EXPERIMENTAL STUDY

Effects of leptin on secretion of LH and FSH from primary cultured female rat pituitary cells

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Abstract

Background: Leptin, which is the product of the *obese* gene, is believed to play important roles in pubertal development and reproductive function in females. In a study using adult male rats, it was found that leptin stimulated secretion of gonadotropin from the pituitary in a dose-related manner. However, there has been no such study in female rats.

Objective: To investigate the effects of leptin on the production of LH and FSH from the pituitary in female rats, using primary cultured pituitary cells.

Methods: In this study, we determined body weight, serum leptin concentration and serum estradiol (E_2) concentration in female Wistar rats at 3, 5, 6, 7, 9 and 11 weeks of age, and cultured pituitary cells from 6-week-old female Wistar rats with leptin (0 – 10^{-7} mol/l) and GnRH (0 or 10^{-8} mol/l). Then basal and GnRH-stimulated extra- and intracellular LH and FSH were assayed by RIA.

Results: Serum leptin concentration increased with increases in body weight and E_2 concentration. The pubertal serum leptin concentration was about 10^{-10} mol/l. At a lower or moderate concentration, leptin produced dose-related increases in both basal and GnRH-stimulated extra- and intracellular LH and FSH in pituitary cells. At a concentration of 10^{-10} mol/l, leptin significantly ($P < 0.05$) stimulated both basal and GnRH-stimulated extra- and intracellular LH and FSH. However, at greater concentrations, these effects diminished.

Conclusions: These results indicated that leptin induced pituitary cells to produce and secrete both LH and FSH, with or without GnRH. The concentration of leptin that induced the greatest production of gonadotropins by pituitary cells was 10^{-10} mol/l, which was the same as the physiological pubertal concentration. Leptin may be involved in the onset of puberty. It is also conceivable that leptin may be a cause of ovulatory failure, not only in weight loss but also in weight gain.

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Introduction

Leptin is a product of the *obese* gene (1). It is well known that leptin is secreted from adipose tissues and that it has an important role in the control of energy homeostasis (2–5). Leptin receptors can be found throughout the body (6), including the hypothalamo–pituitary axis (7, 8), which is the center of reproductive function. Leptin has been reported to play important roles in reproductive function as well as in energy homeostasis.

In *ob/ob* mice, chronic leptin treatment restored puberty and fertility (9, 10). In normal prepubertal girls, the serum leptin concentration increases earlier than that of other reproductive hormones related to puberty (11–13). In normal prepubertal female mice,

the reproductive tract showed earlier maturation with leptin injection (14–17). In normal adult female rats, treatment with anti-leptin serum caused a decrease in luteinizing hormone (LH) pulsatility (18). In contrast, hyperleptinemia originating from extreme obesity has been reported to interfere with pituitary function (19). These reports indicate that leptin has an important role in reproduction through the hypothalamo–pituitary axis.

In a study using adult male rats, it was found that leptin induced secretion of LH and follicle-stimulating hormone (FSH) from the pituitary in a dose-dependent manner (20). However, there has been no such study in female rats. In this study, we therefore investigated the effects of leptin on the production of LH and FSH from the pituitary in female rats, using primary cultured pituitary cells.

Materials and methods

Animals

Female Wistar rats were used in this study. The body weight of each rat was measured, and blood samples were taken from each rat for assays of serum estradiol (E_2) and leptin concentrations at 3, 5, 6, 7, 9 and 11 weeks of age.

Primary cell culture of the rat anterior pituitary

Six-week-old female rats weighing 130–145 g were decapitated, and their pituitaries were excised. The pituitaries were cut into small pieces and washed in Dulbecco's modified Eagle's medium (DMEM; Nissui Co., Tokyo, Japan).

