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Sex influences in behavior and brain inflammatory and oxidative alterations in mice submitted to lipopolysaccharide-induced inflammatory model of depression

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ABSTRACT

Peripheral inflammation induced by lipopolysaccharide (LPS) causes a behavioral syndrome with translational relevance for depression. This mental disorder is twice more frequent among women. Despite this, the majority of experimental studies investigating the neurobiological effects of inflammatory models of depression have been performed in males. Here, we sought to determine sex influences in behavioral and oxidative changes in brain regions implicated in the pathophysiology of mood disorders (hypothalamus, hippocampus and prefrontal cortex - PFC) in adult mice 24 h post LPS challenge. Myeloperoxidase (MPO) activity and interleukin (IL)-1β levels were measured as parameters of active inflammation, while reduced glutathione (GSH) and lipid peroxidation as parameters of oxidative imbalance. We observed that male mice presented behavioral despair, while females anxiety-like alterations. Both sexes were vulnerable to LPS-induced anhedonia. Both sexes presented increased MPO activity in the PFC, while male only in the hippocampus. IL-1β increased in the PFC and hypothalamus of animals of both sexes, while in the hippocampus a relative increase of this cytokine in males compared to females was detected. GSH levels were decreased in all brain areas investigated in animals of both sexes, while increased lipid peroxidation was observed in the hypothalamus of females and in the hippocampus of males after LPS exposure. Therefore, the present study gives additional evidence of sex influence in LPS-induced behavioral alterations and, for the first time, in the oxidative changes in brain areas relevant for mood regulation.

1. Introduction

Depression is a common chronic-recurrent mental disorder. Recently, the World Health Organization reported that globally > 300 millions of people of all ages suffer from depression, highlighting depression as the leading cause of disability worldwide (WHO | Depression, 2015). Depression causes great social and economic costs. For example, in the United States a survey revealed that 8.3% of all years lived with disability (YLDs) were associated to depression, with an enormous economic burden in the order of 210.5 billions of dollars each year (Greenberg et al., 2015). Furthermore, there is a well-established overall gender difference in the prevalence of depression, with women outnumbering men 2:1. Additionally, the onset of depression in women occurs at younger ages with women also presenting more recurrent episodes (Schuch et al., 2014).

In the last decades, compelling evidences revealed an important contribution of immune-inflammatory alterations to the pathophysiology of depression (Miller et al., 2009; Rosenblat et al., 2014). Indeed, recent meta-analyses have confirmed that depressed patients present increased serum levels of inflammatory markers, such as interleukin-1β (IL-1β), IL-6, tumor necrosis factor (TNF)-alpha and IL-10, and markers of cell activation, such IL-2 receptors (sIL-2Rs) and neopterin (Farooq et al., 2016; Köhler et al., 2017). Furthermore, the systemic injection of cytokines or of the bacterial endotoxin lipopolysaccharide (LPS) induce depressive symptoms in health humans and depressive-like behavior in rodents (Suarez et al., 2004; Vogelzangs et al., 2016).

In preclinical approaches, LPS-based models of depressive-like

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behavior are accompanied by time-dependent alterations related to sickness behaviors (behavioral inhibition, anorexia, weight loss, fatigue, hyperalgesia, malaise symptoms, neurocognitive impairment and anxiety) (Dantzer et al., 2008; Maes et al., 2012) and depressive-like behavior. In this regard, some studies (Custódio et al., 2013; Dantzer et al., 2008; Frenois et al., 2007) have demonstrated that most behavioral alterations elicited by the acute (1.5–2 h) exposure to LPS compose the spectrum characteristic of sickness behavior, in which a marked pyrexia, anorexia and locomotor inhibition occurs. On the other hand, 24 h later, the emergence of depressive-like behavior characterized by despair-, anhedonia- and anxiety-like behaviors takes place. Twenty-four h after LPS exposure motor activity, food and drink consumption returns to normal (Dantzer et al., 2008).

The influence of sex in the behavioral responses to LPS is not fully understood. In this regard, there are studies pointing to a greater sensibility of female animals to LPS and cytokine effects in some behavioral aspects, such as sexual activity as well as sucrose and food consumption (Avitsur and Yirmiya, 1999; Merali et al., 2003). On the other hand, other studies reported better coping strategies of females in despair-like conditions, such as forced swimming test (Pitychoutis et al., 2009). Furthermore, the findings in the literature about sex influences in LPS-induced behavioral effects changes markedly accordingly to the dose of LPS used, time of assessment and murine specie tested (Badalà et al., 2008; Cai et al., 2016).

