Journal of Psychiatric Research 94 (2017) 47–53

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

The impact of chronic mild stress on long-term depressive behavior in rats which have survived sepsis

Amanda V. Steckert [a], Diogo Dominguini [a], Monique Michels [b], Helena M. Abelaira [a], Débora B. Tomaz [a], Beatriz Sonai [b], Airam B. de Moura [a], Danyela Matos [a], Júlia B.I. da Silva [a], Gislaine Z. Réus [a], Tatiana Barichello [a, c, d, e], João Quevedo [a, c, d, e], Felipe Dal-Pizzol [b, *]

[a] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[b] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[c] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UT Health), Houston, TX, USA
[d] Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UT Health), Houston, TX, USA
[e] Neuroscience Graduate Program, The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX, USA

ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form
22 May 2017
Accepted 15 June 2017

Keywords:
Brain
Inflammation
Oxidative damage
Sepsis
Sickness behavior

ABSTRACT

The present study was created to investigate the effects of chronic mild stress (CMS) on the depressive behavior and neurochemical parameters of rats that were subjected to sepsis. Wistar rats were subjected to a CMS protocol, and sepsis was induced by cecal ligation and perforation (CLP). The animals were then divided into 4 separate groups; Control + Sham (n = 20), Control + CLP (n = 30), CMS + Sham (n = 20) and CMS + CLP (n = 30). Body weight, food and water intake and mortality were measured on a daily basis for a period of 10 days after the induction of sepsis. Locomotor activity, splash and forced swimming tests were performed ten days after CLP. At the end of the test period, the animals were euthanized, and the prefrontal cortex and hippocampus were removed to determine the levels of cytokines and oxidative damage. Our results show that there was no significant interaction between CMS and CLP in relation to locomotor activity and the forced swimming test. However, we did observe a significant decrease in total grooming time in the Control + CLP and CMS + Sham groups, with the CMS + CLP group showing behavior similar to that of the control animals. This was found to be related to a decrease in the levels of brain cytokines, and not to oxidative damage parameters. Collectively, our results suggest that a previous stress caused by CMS can protect the brain against the systemic acute and severe stress elicited by sepsis.

1. Introduction

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). Inflammatory response that is evoked during sepsis may lead to acute and long-term brain dysfunctions that are associated with higher rates of mortality and morbidity (Wilson and Young, 2011; Steckert et al., 2013; Bozza et al., 2013). However, the mechanisms of action that drive these dysfunctions are poorly understood.

Systemic inflammation can induce an adaptive response collectively known as sickness behavior (SB) in animals (Dantzer, 2009; Hennessy et al., 2014), and a similar response is also seen in humans (Lekander et al., 2016). In this context, sepsis-associated brain dysfunction (both acute and chronic) does not appear to fall within the spectrum of adaptive stress response, since it is associated with neuronal death and cognitive impairments (Bozza et al., 2013). Reactions to aberrant stress constitute a group of responses that are somehow deficient or exaggerated in certain individuals.

* Corresponding author. Universidade Do Extremo Sul Catarinense, Criciúma, 88806-000, SC, Brazil.
E-mail address: fdpizzol@gmail.com (F. Dal-Pizzol).

http://dx.doi.org/10.1016/j.jpsychires.2017.06.006
0022-3956/

Responses that are typically adaptive under normal circumstances become maladaptive (Singer et al., 2004), and have been proposed as a trigger for brain dysfunction (Maclullich et al., 2008).

It has been demonstrated that aberrant stress responses can be elicited more robustly in the presence of a vulnerable brain. Experimentally, this was done using the ME7 model of prion disease that induces neurodegeneration, and also by exposing these animals to low doses of systemically administered lipopolysaccharide (LPS) (Murray et al., 2011; Griffin et al., 2013). In previous studies involving vulnerable brain, low doses of systemic LPS were able to induce a robust brain inflammatory response and cognitive deficits, mainly by priming microglia and astrocytes (Cunningham et al., 2005; Hennessy et al., 2015). This experimental model is clinically relevant, since prior cognitive impairment is one of the most important risk factors for acute brain dysfunction (Davis et al., 2015). However, it is important to distinguish between severe systemic inflammation, such as the type that occurs during sepsis which leads to brain dysfunction in critically ill patients, and the types that are seen during milder infections, which are sufficient to cause delirium in the aged and demented population. The ME7 model actually mimics this last condition. In addition, not only is prior cognitive impairment associated with brain dysfunction in the context of critical care, but also in less severe forms of brain dysfunction, such as those found in major depression (Xiong et al., 2006; Foss-Nieradko et al., 2012; Stenman et al., 2013).

