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LIPOPOLYSACCHARIDE INDUCES PAIRED IMMUNOGLOBULIN-LIKE RECEPTOR B (PIRB) EXPRESSION, SYNAPTIC ALTERATION, AND LEARNING–MEMORY DEFICIT IN RATS

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Abstract—Some typical immune proteins are expressed in the nervous system, among which the paired-immunoglobulin-like receptor B (PirB) is a receptor for major histocompatibility complex class I antigen (MHC-I), but may play a physiological role in the brain for neuronal circuitry stability by inhibiting synaptic plasticity. Chronic neuroinflammation is common to many neurodegenerative diseases and is often associated with neuronal/synaptic damage and dysfunction. Here we examined the expression of PirB in the rat brain following intracerebral application of lipopolysaccharide (LPS), which has been shown to induce proinflammatory changes and cognitive deficits in rodents. One month after unilateral intrahippocampal LPS injection (10 μg in 4 μl phosphate-buffered saline, PBS), increased protein levels and immunoreactivity of PirB were detected in the ipsilateral hippocampal formation and cortex of the experimental group relative to vehicle (PBS) control. The increased PirB labeling was localized to astrocytes and neurons. Reduced synaptophysin protein levels and immunoreactivity were also found in the ipsilateral hippocampal formation and cortex in LPS-treated rats relative to controls. Morris water maze tests indicated that hippocampus-dependent spatial learning and memory were impaired in LPS-treated animals. Our findings add new experimental data for an upregulation of immune proteins in neuronal and glial cells in the brain in a model of endotoxin-induced neuroinflammation, synaptic alteration, and cognitive decline. The results suggest that PirB modulation may be involved in the pathological process under neurodegenerative conditions. **© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: inflammation, bacterial endotoxin, hippocampus, neuroplasticity, Morris water maze.

The central nervous system can mount immune-like and inflammatory response to exogenous pathogens, as well as endogenous molecules, in conditions including neural stress, trauma, or degeneration. In fact, inflammatory mechanisms are thought to contribute to the pathophysiology in brain infection, traumatic injury, epilepsy, and chronic neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (Akiyama et al., 2000; Schmidt et al., 2005; Sastre et al., 2006; Qian et al., 2010; Tansey and Goldberg, 2010; McGeer and McGeer, 2010; Ownby, 2010; Aronica and Crino, 2011). For example, in AD, the entorhinal cortex and hippocampus appear to be vulnerable to chronic neuroinflammation; these regions exhibit a high degree of glia cell activation in the early disease stage but show a great extent of atrophy with disease progression (Terry et al., 1991; Heinonen et al., 1995; Hauss-Wegrzyniak et al., 1998, 2002; Cagnin et al., 2001; Masliah et al., 2001; Rosi et al., 2004). It is considered that synaptic damage may occur during the early phase of chronic neurodegeneration and may lead to cognitive impairment and loss of other neuronal function (Arendt and Brückner, 2007; DeKosky and Scheff, 1990; Jacobsen et al., 2006; Russo et al., 2011). Therefore, understanding the interplay of immune/inflammatory modulation/alteration with synaptic changes is of potential pathogenic relevance to human neurodegenerative disorders.

Many typical immune system molecules are found in the nervous system. Specifically, major histocompatibility complex class I (MHC-I) antigen may serve as a ligand for the paired-immunoglobulin-like receptor B (PirB) in the brain (Syken et al., 2006). In addition to their known immune functions, MHC-I and PirB may play critical nonimmune roles in the brain (Boulanger, 2009; Goddard et al., 2007; Huh et al., 2000). Specifically, MHC-I and PirB appear to modulate synaptic plasticity and neuronal circuitry stability (Datwani et al., 2009; Syken et al., 2006; Glynn et al., 2011). Data also suggest that PirB is a high-affinity receptor for Nogo, MAG, and OMgp in mammalian brain and influences axon regeneration after injury (Jasvinder et al., 2008). Thus, PirB expression could be linked to synaptic and/or neuronal circuitry integrity, plasticity, and functionality in the brain.

