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Effects of postnatal bromocriptine injection on thyroid function and prolactinemia of rats at adulthood

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ABSTRACT

Previously, we demonstrated that maternal prolactin inhibition at the end of lactation, using bromocriptine (BRO), leads to an increase in leptin transfer via milk and induces the adult progeny to present hypothyroidism, leptin resistance and metabolic syndrome (obesity, hyperglycemia, hypertriglyceridemia, lower HDL). To test if these alterations are due to direct BRO action on the pups, in the present study we evaluated the long-term effects of direct injection of BRO (0.1 μg/once daily) in male Wistar rats from postnatal (PN) day 1 to 10 (early treatment) or from PN11 to 20 (late treatment) on: food intake, body mass, cardiovascular parameters, hormone profile, hypothalamic leptin signaling, glucose homeostasis and thyroid hormone-dependent proteins. The respective controls were injected with methanol-saline. Offspring were killed at adulthood (PN180). Adult PN1-10 BRO-treated animals had lower food intake, hypoprolactinemia, lower leptin action (lower OBR-b, STAT-3 and SOCS-3 mRNA levels in the arcuate nucleus), lower TRH-TSH-thyroid axis as well as lower thyroid hormone markers. On the other hand, adult animals that were BRO-treated during the PN11-20 period showed hyperphagia, higher blood pressure, higher prolactinemia and OBR-b, higher TRH and plasma T3, hypercorticosteronemia as well as higher Dio2 and UCP1 mRNA expression in the brown adipose tissue. Glucose homeostasis was not changed treatment in either period. Our data show that early and late dopamine overexposure during lactation induces diverse metabolic disturbances later in life, increasing the risk of thyroid dysfunction and, consequently, changes in prolactinemia.

1. Introduction

Several epidemiological studies have shown that nutritional and metabolic modifications that occur in early life have late-emerging long-term effects on hormonal and metabolic homeostasis, findings that have been commonly referred to as metabolic programming (Barker et al., 1993). These programming effects, including those on hormonal regulation, were reproduced by experimental studies that were carried out during periods of relevant developmental plasticity, even after birth (Gluckman and Hanson, 2007; Cottrell and Ozanne, 2008). In rodents, significant cognitive and neurological development still occurs postnatally, during the critical period of lactation (de Moura and Passos, 2005; Miñana-Solis and Escobar, 2006). Therefore, it is plausible that changes at this stage of life can cause late-emerging long-lasting physiological modifications that may either guarantee a better adaptation to the prevailing environmental conditions that resulted in the programming effect to begin with or that can also lead to the development of diseases at adulthood if the initial conditions were substantially changed (Moura et al., 2008).

Dopamine is a natural catecholamine released by the brain and it is an important neurotransmitter in the central nervous system (CNS). Dopamine can induce pituitary suppression, inhibiting hormones such as prolactin, TSH and GH, and can also inhibit thyroid hormones production (Leblanc et al., 1976; de Zegher et al., 1993; Filippi et al., 2006; Haugen, 2009). These hormones are essential for immune regulation, growth, metabolism and neurodevelopment (Devins et al., 1992; Wit and van Unen, 1992; Leviton et al., 1999). Dopamine, through its action on different kinds of receptors, controls several aspects of SNC activity, such as cognition, emotion, control of locomotion and food intake behavior, as well as those related to reward (Missale et al., 1998). Dopaminergic receptors have been classified into two subtypes: D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors. The D2-like receptors inhibit adenylyl cyclase and are the targets for most of the common drugs used to treat Parkinson's disease. Bromocriptine (BRO) is a D2 agonist that inhibits PRL production and release, and is used in the management of prolactinomas (Seeman and Van, 1994), to treat type 2 diabetes (Liang et al., 2015) and for the suppression of lactation

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(Verma et al., 2006; Bernard et al., 2015). In addition, dopamine has not only been used to treat shock in adults, but also in neonates (Noori and Seri, 2012; Saini et al., 2014), who have more plastic, therefore susceptible, dopaminergic neurons than adults.

Our group demonstrated that maternal PRL inhibition due to the treatment with BRO at the end of lactation programs for several changes in the adult offspring. The weaned pups (PN21) are malnourished and hyperleptinemic due to the additional transfer of leptin through the milk (Bonomo et al., 2005). At adulthood, we observed higher visceral fat mass, hyperinsulinemia, increase of blood glucose and triglycerides, leptin resistance, hypothyroidism, hypoprolactinemia, increased catecholamine adrenal content and serum corticosterone (Bonomo et al., 2007, 2008; Moura et al., 2009). Also, we found higher neuropeptide Y (NPY) and lower cocaine- and amphetamine-regulated transcript (CART) (Younes-Rapozo et al., 2012), adipocyte hypertrophy and higher liver oxidative stress (Peixoto-Silva et al., 2014), as well as higher levels of anxiety-like behavior, impaired learning and memory performance (Fraga et al., 2011). It is conceivable that the metabolic and hormonal changes previously observed when BRO was administered in lactating dams are associated with either an increase in the leptin milk transfer (Bonomo et al., 2005) or in BRO milk transfer, since it was demonstrated that, in humans, BRO can be transferred via breast milk (Kulski et al., 1978; Katz et al., 1985).

