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Effect of Leptin on LH Levels and Hypothalamic Release of GnRH. Its Relationship with the Hypothalamic Neurotransmitter Amino Acids System in Adult Male Rats

Osvaldo J. Ponzo, Roxana Reynoso, Dora Rondina, Berta Szwarcfarb, Silvia Carbone, Pablo Scacchi,[†] and Jaime A. Moguilevsky[*]

Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires and Universidad Favaloro, Buenos Aires, Argentina

ABSTRACT

The objective of the present paper was to determine the effect of leptin on the reproductive axis in adult male rats, as well as the hypothalamic mechanisms involved in this effect. For this purpose, we studied the in vivo effect of leptin in adult male rats on serum LH levels, and the in vitro effect on hypothalamic GnRH and amino acid neurotrasmitter release. For in vivo experiments, animals were injected i.p. with leptin at a dose of 30, 100 and 300 μg/kg. In the in vitro experiments, hypothalamic samples were incubated for 60 min in Earle's medium with leptin: 10^{-9}, 10^{-10} and 10^{-12} M for GnRH determination, and 10^{-10} M for amino acids evaluation. Finally, we studied the effect of the lowest effective leptin dose on plasma LH levels in peripubertal male rats to compare the effect between this group and adults. Leptin induces significant decreases of serum LH levels with the different studied doses ($p < 0.01$ vs. control) in adult male rats, while in peripubertal male rats, it induced a significant ($p < 0.01$ vs.

[†]Established Investigator. National Research Council. Buenos Aires, Argentina.
[*]Correspondence: Dr. Jaime A. Moguilevsky, Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, (1121) Buenos Aires, Argentina; Fax: 54-11-4981-9921; E-mail: jmoguile@fmed.uba.ar.

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control) increment in serum LH levels. On the other hand, in vitro leptin in adult male rats, significantly decreases GnRH release as well as the hypothalamic release of glutamate (GLU). In contrast, leptin increased the GABA release by this hypothalamus in these animals. These results indicate that leptin has an inhibitory effect on the GnRH–LH axis in adult male rats and this effect appears to be connected with an inhibition of hypothalamic release of GLU (the excitatory amino acid) and a stimulatory effect on GABA release (the inhibitory amino acid). On the other hand, in peripubertal male rats, leptin showed a stimulatory effect.

Key Words: Leptin; GABA; Excitatory amino acids; GnRH; LH; Male rats.

INTRODUCTION

Leptin, a peptide hormone secreted by adipocytes, appears to be linked to the different neuroendocrine processes involved in reproduction as well as in the onset of puberty (1–5). It has been proposed that leptin could initiate the neuroendocrine processes involved in the onset of puberty, since its administration into immature mice accelerates vaginal opening, the appearance of estrus and the initiation of the estrus cycle (2).

Previous reports of our laboratories indicate that the adipocyte hormone is able to stimulate the reproductive axis during the prepubertal stage in female rats (6,7). These reports also demonstrated that leptin acts at hypothalamic levels modifying the amino acid neurotransmitter systems that control the GnRH secretion.

There are several pieces of evidence that in women and female animals leptin not only acts on the onset of puberty, but also in the regularity of sexual cycles as well as in reproductive function (8–12).

The role of leptin in adult males is not as clear since excitory and inhibitory effects have been reported (13,14). The aim of the present investigation was to study in adult male rats, the effect of leptin treatment on: 1) serum LH levels, 2) the hypothalamic release of GnRH, 3) the hypothalamic release of excitatory and inhibitory amino acid neurotransmitters, which are involved in the control of GnRH secretion (15–17). Also, we investigated the leptin effect on peripubertal male rats to study the effect on serum LH levels to compare this with those in adult rats.

MATERIALS AND METHODS

Animals

Adult Wistar male rats (75 days old) 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). Animals were food ad libitum and had access to the food until the moment of sacrifice. In a last experiment we used peripubertal (30 days old) male rats maintained under the same environmental condition. There were 8 to 10 animals per group in every experiment.