There is currently nothing in the literature describing the possible mechanism that may explain the interaction between these less severe forms of brain dysfunction and severe systemic inflammation. We have previously characterized depressive behaviors in rats that have survived sepsis (Tuon et al., 2007), thus in this study, we used the chronic mild stress (CMS) paradigm as a model of major depression (Hill et al., 2012) and characterized its effects in relation to acute (mortality and SB) and long-term (depressive behavior, brain inflammation and oxidative damage) outcomes after severe sepsis. We hypothesized that CMS would negatively impact outcomes in septic animals.

2. Materials and methods

2.1. Animals

Male Wistar rats (60 days old, weighting 220–310g, n = 100) were obtained from Universidade do Extremo Sul Catarinense (UNESC) breeding colony. Animals were caged in groups of 5 with water and food *ad libitum* and were maintained on a 12 h light–dark cycle (lights on at 7:00 a.m.), at a temperature of 23 °C ± 1 °C. These conditions were maintained constant throughout the experiments. All experimental procedures were performed in accordance with the approval of the local Ethics Committee of Animals Use (Protocol 001/2015-1) and conformed to international regulations.

2.2. Experimental procedure

The experimental design is shown in Fig. 1 (supplementary material).

2.3. Chronic mild stress protocol

Chronic mild stress (CMS) protocol was adapted from Gamaro et al. (2003) and Garcia et al. (2009). Animals were divided in control (n = 50) and CMS (n = 50) groups. The stressors used in this protocol were: (a) 24 h of water deprivation; (b) 24 h of food deprivation; (c) flashing light for 3 h; (d) isolation (1–3 days); (e) 2 h of restraint, and (f) 2 h of restraint at 4 °C. Animals were submitted to stressors during 40 days. The distribution of stressors during the 40 days is summarized in Table 1 (supplementary material).

To evaluate the efficacy of CMS induction anhedonia was determined as the amount of sweet food consumption after CMS induction. Briefly, rats were placed in a box (40 × 15 × 20 cm) divided into 9 equal rectangles. Ten Froot Loop(s) (Kellogg's®) were placed in one extremity of the box. In the training trials, rats were exposed to the environment for 3 min, once a day, during 5 consecutive days to become familiarized with the food. After, animals were exposed to 2 test sessions (3 min each, on consecutive days) during which the number of ingested Froot Loop(s) was measured (Gamaro et al., 2003; Frey et al., 2006). Anhedonia test took 7-days in total, and after this time frame sepsis was induced as described below.

2.4. Sepsis induction

Rats were subjected to cecal ligation and perforation (CLP), as previously described by Fink and Heard (Fink and Heard (1990). Briefly, animals were anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under aseptic conditions, a 3-cm midline laparotomy was performed to allow exposure of the cecum with the adjoining intestine. The cecum was tightly ligated with a 3.0-silk suture at its base, below the ileocecal valve, and perforated once with a 14-gauge needle. The cecum was then gently squeezed to extrude a small amount of feces from the perforation site, returned to the peritoneal cavity, followed by the closure of the laparotomy with 4.0-silk sutures. The rats were resuscitated with normal saline (50 ml/kg subcutaneously) immediately and 12 h after CLP. All animals were returned to their cages with free access to water and food. In the sham-operated group, the rats were submitted to all surgical procedures, but the cecum was neither ligated nor perforated. After surgery, both groups received 30 mg/kg ceftriaxone and 25 mg/kg clindamycin subcutaneously every 6 h for a total of 3 days. Rats were divided in 4 experimental groups: 1) Control + Sham (n = 20), 2) Control + CLP (n = 30), 3) CMS + Sham (n = 20), and 4) CMS + CLP (n = 30).

2.5. Sickness behavior and survival studies

SB was assessed by changes in body weight, water and food intake, and locomotor activity (Dantzer, 2001; Dantzer and Kelley, 2007; Dantzer et al., 2008), daily for 8 days after sepsis induction in all groups. Additionally, animals were observed at regular intervals (24 h) to the determination of mortality until 10 days after sepsis.

2.6. Depressive behavioral tests

Ten days after sepsis induction depressive-like behavior was assessed in survivors animals: Control + Sham (n = 15), Control + CLP (n = 6), CMS + Sham (n = 13), and CMS + CLP (n = 8). This time point was chose since we had previously demonstrated that depressive-like behavior was more evident 10 days after sepsis induction (Tuon et al., 2007).

2.7. Exploratory activity in the open field apparatus

The test was performed in a 40 × 60 cm open field surrounded by 50 cm high walls. The floor of the apparatus was constructed with wood and divided into 9 equal rectangles by black lines. The rats were gently placed in one of the corner squares at the start of the test and crossings of the black lines and rearings (exploratory