Lipopolysaccharide (LPS), an endotoxin of gram-negative bacteria, is a strong inducer of innate immunity, but it is used to experimentally induce chronic inflammation in the rodents, including in the central nervous system. Previous studies have shown that intrahippocampal injection of LPS could potentiate the expression of inflammation-related molecules, including glial fibrillary acid protein (GFAP), inducible nitric oxide synthase (iNOS), cycloxygenase (COX)-2, and interleukins (IL) (Quan et al., 1994; Jain

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Abbreviations: AD, Alzheimer's disease; BSA, bovine serum albumin; GFAP, glial fibrillary acid protein; LPS, lipopolysaccharide; MAP-2, microtubule-associated protein-2; MHC-I, major histocompatibility complex class I; PBS, phosphate-buffered saline; PirB, paired-immunoglobulin-like receptor B, TBS-T, Tris-buffered saline containing 0.1%Tween 20.

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et al., 2002; Dai et al., 2006; Herber et al., 2006; Pang et al., 2006; Sparkman et al., 2006; Russo et al., 2011).

The present study was aimed to determine if intrahippocampal LPS injection might induce PirB expression and synaptic changes. The effects of LPS were determined in adult rats by biochemical and anatomical analysis of PirB expression and the presynaptic marker synaptophysin. Behavioral tests were also used to assess learning and spatial memory in experimental rats following LPS treatment.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley adult rats weighing 180–200 g were obtained from the animal center of Central South University (Changsha, China). The animals were housed in temperature- (20–26 °C), humidity- (30–60%), and lighting- (12:12-h light/dark cycle) controlled rooms, in groups of two per cage. Food and water were freely available. The experimental protocol was approved by the Animal Care and Use Committee of the Central South University, which was in accordance with National Institutes of Health guidelines for use of experimental animals. Every effort was made to minimize animal use and suffering during experiment.

Surgery, treatment, and histological processing

Rats were placed on a stereotaxic apparatus (Kopf 921, David Kopf Instruments, Tujunga, CA, USA) under deep anesthesia (chloride hydrate, 340 mg/kg i.p.). LPS (from *Escherichia coli*, serotype 055:B5, purchased from Sigma Chemical, St. Louis, MO, USA) was dissolved (2.5 μg/μl) in sterile 0.1 M phosphate-buffered saline, pH 7.4 (PBS), and 4 μl (10 μg) LPS was injected slowly into the right hippocampal CA3 region via a microsyringe (Third syringe factory, Shanghai, China) mounted on the stereotaxic holder, with the injection performed over 10 min to minimize the reflux along the injecting track (Dai et al., 2006). Control groups were injected with an equivalent volume of PBS. The rats were closely monitored during recovery on a thermal blanket at 34–35 °C. All injections were performed during daytime (9:00–12:00 AM). A total of 68 rats were used, with a half of the animals being treated with either LPS or the vehicle PBS.

Previous studies have shown that LPS treatment may induce neuroinflammation, neuronal pathology, and cognitive deficits in days to several months, often evident around one month (Hauss-Wegrzyniak et al., 2002; Herber et al., 2006; Pang et al., 2006; Lee et al., 2008; Chen et al., 2011). Therefore, in the present study, we explored the experimental effects one month after intrahippocampal LPS injection. Rats were reanesthesized and perfused transcardially with PBS, followed by 4% phosphate-buffered paraformaldehyde (pH 7.4). Brains were removed from the skull and postfixed for 4 h in the above perfusion fixative, rinsed with several changes of PBS, and immersed in 30% sucrose in PBS for cryoprotection at room temperature until they sank. Thirty-five-micrometer-thick coronal sections were cut in a cryostat (Shandon SME Cryostat, England), with every 10th of sections collected for Cresyl Violet staining and for immunohistochemical studies.