Drugs and Doses

In the in vivo experiments with adult male rats, leptin (Sigma Chemical Co St. Louis, Mo, USA) was administered at doses of 30, 100 and of 300 μg/kg i.p. in a single injection, 60 min before sacrifice. In peripubertal rats, we used only minimal doses (30 ug/kg). Control animals only received the vehicle.

In the in vitro experiments,the adipocyte hormone was added to the incubation medium at 10^{-9}, 10^{-10} and 10^{-12} M for the GnRH release study, and 10^{-10} for amino acid release study.

Tissue Processing

Animals were killed by decapitation at 14.00–16.00 h. After decapitation, serum was collected for LH determinations. In adult animals, the brains were rapidly removed, and the hypothalamus dissected out with a single razor blade and weighed. The hypothalamic samples containing the anterior preoptic and medial basal areas (APOA-MBH) were dissected with the help of a stereomicroscope. The hypothalamic samples were bordered laterally by the hypothalamic sulci; rostrally, 3 mm anterior to the optic chiasma; caudally, by the mammillary bodies; the depth was 3–4 mm. The thicknesseach sample was less than 2 mm. This tissue included the median eminence.

After dissection, the APOA-MBH were put into plastic chambers (similar hypothalamic samples were used in each incubation) containing 300 μl of Earle's medium with glucose (1 mg/ml) and bacitracin (20 mM). The pH was adjusted to 7.4. Each chamber was incubated in a Dubnoff shaker at 37°C with constant shaking (60 cycles/min) under an atmosphere of 95% O_2, 5% CO_2. After 20 min of pre-incubation period the medium was discarded and fresh medium added. The samples were incubated for 60 min, the medium was collected (basal samples) and immediately new medium with leptin was added for another 60 min. The collected samples were immediately frozen at −80°C for GnRH determination by RIA and amino acids by HPLC. The viability of tissue at the end of incubation was determined by addition of KCl 56 mM in the medium, eliciting an increase in GnRH and amino acid release.

LH and GnRH Determinations

LH was determined in serum using a double antibody radioimmunoassay technique. The material for these assays was kindly provided by the NIAMDD rat pituitary program. A NIDDK-rLH-RP-3 reference preparation was employed. All samples were run in the same assays. Detection limit was 0.5 ng/ml and the intra-assay coefficient of variation was 5.7%. Results were expressed as ng/ml serum. Gn-RH determination was performed on samples of the incubation medium by RIA and the results were expressed as pg/mg tissue. All samples were run in the same assays. Detection limit was 0.2 pg/100 ul.

Amino Acid Determinations

The concentrations of GLU and GABA were determined by HPLC after derivatization with phenylisothiocyanate and UV detection at 254 nm, as previously

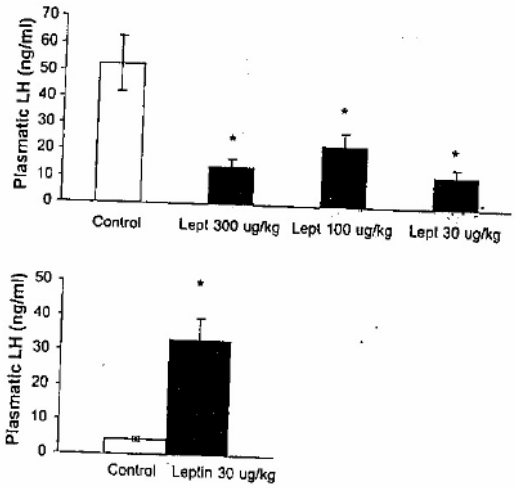

Figure 1. Effect of leptin on serum LH levels in adult and peripubertal male rats. Each column represent the mean ± SEM of 8–10 determinations. *$p < 0.01$ vs. control.

described (18). The drugs used did not interfere in the derivatization process. Mean inter- and intra-assay coefficients of variation were 4.0 and 8.0%, respectively. The detection limit was 10 pM for GLU and 5 pM for GABA. The mobile phase consisted of 0.57 M sodium acetate buffer (pH 6.5) containing 10% acetonitrile (Sintorgan, Buenos Aires, Argentina). Amino acid standards, GLU and GABA were obtained from Sigma Chemical Co St. Louis, Mo, U.S.A. Results are expressed as pM/100 µl medium.

