Developmental changes in hypothalamic leptin receptor: relationship with the postnatal leptin surge and energy balance neuropeptides in the postnatal rat


Institute of Metabolic Science, Metabolic Research Laboratories, University of Cambridge, Addenbrooke’s Hospital, Cambridge, UK; and Division of Obesity and Metabolic Health, University of Aberdeen, Rowett Institute of Nutrition and Health, Bucksburn, Aberdeen, UK

Submitted 11 August 2008; accepted in final form 22 December 2008

Cottrell EC, Cripps RL, Duncan JS, Barrett P, Mercer JG, Herwig A, Ozanne SE. Developmental changes in hypothalamic leptin receptor: relationship with the postnatal leptin surge and energy balance neuropeptides in the postnatal rat. Am J Physiol Regul Integr Comp Physiol 296: R631–R639, 2009. First published January 14, 2009; doi:10.1152/ajpregu.90690.2008.—In the adult brain, leptin regulates energy homeostasis primarily via hypothalamic circuitry that affects food intake and energy expenditure. Evidence from rodent models has demonstrated that during early postnatal life, leptin is relatively ineffective in modulating these pathways, despite the high circulating levels and the presence of leptin receptors within the central nervous system. Furthermore, in recent years, a neurotrophic role for leptin in the establishment of energy balance circuits has emerged. The precise way in which leptin exerts these effects, and the site of leptin action, is unclear. To provide a detailed description of the development of energy balance systems in the postnatal rat in relation to leptin concentrations during this time, endogenous leptin levels were measured, along with gene expression of leptin receptors and energy balance neuropeptides in the medial basal hypothalamus, using in situ hybridization. Expression of leptin receptors and both orexigenic and anorexigenic neuropeptides increased in the arcuate nucleus during the early postnatal period. At postnatal day 4 (P4), we detected dense leptin receptor expression in ependymal cells of the third ventricle (3V), which showed a dramatic reduction over the first postnatal weeks, coinciding with marked morphological changes in this region. An acute leptin challenge robustly induced suppressor of cytokine signaling-3 expression in the 3V of P4 but not P14 animals, revealing a clear change in the location of leptin action over this period. These findings suggest that the neurotrophic actions of leptin may involve signaling at the 3V during a restricted period of postnatal development.

neonate; hypothalamus; development

LEPTIN, derived predominantly from adipocytes, is a key regulator of energy homeostasis and neuroendocrine function (3). In the adult, leptin acts to promote negative energy balance, through an inhibition of feeding and concurrent stimulation of energy expenditure, mainly through the modulation of central energy balance pathways (38). These central effects of leptin are mediated largely through the hypothalamus, with the arcuate nucleus (ARC) considered of primary importance (18). Within the ARC, two distinct populations of neurons are key in mediating the first-order actions of leptin (38). One population coexpresses the anorexigenic neuropeptides, proopiomelanocortin (POMC), and cocaine and amphetamine-regulated transcript (CART), whereas the other coexpresses the orexigenic neuropeptide Y (NPY), and agouti-related protein (AgRP). Leptin acts via the long, signaling receptor isoform (ObRb) to increase the activity of POMC/CART and decrease that of NPY/AgRP neurons.

In addition to the well-defined actions of leptin in the regulation of energy balance in adult animals, a developmental role for leptin is becoming increasingly apparent (14, 40). In both rats and mice, there is a surge in circulating leptin concentrations during the early postnatal period (5, 22, 31, 32, 37, 45), which is independent of body fat mass. Despite this increase in leptin concentrations, animals maintain a high level of food intake at this time, demonstrating resistance to the anorexigenic effects of leptin and presumably enabling the rapid growth observed at this age. Furthermore, metabolic responses to leptin are absent during early life, with a lack of effect on energy expenditure and food intake until after the second postnatal week (4, 30). This occurs despite the fact that mRNA expression of several leptin target genes in the ARC are altered by leptin administration in the neonatal rat (36), suggesting an absence of functional downstream signaling to energy balance pathways.

The production of leptin within the brain itself, and widespread distribution of leptin receptors (31, 32), support a role for leptin as a general factor involved in brain development, and not just in those regions involved in body weight regulation. Leptin receptors have been identified as early as embryonic day 10.5 (E10.5) in the mouse brain (42). In the rat, levels of ObRb and the degree of leptin-binding increase between E18 and early postnatal life (16). Access of circulating leptin to central nervous system (CNS) targets involves regulated transport across the blood-brain barrier (BBB) (8), and recently, developmental changes in the expression of leptin receptors at the BBB and within the cerebral microvessels have been described (34). Leptin receptors are up-regulated in the cerebral microvessels of 1-wk-old rats compared with 2-mo-old animals, although the functional significance of this increased expression is unclear, as transport rates are reportedly unaffected (34).

