Postnatal intracerebroventricular exposure to neuropeptide Y causes weight loss in female adult rats

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Varma, Amit, Jing He, Lisa Weissfeld, and Sherin U. Devaskar. Postnatal intracerebroventricular exposure to neuropeptide Y causes weight loss in female adult rats. Am J Physiol Regul Integr Comp Physiol 284: R1560–R1566, 2003.—We investigated the effect of repetitive postnatal (2–7 days) intracerebroventricular administration of neuropeptide Y (NPY) on food intake and body weight gain in the 3- to 120-day-old Sprague-Dawley rats. NPY caused a 32% transient increase in body weight gain with elevated circulating insulin concentrations within 24 h. This early intervention led to the persistence of hyperinsulinemia and relative hyperleptinemia with euglycemia in the 120-day-old female alone. This perturbation was associated with 50% suppression in adult female hypothalamic NPY concentrations and a 50–85% decline in NPY immunoreactivity in the paraventricular and arcuate nuclei. This change was paralleled by a ~20% decline in food intake and body weight gain at 60 and 120 days. However, when exogenous NPY was stereotaxically reinjected into the paraventricular nucleus of the ~120-day-old adult females who were pretreated with NPY postnatally, an increase in food intake and body weight gain was noted, attesting to no disruption in the NPY end-organ responsivity. We conclude that postnatal intracerebroventricular NPY has long-lasting effects that predetermine the resultant adult phenotype in a sex-specific manner.

The energy homeostasis is maintained by a highly complex and integrated neurohormonal system that minimizes fluctuations in energy balance (4, 18, 27). Essential elements contributing toward this balance include systemic hormones secreted sometimes in proportion to the body fat mass (4, 18, 27) and the central nervous system (CNS) targets on which these hormones act (4, 18, 27). Of the CNS targets, a complex array of hypothalamic neuropeptides constitutes the appetite regulation system (4, 18, 27). Of these neuropeptides, NPY, a 36-amino acid orexigenic peptide, is synthesized in the arcuate and dorsomedial nuclei, transmitted to the paraventricular nucleus, and released at the nerve terminals (4, 18, 27). In the adult rat, NPY has been observed to play a key role in mediating hyperphagia with resultant obesity (18, 21, 27, 28). Various hormones orchestrate the synthesis and release of this neuropeptide (4, 18, 27). Particularly, pancreatic insulin and leptin released by adipocytes have been reported to suppress the synthesis and release of hypothalamic NPY, thereby potentially forming a feedback system in regulating appetite, feeding behavior, and energy balance (4, 18, 27). Our previous investigations demonstrated that perturbations in the fetal metabolic milieu as encountered in the fetus of a diabetic rat or an intrauterine growth-restricted (IUGR) fetus can also alter postnatal brain NPY mRNA and peptide concentrations (13, 19). Whereas such studies have demonstrated the presence and regulation of NPY in the fetal and neonatal hypothalamus (13, 19, 26), the exact functional role of this peptide during the early stages of development remains to be ascertained. The functional relevance of a postnatal increase in hypothalamic NPY as seen in the IUGR fetus/newborn is unknown. We hypothesized that high levels of intracebroventricular NPY during the critical stages of postnatal development will have an immediate positive effect on body weight gain, reflecting increased milk intake and a permanent influence on adult appetite, feeding behavior, and body weight gain pattern. To test this hypothesis, we undertook the present investigation and examined the effect of intracerebroventricular NPY on newborn body weight gain and adult food intake and body weight gain pattern.

Materials and Methods

Animals. Gestationally timed pregnant Sprague-Dawley rats (Taconic Farms, Germantown, NY) were housed in individual cages, exposed to 12:12-h light/dark cycles at 21–23°C, and allowed access to standard rat chow (Purina, St. Louis, MO) ad libitum. As approved by the Magee-Womens Research Institute’s Animal Care and Use Committee, the National Institutes of Health guidelines were followed. The animals were allowed to deliver, and the number of pups in a litter was culled or expanded to 10 to minimize the effect of litter size on postnatal nutrition and body weight.

