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Developmental changes in the hypothalamic mRNA expression levels of brain-derived neurotrophic factor and serum leptin levels: Their responses to fasting in male and female rats

Takeshi Iwasa*, Toshiya Matsuzaki, Kiyohito Yano, Munkhsaikhan Munkhzaya, Altankhuu Tungalagsuvd, Maira Yiliyasi, Akira Kuwahara, Minoru Irahara

Department of Obstetrics and Gynecology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-Cho, Tokushima 770-8503, Japan

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ABSTRACT

The actions and responses of hypothalamic appetite regulatory factors change markedly during the neonatal to pre-pubertal period in order to maintain appropriate metabolic and nutritional conditions. In this study, we examined the developmental changes in the hypothalamic mRNA levels of brain-derived neurotrophic factor (BDNF), which is a potent anorectic factor and the changes in the sensitivity of the hypothalamic expression of this factor to fasting during the neonatal to pre-pubertal period. Under fed conditions, hypothalamic BDNF mRNA expression decreased during development in both male and female rats. Similarly, the serum levels of leptin, which is a positive regulator of hypothalamic BDNF expression, also tended to fall during the developmental period. The serum leptin level and the hypothalamic BDNF mRNA level were found to be positively correlated in both sexes under the fed conditions. Hypothalamic BDNF mRNA expression was decreased by 24 h fasting (separating the rats from their mothers) in the early neonatal period (postnatal day 10) in both males and females, but no such changes were seen at postnatal day 20. Twenty-four hours' fasting (food deprivation) did not affect hypothalamic BDNF mRNA expression in the pre-pubertal period (postnatal day 30). On the other hand, the rats' serum leptin levels were decreased by 24 h fasting (separating the rats from their mothers at postnatal day 10 and 20, and food deprivation at postnatal day 30) throughout the early neonatal to pre-pubertal period. The correlation between serum leptin and hypothalamic BDNF mRNA levels was not significant under the fasted conditions. It can be speculated that leptin partially regulates hypothalamic BDNF mRNA levels, but only in fed conditions. Such changes in hypothalamic BDNF expression might play a role in maintaining appropriate metabolic and nutritional conditions and promoting normal physical development. In addition, because maternal separation induces a negative energy balance and short- and long-term stress responses, it is also possible that reductions in hypothalamic BDNF mRNA levels in the early neonatal period (postnatal day 10) may be partially induced by stress responses of the maternal deprivation.

1. Introduction

The actions and responses of hypothalamic appetite regulatory factors change markedly during the neonatal to pre-pubertal period in order to maintain appropriate metabolic and nutritional conditions. We have shown that the basal expression levels of some hypothalamic orexigenic factors and their responses to fasting change during the neonatal to pre-pubertal period (Iwasa et al., 2015, 2016). On the other hand, as far as we know, there are only limited data about the developmental changes in the expression of hypothalamic anorexigenic factors.

Brain-derived neurotrophic factor (BDNF), which is a neurotrophin, is widely expressed in the central nervous system (Lewin and Brade, 1996) and is known to be involved in the development and maintenance of the nervous system (Huang and Reichardt, 2001). Recently, it has been shown that hypothalamic BDNF plays important roles in the regulation of appetite and energy metabolism. BDNF mRNA is expressed at high levels in the ventromedial nucleus of the hypothalamus (VMH) and paraventricular nucleus (PVN), areas that are associated with appetite and metabolism (Wang et al., 2007a,b; Xu and Xie, 2016). The chronic intracerebroventricular infusion of BDNF reduced the appetite and

* Corresponding author.
E-mail address: iwasa.takeshi@tokushima-u.ac.jp (T. Iwasa).

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body weight of adult rats (Lapchak and Hefti, 1992), and the direct injection of BDNF into the VMH and PVN reduced food intake and increased energy expenditure (Wang et al., 2007a,b; Xu and Xie, 2016). In addition, 24 h food deprivation reduced the VMH BDNF mRNA levels of rats (Liu et al., 2014), and the injection of leptin, which is a potent anorectic factor derived from adipose tissue, increased the VMH BDNF mRNA levels of mice (Komori et al., 2006). These results suggest that hypothalamic BDNF is a potent anorectic factor and that the BDNF mRNA level might be partially regulated by leptin. Human studies have also indicated that the BDNF pathway plays a pivotal role in the energy regulation system (Cordeira and Rios, 2011). We speculate that, as has been found for other factors, the basal hypothalamic expression levels of BDNF and their responses to fasting change during development because the serum leptin level varies markedly during this period (Iwasa et al., 2014). In the present study, the developmental changes in the hypothalamic BDNF mRNA level and the changes in their sensitivity to fasting during the neonatal to pre-pubertal period were examined. In addition, the relationship between hypothalamic BDNF mRNA and serum leptin levels were evaluated under fed and fasted conditions.

