Programming of rat adrenal medulla by neonatal hyperleptinemia: adrenal morphology, catecholamine secretion, and leptin signaling pathway


1Departamento Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes and 2Departamento Nutrição Aplicada, Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil

Submitted 14 December 2009; accepted in final form 1 February 2010

Leptin is a hormone released mainly by white adipocytes but also by other tissues such as placenta, mammary gland, muscle, and brain (3). The hypothalamus is the most important responsive tissue to leptin where it promotes satiety and increases energy expenditure (1). The leptin actions on the central nervous system (CNS) are mediated by binding to the long form of the leptin receptor (OBRb) and activation of the janus tyrosine kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) intracellular pathway. The activation of the JAK2/STAT3 pathway stimulates the suppressor of cytokine signaling 3 (SOCS3) expression, an inhibitor of the leptin signaling pathway (43, 2). Leptin signaling proteins were reported in several other tissues, such as pituitary (56, 42), liver (45), muscle (46), and thyroid (36, 18, 42). Leptin receptors (OB Rs) were also found in adrenal glands of humans, rats, pigs, and bovines, suggesting a direct regulation of cortex and medullary adrenal tissues by leptin (31).

Leptin serum concentration may be changed in some physiological states and diseases. Starvation reduces leptinemia while feeding increases its serum concentration (30). Chronic stress also induces an increase in leptin levels, since glucocorticoids increase leptin expression and release (31). Obese humans and animals are recognized to present hyperleptinemia because of the large amount of adipose tissue (8). However, in obesity, the capacity of leptin to decrease food intake is impaired, since individuals frequently develop central leptin resistance (20). Leptin resistance has been associated with a reduction of expression and/or activation of the OBRs, JAK2, and STAT3 as well as an increase in SOCS3 expression (43). Besides, changes in leptin transport across the blood-brain barrier may decrease its action in the CNS (4). Leptin resistance is well characterized in CNS, but few data are available about its occurrence in the peripheral tissues. We recently showed this resistance in the thyroid of leptin-programmed rats (18). Besides, a selective leptin resistance has been described in animals that present resistance to the anorexigenic effect and preservation of the renal sympathetic and arterial pressure responses to leptin (41).

Catecholamines from adrenal medulla have an important contribution to energy metabolism and cardiovascular function. They regulate intermediate metabolism affecting glucose production and utilization, fat storage, and protein metabolism (9). The catecholamine synthesis is regulated mainly by tyrosine hydroxylase (TH), which is activated by tyrosine phosphorylation (19). There are few reports about the relationship between leptin and adrenal catecholamines; most of them were performed in cultured cells. It was shown that leptin stimulates the function of adrenal chromaffin cells through activation of catecholamine synthesis and secretion (49, 57). However, the intracellular mechanisms involved in OBR signaling in adrenal medulla of rats are still unclear, whereas it is well recognized that leptin stimulates the sympathetic nervous system (SNS), which may result in adverse changes in cardiovascular parameters (41). Thus, hyperleptinemia in obesity may be one of the causes of the obesity-associated hypertension, characterized by central hypothalamic leptin resis-
E942 HYPERLEPTINEMIA AND ADRENAL MEDULLARY FUNCTION

**Fig. 1. Programming of the leptinemia and catecholamine content.** Leptinemia (A) and absolute (B) and relative (C) adrenal catecholamine content of control (C, n = 9–11) and leptin-programmed (L, n = 11–14) rats at 150 days old. BW, body wt. Results are expressed as means ± SE. *P < 0.05.

