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Time course of the effects of lipopolysaccharide on prepulse inhibition and brain nitrite content in mice

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ABSTRACT

The systemic administration of lipopolysaccharide (LPS) induces time-dependent behavioral alterations, which are related to sickness behavior and depression. The time-course effects of LPS on prepulse inhibition (PPI) remain unknown. Furthermore, the time-dependent effects of LPS on central nitrite content had not been investigated. Therefore, we studied alterations induced by single LPS (0.5 mg/kg, i.p.) administration to mice on parameters, such as PPI, depressive- and anxiety-like behaviors, working memory, locomotor activity and motor coordination, 1.5 and 24 h post-LPS administration. IL-1β and TNFα in the blood and brain as well as brain nitrite levels were evaluated in the prefrontal cortex (PFC), hippocampus (HC) and striatum (ST). An overall hypolocomotion was observed 1.5 h post-LPS, along with depressive-like behaviors and deficits in working memory. Increments in IL-1β content in plasma and PFC, TNFα in plasma and decreases in nitrite levels in the ST and PFC were also verified. Twenty-four hours post-LPS treatment, depressive-like behaviors and working memory deficits persisted, while PPI levels significantly reduced along with increases in IL-1β content in the PFC and a decrease in nitrite levels in the HC, ST and PFC. Our data demonstrate that a delayed increase (i.e., 24 h post-LPS) in PPI levels ensue, which may be useful behavioral parameter for LPS-induced depression. A decrease in nitrergic neurotransmission was associated with these behavioral findings.

1. Introduction

Over-activation of the innate immune system and abnormal secretion of inflammatory mediators (e.g., cytokines) have been implicated in the physiopathology of depression (Dellagioia et al., 2012; Dunjic-Kostic et al., 2012; Dunn and Swiergiel, 2005; Dunn et al., 2005) and it has been proposed that treatment with lipopolysacharide (LPS) or interleukin-1 (IL-1) could induce time-dependent depressive-like behavioral in rodents (Dunn et al., 2005).

Whether depression is a form or consequence of sickness behavior has been highlighted as a question of considerable translational importance (Maes et al., 2012a). In fact, there are considerable phenomenological similarities between sickness behavior and depression; behavioral inhibition, anorexia, weight loss, anhedonia, physio-somatic (fatigue, hyperalgesia, malaise) symptoms, anxiety and neurocognitive impairment can be characteristic of both conditions.

Some studies have identified time-related behavioral alterations induced by systemic LPS administration (Dantzer et al., 2008; Fu et al., 2010). It has been suggested that short-term behavioral alterations (i.e., during the peak secretion of cytokines) are related to sickness behavior, while long-term depressive behavior is pathophysiologically linked to depression (Dantzer et al., 2008).

Possibly the most compelling evidence implicating cytokines in depressive illness comes from the induction of depressive-like behavior following treatment with endotoxin or recombinant cytokines, such as interleukin-2 (IL-2) and interferon-α (IFN-α) (Capuron and Dantzer, 2003). This includes alterations in neurotransmitter function and neuroendocrine output (Dunn, 2006)

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similar to those observed in depressed patients (Anisman et al., 2002; Capuron and Dantzer, 2003). Moreover, healthy volunteers treated with LPS report a decrease in positive mood and increase in anxiety (Grigoleit et al., 2011; Kullmann et al., 2012). Taken together, these studies support the utility of the LPS-induced animal model of depression.

Animal models of depression, such as the separation-induced model, promote traditional depressive-like behavior together with a decrease in prepulse inhibition (PPI) levels (Martin and Brown, 2010). This decrease in PPI levels observed in preclinical models of depression may be related to stress-induced increases in corticotropin-releasing hormone levels (Tejeda et al., 2010), which are observed in depressed subjects (Bangasser and Valentino, 2012). There is also evidence to suggest cytokines may impair PPI (Mizuno et al., 2007). To our knowledge, changes in PPI levels 24 h post systemic LPS treatment have not been investigated.

Nitrergic signaling is involved in the neurobiology of stress-related psychiatric disorders such as anxiety and depressive disorders (Zhou et al., 2007). In the chronic mild stress depression model hippocampal production of nitric oxide (NO) by neuronal nitric oxide synthase isoform (nNOS) is altered (Palumbo et al., 2007; Zhou et al., 2007). However, an association between LPS-induced depression and central alterations in nitrite levels has yet to be reported.

Following previously reported LPS-induced time-dependent behavioral alterations (Dantzer et al., 2008), we investigate the time-course of behavioral changes (depressive/anxiety-like behaviors and alterations in PPI levels) induced by a single systemic administration of LPS to adult mice, as well as the associated changes in cytokine (IL-1β and TNFα) and nitrite content in brain areas putatively related to affective symptoms (Drevets et al., 2008); the prefrontal cortex (PFC), hippocampus (HC) and striatum (ST).

