Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring

Isabela Teixeira Bonomo, Patricia Cristina Lisboa, Analaura Ribeiro Pereira, Magna Cottini Fonseca Passos and Egberto Gaspar de Moura

Department of Physiology, Institute of Biology Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Av. 28 de setembro, 87 Rio de Janeiro, RJ, 20551-030, Brazil

1Department of Applied Nutrition, Institute of Nutrition, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

(Requests for offprints should be addressed to P C Lisboa; Email: pclisboa@uerj.br)

Abstract

Maternal malnutrition during lactation reduces prolactin (PRL) and milk production, alters milk composition, and programs the body weight of the offspring. Our study aimed to evaluate the long-term effects of maternal hypoprolactinemia at the end of lactation on food ingestion, body weight, amount of retroperitoneal white adipose tissue (RPWAT), leptinemia, and anorectic leptin effect in the adult offspring. Lactating rats were treated with bromocriptine (BRO), a PRL inhibitor, 1 mg twice a day, or saline (C – control) for the last 3 days of lactation. The body weight and food intake were monitored, and after sacrifice at 180 days, the RPWAT was weighted. In a second experiment, the anorectic leptin effect was tested on 180-day-old animals. Adult offspring whose mothers were BRO-treated showed higher body weight (10%), higher amount of RPWAT (2.3 times), higher total body fat (+39%), and hyperleptinemia (2.9 times) when compared with C, although food intake did not alter. After injection of leptin, the food ingestion at 2, 4 and 6 h was unaffected in BRO animals, confirming a resistance to the anorectic effect of leptin. Since the maternal PRL inhibition during lactation programs a higher body weight with no alteration of food ingestion, we suggest a hypometabolic state. The leptin anorectic resistance can be due to the hyperleptinemia. We suggest that PRL changes during lactation can regulate body weight during adulthood.


Introduction

Our group has shown that adverse situations during lactation, such as malnutrition and hormonal changes, could permanently affect the nutritional and hormonal status of the progeny (Passos et al. 2000, 2002, 2004, Cravo et al. 2002, Teixeira et al. 2002, 2003, Vicente et al. 2004, Lins et al. 2005). This association has been denominated as metabolic imprinting or programming, which is defined as a biological phenomenon that determines relationship between physical and chemical stimuli in early life and future functional status (Lucas 1994, Waterland & Garza 1999, Barker 2003, Moura & Passos 2005). Barker et al. (1993) has associated low birth weight with diseases related to the metabolic syndrome (diabetes, obesity, and hypertension) during adulthood.

The body-weight modulation is performed by many factors, including hormones, such as leptin. Leptin is a hormone secreted by adipose tissue, which reduces the food intake and increases the energy expenditure, maintaining the body-weight homeostasis (Zhang et al. 1994, Friedman & Halaas 1998). Maternal energy malnutrition during lactation programs overweight in the adult offspring, without change in food consumption (Passos et al. 2000). Those animals presented a leptin resistance to their anorexigenic effect (Passos et al. 2004).

During lactation, in rats, malnutrition causes hypoprolactinemia (Lisboa et al. 2006), explaining the failure in milk synthesis and changes in their macronutrient, iodine, and thyroid hormone content (Passos et al. 2000, 2001a, b). Previously, we reported that the milk production suppression through the inhibition of prolactin (PRL) synthesis with administration of an agonist of dopaminergic receptor type 2, bromocriptine (BRO), causes malnutrition in neonatal pups and changes the transfer of leptin through the milk (Bonomo et al. 2005).

Therefore, in this work, we investigated the consequences of maternal hypoprolactinemia during lactation upon body weight, food intake, leptin, and leptin anorectic action in the adult life of the offspring.

Material and Methods

Three-month-old Wistar rats were maintained in a room under a 7 h light:19 h darkness cycle and controlled temperature (23/24 °C). Virgin female rats were caged with one male rat at a ratio of 2:1. After mating, each female was...
placed in an individual cage with water and food available ad libitum until parturition. The use of the animals was according to the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro, which based their analysis on the principles described in the Guide for the Care and Use of Laboratory Animals (Bayne 1996).

