Epilepsy & Behavior 41 (2014) 158–163

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Involvement of the nitrergic system in the proconvulsant effect of social isolation stress in male mice

Shayan Amiri [a,b,1], Armin Shirzadian [a,b,1], Arya Haj-Mirzaian [a,b], Muhammad Imran-Khan [c], Maryam Rahimi Balaei [d], Nastaran Kordjazy [a,b], Ahmad Reza Dehpour [a,b], Shahram Ejtemaei Mehr [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, P.O. Box 13145-784, Tehran, Iran
[c] International Campus, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Human Anatomy and Cell Science, College of Medicine, Faculty of Health Science, University of Manitoba, Winnipeg, MB, Canada

ARTICLE INFO

Article history:
Received 26 July 2014
Revised 26 September 2014
Accepted 29 September 2014
Available online xxxx

Keywords:
Social isolation
Nitric oxide
PTZ-clonic seizure model
Mice
Stress

ABSTRACT

Social isolation stress (SIS) in adolescence is accompanied by neurobehavioral disturbances and pathophysiological changes in certain regions of the CNS such as the hippocampus. In this study, we tested whether SIS impacts seizure susceptibility in postnatal male mice due to a role of hippocampal nitric oxide (NO). To do this, we used the pentylenetetrazole (PTZ) model of clonic seizures, open-field test, hole-board test, forced swimming test, and plasma corticosterone assay. We aimed to evaluate if 4 weeks of SIS is capable of decreasing seizure threshold along with altering affective and neuroendocrine responses in isolated conditioned (IC) animals in comparison with socially conditioned (SC) animals. In addition, we applied subeffective doses of NO precursor L-arginine (25, 50, and 100 mg/kg) and NOS inhibitors 7-NI (15 and 40 mg/kg), aminoguanidine (50 and 100 mg/kg), and L-NAME (10 and 15 mg/kg) to both IC and SC groups prior to the determination of seizure threshold. Injection of a single dose of all mentioned drugs did not induce changes in seizure threshold of SC mice. On the other hand, L-NAME and 7-NI, but not aminoguanidine, modulated the proconvulsant effect of SIS, while L-arginine augmented the latter effect. We also measured the hippocampal nitrite levels after the administration of the aforementioned drugs. Social isolation stress increased the nitrite levels in comparison with those in SC mice, whereas 7-NI and L-NAME, unlike aminoguanidine, mitigated the effect of SIS. Additionally, L-arginine boosted the effects of SIS on nitrite production. In summary, we showed that SIS enhanced seizure susceptibility in the PTZ model of clonic seizures through the activation of the nitrergic system in the hippocampus. Also, we proved that nNOS, but not iNOS, accounts for these changes following SIS.

1. Introduction

People with epilepsy (PWE) experience greater psychosocial challenges compared with the general population, thereby contributing to poor quality of life [1]. Among psychosocial problems, stress and social isolation have been reported as the most determinant factors which affect the severity of epilepsy and social functioning of PWE, respectively [2,3]. Previous studies have reported that social isolation stress (SIS) in the adolescent period induces considerable psychobiological abnormalities, neurobehavioral disturbances, and hypothalamic–pituitary–adrenocortical (HPA) axis malfunction [4,5]. In addition, the social isolation paradigm has been suggested as a reliable animal model for the investigation of neurobehavioral changes in psychiatric disorders similarly seen in humans [6]. Under chronic stress circumstances, the neurotoxic action of excitatory neurotransmitters such as glutamate causes an overproduction of nitric oxide (NO) via the excessive activity of nitric oxide synthase (NOS) [7,8].

Nitric oxide contributes to a variety of physiological and pathophysiological processes in the hippocampus (HIPP), such as learning, memory, depression, and seizure susceptibility [9–13]. Among NOS isoforms, both iNOS (inducible NOS) and nNOS (neuronal NOS) have been reported to increase the NO levels in the HIPP in response to stressful paradigms [14,15]. In addition, early stressful life events have negative enduring effects on the HIPP which are relevant to increased susceptibility to seizures in adulthood [16]. Recently, it has been demonstrated that endogenous NO is a key factor for initiation of seizure-like events [17]. In another study, Watanabe et al. showed that elevated NO levels in the murine brain are associated with increased seizure susceptibility in the pentylenetetrazole (PTZ) model of convulsive seizures. They also showed that PTZ-induced convulsive seizure is sensitive to small changes of NO levels in the brain; therefore, it is a valid animal model for the evaluation of epileptic activity [13,18]. Surprisingly, there are few studies about the effects of social isolation,

