Neuroscience 234 (2013) 146–157

NEONATAL LIPOPOLYSACCHARIDE EXPOSURE INDUCES LONG-LASTING LEARNING IMPAIRMENT, LESS ANXIETY-LIKE RESPONSE AND HIPPOCAMPAL INJURY IN ADULT RATS

K.-C. WANG,[a,b] L.-W. FAN,[c†] A. KAIZAKI,[d] Y. PANG,[c] Z. CAI[c] AND L.-T. TIEN[b*†]

[a] *Department of Anesthesiology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei City, Taiwan, ROC*

[b] *School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan, ROC*

[c] *Department of Pediatrics, Division of Newborn Medicine, University of Mississippi Medical Center, Jackson, MS 39216, USA*

[d] *Department of Pharmacology, Toxicology and Therapeutics, Division of Toxicology, School of Pharmacy, Showa University, Shingawa-ku, Tokyo 142-8555, Japan*

Abstract—Infection during early neonatal period has been shown to cause lasting neurological disabilities and is associated with the subsequent impairment in development of learning and memory ability and anxiety-related behavior in adults. We have previously reported that neonatal lipopolysaccharide (LPS) exposure resulted in cognitive deficits in juvenile rats (P21); thus, the goal of the present study was to determine whether neonatal LPS exposure has long-lasting effects in adult rats. After an LPS (1 mg/kg) intracerebral (i.c.) injection in postnatal day 5 (P5) Sprague–Dawley female rat pups, neurobehavioral tests were carried out on P21 and P22, P49 and P50 or P70 and P71 and brain injury was examined at 66 days after LPS injection (P71). Our data indicate that neonatal LPS exposure resulted in learning deficits in the passive avoidance task, less anxiety-like (anxiolytic-like) responses in the elevated plus-maze task, reductions in the hippocampal volume and the number of neuron-specific nuclear protein (NeuN)+ cells, as well as axonal injury in the CA1 region of the middle dorsal hippocampus in P71 rats. Neonatal LPS exposure also resulted in sustained inflammatory responses in the P71 rat hippocampus, as indicated by an increased number of activated microglia and elevation of interleukin-1β content in the rat hippocampus. This study reveals that neonatal LPS exposure causes persistent injuries to the hippocampus and results in long-lasting learning disabilities, and these effects are related to the chronic inflammation in the rat hippocampus.

Key words: lipopolysaccharide, chronic inflammation, hippocampus, learning dysfunction, anxiolytic behavior.

INTRODUCTION

Maternal, placental or neonatal infection/inflammation has been shown to induce neonatal brain injury and it may be associated with the consequent neurological disorders and functional disability in later life (Hagberg et al., 2002, 2012; Volpe, 2003). Increasing evidence indicates that acute inflammation can be shifted to a chronic inflammatory state and/or adversely affect brain development (Williamson et al., 2011; Hagberg et al., 2012). Using this neonatal rat model, we have found that neonatal exposure to lipopolysaccharide (LPS) resulted in sensory, motor, emotional and cognitive impairment in juvenile rats (P21) (Fan et al., 2005, 2008).

Our previous studies have shown that neonatal exposure (postnatal day 5 (P5)) to LPS through an intracerebral (i.c.) injection in rats can produce chronic inflammation and injury in the nigrostriatal dopaminergic system, as indicated by the phenotypic suppression of tyrosine hydroxylase expression from neurons in the substantia nigra (SN), impairment of nigrostriatal neuron connectivity and neurobehavioral deficits in the LPS-exposed rats (Fan et al., 2005, 2008, 2011a,b). On the other hand, neonatal LPS exposure did not cause actual death of dopaminergic neurons in the SN and the LPS-induced motor dysfunction was spontaneously recoverable by adult ages (P70) (Fan et al., 2011a,b). We also observed that neonatal LPS exposure (P5) lead to learning and memory deficits in the passive avoidance task, less anxiety-like (anxiolytic-like) responses in the elevated plus-maze task, and axonal injury in the CA1 region of the middle dorsal hippocampus in the juvenile male and female rats (P21) (Fan et al., 2005, 2008). However, it is unclear whether neonatal exposure to LPS can produce chronic inflammation and neuronal damage in other brain regions, such as the hippocampus, and cause hippocampal-related neurological disability in adults. Thus, the present study was designed to examine whether neonatal LPS exposure can produce chronic inflammation and persistent neuronal injury in the

*Corresponding author. Address: School of Medicine, Fu Jen Catholic University, 510 Zhongzheng Road, Xinzhuang District, New Taipei City 24205, Taiwan, ROC. Tel: +886-(2)-2905-3451; fax: +886-(2)-2905-2096.

E-mail address: 068154@mail.fju.edu.tw (L.-T. Tien).

† These authors contributed equally to this work.

Abbreviations: DAPI, 4', 6-diamidino-2-phenylindole; ELISA, enzyme-linked immunosorbent assay; i.c., intracerebral; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; MAP1, microtubule-associated protein 1; NeuN, neuron-specific nuclear protein; NIH, national institutes of health; P5, postnatal day 5; SN, substantia nigra; TNFα, tumor necrosis factor-α.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.12.049

hippocampus, and long-lasting cognitive deficits in adult rats.

Our preliminary data showed that neonatal LPS infection induced great learning impairments in P49 female rats. To eliminate a possible effect of gender difference, female rats were used in this study. The hippocampal formation mediates not only processes associated with learning and memory but also anxiety and fear (Spolidorio et al., 2007; Zarrindast et al., 2012). The abnormal neurobehavioral performance in avoidance learning and memory, and locomotor activity has also been linked with dopaminergic neuronal injury in many studies (Nishii et al., 1998; Denenberg et al., 2004). Therefore, the learning and memory task, behaviors of locomotion and anxiety-like response were determined in this study.