After birth, excess pups were removed, so that only six male pups were kept per dam, because it has been shown that this procedure maximizes lactation performance (Fishbeck & Rasmussen 1987).

Twelve lactating rats were separated into the following groups: BRO − treated with 1 mg bromo-α-ergocriptine (BRO; Novartis, SP, Brazil), twice a day, for the last 3 days of lactation before death and C − control group, which received saline treatment for the same time (Bonomo et al. 2005). We tested several days of PRL inhibition and 3 days was the maximum period that permitted the pups’ survival through to adulthood (data not shown). Table 1 shows the body weight of the pups before and during maternal BRO treatment.

After weaning (21 days of lactation), three pups of each dams’ group had their body weight and relative food intake (g/100 g BW) monitored every 4 days until 180 days, when the rats were killed by decapitation. Trunk blood was collected and the retroperitoneal white adipose tissue (RPWAT) was dissected out and immediately weighed.

**Body fat determination**

The rats were eviscerated and the carcass was weighed, autoclaved for 1 h, and homogenized on distilled water (1:1; Leshner & Litwin 1972). The homogenate was stored at −20 °C for analysis. Three grams of homogenates were used for determining the fat mass gravimetrically (Stansbie 1976, Toste et al. 2006). The samples were hydrolyzed on a shaking water bath at 70 °C for 2 h with 30% KOH and ethanol. The total fatty acids and free cholesterol were removed by washing three times with petroleum ether. After drying overnight in a vacuum, all tubes were weighed and data were expressed as grams of fat per 100 g carcass.

**Leptin anorectic effect test**

**Peptide** Recombinant mouse leptin (PeproTech, Rocky Hill, NJ, USA) was dissolved in saline (0.9% wt/vol) and injected as a bolus at a dose of 0.5 mg/kg body weight intraperitoneally (Martin et al. 2000, Passos et al. 2004).

**Feeding study** C and BRO adult (180 days old) offspring were divided into the following groups: leptin (Clep and BROLep) or saline (Csal and BROsal). All groups were food-deprived for 24 h with free access to water before the test. After injection, the rats were returned individually to their cages and provided with the standard diet. Food intake was measured by weighing the food, 2, 4, and 6 h after leptin or saline injections. In order to check the effectiveness of the acute leptin injection, we measured leptinemia after 2 h and 24 h in both groups.

**Serum leptin**

Blood samples of 180-days old C and BRO offspring were centrifuged and serum was stored at −20 °C until assayed. Leptin was determined by RIA kit (Linco Research, St Charles, MO, USA). This kit has an assay sensitivity of 0.5 ng/ml and a range of detection from 0.5 to 50 ng/ml. All measures were done in one assay. The intra-assay variation was 6-9%.

**Statistical analysis**

Data are reported as means±S.E.M. Two-way ANOVA and Newman–Keuls multiple comparison were used to analyze food intake in response to the leptin acute administration, and the Student’s t-test to analyze the other experimental observations, with significance level set at P<0.05.

**Results**

During maternal BRO treatment, pups presented a significant low body weight gain (day 2, 6%; day 3, 8%), as demonstrated in Table 1.

The body weight of offspring whose mothers were injected with BRO for the last 3 days of lactation was significantly higher than the controls from day 133 until 180 days, reaching about 10% of overweight (P<0.05; C, 397.4±8.9 vs BRO, 438.2±20.8; Fig. 1). However, these rats did not show changes in relative food intake since weaning until adult life (C, 4.4±0.3 vs BRO, 4.0±0.7; Fig. 2).

We also detected on 180-days-old group, a higher amount of RPWAT (2.3 times, P<0.05; C, 14.7±2.8 vs BRO, 33.3±2.6; Fig. 3) and total body fat (+39%, P<0.05; C, 7.4±0.5 vs BRO, 10.3±0.7; Fig. 4), confirming that the overweight is due, at least in part, to an increase in adiposity.