2. Materials and methods

2.1. Drugs

Lipopolysaccharide (LPS) from *Escherichia coli*, strain 055:B5 (Sigma-Aldrich Corp., St Louis, USA) and ketamine (Cristália Chemical and Pharmaceutical Products, Itapira, Brazil) were used. Drugs were freshly prepared. All other chemicals used were of analytical grade.

2.2. Animals

The experiments were performed in male Swiss mice (weighting 20–30 g) obtained from the Animal House of Federal University of Ceará. The animals were housed 10 per cage in standard polycarbonate cages (42 × 20.5 × 20 cm) and standard environmental conditions (22 ± 1 °C; humidity 60 ± 5%; reversed 12-h light/dark cycle with lights on at 19:00) with access to food (FRI-LAB Rat II, FRI-Ribe) and water ad libitum. All experimental procedures were conducted between 8:00 and 14:00 h and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH, 2011) and the Brazilian College of Animal Experimentation (COBEA). The research protocol was approved by the local ethical committee of Federal University of Ceará.

2.3. Experimental design

The dose of LPS was chosen based on previous studies evaluating LPS-induced behavioral and neurochemical alterations in mice (Kessler et al., 2003; Lejuez et al., 2011). In the case of ketamine, the dose selection was based on studies using this drug as an animal model of schizophrenia (de Araujo et al., 2011). The animals were randomly divided into two experimental groups: LPS-treated group (LPS 0.5 mg/kg, i.p., dissolved in 0.2 ml of volume, $n=32$) and control group ($n=32$, i.p., injected with 0.2 ml of vehicle - sterile endotoxin-free PBS). To avoid potential influence of behavioral testing on cytokine levels, cytokine assays and behavioral testing were performed on different animals (Bossu et al., 2012). Behavioral testing and cytokine analyses were performed in controls and after LPS challenge at two time-points (1.5 and 24 h). Different animals were used at each time-point. One additional group of mice ($n=8$) received a single sub-anesthetic dose of ketamine (20 mg/kg, i.p.) and PPI levels were evaluated. The behavioral assessments were performed on four distinct different animal group; (1) open field, plus maze and rota rod tests in that order; (2) forced swimming test; (3) the Y-maze and (4) PPI determinations. In all behavioral determinations, the rater was blind to the experimental treatment.

After each time-point of observation the animals used for cytokines and nitrite determinations were killed by cervical dislocation. Blood was immediately collected, centrifuged and the plasma isolated for posterior analyses. The brain areas dissected were: prefrontal cortex (PFC), hippocampus (HC) and striatum (ST). All biological material was immediately stored at −70 °C until assay.

2.4. Behavioral determinations

2.4.1. Open field test (OFT)

To analyze the effects of LPS treatment on locomotor activity, animals were evaluated in an open field test. The arena was made of acrylic (40 × 60 × 50 cm) with the floor divided into nine equal squares. The exploratory activity of the animal was registered during 5 min (Archer, 1973). The number of squares crossed by the animal and number of groomings (stereotyped behavior) and rearings (vertical exploratory activity) were observed. The experiments were conducted in a sound-attenuated room, under low-intensity red light.

2.4.2. Rota rod test (RRT)

The rota rod test was used to evaluate motor coordination. In this test, animals were placed with the four paws on a rotating swivel 25 cm above the floor, turning at 12 rpm (Ugo Basile, Italy). For each animal, the number of falls (up to three falls) during 1 min was registered (Dunham and Miya, 1957).

2.4.3. Forced swimming test (FST)

The mice were individually placed into an acrylic cylinder (25 cm height, 10 cm diameter) containing 8 cm of water maintained at 22–24 °C. After 1 min of habituation the time of immobility (s) of the animals was rated during 5 min in a total time of 6 min inside the cylinder. Immobility was defined as the absence of active, escape-oriented behaviors such as swimming, jumping, rearing, sniffing, or diving (Porsolt et al., 1978). Any mouse appearing to have difficulty keeping its head above water was removed from the cylinder and excluded from the study. The procedure has been validated in our laboratory by demonstrating that imipramine treatment (10 mg/Kg, i.p.) dramatically decreases immobility time (data not shown).

2.4.4. Elevated plus maze test (EPM)

The elevated plus maze consisted of two open (30 × 5 cm) and two darkened, closed arms (30 × 5 × 15 cm) emanating from a common central platform (5 × 5 cm) to form a plus shape. The entire apparatus was raised 45 cm above its base, and the test was made under dim red light (2 × 60 W). The test commenced by placing a mouse on the central platform, facing an open arm. A 5 min observation period was used, during which total arm entries, and the amount of time spent by the animals in open