Epidemiological and experimental studies have shown a strong correlation between stressful events (nutritional, hormonal, or environmental) in early life and the development of adult chronic diseases such as obesity, diabetes, and cardiovascular failure (5, 11, 12). This phenomenon is known as programming. The development of the sympathoadrenal system of rats may be influenced by environmental changes during critical periods of life like gestation and lactation (58). Undernutrition (21), handling, low temperature, and changes in litter size during lactation alter the function of the SNS and adrenal medulla at adulthood in a permanent manner (58). Maternal malnutrition is associated with higher leptinemia in the pups at weaning (50). We have shown that hyperleptinemia during lactation increases adrenal catecholamines (54) and programs for hyperleptinemia and central leptin resistance with lower expression of hypothalamic OBR at adulthood (53). In addition, adult offspring showed a higher content of catecholamines in adrenal medullas with higher expression of TH and higher ex vivo catecholamine secretion stimulated by caffeine (54). Changes in catecholamine levels and leptin sensitivity were followed by higher systolic blood pressure and increased heart rate at adulthood (54). We have suggested that the changes in leptin and catecholamine levels in early life could contribute to the development of adult chronic diseases like hypertension and higher risk for diabetes mellitus.

The purpose of the present study was to elucidate the leptin sensitivity in adrenal medulla that could contribute to the higher

---

**Fig. 2. Programming of the adrenal morphology and mass.** Photomicrographs of adrenals from control (A; n = 5) and leptin-programmed (B; n = 5) adult rats (150 days old). Staining was performed with hematoxylin-eosin (HE). Images were captured at ×2.5 objective. Cortex (C) and medullary (M) regions are identified. Adrenal mass of control (n = 20) and leptin (n = 23) groups are presented graphically in C. The results are expressed as means ± SE. *P < 0.05.
catecholamine production previously observed in leptin-programmed rats, since they were hyperleptinemic but leptin resistant at least in the hypothalamus. Thus, to address the question whether the higher catecholamine production and secretion were due to a direct leptin effect or to lack of leptin action in the adrenal medullas, we evaluated the following in the adult offspring that were leptin treated on lactation: leptinemia, adrenal catecholamine content, expression of OBR, STAT3, JAK2, SOCS3, and STAT3 phosphorylation in isolated medullas, and catecholamine in vitro release induced by leptin and adrenal morphology. To determine the isolated contribution of the hyperleptinemia upon the adrenal medullary function at adulthood, we performed another experiment in which adult normal rats were leptin treated during six successive days to induce hyperleptinemia, and we analyzed the same adrenal parameters.

**MATERIALS AND METHODS**

**Animals.** The use of animals in our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEA/187/2007).

---

**Fig. 3.** Programming of the leptin signaling pathway in rat adrenal medulla. Content of the long form of leptin receptor (OBRb; **A**), janus tyrosine kinase 2 (JAK2; **B**), signal transducer and activator of transcription 3 (STAT3; **C**), phosphorylated (p) STAT (D), pSTAT3/STAT3 (E), and suppressor of cytokine signaling 3 (SOCS3; **F**) in medulla from control (n = 6) and leptin-programmed (n = 6) adult rats at 150 days old. Representative blots of each protein (G) are shown. Actin content was used as control loading. Results are expressed as means ± SE. *P < 0.05.
The handling of experimental animals followed the principles adopted in the United Kingdom and Brazil according to the Brazilian Law no. 11.794/2008 (17, 32).

Experiment 1: Experimental model of programming by neonatal hyperleptinemia. Wistar rats were kept in an environmentally controlled room (23 ± 3°C and 12:12-h light-dark cycle; lights on from 7:00 A.M. to 7:00 P.M.). Three-month-old nulliparous female rats were placed with male rats in a 3 to 1 ratio. After mating, pregnant rats were housed in individual standard rat cages with free access to water and food until the pups’ birth (13, 52, 54).

At birth, all of the litters were adjusted to six males for each dam, since it has been shown that maximizes lactation performance (23). Litters that did not have at least six male pups were discarded. Pups received 50 μl of saline (group C) or murine leptin (group L) (8 μg/100 g of body wt, saline diluted; Prepotech) subcutaneously daily during the first 10 days of lactation. All of the injections were made from 4:00 to 5:00 P.M. Body weight was monitored daily during lactation and every 7 days after weaning until death when rats were 150 days old. Blood samples and adrenal glands were collected. Blood samples (1,000 g, 4°C, 20 min), and serum was kept individually at −20°C until leptin quantification assay.