The adult rats of BRO-treated dams presented hyperleptinemia (2.9 times, P<0.05; C, 5.8±1.3 vs BRO, 17.0±1.7; Fig. 5). To check this high leptin serum levels in BRO group, we performed an anorectic test of the leptin effect. The test effectiveness of the anorectic effect of leptin is showed by the data of C group, where leptin injection inhibited more than

**Table 1** Body weight of pups during maternal BRO or saline treatment. Values are given as the means±S.E.M.

<table>
<thead>
<tr>
<th>Days of lactation</th>
<th>C (g)</th>
<th>BRO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>50.4±1.2</td>
<td>48.0±0.8</td>
</tr>
<tr>
<td>20</td>
<td>53.1±1.1</td>
<td>50.0±0.7*</td>
</tr>
<tr>
<td>21</td>
<td>55.5±1.3</td>
<td>51.2±0.6*</td>
</tr>
</tbody>
</table>

n=5 animals/group, *P<0.05.


www.endocrinology-journals.org
50% of the food intake when compared with group CsAl, in all the three periods studied (Fig. 6A, Table 2). However, leptin-injected BRO group showed unaffected food intake after 2, 4, and 6 h (Fig. 6B, Table 2), which demonstrates a resistance to the leptin anorexigenic action. We also measured leptinemia after 2 h of acute saline or leptin administration and after 24 h in both groups, as shown in Table 3. Clep and BROlep presented high serum leptin after 2 h (+1.24 times and +100% respectively) and unchanged levels after 24 h.

Discussion

We showed previously that hypoprolactinemia at the end of lactation causes malnutrition (Bonomo et al. 2005), because there was a marked decrease in milk production. Other authors showed a different kind of programming by energy malnutrition during lactation (Waterland & Garza 1999, Godfrey & Barker 2000, Vickers et al. 2000, Passos et al. 2000, 2002, 2004, Breier et al. 2001, Teixeira et al. 2002, Vicente et al. 2004). Here, we showed that maternal hypoprolactinemia at the end of lactation caused overweight, higher visceral fat, hyperleptinemia, and hypothalamic leptin resistance in their adult offspring.

The higher body weight with normal food intake detected in BRO adult rats is similar to our previous data in adult animals whose mothers were submitted to energy restriction during lactation (Passos et al. 2002, Teixeira et al. 2002). In the present work, we showed that the overweight developed by the adult offspring of BRO-treated dams was due, at least partially, to a higher amount of central adiposity.

In obese individuals, higher leptin levels do not produce the expected satiety and increase in energetic expenditure. This unexpected leptin effect is explained through leptin resistance, caused by downregulation of their hypothalamic receptors (Considine et al. 1996, Martin et al. 2000), by a reduced blood–brain barrier transport (Burguera et al. 2000, Banks 2001) or by an impairment of the intracellular transduction pathway (Widdowson 1997, Wilson et al. 1999, Ahima & Flier 2000, Jequier 2002).

Concerning the leptin anorectic test in adult BRO offspring, since we did not detect a decrease in food consumption after leptin acute injection, as observed for the adult control rats, it is reasonable to believe that adult BRO rats did have a hypothalamic leptin resistance. The leptin resistance observed in BRO adult rats may be explained by their hyperleptinemia. These programmed rats also presented higher serum leptin levels at weaning (Bonomo et al. 2005), similar to the changes observed in leptinemia of pups whose...
mothers were malnourished during lactation (Teixeira et al. 2002). When the pups of energy-malnourished mothers become adults, they also presented leptin resistance (Passos et al. 2004). In the same way, other models of neonatal leptin imprinting (Cravo et al. 2002, Lins et al. 2005, Toste et al. 2006) are associated with leptin resistance. It seems that in the present model, higher neonatal leptinemia also imprints for leptin resistance at adulthood. Our data support the hypothesis of a central role of leptin on the body-weight control mechanisms imprinted at the beginning of life, especially on lactation.

