Please cite this article in press as: Haj-Mirzaian A et al. Lithium attenuated the depressant and anxiogenic effect of juvenile social stress through mitigating the negative impact of interlukin-1β and nitric oxide on hypothalamic–pituitary–adrenal axis function. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.12.024

Neuroscience xxx (2015) xxx–xxx

LITHIUM ATTENUATED THE DEPRESSANT AND ANXIOGENIC EFFECT OF JUVENILE SOCIAL STRESS THROUGH MITIGATING THE NEGATIVE IMPACT OF INTERLUKIN-1β AND NITRIC OXIDE ON HYPOTHALAMIC–PITUITARY–ADRENAL AXIS FUNCTION

A. HAJ-MIRZAIAN,[a,b†] S. AMIRI,[a,b†] N. KORDJAZY,[a,b†] M. MOMENY,[a,b] A. RAZMI,[c] M. RAHIMI-BALAEI,[d] H. AMINI-KHOEI,[a,b] A. HAJ-MIRZAIAN,[a,b] H. MARZBAN,[e] S. E. MEHR,[a,b] S. H. GHAFFARI[f] AND A. R. DEHPOUR[a,b]*

[a] *Experimental Medicine Research Center, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

[b] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

[c] *Institute of Medicinal Plants, Academic Center for Education, Culture and Research (ACECR), Supa Boulevard, Pouleh Kordan, P.O. Box 31375-1369, Karaj, Iran*

[d] *Department of Human Anatomy & Cell Science, Faculty of Medicine, University of Manitoba, Room 124, BMSB, 745 Bannatyne Avenue, Winnipeg, MB R3E 0J9, Canada*

[e] *Department of Human Anatomy & Cell Science, Faculty of Medicine, University of Manitoba, Winnipeg, MB R3E 0J9, Canada*

[f] *Hematology, Oncology & Stem Cell Transplantation Research Center, University of Tehran Medical Sciences, Shariaty Hospital, Tehran, Iran*

Abstract—The neuroimmune-endocrine dysfunction has been accepted as one of fundamental mechanisms contributing to the pathophysiology of psychiatric disorders including depression and anxiety. In this study, we aimed to evaluate the involvement of hypothalamic–pituitary–adrenal (HPA) axis, interleukin-1β, and nitrergic system in mediating the negative behavioral impacts of juvenile social isolation stress (SIS) in male mice. We also investigated the possible protective effects of lithium on behavioral and neurochemical changes in socially isolated animals. Results showed that experiencing 4-weeks of juvenile SIS provoked depressive and anxiety-like behaviors that were associated with hyper responsiveness of HPA axis, upregulation of interleukin-1β, and nitric oxide (NO) overproduction in the pre-frontal cortex and hippocampus. Administration of lithium (10 mg/kg) significantly attenuated the depressant and anxiogenic effects of SIS in behavioral tests. Lithium also restored the negative effects of SIS on cortical and hippocampal interleukin-1β and NO as well as HPA axis deregulation. Unlike the neutralizing effects of L-arginine (NO precursor), administration of L-NAME (3 mg/kg) and aminoguanidine (20 mg/kg) potentiated the positive effects of lithium on the behavioral and neurochemical profile of isolated mice. In conclusion, our results revealed that juvenile SIS-induced behavioral deficits are associated with abnormalities in HPA-immune function. Also, we suggest that alleviating effects of lithium on behavioral profile of isolated mice may be partly mediated by mitigating the negative impact of NO on HPA-immune function. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: juvenile social isolation stress, lithium, nitric oxide, interlukine-1β, hypothalamic–pituitary–adrenal axis, depressive-like behaviors.

INTRODUCTION

Depression, a condition accounting for enormous social and financial burden, is characterized by despair behaviors, anhedonia, and depressed mood. Adolescence is a pivotal period for brain development accompanied with cortico-limbic maturation (Sisk and Foster, 2004). Ample evidence indicated that experiencing adversity namely by social stressors negatively alters brain function and leads to the occurrence of psychiatric disorders including depression and anxiety (Andersen and Teicher, 2008; Romeo, 2010). Juvenile social isolation stress (SIS) has been shown to produce behavioral and neurochemical changes in social mammals reminiscent of various symptoms observed in depressed patients (Fone and Porkess, 2008; Nestler and Hyman, 2010). A large body of evidence reported that applying SIS to animals provoked behaviors relevant to depression and anxiety (Grippo et al., 2007; Fone and Porkess, 2008; Amiri et al., 2015b).

*Correspondence to: A. R. Dehpour, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran. Tel: +98-2188973652; fax: +98-2166402569.
E-mail addresses: arya.mirzaian@gmail.com, a-hmirzaian@student.tums.ac.ir (A. Haj-Mirzaian), from.here.till.there@gmail.com (S. Amiri), nastaran.kordjazy@gmail.com (N. Kordjazy), m.momeni@yahoo.com (M. Momeny), a.razmi@yahoo.com (A. Razmi), maryam_rahimib@yahoo.com (M. Rahimi-Balaei), aminikhoyi@gmail.com (H. Amini-Khoei), arvin.mirzaian@gmail.com (A. Haj-Mirzaian), Hassan.Marzban@my.umanitoba.ca (H. Marzban), ejtemam@gmail.com (S. E. Mehr), shghaffari@tums.ac.ir (S. H. Ghaffari), dehpour@yahoo.com (A. R. Dehpour).
† The authors contributed equally in this work.

