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Evidence That Physiological Levels of Circulating Leptin Exert a Stimulatory Effect on Luteinizing Hormone and Prolactin Surges in Rats

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Increasing evidence suggests that leptin, an adipocyte-derived hormone, may positively regulate the reproductive axis, and serve as a critical metabolic signal linking nutrition and the reproductive function. However, along this line there remains an as-of-yet unresolved important issue whether physiological levels of circulating leptin exert a stimulatory effect on the reproductive axis. It is also unknown whether hyperleptinemia affects the reproductive function. In this study, we attempted to examine these unexplored issues, employing as an indicator the estradiol/progesterone-induced luteinizing hormone (LH) and prolactin (PRL) surges in ovariectomized female rats. Experiments were performed on normally fed, 3-day starved, 3-day starved + murine leptin (100 μg/kg/day), and normally fed + murine leptin (300 μg/kg/day) groups. Leptin was administered utilizing osmotic minipumps during 3 days immediately before experimentation. From 11:00 to 18:00 h, blood was collected every 30 min to measure LH and PRL. The 3-day starvation completely abolished both LH and PRL surges, but 3-day starved + leptin (100 μg/kg/day) group, whose plasma leptin levels (3.7 ± 0.4 ng/ml) were similar to those in normally fed group (3.4 ± 0.5 ng/ml), showed a significant recovery of the hormonal surges. On the other hand, the magnitudes of LH and PRL surges in normally fed + leptin (300 μg/kg/day) group, whose leptin levels were 10.8 ± 1.5 ng/ml, were statistically the same as those in normally fed group. These results indicate for the first time that physiological concentrations of circulating leptin exert a stimulatory effect on the steroid-induced LH and PRL surges in the rat. It was also suggested that mild hyperleptinemia of 3 days' duration may not significantly affect the hormonal surges.

Leptin, the protein encoded by the *ob* (obese) gene, is a putative homeostatic signal controlling food intake, body weight, and energy expenditure (1, 2). In addition to such a primary effect on the metabolism, leptin is also known to stimulate the reproductive function in both rodents (3–11) and humans (12–15). In this connection, we recently reported for the first time that leptin may play a physiologically relevant role in the generation of E_2/P-induced LH and PRL surges in adult female rats (16). We further found that this stimulatory effect of leptin on the hormonal surges is, at least in part, mediated through the melanocortin 4 receptor in the brain, as is leptin's effect on feeding behavior (17).

However, to the best of our knowledge, no previous study has examined whether fluctuations in circulating leptin levels within physiological ranges can significantly affect the reproductive axis in any species. In order to address this as-of-yet unresolved but important issue, in the present study we compared the E_2/P-induced LH and PRL surges between normally fed and leptin-supplemented starved OVX rats. The latter group was made normoleptinemic by a continuous infusion of leptin. In addition, utilizing the same infusion technique, we also tested the effect of short-term hyperleptinemia on the steroid-induced hormonal surges. We became interested in the latter protocol as well, in view of the preexisting studies which reported impaired reproductive function in genetically obese strains of rats (18–21). No previous study has tested the effect of hyperleptinemia produced by exogenous leptin administration on the rat reproductive function.

Abbreviations used: E_2, estradiol; P, progesterone; LH, luteinizing hormone; PRL, prolactin; OVX, ovariectomized; PBS, phosphate-buffered saline; RIA, radioimmunoassay; BW, body weight.

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TABLE 1

BW and Plasma Leptin Levels in the 4 Experimental Groups

Group	Number of rats	BW (g) 72 h before[1]	BW (g) Day of Experiment[2]	Percent change in BW	Plasma leptin[3] (ng/ml)
Normally fed + PBS	8	248 ± 8	258 ± 7	+4 (±1)	3.4 ± 0.5
Starved + PBS	7	252 ± 7	212 ± 6	−16 (±3)	<0.5
Starved + leptin (100 μg/kg/day)	7	254 ± 10	211 ± 8	−17 (±3)	3.7 ± 0.4
Normally fed + leptin (300 μg/kg/day)	8	251 ± 8	218 ± 6	−13 (±2)	10.8 ± 1.5

[1] BW was measured at 08:00 h of the day 72 h before experiment.
[2] BW was measured at 08:00 h of the day of experiment.
[3] Leptin was measured in the samples obtained at 11:00 h of the day of experiment.

