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The expression of orexigenic and anorexigenic factors in middle-aged female rats that had been subjected to prenatal undernutrition

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ABSTRACT

Fetal growth retardation, which affects short- and long-term fetal brain development, is associated with metabolic, hematological, and thermal disturbances, which can increase the risk of metabolic syndrome later in life. Orexigenic and anorexigenic factors regulate food intake and energy expenditure. We studied how the expression of these factors was affected by food deprivation (FD) in middle-aged female rats that had been subjected to prenatal undernutrition. Eight pregnant rats were divided into two groups, the normal nutrition (NN) ($n = 4$) group and the undernutrition (UN) ($n = 4$) group, which received 50% (approximately 11 g) of the daily food intake of the normal nutrition rats from day 13 of pregnancy to delivery. The pups from these dams were defined as the maternal NN (mNN) and maternal UN (mUN) groups, respectively. After weaning, all of the pups were housed and allowed ad libitum access to food and water. At the age of 6 months, both groups of pups were sub-divided into three groups. One group was allowed to consume normal amounts of food (Fed), and the other two groups were subjected to 24 h or 48 h FD ($n = 7$–8 per group). The rats' serum leptin levels and hypothalamic mRNA expression levels of various orexigenic or anorexigenic factors were measured. In both the mNN and mUN rats, the serum leptin levels of the 24 h and 48 h FD groups tended to be lower than those of the Fed group, and the serum leptin levels of the 24 h FD mUN rats and the Fed mUN rats differed significantly. The hypothalamic neuropeptide Y (NPY) mRNA expression levels of the 24 h and 48 h FD groups were significantly higher in the mUN rats than in the mNN rats. In addition, among the mUN rats the hypothalamic NPY mRNA expression levels of the 48 h FD group were significantly higher than those of the Fed group. In both the mNN and mUN rats, prepro-orexin mRNA expression was lower in the 48 h FD group than in the corresponding Fed group. Among the mUN rats, the 48 h FD group exhibited significantly lower hypothalamic proopiomelanocortin (POMC) mRNA expression than the Fed group, and a similar tendency was seen among the mNN rats. Among the mNN rats, the 24 h FD group displayed significantly higher hypothalamic leptin receptor (OBRb) mRNA levels than the Fed group. However, no such differences were seen among the mUN rats. As a result, the hypothalamic OBRb mRNA expression levels of the mUN rats in the 24 h and 48 h FD groups were lower than those of the corresponding mNN rat groups. These findings indicate that rats that are subjected to prenatal undernutrition exhibit upregulated expression of orexigenic factors and are more sensitive to FD in middle age, which might increase their risk of developing metabolic disorders in later life.

1. Introduction

Maternal protein restriction induces fetal growth retardation (FGR), which influences short- and long-term fetal brain development and can result in metabolic, hematological, and thermal disturbances that promote the development of metabolic syndrome in later life (Barker et al., 2002; Wang et al., 2015). In animal models, low birth weight offspring display reduced circulating leptin levels and downregulated hypothalamic leptin signaling, which alter orexigenic and anorexigenic regulatory mechanisms. Furthermore, they tend to exhibit hyperleptinemia and leptin resistance, which is associated with obesity in adulthood (Desai et al.,