Over recent years, numerous studies have added to a body of evidence showing that the effect of leptin in early life is quite distinct from that in the adult. Leptin has a profound effect on the proliferation, maintenance, and differentiation of neuronal and glial cells and is required for the formation of neuronal circuitry involved in functions ranging from cognition and memory to energy homeostasis (41). In the rodent, hypotha-
Hypothalamic energy balance circuits are still forming during the early postnatal period, and in the leptin-deficient ob/ob mouse, there is reduced axonal projection from the ARC to the downstream paraventricular nucleus in the neonate, which can be rescued by administration of leptin, exclusively during this perinatal period (12). The stimulation of neurite outgrowth from ARC explants of neonatal mice indicates the presence of functional leptin receptors at this age (13). However, the identity and precise location of cells responding to leptin during this early period in vivo remains unknown. The aim of the present study was to determine whether there are developmental changes in the site of leptin response during the early postnatal period, which may explain its differential effects during this time and to describe, in detail, the postnatal development of hypothalamic leptin receptor and energy balance neuropeptide systems, in relation to endogenous leptin.

We present data showing the postnatal ontogeny of hypothalamic leptin receptor and ARC neuropeptide gene expression of the neonatal rat, using in situ hybridization. Furthermore, we show that ependymal cells of the third ventricle (3V) express high levels of functional leptin receptor at postnatal day 4 (P4), which subsequently disappear over the first 2 postnatal weeks, and coincide with morphological changes in this hypothalamic region. These results show that leptin target sites change markedly during the early postnatal period and further define a restricted period of leptin action on hypothalamic development.

METHODS

Materials. Unless otherwise stated, all chemicals and reagents were obtained from Sigma (Dorset, UK).

Animals. All procedures involving animals were carried out with the approval of the UK Home Office Animals (Scientific Procedures) Act, 1986. Virgin female Wistar rats were mated, and following confirmation of a vaginal plug, pregnant dams were housed individually for the remainder of gestation. All animals were maintained at 22°C on a 12:12-h light-dark cycle (lights on at 0600) and were given ad libitum access to water and standard rat chow (Arie Blok, Woerden, The Netherlands). After birth [day of birth designated as postnatal day 1 (P1)], litter size was standardized to eight pups per dam on P3.

Postnatal hypothalamic ontogeny. For initial ontogeny studies, male offspring were used at 10 time points across the early postnatal period (P4, 7, 10, 11, 12, 13, 14, 15, 16 and 19; weaning in our laboratory occurs at P21), and individual body weights recorded on the day. Pups were taken directly from their mother between 1000 and 1200 and killed by CO2 asphyxiation. Trunk blood was collected, and blood glucose measurements were taken using a blood glucose analyser. Trunk blood was collected, and blood glucose measurements were taken using a blood glucose analyzer. After birth, all offspring were weighed at 80°C until sectioning. For these studies, 6–8 animals, derived from at least four litters, were used at each time point.

Postnatal leptin challenge and tissue collection. Recombinant rat leptin was purchased from PeproTech EC (London, UK). At P4 and P14, male offspring received a single intraperitoneal (i.p.) injection of either 3 μg/g body wt leptin or saline solution, between 1000 and 1200 (~30 μl in P4 neonates of 1 mg/ml solution or 45 μl in P14 animals of 2 mg/ml solution). This dosage was chosen as it has been reported previously to effectively induce leptin receptor signaling and alter neuropeptide expression in young rat pups (36, 43). Pups were then returned to their mother, and subsequently killed 60–90 min later. Trunk blood and brain tissue were collected as described above, however, in this study, both P4 and P14 brains were carefully dissected from the skull and frozen on powdered dry ice. Within each group, 7–8 animals from at least 4 different litters were used for this study.

Circulating hormone levels. Serum leptin concentrations were measured using an ELISA assay from CrystalChem (Downers Grove, IL), performed using the manufacturer’s instructions. Serum insulin concentrations were measured using an ultra-sensitive rat/mouse ELISA assay from Mercodia (Uppsala, Sweden).

