Journal of Neuroimmunology 288 (2015) 40–46

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Behavioral and systemic consequences of long-term inflammatory challenge

Christina W. Fischer [a,*], Betina Elfving [a], Sten Lund [b], Gregers Wegener [a]

[a] Translational Neuropsychiatry Unit, Aarhus University, Skovagervej 2, 8240 Risskov, Denmark
[b] Department of Internal Medicine and Endocrinology, Aarhus University Hospital, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 14 August 2015
Accepted 19 August 2015

Keywords:
Cytokines
Sickness behavior
Depression-like behavior
Lipopolysaccharide

ABSTRACT

Inflammatory reactions are involved in a diversity of diseases, including major depressive disorder. Cytokines act as intercellular signaling molecules and mediators of inflammation between the periphery and the brain. Within the brain, evidence from animal studies of acute inflammation has shown that elevated cytokine levels are linked to behavioral responses of sickness and depression-like behavior. Although chronic inflammation is more translational to human depression than acute studies, little is known on central cytokine expression and associated behavioral responses following chronic immune challenges.

The present study assessed behavioral changes and a selection of cytokines in the brain and in the blood in rats randomized to receive a single or 8 week administration with either lipopolysaccharide (LPS, 600 μg/kg, i.p.) or saline.

Acute and long-term LPS treatments caused similar sickness and depression-like behavior. Chronic LPS administration did not have an effect on blood cytokine levels, indicating endotoxin tolerance, whereas increased fasting blood glucose was observed, indicating insulin resistance, a metabolic consequence of chronic inflammation. While a single LPS injection produced a generalized cytokine response in the brain, long-term LPS administration produced a specific central cytokine response with increased interleukin (IL)-1β and interferon (IFN)-γ. These cytokines can explain the behavioral changes observed, and could indicate microglia activation, although future studies are needed to uncover this assumption.

Taken together, although the behavioral outcome was similar between acute and chronic LPS administration, the central cytokine response was distinct. As the long-term LPS paradigm also posed a metabolic demand, this setting may reflect a more translational insight into inflammatory reactions in human depression, and could prove useful for assessing cytokine down-stream effects and experimental antidepressant drug products.

1. Introduction

Chronic inflammation is linked to several diseases, including psychiatric disorders (Dantzer et al., 2008) and the metabolic syndrome, a constellation of risk factors for cardiovascular disease that includes hyperglycemia and hypertension (Eckel et al., 2005). In a subset of patients with major depressive disorder, inflammation is evident as increased levels of inflammatory factors, especially the cytokines interleukin (IL)-6, and tumor necrosis factor (TNF)-α (Dowlati et al., 2010; Liu et al., 2012).

Cytokines in the blood signal to the brain through neural and humoral routes, which can lead to changes in mood and behavior (Rivest et al., 2000; Dantzer et al., 2008). The biological mechanisms behind cytokine-induced behavioral changes have been studied in animal models of systemic immune activation, using *e.g.* lipopolysaccharide (LPS). LPS is a component of the cell wall of Gram-negative bacteria that stimulates the innate immune system in a similar manner to bacterial infection and metabolic endotoxemia (Alexander and Rietschel, 2001; Cani et al., 2007). LPS activates the innate immune response *via* toll-like receptor (TLR)-4 on host immune cells, including macrophages, and signals through nuclear factor kappa beta to induce pro-inflammatory gene expression (Alexander and Rietschel, 2001). This subsequently results in increased levels of cytokines in the brain, such as IL-1β, and leads to behavioral signs of sickness (anorexia and reduced activity) and depression-like behavior (Hart, 1988; Dantzer et al., 1998).

This has especially been studied during the acute phase of inflammation, as repeated LPS exposure in animal studies are known to cause LPS tolerance, a defense mechanism in which immune cells re-exposed to the same endotoxin lower their cytokine response (Cavaillon and Adib-Conquy, 2006). However, despite endotoxin tolerance, chronic LPS exposure in rodents has still been shown to induce molecular changes in the brain and in the periphery. For instance, repeated LPS administration induced microglia activation in the brain (Borges et al.,

* Corresponding author at: Skovagervej 2-8240 Risskov, Denmark.
E-mail addresses: Christinawfischer@gmail.com (C.W. Fischer), betina.elfving@clin.au.dk (B. Elfving), SL@dadlnet.dk (S. Lund), wegener@dadlnet.dk (G. Wegener).

http://dx.doi.org/10.1016/j.jneuroim.2015.08.011
0165-5728/

2012), and depression-like behavior in mice, indicated by a reduced sucrose preference (Kubera et al., 2013). However, the exact central cytokines involved in producing depression-like behavior is unknown (McCusker and Kelley, 2013).

Interestingly, chronic LPS exposure has further been shown to cause hyperglycemia and insulin resistance (Cani et al., 2007), hypertension (Wu et al., 2012) and coronary vessel disease (Smith et al., 2006). These findings indicate that chronic LPS exposure in rodents could provide a model for studying depression and metabolic abnormities, and inflammatory pathways have indeed been suggested as a link for the comorbidity between these disorders (Capuron et al., 2008).

Thus, the aim of this study was to investigate the effect of chronic LPS on behavior and the central cytokine expression, together with fasting blood glucose levels to assess insulin sensitivity. This was compared to a single LPS injection, in order to differentiate between the acute *versus* chronic responses.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (n = 60) were obtained from Taconic A/S (Denmark), weighing 295.5 ± 1.9 g at the beginning of the experiment. Animals were allowed one week of habituation before entering the experiment. Animals were housed in pairs in a 12 h daily cycle (light on at 06:00) with *ad libitum* access to food and water. All experimental procedures were approved and monitored by the Danish National Committee for Ethics in Animal Experimentation (permission id 2007/561-1378).