MATERIALS AND METHODS

All the following experiments were conducted in accordance with the Guidelines for Animal Experimentation, Hirosaki University.

Female rats (220–240 g) of the Wistar strain were used. They were housed in an air-conditioned room with controlled lighting (light 08:00–20:00 h), and were given free access to laboratory chow and tap water unless otherwise indicated. Animals were OVX under light ether anesthesia about two weeks before experimentation. Four experimental groups were prepared; (a) normally fed + PBS, (b) 3-day starved + PBS, (c) 3-day starved + murine leptin (100 μg/kg/day), and (d) normally fed + murine leptin (300 μg/kg/day). Groups (c) and (d) received a continuous 3-day infusion of the indicated doses of recombinant murine leptin (donation from NIDDK) dissolved in 0.01 M PBS (pH 7.4) via an osmotic minipump (Alzet, model 2001, Alza Corp., Palo Alto, CA) at the rate of 1.0 μl/h. Seventy-two h before the commencement of experimentation, the osmotic minipump was placed subcutaneously in the back region under light ether anesthesia. The minipumps were activated by immersion in PBS at room temperature 12 h before the implantation, so that the infusion started immediately upon implantation of the pumps. Groups (a) and (b) were implanted with the same osmotic minipump loaded with the vehicle (PBS) only. In our preliminary study, we found that plasma leptin concentrations of starved OVX rats receiving a continuous infusion of 100 μg/kg/day of murine leptin were 4.0 ± 0.5 (n = 6), 3.7 ± 0.4 (n = 6), and 3.5 ± 0.4 (n = 6) ng/ml after 24, 48, and 72 h of starvation, respectively. Since these levels of plasma leptin were statistically the same as those in normally fed OVX rats (3.7 ± 0.5 ng/ml) which we previously reported (22), 100 μg/kg/day of leptin was chosen as a dose that mimics the physiological level of leptin in the general circulation. Based on these data, the 3 times higher dose of leptin (300 μg/kg/day) was also employed in this study expecting to achieve a supra-physiological level of plasma leptin.

Two days prior to the experiment, under light ether anesthesia, the animals were implanted with a jugular vein catheter filled with heparin solution, and also implanted subcutaneously with a single Silastic capsule containing 300 μg/ml of E_2-17β (Sigma Chemical Company, St. Louis, MO) in the same manner as in our previous reports (16, 17, 23). At about 08:00 h on the day of the experiment, the jugular vein catheter was exteriorized for frequent blood sampling. At 09:00 h, 5 mg per rat of P (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was injected intramuscularly. Blood samples (200 μl) were collected every 30 min over a total period of 420 min (11:00–18:00 h). At 11:00 h, 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 EDTA-2Na (2.5 mg/ml)-containing tubes, centrifuged, and the plasma was stored at −70°C until assayed for leptin, LH, and PRL.

Plasma leptin levels were determined by a rat leptin RIA kit produced by Linco Research (St. Louis, MO). In this kit, murine leptin crossreacts with the rat counterpart at 100%. The sensitivity of the assay was 0.5 ng/ml. LH and PRL levels were determined by RIA using the reagents kindly donated by Dr. A. F. Parlow (NIDDK). Rat LH-RP-3 and PRL-RP-3 were used as the standards. Sensitivity of the LH assay was 0.2 ng/ml, and that of PRL assay was 0.8 ng/ml. Both the intra- and interassay coefficients of variation were less than 10% in all the three assays.