The cells were dissociated by treatment with 0.4% collagenase plus DNase at 37 °C for 2 h and then incubated with pancreatin at 37 °C for 7 min, according to the method previously reported (21). The cells were seeded at 10^6 cells/ml in 24-well culture dishes (Falcon Plastics, Los Angeles, CA, USA) and cultured for 48 h in DMEM containing 10% fetal calf serum (FCS) at 37 °C under 5% CO_2 –95% O_2 -humidified air. The FCS was treated with dextran-coated charcoal to remove endogenous steroids before use. After culture for 48 h, the cells were washed three times with serum-free DMEM containing 0.1% bovine serum albumin and then incubated for 20 h in serum-free DMEM medium with leptin (0 – 10^{-7} mol/l).

For determination of basal extracellular gonadotropin concentrations, the supernatants were collected and assayed by RIA. The cells were then washed three times with serum-free DMEM and incubated in the DMEM medium with the same concentration of leptin as that stated above and with or without 10 nmol/l gonadotropin-releasing hormone (GnRH) (Sigma, St Louis, MO, USA). The cultures were then incubated for 4 h at 37 °C. The supernatants were aspirated and assayed by RIA for GnRH-stimulated extracellular LH and FSH release.

The cells were then washed with serum-free DMEM. One milliliter of Tris–HCl was added, the cells were removed from the dishes with a rubber remover, and the samples were sonicated (20 kHz, 160 W) at 30 s intervals for 5 min. They were then centrifuged at 10 000 *g* for 10 min at 4 °C, and the supernatants aspirated and assayed by RIA for intracellular LH and FSH content.

Measurement of hormones

Leptin was measured using a Rat Leptin RIA kit (LINCO Research Inc., St Charles, MO, USA). The intra- and interassay variances were 2.4% and 4.8% respectively. E_2 was assayed using an Estradiol Kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The

intra- and interassay variances were 7.0% and 8.1% respectively. LH and FSH were measured by double-antibody RIA with NHPP kits.

Statistical analysis

All data were calculated using the ANOVA program, and the significance of differences was examined by Student's *t*-test. Changes in body weight, serum leptin concentration and serum E_2 concentration are expressed as means $\pm$ S.E.M. Concentrations of LH and FSH are expressed as the means $\pm$ S.E.M., with percentage of the control value.

Results

Changes in body weight, serum leptin concentration and serum E_2 concentration

Changes in body weight, serum leptin concentration and serum E_2 concentration are shown in Fig. 1. The mean body weight increased from 3 to 11 weeks of age (Fig. 1A). Serum leptin concentrations increased in parallel with age and body weight. The mean concentration of leptin in rats at 6 weeks of age (1.41 ± 0.15 ng/ml) was significantly ($P < 0.05$) greater than that in rats at 3 weeks of age (0.91 ± 0.14 ng/ml) and lower than that in rats at 11 weeks of age (2.32 ± 0.23 ng/ml; Fig. 1B). The pubertal serum leptin concentration was approximately 10^{-10} mol/l. Serum E_2 concentrations also increased in parallel with age, body weight and serum leptin. The mean serum E_2 concentration in rats at 6 weeks of age (17.8 ± 1.4 pg/ml) was significantly ($P < 0.05$) higher than that in rats at 3 weeks of age (2.7 ± 1.4 pg/ml) and lower than that in rats at 11 weeks of age (45.7 ± 6.8 pg/ml; Fig. 1C).

Effects of leptin on basal extra- and intracellular LH and FSH from cultured pituitary cells

Figure 2 shows the effects of leptin on basal concentrations of extra- and intracellular LH from cultured pituitary cells. Basal extracellular LH concentration increased with increases in leptin concentration up to 10^{-10} mol/l (126%, $P < 0.05$ compared with control). However, at greater concentrations, these effects diminished (Fig. 2A). Basal intracellular LH content also increased with increases in leptin concentration up to 10^{-10} mol/l (136%, $P < 0.05$ compared with control; Fig. 2B), but these effects also diminished with greater concentrations.

Similarly, both basal extracellular and basal intracellular FSH concentrations also increased with increases in leptin concentration up to 10^{-10} mol/l (106 and 160%, respectively; $P < 0.05$ compared with control). However, at greater concentrations, these effects also diminished (Fig. 3).