It is well estabished that depression is accompanied by increased levels of reactive oxygen- (ROS) and nitrogen- (RNS) species, such as superoxide, peroxynitrite and hydrogen peroxide, and oxidative damage (Maes et al., 2011a). These pro-oxidative alterations are triggered in some patients by inflammation and mitochondrial metabolic processes. In clinical samples, some studies pointed that sex could be a determining factor for ROS and RNS levels in health subjects (Bellanti et al., 2013; Massafra et al., 2002), however little is known about the influence of sex in a depressive subset of patients, that is, those patients with depression induced by inflammatory alterations. Also, regarding animal models, several studies demonstrated the contribution of oxidative stress in the molecular signature of depressive-like alterations (Lucca et al., 2009; Zhang et al., 2009). However, most of them were conducted in male animals, thus not assessing the possible influence of sex in the oxidative response triggered by depressive-like conditions induced by inflammatory challenge. This issue regarding sex influences in depression deserves a better comprehension, since it will contribute to the development of personalized treatments against this mental disorder.

Therefore, taking into account the important and poorly understood influence of sex in the manifestation of depressive-like phenomenology by LPS immune challenge, in this study, we investigated the behavioral (despair-, anhedonia- and anxiety-like) alterations in male and female mice 24 h post-LPS administration, a time related to the emergence of depressive-like phenotype without locomotor bias (Custódio et al., 2013). Also, we evaluated the influence of sex in brain inflammatory alterations, namely myeloperoxidase (MPO) activity and IL-1β, and, to our knowledge for the first time, in the oxidative stress parameters reduced glutathione (GSH) and lipid peroxidation in relevant brain areas for mood regulation: prefrontal cortex (PFC), hippocampus and hypothalamus.

2. Materials and methods

2.1. Drugs

Lipopolysaccharide (LPS) from *Escherichia coli*, strain 055:B5 (Sigma e Aldrich Corp., St Louis, (USA) was used. The drugs were made up freshly for the study. All other chemicals used were of analytical grade.

2.2. Animals

Male and female adult Swiss mice (weighing 25 to 30 g) obtained from the Animal house of the Federal University of Ceara were used. Animals were housed 10 per cage under standard polycarbonate cages (42 × 20.5 × 20 cm) and at normal environmental conditions (22 ± 1 °C, humidity of 60 ± 5%; reversed 12 h light cycle/darkness with lights on at 18:00) with access to food (FRILAB Mouse II, FRIRibe) and water ad libitum. All experimental procedures were performed between 8:00 AM and 02:00 PM and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH, 2011) and the Brazilian law for scientific use of animals from the Brazilian College of Animal Experimentation (COBEA). This research protocol was approved by the local ethics committee of the Federal University of Ceara.

2.3. Experimental protocol

The animals were randomly divided into four experimental groups: male LPS-challenged group, male control-group, female LPS-challenged group, female control-group (n = 24 mice/group). LPS-challenged groups received an intraperitoneal injection of 0.5 mg/kg LPS dissolved in 0.2 ml sterile endotoxin-free SF 0.9% - saline (as vehicle). Control animals received saline, 0.1 ml/10 g weight. Twenty-four hours after LPS or saline exposure, the behavioral tests and sample collection were performed. The behavioral tests were conducted in the order of the less stressful to the more stressful, i.e., open field, elevated plus maze and forced swimming test. A different set of animals was used to perform sucrose preference test due the potential stressful conditions of food and water deprivation involved in this test. In all behavioral determinations, two raters blinded to the experimental treatment performed the tests. Two independent experiments were performed to ensure the reproducibility of the data. To avoid some potential confounding effect of the behavioral testing in the neurochemical parameters, a different set of animals was used to the removal of brain structures. The animals were killed by decapitation, and the brain areas were quickly dissected namely prefrontal cortex (PFC), hippocampus and hypothalamus. All samples were immediately stored −70 °C until assay. The dose of LPS used and the time of assessment was based on previous studies demonstrating depressive-like behavior and neuroinflammatory alterations induced by LPS in mice (Custódio et al., 2013; Frenois et al., 2007).

2.4. Behavioral determinations

2.4.1. Forced swimming test (FST)

The animals were placed individually in an acrylic cylinder (25 cm of height × 10 cm of diameter) containing 8 cm of water at 24 °C. After a habituation period of 1 min, the immobility time (in seconds) of the animals was evaluated for 5 min. Immobility was defined as the absence of targeted escape behavior, such as swimming, jumping, lifting, smell or diving (Porsolt et al., 1977). Any mouse seeming to have trouble keeping its head out of the water was removed from the cylinder and excluded from the analysis. In this study, two experienced evaluators blinded to the treatment group independently assessed the mice behavior.

2.4.2. Sucrose preference test (SPT)

The test was performed as described previously (Mao et al., 2014). Briefly 72 h before testing, rats were trained to adapt sucrose solution at 10% (w/v). Two sucrose solution bottles were placed in each cage, and 24 h later one of them was replaced by a bottle with water for 24 h. After adaptation, mice were deprived of food and water for 24 h. The test section was conducted to 9:00 AM in which the rats were housed in