Int. J. Devl Neuroscience 48 (2016) 80–83

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Short communication

Prenatal undernutrition results in greater lipopolysaccharide-induced changes in hypothalamic TNF-α expression, but does not affect the equivalent changes in the serum levels of luteinizing hormone and testosterone, in adult male rats

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ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form 29 October 2015
Accepted 3 December 2015
Available online 7 December 2015

Keywords:
IUGR
LH
Testosterone
LPS

ABSTRACT

Immune stress can cause reproductive dysfunction. Some hypothalamic factors such as pro-inflammatory cytokines play pivotal roles in reproductive disorders under immune stress conditions. Recently, it has been reported that prenatal undernutrition affects not only metabolic functions, but also the responses of physiological functions to immune stress in adulthood. In this study, the long-term effects of prenatal undernutrition on the responses of hypothalamic pro-inflammatory cytokine (interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6) expression; reproductive endocrine factors; i.e., the serum levels of gonadotropins and testosterone; and hypothalamic kisspeptin expression to lipopolysaccharide (LPS) were examined in male rats. Pregnant rats were divided into two groups; i.e., the normally nourished group and the undernourished (50% food restricted) group. The offspring of the normally nourished mothers (control) and undernourished mothers (the intrauterine growth restriction [IUGR] group) were sub-divided into saline-injected and LPS (500 μg, i.p.)-injected groups at 10 weeks of age. The rats' hypothalamic pro-inflammatory cytokine levels and serum luteinizing hormone (LH) and testosterone levels were measured and compared between the control and IUGR groups. The hypothalamic pro-inflammatory cytokine mRNA levels of the LPS-injected rats were significantly higher than those of the saline-injected rats in both the control and IUGR groups. The changes in the hypothalamic expression level of TNF-α, but not those of the other cytokines, induced in response to LPS were more marked in the IUGR group than in the control group. On the other hand, although the serum LH and testosterone levels of the LPS-injected rats were significantly lower than those of the saline-injected rats in both the control and IUGR groups, their levels did not differ between the control and IUGR groups under the LPS-injected conditions. These results suggest that prenatal undernutrition results in more marked LPS-induced changes in hypothalamic TNF-α expression, but does not alter the effects of LPS on the serum levels of LH or testosterone, in adult male rats.

1. Introduction

Immune stress can cause various reproductive disorders, such as amenorrhea and sexual behavioral disorders. Some hypothalamic factors such as pro-inflammatory cytokines play pivotal roles in these reproductive disorders under immune stress conditions. For example, the central injection of pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, attenuates the activity of the reproductive endocrine system, and the downregulation of these factors aids the maintenance of reproductive function under immune stress conditions (Yoo et al., 1997; Watanobe and Hayakawa, 2003; Matsuwaki et al., 2006; Iwasa et al., 2015a).

It has been suggested that prenatal undernutrition affects the development of metabolic and physiological functions after birth and that such changes are associated with the pathogenesis of metabolic-related diseases in adulthood (Godfrey and Barker, 2000; Breier et al., 2001; Gluckman and Hanson, 2004). It has been

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http://dx.doi.org/10.1016/j.ijdevneu.2015.12.001
0736-5748/

established that changes in hypothalamic function play pivotal roles in the metabolic and physiological alterations induced by prenatal undernutrition in animal models (Yura et al., 2005; Delahaye et al., 2008). Recently, it has also been reported that prenatal undernutrition affects the development of the immune system (Chandra, 2002). For example, prenatal undernutrition impaired immunological functions, increased the risk of infection-induced sepsis, and increased the activity of the innate immune system in human and animal models (Ferguson, 1978; Chandra, 1981; Simchen et al., 2000; Equils et al., 2005). Although, as noted above, the relationships between prenatal undernutrition and peripheral responses to immune stress in adulthood have been clarified, the effects of prenatal undernutrition on the responses of hypothalamic functions to immune stress have not been fully elucidated. Recently, we reported that prenatally undernourished male rats exhibited stronger febrile responses, which are mainly regulated by the hypothalamus, to lipopolysaccharide (LPS) in adulthood than prenatally normal nourished rats (Iwasa et al., 2015a). In addition to the febrile response, the reproductive endocrine system is also mainly regulated by the hypothalamus, and its function is affected by immune stress (Iwasa et al., 2014a). Therefore, we hypothesized that the changes in the hypothalamic expression levels of pro-inflammatory cytokines induced in response to immune stress in adulthood are exaggerated by prenatal undernutrition and that prenatal undernutrition also influences the responses of the reproductive endocrine system to immune stress in adulthood. To evaluate this hypothesis, the changes in the hypothalamic expression levels of pro-inflammatory cytokines, serum luteinizing hormone (LH, a gonadotropin), and testosterone induced in response to immune stress in adulthood were compared between rats that had been subjected to undernutrition or normal nutrition during the prenatal period. As the hypothalamic expression level of kisspeptin (Kiss1), which is a positive regulator of gonadotropins, is also sensitive to immune stress, the rats'hypothalamic Kiss1 levels were also evaluated.

