ELSEVIER

Journal of Neuroimmunology 159 (2005) 106–112

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

Maternal poly I:C exposure during pregnancy regulates TNFα, BDNF, and NGF expression in neonatal brain and the maternal–fetal unit of the rat

John H. Gilmore*, L. Fredrik Jarskog, Swarooparani Vadlamudi

UNC Schizophrenia Research Center, Department of Psychiatry, CB #7160, University of North Carolina, Chapel Hill, NC 27599-7160, United States

Received 27 August 2004; received in revised form 8 October 2004; accepted 8 October 2004

Abstract

Maternal infection during pregnancy is associated with increased risk for neurodevelopmental disorders. Polyriboinosinic–polyribocytidilic acid (poly I:C) or saline was administered to rats to model maternal infection; levels of TNFα, brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) were determined by ELISA. TNFα was significantly increased in maternal plasma, placenta, and amniotic fluid, while it was significantly decreased in fetal liver/spleen and neonatal brain. NGF and BDNF were significantly decreased in the placenta and fetal liver/spleen. There was no change in BDNF or NGF in the fetal or neonatal brain. Changes in TNFα, BDNF, and NGF after maternal exposure to poly I:C represent a potential mechanism through which maternal infection increases risk for neurodevelopmental disorders.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Infection; Placenta; Amniotic fluid; Cortex; Schizophrenia; Animal model

1. Introduction

Prenatal exposure to maternal infection is a risk factor for neurodevelopmental disorders, including schizophrenia (Brown et al., 2000), mental retardation (Rantakallio and Von Wendt, 1985), and autism (Ciaranello and Ciaranello, 1995). Little is known about the mechanism through which maternal infection acts on the developing brain to increase risk for these neurodevelopmental disorders. Because a variety of bacterial and viral maternal infections can increase risk, we have hypothesized that cytokines generated in response to infection represent a common mechanistic pathway through which early brain development is altered (Gilmore and Jarskog, 1997; Gilmore et al., 2004).

There have been several recent attempts to model the long-term consequences of maternal infection during pregnancy on behavior relevant to schizophrenia. These models include infection with human influenza virus (Shi et al., 2003), exposure to lipopolysaccharide (LPS), a bacterial cell wall endotoxin (Borrell et al., 2002; Fortier et al., 2004), and exposure to polyriboinosinic–polyribocytidilic acid (poly I:C), which mimics viral RNA (Shi et al., 2003; Zuckerman and Weiner, 2003; Zuckerman et al., 2003). These models suggest that maternally generated cytokines are likely mediators of the abnormal brain development that leads to long-term behavioral changes. In the influenza model, no virus is detected in the fetal brain (Shi et al., 2003), suggesting that the immune response to the infection plays a major role in the mechanism of action. In the poly I:C and LPS models, no infectious agent is present, implicating the cytokine response. Maternal LPS exposure does increase cytokine expression in the maternal blood, placenta, and amniotic fluid of rodents (Fidel et al., 1994; Urakubo et al., 2001; Gayle et al., 2004). There is increasing evidence of interactions between inflammatory cytokines and neurotrophins in the nervous system, and we have recently demonstrated that maternal LPS exposure also alters brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the developing brain and maternal–fetal unit (Gilmore et al., 2003). While poly I:C increases

* Corresponding author. Tel.: +1 919 966 6971; fax: +1 919 966 9604.
E-mail address: jgilmore@med.unc.edu (J.H. Gilmore).

0165-5728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2004.10.008

cytokine production by cells of the immune system (Kimura et al., 1994), the effect of poly I:C on cytokine or neurotrophic factor production during pregnancy is not known.

We studied the regulation of TNFα, BDNF, and NGF expression in maternal plasma, placenta, amniotic fluid, fetal liver/spleen, fetal brain, and neonatal cortex in a rodent model of maternal infection that utilizes poly I:C. We hypothesized that maternal exposure to poly I:C will regulate the expression of TNFα, BDNF, and NGF in a manner similar to that previously observed after maternal LPS exposure.