Abbreviations: AG, aminoguanidine; FST, forced swimming test; GC, glucocorticoid; HBT, hole-board test; HIPP, hippocampus; HPA, hypothalamic–pituitary–adrenal axis; hprt1, hypoxanthine phosphoribosyl transferase1; i.p., intraperitoneal; iNOS, inducible NOS; IC, isolation condition; IL-1β, interlukine-1β; L-arg, L-arginine; L-NAME, NG-nitro-L-arginine methyl ester; NMDA, N-Methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; OFT, open-field test; PFC, pre-frontal cortex; PND, Postnatal day; SC, social condition; SIS, social isolation stress.

http://dx.doi.org/10.1016/j.neuroscience.2015.12.024
0306-4522/

Lithium, a well-known mood stabilizer, has been reported to exert antidepressant properties in preclinical and clinical studies (Austin et al., 1991; Gray and McEwen, 2013). In this context, advantageous effects of lithium on stress-induced psychiatric disorders have been well documented in the literature (Kovacsics and Gould, 2010). Despite several mechanisms of action identified for the therapeutic effects of lithium, the exact mechanisms through which lithium modulates the negative impacts of stress remains elusive. Our previous studies showed that the antidepressant effect of lithium is partly associated with nitric oxide (NO) modulation (Ghasemi et al., 2008, 2009). It has been also demonstrated that lithium exerts anti-inflammatory effects and prevents interleukin-1β (IL-1β) overproduction (Albayrak et al., 2013). On the other hand, it has been proposed that lithium is able to modulate the hypothalamic–pituitary–adrenal (HPA) axis dysregulation in the variety of mood disorders (Bschor et al., 2002; Boku et al., 2009). Evidence is accumulating that nitric oxide synthase (NOS) overactivity, IL-1β overproduction, and HPA-axis dysfunctions are involved in the pathophysiology of psychiatric disorders including depression and anxiety (McEwen, 2005; Dantzer, 2009).

Several lines of research suggested that dysregulation of the neuroimmune-endocrine system is one of the fundamental mechanisms that underlie psychiatric disorders (Pariante and Lightman, 2008). Evidence indicates that experiencing social isolation during adolescence correlates with HPA axis hyper responsiveness and influences brain regions related to mood disorders such as the pre-frontal cortex (PFC) and hippocampus (HIPP) (Tsigos and Chrousos, 2002; Serra et al., 2005; Pariante and Lightman, 2008; Hawkley et al., 2012; Gądek-Michalska et al., 2013a). Disruption of the HPA axis activity after chronic stress exposure might be a result of inflammatory cytokines, including IL-1β and NO overproductions (Gądek-Michalska et al., 2013b). In addition, we have recently shown that inducible NOS (iNOS) activity is associated with co-occurrence of anxiety and depressive-like behaviors following juvenile SIS (Amiri et al., 2015b).

Considering the protective properties of lithium in modulating the negative effects of stress and also the putative role of glucocorticoids (GCs), NO, and IL-1β in depression and anxiety development, we aimed to investigate the effect of lithium on the depressive and anxiety-like behaviors in animals exposed to juvenile SIS paradigm using behavioral tests. Although the antidepressant and anxiolytic effects of lithium has been established by past studies, the possible effect of this drug on social stressors during adolescence, which is a critical period in developing the brain of animals, has not been illustrated yet. Also, our goal is to evaluate the possible involvement of HPA axis, nitrergic system, and IL-1β in mediating the protective effect of lithium on mood disorders in socially isolated mice.

EXPERIMENTAL PROCEDURES

Animals

In the current study, we used male NMRI (Naval Medical Research Institute) mice (Pasteur Institute, Tehran, Iran), weighing 10–14 g and on postnatal day (PND) 21–25. Each experimental group consisted of 6–8 animals. The 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, social condition (SC), and isolated condition (IC). Socially conditioned mice were housed in groups (six mice per cage) in Plexiglas boxes (25 × 25 × 15 cm), while 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 contact. The cages of IC mice were cleaned weekly by the same experimenter to avoid minimum handling and social contact. All procedures in our study were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 or the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, and we also adhered to institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS).

Drugs

The following drugs were used in this study: lithium chloride (Merck, Darmstadt, Germany), NG-nitro-L-arginine methyl ester (L-NAME, non-selective NOS inhibitor), aminoguanidine (AG, selective iNOS inhibitor), and L-arginine (L-arg, NO precursor) (Sigma, St Louis, MO, USA). All drugs were dissolved in sterile saline and were administered in a volume of 5 ml/kg mouse weight. All drugs were administered intraperitoneally (i.p.) to animals. Doses and administration time of each drug were chosen according to our pilot treatments and previous published studies (Ghasemi et al., 2008; Amiri et al., 2015b). The efficacy and bioavailability of applied drugs have been documented in the literature suggesting the ability of them to pass through the blood–brain barrier (Gyulai et al., 1991; Sakuma et al., 1992; Cockroft et al., 1996; Tsuji et al., 2000).

Forced swimming test (FST)

We used FST as a behavioral test in which the prolonged immobility time presents the despair behavior reflecting the depressive-like symptoms (Porsolt et al., 1977). Mice were individually placed in an open glass cylinder (diameter: 10 cm, height: 25 cm) containing 19 cm water (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 water and made only those necessary movements to keep its head above water.

Splash test

In rodents, lack of motivation to engage in self-care can be assessed by the splash test. In this test, grooming behavior of mice, which can be considered as an indirect measure of palatable solution intake was

Please cite this article in press as: Haj-Mirzaian A et al. Lithium attenuated the depressant and anxiogenic effect of juvenile social stress through mitigating the negative impact of interlukin-1β and nitric oxide on hypothalamic–pituitary–adrenal axis function. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.12.024