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Leptin increases luteinizing hormone secretion of fasting female rats

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Summary

Purpose: To investigate whether leptin acts directly on the anterior hypophysis by influencing gonadotropin secretion in vivo. *Materials and Methods:* Cycling female rats were catheterised for frequent blood sampling and were either fasted or allowed free access to food. Stereotactic lesion of the medial preoptic area (MPOA) of the hypothalamus was performed in order to eliminate gonadotropin releasing hormone (GnRH) production. Leptin was administered at a dose of one mg/kg i.v. and blood samples were taken just before leptin administration and then after 30, 60, 90, 120, and 180 minutes. Plasma gonadotropin levels were determined. With completion of sampling, the brains were removed and the localisation of the lesions was verified histologically. *Results:* Leptin at one mg/kg induced an increase in luteinizing hormone (LH) secretion in fasting rats, both in those with a lesion and those with intact medial preoptic area with a peak occurring 90 minutes after infusion. The augmenting effect was more prominent when the hypothalamus was intact. There was no effect in fed animals with or without lesion. Similarly, no effect was observed on follicle stimulating hormone (FSH) levels in any of the experimental groups. *Conclusions:* Leptin acts directly on the hypophysis enhancing LH but not FSH secretion. Nutritional state influences leptin's effect on the hypothalamus and the hypophysis.

Key words: Leptin; Rats; Medial preoptic area; Hypophysis; Gonadotropins.

Introduction

Leptin is a hormone that is mainly produced in fat tissue that influences the reproductive axis [1, 2]. In the infertile, genetically obese ob/ob mice, fertility is restored by leptin administration [3]. Leptin may prevent the effect of fasting on various aspects of reproduction as the onset of puberty [4-9], the length of the menstrual cycle [10, 11], the levels of gonadotropins and gonadal steroids [12], and the pulsatile secretion of the luteinising hormone (LH) in rodents and primates [13, 14].

The mechanism by which leptin influences the reproductive axis is still unclear. There is evidence for an indirect action of leptin on the gonadotropin-releasing hormone (GnRH) neurons, through intermediate neurons that produce neuropeptides as cocaine- and amphetamine-regulated transcript (CART) peptide [15], galanin–like peptide [16], and melanin-concentrating hormone (MCH) [17]. Leptin administration in the arcuate nucleus of the hypothalamus, the site of action of neuropeptide Y (NPY), increases GnRH pulsatility [15]. There is also evidence for a direct action of leptin on the (GnRH) producing neurons. *In vitro*, leptin administration stimulates GnRH release from a GnRH-secreting neuronal cell line [18]. *In vivo,* leptin administration in the medial preoptic area (MPOA) and the median eminence results in an increase of LH release [19]. Leptin may act directly at the level of the anterior hypophysis as well [20]. Leptin receptors have been identified in gonadotropin-producing cells [21] and leptin was found to stimulate LH and FSH release from rat pituitary extracts *in vitro* [12]. In the rat, GnRH-secreting neurons are concentrated in the MPOA of the hypothalamus. Lesion of the above area eliminates GnRH release [22]. Therefore, from all the above *in vitro* [18] and *in vivo* [19] studies, it is evident that leptin increases LH release acting on MPOA and the median eminence, but only *in vitro* investigations [12, 20, 21] suggest that leptin augments LH release acting also directly on the anterior hypophysis.

Main objective of the present study was to investigate *in vivo* whether leptin, when administered in MPOA lesioned female rats can act directly on the anterior hypophysis by affecting gonadotropin secretion, since *in vivo* experiments have the advantage of approaching subject experimentation holistically, as the nature and properties of a chemical or humoral tool cannot be considered independently of the system it is to be tested in.

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Materials and Methods

Experiments have been conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and were approved by the Ethics Committee of the School of Medicine, Aristotle University of Thessaloniki. Attention was paid to minimize pain and discomfort to the animals.

Experimental protocols

Female Wistar rats, weighing 220-240 g, were housed in an environment controlled for temperature, humidity, and light (12:12 h light/dark cycle). Animals had free access to food and water until the beginning of the experiment. Daily vaginal smears were taken to document at least two consecutive four-day oestrus cycles.