2. Materials and methods

Sixteen pregnant Sprague-Dawley rats (Charles River Japan, Inc., Tokyo, Japan) were housed individually under controlled lighting (14 h light, 10 h darkness) and temperature (24 °C) conditions. All animal experiments were conducted in accordance with the ethical standards of the University of Tokushima. Standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) was used in this experiment. The pregnant rats were divided into the normally nourished ($n = 8$) and undernourished ($n = 8$) groups. The undernourished dams received about 50% of the daily food intake of the normally nourished dams from days 15 to 21 of pregnancy and were then allowed to feed ad libitum during the lactation period. Daily food intake was calculated based on daily assessments of ad libitum fed pregnant rats in a preliminary experiment. The day on which the pups were delivered was defined as day 0. The pups were randomly assigned to dams (10–12/dam) and were fostered until weaning (postnatal day 21). Half of the pups from the normally nourished mothers were assigned to normally nourished mothers, and the remainder were assigned to undernourished mothers. The pups from the undernourished mothers were also assigned in the same way. The pups from the normally nourished mothers and those from the undernourished mothers were fostered separately until weaning. After being weaned, the pups were separated by sex and housed at 3–4 animals per cage. Only male rats were used in these experiments. At 10 weeks of age, the offspring of the normally nourished mothers (control) and the undernourished mothers (the intrauterine growth restriction [IUGR] group) were sub-divided into saline-injected and LPS-injected groups. The rats were injected with saline or LPS (500 μg/kg, i.p.), and their brains and blood were collected 6 h later (control-saline, $n = 8$; control-LPS, $n = 6$; IUGR-saline, $n = 8$; IUGR-LPS, $n = 6$). Their serum LH and testosterone levels were measured as described previously (Iwasa et al., 2015b). Briefly, the rats' serum LH levels were measured in duplicate using a radioimmunoassay (RIA) (rat LH [I-125] RIA kit, Institute of Isotopes Co., Ltd., Tokyo, Japan). On the other hand, their serum testosterone levels were examined using an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany). The hypothalamic block was dissected, and total RNA was isolated from the hypothalamus as described previously (Iwasa et al., 2015b). cDNA was synthesized with oligo (deoxythymidine) primers using the SuperScript® III first-strand synthesis system (Invitrogen™) for the real-time polymerase chain reaction (PCR). The real-time PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and SYBR® green. The relative quantification of the mRNA expression levels of IL-1β, TNF-α, IL-6, and Kiss1 was performed as described previously (Iwasa et al., 2014b, 2015a). The mRNA expression levels of these molecules were normalized to the mRNA expression level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The serum LH, testosterone, and hypothalamic mRNA levels of the examined molecules were compared using two-way ANOVA followed by Tukey–Kramer post-hoc analysis and the Student's *t*-test at key points. *P*-values of <0.05 were considered to indicate significant differences. Data are expressed as mean ± standard error of the mean (SEM) values.

3. Results

At 10 weeks of age, the mean body weights of the control and IUGR groups were 342 ± 20 and 333 ± 11 g, respectively, and there was no significant difference between the two groups. The rats' serum LH levels were significantly lower under the LPS-injected conditions than under the saline-injected conditions in both the control and IUGR groups (Fig. 1A). Similarly, the rats' serum testosterone levels were significantly lower under the LPS-injected conditions than under the saline-injected conditions in the both control and IUGR groups (Fig. 1B). The hypothalamic mRNA levels of pro-inflammatory cytokines; i.e., IL-1β, TNF-α, and IL-6, were significantly higher under the LPS-injected conditions than under the saline-injected conditions in both the control and IUGR groups (Fig. 1C). The changes in hypothalamic TNF-α expression induced in response to LPS in the IUGR group were significantly greater than those seen in the control group (two-way ANOVA; $F(1,27) = 6.49$, $P = 0.02$). Under the LPS-injected conditions, the TNF-α mRNA level of the IUGR group was significantly higher than that of the control group (Tukey–Kramer post-hoc analysis), whereas none of the other pro-inflammatory cytokines exhibited such tendencies. Hypothalamic Kiss1 mRNA expression did not differ between the saline-injected and LPS-injected conditions in either the control or IUGR groups.

4. Discussion

In the present study, we found that prenatal undernutrition does not affect the responses of LH or testosterone to LPS-induced immune stress in adulthood. Although LPS upregulated hypothalamic TNF-α expression in prenatally undernourished rats, it did not affect the hypothalamic mRNA expression levels of IL-1β, IL-6, or Kiss1. These results suggest that although prenatal undernutrition partially alters the hypothalamic responses of pro-inflammatory cytokines to immune stress, such changes might not affect reproductive endocrine responses.