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Original

Leptin regulates gonadotropins and steroid receptors in the rats ovary

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Abstract

The leptin hormone is important to satiety and an important link between the nutritional status and reproductive processes. Owing to the contradictory effects of leptin on the ovary and the failure to clarify the precise mechanism by which leptin affects the ovary, our aim was to contribute to evaluation if leptin can directly regulate the gene expression of leptin itself and its receptors, and the expression of several genes related to the ovary function by a model of tissue culture. Ovaries from Wistar dams were used at 90 days of age and were submitted to medium with presence and absence of leptin. The results can demonstrate that leptin regulates gonadotropins and steroid receptors, which could suggest that the ovarian leptin role could be secondary to the changes in these receptorsê expression in rats.

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LA LEPTINA REGULA LAS GONADOTROPINAS Y LOS RECEPTORES DE ESTEROIDES EN EL OVARIO DE LAS RATAS

Resumen

La hormona leptina es importante en la sensación de la saciedad y un vínculo importante entre el estado nutricional y los procesos reproductivos. Debido a los efectos contradictorios de la leptina en el ovario y la falta de esclarecimiento del mecanismo exacto por el cual la leptina afecta el ovario, nuestro objetivo es contribuir a la evaluación si la leptina puede regular directamente la expresión del gen de la leptina sí mismo y sus receptores, y la expresión de varios genes relacionados con la función del ovario por un modelo de cultivo de tejidos. Los ovarios de las presas Wistar fueron usadas en los 90 días de edad y se sometieron a medio con presencia y ausencia de leptina. Los resultados pueden mostrar que la leptina regula las gonadotropinas y los receptores de esteroides, lo que podría sugerir que la función ovárica de la leptina podría ser secundario a los cambios en la expresión de sus receptores en ratas.

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Palabras clave: *Ovario. Leptina. Gonadotropinas. Fertilidad. Nutrición.*

Abbreviations

Ob-Rb: Long isoform of leptin receptor.
Ob-Ra, Ob-RcÖOb-Rf: Short isoforms of leptin receptor.
FSH: Follicle stimulating hormone.
IGF-I: Insulin-like growth factor type 1.
BCL-2: B-cell lymphoma 2.
Bax: Bcl-2-associated X protein.
DMEM: Dulbecco's Modified Eagle Medium.
ER: Estrogen receptor alpha.
ER: Estrogen receptor beta.
AR: Androgen receptor.
LHR: Luteinizing hormone receptor.
FSHR: Follicle stimulating hormone receptor.
cDNA: Complementary deoxyribonucleic acid.
RT: Reverse transcriptase.
PCR: Polymerase chain reaction.

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Introduction

Leptin, the product of obese gene, is an important satiety hormone[1]. Now it is known as an important link between the nutritional status and reproductive processes[2]. Although leptin is mainly produced and secreted to the bloodstream by white adipocytes, this is not the only potential source of the hormone. Placenta, gastric mucosa, bone marrow, mammary epithelium, skeletal muscle, pituitary, hypothalamus, bone, prostate, testis, uterus and ovaries have also been shown to be able to produce small amounts of leptin[3].

The OB-R is a transmembrane receptor. Several isoforms of the receptor, resulting from alternative spli-

cing, convey differing biological activity and are involved in mediating leptin's actions in the brain and peripheral organs. The Ob-Rb is expressed abundantly in the hypothalamic arcuate, ventromedial, and dorsomedial nuclei and is the predominant signaling form of the receptor. The Ob-Ra, Ob-Rc...Ob-Rf are distributed in almost all peripheral tissues, including theca and granulosa cells and oocytes in the ovary[3].