Postnatal studies. The litters (n = 400 pups from 40 litters) of pups were arbitrarily divided into two major groups, of which one of the groups received intracerebroventricular NPY (1 μg NPY/2.5 μl dose) daily between 2 and 7 days of age.
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(n = 200 pups), and the second group received 2.5 μl of vehicle (n = 200 pups). The study design accounted for interlitter and intralitter variations. The 2–7 days of age were chosen for intervention, because this is the critical period of hypothalamic development that has previously been observed to have permanent effects lasting into adulthood (7). All intracerebroventricular injections were performed using the conventional stereotaxic coordinates for the adult rat lateral ventricle (11, 22) that were adapted to the postnatal rat by initial trial and error followed by confirmation of the site of injection by instillation of India ink. The coordinates were subsequently set at 0.4 mm lateral to the midline, 0.4 mm posterior to the bregma, and 2.5 mm ventral to the skull surface. Individual pups in each litter were weighed daily between 8 and 10 AM. On weaning of the pups at 21 days, body weight was assessed once every 10 days until 120 days of age. Food intake was measured over a 24-h period by weighing the rat chow at the beginning and end of the 24-h period, accounting for spillage and evaporation.

Female adult studies. At 120 days of age, the female animals in the neonatal NPY- or vehicle-treated groups were further divided into two groups each, one that received 10 μg of NPY/2.5-μl dose and the other that received an equal volume of vehicle, thereby leading to a total of four adult experimental groups. These four groups were NPY-NPY, NPY-vehicle, vehicle-NPY, and vehicle-vehicle. Stereotaxic coordinates used were with the incisor bar at 3.0 mm above the interaural line, 0.4 mm posterior to the bregma, 0.4 mm lateral to the midline, and 7.5 mm ventral to the skull surface, to ensure delivery into the paraventricular nucleus (PVN) (11, 22). All animals were anesthetized with a combination of ketamine (40 mg/kg) and xylazine (8 mg/kg). The 120-day-old animals subjected to stereotaxic surgery were weighed before surgery and every day between 8 and 10 AM, spanning a total of 3 days. In addition, food intake was measured over a 24-h period daily spanning a 3-day period beginning on 121 days and lasting through 123 days.

Plasma assays. Animals were euthanized with intraperitoneal pentobarbital sodium (100 mg/kg), blood was collected from the left ventricle, and the plasma was separated and aliquoted for measurement of glucose by the glucose oxidase method (Sigma Diagnostics, St. Louis, MO; sensitivity = 0.1 mM with an intra-assay coefficient of variation = 1.2%). Insulin and leptin were quantified by double antibody radioimmunoassays using rat standards and anti-rat insulin or leptin antibodies (Linco Research, St. Charles, MO; sensitivity: insulin = 0.1 ng/ml, leptin = 0.5 ng/ml). Leutinizing hormone (LH) was also assessed by a radioimmunoassay using rat-specific standards and antibodies (sensitivity = 0.005 ng/ml).

Hypothalamic tissue assays. The hypothalamus was obtained as a frontal slide by vertical cuts 1 mm anterior to the body of the optic chiasm and 1 mm posterior to the mammillary bodies. The tissue block was weighed, pooled in the case of newborn animals (5 pups = n of 1), and extracted in four volumes of 0.1 N HCl (wt/vol). The extract was sonicated for 10 s (Sonic dismembrator, Fisher Scientific) using 10-W output power. The sonicated acid extracts were centrifuged at 10,000 rpm for 10 min to remove the tissue debris. The supernatant was freeze-dried. The freeze-dried extracts were reconstituted in 0.05 M Tris-HCl buffer containing 0.1% bovine serum albumin (pH 7.8) for NPY measurements by RIA. NPY was assessed by an RIA that employed a polyclonal rabbit anti-rat NPY antibody and rat NPY standards (Peninsula Laboratories, Belmont, CA). NPY was expressed as picograms per unit hypothalamic protein measured by the Bradford’s dye binding assay (19) and as nanograms per gram hypothalamic wet weight (19).

