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Prenatal undernutrition decreases the anorectic response to septic doses of lipopolysaccharides in adulthood in male rats

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ABSTRACT

Prenatal undernutrition affects some physiological functions after birth, and such changes are associated with the pathogenesis of various diseases. Recently, we have reported that prenatally undernourished male rats exhibited stronger febrile and anorectic responses to immune stress induced by moderate-dose lipopolysaccharide (LPS) treatment in adulthood. In the present study, we evaluated the effects of prenatal undernutrition on stress responses to the administration of a septic dose (3 mg/kg) of LPS in later life, mainly focusing on changes in hypothalamic proinflammatory cytokine expression. We also evaluated the expression of hypothalamic and peripheral reproductive factors because it has been suggested that the stress responses of reproductive functions are affected by prenatal and neonatal stress and nutritional conditions. As a result, we found that prenatal undernutrition attenuated the anorectic response to septic-dose LPS treatment in adulthood in male rats. In addition, it attenuated the LPS-induced suppression of serum testosterone levels and the changes in hypothalamic proinflammatory cytokine (interleukin (IL)-1β, tumor necrosis factor-α, and IL-6) expression induced by septic-dose LPS treatment in adulthood. These results suggest that prenatal undernutrition attenuates stress and reproductive responses under severe immune stress conditions. The downregulation of hypothalamic stress-related factor expression might be involved in such attenuated stress responses, which could be one of the protective mechanisms used to prevent excessive immune responses and aid survival.

1. Introduction

Epidemiological and experimental evidence suggests that prenatal undernutrition affects various physiological functions after birth and that such changes are associated with the pathogenesis of certain diseases, such as diabetes, hypertension, and ischemic heart disease, in adulthood (Godfrey and Barker, 2000; Breier et al., 2001; Gluckman and Hanson, 2004). These phenomena can be reproduced in animal models, e.g., in mice the offspring of undernourished mothers exhibit obesity and insulin resistance in adulthood (Yura et al., 2005), and it has been established that changes in hypothalamic functions play pivotal roles in such prenatal undernutrition-induced physiological changes (Yura et al., 2005; Breton et al., 2008; Delahaye et al., 2008). It has been reported that prenatal undernutrition also affects the development of the immune system in humans and animals. For example, humans that have experienced intrauterine growth retardation (IUGR) and animals that are exposed to prenatal undernutrition have impaired immunological functions (Ferguson, 1978; Chandra, 1981, 2002). Similarly, infants that are small-for-gestational-age are at increased risk of infection-induced sepsis during the neonatal period and childhood (Smichen et al., 2000), and IUGR rats exhibit increased innate immunological activity (Equils et al., 2005). Recently, we have reported that prenatally undernourished male rats displayed stronger febrile and anorectic responses to immune stress induced by the injection of a moderate dose of lipopolysaccharides (LPS) in adulthood than prenatally normally nourished rats (Iwasa et al., 2015).

Similar to prenatal undernutrition, prenatal and neonatal stress also have long-lasting effects on stress responses to immune challenges. It has been shown that in rats a single injection of low-dose LPS during the perinatal period induced attenuated febrile responses to low-dose immune challenges in adulthood (Ellis et al., 2005; Mouihate, 2012). Interestingly, it has been reported that the neonatal injection of low-dose LPS also attenuates immune responses to septic doses of LPS in later life (Spencer et al., 2010), suggesting that neonatal stress induces tolerance to several grades of homotypic stress in later life. Therefore, it can be assumed that prenatal undernutrition also affects stress responses to various grades of stress in later life, mainly by altering hypothalamic stress responses. In the present study, we evaluated the effects of prenatal undernutrition on stress responses to the administration of septic doses of LPS in adulthood, mainly focusing on changes in hypothalamic

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Table 1
Primer sequences, product sizes and annealing temperature.

Primer	Sequence	Product size (bp)	Annealing T (°C)
IL-1β forward	GCT GTG GCA GCT ACC TAT GTC TTG	120	61
IL-1β reverse	AGG TCG TCA TCA TCC CAC GAG		
TNF-α forward	AGC CCT GGT ATG AGC CCA TGT	109	65.5
TNF-α reverse	CCG GAC TCC GTG ATG TCT AAG T		
IL-6 forward	TCC TAC CCC AAC TTC CAA TGC TC	79	67
IL-6 reverse	TTG GAT GGT CTT GGT CCT TAG CC		
Kiss1 forward	ATG ATC TCG CTG GCT TCT TGG	91	65
Kiss1 reverse	GGT TCA CCA CAG GTG CCA TTT T		
GAPDH forward	ATG GCA CAG TCA AGG CTG AGA	64	70
GAPDH reverse	CGC TCC TG GAA GAT GGT GAT		

proinflammatory cytokine expression. We evaluated the anorectic response as a behavioral stress marker because the hypothalamic immune system plays pivotal roles in such stress responses (Plata-Salaman, 2001; Iwasa et al., 2014). We also assessed the expression of hypothalamic and peripheral reproductive factors because it has been reported that the stress responses of reproductive functions might also be affected by prenatal and neonatal stress and/or nutritional conditions (Iwasa et al., 2009, 2010, 2017).