The effects of leptin on the ovary are contradictory and both stimulatory and inhibitory actions on ovarian function have been described. As negative actions we can mention: (i) leptin can directly suppress estrogen production stimulated by FSH and IGF-I in ovarian granulosa cells of rat[4], (ii) acute administration of leptin to immature gonadotrophin-primed rats inhibits ovulation[5] and (iii) in vivo, leptin deficiency (ob/ob animals) is associated with delayed vaginal opening, subnormal uterine weight and altered folliculogenesis (reduced number of follicles and evidence of increased granulosa cell apoptosis and follicular atresia)[6]. Likewise, leptin is able to produce some positive effects: (i) leptin accelerates the onset of puberty in rodents[7], (ii) leptin induces ovulation in eCG/hCG-primed rats[8], (iii) leptin stimulates aromatase protein expression and activity[9], (iv) leptin increases insulin and gonadotropin-stimulated follicular progesterone, testosterone and estradiol production in a dose-dependent manner[10] and (v) leptin accelerates follicular maturation by attenuating follicular atresia and increasing the ratio of BCL-2/Bax[7].

Nevertheless, the precise mechanism by which leptin affects the ovary is unknown. In this paper we aimed to evaluate if leptin can directly regulate the gene expression of leptin itself and its receptors, the expression of several genes related to the ovary function such as estrogen, androgen, follicle stimulating hormone, luteineizing hormone receptors and aromatase.

Methods

The study design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro. Six Wistar female rats were kept under controlled conditions and free access to food and water until adult age. At the proestrus stage of the oestrous cycle, animals were anesthetized with thiopental (0.2 mg/g body weight, ip). The ovaries were excised and maintained in DMEM supplemented with 10% fetal bovine serum and 1 ng/mL of gentamycin for one hour. Ovaries were then incubated with the same medium above described in a final volume of 5mL in either the presence (L group; left ovary) or the absence (C group; right ovary) of human recombinant leptin (16 ng/mL DEMEM) at 37 ºC in a humidified atmosphere (5%CO2:95%O2) for 3h. Both optimal concentration and time response to leptin was previously standardized (data not showed). At the end of the incubation time, RNA was extracted by using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Then 1 µg RNA was used in a 20-µL cDNA reaction using oligo-dT and the superscript III cDNA synthesis system (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The gene expression of leptin and their OBRa and OBRb isoforms receptors, aromatase enzyme and the ER_ and ERβ, AR, LHR FSHR were evaluated by Real Time Polymerase Chain Reaction in triplicates. β actin gene was used as internal control.

The PCR primers used were the following: Leptin sense (5ê-gacatttcacacacgcagtc-3ê) antisense (3êgaggaggtctcgcaggtt5ê); OBRa sense (5'-taccaacctcccaacagtcc-3') antisense (3'agcatatgccccaactgaac5'); OBRb sense (5'-ctgaagaaaatcacggggaa-3') antisense (3'gaacagacagtgagctggg5'); Aromatase sense (5'-ctccctgaagacacacagca-3') antisense (3'gggttcagcatttccaaaaa5'); AR sense (5'-ggcaaaggcactgaagagac-3') antisense (3'cccagagctacctgcttcac5'); ER sense (5'-gaagctgaaccacccaatgt-3') antisense (3'caatcatgtgcaccagttcc5'); ER sense (5'-cctgcagggagaagagtttg-3') antisense (3'atcttg tccaggactcggtg5'); LHR sense (5'-atggccatcctcatcttcac-3') antisense (3'tggattggcacaagaattga5'); FSHR sense (5'-ctcatcaagcgacaccaa-3') antisense (3'ggaaaggattggcacaag5') and actin sense (5'-ctccggcatgtgcaa-3') antisense (3'- cccaccatcacaccct-5ê).

The data were reported as mean ± SEM. Statistical significance of experimental observations was determined by Student t test. The level of significance was set at P<.05. Using an in vitro system tissue culture, six ovaries from rats at the pro estrus stage were treated with 16ng/ml of leptin for 3h. The gene expression was evaluated by RT-Real time PCR and the results show that leptin upregulated the expression of OBRb and OBRa while downregulated the expression of leptin itself, Er_, Er_, AR, aromatase, FSHR, LHR (fig. 1).

