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Research Report

Estrogen-dependent post-translational change in the nitric oxide system may mediate the leptin action on LH and prolactin secretion

*B. Del Bianco-Borges, C.R. Franci**

Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil

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ABSTRACT

Gonadotrophin-releasing hormone (GnRH) neurons do not express the leptin receptor (OB-R) in the medial preoptic area (MPOA) and the medial basal hypothalamus (MBH). We assessed whether the effect of leptin on the secretion of luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) in proestrus could be mediated by nitric oxide (NO) under estrogen modulation. Female rats were treated with an estrogen antagonist (tamoxifen s.c. 3 mg/rat) or vehicle during metestrus and diestrus. At proestrus, they received leptin (3 or 10 μg/μl) or intracerebroventricular saline at 11:00 am and were decapitated at 5:00 pm. The following were analyzed in this work: plasma LH, FSH and PRL levels (radioimmunoassay); neuronal NO-synthase (nNOS) and OB-R transcription (RT-PCR); nNOS and phosphorylated nNOS (pnNOS) translation levels (western blotting); and pSTAT3 immunoreactivity. Tamoxifen reduced the plasma LH and PRL levels and decreased the nNOS mRNA and pnNOS expression in the MPOA. Three micrograms of leptin increased the LH secretion and pnNOS protein levels in the MPOA and MBH. Ten micrograms of leptin decreased the transcription, translation and phosphorylation of nNOS in the MPOA. In the MBH, 10 μg of leptin increased the protein expression of nNOS but not the mRNA expression neither pnNOS protein. Tamoxifen did not change either the mRNA or protein expression of nNOS or the phosphorylation of nNOS but decreased the number of cells that contained pSTAT3 immunoreactivity in both areas. In conclusion, the stimulatory effect of leptin on the secretion of LH and PRL on the afternoon of proestrus may be mediated by estrogen-dependent post-translational changes in the nNOS in the MPOA and MBH.

*Corresponding author. Fax: +55 16 36330017.
E-mail addresses: brunodbb@yahoo.com.br (B. Del Bianco-Borges), crfranci@fmrp.usp.br (C.R. Franci).

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1. Introduction

Gonadotropin-releasing hormone (GnRH) is released from nerve terminals in the median eminence to regulate the release of luteinizing and follicle stimulating hormones (LH and FSH, respectively) from the anterior pituitary (Herbison, 2008). In females, these hormones promote follicle development and the production of steroids, such as estrogen and progesterone, among other factors (Fink, 1986). Estrogen acts on the brain to modulate GnRH secretion during the follicular phase of the ovarian cycle through two types of receptors: estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β). However, GnRH neurons express ER-β but not ER-α, which is mainly responsible for positive feedback, indicating that estrogen acts indirectly on GnRH neurons to bring about their activation (Herbison, 2008).

Several neurons networks communicate with the GnRH neurons, allowing an interaction between reproductive function and other functions in the body (Dudas and Merchenthaler, 2006; Herbison, 1998). For example, the success of fertilization depends on the interaction between energy homeostasis and the reproductive axis (Schneider, 2004). One possible mediator of this interaction is leptin, a hormone that is mainly produced by adipose tissue but is also produced by other tissues (Del Bianco-Borges et al., 2010; Morash et al., 1999; Ur et al., 2002). Leptin activates several intracellular signal transduction pathways, but the JAK/STAT pathway is the most well-known pathway. Leptin binds to its receptor and causes phosphorylation of the receptor-associated JAK proteins, which in turn induce the expression of binding sites for cytoplasmic STAT. Once bound to the receptor, STAT molecules are phosphorylated, dimerized and then translocated to the nucleus, where they modify gene transcription (Fruhbeck, 2006).