Immunohistochemical analysis. The 3- (n = 7) and 120-day-old rats (n = 5) for each NPY- and vehicle-treatment group were anesthetized by a combination of ketamine (40 mg/kg) and xylazine (8 mg/kg), and their brains were perfused and fixed as previously reported (13). Serial rostrocaudal floating microtome coronal brain sections were obtained (35 μm) and subjected to immunohistochemical analysis as described previously (13). A rabbit anti-rat NPY (1:8,000; Peninsula Laboratories) IgG served as the primary antibody. PBS alone, PBS buffer containing appropriate dilutions of normal rabbit serum and lacking the primary antibody, preimmune serum, and the peptide preabsorbed anti-NPY antibody were used as appropriate controls. Sections containing the hypothalamic region were subjected to image analysis under a ×40 magnification using the Simple C-32 software program (C-imaging series SIMPLE 32 Compix Imaging Systems, Cranberry, PA). After subtracting the background, a gray scale was developed based on the intensity of the immunoreactivity. This gray scale provided the relative intensity of the NPY immunoreactivity. In addition, the area of immunoreactivity was circumscribed and measured. The measured intensity multiplied by the area of NPY immunoreactivity equaled the total amount of NPY immunoreactivity observed and was expressed in arbitrary units per section. A total of six sections per brain were analyzed to obtain the mean value of an n = 1.

Data analysis. n = 1 in the postnatal stage represented pooled pups (usually 5) from a single litter, each n = 1 arising from a separate litter for all experimental assays. Data are expressed as means ± SE. The Wilcoxon-Mann-Whitney test was used to make intergroup comparisons, whereas simultaneous intergroup and intertime comparisons were validated by the Friedman’s test. Significance levels were computed based on exact methods, accounting for the small sample sizes.

RESULTS

By injecting intracerebroventricular NPY on a daily basis between day 2 and day 7 of life, we observed an immediate increase in body weight gain within 24 h of initiating treatment compared with the vehicle treatment group (NPY = 1.58 ± 0.07 g vs. vehicle = 1.2 ± 0.03 g; P = 0.0012). This 32% increase in body weight resolved in 48 h and was no longer evident throughout the suckling period (Fig. 1). By day 21, body weight in NPY-treated rats had returned to that of vehicle-treated rats. Female rats that received vehicle treatment gained significantly more weight than those that received NPY treatment during weeks 2 and 7 of age (P < 0.001 by the Wilcoxon-Mann-Whitney test).

Fig. 1. Body weight gain during the suckling phase. Body weight gain (body weight minus the baseline body weight before intracerebroventricular injections) in grams per day (g/d) on days 2 to 21 are shown in suckling postnatal pups that received intracerebroventricular peptide Y (NPY; n = 20 per age) or vehicle (n = 20 per age) daily from days 2 to 7. *P < 0.001 vs. the age-matched vehicle control.
the suckling phase despite ongoing NPY administration until day 7 (Fig. 1). However, beginning on day 60, a significant decline in body weight gain and food intake was observed in females (Fig. 2, A and C) that persisted through 120 days. In contrast, no statistically significant change in either the food intake or body weight gain pattern was observed in males (Fig. 2, B and D).

The newborn animals that received NPY demonstrated hyperinsulinemia at 3 days of age (P = 0.04), with no change in glucose and leptin (Table 1) concentrations. This hyperinsulinemia persisted until 120 days of age in the female progeny (P = 0.04) (Table 1), with continuing euglycemia (Table 1). The leptin levels in the NPY-treated group were not statistically different from the vehicle group (Table 1). However, given the decline in female adult body weight of the NPY treatment group, plasma leptin concentrations at this age in females, if expressed per unit body weight (g), are significantly increased in the NPY (≈35%) vs. the vehicle group (≈19%; P < 0.05). Similar changes were absent in the male progeny (Table 1). LH synthesized and released into the circulation by the pituitary in response to hypothalamic LH-releasing hormone (LHRH) and measured as plasma LH concentrations was altered in response to exogenous NPY. Neonatal exogenous intracerebroventricular NPY administration increased plasma LH concentrations at 21, 60, and 120 days, particularly in the females (Table 2). The 120-day-old and not the 21-day-old males expressed a similar increase in plasma LH concentrations (P = 0.04) (Table 2). LH in 35- and 60-day-old male adults was not assessed.

To determine the possible mechanisms by which this negative effect on adult food intake and body weight gain occurred, we examined the total hypothalamic NPY concentrations and NPY immunoreactivity in both the paraventricular (PVN) and arcuate (ARC) nuclei. Total hypothalamic NPY concentrations increased at 3 days in response to exogenous NPY administration.