In situ hybridization. Brain sections were cut on a Leica CM1900 cryostat (Leica Microsystems AG, Germany) and tissue thaw-mounted onto poly-l-lysine coated slides (Polysine, Menzel Glaser, Braunschweig, Germany). Coronal sections of 14-μm thickness containing the full extent of the ARC [according to the atlas of the rat brain of Paxinos and Watson (35)] were collected and subsequently stored at −80°C until processing.

Ribsprobes for the leptin receptor ObR (generated from PCR primers directed at the extracellular domain common to all leptin receptor isoforms and thus providing a measurement of total leptin receptor) and ObR (which recognizes specifically the long, signaling isoform of the leptin receptor), the neuropeptides POMC, CART, NPY, and AgRP, suppressor of cytokine signaling 3 (SOCS3), and nestin were generated as described elsewhere in detail (2, 9, 25, 27–29, 33). For processing of brain sections, slides were first fixed in 4% paraformaldehyde, acetylated (for all ribsprobes except NPY), washed in 0.1 M phosphate buffer and dehydrated through a graded ethanol series and vacuum-dried. Slides were then incubated overnight at 58°C with 35S-labeled complementary RNA probes at concentrations of 1.5–2 × 105 cpm/ml. The following day, slides were treated with RNase A at 37°C for 30 min and washed in a series of saline sodium citrate (SSC) washes with a final high-stringency wash in 0.1 × SSC at 60°C for 30 min to remove unhybridized probe, and finally dehydrated in ethanol and air-dried. Slides were exposed to Kodak BioMax MR autoradiography film (Amersham Pharmacia Biotech, Little Chalfont, Bucks, UK), together with a [14C] microscale (Amersham), for a period of 5–21 days, depending on the ribsprobe applied.

Light microscopy. Following initial analysis of leptin receptor and SOCS3 mRNA expression in the postnatal hypothalamus by autoradiography, we sought to confirm whether the mRNA expression observed around the 3V region was localized to the ependymal cells. Furthermore, we wished to see whether the leptin-induced SOCS3 signal could be localized within cells of a neuronal phenotype within the ependymal layer, as autoradiographic films had shown that nestin expression was also particularly dense over this region. Toward these ends, a subset of radioactively labeled ObR, SOCS3, and nestin in situ slides were coated with autoradiographic emulsion (LM-1, Amersham Pharmacia Biotech) according to the manufacturer’s instructions, exposed at 4°C for 12 wk, developed and counterstained with toluidine blue. Images were captured using a digital camera, and Image ProPlus software (Media Cybernetics, Wokingham, Berkshire, UK).

Quantification of gene expression. Hypothalamic mRNA expression was quantified using the Image ProPlus system (Media Cybernetics, Wokingham, Berkshire, UK), and integrated optical densities for each area of interest were calculated. For hypothalamic ontogeny studies, data are expressed relative to P4 values. In the case of leptin-injected neonates, mRNA expression is presented relative to P4 saline-injected animals.

Statistical analyses. Comparisons between groups were made by one- or two-way ANOVA as appropriate, using Statistica v. 7 software (Statsoft), with a level of significance set at P < 0.05. Data are presented as means ± SE (except in the case of serum insulin data, which are presented as geometric means ± 95% confidence intervals since values were not normally distributed and so were log transformed prior to analysis).
RESULTS

Postnatal growth and hormone levels. In agreement with previous studies in rats (37) and mice (5, 22, 45), there is a significant rise in circulating leptin during the postnatal period (Fig. 1A), which is not related to body weight. Pup body weight increased linearly from 9.26 ± 0.47 g at P4 to 44.79 ± 1.88 g in P19 animals. In addition, the leptin surge in these animals does not appear to be associated with circulating concentrations of glucose or insulin, as neither of these variables were found to change significantly over the postnatal period (Fig. 1, B and C).

Ontogeny of leptin receptor expression in the ARC and ventromedial hypothalamus. At P4, the expression of leptin receptors (total ObR and also ObRb) in the ARC and ventromedial hypothalamus (VMH) is low and begins to increase toward the end of the second postnatal week (Fig. 2). At P14 in the ARC, both ObR (Fig. 2A) and ObRb (Fig. 2C) show a significant increase from P4 levels (P < 0.001). In the VMH (Fig. 2, B and D for ObR and ObRb, respectively), the same pattern is evident. In each of these regions, differences are apparent between the ObR (Fig. 2, A and B) and ObRb (Fig. 2, C and D). ObR expression was consistently elevated between P14 and P19, whereas ObRb mRNA expression was found to peak at P15 before declining at P16.