EXPERIMENTAL PROCEDURES

Chemicals

Unless otherwise stated, all chemicals used in this study were purchased from Sigma (St. Louis, MO, USA). Monoclonal mouse antibodies against neuron-specific nuclear protein (NeuN) or microtubule-associated protein 1 (MAP1), and OX42 (CD11b) were purchased from Millipore (Billerica, MA, USA) and Serotec (Raleigh, NC), respectively. Enzyme-linked immunosorbent assay (ELISA) kits for immunoassay of rat interleukin-1β (IL-1β) (RLB00), interleukin-6 (IL-6) (R6000B) and tumor necrosis factor-α (TNFα) (RTA00) were purchased from R&D Systems (Minneapolis, MN, USA).

Animals

Timed pregnant Sprague–Dawley rats arrived in the laboratory on day 19 of gestation. Animals were maintained in an animal room on a 12-h light/dark cycle and at constant temperature (22 ± 2 °C). The day of birth was defined as postnatal day 0 (P0). After birth, the litter size was adjusted to twelve pups per litter to minimize the effect of litter size on body weight and brain size. All procedures for animal care were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center or Fu Jen Catholic University. Every effort was made to minimize the number of animals used and their suffering.

Surgery procedures and animal treatment

Intracerebral injection of LPS to 5-day old Sprague–Dawley female rat pups was performed as previously described (Cai et al., 2003; Pang et al., 2003; Fan et al., 2005). Under light anesthesia with isoflurane (1.5%), LPS (1 mg/kg, from Escherichia coli, serotype 055: B5) in sterile saline (total volume of 2 μl) was administered to the rat brain at the location of 1.0 mm posterior and 1.0 mm left to the bregma, and 2.0 mm deep to the skull surface in a stereotaxic apparatus with a neonatal rat adapter. The dose of LPS was chosen based on the previously reported results which produced brain injury (Cai et al., 2003; Pang et al., 2003; Fan et al., 2005). The injection site was located at the area just above the left cingulum. The control rats were injected with the same volume of sterile saline. All animals survived the intracerebral injection.

Each dam had the same litter size (12 pups) and equal numbers of LPS-treated and saline-treated rat pups were included in a litter. The pups were weaned at P21 and four rats (two LPS-treated and two saline-treated) per cage were housed after weaning. Thirty-six rats (18 rats from each group) were used in the present study. Equal numbers of rat pups (six pups) were included in LPS or saline injection group for behavioral tests for three experiment groups (P21, P49, P70). Sixty-six days after the injection (P71), rats were sacrificed by transcardiac perfusion with normal saline followed by 4% paraformaldehyde for brain section preparation. Frozen coronal brain sections at 10 μm of thickness from six rats of each group were prepared in a cryostat for immunohistochemistry staining. The other six rats from each group were used for preparation of free-floating coronal brain sections at 40 μm of thickness in a sliding microtome (Leica, SM 2000R, Wetzlar, Germany) for stereological estimates of the size of the cerebrum, ventricle, white matter, and hippocampus. For determination of the content of pro-inflammatory cytokines in the hippocampus, six P71 rats from each group were sacrificed by decapitation to collect fresh brain tissue.

Behavioral testing

The behavioral tests were performed as previously described (Fan et al., 2005, 2008, 2011a,b) with modifications. As shown in Fig. 1, three groups were included in the present study. Group 1: 16 days after the injection (P21), rats (six rats in each group) were performed by open field, elevated plus-maze and passive avoidance (learning trial) tests. The memory trials were tested on P22, P50 and P71. Group 2: 44 days after the injection (P49), rats (six rats in each group) were performed by open field, elevated plus-maze and passive avoidance (learning trial) tests. The memory trials were tested on P50 and P71. Group 3: 65 days after the injection (P70), rats (six rats in each group) were performed by open field, elevated plus-maze and passive avoidance (learning trial) tests. The memory trials were tested on P71.

Locomotion (open field)

This test measures the activity and habituation response of animals on placement in a novel environment (Hermans et al., 1992). Locomotor activity was measured on P21, P49 or P70, using the ANY-maze Video Tracking System (Stoelting Co., Wood Dale, IL, USA). Pups were placed in the activity chambers ($42 \times 25 \times 40\ cm^3$) in a quiet room with dimmed light. The total distance traveled by the animal was recorded during a 10-min testing period (Fan et al., 2005, 2008, 2011a,b). The number of rearing events including exposure rearing responses (body inclined vertically with hindpaws on the floor of the activity cage and forepaws on the wall of the chamber) and sniffing-air responses (rearing in the open area of the active chamber) were counted during the first 5-min testing period. The summation of exposure rearing and sniffing-air responses reflect vertical activity which has been used apart from locomotion, as a reliable criterion for motor activity during their exposure to novelty (Antoniou et al., 2004).

Passive avoidance

Passive avoidance involves the learned inhibition of a natural response and gives information about learning and memory capabilities (Olton, 1973; Rodier, 1977; Hermans et al., 1992). The passive avoidance procedure consists of two sessions (Fan et al., 2005, 2008). In the first session (P21, P49 or P70), rats were trained in a step-down type of passive avoidance apparatus. The experimental chamber ($30 \times 30 \times 40\ cm^3$) was made of plexiglass. The floor of the chamber was made of