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Lithium attenuates the proconvulsant effect of adolescent social isolation stress via involvement of the nitrergic system

Shayan Amiri [a,b,c,1], Arya Haj-Mirzaian [a,b,1], Hossein Amini-khoei [a,b], Armin Shirzadian [a,b], Maryam Rahimi-Balaei [d], Ali Razmi [e], Hugo Bergen [d], Mojgan Rastegar [c], Nastaran Kordjazy [a,b], Arvin Haj-Mirzaian [a], Shahram Ejtemai-Mehr [a,b], Ahmad Reza Dehpour [a,b,*]

[a] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, College of Medicine, Faculty of Health Science, University of Manitoba, Winnipeg, Manitoba, Canada
[d] Department of Human Anatomy and Cell Science, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
[e] Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

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ABSTRACT

In this study, we tested whether acute administration of lithium mitigates the deleterious effect of adolescent social isolation stress (SIS) on seizure susceptibility. In comparison with socially conditioned (SC) mice, isolated conditioned (IC) mice exhibited an increase in seizure susceptibility to pentylenetetrazole. Acute administration of lithium (10 mg/kg) reversed the proconvulsant effect of SIS in IC mice, but this effect was not observed in SC mice. Coadministration of subthreshold doses of lithium (3 mg/kg) with nitric oxide synthase (NOS) inhibitors reversed the effect of SIS on seizure susceptibility and decreased hippocampal nitrite levels in IC animals. In addition, a subthreshold dose of a nitric oxide precursor reduced the protective effect of lithium on seizure susceptibility and increased nitrite levels in the hippocampus of IC mice. These results suggest that lithium exerts a protective influence against the proconvulsant effect of adolescent SIS via a nitrergic system that includes activation of neuronal NOS in the hippocampus.

1. Introduction

Experiencing aversive events in early stages of life affects the normal development of the brain and increases the risk of vulnerability to neuropsychiatric disorders in later life [1–4]. Adolescence is considered as a period in which exposure to stressful stimuli (i.e., psychosocial stress) promotes disturbances in mental health including mood and anxiety disorders [5,6]. Social isolation stress (SIS) in adolescent animals that are normally social induces behavioral and neurochemical changes [7]. It has been proposed that SIS is a valid animal model to investigate the neurobehavioral changes in psychiatric disorders that are observed in humans [8,9]. Recently, we showed that applying SIS to adolescent mice is associated with an increase in seizure susceptibility to pentylenetetrazole (PTZ) in adulthood [10]. We also demonstrated that the nitrergic system plays a role as an underlying mechanism in mediating the proconvulsant effect of SIS [10]. A recent study showed that PTZ induces seizure activity and this effect is mediated by overproduction of nitric oxide (NO) in certain areas of the brain [11]. Under physiological circumstances, NO has a modulatory effect on seizure susceptibility, while excessive production of NO in pathological conditions (such as exposure to chronic stress) has been reported to decrease the seizure threshold induced by PTZ [10,11]. Prolonged stressful conditions induce neuronal changes via increase of excitatory amino acids, and consequently, overproduction of nitric oxide (NO) is triggered by over-expression of nitric oxide synthase (NOS), mainly inducible (iNOS) and neuronal (nNOS) isoforms, in different areas of the brain including the hippocampus (HIPP) [12–14]. Accumulating data indicate that HIPP plays an important role in the development of epileptogenesis [15].

Lithium is a well-known mood stabilizer that possesses antioxidant and neuroprotective properties [16]. It has been also used as an antiepileptic agent in preclinical [17,18] and clinical studies [19,20]. Our previous studies using the pentylenetetrazole (PTZ) model of clonic seizures have shown that the anticonvulsant effect of lithium is at least partly mediated through NO [21,22]. Stressful events in early life are associated with an increased risk of epileptogenesis [15], and it has been

Abbreviations: SIS, social isolation stress; PTZ, pentylenetetrazole; SC, social condition; IC, isolation condition; NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible NOS; nNOS, neuronal NOS; HIPP, hippocampus; PND, postnatal day; L-NAME, NG-nitro-L-arginine methyl ester; AG, aminoguanidine; 7-NI, 7-nitroindazole; L-arg, L-arginine; FST, forced swimming test; OFT, open-field test; HBT, hole-board test; i.p., intraperitoneal.

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, P.O. Box: 13145-784, Iran. Tel.: +98 2188973652; fax: +98 2166402569.
E-mail address: dehpour@yahoo.com (A.R. Dehpour).

[1] Please note that the first two authors contributed equally in this work.

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shown that lithium exerts protective properties against the negative impact of stress on the HIPP [16,23]. In addition, previous studies have reported that lithium mitigates the impact of exposure to chronic stress on promoting depressive-like behaviors and aggression and the deficits in memory associated with chronic stress [24–26].

Considering the protective properties of lithium in modulating the negative effects of stress, we examined the effect of lithium on seizure susceptibility in mice exposed to SIS during adolescence. We also investigated the possible involvement of the nitrergic system in mediating the effect of lithium on seizure vulnerability in socially isolated animals.