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.
E-mail addresses: ejtemam@gmail.com, ejtemam@tums.ac.ir (S.E. Mehr).
[1] Please note that the first two authors are considered as the first author.

http://dx.doi.org/10.1016/j.yebeh.2014.09.080
1525-5050/

as a chronic stress model, on seizure vulnerability in animals [19,20]. Therefore, considering the increased activity of NOS by chronic stress in the HIPP (an important part of the limbic system in epileptogenesis) [2,21], we hypothesized that hippocampal NO levels are correlated with seizure vulnerability changes in socially isolated animals. In this study, firstly, we determined whether SIS could induce stress-related behavioral and neuroendocrine changes in animals. Secondly, we investigated the relationship between SIS and seizure susceptibility by using NOS inhibitors such as aminoguanidine (AG) (a specific iNOS inhibitor), 7-nitroindazole (7-NI) (a specific nNOS inhibitor), and NG-nitro-L-arginine methyl ester (L-NAME) (a nonspecific NOS inhibitor) as well as the NO precursor, L-arginine (L-arg). Our aim was to determine whether the nitrergic system is involved in seizure susceptibility as an underlying mechanism.

2. Materials and methods

2.1. Animals

Male NMRI mice (Pasteur Institute, Tehran, Iran), weighing 10–14 g and in the postnatal stage (PND: 21–25), were used throughout the study. Animals were housed under standard conditions (temperature: 22 ± 2 °C, humidity: 50 ± 10%, 12-h light–dark cycle, and free access to food and water) for 28 days in either of 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 isolated conditioned mice were housed individually in Plexiglas boxes (24 × 17 × 12 cm). Wood shavings were used as bedding for animals. 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 (NIH publication # 80-23) 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 and treatments

The following drugs were used in this study: pentylenetetrazole (PTZ), L-arginine, NG-nitro-L-arginine methyl ester (L-NAME), aminoguanidine (AG), and 7-nitroindazole (7NI). The drugs were purchased from Sigma, UK. 7-Nitroindazole was suspended in 1% aqueous solution of Tween 80, and all other drugs were dissolved in saline and were administered in the volume of 10-ml/kg mouse weight. To assess clonic seizures in experimental animals, 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 [22,23].

2.3. Open-field test (OFT)

The open-field test was used to evaluate the locomotion and anxiety behavior of animals in response to SIS. The open-field apparatus was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm) which was dimly illuminated. Each mouse was placed gently on the center square (30 cm × 30 cm), and 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. Each animal was used in only one experiment.

2.4. Hole-board test (HBT)

The hole-board test was used to evaluate the anxiety of subjects [24]. The apparatus consisted of a white Plexiglas square (50 cm × 50 cm) with 16 equidistant holes (3 cm in diameter) and 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. Each animal was used in only one experiment.

2.5. Forced swimming test (FST)

Mice were individually placed in an open glass cylinder (diameter: 10 cm, height: 25 cm) containing 19 cm of water at 25 ± 1 °C [25,26]. 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. Determination of clonic seizure threshold

In order to measure the clonic seizure threshold in mice, we used the method that was previously described. 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 (began with running and then loss of righting ability) 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 [27].

2.7. Hippocampal nitrite assay

To determine the NO levels in the hippocampus, we measured nitrite levels as the result of the NO end product [28]. The animals were decapitated under mild anesthesia, and then the hippocampi were dissected on ice cold surface and immediately immersed in liquid nitrogen. Tissue homogenates were prepared, and nitrite levels were determined by using a colorimetric assay based on the Griess reaction. Initially, 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.

2.8. Corticosterone assay

To assess HPA axis activity in mice, we measured basal and postacute (after 60 min) stress levels of corticosterone (CORT) in plasma using the method which was previously described [29]. Blood samples were centrifuged at 3000 *g* for 10 min at 4 °C, and plasma samples were then stored at −20 °C until the assay day. We evaluated the HPA axis response to acute stress by applying the forced swim stress which is known as a strong stressor for rodents [30]. Corticosterone concentrations were measured by a commercial ELISA kit (Biospes, China).

2.9. Statistical analysis

Comparison between the groups was analyzed using *t*-test and one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant. The factors were housing [social condition (SC) and isolation condition (IC)] and treatments [control: no treatment and treatment: drug-administered animals by a single i.p. injection] for all assessments except CORT values. For the evaluation of CORT, treatment factor was the same as mentioned above, and in the case of housing factors, subjects were divided into four groups: 1) unstressed SC, 2) SC + stress (SC mice which experienced the FST prior to plasma collection), 3) unstressed IC, and 4) IC + stress.