Behavioural Brain Research 250 (2013) 123–132

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Adolescent fluoxetine treatment decreases the effects of neonatal immune activation on anxiety-like behavior in mice

Jafar Majidi-Zolbanin[a], Maryam Azarfarin[b], Hanieh Samadi[b], Mohsen Enayati[c], Ali-Akbar Salari[a,b,d,e,*]

[a] Laboratory of Immunology, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Laboratory of Physiology, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Department of Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

HIGHLIGHTS

- We investigated for the first time effect of neonatal infection on anxiety in mice.
- Neonatal immune activation increased anxiety levels in adult male and female mice.
- The open field, elevated plus-maze and light–dark box were used in this study.
- Adolescent fluoxetine reduced the effects of neonatal infection on anxiety in mice.

ARTICLE INFO

Article history:
Received 28 March 2013
Received in revised form 29 April 2013
Accepted 3 May 2013
Available online 10 May 2013

Keywords:
Neonatal infection
Sex differences
Adolescent
Fluoxetine
Anxiety
HPA axis

ABSTRACT

Experimental studies have shown conflicting effects of neonatal infection on anxiety-like behaviors and hypothalamic-pituitary-adrenal (HPA) axis activity in adult rats. We investigated for the first time whether neonatal exposure to lipopolysaccharide (LPS) is associated with increased levels of anxiety-like behaviors in mice. Moreover, there have been several studies showing that adolescent fluoxetine (FLX) treatment can influence HPA axis development and prevent occurrence of psychiatric disorders induced by common early-life insults. In the present study, we also investigated the effects of adolescent FLX exposure following neonatal immune activation on anxiety-like behavior in mice. Neonatal mice were treated to LPS (50 μg/kg) or saline on postnatal days (PND) 3 and 5, then male and female mice of both neonatal intervention groups received oral administration of FLX (5 and 10 mg/kg/day) or water via regular drinking bottles during the adolescent period (PNDs 35–65). The results showed that postnatal immune challenge increased anxiety-like behavior in the open field, elevated plus-maze and light–dark box in adult mice (PND 90). Furthermore, the adolescent FLX treatment inhibited the anxiety-like behavior induced by neonatal infection in both sexes. However, this study indicates the negative effects of the FLX on normal behavioral symptoms in male control mice. Taken together, the current data provide experimental evidence that neonatal infection increases anxiety levels in male and female mice in adulthood. Additionally, the findings of this study support the hypothesis that an early pharmacological intervention with FLX may be an effective treatment for reducing the behavioral abnormalities induced by common early-life insults.

Abbreviations: HPA, hypothalamic-pituitary-adrenal; LPS, lipopolysaccharide; COR, corticosterone; SSRI, selective serotonin reuptake inhibitor; FLX, fluoxetine; PND, postnatal day; OF, open field; IZT, inner zone time; IZE, inner zone entries; EPM, elevated plus-maze; OAT, open arm time; OAE, open arm entries; LMA, locomotor activity; LDB, light–dark box; LD, latency of entry into the dark compartment; LL, latency of entry into the light compartment; LCT, light compartment time; LCE, light compartment entries; ANOVA, analysis of variance; LPS vs. saline, pre-treatment; FLX vs. vehicle, post-treatment; CON animals, mice treated to saline during neonatal; LPS animals, mice treated to LPS during neonatal; Poly I:C, polyinosinic–polycytidylic acid; PFC, prefrontal cortex; 5-HT1A, 5-hydroxytryptamine1A; GR, glucocorticoid receptors; BDNF, brain derived neurotrophic factor.

* Corresponding author at: Laboratory of Physiology, Drug Applied Research Center, Tabriz University of Medical Sciences, P.O. Box 51656-65811, Tabriz, Iran. Tel.: +98 919 4099673; fax: +98 919 4099673.
E-mail address: aa.salari@yahoo.com (A.-A. Salari).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.05.003

1. Introduction

Anxiety is one of the most common neuropsychiatric disorders, which can lead to reduced quality of life and affect cognitive functioning in humans. This disorder is a complex emotional condition related to heightened physiological and behavioral arousal after exposure to stressful events. Gender differences have long been recognized in basic neural processes, behaviors and in response to antipsychotic drugs [1,2]; however the factors mediating these differences are not well understood. The real causes of anxiety-like behaviors are still unknown, but could be rooted in multifactorial dysfunctions including both environmental and genetic disorders [3]. In support of this hypothesis, we and others have provided important experimental evidence about the neurodevelopmental origins of anxiety disorder in rodents [4–6]. In this context, previous studies have shown that neonatal infection resulted in long-lasting physiological consequences and abnormal hypothalamic-pituitary-adrenal (HPA) axis activity in response to stress in adulthood [7,8]. Furthermore, the studies conducted to date have shown conflicting findings of the effects of neonatal exposure to lipopolysaccharide (LPS) on the development of anxiety-related behaviors (e.g., increase [9,10], decrease or no alteration [11–13]) in rats. It has been reported that an immune challenge stimulates production of pro-inflammatory cytokines and corticosterone (COR) in neonate rodents [14]. These agents play an important role in regulation of neuronal functions and have proposed to have the adverse impacts on fetal brain development [4].

The development of the therapeutic strategies against neuropsychiatric diseases, such as anxiety and depression with the origin of genetic disorders is an important topic for researchers. While the majority of studies have investigated the effects of antidepressant and antipsychotic drugs for the treatment of psychiatric illnesses during adulthood, a few studies have recently focused on the effects of early pharmacological intervention during the prodromal phase of neuropsychiatric disorders with neurodevelopmental origin, like schizophrenia and depression. Serotonergic agents are known as a first-line treatment option for human anxiety disorder and play a crucial role in cell proliferation, neurogenesis, synaptogenesis and survival of newborn neurons in the brain [15]. It is also well documented that selective serotonin reuptake inhibitors (SSRIs), like fluoxetine (FLX), are effective in the treatment of anxiety disorders in humans [16]. Moreover, several reports have shown the protective effects of antidepressant drugs, like FLX, against the emergence of behavioral abnormalities in the offspring following prenatal and neonatal immune activation, maternal stress and neonatal maternal separation [7,17–28].

