Sensitivities of mRNA expression levels of Kiss1 and its receptor, Kiss1r, to nutritional status are changed during the developmental period in female rats

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Abstract

Decreased activity of kisspeptin, the product of the hypothalamic Kiss1 gene, is the major cause of the suppression of reproductive function in subnutritional conditions. The sensitivities of the endocrine and the hypothalamic neuronal systems to nutritional status develop during the neonatal period. We examined the developmental changes in the sensitivity of hypothalamic mRNA expression of Kiss1 and its receptor, Kiss1r, to nutritional status in female rats. Kiss1 mRNA expression was reduced by 24 h food deprivation (24 h FD) at postnatal day 25, but not at postnatal day 5 or 15. Kiss1r mRNA expression was reduced by the 12 or 24 h FD at postnatal days 5 and 25, but not at postnatal day 15. Kiss1r mRNA level was found to be correlated with the plasma leptin level, and the administration of leptin, which increased the serum leptin concentration above the physiological range, restored the acute FD-induced suppression of Kiss1r mRNA expression. These data suggest that the hypothalamic Kiss1 and Kiss1r mRNA expression is differentially affected by the nutritional condition at different age points. It is speculated that the sensitivity of Kiss1 mRNA, which is expressed in kisspeptin neuron, to nutritional status develops during the neonatal period. On the other hand, it seems that the sensitivity of Kiss1r mRNA, which is expressed in GnRH neuron, to nutritional status has been already established during the early neonatal period. These data also show that hypoleptinemia plays a role in the reduction of hypothalamic Kiss1r mRNA expression under subnutritional conditions.


Introduction

Reproductive function is highly sensitive to metabolic and nutritional status. Both pulsatile secretion of LH and the LH surge are inhibited by short-term food deprivation (FD) in ovariectomized estradiol (E2) and/or progesterone-primed female rodents (McClure & Saunders 1985, Cagampang et al. 1991, Wade & Schneider 1992, Kohsaka et al. 2001). Similarly, the estrous cycle is disrupted (Knuth & Friesen 1983) and sexual maturation is delayed by chronic food restriction in gonadal intact female rats (Castellano et al. 2005). Recently, kisspeptin, the product of the Kiss1 gene, and its receptor, Kiss1r, a product of the G protein-coupled receptor 54 (GPR54) gene, have emerged as essential gatekeepers of reproduction. GnRH and the gonadotrophin release are stimulated and sexual maturation is accelerated by the administration of kisspeptin in several species (Gottsch et al. 2004, Irwig et al. 2004, Navarro et al. 2004, 2005, Messager et al. 2005, Shahab et al. 2005, Roseweir & Millar 2009). On the other hand, hypogonadotropic hypogonadism and delayed sexual maturation are induced by loss-of-function point mutations and deletion of GPR54 in humans and mice (de Roux et al. 2003, Seminara et al. 2003). Experimental evidence suggests that a decrease in the activity of kisspeptin is the main cause of the inhibition of reproductive function observed in subnutritional conditions. Hypothalamic Kiss1 mRNA expression is reduced in acute food-deprived female rodents (Castellano et al. 2005, Luque et al. 2007, Brown et al. 2008) and streptozotocin-induced diabetic male rats (Castellano et al. 2006), and hypogonadotropic hypogonadism can be restored in these animals by the administration of kisspeptin (Castellano et al. 2005, 2006, Luque et al. 2007). On the other hand, the effects of FD on Kiss1r are disputed. Although hypothalamic Kiss1r mRNA expression is increased by 72 h FD in prepubertal rats (Castellano et al. 2005), its expression levels are reduced by 12–48 h FD in adult mice (Luque et al. 2007).

It is suggested that leptin transmits signals related to energy balance to kisspeptin neurons through its receptor, Ob-R (Smith et al. 2006, Castellano et al. 2009, Roa et al. 2010) and acts as a positive regulator of the hypothalamic kisspeptin system. Kiss1 mRNA expression is lower in leptin-deficient
ob/ob mice than in wild-type mice (Smith et al. 2006, Castellano et al. 2009, Roa et al. 2010), and the reduced Kiss1 mRNA expression seen in streptozotocin-induced diabetic male rats can be normalized by central leptin infusion (Castellano et al. 2006, 2009, Roa et al. 2010). In addition, the mRNA expression levels of Kiss1 and Kiss1r in primary cultures of human fetal GnRH-secreting neuroblasts are increased by the addition of leptin (Morelli et al. 2008).

