Effect of leptin on LH and FSH release in ovariectomized rats

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تأثير الليبتين على إطلاق الهرمون المُلَوْتن LH والهرمون المنبِّه للجريب FSH في الفئران المستأصلة المبايض

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الخلاصة: إن الهدف من هذا العمل هو مقارنة إطلاق الهرمون المُلَوْتن (LH) والهرمون المنبِّه للجريب (FSH) إطلاقاً محرَّضاً بالاستراديول والبروجستيرون في إناث الفئران الموضوعة على غذاء عادي وفي تلك المعرَّضة للمَخْمَصة مع المعالجة بالليبتين بعد استئصال المبيضين، إلى جانب دراسة تأثير فرط الليبتين في الدم على إطلاق الهرمونات المحرَّض بالستيروئيدات لدى الفئران الموضوعة على غذاء عادي بعد استئصال المبيضين. لقد أدت المَخْمَصه لمده ثلاثه أيام إلى زوال تام لإطلاق كل من الهرمون المُلَوْتن LH والهرمون المنبِّه للجريب FSH المحرَّض بالستيروئيدات. وقد لوحظت استعادة هامة لإطلاق الهرمونات في المجموعة المعرَّضة للمَخْمَصة والمعالجة بالليبتين. ومن جهة أخرى كانت مستويات إطلاق الهرمون المُلَوْتن LH والهرمون المنبِّه للجريب FSH لدى الحيوانات التي وضعت على غذاء عادي مع المعالجة بجرعات أعلى من الليبتين تعادل من وجهة نظر إحصائية تلك المستويات لدى الحيوانات الموضوعة على غذاء عادي وبدون المعالجة بالليبتين. إن هذه الملاحظات تشير إلى أن التراكيز الفيزيولوجية لليبتين الجائل في الدوران ذات تأثير منبه على إطلاق الهرمون المُلَوْتن LH والهرمون المنبِّه للجريب FSH المحرَّض بالستيروئيدات.

ABSTRACT We compared the estradiol/progesterone-induced luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release between normally fed and leptin-supplemented starved ovariectomized female rats and studied also the effect of hyper-leptinaemia on the steroid-induced hormonal release in normally fed ovariectomized rats. Three days' starvation completely abolished steroid-induced LH and FSH release. Significant recovery of the hormonal release was shown in the leptin-supplemented starved group. The magnitudes of LH and FSH release in the normally fed animals with a higher dose of leptin were statistically the same as those in the normally fed group without leptin. These observations indicate that physiological concentrations of circulating leptin exert a stimulatory effect on steroid-induced LH and FSH release.

Effet de la leptine sur la libération de LH et de FSH chez des rates ovariectomisées

RESUME Nous avons comparé la libération d'hormone lutéinisante (LH) et d'hormone folliculostimulante (FSH) induite par la progestérone/l'œstradiol entre des rates normalement nourries et des rates ovariectomisées, à jeun et ayant reçu un supplément de leptine, et également étudié l'effet de l'hyperleptinémie sur la libération d'hormone induite par les stéroïdes chez des rates ovariectomisées et normalement nourries. Un jeûne de trois jours supprimait complètement la libération de LH et de FSH induite par les stéroïdes. Une reprise significative de la libération d'hormone était montrée dans le groupe maintenu à jeun et ayant reçu un supplément de leptine. L'importance de la libération de LH et de FSH chez les animaux normalement nourris recevant une dose plus élevée de leptine était statistiquement la même que chez les animaux normalement nourris sans leptine. Ces observations montrent que les concentrations physiologiques de leptine circulante exercent un effet stimulant sur la libération de LH et de FSH induite par les stéroïdes.

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Introduction

Leptin, the satiety producing hormone secreted by fat cells [*1,2*], is known to stimulate reproductive function in both rodents [*3,4*] and humans [*5,6*]. It may play a role in the generation of the estradiol/progesterone-induced luteinizing hormone (LH) surge in adult female rats [*7*]. This stimulatory effect of leptin on the hormonal surge is, at least in part, mediated through the melanocortin 4 receptor in the brain [*8*].

However, no previous study has examined whether fluctuations in circulating leptin levels within physiological ranges can significantly affect the reproductive axis in any species. The aim of this study was to compare the estradiol/progesterone-induced LH and follicle-stimulating hormone (FSH) release between normally fed and leptin-supplemented starved ovariectomized female rats. The effect of hyperleptinaemia on the steroid-induced hormonal release was also tested.

