ELSEVIER

Journal of Neuroimmunology 166 (2005) 39 – 46

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

The effects of repeated endotoxin exposure on rat brain metabolites as measured by ex vivo ^{1}HMRS

Sulie L. Chang[a,*], Christine C. Cloak[b], Lorenc Malellari[a], Linda Chang[b]

[a]Department of Biology, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA
[b]Department of Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96813, USA

Received 5 January 2005; received in revised form 15 April 2005; accepted 29 April 2005

Abstract

Abnormalities in brain chemistry induced by acute or chronic treatment with LPS were studied in the rat model. Ex vivo brain metabolites were measured using proton magnetic resonance spectroscopy, whereas serum corticosterone levels were determined using radioimmunoassay. We observed increased lactate levels in all measured brain regions and decreased choline in the hypothalamus after chronic LPS treatment. Acute LPS treatment led to an elevation of corticosterone, whereas chronic LPS treatment led to attenuation of the HPA response. These findings suggest that chronic inflammation induced by LPS could lead to cell loss/dysfunction, and hence, desensitization of the HPA axis, particularly in the hypothalamus.

Keywords: Lipopolysaccharide; ^{1}HMRS; Lactate; Choline; Hypothalamus; Corticosterone

1. Introduction

During a bacterial infection, an endotoxin, such as lipopolysaccharide (LPS), can trigger an immune response in the host. Prior exposure to sub-lethal doses of LPS allows an animal to survive a subsequent lethal dose of the same endotoxin (Harbuz et al., 2002; West and Heagy, 2002). This phenomenon is called endotoxin tolerance and, while it has long been thought that endotoxin tolerance may be a defense mechanism, some researchers now believe that it may actually be a dysfunction of the host's immune system (Curley, 1996; Ayala et al., 2000; Varma et al., 2001; Song et al., 2002).

The hypothalamic–pituitary–adrenal (HPA) axis is activated in response to an immune challenge (Elenkov et al., 1992; Hadid et al., 1999; Beishuizen and Thijs, 2003), and is strongly activated by LPS (Beishuizen and Thijs, 2003). Corticosterone, a glucocorticoid stress hormone with anti-inflammatory effects, is released by the adrenal gland in response to activation of the HPA axis (Elenkov et al., 1992; Giovambattista et al., 1997; Hadid et al., 1999). Repeated exposure to an escalating dosage of LPS can result in desensitization of the HPA axis (Chautard et al., 1999; Beishuizen and Thijs, 2003), which could inhibit the anti-inflammatory processes and result in a prolonged inflammatory response.

Systemic inflammation has been linked to the pathogenesis of several neurodegenerative disorders (Harms et al., 1997; Liu et al., 2002; Barcia et al., 2004), and patient's suffering from such disorders exhibits alterations in several brain metabolites, including choline and lactate (Harms et al., 1997; Govindaraju et al., 2000). An alteration in choline indicates membrane turnover (Tong et al., 2004), and changes in lactate may reflect an oxygen deficiency in a particular tissue (LaManna et al., 1996; Govindaraju et al., 2000).

Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) are useful tools to measure changes in the brain. While MRI gives a better understanding of the morphological changes in the brain, MRS allows quantification of specific metabolites, such as choline and lactate, in the brain

* Corresponding author. Tel.: +1 973 761 9456; fax: +1 973 275 2489.
E-mail address: changsul@shu.edu (S.L. Chang).

0165-5728/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2005.04.021

(Gasparovic et al., 1999; Schuhmann et al., 2003; Bo et al., 2004; Geurts et al., 2004; Jones and Waldman, 2004).

Using ex vivo proton MRS (^{1}HMRS), we tested the hypothesis that both acute and chronic exposure to LPS will increase lactate levels, whereas chronic LPS treatment will decrease choline in the hypothalamus (indicating cell loss). We also hypothesize that corticosterone will be elevated following acute LPS exposure and activation of the HPA axis, but attenuated after chronic LPS treatment, indicating desensitization of the HPA axis.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats (225–250 g) were obtained from Harlan Inc., Indianapolis, IN. Animals were pathogen free as confirmed by serology, bacteriology, and parasitological analyses. They were housed 2 or 3 per transparent plastic cage with chopped corn bedding in a temperature/humidity controlled environment and a 12-h light:dark cycle. They were fed a standard rat diet and water ad libitum, and were allowed a period of 5 to 7 days to adapt to the environment before any treatment was given. This animal study was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Seton Hall University.