Table 1. Plasma insulin, leptin, and glucose concentrations

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<th>3 Days</th>
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<th>120 days</th>
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<tr>
<td></td>
<td>Vehicle</td>
<td>NPY</td>
<td>Female</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>2.6 ± 0.50</td>
<td>3.89 ± 0.30*</td>
<td>2.83 ± 1.00</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>1.33 ± 0.10</td>
<td>1.17 ± 0.10</td>
<td>6.37 ± 1.00</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>6.20 ± 0.50</td>
<td>5.20 ± 0.20</td>
<td>9.90 ± 0.80</td>
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Values are means ± SE. NPY, neuropeptide Y. *P < 0.05 vs. age-matched vehicle-treated group.

Fig. 2. Body weight gain and food intake in female and male adults. Body weight gain (body weight minus the baseline body weight before intracerebroventricular injections) at 35 (n = 20 females; n = 9 males), 60 (n = 19 females; n = 9 males), and 120 (n = 14 females; n = 9 males) days of age is shown in the female (A) and male (B) progeny. *P < 0.007; **P < 0.0002. Food intake in grams per day is depicted in the female (C) and male (D) progeny. *P < 0.05. n At each age in each group is the same as for body weight gain measurements.
ministration ($P = 0.04$); however, at 120 days, whereas no significant change was observed in the males, a threefold decline was noted in the females ($P = 0.04$) (Table 3).

Specific examination of the hypothalamic nuclei revealed no difference in the ARC nuclear NPY immunoreactivity (Fig. 3, E, F, M, and N), but the PVN nuclear NPY content was higher at 3 days of age (NPY = 122.2 ± 28 arbitrary units vs. vehicle = 33.6 ± 6; $P < 0.05$) despite ongoing administration of exogenous NPY (Fig. 3, A–D, M, and N). In contrast, at 120 days of age, long after cessation of exogenous NPY delivery, a decline in PVN (NPY = 27.3 ± 15 vs. vehicle = 181.2 ± 7; $P < 0.05$) and ARC nuclear (NPY = 160 ± 12 vs. vehicle 324.4 ± 32; $P < 0.05$) NPY immunoreactivity was observed (Fig. 3, K–N) in the females. No similar change in ARC and PVN NPY content was observed in the males. Although a decline in dorsomedial nuclear (DMN) NPY concentrations was observed in females, no similar change was noted in males.

To determine whether the adult NPY responsivity was altered due to neonatal administration of NPY, we undertook a second hypothalamic administration of NPY or vehicle in the 120-day-old adult females. We observed that the vehicle-NPY (neonatal vehicle but adult NPY treatment) and NPY-NPY (neonatal and adult NPY treatment) groups demonstrated similar changes. The change was of a relatively higher body weight (Fig. 4A) and food intake (Fig. 4B) compared with the vehicle-vehicle and NPY-vehicle counterparts, achieving statistical significance at 72 h after the hypothalamic instillation. These observations suggest that the neonatal intervention with intracerebroventricular administration of NPY does not interfere with the end organ responsivity to NPY in relation to appetite control, feeding behavior, and body weight gain.

**DISCUSSION**

We had hypothesized that postnatal administration of exogenous NPY would lead to immediate and permanent changes in food intake and body weight gain. In adults, NPY causes hyperphagia and obesity (21), hence we anticipated an increase in body weight gain in the newborn with a persistence of increased food intake and body weight in the adult. However, in response to postnatal administration of pharmacological doses of NPY, endogenous hypothalamic concentrations of the peptide were negatively affected. During the exogenous administration period, as expected, the postnatal hypothalamic NPY concentrations were high. However, in the female adult hypothalamus, postnatal administration of NPY permanently altered the endogenous concentrations of the peptide. This was seen as a decline in the PVN, DMN, and ARC nuclear NPY immunoreactivity. In contrast to this change in hypothalamic NPY concentrations, no decrease in NPY responsivity was observed secondary to 10 μg of NPY administered directly into the PVN.

These hypothalamic alterations were associated with certain peripheral hormonal changes. Postnatal administration of intracerebroventricular NPY caused hyperinsulinemia (1) that persisted in female adults. This increase in circulating insulin may reflect the end result of a parasympathomimetic effect of centrally administered NPY (4). Hyperinsulinemia did not affect the circulating glucose concentrations but may have relatively increased plasma leptin concentrations and suppressed the hypothalamic NPY content. Previous studies in adult rats have revealed suppression of hypothalamic NPY synthesis due to hyperinsulinemia (2, 17, 20) and hyperleptinemia (2). Thus central administration of exogenous NPY postnatally could suppress the net hypothalamic NPY concentrations either directly or indirectly via the action of insulin and/or leptin. This suppression of NPY concentrations particularly in the ARC and PVN caused a decline in food intake and body weight. DMN neuronal cells expressing NPY are known to neither respond to signals of energy balance (metabolic/hormonal) during development nor alter food intake (6).