Results were expressed as the mean ± S.E.M. One-way or two-way ANOVA followed by Scheffe's post-hoc test was used to analyze the data. Differences were considered significant if P was smaller than 0.05.

RESULTS

Table 1 shows the data of BW and plasma leptin levels in the 4 groups examined in this study. At 08:00 h of the day on which an osmotic minipump was implanted to every animal, BW was statistically the same among all groups. The percent reduction in BW after 3 days' starvation was similar in both starved + PBS and starved + leptin (100 μg/kg/day) groups. In agreement with the established anorexigenic effect of leptin (1, 2), normally fed + leptin (300 μg/kg/day) group also lost BW, even though the percent decrease in BW tended to be smaller than those in starved + PBS and starved + leptin (100 μg/kg/day) groups. With respect to plasma leptin concentrations, the level in starved + PBS group was below the assay sensitivity. The continuous infusion 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 3.2 times higher level of the hormone than that in normally fed + PBS group.

Figure 1 shows the temporal changes in plasma LH and PRL in the 4 groups examined in this study. With respect to LH levels [Fig. 1(a)], normally fed + PBS group showed significantly higher levels of the hormone (LH surge) during the period of 13:30–18:00 h as compared to their 11:00 h value. In agreement with our previous studies (16, 17), the 3-day starvation completely abolished LH surge (starved + PBS group). The

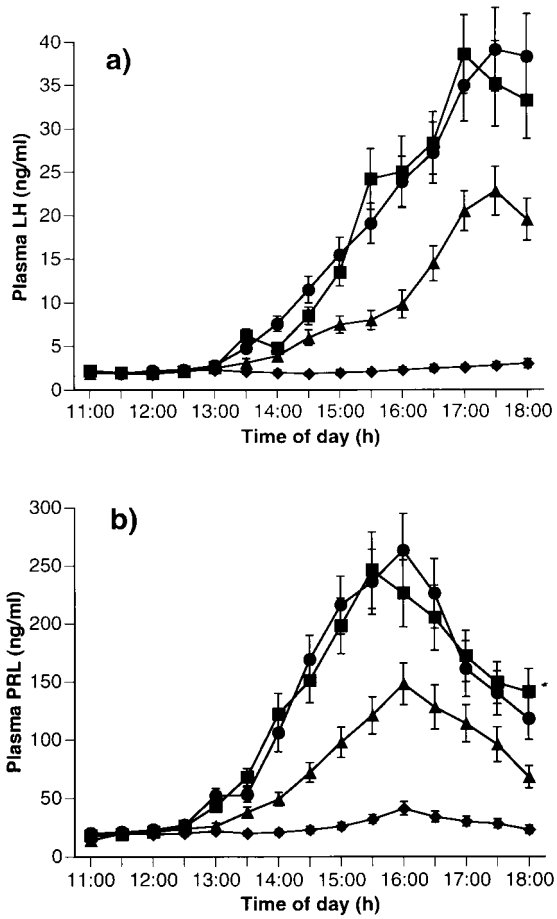

FIG. 1. Effects of systemic leptin infusion on steroid-induced LH and PRL surges in normally fed and 3-day starved OVX rats. The number of rats examined was 7–8 per group. ●——●, normally fed + leptin (300 μg/kg/day); ■——■, normally fed + PBS; ▲——▲, starved + leptin (100 μg/kg/day); ◆——◆, starved + PBS. Where standard errors are not shown, they were smaller than the symbols. For further details, see text.

abolished LH surge was significantly reinstated by the 3-day infusion of 100 μg/kg/day of leptin in the face of the sustained starvation [starved + leptin (100 μg/kg/day) group]. However, the magnitude of LH surge in this group was still significantly smaller than that in normally fed + PBS group during the period of 15:00–18:00 h. On the other hand, in normally fed + leptin (300 μg/kg/day) group, both the temporal profile and magnitude of LH surge were essentially the same as those in normally fed + PBS group.