Figure 1 Changes in body weight, serum leptin concentration and serum estradiol (E_2) in relation to age in female Wistar rats. (A) Changes in body weight ($n = 8$). Body weight steadily increased from 3 to 11 weeks of age. (B) Changes in serum leptin concentrations ($n = 8$). Serum leptin concentrations increased in parallel with age and body weight and increased significantly ($P < 0.05$) in rats from 3 weeks of age (0.91 ± 0.14 ng/ml) to 6 weeks of age (1.41 ± 0.15 ng/ml), and in rats from 6 weeks of age to 11 weeks of age (2.32 ± 0.23 ng/ml). The pubertal serum leptin concentration was approximately 10^{-10} mol/l. (C) Changes in serum E_2 ($n = 8$). Serum E_2 concentrations increased in parallel with age, body weight and serum leptin concentration, and increased significantly ($P < 0.05$) in rats from 3 weeks of age (2.7 ± 1.4 pg/ml) to 6 weeks of age (17.8 ± 1.4 pg/ml), and in rats from 6 weeks of age to 11 weeks of age (45.7 ± 6.8 pg/ml). Each bar and vertical line represents the mean $\pm$ S.E.M. * $P < 0.05$.

Effects of leptin on GnRH-stimulated extra- and intracellular LH and FSH from pituitary cells

Likewise, both GnRH-stimulated extra- and intracellular LH concentrations increased with increases in leptin

Figure 2 Effects of leptin on basal LH secretion and production. (A) Effect of leptin on basal extracellular LH ($n = 12$). (B) Effect of leptin on basal intracellular LH ($n = 5$). Both basal extracellular and basal intracellular LH concentrations increased with increases in leptin concentration up to 10^{-10} mol/l (126 and 136% respectively; $P < 0.05$ compared with control). However, these effects diminished at higher concentrations. Each bar and vertical line represents the mean $\pm$ S.E.M. with percentage of the control value. Control values of basal extra- and basal intracellular LH were 2.84 ± 0.23 and 21.19 ± 1.99 ng/ml respectively. * $P < 0.05$.

concentration up to 10^{-10} mol/l (150 and 133%, respectively; $P < 0.05$ compared with control). However, at greater concentration, these effects also diminished (Fig. 4).

Finally, both GnRH-stimulated extra- and intracellular FSH concentrations also increased with increases in leptin concentration up to 10^{-10} mol/l (107 and 113%, respectively; $P < 0.05$ compared with control), but these effects also diminished at greater concentrations (Fig. 5).

Figure 3 Effects of leptin on basal FSH secretion and production. (A) Effect of leptin on basal extracellular FSH ($n = 12$). (B) Effect of leptin on basal intracellular FSH ($n = 5$). Both basal extra- and intracellular FSH concentrations increased with increases in leptin concentration up to 10^{-10} mol/l (106 and 160% respectively; $P < 0.05$ compared with control), but these effects also diminished at greater concentrations. Each bar and vertical line represents the mean $\pm$ S.E.M. with percentage of the control value. Control values of basal extra- and intracellular FSH were 2.87 ± 0.05 and 4.77 ± 0.47 ng/ml respectively. * $P < 0.05$.

Discussion

Our findings demonstrate that leptin stimulates LH and FSH secretion from the cultured female rat pituitary, but that the effects are dose-related. Leptin stimulated both extra- and intracellular gonadotropins from pituitary cells with or without GnRH in the pubertal (6–7 weeks of age) concentration range, but did not stimulate at greater concentrations.

It is well known that the state of nutrition is an important factor in the reproductive function and the onset of puberty (22–28). Particularly in girls, body weight can be a better predictor of the pubertal

Figure 4 Effects of leptin on GnRH-stimulated extra- and intracellular LH. (A) Effect of leptin on GnRH-stimulated extracellular LH ($n = 7$). (B) Effect of leptin on GnRH-stimulated intracellular LH ($n = 7$). Both GnRH-stimulated extra- and intracellular LH concentrations increased with increases in leptin concentration up to 10^{-10} mol/l (150 and 133% respectively; $P < 0.05$ compared with control), but these effects also diminished at greater concentrations. Each bar and vertical line represents the mean $\pm$ S.E.M. with percentage of the control value. Control values of GnRH-stimulated extra- and intracellular LH were 9.63 ± 0.81 and 4.34 ± 0.35 ng/ml respectively. * $P < 0.05$.

development than age. It has been proposed that the attainment of a critical body weight and a signal related to energy stores may determine the onset of puberty. However, the details are still not clear.