Right adrenal glands for catecholamine content quantification were homogenized in 10% acetic acid and centrifuged (1,120 g, 4°C, 20 min), and serum was kept at 4°C and 12:12-h light-dark cycle; lights on from 7:00 A.M. to 7:00 P.M.). When they were 144 days old, a single dose of 200 μl of saline (group CC) or murine leptin (group LC) (8 μg/100 g of body wt, saline diluted; Prepotech) was injected subcutaneously during six successive days. All of the injections were made from 4:00 to 5:00 P.M. On the 6th day, rats were killed (2 h after the last injection) using a rodent guillotine, and blood samples and adrenal glands were collected (14, 37).

Adrenal glands for catecholamine content quantification, study of the leptin signaling pathway, had their medullas dissected (to avoid cortex contamination), and were immediately frozen in liquid nitrogen for Western blotting assays. Five right adrenals per group were fixed in Bouin and prepared for histological analysis. For in vitro studies, left adrenal glands were weighed, and fresh isolated adrenal medullas were submitted to stimulus with leptin.

Thirty controls (5 litters) and 36 leptin-injected animals (6 litters) were used. For each experimental procedure at adulthood, animals from at least five different litters per group were used to avoid litter effects.

Experiment 2: Experimental model of chronic hyperleptinemia at adulthood. Wistar rats were kept in an environmentally controlled room (23 ± 3°C and a 12:12-h light-dark cycle; lights on from 7:00 A.M. to 7:00 P.M.). When they were 144 days old, a single dose of 200 μl of saline (group CC) or murine leptin (group LC) (8 μg/100 g of body wt, saline diluted; Prepotech) was injected subcutaneously during six successive days. All of the injections were made from 4:00 to 5:00 P.M. On the 6th day, rats were killed (2 h after the last injection) using a rodent guillotine, and blood samples and adrenal glands were collected (14, 37).

Adrenal glands for catecholamine content quantification, study of the leptin signaling pathway, and in vitro studies were processed in the same way reported for experiment 1. Ten animals were used for each group.

Catecholamine release assay. The experiment was performed in culture cell plates (96 wells) at 25°C as described previously (33, 54, 22) with some modifications. After dissection, the medullas were maintained in standard Krebs-Hepes solution, pH 7.2, for 1 h. This solution contained (in mM): 154.26 Cl−, 143.4 Na+, 2.5 Ca2+, 1.18 Mg2+, 1.2 SO42−, 5.9 K+, 25 HEpES, and 0.5% BSA and glucose (85 mg/dl). Medullas were incubated for 5 min in 200 μl of Krebs solution to evaluate basal secretion. Next, medullas were stimulated by 10−5 M of leptin for 10 min (200 μl/well; Prepotech). At the end of incubations, we added 20 μl of concentrated acetic acid in the wells for better catecholamine conservation. Medium aliquots were collected and kept at −20°C until catecholamine measurement.

Catecholamine quantification. The total catecholamines (epinephrine and norepinephrine) were quantified by the trihydroxyindole method (27). We used 50 μl of the supernatant of the homogenized glands to quantify catecholamine content or 50 μl of incubation solutions to determine catecholamines released from isolated medullas. Epinephrine was used as the standard. Briefly, 50 μl of the standard/supernatant/medium was mixed with 250 μl of 0.5 M buffer phosphate, pH 7.0, and 25 μl of 0.5% potassium ferricyanate, followed by incubation (20 min; ice bath). The reaction was stopped with 500 μl of 60 mg/ml ascorbic acid/10 N NaOH (1:19 proportion). The parameters used in the fluorometer (Victor3; PerkinElmer) were 420 nm to excitation and 510 nm to emission. Results were obtained by plotting the values into a linear regression of the standard epinephrine curve.