Ependymal leptin receptor expression during early postnatal life. In the course of initial in situ hybridization ontogeny studies, we identified striking differences in the distribution of ObR over the first weeks of life. At P4, we observed dense ObR mRNA expression in the ependymal layer of the 3V (Fig. 3 and Fig. 4). This expression was reduced by P7 and had virtually disappeared by 2 wk of age (Fig. 3), revealing a highly significant effect of postnatal age on ObR mRNA expression in this region (P < 0.001). To verify that this 3V ObR was localized to ependymal cells, we further investigated the distribution of ObR in a subset of ages using emulsion-coated slides. At P4, the 3V ependymal layer displayed dense ObR mRNA signal, which showed a dramatic reduction with increasing age. This expression was replaced by the more adult-like hypothalamic distribution by P19, where ObR expression in the ARC and VMH is clearly visible (Fig. 4B). The decline in ObR mRNA expression within the 3V was also observed to coincide with a progressive thinning of the ependymal layer (Fig. 4C).

ARC neuropeptide profiles during postnatal development. In the ARC, both anorexigenic (POMC and CART) and orexigenic (NPY and AgRP) neuropeptide expression is low at P4 but increased significantly after the second postnatal week (Fig. 5). Importantly, during the period between P7 and P13 when leptin levels are elevated, there is an apparent lack of effect on neuropeptide mRNA expression. In contrast to the gradual rise in expression of POMC, NPY, and AgRP genes, where P19 expression levels are around 3–5-fold higher than those in the P4 ARC, CART mRNA expression (Fig. 5B) markedly increases in the ARC during this period, with an ~60-fold increase in mRNA expression between P4 and P19.

Functionality of ependymal leptin receptor. To investigate whether 3V leptin receptors are functional in the neonatal rat brain, postnatal rats at P4 and P14 (corresponding to ages at which 3V leptin receptor was maximal or virtually undetectable, based on the earlier experiments) were given an acute leptin challenge. In this experiment, as in the initial ontogeny studies, leptin receptor expression in the 3V was evident in P4 animals but was virtually absent at P14 (data not shown). To validate leptin injections, leptin concentration was determined in terminal serum samples. In saline-treated offspring, leptin levels were 5.20 ± 0.77 and 7.43 ± 0.99 ng/ml at P4 and P14, respectively. Leptin levels were raised ~1,000-fold in leptin-
treated animals, with levels reaching 5,695 ± 393 ng/ml in P4 animals and 7,392 ± 867 ng/ml at P14.

To identify functional leptin-receptor signaling, induction of SOCS3 mRNA was used as a marker of receptor activation. SOCS3 is a negative regulator of leptin signaling and is robustly induced in response to leptin (11). We demonstrated a striking induction of SOCS3 expression in the 3V at P4 in response to intraperitoneal leptin, which is virtually absent in this region at P14 (Fig. 6, A and B and Fig. 7). Semiquantification of autoradiographs (as shown in Fig. 6B) indicated an approximate 25-fold induction of SOCS3 in the 3V at P4 in response to leptin challenge. SOCS3 expression in the ARC was induced in response to leptin at both P4 and P14 (effect of treatment P < 0.01 at both ages; Fig. 6C and Fig. 7). In addition, at P4 SOCS3 gene expression in the VMH was absent, but dramatic induction was observed at P14 in this brain region, with signal intensity increasing over 5-fold from saline values (Fig. 6, A and D).

In an attempt to determine which cell type is leptin responsive in the 3V of P4 neonates, studies were carried out assessing the distribution of SOCS3 expression in relation to that of the neuronal marker nestin. As shown in Fig. 8, there is dense nestin expression in virtually all cells of the ependymal layer at P4, similar to that seen in the case of ObR. As such, the SOCS3 expression that is seen in a subset of 3V cells (Fig. 8D) will necessarily colocalize with nestin and ObR, hence the precise phenotype of these responsive cells remains unclear.

**DISCUSSION**

Although it has become increasingly clear that leptin is critical for both the initial establishment and ongoing regulation of hypothalamic circuitry and neuroendocrine function, the precise way in which leptin signals are received and the site of leptin action during early life are not well understood.