Morris water maze test

Morris water maze was tested following a 4-days training period before animal perfusion. On the first day, rats were given a trial to familiarize with the environment. The maze (SMART Small Animals Behavior Analysis System, Shen Zhen Rui Wo De Life Science and Technology Company, Shenzhen, China) consisted of a black circular pool (120 cm of diameter and 40 cm high) filled with warm water (26 °C, 30 cm deep). The pool was divided into four quadrants. An escape platform (10 cm diameter) was placed near the middle of one of the quadrants, 2.0 cm below water surface. For training, a rat was placed in the water randomly at one of the four starting positions and was given 60 s to reach the platform. If the animal failed to locate the platform, it was carefully guided to the platform and stayed on it for 10 s. The rat was then taken out of the platform and allowed to rest for 15 s. This was followed by another round of training. The latency and swimming distance to find the platform were measured. The rats were trained twice each day, with a total of four trials. The final test consisted of a single trial, during which the platform was removed. The time spent in the target location was measured. All data were collected and processed by a SMART Small Animals Behavior Analysis System, which includes the audio–video equipment and a computer equipped with an analysis-management system.

Immunohistochemistry

Sets of free-floating sections were soaked in 1% H_2O_2 in PBS for 20 min to inactivate endogenous peroxidase activity and preincubated in a solution containing 5% bovine serum albumin (BSA) and 0.2% Triton X-100 in PBS for 1 h. The sections were then incubated with primary antibodies overnight at 4 °C in PBS containing 0.2% Triton X-100 and 1% BSA. The following primary antibodies were used: (1) A20 (ILT-5, goat polyclonal antibody, Santa Cruz Biotechnology, sc-9608, 1:500), specific for the cytoplasmic domain of PirB (Syken et al., 2006); (2) GFAP, a marker of astrocytes (rabbit anti-GFAP polyclonal antibody, Z0334, Dako, USA, 1:200), and (3) synaptophysin (rabbit anti-synaptophysin polyclonal antibody, AB9272, Millipore, Temecula, CA, USA, 1:1000), a presynaptic axon terminal protein. The sections were washed and reacted with approximated biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA, USA, 1:200). Immunoreaction product was visualized per the avidin–biotin peroxidase protocol (ABC kit, Vector Laboratories, 1:400). Sections were then washed and mounted on gelatinized slides, dehydrated, cleared, and coverslipped. For controls, series of sections were processed in the absence of the primary antibodies, which did not yield specific immunoreactivity.

Double immunofluorescence was used to study colocalization of PirB labeling with the neuronal marker microtubule-associated protein-2 (MAP-2, rabbit anti-MAP-2 polyclonal antibody, AB5622, Chemicon, USA, 1:500) and synaptophysin, the glial marker GFAP, or the microglial marker CD11b (mouse anti rat CD11b, Abcam, USA, ab1211, 1:1000), respectively. Nonspecific binding sites were blocked by a preincubation of the sections with 5% normal donkey serum for 30 min at room temperature. Sections were further incubated with PirB and MAP-2, PirB and GFAP, and PirB and CD11b antibodies in PBS containing 0.2% Triton X-100 and 5% normal donkey serum. The immunofluorescences were visualized using Cy™3-conjugated donkey anti-goat IgGs (Jackson Immunoresearch Laboratories, USA, diluted 1:100) for PirB, and Cy™2-conjugated donkey anti-rabbit IgGs and Cy™2-conjugated donkey anti-mouse IgGs (Jackson Immunoresearch Laboratories; diluted 1:100) for GFAP, synaptophysin, and CD11b, respectively. Finally, the sections were washed in PBS, mounted on gelatinized slides, and coverslipped with the antifading medium containing 0.01% 1,4-diazabicyclo[2.2.2] octane (DABCO, Fluka, Buchs, Switzerland).

Immunoblot

Hippocampi and cortices ipsilateral and contralateral to the LPS or PBS injection side were harvested following decapitation (n=6/group). Tissue was weighed and homogenized using a protein extraction kit (CW0883, Kangwei Century Company, Beijing). A total of 20 μg protein from each sample was separated electrophoretically in 10% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride membranes. Membranes were blocked

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