A similar finding was observed for plasma PRL levels as for the LH levels [Fig. 1(b)]. In agreement with our previous observations (16, 17), normally fed + PBS group had significantly higher levels of PRL between 13:00–18:00 h than at 11:00 h (PRL surge), and starved + PBS group did not show a significant PRL surge. However, the simultaneous administration of 100 μg/kg/day of leptin to starved rats resulted in a significant recovery of PRL surge [starved + leptin (100 μg/kg/day) group], even though the magnitude of PRL surge in this group was significantly smaller than that in normally fed + PBS group between 14:00–18:00 h. Finally, similarly to what was observed for LH surge [Fig. 1(a)], the temporal pattern and magnitude of PRL surge were essentially the same between normally fed + PBS and normally fed + leptin (300 μg/kg/day) groups.

DISCUSSION

Recent evidence suggests that leptin may be a prerequisite for the full expression of normal neuroendocrine function, especially for growth hormone (24–27) and LH (3–11, 16, 17) secretion in rodents. Our previous data (16, 17) as well as results from other laboratories (3–11) support the concept that leptin may be a critical metabolic signal linking nutrition and the reproductive function. However, along this line there is a crucial question which remains to be answered. That is whether physiological concentrations of circulating leptin cause a significant stimulation of the reproductive axis. In the present study, we addressed this question by examining the E_2/P-induced LH and PRL surges in 3-day starved OVX rats whose plasma leptin levels were made similar to those of the normally fed group through a continuous leptin infusion. The results were that the supplementation of 100 μg/kg/day of leptin to starved rats resulted in a significant recovery of both LH and PRL surges as compared to those in starved + PBS group. These findings are the first to clearly indicate that physiological concentrations of circulating leptin significantly stimulate E_2/P-induced LH and PRL surges in female rats. Even so, it is worth noting that the magnitudes of the hormonal surges in starved + leptin (100 μg/kg/day) group were still significantly smaller than those in normally fed + PBS group. This observation strongly suggests that the attainment of normal circulating levels of leptin alone may not be sufficient for starved rats to fully recover the LH and PRL surges, and other metabolic ingredient(s) which diminish(es) during starvation may also play a significant role. In a recent report of Schneider *et al.* (28), it was 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 acid. We consider that this important finding of Schneider *et al.* (28) may, at least in part, account for the subnormal surges of LH and PRL in starved + leptin (100 μg/kg/day) group, because the 3-day starvation must have significantly decreased the reservoir of metabolic fuels.

Another objective of this study was to test whether the reproductive function is affected by hyperleptinemia induced by exogenous leptin administration. As we are not aware of any previous study investigating this issue, we thought it worth trying in view of the re-

ported impairment of gonadal function in genetically obese rats (18–21). In the present study, plasma leptin levels in normally fed + leptin (300 μg/kg/day) group (10.8 ± 1.5 ng/ml) were similar to the reported levels of the hormone (8.6 ± 0.9 ng/ml) in the Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat (21), which is a model of non-insulin dependent diabetes mellitus associated with mild obesity. The results we obtained were that normally fed + PBS and normally fed + leptin (300 μg/kg/day) groups showed statistically the same magnitude of surge for both LH and PRL. These data suggest that hyperleptinemia of 3 days' duration in the range of about 10 ng/ml may not significantly affect the hormonal surges in female rats. Even so, based on this finding alone we can not negate a possible detrimental effect of hyperleptinemia on the reproductive axis, since it is very likely that the reported impairment of gonadal function in genetically obese rats (18–21) ensues as a result of protracted hyperleptinemia. Thus, for the purpose of clarifying the possible depressive action of hyperleptinemia on the reproductive function, a study utilizing genetically obese strains of rodents appears to be feasible.

In summary, this study demonstrated for the first time that physiological levels of leptin in the general circulation exert a significant stimulatory input on the steroid-induced LH and PRL surges in female rats. It was also suggested that mild hyperleptinemia of 3 days' duration may not affect the hormonal surges.

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