Methods

Thirty-two (32) adult female albino rats (252 ± 5 g) were used. They were given free access to standard rat food (Ralston Purina, Richmond, United States of America) and tap water unless otherwise indicated. Animals were ovariectomized under light ether anaesthesia about two weeks before experimentation. Four experimental groups (each consisting of eight rats) were prepared: group a, normally fed + phosphate-buffered saline (PBS); group b, 3-days starved + PBS; group c, 3-days starved + leptin (100 µg/kg/day); and group d, normally fed + leptin (300 µg/kg/day). Groups c and d received subcutaneously the indicated doses of leptin (Medipan Diagnostica Entwicklungs- und Vertriebs) dissolved in 0.01 M PBS (pH 7.4). Groups a and b were injected subcutaneously with PBS only. The dose of 100 µg/kg/day of leptin was chosen to mimic the physiological level of leptin in the general circulation [*9*]. A three times higher dose of leptin (300 µg/kg/day) was used in the normally fed rats as hyperleptinaemia was anticipated

Two days prior to the experiment, under light ether anaesthesia, the animals were implanted with a jugular vein catheter filled with heparin solution and also injected subcutaneously with a single dose of estradiol (300 µg/rat) [*10*] in the form of ovocyclin P. At about 08:00 on the day of the experiment, the jugular vein catheter was exteriorized for frequent blood sampling. At 0900, 5 mg/rat of progesterone [*10*] in the form of Primolut N (norethisteron) was injected intramuscularly. Blood samples (200 µL) were collected every hour over a total period of 7 hours (from 11:00 to 18:00). At 11:00, an additional 200 µL of blood was drawn to measure leptin as well. To prevent the loss of circulating plasma volume, 0.9% NaCl was injected intravenously immediately after each blood collection in the same volume as that drawn.

The blood was collected in centrifuge tubes containing EDTA-2Na (2.5 mg/mL), and the subsequently separated plasma was stored in a refrigerator until assayed for leptin, LH and FSH.

Plasma leptin levels were determined by using an enzyme immunoassay kit for the determination of leptin in serum and plasma (Medipan Diagnostica Entwicklungs- und Vertriebs GmbH, Code 3401). The sensitivity of the assay was 0.45 ng/mL. LH and FSH plasma levels were determined by radioimmunoassay using reagents produced by Genzyme Diagnostics' Medix Biotech subsidiary. The sensitivity of the

LH assay was 0.2 μIU/mL and that of FSH assay was 0.8 μIU/mL.

Results were expressed as mean ± standard deviation. One-way or two-way ANOVA followed by Scheffe's post hoc test was used to analyse the data. Differences were considered significant if P was less than 0.05.

Results

Table 1 shows the data of the body weight and plasma leptin levels in the four groups. Seventy-two hours before the experiment, body weight was statistically the same among all groups. The reduction in body weight after 3 days' starvation was similar in both the starved + PBS and starved + leptin (100 μg/kg/day) groups. Loss of body weight was also seen in the normally fed + leptin (300 μg/kg/day) group. With respect to plasma leptin concentrations, the level in the starved + PBS group was below the assay sensitivity. The subcutaneous injection of 100 μg/kg/day of leptin to starved rats restored the hormone level to that of normally fed + PBS group. The administration of 300 μg/kg/day of leptin to normally fed rats produced a three-times higher level of the hormone than that in normally fed + PBS group.

Tables 2 and 3 and Figures 1 and 2 show the changes in LH and FSH in the four groups tested in this work. With respect to LH levels (Table 2 and Figure 1), the normally fed + PBS rats showed significantly higher levels of the hormone (LH release) between 13:00 and 18:00 as compared to their 11:00 values. The 3 days' starvation completely abolished LH release (starved + PBS group). The abolished LH release was significantly reinstated by the 3 days' administration of 100 μg/kg/day of leptin in the face of sustained starvation.

Table 1 **Body weight and plasma leptin levels in the four experimental groups**

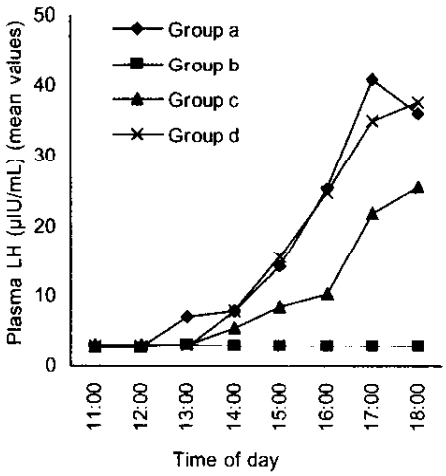
Group	Body weight (g) (mean ± s)		Plasma leptin (ng/mL) (mean ± s)
	72 hours before the experiment	Day of the experiment	
Normally fed + PBS	247.5 ± 4.2	256.5 ± 4.1	3.5 ± 0.4
	$t = 5.494^a$; $P > 0.05$		
Starved + PBS	252.3 ± 4.6	213.3 ± 4.7	–[b]
	$t = 21.276^a$; $P < 0.001$		
Starved + leptin (100 μg/kg/day)	254.3 ± 4.1	209.4 ± 3.3	3.6 ± 0.4
	$t = 20.253^a$; $P < 0.001$		$t = 0.146^c$; $P > 0.05$
Normally fed + leptin (300 μg/kg/day)	254.9 ± 4.5	216.1 ± 4.1	10.8 ± 1.8
	$t = 19.822$; $P < 0.001$		$t = 11.915^c$; $P < 0.001$