To date, all studies have investigated the impacts of postnatal immune challenge on anxiety-like behaviors in rats exclusively. Hence, this can be a problematical issue given that many researchers have reported a series of differences in a broad range of neuropsychiatric disorders, like anxiety, in rats and mice [29]. Therefore, the aim of the present study was to investigate the effects of neonatal immune activation on anxiety-like behaviors in adult mice, and then whether a chronic regimen of FLX can decrease the effects of neonatal infection on anxiety-like behavior in male and female mice.

2. Materials and methods

2.1. Ethics

All procedures described in this study had been approved by the Research and Ethics Committee of Tabriz University of Medical Sciences (GN-90-32), and are in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (NIH; Publication No. 85-23, revised 1985).

2.2. Subjects

Adult NMRI mice, 9–10 weeks old were purchased from the animal house of Pasteur Institute of Iran and were maintained in the standard polycarbonate cages in a room with a 12:12 h light/dark cycle (lights on at 8:00 A.M.), controlled temperature ($23 \pm 1\,^{\circ}C$) and had free access to food and water. These conditions were kept as a standard housing condition in all stages of experiments. After a 2-week period of acclimatization to the new animal holding room, in order to facilitate of mating, male and female mice were kept together one-by-one in a cage. Successful mating was confirmed via the presence of vaginal plug and that day was referred to as gestational day 0 [4]. Once a pregnant female was identified, it was removed from the breeding cage and housed individually in a standard cage. All pregnant mothers were allowed for normal delivery and the day of birth was designated as postnatal day (PND) 0. One day after birth, all litters were culled to 6 pups per mother (3 female and 3 male). On the day 21, litters were weaned by removal of the mother and then were housed with same sex litter-mates (3 animals per cage). A total of fifty litters were used during this study in four stages, each of which included 13 or 11 litters. Only one mouse per sex per dam was used for each of the experiment to avoid the litter-effect.

2.3. Neonatal treatment

A summary of the experimental design is shown in Fig. 1. The pups were divided into 2 main clusters (each of which consisted of 6 main groups in experimental group 2; 8 male and 8 female mice in each group): Cluster 1, saline injected mice and Cluster 2, LPS injected mice. In the present work, the dams were removed from their pups for approximately 10 min and the pups were weighed and received subcutaneously (in the interscapular region) injection of LPS (*Escherichia coli* 0111:B4, Sigma Co, USA; 50 μg/kg) or vehicle solution (1 ml/kg) on the PNDs 3 and 5. The dose and timing of LPS treatment were chosen based on the previous studies [9,10]. LPS was dissolved in saline (0.9% NaCl) and injections were performed between 14:00 and 15:00 P.M. Each injection was performed through a needle (27-gauge) connected by polyethylene tubing to a 10-μl Hamilton syringe. Newborn mice were returned to their housing immediately following saline or LPS administration.

2.4. Fluoxetine treatment

The adolescent FLX hydrochloride (0060108; TEMAD Co., Tehran, Iran) treatment (5 and 10 mg/kg/day) was initiated on the PND 35 and lasted for 30 days (PND 65) [7]. In order to prevent the possible confounding factors of isolation housing and oral gavage or injection (as an invasive or stressful procedure), the mice were kept in groups of 3 animals in the cages and the FLX was administered via regular drinking bottles (the bottles were opaque to protect the drug from light) in mice during the adolescent period [21]. Before the experiment began, we recorded the average water consumption according to the body weight per cage per day for each mouse in the laboratory. Furthermore, FLX was dissolved in the drinking water and its concentration was calculated at four-day intervals according to the average liquid consumption and body weight per cage in our laboratory. The mice did not receive any source of water except for the drinking water containing FLX solutions. Therefore, they were motivated by thirst to drink the drug solutions. To examine possible effects of the chronic FLX treatment, the liquid consumption of the control mice was measured every 4 days which showed no significant change in the liquid intake compared with the drug-receiving mice.

2.5. Behavioral tests

Behavioral assessments began at the PND 90, 25 days after the termination of drug treatment. The following behavioral tests are widely accepted models for measuring anxiety-like behaviors in mice. All behavioral parameters were recorded by blind observers to the treatment. In addition, all behavioral tests were conducted in a quiet room during the light period (between 13:00 and 17:00 h) under bright and moderate illumination (150 lux) and the mice were kept in the room for at least 1 h before the assessment. At the end of each test session, the arena and the objects were carefully cleaned with 70% ethanol and the cage was transferred back to the colony room. In all experiments, each male or female mouse was tested only once in the one test. Furthermore, the female mice were not monitored for estrus cycle since it is an invasive and stressful procedure that can impose dramatic changes in the animal responses to other stressors [7]. In addition, it was not possible to provide the adult male mice with a stressor that completely mimics the stress applied to female mice from the repeated samplings of vaginal smears.

2.5.1. Open field

The open field (OF) apparatus consisted of a white wooden box (40 cm × 40 cm × 20 cm) with 16 squares (10 cm × 10 cm; 12 outer and 4 inner) which was directly illuminated by a 100 W bulb placed 90 cm above the center of the apparatus floor. The test period was initiated when a single mouse was placed in the middle of the apparatus and allowed to move freely for 5 min, and an observer recorded the time spent and number of entries (an entry was defined as all four paws) to the inner squares. Decreased the time spent (IZT) and number