Short communication

Stimulation of prolactin secretion by chronic, but not acute, administration of leptin in the rat

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Abstract

Leptin, the product of obese (ob) gene, has been reported to affect the secretion of all six anterior pituitary hormones, but data are especially scarce regarding the interplay between leptin and prolactin (PRL). Thus, in this study we examined and compared in vivo the effects of acute and chronic administrations of recombinant mouse leptin on PRL secretion in male rats. Normally-fed and 3-day-fasted rats received an intraperitoneal bolus injection of leptin [1.0 mg/kg body weight (BW)] or vehicle only. The leptin treatment was without effect on plasma PRL levels up to 5 h postadministration. Food deprivation for 3 days significantly decreased both PRL and leptin levels. This decrease in plasma PRL was prevented by a 3-day constant infusion of 75 µg/kg BW/day of leptin, which maintained plasma leptin levels similar to those of normally-fed rats. The administration of three times the higher dose of leptin (225 µg/kg BW/day) to fasted rats led to further increases in both PRL and leptin in the plasma. Thus, a dose-dependent stimulatory effect of chronic leptin treatment on PRL secretion was indicated. This study demonstrates that chronic, but not acute, administration of leptin stimulates PRL secretion in the rat. © 2000 Elsevier Science B.V. All rights reserved.

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The obese gene product, leptin, is a hormone produced by adipocytes which plays a key role in the regulation of food intake and energy expenditure [4,13,23,40]. Besides its well known role in body weight homeostasis, leptin has recently been shown to play a significant role in the regulation of neuroendocrine function. Increasing evidence suggests that leptin may affect the secretion of all six anterior pituitary hormones. Of these, gonadotropins and growth hormone have thus far been studied in more detail than the other pituitary hormones from the viewpoint of their interaction with leptin (for review, see Ref. [5]).

On the other hand, regarding the interplay between leptin and prolactin (PRL), relevant data are especially scarce. A stimulatory effect of leptin on PRL secretion may be indirectly suggested from the evidence that leptin partially restores lactation in the ob/ob mouse [6], a strain which does not produce leptin. There exist a few studies which reported a direct stimulatory effect of leptin or its fragment of PRL secretion in vivo [10] and in vitro [39], but conflicting in vivo [14] and in vitro [33] data have also been reported. We have also found that an intracerebroventricular (icv) administration of anti-leptin antisera significantly delayed the onset of preovulatory PRL surge, and icv and systemic treatments of leptin to fasted rats resumed the hormonal surge to normality [20,35,37].

In spite of these preexisting studies, it still remains to be elucidated whether leptin can acutely affect PRL secretion in vivo, and also whether acute and chronic administrations of leptin exert differential effects on PRL release. Thus, in the present study we examined and compared the effects of acute and chronic administrations of leptin to freely-moving male rats in order to better understand a possible physiological role of the adipocyte-derived hormone in regulating PRL secretion in the rat.

Animals: Adult male rats of the Wistar Strain [body...
weight (BW), 240–250 g] were used. They were housed at constant room temperature and humidity under a 12-h light–dark cycle (light on at 08:00 h and off at 20:00 h). Food and water were available ad libitum, unless otherwise indicated.

**Acute leptin treatment:** For this experiment, two groups were prepared, i.e. normally-fed and 3-day-fasted rats. The latter group had started fasting 72 h before the injection of leptin as described below. Two days prior to the experiment, all animals were implanted with a jugular vein catheter filled with heparin solution under light ether anesthesia. At about 08:00 h on the day of the experiment, an extension tube for blood sampling was connected to the jugular vein catheter. After leaving the animals undisturbed for about 4 h, the experiment was started at approximately 12:00 h. Throughout the experimental period, they were kept under a conscious, freely-moving condition without apparent stress. Reombinant mouse (rm) leptin (1.0 mg/kg BW) dissolved in vehicle [0.01 M phosphate-buffered saline (PBS)], or the vehicle only, was administered intraperitoneally (ip) as a bolus injection. Two separate groups from both normally-fed and 3-day-fasted rats were given rm leptin or vehicle, respectively. Blood samples (400 μl) were collected 30 min before, immediately before, and thereafter at 30-min intervals up to 300 min after the injection. To prevent anemia and also the loss of circulating plasma volume, red blood cells were resuspended in 0.9% NaCl and returned to the animals after each blood collection. The blood was immediately placed in EDTA-2Na (2.5 mg/ml blood)-containing tubes, centrifuged, and the plasma was kept frozen at −20°C until assayed for PRL and leptin.