Serum leptin measurement. The hormonal analysis was done in a single assay. Serum leptin concentration was measured by a rat leptin specific radioimmunoassay kit (Linco Research). This kit measures both rat and mouse leptin with an assay sensitivity of 0.5 ng/ml, and the intra-assay coefficient of variance was 6.9%.

Western blotting. To achieve an adequate protein concentration in the tissue extracts, it was necessary to make a pool of adrenal medulla to compound each sample for Western blotting. In the experiment of programming, we homogenized four medullas (from 2 animals) in 350 μl of ice-cold lyses buffer (50 mM HEPES, 1 mM MgCl2, 10 mM EDTA, and 1% Triton X-100, pH 6.4). In the experiment of adult chronic hyperleptinemia, we used two medullas (one animal) to make 200 μg of homogenate in the same lyses buffer. Inhibitor cocktail (1 mg/ml aprotinin, 1 mg/ml leupeptin, and 1 mg/ml soybean trypsin inhibitor) was added in the lyses buffer at 0.1%. Homogenates were centrifuged at 4°C and 1,120 g for 15 min. Protein concentration of the supernatants was determined using the BCA Protein Assay Kit (Thermo Scientific), and samples were denatured in sample buffer (50 mM Tris-HCl, pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 10% glycrol, and 0.001% bromphenol blue) and heated at 95°C for 5 min.

The supernatants were analyzed by the SDS-PAGE method, using a 10% polyacrylamide gel and 10 μg of total proteins in each slot of gel, electroblotted in a nitrocellulose membrane (Hybond P ECL)
membrane; Amersham Pharmacia Biotech). Membranes were incubated with TBS containing 5% of nonfat dry milk for 90 min to block nonspecific binding sites. Next, membranes were washed with TBS and incubated overnight with the following primary antibodies (Santa Cruz Biotechnology): anti-OBR (1:500); anti-JAK2 (1:500); anti-STAT3 (1:500); anti-phosphorylated (p) STAT3 (1:500); anti-SOCS3 (1:500); and anti-actin (1:1,000). Furthermore, membranes were washed and incubated with appropriate secondary antibodies conjugated to biotin (0.5% nonfat dry milk TBS diluted; Santa Cruz Biotechnology) for 1 h at room temperature. Membranes were washed and incubated for 1 h at room temperature with streptavidin horseradish peroxidase (HRP) conjugated (Zymed) in the same dilution of the secondary antibody. Immunoreactive proteins were visualized by HRP substrate (ECL-plus; Amersham Pharmacia Biotech) and then exposed to X-ray film. Finally, area and density of the protein bands were quantified by Image J 1.34s software (Wayne Rasband National Institute of Health).

The expression of TH in the adrenal medulla from animals of experiment 2 (hyperleptinemia in adult animals) was also determined in the homogenates. The procedure was very similar to the one used for leptin pathway proteins. Membranes were incubated with primary antibody (monoclonal mouse anti-TH; Sigma-Aldrich) overnight at 4°C (0.5% nonfat dry milk TBS diluted, 1:1,000). Furthermore, membranes were washed and incubated with secondary antibody (goat anti-mouse; Santa Cruz Biotechnology) conjugated with biotin (0.5% nonfat dry milk TBS diluted, 1:1,000) for 1 h at room temperature. Next, there was an incubation with streptavidin HRP conjugated (1:1,000; Zymed) for 1 h. Immunoreactive proteins were visualized by 3,3-diaminobenzidine (Sigma-Aldrich, St. Louis, MO) staining. Finally, area and density of TH bands were also quantified by Image J 1.34s software.

