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Maternal immune activation increases seizure susceptibility in juvenile rat offspring

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ABSTRACT

Epidemiological data suggest a relationship between maternal infection and a high incidence of childhood epilepsy in offspring. However, there is little experimental evidence that links maternal infection with later seizure susceptibility in juvenile offspring. Here, we asked whether maternal immune challenge during pregnancy can alter seizure susceptibility and seizure-associated brain damage in adolescence. Pregnant Sprague–Dawley rats were treated with lipopolysaccharide (LPS) or normal saline (NS) on gestational days 15 and 16. At postnatal day 21, seizure susceptibility to kainic acid (KA) was evaluated in male offspring. Four groups were studied, including normal control (NS–NS), prenatal infection (LPS–NS), juvenile seizure (NS–KA), and "two-hit" (LPS–KA) groups. Our results demonstrated that maternal LPS exposure caused long-term reactive astrogliosis and increased seizure susceptibility in juvenile rat offspring. Compared to the juvenile seizure group, animals in the "two-hit" group showed exaggerated astrogliosis, followed by worsened spatial learning ability in adulthood. In addition, prenatal immune challenge alone led to spatial learning impairment in offspring but had no effect on anxiety. These data suggest that prenatal immune challenge causes a long-term increase in juvenile seizure susceptibility and exacerbates seizure-induced brain injury, possibly by priming astroglia.

1. Introduction

The brain undergoes dramatic and important changes during fetal development and is vulnerable to environmental insults [1]. Accumulating evidence suggests that early exposure to infection contributes to many neurological disorders that are manifested throughout the entire life span [2]. For instance, maternal infection during pregnancy is associated with increased risk of neurological disorders such as schizophrenia, autism, and cerebral palsy in offspring [3,4]. An increasing body of clinical and experimental data suggests that infection or inflammation is an important factor in the pathophysiology of seizure generation, seizure-related brain injury, and epileptogenesis [5–7]. Recently, epidemiological studies indicate that maternal infection during pregnancy is a risk factor for childhood epilepsy [8,9]. Furthermore, intrapartum infection is linked to heightened risk for seizures in the newborn [10]. Our previous study [11] has shown that maternal immune challenge increases seizure susceptibility and exacerbates seizure-induced behavioral deficits in adult offspring. However, the effects of maternal immune challenge on seizure susceptibility in the immature brain as well as the associated behavioral impairment remain largely unexplored experimentally.

Astrocytes, the most numerous glial cells within the central nervous system (CNS), play important roles in brain inflammation [12], regulation of brain tissue homeostasis, and neuronal excitability [13,14]. In recent years, accumulating data have suggested that astrocyte dysfunction contributes to the development of epilepsy, including seizure occurrence, neuronal damage, and behavioral impairment [15–17]. Excessive or prolonged reactive astrogliosis is a recognized response to seizures and a potential contributor to mechanisms of epileptogenesis [18,19]. Increasing evidence has shown that maternal immune challenge can lead to long-lasting astrogliosis in the developing brain, which may contribute to brain damage [20–22]. Thus, prenatal immune challenge may render the CNS more vulnerable to later seizures and the associated brain injury by causing astrocyte dysfunction.

In the present study, lipopolysaccharide (LPS), a cell wall component from Gram-negative bacteria, was administered to pregnant Sprague–Dawley rats to mimic the effects of maternal bacterial infection, which is an established model of maternal immune activation (MIA) [3]. Then, juvenile male offspring were tested for seizure susceptibility in response to kainic acid (KA) on postnatal day (P) 21. Furthermore, we also examined hippocampal astrocyte response and behavioral performance after seizures in the offspring.

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2. Materials and methods

2.1. Animals and drugs

Time pregnant Sprague–Dawley rats were purchased from Shandong University Animal Center and individually housed in an uncrowded, quiet animal facility room on a regular 12-hour light/dark cycle (room temperature: 22 °C, humidity: 45%–55%). Animals were given ad libitum access to food and water. Pregnant dams were monitored for the day of parturition, which was taken as P0, when all litters were culled to 10 pups. Offspring were weaned on P25 and housed four per cage in same-sex groups. Only male offspring were involved, and all experimental care and use of animals were conducted in compliance with the guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Lipopolysaccharide (*Escherichia coli*, serotype 055:B5) and KA were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA). All compounds were administered between 09:00 and 10:00 h at 1 ml/kg unless otherwise stated.

2.2. Maternal administration of LPS

Pregnant rats were injected intraperitoneally with LPS dissolved in saline (200 μg/kg) or normal saline (NS) at gestational days (GD) 15 and 16. Our previous work and the study by Ghiani et al. have shown that the dose of LPS used here has successfully induced cytokine release and fever in pregnant rats, without interfering with maternal care [11,23]. In addition, survival of dams and litters after administration of 100 μg/kg LPS is 100% at GD15/16. Control rats were administered comparable volume of normal saline (NS).