We describe here in detail the postnatal development of ARC energy balance neuropeptides in relation to circulating leptin levels and leptin receptor expression and show for the first time in the rat that there is a critical window during early postnatal life in which functional leptin receptor is present in the 3V of the hypothalamus. These findings are in agreement with a recent study in mice, in which phosphorylation of the signal transducer and activator of transcription 3 (STAT3) leptin signaling pathway was investigated during the early postnatal period. In this study, leptin-induced phosphorylation of STAT3 protein in the ARC was found to increase over the first 2 wk of life, being weak at P1 and P5 but indistinguishable
from adult mice by P15. Furthermore, a distinct group of subependymal cells of unknown phenotype was described that responded to leptin between P5 and P13 (23). The results of the present study corroborate these previous findings and provide a substrate basis for the effects of leptin in this region in the neonatal rodent. As was the case in the study of Frontini and colleagues (23), we were also unable to define a specific phenotype for these leptin-responsive cells. However, given the dense expression of nestin at this age, we speculate that a subset of neuronal precursor cells are those which are responding to leptin treatment in these young neonates.

Ontological mapping of hypothalamic neuropeptides has been reported previously (4), but in the present study, the use of in situ hybridization as opposed to whole hypothalamic RT-PCR enables demonstration of mRNA expression changes within discrete nuclei during this period of development. Our
results are in agreement with a recent report in neonatal rats, in which POMC, NPY, AgRP, and CART all increase over the first postnatal weeks in the ARC (17). In the present study, the patterns of mRNA expression of these same candidate genes were determined in relation to endogenous leptin concentrations. Interestingly, we found that the onset of significantly increased leptin receptor and neuropeptide expression in the ARC corresponds to the decline in circulating leptin. Whether the reducing leptin concentrations are triggering these changes in expression is not addressed in the present study but would be of interest for future investigations.

The transiently increased leptin concentrations during early postnatal life are thought to be derived predominantly from adipose tissue, as leptin gene expression within adipose depots

Fig. 6. Suppressor of cytokine signaling-3 (SOCS-3) induction in the postnatal hypothalamus in response to an acute leptin injection (3 μg/g ip). Representative images of saline- or leptin-injected animals at P4 or P14 are shown in (A), and semiquantification of SOCS-3 mRNA induction in the 3V (B), ARC (C), and VMH (D). Solid bars denote saline-injected animals; open bars denote leptin-injected animals. Significant differences in SOCS3 mRNA expression are indicated as **p < 0.01, ***p < 0.001, compared with P4 saline-injected values, or P14 saline-injected animals in the case of VMH data.

Fig. 7. Representative brightfield photomicrographs illustrating the hypothalamic regions of interest (A) and the corresponding darkfield photomicrographs of emulsion-coated sections (B) demonstrating the change in distribution of SOCS3 induction by leptin at P4 and P14. At P4, a high density of silver grains is apparent over the 3V in leptin-injected animals, and this effect is absent at P14. Induction of SOCS3 is apparent in the ARC at both ages. Scale bars = 100 μm.
has been shown to exhibit a postnatal surge, which mirrors the circulating hormone concentrations (5, 22, 37). Although less well studied, the regulation of leptin secretion in the neonatal rodent also does not appear adult-like. In the adult rat, circulating leptin levels are modulated by nutritional state (3). Conversely, the increased leptin concentrations in the neonate do not appear to correlate with circulating glucose or insulin levels. In addition, a recent report in which rat pups are raised on a high-carbohydrate diet and develop marked hyperglycemia and hyperinsulinemia, the circulating leptin concentrations in these animals are significantly reduced compared with control-fed pups, being barely detectable at P12 (39). This likely indicates a dissociation of leptin secretion from these typically stimulatory factors during early life.

The leptin dose chosen in the present studies was based on that used previously in the literature (36, 43). However, the serum leptin concentrations achieved by using this dose are clearly nonphysiological, being elevated ~1,000-fold from saline-injected controls. Few studies in which similar doses of leptin were administered have measured directly the resulting circulating levels. It is recognized that much lower doses of leptin would be required to induce physiologically relevant changes in blood leptin levels. Future studies clearly need to address this issue; however, much of the existing data in the literature is based on similarly supra-physiological hormone levels. Also of note, there was a discrepancy in the leptin concentrations between endogenous circulating levels (in the ontogeny studies) and those in the saline-injected animals. In the latter group, leptin concentrations were elevated, perhaps relating to a stress-induced response in these animals.