2. Materials and methods

2.1. Animals

Eight pregnant Wistar rats were purchased (Charles River Japan, Inc., Kanagawa, Japan) and housed individually under controlled lighting (12 h light, 12 h darkness; lights turned on at 0800 and turned off at 2000) and temperature (24 °C) conditions with free access to water. All animal experiments were conducted in accordance with the ethical standards of the University of Tokushima. The pregnant rats were divided into normally nourished (NN, n = 4) and undernourished (UN, n = 4) groups. The undernourished dam received about 50% (11.5–12.5 g/day) of the daily food intake of the normally nourished dam from days 14 to 21 of pregnancy and then was allowed to feed ad libitum during the lactation period. The day when the pups were delivered was defined as postnatal day 0. To control the litter size to 10–12 per dam, pups were culled or moved to other dams and were fostered until weaning. The pups were weaned at postnatal day 21 and housed at 3–4 animals per cage. Only male rats were used in this study.

2.2. Effects of the injection of a septic dose of LPS on body weight, food intake, and the expression of peripheral and hypothalamic factors

At nine weeks of age, the offspring of the normally nourished dam (the NN group) and undernourished dam (the UN group) were divided into three groups each; i.e., into saline-injected (control), 6 h LPS-injected (6 h), and 24 h LPS-injected (24 h) groups (n = 7–8 per each group). In the control groups, saline was intraperitoneally injected at 0900, and samples (brain and blood) were harvested after the rats had been decapitated at 24 h after the injection procedure (0900 on the next day). In the LPS-injected groups, LPS (055:B5, 3 mg/kg) was intraperitoneally injected at 0900 (24 h groups) or 0300 (6 h groups), and then body weight (BW) and food intake (FI) were measured at 6 h (6 h and 24 h groups) and 24 h (24 h groups) after the injection procedure. The samples were harvested after decapitation at 0900 in both groups.

2.3. Hormone assay

Serum leptin levels were measured using radioimmunoassay kits (multi-species leptin RIA kit, Linco Research Inc., MO, USA). The sensitivity of the assay was 1.0 ng/mL, and its inter- and intra-assay coefficients of variation (CV) were 3.2% and 7.8%, respectively. The serum luteinizing hormone (LH) level was measured using a radioimmunoassay (rLH [I-125] RIA kit, Institute of Isotopes Co., Ltd., Tokyo, Japan). The sensitivity of the assay was 0.8 ng/ ml, and its inter- and intra-assay CV were 7.7% and 6.5%, respectively. The serum follicle stimulating hormone (FSH) level was measured using a radioimmunoassay (rFSH [I-125] RIA kit, Institute of Isotopes Co., Ltd., Tokyo, Japan). The sensitivity of the assay was 0.09 ng/ml, and its inter- and intra-assay CV were 10.3% and 8.4%, respectively. Serum testosterone levels were measured by a commercial laboratory (SRL, Tokyo, Japan) using an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany).

2.4. Quantitative real-time polymerase chain reaction

Whole hypothalamic explants were dissected from the frozen brains, as described previously (Iwasa et al., 2014). Briefly, the brain sections were dissected out via an anterior coronal cut at the posterior border of the mammillary bodies, parasagittal cuts along the hypothalamic fissures, and a dorsal cut 2.5 mm from the ventral surface. Total RNA was isolated from the hypothalamic explants and visceral fat using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen GmbH, Hilden, Germany). Then, cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript III first-strand synthesis system for the real-time polymerase chain reaction (PCR; Invitrogen Co.). The PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. The mRNA levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6, and Kiss1 (a positive regulator of gonadotropin-releasing hormone secretion) were measured. The mRNA expression level of each factor was normalized to that of the GAPDH level. Dissociation curve analysis was also performed for each gene at the end of the PCR. Each amplicon generated a single peak. The primer sequences, product sizes, and annealing temperatures are shown in Table 1. The PCR conditions were as follows: initial denaturation and enzyme activation were performed at 95 °C for 20 s, followed by 45 cycles of denaturation at 95 °C for 3 s, and annealing and extension for 30 s.

2.5. Statistical analysis

Data analysis was performed using one-way ANOVA (for intra-group comparisons) followed by the Tukey-Kramer test or Kruskal-Wallis test followed by the Steel-Dwass test. Two-way ANOVA or Student's unpaired *t*-test and Mann-Whitney U test were used to confirm the significance of differences between the groups.

3. Results

The BW (two-way ANOVA; $F(2,74) = 0.56$, $P = 0.46$) changes and FI (two-way ANOVA; $F(2,74) = 0.49$, $P = 0.48$) after LPS injection did not differ between NN and UN groups. The BW changes seen at 6 h and