Results

Our results showed that leptin affects the ovary by directly altering the expression of several important genes related to the ovarian function. The addition of leptin to the culture medium resulted in an increase in the gene expression of leptin receptors isoforms and a decrease in the gene expression of leptin itself. Recently we have published, using a similar in vitro model, that in the ventral prostate lobe of adult rats leptin is also capable of regulating the expression of its receptors isoforms and the hormone itself[11] but since the results are different, the leptin regulation seems to be tissue specific. Our results are not in agreement with those of Duggal et al[12]. in which the authors evaluated the expression of the ovarian leptin receptors and the serum levels of the hormone throughout the estral cycle. It was shown that the pro-estrus stage presents the highest leptin serum levels and the lowest expression of OBRa and OBRb. However we can not forget that in an in vitro model some regulators factors are missed and this could account for the different responses.

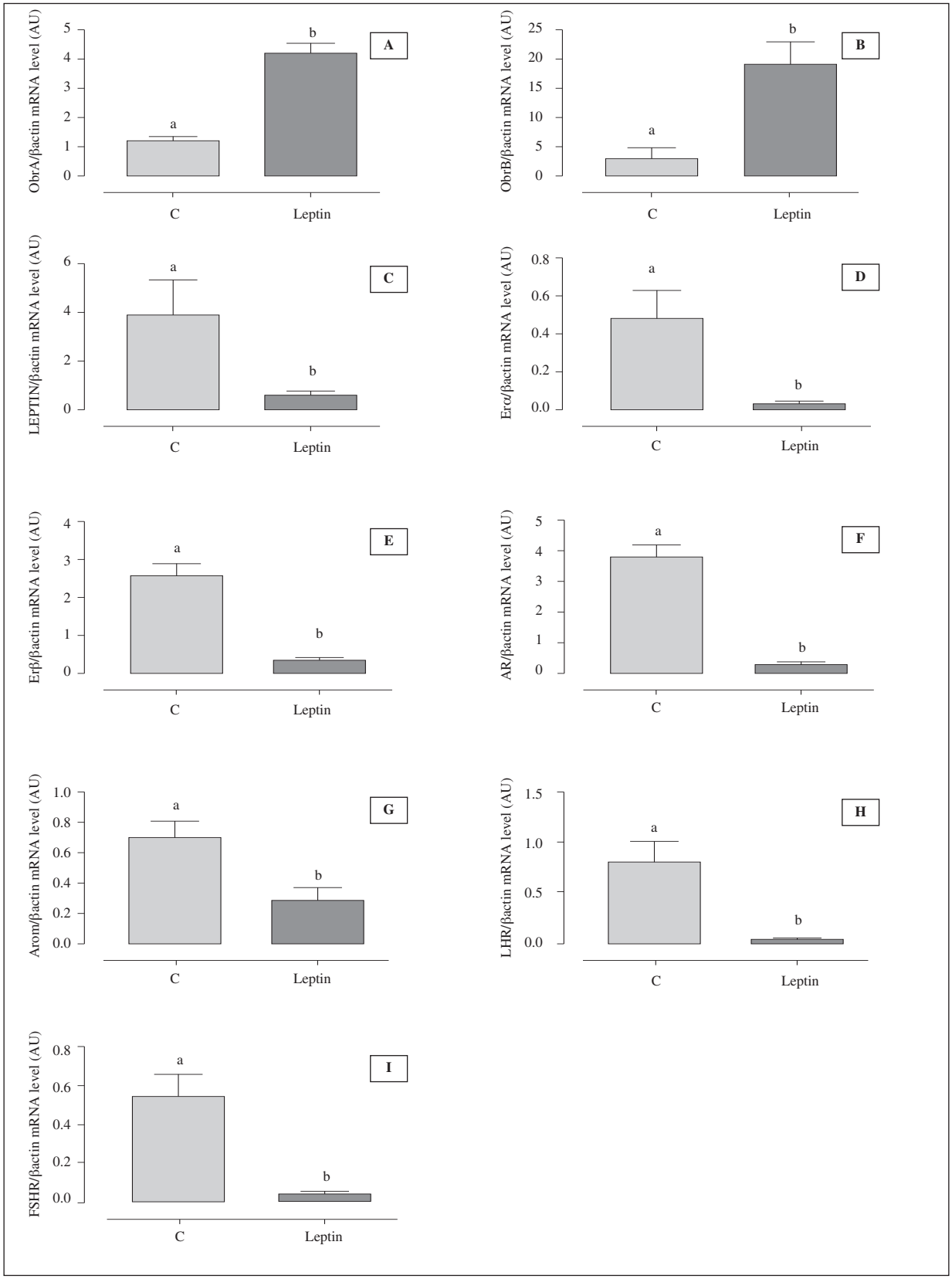

Fig. 1.—Gene expression of short isoform leptin receptor OBRa (A), long isoform leptin receptor (ObRb), (B) leptin (C), estrogen receptor (D), estrogen receptor (E), androgen receptor (AR) (F), aromatase (G), luteinizing hormone receptor (H) and follicle stimulating hormone receptor (I) in the rat ovary after leptin (16 ng/mL) treatment for 3 hours (L) or not (C). actin was used as an internal control. Primer sequences are listed in the text. Data are represented as mean±SEM of 6 tissues. Different letters mean statistically difference.

Gonadotropins are obligatory for the maintenance and development of growing follicles. FSH binds exclusively to FSH receptors in granulosa cells, whereas LH binds its cognate receptors in thecal cells. This two-cell, two-gonadotropin-mediated control of follicular growth appears to ensure continuous growth of small follicles at all stages of the reproductive cycle and pregnancy[13]. So, it makes sense that a decrease in both FSHR and LHR expression would decrease the ovarian response to these gonadotropins and affects the follicle growth and fertility.

Discussion

Sex steroids play important roles in the growth and differentiation of reproductive tissues and in the maintenance of fertility. Androgens, primarily androstenedione and testosterone, are produced by theca cells in response to LH. Androgens act via receptors AR localised to granulosa cells, stromal cells, human theca cells and more recently, to oocytes. In the early stages of folliculogenesis, androgens appear to promote follicular growth by enhancing follicular recruitment[14].