**Chronic leptin treatment:** For this experiment, four groups were prepared, i.e. normally-fed, fasted (3 days), fasted+rm leptin (75 μg/kg BW/day), and fasted+rm leptin (225 μg/kg BW/day) rats. The administration of rm leptin was performed utilizing Alzet osmotic minipumps (model 2001) which can deliver 24 μl/day for up to 7 days (Alzet, Palo Alto, CA). The minipumps were filled with 0.01 M PBS (vehicle) containing rm leptin which was adjusted for a daily delivery of 75 or 225 μg/kg BW. Normally-fed and fasted groups were treated with the same type of minipump which was loaded with the vehicle only. The minipump, one for each animal, was implanted subcutaneously in the back under light ether anesthesia 72 h before sacrifice. The dosage of 75 μg/kg BW/day of rm leptin was chosen based on our previous report that 100 μg/kg BW/day of rm leptin endowed fasted adult female rats with physiological levels of plasma leptin up to at least 72 h postadministration [37]. In consideration of the reports by us [36] and others [9] that male rats have significantly lower levels of plasma leptin than females, in this study we adopted the dose of 75 μg/kg BW/day of rm leptin expecting it to produce a physiological level of plasma leptin in fasted male rats. Thus, the three times higher dosage of rm leptin (225 μg/kg BW/day) was chosen in order to achieve a supraphysiological concentration of plasma leptin. For the purpose of determining the temporal change in plasma leptin levels in the four groups after implanting the minipump, tail blood (300 μl) was collected for leptin assay immediately before and 12, 24, and 48 h after the implantation. Seventy-two hours after the minipump implantation, animals were sacrificed by rapid decapitation, and trunk blood was collected to measure both leptin and PRL. All these blood samples were collected into tubes containing EDTA-2Na (2.5 mg/ml blood), and their subsequent treatment was done in the same manner as described above.

**Assays:** PRL levels in plasma were measured by RIA using the reagents kindly donated by Dr. A.F. Parlow (NIDDK). Rat PRL-RP-3 was used as the standard, and the sensitivity of the assay was 0.8 ng/ml. Plasma leptin levels were determined by the rat leptin RIA kit produced by Linco Research (St. Louis, MO). In this kit, rm leptin crossreacts with the rat counterpart at 100%. It was reported by the manufacturer that the sensitivity of the rat leptin RIA system is 0.5 ng/ml. However, we found in our preliminary study that the use as the standard of the recombinant rat leptin produced by R&D Systems, Inc. (Minneapolis, MN, USA) yields a better sensitivity of the assay. Thus, in our leptin assay we used as the standard the R&D Systems’ rat leptin in place of a standard preparation reported by the manufacturer. Sensitivity of leptin in our modified assay system was 0.3 ng/ml. For both PRL and leptin, samples from individual rats were analyzed within the same assay. Both the intra- and interassay coefficients of variation were less than 10% in the two assays.

**Statistical analyses:** 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, as appropriate. Differences were considered significant if P was smaller than 0.05.

Fig. 1 shows the effects of ip administration of rm leptin (1.0 mg/kg BW) or vehicle only on plasma leptin and PRL levels in normally-fed and 3-day-fasted rats. Vehicle alone was without effect on plasma leptin concentrations in both normally-fed and fasted groups during the entire period of observation (normally-fed group, 1.3±0.2–1.5±0.3 ng/ml; fasted group, 0.3±0.1–0.4±0.1 ng/ml). As expected, ip administration of rm leptin produced a marked elevation of plasma leptin in both normally-fed and fasted groups. In both groups, plasma leptin reached its peak 30 min postadministration (normally-fed group, 1240±165 ng/ml; fasted group, 1290±182 ng/ml) and gradually declined thereafter [Fig. 1(a)]. Fig. 1(b) shows the temporal change in PRL levels in the same plasma samples in which leptin was measured. The ip administration of vehicle alone did not significantly affect plasma PRL levels in both normally-fed and fasted groups during the entire period of observation. This lack of effect on PRL secretion was also true for the leptin treatment, which however caused the above-mentioned robust rise in plasma
Fig. 2. Temporal profile of plasma leptin in normally-fed, fasted, and leptin-supplemented fasted male rats up to 72 h after commencement of the respective treatments. The number of rats examined was 7–9 per group. ○, normally fed+PBS; △–△, fasted+PBS; ●, fasted+leptin (75 μg/kg BW/day); ▲–▲, fasted+leptin (225 μg/kg BW/day). ★, P<0.01 vs. the remaining three groups. +, P<0.05 vs. normally fed+PBS and fasted+leptin (75 μg/kg BW/day) groups. For further details, see text.