The first day of the experiment, rats that were found to be on oestrous were selected. Under ketamine (50 mg/kg) and xylazine (10 mg/kg) anaesthesia, animals were implanted with a jugular vein catheter for frequent blood sampling according to the technique described by Thrivikraman *et al.* (24). The catheter is a piece of polyethylene tubing ending in a segment of silastic tubing. [(15-cm long piece of Clay Adams brand PE-50 tubing (I.D. 0.023 in.3 O.D. 0.038 in., wall 0.008 in.) and a 3.5–4.0 cm long piece of Dow Corning medical grade silastic tubing (I.D. 0.0253O.D. 0.047 in., wall 0.011 in.)] The catheter was filled with heparinised saline (20 units /ml, sterile, prepared by mixing two ml of heparin solution (1,000 USP units/ml) in 100 ml saline) [24].

The rats were divided into eight (A to H) experimental groups. In groups A-D, rats had free access to food and water, while in groups E-H, they were fasted until the end of the experiment. After catheterization, the animals were left to recover in their home cages until the next day.

On the second experimental day, a blood sample (tx)(400 μl) was taken and replaced with an equal volume of saline through the vein catheter. Subsequently, using a stereotaxic device Horsley-Clarke type and under anaesthesia as described above, electrolytic lesion of the MPOA was performed to the rats belonging to groups C, D, G, and H while animals in groups A, B, E, and F received a similar operation with the hypothalamus remaining intact. Stereotaxic coordinates for the electrolytic lesion were taken from the atlas of Paxinos and Watson [25]. Target coordinates of the MPOA were (mm): AP -0.8, posterior to the bregma, 0.5 lateral from the midline, and 8.8 ventral to the scull surface. Following the operation, the animals were left 24 hours to recover in their home cages.

On experimental day 3, blood sampling (400 μl) was performed at 0 min (t0). In groups B, D, F, and H, leptin (one mg/kg i.v.) [26] was administered through the catheter, while the rest of the groups received saline. Blood samples (400 μl each) were subsequently taken at 30, 60, 90, 120, and 180 minutes (t30, t60, t90, t120, and t180, respectively) after leptin administration. During blood sampling, the rats were conscious, freely moving, and had free access to water, but were deprived of food. To the end of the experiment, rats were sacrificed by decapitation. The brain was removed and the localization of the lesion was verified histologically.

Blood sample analysis

Blood samples were centrifuged for 15 min at 1,040 g and the serum was stored at -20°C until assayed. Only samples from animals in which complete bilateral lesion of the medial preoptic area was verified histologically were included in the study.

For the determination of LH and FSH levels, enhanced chemilluminesence was used. Interassay variation was 5.7% and specificity was 99%.

Statistical analysis

For the comparison of hormone levels' fluctuations within each group, the Friedman test was first applied. Differences were considered significant if p-value was less than 0.05. The statistical analysis for differences in the hormone levels between groups for each sampling time was performed using the Kruskal-Wallis test. As the results were significant in all cases ($p < 0.0001$), further comparison between groups in pairs was performed with the use of Mann-Whitney U test. For all statistical analyses, SPSS 14.0 was used.

Results

In group A (fed, no lesion, no leptin), hormone levels were not significantly different between the different intervals from the time of administration. ($p = 0.077$ for LH, $p = 0.450$ for FSH). Similarly, in group B (fed, no lesion, leptin) no significant differences were observed ($p = 0.061$ for LH, $p = 0.384$ for FSH), while comparison with group A showed that leptin administration did not affect hormone levels. In group C (fed, lesion, no leptin), LH was not significantly affected ($p = 0.054$) and FSH was significantly decreased ($p = 0.012$) after induction of the lesion. In group D (fed, lesion, leptin) the same effect was observed ($p = 0.086$ for LH, $p = 0.003$ for FSH) (Figures 1, 2).

In the groups that were subjected to fasting (E-H), a significant reduction in both LH and FSH levels between the first two samples (tx, t0) ($p < 0.05$ for both LH and FSH) was observed (Figures 1, 2).