2. Materials and methods

2.1. Animals

Experimental protocols were approved by the UNC Institutional Animal Care and Use Committee. For the acute study, gestational day 16 (E16), timed-pregnant Sprague–Dawley rats (Charles River, Raleigh, NC) were injected i.p. with poly I:C (20 mg/kg; Sigma, St. Louis. MO) or saline. The dose of 20 mg/kg was chosen, as it was the highest dose used by Shi et al. (2003). Animals were anesthetized with ether and decapitated after blood was obtained by cardiac puncture, 2, 8, and 24 h after injection. The uterine horns containing E16 pups were surgically removed. Amniotic fluid was aspirated with a syringe; placenta, fetal liver/spleen, and fetal whole brain were dissected and immediately frozen on dry ice. Maternal plasma was obtained after centrifuging EDTA-treated blood samples for 6 min at 2200 rpm. Three pups from three separate litters (n=9) were used for each treatment and control group; there were three samples per group for maternal plasma. For fetal liver/spleen, two samples from each litter were pooled because of small sample weight, giving an N of nine pooled samples/group.

For the subacute study, timed-pregnant rats received a single i.p. injection with 10 mg/kg of poly I:C or saline on E16. We found that repeated or higher doses were associated with miscarriage or maternal death. Pups were decapitated on day 1 or 7 after birth. Whole brain was obtained on day 1, and frontal cortex was dissected on day 7. Three pups from three separate (n=9) litters were used for the treatment and control groups.

To look at the effect of a different immune challenge on TNFα levels in neonatal cortex at day 7 after birth, we used the samples obtained in our previous study, in which pregnant rats were exposed to LPS (0.1 mg/kg) i.p. or saline on E14, E15, and E16 (n=18/condition; Gilmore et al., 2003).

2.2. Sample preparation

All specimens were stored at −80 °C. The brain tissue, placenta, and liver/spleen were placed in 10–15 volumes of Tris–HCl buffer (50 mM, pH 7.4) with NaCl (0.6 M), Triton X-100 (0.2%), and BSA (0.5%) containing freshly dissolved protease inhibitors: benzamidine (1 mM), benzethonium chloride (0.1 mM), and phenylmethylsulfonyl fluoride (0.1 mM). Samples were homogenized (PowerGen 125, Fisher Scientific, Pittsburgh, PA) on ice for 30 s and sonicated (Sonic Dismembrator 60, Fisher Scientific) for 10 s at 10 mV. Samples were centrifuged at 12,000 rpm for 20 min at 4 °C, and the supernatants were aliquoted and frozen at −80 °C until the assays were performed.

2.3. ELISA

Neurotrophin levels were determined using a two-site ELISA according to the directions of the manufacturer for human BDNF, rat NGF (DuoSet ELISA development kit, R&D Systems, Minneapolis, MN), rat TNFα (Biosource, Camarillo, CA) for amniotic fluid and brain, and rat TNFα (DuoSet ELISA development kit, R&D Systems) for placenta, liver/spleen, and plasma. Optical density was measured at 450 nm using a microplate reader (Vmax, Molecular Devices, Sunnyvale, CA). Preliminary experiments were done to determine the optimal sample dilution for the standard curve of each assay—fetal brain: 1:15 for BDNF and NGF; placenta: 1:10 for BDNF; liver/spleen: 1:15 for BDNF and TNFα; amniotic fluid: neat for BDNF and NGF; maternal plasma: neat for BDNF and TNFα; and neonatal cortex: 1:10 for BDNF and NGF. The limits of detection for BDNF, NGF, and TNFα were 39.1, 15.6, and 4 pg/ml (Bio Source), and 62.5 pg/ml (R&D Systems), respectively. The mean intra-assay coefficients of variance for BDNF, NGF, and TNFα were 2.56%, 3.42%, and 3.75%, respectively. Samples and standards were run in duplicate. Poly I:C or LPS and control samples were run in the same assay on the same plate.

2.4. Statistical analysis

Statistical analyses were performed using Prism 4.0 (GraphPad Software, San Diego, CA). A two-way ANOVA was used to determine the effect of both treatment and time in the acute study. Bonferroni posttests were performed if overall treatment effects were significant. To compare the treatment and saline conditions for postnatal brain samples, t-tests were used. Significance was set at 0.05 using a two-tailed test for all analyses.

3. Results

Maternal exposure to poly I:C significantly increased TNFα protein levels in the maternal plasma (treatment p=0.0031) and placenta (treatment p=0.0498), with a maximum effect at 2 h after exposure (Fig. 1; TNFα levels in the control maternal plasma were all below the level of detection). TNFα was increased in the amniotic fluid at a

Download English Version:

https://daneshyari.com/en/article/9194760

Download Persian Version:

https://daneshyari.com/article/9194760

Daneshyari.com