[a]Denotes statistically significant change in body weight as compared to that of 72 hours before the experiment.
[b]Plasma leptin was below the assay sensitivity.
[c]Denotes statistically significant difference in plasma leptin level as compared to that in normally fed + PBS.
PBS = phosphate buffered saline.
s = standard deviation.
There were 8 rats in each group.

Table 2 **Effect of leptin on steroid-induced luteinizing hormone (LH) release in normally fed and 3-day starved ovariectomized female rats [mean values (μIU/mL)]**

Time of day	Group a Normally fed + PBS		Group b Starved + PBS		Group c Starved + leptin		Group d Normally fed + leptin		*F*	*P*	Scheffe test
	Mean	*s*	Mean	*s*	Mean	*s*	Mean	*s*			
11:00	2.95	0.37	2.66	0.48	3.16	0.40	3.01	0.28	2.32	> 0.05	
12:00	2.99	0.45	2.75	0.42	3.14	0.41	3.01	0.31	1.30	> 0.05	
13:00	7.05[a]	0.42	3.04	0.44	3.14	0.35	3.00	0.29	219.00	< 0.001[b]	Values for groups b, c and d were all sigificantly less than group a
14:00	8.01[a]	0.49	2.90	0.43	5.54	0.70	7.93[a]	0.48	162.1	< 0.001[b]	All significant except group a compared with group d
15:00	14.28[a]	0.53	3.00	0.51	8.31[a]	0.73	15.56[a]	0.69	689.9	< 0.001[b]	All significant
16:00	25.38[a]	1.04	2.93	0.41	10.30[a]	1.24	24.63[a]	1.42	811.3	< 0.001[b]	All significant except group a compared with group d
17:00	40.85[a]	1.86	2.98	0.53	21.88[a]	1.33	35.03[a]	0.95	1403	< 0.001[b]	All significant
18:00	35.98[a]	1.31	2.95	0.47	25.53[a]	1.80	37.73[a]	1.07	1299	< 0.001[b]	All significant except group a compared with group d

[a]*Derotes statistically significant difference compared to LH plasma level at 11:00 in each group.*
[b]*Derotes statistically significant difference between groups.*
PBS = phosphate buffered saline.
s = *standard deviation.*
There were 8 rats in each group.



Group a: Normally fed + PBS

Group b: Starved + PBS

Group c: Starved + leptin (100 µg/kg/day)

Group d: Normally fed + leptin (300 µg/kg/day)

Figure 1 **Effect of leptin on LH release in ovariectomized rats**

However the magnitude of LH release in this group was still significantly smaller than that in the normally fed + PBS group between 15:00 and 18:00. On the other hand, in the normally fed + leptin (300 µg/kg/day) group, the magnitude of LH release was essentially the same as in the normally fed + PBS group.

A similar finding was observed for plasma FSH levels (Table 3 and Figure 2). The normally fed + PBS group had significantly higher levels of FSH between 13:00 and 18:00 than at 11:00. The starved + PBS group did not show a significant change. However, the simultaneous administration of 100 µg/kg/day of leptin to starved rats resulted in a significant recovery of FSH level. Finally, the changes in FSH levels were the same between normally fed + PBS and normally fed + leptin in higher dose group.

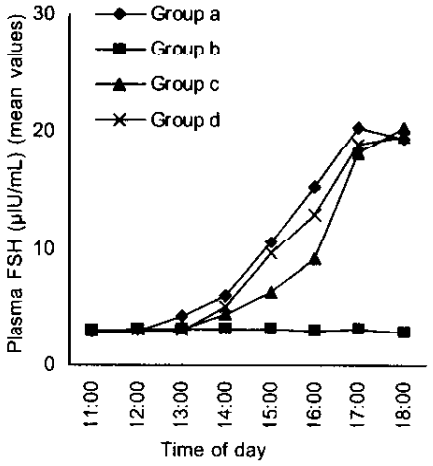
Discussion

Recent studies suggest that leptin may be a prerequisite for the full expression of normal neuroendocrine function, especially for growth hormone [*11,12*] and LH [*3,4, 7,8*] secretion in rodents. Previous studies [*3,4*] support the notion that leptin may be a critical metabolic signal linking nutrition and reproductive function. However, no previous study has tested whether physiological concentrations of circulating leptin cause a significant stimulation of the reproductive axis. The results of the present work show a significant reduction in body weight in normally fed animals receiving higher doses of leptin. This could be secondary to the anorexigenic effect of leptin [*1,2*]. In the present work we examined the estradiol/progesterone-induced LH and FSH release was examined in 3-days starved ovariectomized rats whose plasma leptin levels were made similar to those of the normally fed group. The results were that the supplementation of 100 µg/kg/day of leptin when given to starved rats resulted in a significant recovery of both LH and FSH release as compared to those in the starved group. These findings indicate that physiological concentrations of circulating leptin significantly stimulate estradiol/progesterone-induced LH and FSH release in female rats. It was observed that the magnitudes of the hormonal release in the starved + leptin (100 µg/kg/day) group were still significantly smaller than those in the normally fed + PBS group. This observation suggests that the attainment of