2.3. KA seizure susceptibility testing

Here, we used an established rat model of KA-induced limbic seizures, yet through an entirely different mechanism of action as lithium–pilocarpine, as we used in our previous work [11]. At P21, male offspring of LPS- or NS-treated mothers were injected i.p. with KA (7.5 mg/kg). Control rats received comparable volume of NS. Four experimental groups were studied, including normal control (NS–NS, n = 20/group), prenatal infection (LPS–NS, n = 20/group), juvenile seizure (NS–KA, n = 32/group), and "two-hit" (LPS–KA, n = 36/group) groups. Following administration of KA, animals were monitored continuously for 3 h. Time of latency to onset of forelimb clonus (FLC) was used as a marker of seizure susceptibility, which was documented and analyzed by an observer blind to the treatment conditions. Seizures were characterized according to the published criteria [24] as follows: 0, no response; I, wet dog shake (WDS) and/or behavioral arrest; II, WDS, staring, pawing, and clonic jerks; III, WDS, staring, pawing, clonic jerks, rearing, and falling; and IV, continuous grade III seizures for longer than 30 min. Only animals with grade IV seizures were included in this study.

Three days after KA application, six rats from each group were sacrificed for examination of astroglial response by glial fibrillary acidic protein (GFAP) immunohistochemistry. The remainder (n = 12/group) remained undisturbed until they underwent behavioral tests beginning at P30.

2.4. GFAP immunohistochemistry

Rats were anesthetized, sacrificed, and perfused transcardially with chilled phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed and immersed in 4% paraformaldehyde for 24 h at 4 °C before paraffin embedding. Embedded brains were sectioned coronally with a microtome into 5-micrometer thick sections and collected on gelatin-coated microscope slides. Following dewaxing and dehydration, brain sections were treated with 0.3% hydrogen peroxide to block endogenous peroxidase activity. After three washes in PBS, the sections were pre-exposed to 5% bovine serum albumin for 30 min and incubated with primary antibodies against GFAP (mouse antirat 1:200; Millipore Ltd., Billerica, MA, USA) overnight at 4 °C. Control sections were incubated in PBS. Subsequently, all sections were incubated with horseradish peroxidase-conjugated goat antimouse (1:200; ZSGB-BIO, China) secondary antibody for 2 h at room temperature. After a further wash in PBS, stainings were developed with diaminobenzidine substrate. Then, sections were counterstained with hematoxylin (blue) for contrast and mounted with permanent mounting medium. Then, sections were examined under brightfield microscopy (Olympus, Tokyo, Japan) by an examiner blind to the treatment of the animals. Average optical density (AOD) of GFAP staining was measured using Image-Pro Plus 5.0 (Media Cybernetics, Silver Spring, Maryland, USA). Values of background stainings within subfields of interest were subtracted from the immunoreactive intensities. Five slices (ten hippocampi) from each rat were randomly selected for quantification. The average AOD of the left and right hippocampi was calculated providing one single value per slice. For each rat, the data of the five slices were averaged. The averaged value per animal was used for statistical analyses. We only analyzed the CA1 region because our preliminary experiment showed significant effects within the CA1 area after KA-induced seizures.

2.5. Elevated plus maze (EPM)

Anxiety was examined using the EPM constructed with black plastic at P30. The apparatus, elevated 50 cm from the ground, consisted of two open arms (50 cm × 10 cm), two enclosed arms (50 cm × 10 cm × 30 cm), and a central platform (10 cm × 10 cm). After being placed in the central platform (facing an open arm), a rat was allowed to explore freely for 5 min. An entry was defined as having all four paws in the arm. The time spent in open arms/total times (anxiety score) was used as a measure of anxiety. The maze was cleaned thoroughly with 70% ethanol before each trial.

2.6. Y-maze test

Spontaneous alternation in the Y-maze was used to evaluate hippocampus-dependent spatial learning [24]. The Y-shaped acrylic maze consisted of three identical arms (60 × 17.5 × 3.5 cm). Each rat was placed in the start arm and then released to choose one of the other arms. The rat was trapped within the new arm for 30 s and then placed back in the start arm for 30 s and released to choose one of the other arms again. If the animal selected a different arm, it was scored as alternating. Rats were tested on alternate days beginning on P45 until P73. The percent alternation over the duration of testing was calculated for each animal.

2.7. Statistical analysis

SPSS 17.0 was used for all statistical analysis. Values are expressed as mean ± SEM for each group. Between group difference in seizure susceptibility (time of latency to onset of forelimb clonus) was analyzed using the independent t-test. Two-way (prenatal treatment × postnatal treatment) analysis of variance (ANOVA) followed by Least − Significant Difference post hoc tests was performed to compare multiple groups for GFAP immunostaining, and Y-maze and elevated plus maze test data. The level of statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Prenatal LPS increases juvenile seizure susceptibility to KA

Maternal LPS exposure during pregnancy resulted in the shortening of the latency to FLC in rat offspring of LPS-treated mothers (LPS–KA) compared to NS–KA animals ($t_{66} = 2.05$, $p < 0.05$; Fig. 1).

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