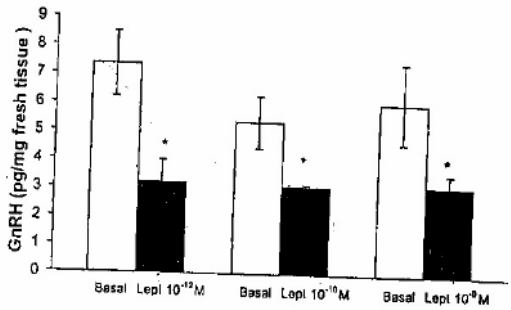

Figure 2. Effect of leptin on GnRH release by APOA-MBH in adult male rats. Each column represent the mean ± SEM of 8–10 determinations. *$p < 0.01$ vs. respective basal.

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 (19). Where appropriate, Student's *t* test was used when comparing only two treatments; $p < 0.01$ was considered significant.

RESULTS

Figure 1 shows the effect of in vivo leptin treatment on the serum LH levels. As can be seen a significant ($p < 0.01$) decrease of LH was observed in serum with the different studied doses. (*Control:* 52.45 ± 10.1, *Leptin 30 ug/kg:* 11.8 ± 3.2, *Leptin*

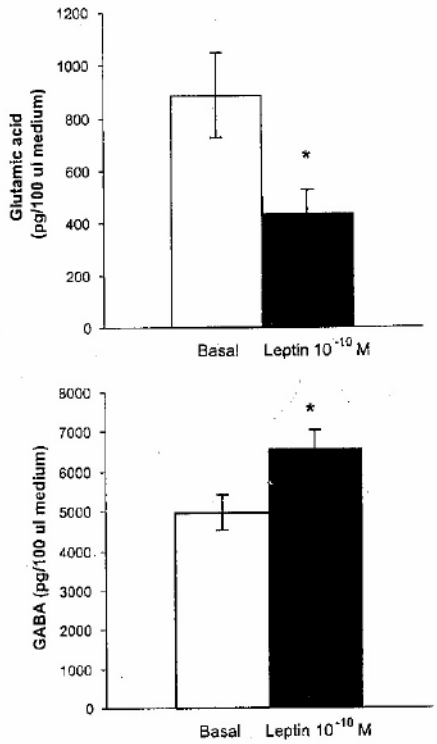

Figure 3. Effect of leptin on hypothalamic release of Glutamate and GABA in adult male rats. Each column represents the mean ± S.E.M of 8–10 determinations. *$p < 0.01$ vs. respective basal.

100 ug/Kg: 22.71 ± 5.1, *Leptin 300 ug/kg:* 14.33 ± 2.9 ng/ml). In the group of the peripubertal rats, leptin produced the contrary effect to that seen with adult rats, increasing the serum LH levels (*Control:* 4.3 ± 0.62, *Leptin 30 ug/kg:* 33.02 ± 6.37 ng/ml).

Figure 2 shows the in vitro effect of leptin on the hypothalamic release of GnRH in adult male rats. All the concentrations significantly decrease GnRH release by APO-AMBH. (*Basal:* 7.36 ± 1.14, *Leptin* 10^{-12}*:* 3.2 ± 0.8; *Basal:* 5.35 ± 0.92, *Leptin* 10^{-10}*:* 3.11 ± 0.08; *Basal:* 6.0 ± 1.4, *Leptin* 10^{-9}*:* 3.18 ± 0.4 pg/mg fresh tissue respectively).