It has been reported that the neuronal pathways in the hypothalamus develop rapidly during the neonatal period (Bouret et al. 2004, Bouret 2010). The neuronal projections from the arcuate nucleus (ARC) to the periventricular nucleus (PeN) develop from postnatal day 8, and the mature patterns of innervation are achieved by postnatal day 16 (Bouret et al. 2004). Similarly, neuronal projection of kisspeptin also develops during the neonatal period (Clarkson & Herbison 2006). In addition, experimental data have suggested that the sensitivity of some endocrine and hypothalamic neuronal systems to metabolic and nutritional status develops simultaneously with their neuronal projection during the neonatal period. For example, the sensitivity of hypothalamic orexigenic and anorexigenic factors to leptin during the neonatal period, and the anorectic effects of leptin on appetite and feeding behaviors are acquired during the same period in mice and rats (Mistry et al. 1999, Carlo et al. 2007). Similarly, the adrenal response to FD is established during the neonatal period in mice (Ahima et al. 1998). It has also been reported that an abnormally high or low nutritional status during the fetal or neonatal period permanently affects hypothalamic function and induces metabolic diseases in later life in humans and experimental animals (Barker et al. 1993, Proulx et al. 2002, Yura et al. 2005, Bouret et al. 2008, Delahaye et al. 2008, Remmers et al. 2008, Breton et al. 2009, Kirk et al. 2009). Although the effects of undernutrition on kisspeptin action have been investigated in mice and rats at various reproductive ages, the developmental changes that occur in the sensitivity of the kisspeptin system to nutritional status have not been evaluated.

In this study, we examined the developmental change in the sensitivity of hypothalamic Kiss1 and Kiss1r mRNA expression to nutritional status in female rats. In addition, we have examined whether the acquisition of leptin sensitivity by the kisspeptin system is involved in the developmental changes in its sensitivity to nutritional status because the leptin sensitivity of other hypothalamic factors is established during the neonatal period.

**Materials and Methods**

**Animals**

Sprague–Dawley rats (Charles River Japan Inc., Tokyo, Japan) were used. The day the litters were born was considered as postnatal day 1. The animals were housed in a room with controlled lighting (14 h light:10 h darkness cycle) and temperature (24 °C) and were weaned at postnatal day 21. All animal experiments were conducted in accordance with the ethical standards of the Animal Care and Use Committee of the University of Tokushima.

**Effects of maternal and food deprivation on plasma hormone concentrations and hypothalamic neuropeptide (GnRH, Kiss1, and Kiss1r) mRNA levels at various developmental stages**

To determine the effects of maternal deprivation (MD) and/or FD on hypothalamic peptides mRNA levels at various developmental stages, female rats at postnatal days 5, 15, and 25 of age were divided into three groups: the control, the 12 h MD or FD, and the 24 h MD or FD groups (n=7–8 per group). The rats in all the groups were weighed, and the rats in the 12 and 24 h deprivation groups were separated from their mothers (postnatal day 5 or 15) or were deprived of food (postnatal day 25). At the end of the MD or FD, the animals were weighed and killed by decapitation between 0900 and 1000 h of the light cycle, and their blood and whole brain were collected. The rats in the control group were neither separated from their mothers nor deprived of food and were weighed again after 24 h and then killed by decapitation. The serum was separated by centrifugation and stored at −20 °C, and the whole brain was snap frozen and stored at −70 °C. Before the RNA analysis, hypothalamic explanats, including the median preoptic area, the anterovenetal PeN (AVPV), and the ARC, were dissected out from the frozen brains according to the following methods, as described elsewhere (Castellano et al. 2006). A brain section was dissected out through an anterior coronal cut at 1 mm (postnatal days 5 and 10) or 2 mm (postnatal day 25) anterior from the optic chiasm and a posterior coronal cut at the posterior border of the mammillary bodies. Then, these tissue blocks were cut through two parasagittal cuts along the hypothalamic fissures and dorsally cut at 2·0 mm (postnatal days 5 and 10) or 2·5 mm (postnatal day 25) from the ventral surface.

The plasma leptin and LH (postnatal days 15 and 25) concentrations and the hypothalamic Kiss1, Kiss1r, and GnRH mRNA levels were then measured. As the sample volume for the postnatal day 5 rats was insufficient, the plasma LH concentration could not be measured. In the postnatal day 25 rats, the serum E2 concentration, the ovarian weight, and the uterine weight were also measured because a change in the E2 concentration might have affected the hypothalamic Kiss1 mRNA level.