2.2. LPS treatment and tissue collection

Forty rats were divided into 4 groups: Group 1, Saline/Saline (S+S); Group 2, Saline/LPS (S+L); Group 3, LPS/Saline (L+S); and Group 4, LPS/LPS (L+L). Groups 3 and 4 were treated chronically with escalating dosages of LPS administered intraperitoneally (i.p.) for a period of 10 days as follows: LPS was given twice a day, in the morning (08:00) and afternoon (17:00), starting with 250 μg/kg on Days 1 and 2, 500 μg/kg on Days 3 and 4, 1 mg/kg on Days 5 and 6, 2 mg/kg on Day 7, 4 mg/kg on Day 8, 8 mg/kg on Day 9, and finally, 16 mg/kg on Day 10. Groups 1 and 2 received injections with an identical volume of saline at the same times for 10 days. On Day 11, the animals in Groups 1 and 3 were given a single injection of saline, Group 4 animals received a single injection of 32 mg/kg LPS, and the animals in Group 2 were given a single acute dose of LPS (32 mg/kg). All the animals were decapitated 2 h later, one animal from each group in sequence. Trunk blood was collected to prepare serum for corticosterone measurement. Brain tissue was collected and dissected over ice immediately following decapitation. All dissections were performed in the same order and timing throughout. The brain was placed ventral side up on a glass dish over an ice bed. A coronal slice was made at the level of the optic chiasm, and the hypothalamus was removed. A second coronal cut was made in the rostral half of the brain, and the olfactory bulbs and frontopolar cortex were removed. The striatum was separated from the frontal cortex. Cortical tissue was removed, and the thalamus and remaining striatum were dissected out and frozen (the rostral and caudal striatal halves were combined). As regions were removed, they were placed in liquid nitrogen, then placed in a −80 °C freezer.

2.3. Acid extraction

The frozen brain regions were weighed and homogenized in five volumes of 0.04 M $HClO_4$ (according to their wet weight), and centrifuged at 15,000 rcf ($\times g$) at 4 °C for 10 min. The supernatant was collected, and the pellets were homogenized and centrifuged again as described above. The supernatants of the two homogenizations were combined, and 3-(trimethylsilyl) propionic [1,d4] acid sodium salt (TSP) was added to a final concentration of 2.5 mM.

2.4. ^{1}HMRS

The brain extract (0.4 ml) was transferred to a 5 mm, series 400, 7 NMR tube (Aldrich, WI), and analyzed in a Bruker 400 MHz NMR (9T) with a 5 mm QNP probe at room temperature. The acquisition parameters were: 30° pulse, 6 μs 4100 Hz spectral width with 128 averages, and 4 s repetition time. The following metabolites were identified by ^{1}H chemical shift positions: *N*-acetyl-aspartate (NAA), glutamate (Glu), γ-aminobutyric acid (GABA), total choline (Cho), total creatine (Cre), lactate (LA), alanine (Ala), and myo-inositol (MI). Following Fourier transformation, phasing, and baseline correction, areas under each peak were integrated to determine the concentration of each metabolite, relative to the value of the TSP standard, taking into account the difference in tissue weight of the sample and the hydrogen atoms responsible for each peak in the spectrum.

2.5. Radioimmunoassay (RIA) for serum corticosterone levels

Serum levels of corticosterone were measured using a commercially available radioimmunoassay (RIA) kit and following the protocol provided by ICN Pharmaceuticals, Inc. (Orangeburg, NY) without modifications. In short, [^{125}I]-tracer and anti-corticosterone (in that order) were added to diluted rat serum. After vortexing the solution, centrifuging it at 1000×*g* for 15 min, and decanting the supernatant, the precipitate was counted on a Wallace Wizard I470 Automatic Gamma Counter.

2.6. Statistical analyses

Statistical analyses of the metabolite concentrations were performed using a two-way analysis of variance (ANOVA). The metabolite of interest (e.g., lactate) was used as the dependent value, whereas the treatments (i.e., repeated,