2. Materials and methods

2.1. Animals

In the current study, male NMRI mice (Pasteur Institute, Tehran, Iran), weighing 10–14 g and on postnatal day (PND) 21-25, were used. Animals were housed under standard conditions (temperature: 23 ± 2 °C; 12-h light–dark cycle; and free access to food and water) for four weeks. Animals were housed under two different conditions: 1) social condition (SC) and 2) isolated condition (IC). Socially conditioned mice were housed in groups (6 mice per cage) in Plexiglas boxes (25 × 25 × 15 cm), and IC mice were housed individually in Plexiglas boxes (24 × 17 × 12 cm). Isolated conditioned mice were housed in a separate room and had olfactory and visual contacts. The cages of IC mice were cleaned weekly by the same experimenter to minimize handling and social contact. All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (HHS publication 85–23, 1985), guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA), and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS). Also, each experimental group contained 6 to 8 animals.

2.2. Drugs

The following drugs were used in this work: lithium chloride (Merck, Darmstadt, Germany), NG-nitro-L-arginine methyl ester (L-NAME), aminoguanidine (AG), 7-nitroindazole (7-NI), L-arginine (L-arg) (Sigma, St Louis, MO, USA) and pentylenetetrazole (PTZ; Sigma, UK). Except for the 7-Nitroindazole, which was suspended in 1% aqueous solution of Tween80, all other drugs were dissolved in saline and were administered in the volume of 5 ml/kg mouse weight. To assess clonic seizure in the subjects, we administered PTZ intravenously (0.5%, i.v.) and all other drugs, intraperitoneally (i.p.). Doses of each drug were chosen according to the pilot treatments, which were published in our previous studies [21,27].

2.3. Open-field test (OFT)

The open-field test was used to elucidate the effects of SIS and treatments on motor function and anxiety behavior [28]. The apparatus consisted of a white opaque Plexiglas box measuring 50 × 50 × 30 cm, which was dimly illuminated. The ground of the box was separated into 16 equal squares. Each mouse was placed gently on the central zone (25 × 25 cm), and its behaviors were recorded by a camera for 5 min and were analyzed by Ethovision software version 8 (Noldus, Netherlands). The surface of the apparatus was cleaned with 70% ethanol after each experiment. The distance moved (horizontal activity), time spent in the central zone, and the number of rearings (vertical activity) were evaluated.

2.4. Hole-board test (HBT)

The hole-board test was used to evaluate the anxiety-like behaviors of animals as described in our previous study [29]. The apparatus was a white Plexiglas panel (50 cm × 50 cm, 2 cm thick) with 16 equal holes each with 3 cm in diameter. The board was positioned 50 cm above the floor. Mice were placed in the center of the board, and the number of head dips was counted in a 5-min period. The apparatus was cleaned with 70% ethanol after each experiment.

2.5. Forced swimming test (FST)

We used FST as a widely used behavioral test in which the prolonged immobility time presents the despair behavior reflecting the depressive-like behaviors [30,31]. Mice were individually placed in an open glass cylinder (diameter: 10 cm; height: 25 cm) containing 19 cm of water at 23 ± 1 °C. Mice were allowed to swim for 6 min, and the immobility time was recorded during the last 4 min of the test. Immobility behavior was considered when the animal remained floating motionless in the water and made only those movements necessary to keep its head above water.

2.6. Splash test

The splash test was carried out by spraying a 10% sucrose solution on the dorsal coat of animal in a familiar cage. The sucrose solution dirtied the coat and induced a grooming behavior. The grooming activity time was recorded for 5 min as an index of self-care and motivational behavior. Moreover, a decrease in grooming activity time is associated with reduced hedonic reactivity in the sucrose preference test and increased immobility in the FST [32–34].

2.7. Determination of clonic seizure threshold

In order to measure the clonic seizure threshold in animals, we used the method that was previously described [21,35]. Briefly, a winged infusion set (30-gauge) was used to infuse the PTZ (0.5%) at a constant rate of 1 ml/min into the tail vein of the freely moving subject. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. The minimal dose of PTZ (mg/kg mouse weight) needed to induce a clonic seizure was considered as the index of seizure threshold. As such, seizure threshold is dependent on the dose and time of PTZ administration.

2.8. Hippocampal nitrite assay

To determine the NO levels in the hippocampus, we measured nitrite levels as the result of the NO end product [36]. The animals were decapitated under mild anesthesia, and then, the hippocampi were dissected on an ice-cold surface and immediately immersed in liquid nitrogen. Tissue homogenates were prepared, and nitrite levels were measured by using a colorimetric assay based on the Griess reaction. Briefly, each well was loaded with 100-μl samples, which were then mixed with 100-μl Griess reagent. Following 10-minute incubation at room temperature, absorbance was measured at 540 nm in an automated plate reader. Concentration of nitrite was determined by reference to a standard curve of sodium nitrite (Sigma, USA) and normalized to the weight of each sample.

2.9. Experiment design and treatments

To determine the effect of SIS on the animal behaviors after exposure to different housing conditions (SC or IC), behavioral experiments were carried out. Firstly, in order to validate the SIS paradigm, we applied a variety of behavioral tests including FST and splash test (for depressive-like behaviors) as well as OFT and HBT (for anxiety-like responses) using different sets of animals for each test. We assessed the effect of SIS on seizure threshold using the PTZ model of clonic seizure.

We then investigated the effect of lithium (3, 5, 10, and 25 mg/kg, i.p., 30 min before tests) on seizure threshold in different sets of SC