**Effects of the co-administration of leptin on FD-induced alterations in plasma hormone concentrations and hypothalamic neuropeptide (Kiss1 and Kiss1r) mRNA levels**

To determine the effects of the co-administration of leptin on FD-induced alterations in plasma hormone concentrations and hypothalamic neuropeptide mRNA levels, the postnatal day 25 female rats were divided into three groups: the control,
the 24 h FD, and the 24 h FD with leptin administration (24 h FD+lep) groups (n=7–8 per group). The rats in the 24 h FD+lep group were given i.p. injections of leptin (3 mg/kg body weight) at 12 and 21 h after the initiation of FD. This dose of leptin is sufficient to maintain the plasma leptin concentration above physiological levels for 13 h in neonatal rats (Proulx et al. 2002). We measured the plasma leptin concentration following exogenous leptin administration at the dose used in this study as a preliminary experiment. The rats in the control and 24 h FD groups were administered i.p. injections of saline at the same time points. At the end of the FD, the animals were killed by decapitation. The blood and whole brain were collected, and hypothalamic explants were dissected out as mentioned above. Then, plasma LH concentrations and hypothalamic GnRH, Kiss1, and Kiss1r mRNA levels were measured.

Hormone assays
The plasma leptin concentration was measured using an I-125 RIA kit (Rat leptin RIA kit, Linco Research Inc., St. Charles MO, USA). The sensitivity of the assay was 0.5 ng/ml. The inter- and intra-assay coefficients of variation (CV) were 4-8 and 2-4% respectively. The plasma LH concentration was measured using an I-125 RIA kit (Rat LH (I-125) RIA kit, Institute of Isotopes Co., Ltd., Tokyo, Japan). The sensitivity of the assay was 0.2 ng/ml. The inter- and intra-assay CV values were 6-6 and 6-5% respectively. The plasma E2 concentration was measured using an I-125 RIA kit (Double Antibody E2 kit, Diagnostic Products Co., Los Angeles, CA, USA). The sensitivity of the assay was 1-4 pg/ml. The inter- and intra-assay CV values were 4-1 and 4-6% respectively.

Quantitative real-time PCR
Total RNA was isolated from the hypothalamus using a TRIzol reagent kit (Invitrogen Co.) and an RNeasy Mini kit (Qiagen GmbH). cDNA was synthesized with oligo (deoxythymidine) primers at 50°C using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Co.). Real-time PCR analysis was performed using the PCR System 7500 (PE Applied Biosystems, Foster City, CA, USA) with SYBR green. Sequence-specific primers were generated on the basis of the published rat sequences using the Primer3 software (Whitehead Institute/MIT Center for Genome Research; http://www.genome.wi.mit.edu). The forward and reverse primers used were as follows: Kiss1: F: 5'-AGC TGC TGC TGC TGC TCC TCT GT-3'; R: 5'-AGG CTT GCT CTC TGC TGC ATG CA-3'; Kiss1r: F: 5'-AGC CTT GAC CGT CAC CAA TTT CT-3'; R: 5'-GGG AAC ACA GTC ACG TAC CA-3'; GnRH: F: 5'-AGC CAA GCC CAA TGG CAA GA-3'; R: 5'-TGG CCA GCT GCC GCT TCA AT-3'; and β-actin: F: 5'-TCA TGA AGT GTG ACC AGC ACG TCA TCC CT-3'; R: 5'-TCA TGA AGT GTG ACC AGC ACG TCA TCC CT-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 (Kiss1, Kiss1r), 58°C for 30 s (GnRH), or 65°C for 30 s (β-actin); and extension at 72°C for 1 min. The copy numbers of the transcripts were normalized against those of β-actin transcripts for each sample.

Statistical analysis
Data were analyzed by one-way ANOVA followed by Fisher’s protected least significant difference test. All results are presented as means±S.E.M. Differences were considered to be statistically significant at P<0.05. Correlation analyses were performed using Spearman’s correlation as appropriate.

Results
Effects of MD and FD on the plasma leptin concentration and hypothalamic neuropeptide (GnRH, Kiss1, and Kiss1r) mRNA levels at various developmental stages
In postnatal day 5 rats, body weight (one-way ANOVA; P<0.001, F(3,47) = 22.38) and plasma leptin concentration (one-way ANOVA; P=0.006, F(3,52) = 6.89) were significantly lower in the 12 h MD and 24 h MD groups than in the control group (Fig. 1). Similarly, the Kiss1r mRNA level was significantly lower in the 24 h MD group (one-way ANOVA; P=0.002, F(3,49) = 5.01) than in the control group. However, the mRNA levels of GnRH (one-way ANOVA; P=0.306, F(3,47) = 1.26) and Kiss1 (one-way ANOVA; P=0.751, F(3,47) = 0.29) were not altered by MD.