2.2. Experimental design

Rats were randomly assigned to groups receiving either a single (acute study) or repeated (chronic study for 8 weeks) injections with either LPS (from *Escherichia coli*, 055:B5, Sigma, Saint Louis, Missouri, USA; 600 μg/kg i.p.) or saline (sterile 0.9% NaCl). All injections were given at around 13:00 and behavioral testing/metabolic assessments were carried out 20 h after injection to avoid the immediate stress response. The LPS dose was chosen as repeated injections between 500 and 750 μg/kg, i.p. resulting in behavioral and central changes (Puntener et al., 2012; Kubera et al., 2013) while a lower dose may result in behavioral tolerance (Engeland et al., 2003; Elgarf et al., 2014; Fischer CW et al., 2015). Separate groups were used for the behavioral studies and molecular measurements in the acute study, to ensure that any changes were not influenced by the stressful forced swim test. The experimental design is illustrated in Fig. 1.

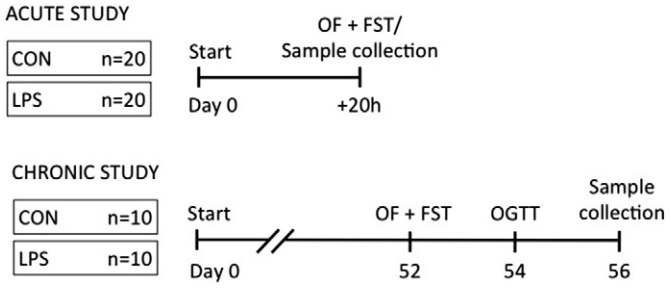

Fig. 1. Experimental design for the acute (top panel) and chronic (lower panel) studies. In the acute study, rats were grouped into either saline (CON) or LPS injection, in which half underwent behavioral testing and the other half were used for tissue analysis. In the chronic study, rats were grouped into saline or LPS treatment for 56 days (8 weeks). Behavioral testing in the open field (OF) and forced swim test (FST) took place 20 h following the injection on day 1 (acute study) or day 52 (chronic study). An oral glucose tolerance test (OGTT) was performed in the chronic study on day 54. Animals were sacrificed 20 h following the injection on day 1 (acute study) or day 56 (chronic study).

2.3. Metabolic measurements

In the acute study, body weight and food intake was monitored before and after the injection. During the chronic study, body weight and food intake was monitored on day 0 and then weekly throughout the experiment period. An oral glucose tolerance test (OGTT) was performed during the chronic study on day 54. After overnight fasting, rats received an oral glucose load (2.5 g/kg body weight) by oral gavage, and blood samples were collected from the tail vein at time 0, 30, 60 and 120 min. Blood glucose was measured at all time points in duplicate by using a "Precision Xtra Plus" glucose monitor (Abbott Laboratories A/S, Denmark), and insulin was measured at time 0 using an ultrasensitive rat insulin ELISA kit (DRG Diagnostics GmbH, Germany) according to the manufacturer's instructions.

2.4. Behavioral tests

Rats were moved to the behavioral room 2 h prior to testing to habituate. Behavioral tests took place between 09:00 and 13:00, approximately 20 h following the single injection in the acute study, or 20 h following the 51th injection in the chronic study (see Fig. 1).

2.4.1. Open field test

Locomotor activity was measured during a 5 min trial in an open field arena (100 cm × 100 cm). The trials were recorded and the total distance moved (cm), percentage of time spent in the center area (25% of arena) and entries into the center area were scored using an EthoVision video tracking system and software (version 8, Noldus Information Technology).

2.4.2. Forced swim test (FST)

Depression-like behavior was evaluated in the FST, directly after the open field test. The rats were placed in cylinders (60 cm high, 24 cm in diameter) filled with 40 cm of tap water at 24 °C ± 1 °C for 10 min. The trial was recorded and the time (s) spent immobile (when the rat makes only the necessary movements to keep its head above the water) was scored afterwards as a readout of depression-like behavior by an experienced observer who was blind to the treatment groups.

2.5. Sample collection

Rats were fasted overnight before sample collection and euthanized by decapitation. The brain was quickly removed and the frontal cortex and hypothalamus dissected and snap frozen on powdered dry ice. Serum was collected into sterile anticoagulant-free tubes with gel (Terumo, Venosafe™, VF-054SAS) from the trunk and allowed to clot for 1 h. This was followed by centrifugation (1000 ×*g*, 10 min, 4 °C) and subsequently the supernatant was transferred to another sterile tube. The liver and epididymal fat pads around the testes (as an indication of fat mass) were dissected and weighed. All organs were stored in −80 °C until further analysis.

2.6. Tissue homogenization

The right frontal cortex and hypothalamus were weighed and rinsed with Cell Wash Buffer (Bio-Rad, United States). The brain samples were then homogenized in 10 volumes of Cell Lysis buffer (Bio-Plex™ Cell Lysis Kit, Bio-Rad, United States) containing protease inhibitor cocktail, using a 4 mm stainless steel ball on a mixer-mill (Retsch) twice for 20 s at 30 Hz. The samples were frozen overnight at −80 °C.

2.7. Cytokine analysis

On the day of analysis, brain homogenates were thawed on ice, sonicated and centrifuged at 4500 ×*g* at 4 °C for 4 min, and the supernatant