Neuroscience 259 (2014) 71–83

POSSIBLE CROSSTALK BETWEEN LEPTIN AND PROLACTIN DURING PREGNANCY

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Abstract—Rodents exhibit leptin resistance and high levels of prolactin/placental lactogens during pregnancy. A crosstalk between prolactin and leptin signaling has been proposed as a possible mechanism to explain the changes in energy balance during gestation. However, it remains unclear if specific neuronal populations co-express leptin and prolactin receptors. Therefore, our present study was undertaken to identify in the mouse brain prolactin-responsive cells that possibly express the leptin receptor (LepR). In addition, we assessed the leptin response in different brain nuclei of pregnant and nulliparous mice. We used a LepR-reporter mouse to visualize LepR-expressing cells with the tdTomato fluorescent protein. Prolactin-responsive cells were visualized with the immunohistochemical detection of the phosphorylated form of the signal transducer and activator of transcription-5 (pSTAT5-ir). Notably, many neurons that co-expressed tdTomato and pSTAT5-ir were observed in the medial preoptic area (MPA, 27–48% of tdTomato cells), the retrochiasmatic area (34–51%) and the nucleus of the solitary tract (NTS, 16–24%) of prolactin-treated nulliparous mice, pregnant mice and prolactin-treated leptin-deficient (*ob/ob*) mice. The arcuate nucleus of the hypothalamus (8–22%), the medial tuberal nucleus (11–15%) and the ventral premammillary nucleus (4–10%) showed smaller percentages of double-labeled cells among the groups. Other brain nuclei did not show significant percentages of neurons that co-expressed tdTomato and pSTAT5-ir. Late pregnant mice exhibited a reduced leptin response in the MPA and NTS when compared with nulliparous mice; however, a normal leptin response was observed in other brain nuclei. In conclusion, our findings shed light on how the brain integrates the information conveyed by leptin and prolactin. Our results corroborate the hypothesis that high levels of prolactin or placental lactogens during pregnancy may directly interfere with LepR signaling, possibly predisposing to leptin resistance.

Key words: gestation, cytokine receptor, hypothalamus, energy balance, STAT3, STAT5.

INTRODUCTION

Prolactin and leptin are hormones with distinct known functions. The main effects of prolactin are related to the development of mammary glands and milk production (Bole-Feysot et al., 1998; Grattan and Kokay, 2008). Leptin plays a pivotal role in the regulation of the energy balance, food intake and glucose homeostasis (Coppari and Bjorbaek, 2012). However, the circulating levels of prolactin or placental lactogens are high during pregnancy, a condition characterized by marked changes in the control of energy balance (Augustine et al., 2008; Ladyman et al., 2010, 2012). The responsible mechanisms for triggering the metabolic changes observed during pregnancy are not well understood but are believed to be hormone-dependent (Grattan et al., 2007; Augustine et al., 2008; Ladyman et al., 2010). A key metabolic feature observed during pregnancy is the development of leptin resistance. For example, acute anorectic effects of leptin are blunted in rats (Grattan et al., 2007) and mice (Ladyman et al., 2012) in the mid-gestation period (G14 and G12, respectively) when compared with non-pregnant animals. In addition, some nuclei are more prone to develop leptin resistance during gestation. Rats and mice in the mid-gestation period show a reduced leptin-induced phosphorylation of signal transducer and activator of transcription-3 (STAT3) in the ventromedial nucleus of the hypothalamus (VMH) (Ladyman and Grattan, 2005; Grattan et al., 2007; Ladyman et al., 2012) but a similar response in the arcuate nucleus of the hypothalamus (ARH) when compared with non-pregnant animals. Recent findings suggest a potential role for prolactin signaling in the induction of leptin resistance during gestation. Chronic intracerebroventricular infusion of prolactin in pseudopregnant rats

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Abbreviations: 3v, third ventricle; AgRP, agouti-related peptide; ANOVA, analysis of variance; AP, area postrema; ARH, arcuate nucleus of the hypothalamus; cc, central channel; DAB, 3,3′-diaminobenzidine; DMH, dorsomedial nucleus of the hypothalamus; f, fornix; KPBS, 0.02 M potassium phosphate-buffered saline; LepR, leptin receptor; LHA, lateral hypothalamic area; MPA, medial preoptic area; MTu, medial tuberal nucleus; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; oc, optic chiasm; OVLT, organum vasculosum of the lamina terminalis; PBS, phosphate-buffered saline; PMV, ventral premammillary nucleus; POMC, proopiomelanocortin; PrlR, prolactin receptor; pSTAT3, phosphorylated form of signal transducer and activator of transcription-3; pSTAT3-ir, pSTAT3 immunoreactivity; pSTAT5, phosphorylated form of signal transducer and activator of transcription-5; pSTAT5-ir, pSTAT5 immunoreactivity; PVH, paraventricular nucleus of the hypothalamus; RCA, retrochiasmatic area; SOCS, suppressors of cytokine signaling; STATs, signal transducer and activators of transcription; VMH, ventromedial nucleus of the hypothalamus; VMHdm, dorsomedial subdivision of the VMH; VMHvl, ventrolateral subdivision of the VMH.

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http://dx.doi.org/10.1016/j.neuroscience.2013.11.050

blunts the acute anorectic effects of leptin, whereas pseudopregnancy itself does not influence leptin sensitivity (Augustine and Grattan, 2008).