We detected an 8% decrement on body weight of BRO pups at 21 days-old, corroborating our previous findings (Bonomo et al. 2005), which characterize neonatal malnutrition. This early malnutrition may be the imprinting factor for adult overweight, as demonstrated by other authors (Waterland & Garza 1999, Godfrey & Barker 2000, Vickers et al. 2000, Passos et al. 2000, 2002, 2004, Breier et al. 2001, Teixeira et al. 2002, Vicente et al. 2004). Thus, we believe that maternal hypoprolactinemia early in a pup’s life plays the relevant role for the nutritional imprinting phenomena detected in our study. Our hypothesis is supported by the fact that milk presents large amounts of PRL, which is probably transferred by the milk and absorbed by the immature newborn gastrointestinal tract (Ellis & Picciano 1995, Ben-Jonathan et al. 2006). However, the function of this milk secreted PRL on the developing offspring is unknown.

The events that could link low maternal PRL imprinting with the higher body weight of the offspring were based on the fact that BRO offspring received higher leptin transfer through the milk (Bonomo et al. 2005) and that leptin administration to pups or to dams are related with the same kind of body weight programming (Lins et al. 2005, Toste et al. 2006).
Table 2 Food intake (g) in leptin anorectic test of BRO and C adult offspring. Values are given as the means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C sal</td>
<td>0.6 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>C lep</td>
<td>0.2 ± 0.1*</td>
<td>0.4 ± 0.2*</td>
<td>0.7 ± 0.2*</td>
</tr>
<tr>
<td>BRO sal</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>BRO lep</td>
<td>0.9 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

n=8 animals/group, *P<0.05.

Another important issue is related to the model of PRL blockade. Here, we only used BRO with the aim of PRL inhibition, but it is difficult to distinguish the hypoprolactinemia effect and a possible direct BRO effect on the pups, as well as the short-term and moderate (8%) undernutrition, much more higher in the maternal malnourished models (20% for a longer period). BRO is a specific dopamine type 2 receptor agonist, mainly found in the pituitary gland (Ben-Jonathan & Hnasko 2001). Therefore, even if BRO is transferred through the milk to the pups, its putative effect is over pups’ PRL itself. Then, we believe that maternal PRL inhibition by BRO is the main imprinting factor. Further studies are being carried out in order to test a possible direct BRO effect in pups.

Finally, our data make evident the crucial role of PRL, even when it is blocked for a short period at the end of lactation, as an important factor that programs body weight regulation and adiposity, suggesting the importance of the PRL effect on milk transfer of other possible imprinting factors, such as leptin.

Acknowledgements

Research was supported by CNPq, CAPES, and FAPERJ. The authors are grateful to Ms Luciene Andrade and Mr Carlos Roberto for their technical assistance. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


www.endocrinology-journals.org
Passos MCF, Vicente LL, Lisboa PC & Moura EG 2004 Absence of anorectic effect to acute peripheral leptin treatment in adult animals whose mothers were malnourished during lactation. Hormone and Metabolic Research 36 625–629.

Stansbie D 1976 Regulation of the human pyruvate dehydrogenase complex. Clinical and Science Molecular Medicine 51 445–452.

Teixeira CV, Passos MCF, Ramos CF, Dutra SCP & Moura EG 2002 Leptin serum concentration, food intake and body weight in rats whose mothers were exposed to malnutrition during lactation. Journal of Nutritional Biochemistry 13 493–498.


Vicente LL, Moura EG, Lisboa PC, Costa AMA, Amadeu T, Mandarim-de-Lacerda CA & Passos MCF 2004 Malnutrition during lactation is associated with higher expression of leptin receptor in pituitary of the adult offspring. Nutrition 20 924–928.


Received in final form 31 October 2006
Accepted 3 November 2006
Made available online as an Accepted Preprint 15 November 2006