Adrenal histological analysis. The adrenal glands were collected and instantly fixed in Bouin’s solution. Next, the whole glands were embedded in paraffin, sectioned 5 μm thick, and stained with hematoxylin-eosin. The sections were observed in a light microscope (Olympus BX40), and digital images were captured with an Optronics charge-coupled device video camera system at ×2.5 objective.

Statistical analysis. Data are expressed as means ± SE. Groups were compared using Student’s unpaired t-test. Graph Pad Prism 4.0 software (GraphPad Software, La Jolla, CA) was used to perform the analysis. Results were considered significant when \( P < 0.05 \).

RESULTS

Experiment 1. Leptin treatment during lactation resulted in lower body mass from the 2nd day of treatment to the 10th day,
reaching at the last day of treatment (~10% lower values ($P < 0.05$) in the leptin group (20.7 ± 0.41 g) compared with the control group (22.8 ± 0.16 g). At adulthood (150 days old), the leptin-programmed rats presented higher body mass (406.1 ± 5.3 g) compared with the control group (378. ± 9.5 g) ($P < 0.05$). Hyperleptinemia during the first 10 days of life also programmed for hyperleptinemia (+78%, $P < 0.05$; Fig. 1A) and higher absolute and relative adrenal catecholamine content (~3-fold increase, $P < 0.05$) at adulthood (Fig. 1, B and C, respectively).

Leptin-programmed animals presented hypertrophy of adrenal glands (Fig. 2, A and B) as well as higher adrenal mass (+20%, $P < 0.05$; Fig. 2C) at adulthood. The analyses of the content of proteins of the leptin signaling pathway in adrenal medulla of the programmed rats are presented in Fig. 3. The L group showed a lower content of OBR (−61%, $P < 0.05$; Fig. 3A) and JAK2 (−29%, $P < 0.05$; Fig. 3B) in the medulla, and no changes were observed in the content of STAT3 and SOCS3 (Fig. 3, C and F, respectively). However, the phosphorylation of STAT3 (Fig. 3D) and the pSTAT3/STAT3 rate (Fig. 3E) were higher in the leptin-programmed group (~2-fold increase, $P < 0.05$). The analysis of the in vitro catecholamine release in isolated adrenal medulla of control and leptin-programmed rats is presented in the Fig. 4. The basal catecholamine secretion was unchanged in the L group (Fig. 4A), but the catecholamine secretion stimulated by leptin was lower in these animals compared with the controls (~22%, $P < 0.05$; Fig. 4B).

Experiment 2. Leptin treatment for 6 days at adulthood (150 days old) did not alter body mass (CC = 352.8 ± 13.33 g; LC = 357.0 ± 10.74 g) but induced hyperleptinemia at the last day of leptin administration (~2-fold increase, $P < 0.05$; Fig. 5A). The LC group did not present alterations in the adrenal mass (Fig. 5B) or in the adrenal catecholamine content (Fig. 5, C and D). However, they displayed a lower content of TH (~17%, $P < 0.05$) in the adrenal gland (Fig. 5E).

The adrenal medullary content of the leptin signaling pathway proteins of control and leptin-treated rats at adulthood is presented in Fig. 6. The treatment did not affect the content of OBR (Fig. 6A), JAK2 (Fig. 6B), STAT3 (Fig. 6C), pSTAT3 (Fig. 6D), or pSTAT3/STAT3 (Fig. 6E). The LC group also did not differ from the CC group in the basal and leptin-stimulated catecholamine release (Fig. 7, A and B).

**DISCUSSION**

Serum leptin concentration varies in normal rodents throughout lactation. In newborn rats, leptin concentration reaches its highest level on the 10th day of life, characterizing a postnatal leptin surge (16). Rat undernutrition during gestation and lactation reduces leptin levels in the neonates (10), whereas...
undernutrition in mice results in higher leptin levels (59). Leptin serum concentration in early life has been postulated to be an important factor for adequate future development in experimental models of programming. Either situation, hyperleptinemia or hyperleptinemia (39, 53), in early life is associated with several adult chronic diseases in rodents such as obesity and diabetes (16). However, none of these studies focused on the contribution of leptin for the adrenal medullary function and the development of those dysfunctions, which was the major goal of the present study.

