mice were left for 10 min in background noise (65 dB), followed by five pulse-alone trials, comprised of a single white noise burst (120 dB, 40 ms). The experimental protocol included 10 blocks of trials consisting of no stimulus (background noise, 65 dB), a startle-pulse (120 dB, 40 ms) alone, or one of two prepulse intensities (72 and 76 dB, 20 ms) presented 100 ms before the startle pulse, in pseudorandom order. Inter-trial intervals were between 12 and 30 s. The peak startle activity for each trial was recorded. We calculated the inhibition of the startle response using the formula: %PPI = 100 − (startle response on prepulse trials/startle response on startle-pulse alone trials) × 100.

2.9. Object recognition task

The object recognition task evaluates recognition memory. In the first day, animals were allowed to freely explore the arena (30 × 30 × 40 cm chamber of black plywood) in the absence of objects for a single 30 min session. On the training day, two identical objects were placed in the arena, equidistantly of its walls, and mice were allowed to explore the environment for 10 min. An experimenter blind to the experimental group of each animal recorded the exploration time for both objects (defined as sniffing or touching the object with the nose and/or forepaws). Simply sitting on top of objects was not counted as exploration time. Animals that did not reach 10 s of exploration of the objects were excluded from the analysis. In the test session, mice explored the environment for 5 min in the presence of one of the familiar objects, as well as a novel object of similar size. The object acting as the familiar object and the one acting as the novel object were counterbalanced across the animals, to ensure that any preference was due to novelty, not to a specific object. The test session was performed 24 h after training, and animals that did not explore the objects for a minimum of 3 s were excluded. The ratio of time of exploration of the novel object/total time of exploration in the test session was used as a measure of recognition memory.

2.10. Statistical analysis

Comparisons between two groups in the analysis of corticosterone, D-serine and glycine levels, SR phosphorylation and NR2a e NR2b expression were performed using a two-tailed Student's *t* test. A one-way repeated measures ANOVA followed by Dunnett's post hoc test was used to evaluate differences between D-serine in different time points and baseline levels in the microdialysis experiment. In the behavioral tests, a two-way ANOVA followed by a Bonferroni post hoc test was used