Table 3 **Effect of leptin on steroid-induced follicle stimulating hormone (FSH) release in normally fed and 3-days starved ovariectomized female rats [mean values (μIU/mL)]**

Time of day	Group a Normally fed + PBS		Group b Starved + PBS		Group c Starved + leptin		Group d Normally fed + leptin		*F*	*P*	Scheffe test
	Mean	*s*	Mean	*s*	Mean	*s*	Mean	*s*			
11:00	2.81	0.56	3.01	0.57	2.98	0.42	3.01	0.28	0.32	> 0.05	
12:00	3.06	0.46	3.08	0.34	3.14	0.49	2.99	0.30	0.19	> 0.05	
13:00	4.20[a]	0.60	3.11	0.45	3.13	0.40	3.01	0.39	11.40	< 0.001[b]	Values for goups b, c and d were all sigificantly less than group a
14:00	5.95[a]	0.33	3.10	0.34	4.39[a]	0.65	4.93[a]	0.42	55.16	< 0.001[b]	All significant except group c compared with group d
15:00	10.83[a]	0.83	3.08	0.29	6.38[a]	0.75	9.64[a]	0.62	217.3	< 0.001[b]	All significant
16:00	15.31[a]	1.24	2.95	0.37	9.20[a]	0.76	12.93[a]	0.26	405.1	< 0.001[b]	All significant
17:00	20.30[a]	1.71	3.11	0.36	18.21[a]	1.25	18.95[a]	0.35	439.9	< 0.001[b]	Values for goups a, c and d were all sigificantly greater than group b
18:00	19.46[a]	1.42	2.83	0.87	20.39[a]	1.30	19.58[a]	0.84	448.8	< 0.001[b]	Values for goups a, c and d were all significantly greater

[a] *Denotes statistically significant difference compared to FSH plasma level at 11:00 in each group.*
[b] *Denotes statistically significant difference between groups.*
PBS = phosphate buffered saline.
s = *standard deviation.*



Group a: Normally fed + PBS

Group b: Starved + PBS

Group c: Starved + leptin (100 µg/kg/day)

Group d: Normally fed + leptin (300 µg/kg/day)

Figure 2 **Effect of leptin on FSH release in ovariectomized rats**

normal circulating levels of leptin alone may not be sufficient for starved rats to fully recover LH and FSH release. Other metabolic factors which diminish during starvation may also play a significant role. Schneider et al [*13*]. suggested that the alleged stimulatory action of leptin on the reproductive axis of hamsters is indirect and requires oxidation of metabolic fuels such as glucose and fatty acids.

Another objective of this work was to examine whether reproductive function is affected by hyperleptinaemia induced by exogenous leptin administration. The results obtained showed that the normally fed + PBS and normally fed + high dose of leptin groups had similar magnitudes of release for both LH and FSH. These data suggest that hyperleptinaemia of 3 days' duration in the range of about 10 ng/mL may not significantly affect hormonal release in female rats.

Leptin may stimulate the release of gonadotropins from the pituitaries. Sir-Petermann et al. [*14*] suggested that leptin modulated hypothalamic-pituitary gonadal axis functions. The effect of leptin occurs through receptor-mediated regulation of hypothalamic protein neuropeptide Y (NPY). The NPY pathway may serve as a communicating bridge between neural processes regulating reproduction and those maintaining energy balance [*15*].

Another mechanism for the effect of leptin on LH and FSH release is through nitric oxide (NO) release. NO is a gaseous transmitter and appears to be involved in the control of LH secretion and in the modulation of LH response after stimulation of luteinizing hormone-releasing hormone (LHRH).

Yu et al. [*16*] provided evidence that leptin acts on both the hypothalamus and pituitary gland through stimulation of NO release by acting on its receptors in both sites, which induces the release of LHRH and LH respectively. Leptin activates NO in the gonadotropes activating guanylate cyclase and cyclooxygenase releasing guanosine 3′, 5′-cyclic monophosphate (cyclic GMP), which causes release of LHRH and change in hypothalamic-pituitary blood flow and has a direct effect on the anterior pituitary gonadotropes [*17*].

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