In group F (fasted, no lesion, leptin), LH levels were significantly increased after leptin administration. The increase became statistically significant 60 minutes after the infusion ($p < 0.001$), reached a peak at 90 minutes ($p < 0.001$) and continued to be significant until the end of the sampling period ($p < 0.001$ at 120 and 180 minutes post-leptin infusion) (Figure 1). No significant difference was observed regarding FSH levels. (Figure 2).

Comparison between groups F (fasted, no lesion, leptin) and E (fasted, no lesion, no leptin) indicated that LH levels in group F were higher 60 ($p < 0.001$), 90 ($p < 0.001$), 120 ($p < 0.001$), and 180 ($p < 0.001$) minutes after leptin infusion (Figure 1). No further reduction of hormone levels was observed when groups G (fasted, lesion, no leptin) and E (fasted, no lesion, no leptin) were compared. (Figure 1)

In group H (fasted, lesion, leptin) LH levels increased after leptin administration. The increase became significant 60 minutes after the infusion ($p = 0.012$), reached a peak at 90 minutes ($p = 0.011$) and then decreased between 90 and 120 minutes ($p = 0.012$) and between 120 and 180 minutes ($p = 0.011$) (Figure 1). FSH levels remained unchanged during the sampling period (Figure 2).

Comparison between groups H (fasted, lesion, leptin) and G (fasted, lesion, no leptin), showed that LH levels were

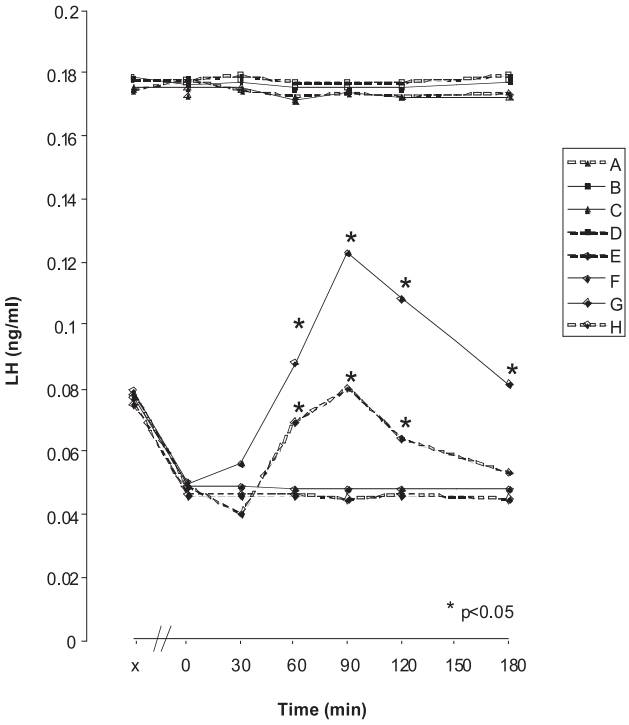

Figure 1. — Effects of leptin (one mg/kg, i.v.) or vehicle (saline) administration on plasma mean values of LH (ng/ml) levels. (n = 8 rats/group, $^{**}p < 0.01$, $^{*}p < 0.05$).