Fig. 3 shows plasma levels of leptin and PRL after ip administration of rm leptin (1.0 mg/kg BW) or vehicle only in normally-fed and 3-day-fasted male rats. The number of rats examined was 7–8 per group. ○–○, normally fed (vehicle); ●–●, normally-fed (leptin); △–△, fasted (vehicle); ▲–▲, fasted (leptin). In this and the following figures, where standard errors are not shown, they were smaller than the symbols.


leptin. Plasma PRL levels in both the vehicle- and leptin-treated normally-fed groups were significantly (P<0.05) higher than those in the two fasted groups between −30 and 300 min.

Fig. 2 shows the temporal profile of plasma leptin levels in normally fed, fasted, and leptin-supplemented fasted groups up to 72 h after commencement of the respective treatments. In normally fed+PBS group, leptin levels remained stable between 0 and 72 h without any significant change. In fasted+PBS group, plasma leptin started to decrease as early as 12 h after commencing fast, and then gradually declined over time. Thus, fasted+PBS group had significantly (P<0.05) lower levels of plasma leptin than normally fed+PBS group at the timepoints of 24, 48, and 72 h. The supplementation of 75 μg/kg BW/day of rm leptin to fasted rats restored plasma leptin to such levels which were slightly higher than but statistically indistinguishable from those in normally-fed+PBS group. The administration of three times the higher dose of rm leptin (225 μg/kg BW/day) to fasted rats produced about three times (P<0.01) the normal level of plasma leptin as early as 12 h postadministration and a consistently higher level (about 4.5 times the normal, P<0.01) from 24 h onward.

In the first part of this study, we examined the effect of ip bolus administration of rm leptin on PRL secretion in conscious, freely-moving male rats. The experiment was done under both normally-fed and 3-day fasted conditions, because we assumed that the metabolic status of the animal, i.e. the background levels of plasma leptin, might modulate plasma PRL response to leptin. However, the
Tena-Sempere et al. [33] reported that $10^{-7}$ M of leptin did not modulate PRL release from incubated anterior pituitaries of fasted male rats within 2 h. It was recently reported by Gonzalez et al. [10] that an icv administration of mouse leptin-(116–130), an active fragment of the native molecule [11], caused a significant rise in serum PRL levels in food-deprived male rats. However, we feel that this study of Gonzalez et al. [10] contains two problems which may preclude us from accepting it as the first undisputed demonstration for leptin’s PRL-releasing activity in vivo. Firstly, the authors used a fragment of mouse leptin instead of the mature protein. Although leptin-(116–130) has been shown to act in a similar manner to the native protein on both food intake and body weight in ob/ob mice [11], it was also reported that the expression of the normal biological activity of leptin requires amino acid residues proximal to position 106 and also sites between residues 106–140 [24]. Moreover, it was recently reported that the effect of leptin-(116–130) on body weight homeostasis is not mediated by its binding to the long form of the leptin receptor, the receptor isoform which is predominantly expressed in the hypothalamus [12]. Thus, the data derived from using the leptin fragment may not necessarily represent all the biological actions of the mature leptin molecule. Secondly, as the authors themselves also stated, the icv dose (15 μg) of leptin-(116–130) which they administered appears to be fairly large, even pharmacological. In our previous studies in which the native leptin molecule was given into the rat cerebroventricle, the dose we chose was 0.15–0.3 nmol on a molar basis [20,35]. With this dose, we observed normalization or a fairly good recovery of prevulatory luteinizing hormone and PRL surges in fasted female rats [20,35]. In the study of Gonzalez et al. [10], they administered icv 9.61 nmol of the leptin fragment, which, on a molar basis, is 32±64 times larger than the dose (0.15–0.3 nmol) which we have previously given via the same route [20,35]. Thus, the data obtained from using this pharmacological dose of leptin fragment must await further evaluation for their physiological meaning. At any rate, our own data in the present study do not favor a PRL-releasing action of acute hyperleptinemia.

