Changes in the Sensitivity of Gonadotrophin Axis to Leptin During Sexual Maturation in Female Rats

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Abstract

OBJECTIVES: The aim of the present paper was to determine the sensitivity of the GnRH-LH axis to leptin administration during sexual maturation in female rats.

METHODS: For this purpose the hypothalamic concentration of GnRH, the pituitary content and the plasmatic levels of LH were determined in prepubertal (15 days of age) and peripubertal female rats (30 days of age), treated with leptin at a dose of 30 µg/kg. i.p. in a single injection, 90 min before sacrifice.

RESULTS: The results indicate that leptin significantly increased the GnRH concentration at 15 days of age (p < 0.01). At 30 days of age the hormone did not significantly modify the hypothalamic GnRH content. Leptin increased the pituitary LH levels, both in prepubertal and peripubertal rats. Nevertheless, while the increase at 15 days of age was around 180%, in peripubertal rats it was about 51.2%. In spite that leptin significantly increased LH plasmatic levels at both ages (p < 0.01), in rats of 15 days of age leptin increased LH in about 244%, at 30 days of age this increase was only about 102%.

CONCLUSION: These results clearly demonstrated that leptin has stimulatory effect on gonadotrophin axis been higher in prepubertal than in peripubertal rats. On these basis, and on the results of previous papers, (in which it has been demonstrated that the hypothalamic control of gonadotrophins by neurotransmitters and neuromodulators also showed qualitative and quantitative changes during sexual maturation), it is proposed that these differences are connected with the maturation of the neuroendocrine mechanisms involved in the regulatory action of leptin on the gonadotrophins axis.
Introduction

For many years considerable efforts have been devoted to the study of the mechanisms responsible for the onset of puberty. There is general agreement that the increase in the activity of hypothalamic GnRH neurones initiates the onset of puberty. This increased activity reflects primarily in the occurrence of the GnRH pulses [1].

The onset of puberty implies the change from a prepubertal type of gonadotrophin secretion, characterized by a low activity of GnRH neurones, to an adult pattern of gonadotrophin secretion, with phasic and synchronous activation of GnRH neurones resulting in GnRH pulses. Since the activity of neurons in these structures is controlled by neurotransmitters and neuromodulators [2] it is clear that these substances participate in the changes of activity of GnRH neurones from prepubertal to adult rats. Moreover, qualitative and quantitative differences between prepubertal, peripubertal and adult rats in the effects of these neurotransmitters on the activity of GnRH neurons have been described [3, 4, 5, 6]. It has been proposed that the onset of puberty implicates modifications in the sensitivity of this axis to the different neurotransmitter systems involved in its regulation and that central receptors mechanisms could be engaged in these modifications [7].

Leptin, a protein hormone secreted by adiposity appears to be bound to the different neuroendocrine processes involved in reproduction [8] as well as in the onset of puberty [9, 10, 11, 12]. In humans, circulating leptin levels increases in children of both sexes before the puberty rise of reproductive hormones. It has been proposed that leptin could initiate the neuroendocrine process involved in the onset of puberty since its administration into immature mice accelerated vaginal opening, the appearance of estrus and the initiation of estrous cycle [12]. It is important to emphasized that endogenous leptin is able to maintain the LH pulsatility as demonstrated in adult female rats that decreased this parameter as showed an impairment of estrous cycles when treated with leptin antiserum [13].

Taking into account that the sensitivity of GnRH to neurotransmitters and neuromodulators changes during the different steps of prepubertal stages until the onset of puberty, the aim of the present paper was to determine the sensitivity of the GnRH - LH axis to leptin administration in prepubertal (15 days of age) and peripubertal rats (30 days of age).

For this purpose the hypothalamic content of GnRH, the pituitary concentration and the plasmatic levels of LH were determined in prepubertal (15 days of age) and peripubertal female rats (30 days of age).

Material and Methods

Animals: Prepubertal (15 days of age) and peripubertal (30 days old) female rats from the Department of Physiology of the Faculty of Medicine, University of Buenos Aires, were used. They had been kept in a light and temperature controlled environment (lights on from 07.00 to 19.00 h, T 22°C).

Drugs: Leptin (Sigma Chemical Co) was used at a dose of 30 µg/kg i.p. in a single injection, 90 min before sacrifice. In previous experiments the minimal doses and schedule of administration of leptin that induces the maximal response on LH and FSH levels were determined.

Blood collection: Animals were killed by decapitation at 16.00–17.00 hs. Blood was collected from the trunk and allowed to clot at 4°C. Samples were centrifuged for 10 min at 2500 rpm and the sera separated and stored frozen until LH assay was carried out. Serum LH was determined in duplicate, using a double antibody radioimmunoassay technique. The reagents for it were kindly provided by the NIAMDD rat pituitary program. Intra and interassay coefficients of variation were 8% and 10%, respectively.

Tissue processing: Anterior-mediobasal hypothalamic were weighed to 0.01 mg, and then homogenized in 20 µl hydrochloric acid 0.1 N per mg of tissue, using an all glass Potter homogenizer refrigerated with ice. Similar samples of hypothalamus were used. The homogenate was centrifuged at 13000 rpm during 2 minutes, and the supernatant obtained was diluted 1:1 and then frozen at −80°C until GnRH assay by RIA. The results were expressed in nanograms per mg of tissue.