Apart from effects on growth, androgens have been shown to enhance the follicle stimulating hormone (FSH)-mediated differentiation of granulosa cells, as indicated by an increase in progesterone and oestradiol production and to play roles in oocyte maturation[15].

One of the most important roles played by androgens in the ovary is in the synthesis of oestrogen. Androgens serve as substrates of P450 aromatase, which mediates the conversion to oestrogens in the granulosa cells, in response to FSH[16]. A decrease in the expression of this enzyme could affect the ovarian function by increasing testosterone while decreasing estrogen concentration in the tissue.

Oestrogens signal via receptors (ER) of which there are two forms, ER_ and ER_[17], with ER_ being the predominant form in the ovary[18]. Distinct roles for each receptor were identified: ER_ inhibited ovulation, most likely via an effect on the hypothalamo-pituitary axis and uterine growth; while ER_ stimulated follicular growth, decreased atresia, induced the expression of specific genes and enhanced the number of oocytes released following ovulation induction[14].

Oestrogen plays a pivotal role as an intrafollicular modulator, facilitating the differentiation of granulosa cells including the induction of receptor systems for FSH, LH and prolactin and it can influence post-receptor mechanisms. Oestrogen controls granulosa cell gap junction formation permiting transfer of nutrients and cytokines to and from the granulosa cells and developing oocytes[19,20].

Conclusion

Considering the important effects of androgens and estrogens in the ovary we can assume that any factor that decreases the expression of these hormones receptors would affect the ovarian function and fertility. We believe this is the first time that a direct effect of leptin regulating gonadotropins and steroid receptors are shown, suggesting that the ovarian leptin role could be secondary to the changes in these receptors expression.

Leptin upregulates its receptors and play important roles in the ovary. The impact of this hormone on ovarian function is determined by the repression or induction of relevant regulatory genes. From the data presented in this paper, it is clear that by downregulating steroids and gonadotropins receptors genes leptin is important for fertility. In absence, or in cases of leptin excess, ovarian function and subsequently fertility, is compromised.

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