Figure 1 Effects of maternal food deprivation (MD) on body weight (A), plasma leptin concentration (B), and hypothalamic neuropeptide (GnRH, Kiss1, and Kiss1r) mRNA levels (C-E) in postnatal day 5 female rats. Values are expressed as the mean±S.E.M. *P<0.05, **P<0.01.
In the postnatal day 15 rats, body weight (one-way ANOVA; \( P \leq 0.001, F (3,59) = 102.86 \)) and the plasma leptin concentration (one-way ANOVA; \( P \leq 0.001, F (3,63) = 34.08 \)) were significantly lower in the 12 h MD and 24 h MD groups than in the control group (Fig. 2). However, the GnRH (one-way ANOVA; \( P = 0.310, F (3,59) = 1.25 \)) and the Kiss1 (one-way ANOVA; \( P = 0.683, F (3,59) = 0.39 \)) mRNA levels were not altered by MD. Similarly, the plasma LH concentration was not altered by MD (one-way ANOVA; \( P = 0.404, F (3,89) = 0.98 \)).

In the postnatal day 25 rats, body weight (one-way ANOVA; \( P < 0.001, F (3,47) = 127.30 \)) and plasma leptin concentration (one-way ANOVA; \( P < 0.001, F (3,47) = 22.95 \)) were significantly lower in the 12 h FD and 24 h FD groups than in the control group (Fig. 3). Similarly, the Kiss1 (one-way ANOVA; \( P = 0.040, F (3,47) = 3.73 \)) and Kiss1r (one-way ANOVA; \( P < 0.001, F (3,47) = 13.54 \)) mRNA levels and the plasma LH concentration (one-way ANOVA; \( P = 0.008, F (3,49) = 6.23 \)) were significantly lower in the 24 h FD group than in the control group. However, the GnRH (one-way ANOVA; \( P = 0.446, F (3,47) = 0.84 \)) mRNA levels were not altered by FD. In addition, the serum E2 concentration (one-way ANOVA; \( P = 0.249, F (3,52) = 1.50 \)), the ovarian weight (one-way ANOVA; \( P = 0.423, F (3,47) = 0.90 \)), and the uterine weight (one-way ANOVA; \( P = 0.530, F (3,52) = 0.66 \)) were not altered, showing that E2 was not associated with changes in the Kiss1 or Kiss1r mRNA level.

In the postnatal day 25 rats, the Kiss1 mRNA expression level was not correlated with the plasma leptin concentration (Fig. 4). On the other hand, the Kiss1r mRNA expression level was positively correlated with the plasma leptin concentration.

Effects of leptin administration on FD-induced alterations in the plasma LH concentration and hypothalamic neuropeptide (Kiss1 and Kiss1r) mRNA levels

In the postnatal day 25 rats, the plasma leptin concentration following exogenous leptin administration at the dose used in this study (3 mg/kg body weight) was significantly increased at each time point (4, 5, 9, and 12 h) compared with that of the saline-administered rats (Fig. 5). Co-administration of leptin restored the 24 h FD-induced reduction of Kiss1r mRNA expression (Kiss1r mRNA expression was significantly higher in the 24 h FD + lep group than in the 24 h FD group; Fig. 6). On the other hand, it did not restore the 24 h FD-induced reduction of Kiss1 mRNA expression or the plasma LH concentration.