Some studies described a similar distribution of the leptin receptor (LepR) and the prolactin receptor (PrlR) in the rodent hypothalamus and brainstem (Chen and Smith, 2004; Scott et al., 2009; Brown et al., 2010, 2011; Sjoeholm et al., 2011). These areas include the VMH and the ARH, both of which contain a large number of prolactin- and leptin-responsive neurons (Scott et al., 2009; Brown et al., 2010). In the VMH, prolactin-responsive cells are mostly distributed in the ventrolateral subdivision of this nucleus (VMHvl), whereas leptin-responsive cells are confined to the dorsomedial subdivision (VMHdm) (Scott et al., 2009; Brown et al., 2010; Ladyman et al., 2010). In the ARH, most prolactin-responsive cells are positive for tyrosine hydroxylase and very few cells co-express neuropeptide Y (NPY)/agouti-related peptide (AgRP) or proopiomelanocortin (POMC) (Chen and Smith, 2004; Kokay and Grattan, 2005; Ma et al., 2005; Sjoeholm et al., 2011). However, leptin-responsive cells in the ARH are mainly composed of NPY/AgRP or POMC-positive neurons (Morton et al., 2006).

A putative co-expression of LepR and PrlR in neurons would permit a crosstalk between leptin and prolactin signaling. This hormone interaction could explain some of the metabolic changes observed during pregnancy (Grattan et al., 2007; Augustine et al., 2008). Both the LepR and PrlR are members of the type I cytokine receptor family and share intracellular signaling pathways. LepR and PrlR are janus kinase 2 (JAK2)-dependent receptors and activate signal transducer and activators of transcription (STATs) as their main intracellular signaling pathway (Bole-Feysot et al., 1998; Myers, 2004). The LepR preferentially recruits STAT3, whereas the PrlR activates STAT5 (Bole-Feysot et al., 1998; Myers, 2004). Once phosphorylated, STATs dimerize and migrate to the nucleus to regulate target gene expression. The activation of STATs induces the expression of proteins known as suppressors of cytokine signaling (SOCS) that are part of a negative feedback loop to inhibit cytokine receptor signaling cascades (Babon and Nicola, 2012). Late pregnant rats show an increased expression of SOCS1 and SOCS3 in the hypothalamus (Anderson et al., 2006, 2008; Steyn et al., 2008). Thus, it is conceivable that high levels of prolactin or placental lactogens increase the activation of PrlR, which in turn increases the expression of SOCS proteins in LepR-expressing neurons (Anderson et al., 2006; Steyn et al., 2008). As a consequence, the ability of leptin to regulate the energy balance is impaired during pregnancy. However, there is no clear evidence of a significant LepR and PrlR co-expression in the mouse brain. Thus, the objective of our present study was to identify prolactin-responsive cells that possibly express the LepR in the mouse brain. In addition, leptin sensitivity in different brain nuclei was compared between pregnant and nulliparous mice.

EXPERIMENTAL PROCEDURES

Animals

Adult female mice with a c57bl/6 background were maintained in standard conditions of light (12-h light/dark cycle), temperature (22 ± 2 °C) and relative humidity (55 ± 15%). All animal procedures were approved by the Ethics Committee on the Use of Animals of the Institute of Biomedical Sciences, University of São Paulo and were performed according to the ethical guidelines adopted by the Brazilian College of Animal Experimentation.

Experiment 1: Validation of the reporter mouse with florescent LepR-expressing cells

To visualize LepR-expressing cells, we bred the LepRb-IRES-Cre mouse (B6.129-Lepr$^{tm2(cre)Rck}$/J, Jackson Laboratories, Bar Harbor, ME, USA) with the Cre-inducible tdTomato-reporter mouse (B6;129S6-Gt(ROSA)26Sor$^{tm9(CAG-tdTomato)Hze}$/J, Jackson Laboratories). Both mouse models have been previously described and validated (DeFalco et al., 2001; Scott et al., 2009; Madisen et al., 2010; Williams et al., 2011; Singireddy et al., 2013). Without any additional staining, the double mutant mouse (LepR-reporter) is expected to express the red fluorescent tdTomato protein in only LepR-expressing cells. To confirm the specificity of tdTomato expression, mice fasted overnight received an intraperitoneal (i.p.) injection of mouse recombinant leptin (5 μg/g, kindly provided by Dr. Parlow, National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). A subset of these mice (n = 3) were perfused 1 h after leptin infusion, and a second group of mice (n = 5) were perfused 2 h after leptin administration. It was previously shown that leptin treatment induces the expression of the phosphorylated form of STAT3 immunoreactivity (pSTAT3-ir) in the brain (Münzberg et al., 2004; Scott et al., 2009). Thus, cells that express pSTAT3-ir following leptin treatment are considered to also express the LepR. We assessed the co-expression of leptin-induced pSTAT3-ir and tdTomato fluorescence in the medial preoptic area (MPA), retrochiasmatic area (RCA), ARH, lateral hypothalamic area (LHA), VMH, dorsomedial nucleus of the hypothalamus (DMH), ventral premammillary nucleus (PMV) and nucleus of the solitary tract (NTS).

Experiment 2: Distribution of prolactin-responsive cells that express the LepR in the mouse brain

We used female LepR-reporter mice to visualize cells that express the LepR. To visualize prolactin-responsive cells, we immunolabeled the phosphorylated form of STAT5 (pSTAT5) after injecting ovine prolactin (i.p., 10 μg/g, Sigma, St. Louis, MO, USA) in nulliparous LepR-reporter mice (n = 7; body weight: 18.1 ± 0.4 g). Pregnant mice already have high prolactin or placental lactogens; therefore, late pregnant LepR-reporter mice (16–18 days of pregnancy) were perfused without any treatment