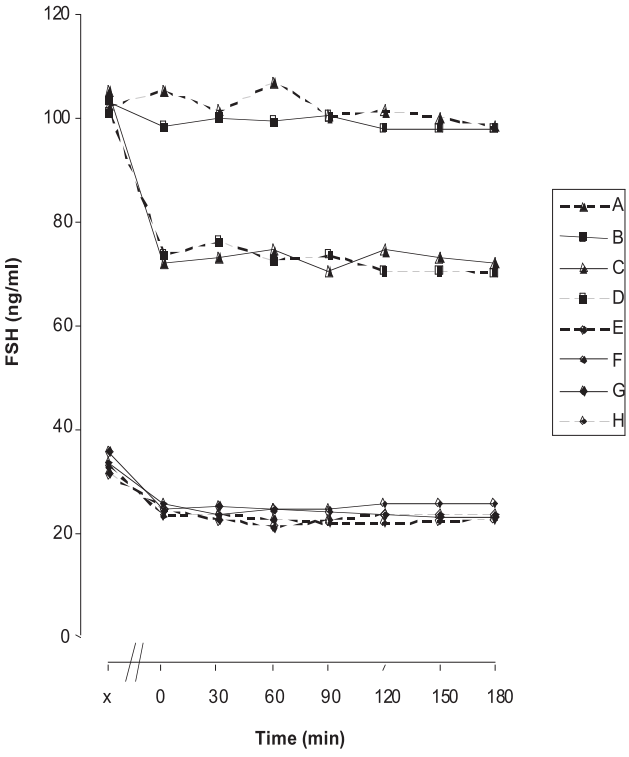

Figure 2. — Effects of leptin (one mg/kg, i.v.) or vehicle (saline) administration on plasma mean values of FSH (ng/ml) levels. (n = eight rats/group, $^{**}p < 0.01$, $^{*}p < 0.05$).

significantly higher 60 ($p < 0.001$), 90 ($p < 0.001$), 120 ($p < 0.001$), and 180 minutes after leptin or saline infusion, in group H rats (Figure 1). FSH levels did not present a significant change (Figure 2). Finally, comparison between groups F (fasted, no lesion, leptin) and H (fasted, lesion, leptin) showed that the increase in LH was more prominent in the rats with intact hypothalamus (group F) 60 ($p < 0.001$), 90 ($p < 0.001$), 120 ($p < 0.001$), and 180 ($p < 0.001$) minutes after leptin administration (Figure 1).

Discussion

The results of the present study are indicative of the fact that in vivo leptin administration may enhance LH secretion in female rats that have been subjected to electrolytic destruction of the MPOA, suggesting a direct action of leptin on the anterior hypophysis. However, no effect of leptin administration on FSH secretion was observed.

The above findings are consistent with previous in vitro experimental data on isolated rat anterior hypophyses, which showed that leptin administration can enhance LH secretion [12]. The same study also revealed a significant effect on FSH release, however to a lesser extend than the one observed on LH. The present findings are in accordance with such a different response to leptin administration. Presumably the concentrations that were used in vitro differed significantly from the ones achieved in vivo in the present study.

In the present study, the evaluation of leptin's action is not performed in the intact rat brain, as the electrolytic lesion of the hypothalamus disrupts the hypothalamic-hypophyseal axis. The objective, however, was the functional isolation of the hypophysis from the hypothalamic influence in vivo. For this purpose, it was essential to eliminate GnRH secretion, by inducing a lesion of the MPOA where the GnRH excreting neurons are situated. As expected, this lesion eventually will result in hypo-gonadotrophic hypogonadism. However, previous experimental data indicated that 24 hours after the lesion, LH levels were the same as prior to the lesion, while FSH levels were mildly decreased [22]. Furthermore, it was chosen not to use ovariectomised animals so as to limit the number of operations and imitate as much as possible the physiological conditions in the reproductive axis function.

The results of the present study show no effect on the fed animals. In contrast, in the fasted animals, leptin administration induced an increase in LH levels. These findings are consistent with a previous study on the direct effect of leptin on the MPOA (19); the author concluded that there was an increase in GnRH and in LH levels observed only in fasted rats and suggested that the stimulatory influence of leptin on the reproductive hormones is likely to be already maximal at the concentration that corresponds to the physiological plasma levels in normally fed female rats. However, other studies have reported that leptin administration induces GnRH release from hypothalamic explants of nor-

mally fed rats [15, 27]. This discrepancy might have been due to the differences between in vivo and in vitro experimental conditions [19].

In the group with intact hypothalamus that received leptin, the effect on LH was more prominent than that observed in the lesioned rats. The above finding suggests that leptin acts both on the hypothalamus and the hypophysis and that in order to exercise its maximal effect it is necessary for the hypothalamus to be intact.

In conclusion, the findings of the present study suggest that leptin, in vivo, acts at the level of the hypophysis to enhance LH secretion. This effect was observed in fasted but not in ad libitum fed rats. However, there was no effect on FSH secretion irrespective of the nutritional state. Further research is therefore necessitated in order to achieve definitve conclusions in the yet unsolved issue of leptin impact on FH secretion.

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