In the second part of this study, we investigated whether chronic leptin treatment affects PRL secretion. The observation that fasting for 3 days significantly lowered plasma PRL levels, is in agreement with the preexisting literature [2,7,30,38]. Moreover, as a novel finding we found that a 3-day constant infusion of leptin to fasted rats at such a dose (75 μg/kg BW/day) which maintained plasma leptin levels similar to those of normally fed rats, reverted plasma PRL levels to normal. Furthermore, the 3-day infusion of three times the larger dose (225 μg/kg BW/day) of leptin led to a further elevation of plasma PRL as well as leptin. These results demonstrate a dose-dependent stimulation of PRL secretion by chronic leptin treatment in the rat.
The elevation of PRL levels by chronic leptin infusion implies that hyperleptinemic subjects may have hyperprolactinemia. It has been reported that obesity per se is not usually associated with increased PRL concentrations [25]. However, in a large population study, a weak, but significant, correlation was revealed between serum PRL levels and body weight [34]. In addition, it was also reported that regularly menstruating women who attained a sizable amount of weight gain over a short period of time, had significantly higher levels of serum PRL than the controls [8]. Thus, it is possible that hyperleptinemia, which accompanies obesity and weight gain, leads to hyperprolactinemia also in humans.

An important question which reasonably emerges from the present data is the neuroendocrine mechanism whereby leptin stimulates PRL secretion. As already discussed above, it is unlikely that leptin directly stimulates PRL release from the pituitary at least at the concentrations which we produced in the plasma by constant leptin infusion. Thus, a probable site where leptin may primarily act to cause a series of events which eventually culminate in stimulated PRL release, is the hypothalamus. A decrease in the secretion of PRL-releasing factors, e.g. dopamine [1], or an increased release of PRL-releasing factors, such as thyrotropin-releasing hormone and vasoactive intestinal peptide [1], may play an intermediary role. However, because of the following reasons, we speculate it more likely that several peptides which originate in the hypothalamic arcuate nucleus [18] may play a primary role in causing the leptin-stimulated PRL secretion. It is well established that the arcuate nucleus is such a site in the hypothalamus that shows the most abundant expression of the leptin receptor [21,26]. The arcuate nucleus contains a high density of neurons that produce neuropeptide Y (NPY), α-melanocyte stimulating hormone (α-MSH), β-endorphin (β-END), agouti-related protein, and several other appetite-regulating factors [18]. Both α-MSH and β-END are the products of the proopiomelanocortin (POMC) gene [31]. It has been reported that the expression of the NPY and POMC genes in the arcuate nucleus is inhibited [27,32] or stimulated [28] by leptin, respectively. Although NPY does not seem to play a significant role in the physiological regulation of PRL secretion [17], both α-MSH [15,16,22] and β-END [19,29] are considered as exerting an excitatory input on PRL release. Thus, it is conceivable that leptin’s stimulatory action on PRL secretion is mediated by these POMC gene products. We recently obtained data suggesting that of the two POMC-derived peptides, only α-MSH, but not β-END, may play a role in mediating the leptin stimulation of PRL secretion in the rat. We found that repeated icv administrations of neutralizing antibody against α-MSH, but not that against β-END, significantly lowered plasma PRL levels which were elevated by chronic leptin infusion (H. Watanobe and H.B. Schiöth, unpublished observations). Recent studies reported that α-MSH acts as a mammotrope-priming agent by rendering these cells much more responsive to physiologically relevant PRL secretagogues [16,22]. This concept may not disagree with our present data that only the chronic, but not acute, administration of leptin was able to stimulate PRL secretion. We speculate so, because the mammotrope may need to be exposed to α-MSH for a sustained, but not a brief, period of time in order for α-MSH to manifest its mammotrope-priming action.

In summary, in this study we demonstrated that chronic, but not acute, administration of leptin stimulates PRL secretion in male rats. PRL seems to be another pituitary hormone which is regulated by the adipocyte-derived hormone.

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References