Leptin is produced by adipocytes (1), and an increase in leptin concentration is believed to serve as a negative feedback signal to the brain, resulting in decreased food intake, increased energy expenditure and resistance to obesity (2–5). In addition, leptin also appears to play important roles in reproductive functions and in pubertal development. In *ob/ob* mice, chronic leptin

Figure 5 Effects of leptin on GnRH-stimulated extra- and intracellular FSH. (A) Effect of leptin on GnRH-stimulated extracellular FSH ($n = 7$). (B) Effect of leptin on GnRH-stimulated intracellular FSH ($n = 7$). Both GnRH-stimulated extra- and intracellular FSH concentrations increased with increases in leptin concentration up to 10^{-10} mol/l (107 and 113% respectively; $P < 0.05$ compared with control), but these effects were not found at greater concentrations. Each bar and vertical line represents the mean $\pm$ S.E.M. with percentage of the control value. Control values of GnRH-stimulated extra- and intracellular FSH were 7.48 ± 0.19 and 3.02 ± 0.06 ng/ml respectively. * $P < 0.05$.

treatment not only reduced food intake and body weight but also restored puberty and fertility (9, 10). In normal prepubertal girls, it was found that the serum leptin concentration increases before those of other reproductive hormones related to puberty (11–13). In the prepubertal period, weight gain in normal female mice was delayed by injection of leptin compared with that in control mice, but the mice injected with leptin showed earlier maturation of the reproductive tract (14–17). In normal adult female rats, anti-leptin serum decreased LH pulsatility (18), and in the adult male rat pituitary, leptin was found to produce a dose-related increase in gonadotropin release at lower or moderate

concentrations (20). These findings suggest that leptin accelerates reproductive function.

In contrast, hyperleptinemia is associated with impaired basal and GnRH-stimulated gonadotropin secretion in humans during the peripubertal or early postpubertal period (19). In cultures of adult male rat pituitary, it was found that gonadotropin production was not induced by leptin at greater concentration (20, 29). It therefore seems that excessive leptin concentration reduces reproductive function.

Our results are consistent with both a positive and a negative correlation between leptin and gonadotropins. At lower or moderate leptin concentrations, leptin was effective for stimulating production and release of LH and FSH. At a pubertal concentration, leptin exerted maximal effect. However, at very high leptin concentrations, these effects diminished. It is likely that leptin is one of the factors critical to the onset of puberty, by which adipose tissue 'informs' the pituitary that the energy stores have attained an adequate level – a so-called critical weight. It is also likely that hyperleptinemia originating from extreme obesity may interfere with pituitary function.

Leptin receptors exist throughout the body (6), including the hypothalamus and the pituitary (7). Because of the density of leptin receptors on the hypothalamo–pituitary axis, leptin might affect the GnRH neuron mainly indirectly (8). In the hypothalamus, it may exert actions on the GnRH neuron both directly by modifying the frequency and magnitude of GnRH pulses (30), and more indirectly through intermediates such as nitric oxide (31), neuropeptide Y (32, 33) and so on. In the pituitary, gonadotropin production might be induced by leptin directly or indirectly through modification of the sensitivity of the pituitary to GnRH. Further investigation of the effects of leptin on the hypothalamo–pituitary axis in reproduction *in vitro* is needed.

In summary, we have demonstrated that leptin has direct effects on pituitary cells. At the physiological pubertal concentration, leptin may stimulate the production and release of LH and FSH. Conversely, it may have inhibitory effects at very high concentrations. Our results indicate that leptin may play important roles in female reproductive function, the process of onset of puberty and ovulatory failure, not only in weight loss but also in weight gain.

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