The origin of hypothalamic neurons, in terms of when during development they arise, has been known for several decades. In the developing brain, the cells of the ventricular zone are the progenitor cells that go on to become the adult central nervous system (1, 20, 24). Injection of radiolabeled thymidine (3H-thymidine) into pregnant rat dams identified that neurons of most hypothalamic nuclei originate between E14 and E18 within the ventricular zone of the hypothalamic third ventricle (6, 7, 10, 20) and that these postmitotic neurons subsequently migrate laterally to populate the surrounding nuclei (10). Morphological changes similar to those reported here, in terms of ventricular thinning over the first postnatal weeks, have been reported previously (44) and suggested that this change may be the result of neuronal migration away from the ventricular zone. In addition, there appears to be a decline in the number of cells both within the ventricular region and also the surrounding ARC and VMH areas with increasing age. The regulation of this reduction in cell number during early postnatal life is not clear.

Consistent with our findings, both leptin binding (16) and leptin receptor gene expression (31) have been demonstrated in the ependymal layer of the third ventricle in the rat during early life. Developmentally, changes in ObRb protein have been shown in the embryonic and postnatal rat, where immunolabeling is clearly observed in the ependymal layer as early as E14 (26). Subsequently, over the course of lactation, ventricular labeling is seen to decrease. We report a similar pattern of redistribution, with a reduction in 3V ObR mRNA expression over the first 2 wk of postnatal life, concurrent with increased VMH and ARC expression. Whether it is the same population of leptin receptor-expressing neuronal progenitors that originate in the ependymal layer and subsequently migrate outward is not known, although the current findings provide some support for this hypothesis. In the present investigation, acute leptin administration at P4 induced a robust SOCS3 signal in the ependymal layer of the third ventricle (Fig. 8A, B) and those adjacent to the 3V. Scale bars = 20 μm. However, the increased leptin concentrations in the neonate do not appear to correlate with circulating glucose or insulin levels. In addition, a recent report in which rat pups are raised on a high-carbohydrate diet and develop marked hyperglycemia and hyperinsulinemia, the circulating leptin concentrations in these animals are significantly reduced compared with control-fed pups, being barely detectable at P12 (39). This likely indicates a dissociation of leptin secretion from these typically stimulatory factors during early life.

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mental events are occurring during a similar window of time. We propose that these developmentally regulated processes may be related and that postnatal leptin may be required for the appropriate differentiation and migration of postmitotic neurons to their surrounding nuclei. However, in vitro experiments to directly determine whether leptin has effects on cell migration are required to confirm this.

The enhanced sensitivity to environmental stimuli during critical periods of development imply that insults or adverse conditions experienced in early life may have long-term effects on subsequent health (19). In the rodent, the perinatal environment can have a vast effect on brain development. A number of studies have shown that changes in the nutritional or hormonal environment during the first weeks of life can affect the development of central energy balance circuits (15, 21) and that this is associated with an increased susceptibility to obesity in later life (45). Leptin has emerged as a candidate factor that may be mediating the effects of early postnatal conditions on CNS development. Studies in mice in which the timing and amplitude of the postnatal leptin surge were altered through undernutrition in fetal life have found long-term changes in central energy balance circuits (45). Furthermore, administration of exogenous leptin to mimic the increased and advanced leptin levels induced by maternal undernutrition was demonstrated to be causally related to altered neuropeptide levels and to predispose offspring to high-fat diet-induced obesity (45).

**Perspectives and Significance**

The findings presented here show that there is a restricted period of functional leptin receptor expression in the ventricular region of the hypothalamus during the early postnatal period. This receptor may provide a substrate basis for the neurotrophic actions of leptin during this early period of life that are important in the establishment of hypothalamic energy balance circuitry. The initially low expression levels and the lack of regulation of neuropeptide mRNA by leptin in the ARC until the end of the second postnatal week also suggest that ARC cells are relatively insensitive to endogenous leptin during this time. This may reflect postnatal changes in neuronal distribution and also suggests a functional uncoupling of leptin receptor to neuropeptide mRNA regulation, until neurons are appropriately positioned in their respective nuclei. Further studies are clearly needed to identify whether leptin itself is required in the stimulation of migration of ventricular progenitor cells and whether ultimate numbers of neurons in hypothalamic nuclei are affected by leptin’s actions in the developing postnatal brain.

**ACKNOWLEDGMENTS**

We would like to thank Adrian Wayman, Delia Hawkes, Ann Flack, Lynn Bell, and Dana Wilson for their excellent technical support, and Jian’an Laan for his assistance with statistical analysis.

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