**Table 2. Plasma leutinizing hormone concentrations**

<table>
<thead>
<tr>
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<th>Female</th>
<th>Male</th>
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<tr>
<td>21 days ng/ml</td>
<td>0.27 ± 0.01(3)</td>
<td>1.64 ± 0.80*(4)</td>
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<tr>
<td>35 days</td>
<td>0.43 ± 0.07(5)</td>
<td>0.26 ± 0.01(5)</td>
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<tr>
<td>60 days</td>
<td>0.23 ± 0.01(5)</td>
<td>3.44 ± 0.20*(5)</td>
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<tr>
<td>120 days</td>
<td>0.93 ± 0.04(5)</td>
<td>0.01(3)</td>
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Values are means ± SE (in ng/ml). $n$ Values in parentheses. *$P < 0.05$ compared with the age-matched vehicle-treated group.

**Table 3. Hypothalamic NPY concentrations expressed per unit protein and unit brain weight**

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<thead>
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<th>Female</th>
<th>Male</th>
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<tr>
<td>120 Days</td>
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<tr>
<td>3 Days</td>
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<tr>
<td>NPY, pg/100 μg protein</td>
<td>6.32 ± 0.80(5)</td>
<td>26.92 ± 1.00*(5)</td>
</tr>
<tr>
<td>NPY, ng/g</td>
<td>0.82 ± 0.10(5)</td>
<td>4.02 ± 0.20*(5)</td>
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Values are means ± SE. $n$ Values in parentheses. *$P < 0.05$ when compared with age-matched vehicle-treated group.
The absence of a comparable effect in the male progeny may be related to a differential effect of sex steroids on NPY synthesis and/or release (8, 10, 23). During the postnatal period, male hypothalamic NPY levels are higher than those of the female. Although gonadal steroids can alter the immediate postnatal hypothalamic synthesis of NPY (9), beginning day 5, a steroid hyposresponsive period occurs (5). Previous investigations in neonatal and adult female rats show that estradiol has a minimal enhancing effect or a negative effect on immunoreactive NPY levels in the PVN (3, 8, 14). In separate studies, testosterone therapy in castrated male rats enhanced the hypothalamic NPY synthesis and neurosecretory release (15). In the context of a previous investigation where the number of NPY-producing neurons are higher in males compared with females (23), in our present study, the augmenting effect of testosterone on the adult male hypothalamic NPY content may contrast the minimal or negative effect by estradiol in females. Thus, in males, despite postnatal exposure to NPY, the adult onset decline in NPY content was perhaps more efficiently countered by the testosterone-induced NPY production compared with the estradiol-induced relative suppression of NPY production seen in females.

In addition, we observed that whereas NPY administration in females led to peripheral changes in insulin and leptin levels, no such alterations were present in males. Again testosterone and/or other related hormones/end products may have had a differing effect from that of estradiol. Future investigations determining the effect of sex steroids on hypothalamic NPY, peripheral insulin, and leptin synthesis and release during the postnatal stages of development leading into adult observations are required to shed light on these sex-related differential observations. However, this absence of a change in the male was not a generalized effect, because postnatal exogenous administration of NPY caused a persistent increase in plasma LH concentrations in the 120-day-old adult female and male, which in turn increases the sex-specific gonadal hormone secretion. Thus the sex-specific effects on endogenous NPY concentrations are not related to exogenous NPY induced differential plasma LH concentrations per se, but rather the LH stimulated sex-specific differences in the type of the gonadal sex steroid. The NPY-induced increase in circulating LH can increase gonadal hormones, thereby explaining the