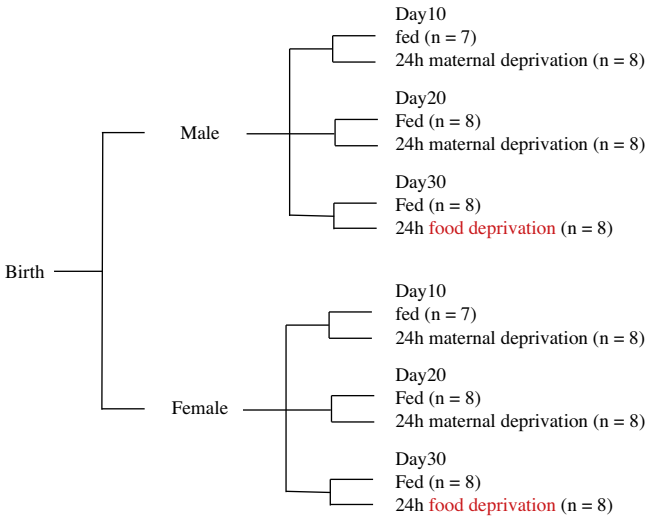

Fig. 1. Experimental design of this study.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats were purchased (Charles River Japan Inc., Tokyo, Japan) and housed under controlled lighting (14 h light, 10 h dark) and temperature (24 °C) conditions. The day on which the pups were born was defined as postnatal day 1. Twelve pups were randomly assigned to each dam on postnatal day 2 and weaned on postnatal day 21. Rats of both sexes were randomly selected from each dam on postnatal days 10 and 20 and divided into the fed and fasting groups (n = 7–8 per group) (n = 7 for the postnatal day 10 fed males, n = 8 in the other groups). Specifically, 3 or 4 rats of each sex were selected from each dam and randomly assigned to the fed and fasting groups on postnatal days 10 and 20. To control litter size to 10–12 per dam, pups were moved to other dams and were fostered until weaning. After weaning, pups of the same sex were housed at 3–4 per cage and were randomly assigned to the fed and fasting groups on postnatal day 30. The fasting groups were subjected to 24 h maternal (postnatal days 10 and 20) or food deprivation (postnatal day 30). It has been reported that 24 h maternal deprivation in the neonatal period induces body weight reduction and increases stress responses, e.g., it activates the hypothalamic-pituitary-adrenal axis (Levine et al., 1991; Smith et al., 1997; Schmidt et al., 2006). The experimental design is summarized in Fig. 1. All animal experiments were conducted in accordance with the ethical standards of the animal care and use committee of the University of Tokushima.

2.2. Hormone assay and quantitative real-time polymerase chain reaction

Serum leptin levels were measured using a 125I-radioimmunoassay (RIA) kit (multi-species leptin RIA kit, Linco Research Inc., MO, USA). Whole hypothalamic explants were dissected from the frozen brains, as described previously (Iwasa et al., 2015, 2016; Munkhzaya et al., 2015). Total RNA was isolated using a TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy mini kit® (Qiagen Gmbh, Hilden, Germany). Five μg of total RNA were used for the cDNA synthesis. 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). Standard curves, which were generated by serially diluting an abundant sample at least 4 times, were used for the relative quantification of each mRNA expression level. The mRNA expression levels of BDNF were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The following forward and reverse primers were used: BDNF: F: 5′- AGC TGA GCG TGT GTG ACA GT −3′, R: 5′- ACC CAT GGG ATT ACA CTT GG −3′; GAPDH: F: 5′- ATG GCA CAG TCA AGG CTG AGA −3′, R: 5′- CGC TCC TGG AAG ATG GTG AT- 3′. The PCR cycling conditions were as follows: initial denaturation and enzyme activation at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 63 °C for 30 s (BDNF) or 65 °C for 30 s (GAPDH), and extension at 72 °C for 1 min. GAPDH mRNA expression was not affected by development or fasting in either sex. In addition, its expression did not differ between the sexes at any of the examined ages.

2.3. Statistical analyses

All data are expressed as mean ± SEM values. The statistical analyses were performed using one-way or two-way analysis of variance (ANOVA) together with the Tukey-Kramer post-hoc test or the Student's *t* test. Correlation analyses were performed using Pearson's (when the data were normally distributed) or Spearman's (when the data were not normally distributed) correlation coefficient as appropriate. Statistical significance was defined as $P < 0.05$.

3. Results

Under the fed conditions, the hypothalamic BDNF mRNA level differed significantly among the examined age groups in both the male (one-way ANOVA; $F(2,23) = 77.8$, $P < 0.01$) and female (one-way ANOVA; $F(2,23) = 7.05$, $P < 0.01$) rats (Fig. 2A). Hypothalamic BDNF mRNA expression fell with age in both sexes. However, under the fed conditions the patterns of change in hypothalamic BDNF mRNA expression seen during development differed between males and females (two-way ANOVA; $F(2,46) = 19.4$, $P < 0.01$). The hypothalamic BDNF mRNA levels of the male rats were significantly higher than those of the females on postnatal days 10 and 20, but not on postnatal day 30 (Student's *t* test) (Fig. 2A). The patterns of change in hypothalamic BDNF mRNA expression seen during development differed between the fed and 24 h fasted conditions in both males (two-way ANOVA; $F(2,46) = 5.93$, $P < 0.05$) and