In rodents, dopamine system development begins in embryonic life and becomes functional during the postnatal period. The first ten days of lactation (PN1 to 10) are characterized by an intense neural proliferation and synaptogenesis and, during this period, the number of dopamine fibers and the quantity of dopamine receptors subtypes (D1R and D2R) are higher than at adulthood. During the subsequent 10 days of life (PN11 to 20) considerable synaptogenesis, gliogenesis and myelinization can be observed, particularly in structures such as the hippocampus and in the reward pathways. Besides, during this period, D2R is higher than D1R, and still higher than D2R at adulthood (Dow-Edwards et al., 1988, 1993; Hughes et al., 1993; Levitt, 1998a,b; Tarazi and Baldessarini, 2000; Ong et al., 2012). Considering the aforementioned information, we hypothesize that BRO can imprint changes in dopaminergic neurons that could have long-term consequences on several hormones related to metabolism, possibly leading to the development of the metabolic syndrome. In fact, we have recently shown, in rats, that the programming effects induced by BRO treatment on the dopaminergic pathway varies as a function of the postnatal period within which the treatment is carried out (Carvalho et al., 2016). When BRO pups were injected during the first ten days of lactation the animals showed, at adulthood, higher tyrosine hydroxylase content in the ventral tegmental area (VTA) and higher DOPA decarboxylase and D2R contents in the nucleus accumbens (NAc). If the BRO treatment was carried out during the last ten days of lactation, the adult animals showed lower TH content in the VTA and lower DAT content in the NAc. These programmed animals also showed, at adulthood, differences in levels of anxiety-like behavior, with anxiogenic effects being observed in PN1-10 BRO-treated animals and anxiolytic effects being observed in PN11-20 BRO-treated ones. To test whether the aforementioned changes are related to long-term effects of BRO treatment on the hormonal profile and metabolic syndrome components, we injected this drug directly in the pups either during the first- (PN1-10) or the second-half (PN11-20) of the lactation period.

2. Materials and methods

This study was conducted under the approval of the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro, which based its decision on the principles promulgated by Brazilian Law no 11.794/2008. The experiments were performed to minimize the number of animals used and any suffering, following the ethical doctrine of the three "Rs" (reduction, refinement and replacement).

Wistar rats were housed in a temperature-controlled (25 ± 1 °C) vivarium with an artificial dark-light cycle (lights on from 07:00 to 19:00). Female rats were mated with male rats at a proportion of 2:1. During pregnancy and lactation, dams were housed in individual cages and had water and a standard pellet diet (commercial control diets for rats) available ad libitum. After birth (defined as postnatal day 1, PN1), litters were culled to six male pups in order to improve the lactation performance.

Ten lactating dams were used and four male pups per litter were randomly chosen for the two experimental sequences described below:

Early postnatal period programming (PN1-10)

At PN1, we randomly chose one pup from a given litter to receive s.c. bromo-ergocriptine (BRO – Novartis, São Paulo, Brazil) injections (once daily from PN1 to PN10; dose: 0.1 μg diluted in methanol-saline (1:1) (Carvalho et al., 2016). A second randomly chosen pup from the same litter received methanol-saline for the same period (CON). Early-treated BRO pups were identified by a non-toxic indelible ink mark on the front left paw, while CON pups were marked on the front right paw.

Late postnatal period programming (PN11-20)

From PN11 to PN20, two more pups in a given litter underwent the same BRO and CON injection procedures indicated above. These late-treated BRO pups were identified by a non-toxic indelible ink mark on the hind left paw, while CON pups were marked on the hind right paw.

The two remaining pups were used in another study and they were not marked.

Body mass (BM) and food intake were monitored, every 4 days, from weaning to PN180 day. At PN180, the offspring were fasted for 12 h and then killed by cardiac puncture under anesthesia (55 mg/kg body mass of ketamine and 10 mg/kg body mass of xylazine) for sample collection. The blood was collected in heparinized tubes, and centrifuged (1500 × *g*/20 min/4 °C) to obtain plasma, which was frozen (−20 °C) until assaying. The tissues were dissected out and immediately frozen (−80 °C).

The whole brain was carefully removed and stored at −80 °C until the removal of the regions of interest. Frozen coronal brain sections were cut using a cryostat (Hyrax C25, Zeiss, Germany) and punches of the hypothalamic paraventricular nucleus (PVN; bregma 0.6 to to 2.1 mm) and the hypothalamic arcuate nucleus (ARC; bregma 2.1 to 3.6 mm) were extracted from the brains using the anatomical references indicated in the Paxinos & Watson (1998) stereotaxic coordinates atlas. The samples were maintained in liquid nitrogen and stored at −80 °C for determination of protein content by Western blotting.

2.1. Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed at PN180. After a 12-h fasting period, 50% glucose was administered in sterile saline (0.9% NaCl) through an oral gavage at 2 g/kg BM. Blood was drawn from the tail tip of each animal so that the plasma glucose concentration could be assessed with the use of a glucometer (Accu-Chek Advantage; Roche Diagnostics, Mannheim, Germany). Blood samples were collected before the glucose was administered and 15, 30, 60, and 120 min after the gavage (Peixoto-Silva et al., 2011).

2.2. Blood pressure and heart frequency

Cardiovascular parameters were measured by the tail-cuff method using a Digital Pressure Meter Letica LE 5000 device (Bioseb, Marseille, France). Animals were trained for at least 2 weeks until the arterial pressure was consistently recorded with minimal restraint and stress during measurements. The first measurement of cardiovascular parameters was discarded and the mean of the three subsequent measurements was recorded.

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