Pituitary glands were dissected and the anterior pituitary separated from the neural lobe, weighed and homogenized in 1 ml isotonic phosphate buffer (disodium phosphate 0.005 M, monosodium phosphate 0.005 M, sodium chloride 0.15 M) pH 7.5. The supernatant was diluted 1:150 in buffer and kept at −20°C until gonadotrophin determination by RIA.

Statistical analysis. The results are expressed as the means ± S.E.M. Significance was assessed by analysis of variance (ANOVA) and Tukey’s multiple range test [14]. Where appropriate, Student’s t-test was used when comparing only two treatments; P < 0.01 was considered significant.

Results

Figure 1 shows the hypothalamic content of GnRH in prepubertal (15 days of age) and peripubertal female rats (30 days old). As it can be seen leptin significantly increased the GnRH concentration at 15 days of age. At 30 days of age the hormone did not significantly modified the hypothalamic GnRH content.
As showed in Figure 2 leptin increased the LH content in both prepubertal and peripubertal rats. Nevertheless while the increase at 15 days of age was around 180% in peripubertal rats was about 51.2%.

Figure 3 shows the plasmatic modifications of LH induced by leptin in both groups. Leptin significantly increased LH plasmatic levels at both ages (p < 0.01), in rats of 15 days of age leptin increased LH in about 244%, while at 30 days of age this increases was about 102%.

**Discussion**

Several recent evidences showed that leptin, the hormone secreted by adiposity, acts as metabolic signals to the Central Nervous System regulating the reproductive functions in humans and in animals. Increased gonadotrophin secretion consistently occurs as results of leptin treatment in ob/ob mice and undernourished animals [15,16] Leptin treatment advances the onset of puberty, which is known to be controlled by hypothalamic release of GnRH. Hypothalamic GnRH release has long been recognized to be deficient in ob/ob mice [15]. Leptin stimulates LH release in estrogen-primed rats indicating stimulatory effects at the levels of the anterior pituitary gland and/or hypothalamus [17]. Culture median eminence – arcuate explants from rats release GnRH in response to leptin [17]. In women, extremes in body mass either obesity or anorexia, are associated with anovulation and infertility. [18,19,20]. Pubertal maturation in food restricted and leptin treated rats is significantly advanced relative to animals fed comparably but not receiving leptin [21] Leptin has been also implicated in the neuroendocrine processes involved in sexual development. In human, circulating leptin increases in children of both sexes before the pubertal rise of reproductive hormones. It was reported two severely obese adults with congenital leptin deficiency that did not undergo pubertal development and had low plasmatic gonadotrophin concentration [22]. Moreover, the treatment with recombinant leptin in a child with congenital leptin deficiency began the nocturnal pulsatile pattern of gonadotrophin secretion consistent with early puberty [23]. In mice leptin treatment accelerated the onset of puberty as measured by vaginal opening, the appearance of estrus and initiation of sexual cycles and
reproductive capacity. [9, 10, 11, 12]. On these basis, it is clear that leptin appear to be one of the neuroendocrine signals involved in the onset of puberty.

Sexual maturation in the female rats implicates qualitative and quantitative modifications of the effect of neurotransmitters on GnRH neurons related to the maturation of different receptors mechanism and/or new interneuronal connections [7]. For instance, serotonin increases gonadotrophin secretion in prepubertal female rats until 20 days of age and this effect disappears in peripubertal rats (30 days of age) [6]. The different stages of maturation of the central nervous system is also responsible for the different effects of sexual hormones on the reproductive axis during prepubertal (from birth to 20 days of age) and peripubertal stage (around 30 days of age) [6].

The results of the present paper demonstrated that leptin has also qualitative and quantitative differences on the reproductive axis in prepubertal and peripubertal female rats. The administration of the adiposity hormone significantly increased hypothalamic GnRH content in prepubertal rats but did not significantly modify this hypothalamic hormone in peripubertal rats. These different effects were accompanied by qualitative differences in the pituitary content and plasmatic concentration of LH. Leptin increased both the pituitary content and the plasmatic levels in prepubertal and peripubertal rats. Nevertheless in both situations the stimulatory effects were significantly higher in prepubertal (15 days of age) than in peripubertal rats (30 days of age) (pituitary content 180%, vs 51.2%; plasmatic concentration 244% vs 102%).

These results clearly demonstrated that leptin has a higher stimulatory effect on gonadotrophin axis in prepubertal than in peripubertal rats. It is difficult to explain these results on the basis of the present knowledge of leptin effects on reproductive axis. The possibility arises that leptin acts during prepubertal stage on this axis, modulating the development of the different neuroendocrine mechanisms involved in the onset of puberty. Once these mechanisms are set, the sensitivity of the hypothalamic-pituitary-gonadal axis to leptin decreases. This hypothesis could be sustained by experimental evidences obtained in rats and in humans. In rats the development of male (tonic) or female (cyclic) type of hypothalamic control of gonadotrophin secretion takes place during the first days of life and is connected with the presence or absence of gonadal steroid during this period of prepubertal stage. [24]. The development of this hypothalamic mechanism of control can be modified either by administration or suppression of sexual hormones until 10 days of life; after this age sexual hormones are unable to modify this mechanism. On the other hand the fact that in humans boys leptin decreased after the development of puberty [10] given additional supported to our hypothesis.

In summary, our results demonstrated that in female rats the sensitivity of the hypothalamic-gonadal axis to leptin decreases before the onset of puberty (peripubertal rats) compared with prepubertal rats. It is proposed that this change in the sensitivity is connected with the maturation of the neuroendocrine mechanisms involved in the onset of puberty. It is clear that more experimental evidences are needed before a conclusion can be reached in this respect.

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