Int. J. Devl Neuroscience 49 (2016) 46–49

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Developmental changes in the hypothalamic mRNA levels of nucleobindin-2 (NUCB2) and their sensitivity to fasting in male and female rats

Takeshi Iwasa*, Toshiya Matsuzaki, Altankhuu Tungalagsuvd, Munkhsaikhan Munkhzaya, Mayila 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

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 9 December 2015
Accepted 9 January 2016
Available online 15 January 2016

Keywords:
NUCB2
Leptin
Food deprivation

ABSTRACT

Nesfatin-1 is a central anorectic peptide derived from the precursor protein nucleobindin-2 (NUCB2). In the present study, the changes in hypothalamic NUCB2 mRNA expression and their responses to food deprivation during the neonatal to pre-pubertal period (postnatal days 10, 20, and 30) were evaluated in male and female rats. The rats' serum leptin levels were also measured because NUCB2 mRNA expression is positively regulated by leptin. In both the female and male rats, hypothalamic NUCB2 mRNA expression tended to fall throughout development. Similarly, higher serum leptin levels were detected on postnatal day 10 than on postnatal days 20 and 30 in both sexes. Hypothalamic NUCB2 mRNA expression was positively correlated with the serum leptin level in both the female and male rats; however, the relationship was not significant in males. The hypothalamic NUCB2 mRNA levels of the fed and 24 h fasted groups did not differ at any time point in either sex. On the other hand, the serum leptin levels of the 24 h fasted group were significantly lower than those of the fed group at all time points in both sexes. It can be speculated that the upregulation of hypothalamic leptin activity might induce a transient increase in hypothalamic NUCB2 mRNA expression during the early postnatal period (postnatal day 10) in both sexes. However, hypothalamic NUCB2 mRNA expression does not become sensitive to a negative energy balance during the neonatal to pre-pubertal period.

1. Introduction

Nesfatin-1 is a peptide derived from the precursor protein nucleobindin-2 (NUCB2) (Oh et al., 2006). NUCB2/nesfatin-1 protein and mRNA are abundant throughout the hypothalamus, and the central injection of nesfatin-1 was found to reduce food intake in rats (Foo et al., 2008; Maejima et al., 2009). On the other hand, the inhibition of endogenous NUCB2/nesfatin-1 expression increased food intake in rats (Oh et al., 2006). NUCB2/nesfatin-1 mRNA and protein expression are downregulated by food restriction and upregulated by refeeding in rats (Oh et al., 2006; Kohno et al., 2008). To summarize, NUCB2/nesfatin-1 acts as an anorectic factor in the hypothalamus, and its activity is affected by nutritional and metabolic status. Recently, it has been reported that hypothalamic NUCB2/nesfatin-1 is targeted by leptin, which is a pivotal anorectic factor derived from adipose tissue. In both in vivo and in vitro experiments, it was demonstrated that the administration of leptin increases NUCB2 mRNA expression in the hypothalamic paraventricular nucleus, which acts as the integrative center for feeding and energy metabolism, and activates neurons in which NUCB2/nesfatin-1 is abundant (Darambazar et al., 2015). In addition, the suppressive effects of leptin on appetite are attenuated in NUCB2 knockdown mice, indicating that NUCB2/nesfatin-1 is a direct target of leptin and that it mediates the anorexigenic effects of leptin. Recently, it was reported that NUCB2/nesfatin-1 also plays a role in other physiological processes. For example, some studies have suggested that nesfatin-1 is involved in the regulation of glucose homeostasis, water intake, gastrointestinal functions, temperature regulation, cardiovascular functions, the onset of puberty, and sleep (Garcia-Galiano et al., 2010; Vas et al., 2013; Stengel, 2015).

Previous studies have indicated that metabolic and nutritional regulatory systems are not in operation in the early postnatal period; i.e., they only develop during the neonatal to pre-pubertal