Leptin treatment on lactation or at adulthood increased body mass gain during the first 10 days of life, which may be associated with high leptin and catecholamine levels at the end of the treatment already demonstrated in this model (54). Thus, as catecholamines and leptin induce lipolysis (28, 7), we have suggested that the pups had a lower content of fat contributing to the lower body mass observed in the leptin group. However, at adulthood, they presented higher body mass that was not associated with changes in fat content, but with an increase of body protein content (53), presumably muscle mass. Corroborating previous data (37), body mass was unchanged by the leptin treatment at adulthood. Probably, the period of treatment of 6 days was not enough to induce detectable changes in body composition and mass.

We have shown that leptin treatment during the first 10 days of lactation also programmed for hyperphagia, central leptin resistance with lower expression of OBRb in the hypothalamus (53), and increased thyroid function at adulthood (51, 52). Recently, we have demonstrated in the same model of programming increased catecholamine production by adrenal medulla with cardiovascular consequences at adulthood, such as higher blood pressure and heart rate in programmed rats (54). We have suggested a contribution of adrenal catecholamines in the development of changes in the cardiovascular parameters. In the present study, we investigated whether the programming of the adrenomedullary function by neonatal hyperleptinemia was a consequence of a direct effect of leptin on the adrenal chromaffin cells.

Leptin-programmed adult rats displayed a higher catecholamine content in adrenal medulla, but the leptin treatment at adulthood did not alter this parameter. In leptin-programmed rats, we have demonstrated a higher expression of TH content in medulla (54) that suggests strongly an increase of catecholamine synthesis in this experimental model since TH is a rate-limiting enzyme for this process. The increase of synthesis and activity of TH can be induced by leptin in vitro, and it seems to be dependent on Ca2+ mobilization and mitogen-activated protein kinase and protein kinase C activity in cultured chromaffin cells (47, 48, 55). On the other hand, leptin treatment at adulthood decreased TH content in the adrenal medullas. Because catecholamine content was unchanged in the LC group, we believe that this alteration in TH levels has no consequences on the basal catecholamine secretion. Nevertheless, it is probable that those animals submitted to a stressful situation could have an inappropriate adrenergic response.

Undernutrition during gestation and lactation induces changes in the development of adrenal chromaffin cells, resulting in increased catecholamine levels at weaning (34, 35). We have also observed that maternal protein restriction only during lactation programs for high catecholamine synthesis and secretion at adulthood (22). Thus, the maternal nutritional status in early life may change leptin levels and consequently program the adrenal medullary function of the offspring in a short- and long-term life.

The fact that all of the leptin signaling proteins have been found in the rat adrenal medulla suggests a direct effect of leptin on this tissue. OBR and JAK2 content were lower in medulla of leptin-programmed rats, and no changes were observed in the rats treated with leptin at adulthood. In the hypothalamus, the leptin action through the activation of the OBR-JAK2-STAT3 pathway is well characterized. However, few studies have demonstrated the leptin signaling pathway in peripheral tissues. Using the same experimental model of programming, Dutra et al. (18) demonstrated lower expression of OBR in hypothalamus and thyroid in leptin of 30-day-old leptin-programmed rats. In the present study, a very similar profile was found with lower expression of OBR in medulla, suggesting leptin resistance also in the periphery, similar to what we have found in the hypothalamus (38, 53). It is well known that hyperleptinemia causes downregulation on OBRs, especially in the hypothalamus. However, there is no previous report of this effect on the adrenal medulla. The same is valid for JAK2 and other proteins of the leptin pathway. We cannot state at the present moment the exact meaning of the rise of pSTAT3 content found in the adrenal medulla of leptin-programmed animals. However, there is evidence of its involvement in signaling pathways of other members of the IL-6 cytokine family, in addition to its participation in leptin sig-