In Fig. 3 the effect of leptin (10^{-10} M) on the hypothalamic release of GLU and GABA in adult rats is represented. As can be seen, the adipocyte hormone induced a significant ($p < 0.01$) decrease in the GLU release by the hypothalamus (*Basal:* 886.1 ± 177.9, *Leptin:* 432.2 ± 29 pM/100 µl medium). In another study leptin induced a significant increase ($p < 0.01$) in hypothalamic GABA release (*Basal:* 4963.1 ± 291.4, *Leptin:* 6538.1 ± 364.8 pM/100 µl medium).

DISCUSSION

Leptin, the adypocyte-derived hormone, plays a key role in body weight homeostasis and as a relevant neuroendocrine mediator in the function of reproductive axis (2–5,8). Thus, leptin plays a role in the regulation of female pubertal development and fertility. Its administration into female immature mice accelerates the vaginal opening and the initiation of estrous cycle (2,3). In women, leptin deficiency induces alterations in sexual cycles and amenorrhea (8). All of the evidence indicates a leptin stimulatory effect on the gonadal axis in females.

The contributions of leptin to the function of the male reproductive system has been less clear and this could be connected with the gender differences in leptin physiology. For instance, serum leptin levels are clearly different in men and in women: the latter have a higher leptin level, a difference already observable in children. In humans, circulating leptin levels increase in children of both sexes before the pubertal rise of reproductive hormones. While in both sexes leptin increase during pubertal development, in girls the hormone maintain its increase to adult levels, but decreases in boys toward the lower adult male levels (9).

The present results show that leptin administration to adult male rats significantly decrease plasma levels of LH. Leptin in vitro also decreases the hypothalamic release of GnRH. These results clearly demonstrate that leptin has an inhibitory effect on the hypothalamic-gonadal axis in adult male rats. In this way, it has been also described that leptin inhibits LH and FSH secretion in incubated hemipituitaries (20). Moreover, in vivo study showed that adult male ewes injected i.c.v. with leptin had a reduction of LH frequency pulsatility (13).

These data indicate an inhibitory effect of leptin on the reproductive axis in adult male rats, that is carried out at different levels of the hypothalamic–pituitary axis. In this respect, it is interesting to note that chronic hyperleptinemia are associated with a hypothalamic hypogonadism (21).

This present results are in discordance with the stimulatory effect seen in results presented by Finn et al. (14), who showed that leptin infusion has a stimulatory effect on monkey reproductive axis at second days of fasting. Also in adult male rats a stimulatory effect on reproductive axis has been described (22). Nevertheless, these

studies cannot be compared with the results of the present report, since there were performed in fasting conditions which appear to modify substantially the effect of leptin on the gonadal axis.

A similar situation is seen in the ob/ob animal models, whose leptin production is absent. In this situation exogenous leptin administration is also able to stimulate the reproductive axis (23).

It is interesting to note that this inhibitory effect could not be seen in peripubertal male rats. Moreover, in these animals we could see a stimulatory effect on LH secretion, even with the minor dose used with adults. In this way, we have demonstrated in previous studies that leptin also stimulated LH secretion in peripubertal female rats (6,7). The present data appear to indicate that there are sexual and age (stage of sexual maturation) differences in the effect of leptin on reproductive axis.

The neuroendocrine mechanisms by which the adipocyte hormone stimulates the gonadotrophin axis in peripubertal rats involved the hypothalamic amino acid neurotransmitter system i.e. increasing the activity of excitatory amino acids (GLU or ASP) and decreasing the release of inhibitory one (GABA) (7). The present result demonstrated that the inhibitory effect of leptin also is exerted on hypothalamic neurotransmitter amino acid system, showing an inhibitory effect on GLU and stimulatory on GABA secretion.

According to the present results and previous publications, in adult male rats the effect of leptin on gonadotrophin secretion would be different than those observed in peripubertal rats. In conclusion, the present results indicated that leptin has a inhibitory effect on reproductive axis in adult male rats, contrarily to the stimulatory effect described for peripubertal rats. Further studies are needed to clarify this difference.

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