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

http://dx.doi.org/10.1016/j.ijdevneu.2016.01.003
0736-5748/

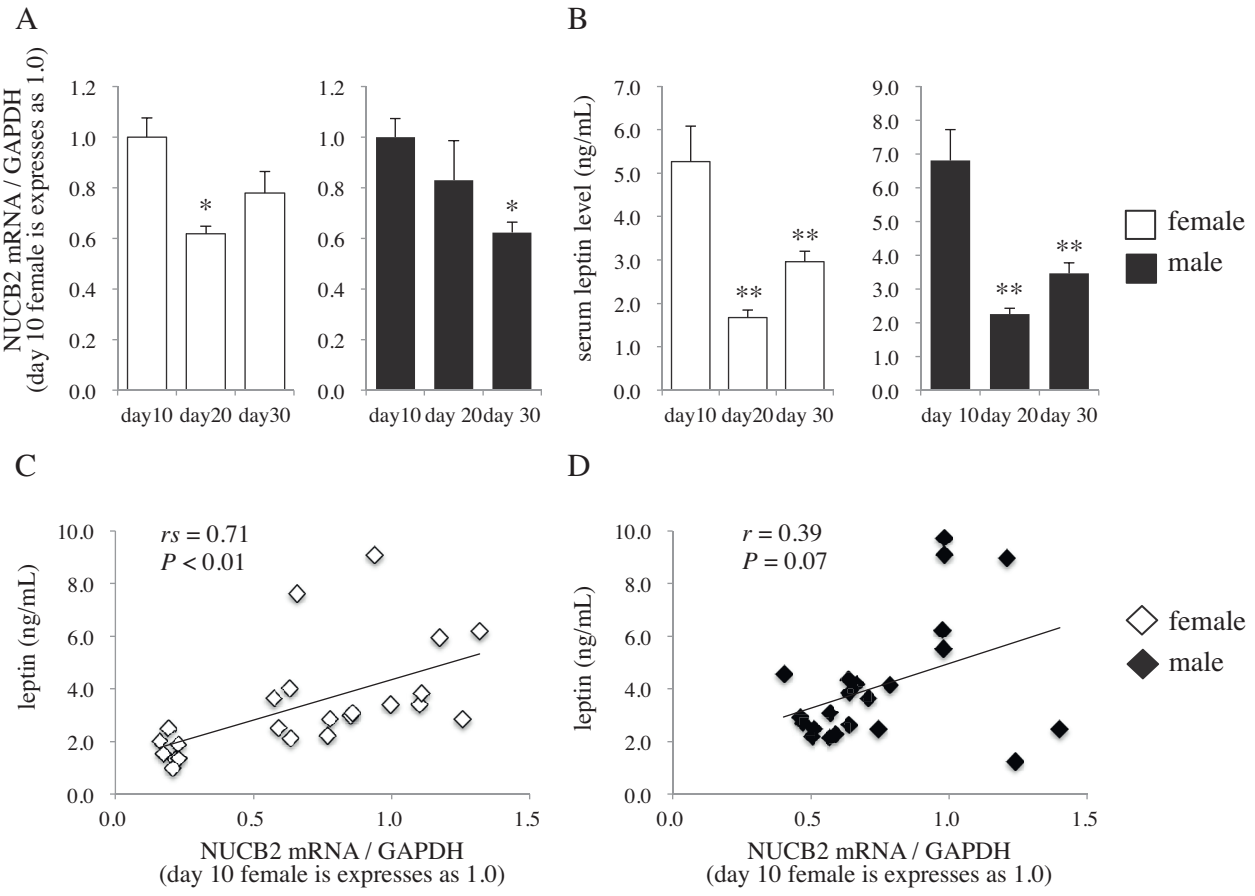

Fig. 1. Hypothalamic NUCB2 mRNA expression (A) and serum leptin levels (B) recorded during the neonatal to pre-pubertal period (postnatal day 10–30) in female (□) and male (■) rats. Data are expressed as mean + SEM values. The correlation between hypothalamic NUCB2 mRNA expression and the serum leptin level in female (C) and male rats (D). NUCB2 mRNA levels were normalized to the GAPDH mRNA level, and the data obtained for females and males on postnatal day 10 were defined as 1.0, respectively. * $P < 0.05$, ** $P < 0.01$ vs. the day 10 values for the same sex (one-way ANOVA together with Dunnett's post-hoc test for intra-sex comparisons and Pearson's correlation co-efficient or Spearman's rank correlation co-efficient for correlation between NUCB2 and leptin).

period. For example, the mRNA levels of some hypothalamic orexigenic factors increase and become responsive to food deprivation during this period (Iwasa et al., 2014a, 2015). In addition, serum leptin levels change markedly during the neonatal to pre-pubertal period. Specifically, the serum leptin level increases transiently in the early neonatal period. This phenomenon is called the leptin surge (Iwasa et al., 2014b).

To the best of our knowledge, the developmental changes in hypothalamic NUCB2/nesfatin-1 levels have not been fully examined. In the present study, the changes in hypothalamic NUCB2 mRNA expression and their responses to food deprivation during the neonatal to pre-pubertal period were evaluated in male and female rats. The rats' serum leptin levels were also measured because, as noted above, NUCB2 mRNA expression is partially regulated by leptin (Darambazar et al., 2015).

2. Materials and methods

All animal experiments were conducted in accordance with the ethical standards of the animal care and use committee of the University of Tokushima. Pregnant Sprague-Dawley rats (Charles River Japan Inc., Tokyo, Japan) were housed under controlled lighting (12 h light: 12 h darkness cycle) and temperature conditions (24 °C). Prior to the sample collection, the rats were killed by decapitation under deep sevoflurane-induced anesthesia. All studies were approved by the animal investigation committee of Tokushima University (permit number: 12116). The day on which the litters were born was defined as postnatal day 1. On postnatal day 2, 10–12 pups were randomly assigned to each dam. The rats used on postnatal day 30 were weaned at postnatal day 21 and housed at 3–4 per cage. Rats of both sexes were randomly selected from each dam, weighed, and divided into fed and fasting groups ($n = 7$–8 per group) on postnatal days 10, 20, and 30. The rats in the fasting groups were subjected to 24 h maternal (postnatal day 10 and 20) or food (postnatal day 30) deprivation. In the maternal deprivation groups, the pups were separated from their mothers for 24 h, and hence, did not receive any milk or food (because they had not been weaned) during this period. Twenty-four hours later, the rats' brains were collected by decapitation and stored at −80 °C after being snap frozen. Whole hypothalamic explants were dissected from the frozen brains, as described previously (Iwasa 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). 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 expression levels of NUCB2 were normalized to that of GAPDH. The following forward and reverse primers were used: NUCB2: F: 5′- GAG GAG ATA AGG AGC GGG AGG C - 3′, R: 5′- ATG TGT CAG GAT TCT GGT GGT TCA - 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: initial denaturation and