It is well known that leptin acts in the control of metabolism, feeding behavior and reproduction (Ahima et al., 2000; Smith et al., 2002). Leptin is a hormone coded by the "ob" gene (Tartaglia, 1997). Mice that are homozygous for a spontaneous mutation of this gene (*ob-/ob-mice*) do not produce leptin and exhibit obesity and infertility; these effects are reversed by the systemic administration of leptin (Chehab et al., 1996). Leptin stimulates the secretion of GnRH and luteinizing hormone (LH) (Smith et al., 2002; Yu et al., 1997a), but GnRH neurons do not express the leptin receptor (Ob-R); a transsynaptic action of leptin on GnRH neurons is a more likely possibility (Donato et al., 2009; Quennell et al., 2009; Reynoso et al., 2007; Watanobe and Schioth, 2001; Yu et al., 1997b). Nitric oxide (NO) is one of the candidates for the mediation of this transsynaptic action (Kosior-Korzecka and Bobowiec ,2006; Reynoso et al., 2007; Watanobe and Schioth, 2001; Yu et al., 1997b).

The synthesis of NO depends on the dynamic regulation of the nitric oxide synthase (NOS) enzyme. There are three genetically different isoforms of NOS that account for NO production: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS) (Bredt and Snyder, 1994). The neuronal isoform constitutes the predominant source of NO in neurons and synapses (Zhou and Zhu, 2009). The activity of nNOS is regulated by posttranscriptional modifications, such as the phosphorylation of amino acid residues (Parkash et al., 2010). NO has a strong influence on several neuronal circuits, including circuits in the neuroendocrine system (Prast and Philippu, 2001). NO-containing neurons are widely distributed within the central nervous system, including regions related to the control of reproduction and sexual behavior (Zhou and Zhu, 2009). The reproductive system is influenced by NO (Bellefontaine et al., 2011; Gyurko et al., 2002), and some neurons express nNOS around the GnRH neurons in the medial preoptic area (MPOA) (Gyurko et al., 2002; Herbison et al., 1996). Some researchers have demonstrated that leptin acts on the reproductive axis, partly through NO (Reynoso et al., 2007; Watanobe and Schioth, 2001; Yu et al., 1997b).

Estrogen may modulate the secretion of leptin from adipocytes and the expression of the leptin receptor (Ob-R) (Clegg et al., 2006). Furthermore, the estrogen and leptin receptors are colocalized in neurons within the areas known to coordinate metabolism and gonadal function, such as the arcuate nucleus (ARC), ventromedial hypothalamus (VMH), and MPOA (Diano et al., 1998); this colocalization suggests an interaction between estrogen and leptin in the brain (Gao and Horvath, 2008). Estrogen may also modulate the content of nNOS in the hypothalamus, MPOA and limbic system (Moreno and Franci 2004; Sica et al., 2009). Taken together, these data suggest that leptin, estrogen and NO may interact.

The aim of this work was to investigate whether an interaction between estrogen and the nitric oxide system in the MPOA and the medial basal hypothalamus (MBH) could mediate the action of leptin on gonadotropin and prolactin (PRL) secretion. Thus, we assessed the following: whether estrogen modulates a possible NO-mediated action of leptin in the MPOA and the medial basal hypothalamus (MBH) on gonadotropin and prolactin (PRL) secretion in proestrus; whether the blockage of estrogen action changes the mRNA/protein expression of nNOS and the protein expression of the phosphorylated form of nNOS (pnNOS) in the MPOA and the MBH; and whether the blockage of estrogen action changes the mRNA expression of OB-R and/or the activation of STAT-3 by leptin in these brain areas.

2. Results

Our results demonstrated that 3 μg and 10 μg of leptin increased the secretion of LH and PRL, respectively, on the afternoon of proestrus. No leptin dose changed the FSH concentration during the same period. However, treatment with TMX reduced the surges of LH, FSH and PRL during the proestrus afternoon. This response was not changed by i.c.v. leptin (Fig. 1).

The microinjection of 3 μg of leptin at 11:00 a.m. on the day of proestrus increased the protein levels of pnNOS, and 10 μg of leptin decreased the mRNA (56%) and protein expression of nNOS and pnNOS protein in the MPOA (Figs. 2 and 4). In addition, treatment with TMX during metestrus and diestrus decreased the mRNA expression (53%) of nNOS and the phosphorylation of nNOS in the MPOA; 3 μg of leptin in animals treated with TMX restored the pnNOS protein level to the level in animals treated with oil plus saline, but did not restore the nNOS mRNA level. However, TMX did not reduce the protein expression of nNOS in this area except in the animals that also received an i.c.v. microinjection of 10 μg of