Fig. 7. Effect of chronic leptin treatment at adulthood on adrenal catecholamine in vitro secretion. Medullary basal catecholamine release (A) and leptin-stimulated catecholamine release (B). Medullas were isolated from control (n = 10) and leptin-treated (n = 10) rats at 150 days old. Data are expressed as means ± SE.
naling (25). In vitro studies have shown that STAT3 can also be activated by insulin (6) and leptin-programmed rats present hyperinsulinemia (53). Besides, STAT3 has been reported to participate in the growth and differentiation of several cell types (29). Thus, the hypertrophy observed in adrenal glands of leptin-programmed rats may be associated, among other factors, with the trophic role of STAT3.

Concerning the role of leptin on catecholamine secretion, it has a stimulatory effect in cultured porcine chromaffin cells that involves a mechanism of activation of the voltage-gated Ca2+ L- and N-type channels (49). Besides, leptin also increases inositol trisphosphate formation that results in Ca2+ release from intracellular stores (47). The increase of cytosolic Ca2+ is a limiting factor for catecholamine secretion in chromaffin cells (26). However, leptin did not affect catecholamine release in adrenomedullary cells of humans (24) and bovines (57). The lower expression of OBR and JAK2 could explain the lower stimulatory effect of leptin on catecholamine secretion in the programmed animals. Because the L group had a lower leptin response and leptin has an acute stimulatory effect on catecholamine secretion (47), there still remains the question why leptin-programmed animals secreted more catecholamine than the controls under caffeine stimulation (54).

Leptin acts in the ventromedial hypothalamus and stimulates SNS outflow (44), and the released catecholamines induce lipolysis in the adipose tissue through β-adrenoreceptor activation to regulate fat and body mass (15). In obesity, a hyperleptinemic condition, the central leptin resistance impairs the leptin control on food intake, but its stimulatory action on SNS activity seems to be preserved or increased (15). In obese mice, a single intracerebroventricular injection of leptin increases renal sympathetic nerve activity despite the lack of leptin sensitivity to control satiety, selecting a selective central leptin resistance in this model of obesity (40). Because leptin-programmed rats are also resistant to the anorexigenic effect of leptin, we suggest that our data concerning catecholamine synthesis and secretion is a consequence of two combined factors: a central stimulatory action of leptin upon the SNS, increasing the sympathetic tonus in the adrenal medullas, and the medullary adrenal hypertrophy. We suppose that the hyperleptinemia of programmed rats could lead to an increase of catecholamine production through SNS activation, and the lower OBR and JAK2 medullary content counterbalance this stimulatory effect to avoid adverse cardiovascular and metabolic consequences. Besides, Molendi-Coste et al. (35) have shown that undernutrition in early life advanced splanchnic neurotransmission maturation during development, and a similar mechanism may be involved in our programming model.

In conclusion, neonatal hyperleptinemia programs for hypertrophy and high catecholamine production of adrenal glands in adult rats. These effects do not seem to be due to a direct action of leptin on medulla, since the leptin signaling pathway is downregulated in this tissue and the leptin treatment at adulthood had no marked effects. Thus, we suggest an important contribution of the SNS to adrenal medullary function and the development of adult chronic diseases in leptin-programmed rats.

ACKNOWLEDGMENTS

We thank Carlos Roberto and Monica Moura for excellent technical assistance.

GRANTS

This research was supported by the National Council for Scientific and Technological Development [Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)], Coordination for the Enhancement of Higher Education personnel [Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)], and the State of Rio de Janeiro Carlos Chagas Filho Research Foundation [Fundaçao Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)]. E. de Moura was the recipient of a FAPERJ fellowship; E. Oliveira was the recipient of a CAPES fellowship; C. R. Pinheiro and E. P. S. Conceição were recipients of CNPq fellowships.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