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2005). Orexigenic and anorexigenic factors in the hypothalamus and peripheral tissues play important roles in the regulation of food intake. Neuropeptide Y (NPY), which is a major orexigenic peptide, is mostly synthesized in the hypothalamic arcuate nucleus (ARC). Food deprivation (FD) increases hypothalamic NPY mRNA expression, which stimulates appetite (Allen et al., 1983; Stanley and Leibowitz, 1985; White and Kershaw, 1990). As the ability of the hypothalamus to synthetize NPY falls with age, NPY release and hypothalamic prepro-NPY (ppNPY) mRNA expression decrease in an age-dependent manner (Gruenewald et al., 1994). Orexins are hypothalamic neuropeptides that localize in the lateral hypothalamus. Orexin-immunoreactive nerve fibers are also present in the ARC, paraventricular nucleus, dorsomedial hypothalamus, and other brain regions including the cerebral cortex, medial thalamic nuclei, circumventricular organs, limbic system, and brain stem. A previous study found that hypothalamic prepro-orexin (ppORX) mRNA expression was upregulated in rats that were subjected to FD for 48 h and then centrally administered orexin A and orexin B. This suggests that orexin also plays a role in the central regulation of feeding behavior (Sakurai et al., 1998; Nambu et al., 1999). Leptin is an anorexigenic factor and is derived from adipose tissue. The OB gene encodes leptin, which reduces food intake, increases energy expenditure, and modulates glucose and fat metabolism through its receptor OBRb in the hypothalamic ARC (Zhang et al., 1994; Friedman and Halaas, 1998). A previous study found that functional hypothalamic leptin receptor (OBRb) expression was increased in 1-day-old FGR rats, and this pattern was reversed in adult FGR rats (Khorram et al., 2015). Proopiomelanocortin (POMC) is another anorexigenic factor. It is predominantly found in the lateral ARC, the neurons of which are activated by leptin (Cowley et al., 2001). This study was conducted to examine the effects of FD on the expression of orexigenic and anorexigenic factors in the hypothalamus in middle-aged female rats that were subjected to prenatal undernutrition.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats (gestational age: 13 days, 280–360 g) were purchased from Charles River Japan, Inc. (Tokyo, Japan) and housed individually. The animal rooms were maintained under controlled lighting (14 h light, 10 h dark cycle) and temperature (24 °C) conditions. All animal experiments were conducted in accordance with the ethical standards of the Animal Care and Use Committee of Tokushima University. In total, 8 pregnant rats and their offspring were used in this study. The pregnant rats were divided into two groups. In the normal nutrition (NN) ($n = 4$) group, the dams were allowed ad libitum access to water and food during the gestation and lactation periods. In the undernutrition (UN) ($n = 4$) group, the dams received 50% (approximately 11 g) of the daily food intake of the NN rats from day 13 of pregnancy to delivery. Thereafter, they were allowed ad libitum access to water and food during lactation period. After birth, the pups from the NN and UN dams were defined as the maternal NN (mNN) and maternal UN (mUN) groups, respectively. Both mNN and mUN pups were weighed and randomly assigned to each dam (10–12 per dam) to avoid any confounding litter size effect. The pups were culled and fostered to other dams until weaning. The day the litters were born was defined as postnatal day (PND) 1. The pups were weaned at PND 21. After weaning, the pups in both groups were housed 3–4 animals per cage and allowed ad libitum access to food and water. Only female pups were used for this experiment. At the age of 6 months, the mNN and mUN rats were sub-divided into three groups. One group was allowed to consume normal amounts of food (Fed), and the other two groups were subjected to 24 h or 48 h FD ($n = 7$–8 per group). The body weights of rats were measured at before and after 24 h, 48 h of FD.

2.2. Serum and tissue collection

After 24 h or 48 h fasting, all of the rats were killed by decapitation between 0900 and 1100 h of the light cycle. Their blood and whole brains were collected. The serum samples were stored at −20 °C, and the brain tissues were stored at −80 °C.

2.3. Hormone assay

Serum leptin levels were measured using an I-125 radioimmunoassay (RIA) kit (rat leptin RIA kit, Linco Research Inc., St. Charles, MO, USA). The sensitivity of the assay was 0.5 ng/ml and its inter-and intra-assay coefficients of variation were 4.8% and 2.4%, respectively.

2.4. Quantitative real-time PCR analysis

Before the RNA analysis, hypothalamic explants including the ARC were dissected out from the rats' frozen brains. The brain sections were dissected via a coronal cut at 2 mm anterior to the optic chiasm and a posterior cut at the posterior border of the mammillary bodies. These tissue blocks were cut 2.5 mm from the bottom of the hypothalamus and then trimmed at 2.5 mm lateral from the midline of each side. Total RNA was extracted from the anterior and posterior blocks using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy® mini kit (Qiagen Gmbh, Hilden, Germany). 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 (RT-PCR; Invitrogen Co.). RT-PCR analysis was performed using the StepOnePlus™ RT-PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. Hypothalamic tissue expression levels were normalized to the mRNA expression levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which is the most stable housekeeping gene in the brain (Vandesompele J et al., 2002). The following forward and reverse primers were used: NPY: F: 5′-GGG GCT GTG TGG ACT GAC CCT-3′, R: 5′-GAT GTA GTG TCG CAG AGC GGAG-3′; ppORX: F: 5′-GCC GTC TCT ACG AAC TGT TG-3′, R: 5′-CGA GGA GAG GGG AAA GTT AG-3′; POMC: F: 5′-CCC GAG AAA CAG CAG CAC TC-3′, R: 5′-AGG GGG CCT TGG ACT GAG AA-3′; OBRb: F: 5′-GCA GCT ATG GTC TCA CTT CTT TTG-3′, R: 5′-GGT TCC CTG GGT GCT CTGA-3'; GAPDH: F: 5′ -ATG GCA CAG TCA AGG CTG AGA-3′, R: 5′ - CGC TCC TGG AAG ATG GTG AT-3′. The PCR conditions were as follows: the 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 at 65 °C for 30 s (NPY), 60 °C for 30 s (ppORX), 65 °C for 30 s (POMC), 63 °C for 30 s (OBRb), or 64 °C for 30 s (GAPDH), and a final extension step of 72 °C for 1 min. The copy numbers of the transcripts were normalized against those the GAPDH transcript for NPY, ppORX, POMC, and OBRb (Iwasa et al., 2011a,b, 2015). Shi et al., reported that mRNA expression and peptide expression of NPY are strongly correlated (Shi et al., 2009). Therefore, we did not estimated peptide expression by Western blot analysis in this experiment.

2.5. Statistical analysis

Statistical analyses were performed using one-way or two-way analysis of variance (ANOVA), and *post hoc* comparisons were carried out using Dunnett's test. All data are presented as mean ± SE values. *P*-values of <0.05 were considered to be statistically significant.