Discussion

In this study, we have shown that the sensitivity of the hypothalamic kisspeptin system to nutritional status in female rats changes during the developmental period. Hypothalamic Kiss1 mRNA expression was reduced by FD at postnatal day 25, but not in the early developmental period (postnatal days 5 and 15). These developmental changes in the sensitivity of Kiss1 mRNA expression to nutritional status resemble their neuronal projection developmental patterns. It has been reported that no kisspeptin neurons were detected in the AVPV or PeN of female mice at postnatal day 15, after which they increased in number from postnatal day 25 until they had reached adult levels at the onset of puberty (Clarkson & Herbison 2006). In addition, the close apposition between kisspeptin fibers and GnRH cell bodies becomes apparent at postnatal day 25 (Clarkson & Herbison 2006). Thus, it is assumed that the sensitivity of the Kiss1 mRNA expression to metabolic and nutritional status develops simultaneously with its neuronal projections during the neonatal period. These developmental patterns of the kisspeptin system resemble those of other endocrine and neuronal systems, i.e. the hypothalamus–pituitary–adrenal axis and the hypothalamic appetite regulatory system (Ahima et al. 1998, Mistry et al. 1999, Carlo et al. 2007). Many epidemiological and experimental studies have indicated that an abnormally high or low nutritional status or putative stress during the plastic period induces long-term alterations in physiological systems (Barker et al. 1993, Proulx et al. 2002, Yura et al. 2005, Bouret et al. 2008, Delahaye et al. 2008, Remmers et al. 2008, Breton et al. 2009, Kirk et al. 2009). Although these alterations are adaptations to environmental conditions, they often increase the risks of certain diseases in later life (Barker et al. 1993). Similarly, we have shown that prenatal undernutrition reduces hypothalamic Kiss1 mRNA expression throughout...
the developmental period and that this alteration retards sexual maturation in female rats (Iwasa et al. 2010).

Furthermore, another group demonstrated that neonatal immune stress reduces hypothalamic Kiss1 mRNA levels during the prepubertal period in female rats (Knox et al. 2009). Therefore, it can be assumed that a negative energy balance in the early developmental period induces alterations in the hypothalamic kisspeptin system and negatively affects sexual maturation and reproductive function in later life. Further epidemiological and experimental studies are needed to clarify this hypothesis.

It has been reported that the sensitivities of some hypothalamic factors to leptin are established in the early developmental period. Therefore, we assumed that the acquisition of leptin sensitivity was involved in the establishment of the sensitivity of Kiss1 mRNA expression to acute food deprivation (FD). However, contrary to our expectations, hypothalamic Kiss1 mRNA expression was not correlated with the plasma leptin level, and the administration of leptin did not restore the reduced Kiss1 mRNA and plasma LH levels induced by acute FD at postnatal day 25. As mentioned above, the plasma leptin concentration observed following exogenous leptin administration at the dose used in this study was significantly increased compared with that of the saline-administered rats. It is disputed whether hypoleptinemia plays a role in the suppression of reproductive hormones under acute food-restricted conditions. For example, the suppression of plasma LH and testosterone levels in acute food-restricted male monkeys is not restored by short-term leptin administration (Lado-Abeal et al. 1999). Our present data also support the hypothesis that hypoleptinemia is not involved in the suppression of reproductive function induced by acute food restriction. As whole hypothalamic blocks containing both the AVPV and ARC were used for the gene expression analyses, the effects of FD and leptin on the Kiss1 mRNA

Figure 3 Effects of food deprivation (FD) on body weight (A), plasma leptin concentration (B), plasma LH concentration (C), ovarian weight (D), uterine weight (E), plasma estradiol concentration (F), and hypothalamic neuropeptide (GnRH, Kiss1, and Kiss1r) mRNA levels (G-I) in postnatal day 25 female rats. Values are expressed as the mean ± s.e.m. *P < 0.05, **P < 0.01.

Figure 4 Correlations between the Kiss1 (A) or Kiss1r (B) mRNA level and the plasma leptin concentration in postnatal day 25 female rats.
expression in individual hypothalamic nuclei could not be examined. Therefore, it remains possible that subtle changes in gene expression in each nucleus might have escaped detection due to the use of whole hypothalamic blocks. In addition, it is possible that leptin regulates Kiss1 mRNA expression in an opposing manner in the AVPV and ARC, similar to estrogen, which stimulates Kiss1 mRNA expression in the AVPV but inhibits it in the ARC (Oakley et al. 2009, Uenoyma et al. 2009). If this is true, the opposing regulatory effects of leptin might have canceled each other out, resulting in no change in the Kiss1 mRNA expression level of the whole hypothalamic block. Further precise examinations, i.e. through in situ hybridization, are needed to clarify the effects of metabolic status and leptin on Kiss1 mRNA expression in each nucleus.

We have shown that hypothalamic Kiss1r mRNA expression was reduced by acute FD at postnatal days 5 and 25 but not at day 15. These findings suggest that the hypothalamic Kiss1r is sensitive to metabolic status from the early neonatal period onward. These developmental changes in the sensitivity of Kiss1r mRNA expression to metabolic status were different from those of Kiss1 mRNA expression. As Kiss1 mRNA is expressed in kisspeptin neurons, it is reasonable that the developmental changes observed in the sensitivity of Kiss1 mRNA expression to metabolic status fit well with the pattern of kisspeptin neuron development during the neonatal period. On the contrary, Kiss1r mRNA is expressed in another neuronal population, i.e. GnRH neurons in the preoptic area. Thus, the developmental changes observed in the sensitivity of Kiss1r mRNA expression to metabolic status do not fit with the developmental pattern of kisspeptin neurons.