![Fig. 3. Immunohistochemical analysis. Low (scale bar = 1.5 mm in A, B, G, H) and high (scale bar = 0.007 mm in C–F, = 0.3 in I–L) power magnification of representative 3-day-old (24 h after initiation of the intracerebroventricular therapy) (A–F) and 120-day-old female (G–L) coronal floating microtome brain sections demonstrating NPY immunoreactivity in the intracerebroventricular vehicle (A, C, E, G, I, K)- and NPY (B, D, F, H, J, L)-treated groups within the paraventricular (PVN), dorsomedial (DMN), and arcuate nuclear (ARC) regions. 3V, third ventricle. Semi-quantification of NPY immunoreactivity in the PVN and ARC nuclear regions in the NPY (n = 6)- and vehicle (n = 6)-treated 3-day-old rats and NPY (n = 5)- and vehicle (n = 5)-treated 120-day-old female rats are shown in M and N. *P < 0.05 vs. the age-matched vehicle control.](http://ajpregu.physiology.org/fig3.png)
sex-specific differential effect on endogenous NPY concentrations in males (testosterone augmentation) vs. females (estradiol inhibition). The enhancing effect of NPY on LH via LHRH has previously been described in the adult (10). At 21 days, whereas an increase in LH is evident in the female, no such effect is seen in the male. Thus, unlike the effect on endogenous NPY, changes in LH via LHRH are sex specific at 21 days, but not so by 120 days, suggesting some time lag in males as opposed to females. Because we did not assess the plasma LH concentration at day 35 and day 60 in males, we could not determine the extent of this time lag.

The significance of our present observations is that postnatal changes in hypothalamic NPY can have long-lasting effects perturbing the adult phenotype. Thus conditions associated with in utero/postnatal changes in insulin concentrations may cause sex-specific changes in the adult. We previously observed that in the fetal rat, hypoinsulinemia of IUGR increases hypothalamic NPY concentrations (13). We also showed that IUGR is associated with an increase in hypothalamic NPY that lasts during the entire suckling phase (13). Recent studies have shown that the IUGR rat progeny develops hyperphagia, visceral obesity with hyperinsulinemia, and hyperleptinemia as an adult (24, 25). Whether the associated increase in hypothalamic NPY noted earlier in life sets the stage for developing hyperphagia and obesity with its complications in later life remains to be determined. Whereas long-term studies of hypothalamic NPY in the IUGR adult offspring do not exist, recent investigations involving early maternal deprivation from day 2 to day 6 demonstrate an increase in hypothalamic NPY concentrations (7). In contrast to the IUGR-associated chronic increase in endogenous NPY concentrations during the postnatal period (13), intermittent delivery of pharmacological doses of exogenous NPY caused a chronic suppression of endogenous NPY. Furthermore, exogenous NPY administration caused postnatal hyperinsulinemia in contrast to the hypoinsulinemia of the IUGR fetal rat (8, 13). Thus, unlike the adult IUGR offspring, our present neonatal NPY intervention led to the opposite phenotype, namely one of a diminution in food intake and loss of body weight. Thus one of the key intermediaries in determining the adult phenotype may comprise of fetal/neonatal endogenous hypothalamic NPY concentrations. Whether administration of lower doses of exogenous NPY similar to the endogenous concentrations observed in the IUGR postnatal rat would have a different effect to that seen in the present investigation needs future exploration.

Our present observations are not dissimilar to what has been reported in the progeny of a streptozotocin-induced diabetic rat mother (19). The fetus of a diabetic mother with fetal hyperinsulinism revealed a decline in endogenous brain NPY content (19). On the other hand, the adult offspring of a diabetic mother demonstrated an opposing effect, namely, an increase in hypothalamic NPY concentrations beginning from the postweaned stage (12) related to developing insulin resistance at the blood-brain barrier. Thus the concentrations of fetal/postnatal endogenous NPY content either as an associated change or an end result of perturbations in circulating insulin and/or leptin concentrations contribute toward the predetermination of the ultimate adult phenotype. These observations collectively make fetal/neonatal nutrition/metabolism extremely important, thereby presenting avenues for intervention before the adult onset of altered eating behavior and phenotype.

We conclude that postnatal exogenous administration of NPY causes central and peripheral changes. The central effect consists of a direct suppression of endogenous hypothalamic NPY concentrations and the indirect effect consists of neonatal hyperinsulinemia and/or hyperleptinemia that in turn chronically suppress hypothalamic NPY concentrations. This effect is permanent, causing a decline in food intake and body weight gain only in female adults. The cause for this sex-specific “neuropeptide imprinting” effect akin to the previously described “hormonal/metabolic imprinting” effect (24, 25) remains to be further investigated.

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