We have also shown that the hypothalamic Kiss1r mRNA level was correlated with the plasma leptin level and that the administration of leptin restored the reduced Kiss1r mRNA expression level induced by acute FD at postnatal day 25, suggesting that hypoleptinemia plays a role in the reduction of Kiss1r mRNA expression induced by acute FD. On the other hand, the administration of a dose of leptin above the physiological range in normal fed rats did not increase the hypothalamic Kiss1r mRNA levels in our preliminary evaluation (1-50±0.19 (control) versus 1-24±0.13 (leptin administration), mean±S.E.M.). Therefore, it is assumed that Kiss1r mRNA expression remains stable when the serum leptin levels are within or above the normal range and that its expression decreases when the plasma leptin level falls below the lower threshold of the normal range. In rodents, the plasma leptin level increases around postnatal days 8–16, which is called the leptin surge (Yura et al. 2005). In this study, the plasma leptin level of the control group at postnatal day 15 (4.04±0.45 ng/ml, mean±S.E.M.) was significantly higher than that at day 5 (0.88±0.14 ng/ml) and day 25 (1.62±0.15 ng/ml). In addition, the plasma leptin level of the 24 h FD group at postnatal day 15 (1.31±0.13 ng/ml, mean±S.E.M.) was also significantly higher than that at day 5 (0.40±0.05 ng/ml) and day 25 (0.78±0.06 ng/ml). We suppose that the plasma leptin level had not fallen below the level required to maintain the hypothalamic Kiss1r mRNA level by postnatal day 15, even after FD. If the MD had been continued for longer than 24 h in the postnatal day 15 rats, we assume that the plasma leptin level would have fallen below the lower threshold of the normal range, causing hypothalamic Kiss1r mRNA expression to decrease. We also speculate that the lower threshold of the plasma leptin level required to maintain Kiss1r mRNA expression is raised during development and that higher plasma leptin levels are required to maintain Kiss1r mRNA expression in postnatal day 25 rats compared with postnatal day 15 rats. However, it is unclear whether alterations in hypothalamic Kiss1r mRNA expression at postnatal day 5 or 25 play a role in fasting-induced suppression of reproductive function because the restoration of the Kiss1r mRNA level by leptin administration did not affect the plasma LH level in this study. Further examinations are needed to clarify the physiological roles of these alterations.

![Figure 5](image-url) Time course of the changes in plasma leptin concentrations after saline or exogenous leptin (3 mg/kg body weight) administration in postnatal day 25 female rats. Values are expressed as the mean±S.E.M. **P<0.01 compared with the saline group.

![Figure 6](image-url) Effects of leptin administration on 24 h food deprivation (FD)-induced alterations in body weight (A), plasma LH concentration (B), and neuropeptide (Kiss1 and Kiss1r) mRNA levels (C and D) in postnatal day 25 female rats. Values are expressed as the mean±S.E.M. *P<0.05, **P<0.01.
In this study, we have focused on the acute changes that occur following MD or FD and leptin administration. However, leptin is regarded as a key signal for the long-term control of energy balance. It has been reported that the chronic negative energy balance caused by diabetes mellitus reduces Kiss1 mRNA expression and that chronic leptin infusion is sufficient to normalize the Kiss1 mRNA level (Castellano et al. 2006). Thus, it remains possible that longer treatment with leptin can rescue Kiss1 mRNA expression under chronic food restriction.

In summary, we have shown that the sensitivity of the hypothalamic mRNA expression of Kiss1 and Kiss1r mRNA to nutritional status is altered during the development in female rats. The sensitivity of hypothalamic Kiss1 mRNA expression to acute FD was not established during the early neonatal period; however, it had become apparent by postnatal day 25. It is speculated that these alterations in the sensitivity of kisspeptin neurons to nutritional status develop simultaneously with their neuronal projections during the neonatal period. On the other hand, the sensitivity of hypothalamic Kiss1r mRNA expression to acute FD may be established in the early neonatal period. In addition, the reduced Kiss1r mRNA expression observed in undernourished conditions may be caused by decreases in serum leptin levels.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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Received in final form 11 August 2010
Accepted 31 August 2010
Made available online as an Accepted Preprint 31 August 2010