TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2013) 43: 557-561
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doi:10.3906/sag-1208-15

Leptin regulation of pubertal maturation in intact and pinealectomized female rats

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Received: 03.08.2012 • **Accepted:** 05.11.2012 • **Published Online:** 29.07.2013 • **Printed:** 19.08.2013

Aim: To explore the roles of leptin and melatonin in early pubertal maturation.

Materials and methods: Wistar female rats were used as experimental animals. Leptin was subcutaneously infused for 28 days through osmotic minipumps starting from postnatal day 15 (preweaning). Pinealectomy was performed on postnatal day 21 (weaning), and the animals were decapitated when estrus was detected by vaginal smearing.

Results: Chronic preweaning, by peripheral infusion of leptin through subcutaneous routes, advanced the onset of puberty in leptin-treated sham and pinealectomized groups as determined by vaginal opening, while causing no significant change in serum estradiol levels and uterus weight. Mean body weights on the day of vaginal opening were significantly lower ($P < 0.01$) in leptin and pinealectomized-leptin groups compared to the control group.

Conclusion: Preweaning leptin administration advances the onset of puberty regardless of body weight, and the pineal gland does not seem to have a modulatory effect on leptin-induced pubertal maturation.

Key words: Leptin, pinealectomy, onset of puberty

1. Introduction

Puberty is one of the most complex and mysterious biological events in mammals. The onset of puberty is not simply dependent on chronological age, but also on nutritional status, weight, psychosocial factors, and environmental contaminants (1–3). During the past decade, early pubertal development and an increased incidence of sexual precocity have been noticed in children, primarily in girls (4), which may cause psychosocial disorders and mammary cancer resulting from early exposure to estrogenic effects (5). Therefore, scientists have recently paid more attention to understanding the mechanisms underlying early onset of puberty in females.

While it has been known that the onset of puberty is physiologically coupled to energetics for a long time, we have only recently understood how this linkage is accomplished at the molecular level. As reproductive functions need huge energy supplies, the brain must perceive metabolic cues informing it that energy reserves are adequate to meet the caloric demands of the reproductive functions. The discovery of leptin, a product of the obese (*ob*) gene (6), has provided an important insight into our understanding of the relationship between energy homeostasis and regulation of reproduction. Leptin is produced by adipose cells and plays an important role in the regulation of body weight and metabolism (7–10). Leptin crosses the blood–brain barrier (BBB) by receptor-mediated transport to exert multiple central nervous system actions, including decreased food intake (11). In addition to this primary function, leptin may also play an important role in the neuroendocrine axis regulating the onset of puberty. Consistent with this hypothesis, *ob/ob* mice with a total deficiency of leptin exhibit infertility, which is restored by leptin treatment (12,13). Leptin has been suggested as being a major determinant of the timing of puberty (14), or to have a permissive effect for the onset of puberty (15).

Melatonin is produced and secreted by the pineal gland with a circadian rhythm characterized by low levels during the day and peak values at night (16,17). Therefore, it is the so-called darkness hormone. It has been known for a long time that there is a connection between the pineal gland and human reproductive functions through clinical observation of the effects of pineal tumors on

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human sexual development (18). It has been suggested that the pineal gland exerts an inhibitory role on pubertal maturation in humans because there is a causal relationship between the onset of puberty and a reduction in pineal melatonin production at the period of pubertal development (19). Melatonin is thought to be involved in the precocious puberty process (20). Melatonin levels were found to be low in precocious puberty (20,21) and high in women with stress-induced, exercise-induced, or functional hypothalamic hypogonadism (22–24). It has been postulated that, before puberty, the decline of serum melatonin represents the activating signal for the hypothalamic pulsatile secretion of gonadotropin-releasing hormone, which is necessary for the activation of the gonadotropic axis and, thereafter, the onset of pubertal changes (25). In rodents and in seasonal breeders, the pineal gland has been shown to convey photoperiodic information through its nocturnal melatonin secretion and to modulate reproductive activity (26–28). In animals, it has been reported that the pineal gland exerts an inhibitory role on pubertal maturation (29,30).

Studies have yielded contradictory results on the possible relationship between melatonin and leptin release. Although a few studies showed that melatonin stimulates leptin release (31,32), our previous studies (33,34) and some other studies (35,36) suggested that melatonin has an inhibitory effect on leptin release. Therefore, we hypothesized that the onset of puberty is advanced by increased leptin, which was potentiated by melatonin reduction near puberty. To test this hypothesis, leptin was given from the postnatal day 15 (preweaning) for 4 weeks, and a pinealectomy was performed on day 21 (weaning). Thus, melatonin secretion was decreased to minimum levels, and the aim was to raise leptin release to a maximum level due to the lack of melatonin. In order to determine whether leptin has effects on postpubertal reproductive properties besides acceleration of puberty, we looked at uterine and ovarian weights and serum estradiol levels.

2. Materials and methods

The experimental protocol was approved by the Fırat University Ethical Committee. Wistar female rats were used in the study. The day the litters were born was considered as day 1. The rats, which were housed under constant conditions of temperature (22 °C) and light (12 h light/12 h dark from 0700 hours), were divided into 2 groups at the beginning of the experiment. The control group (n = 7) received saline only (1 mL/kg), and the experimental groups (n = 14) were subcutaneously infused with rat leptin (Sigma Chemical Co., USA) using Alzet osmotic minipumps model 2004 (Durect Co., USA) from day 15. These minipumps, which delivered 6 μL/day for 28 days, were adjusted to a daily delivery of 1 μg. Half of the experimental group (PNX-leptin) was exposed to pinealectomy on the day 21, as described in our previous study (36,37). The other half (leptin) of the experimental group was only sham-pinealectomized. Subcutaneous implantation of osmotic minipumps and pinealectomy were performed under general anesthesia with ketamine hydrochloride (75 mg/kg) and xylazine (8 mg/kg). Vaginal opening (VO) was monitored daily starting from day 26. The animals were decapitated when the second estrous was observed. Upon decapitation, serum was separated and stored at −20 °C until estradiol was measured. Uteri and ovaries were dissected out and weighed.

Serum estradiol levels were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's procedures (BioSource, USA; Cat. # KAP0621 for serum estradiol). The lowest level of estradiol was 5 pg/mL.

All values were indicated as median (min–max). Differences between medians were calculated by means of one-way analysis of variance followed by a post-hoc Tukey honestly significant different test to determine significant differences among data groups. For all analyses, $P < 0.05$ was accepted as evidence of significance.

3. Results

The onset of puberty was advanced in leptin-treated sham and pinealectomized rats compared to saline-treated rats as determined by VO. VO occurred at day 29 (min–max, 29–31 days) and day 30 (min–max, 29–31 days) in leptin-treated sham and pinealectomized rats, respectively, compared to day 33 (min–max, 31–36 days) in saline-treated rats ($P < 0.01$, $n = 7$ for all groups, Figure 1A). Mean body weights on the day of VO were significantly lower ($P < 0.01$) in leptin (64.2 g; min–max, 60.4–71.6 g) and PNX-leptin (60.9 g; min–max, 52.0–66.8 g) groups compared to the control group (82.3 g; min–max, 76.0–0.5 g) (Figure 1B). There was no significant difference between mean body weights of leptin and PNX-leptin groups on the day of VO.

There were no significant differences between leptin-treated and control rats in terms of uterine (Figure 2A) and ovarian weights (Figure 2B) or serum estradiol levels (Figure 2C). All these parameters also confirmed the beginning of the estrous cycle in the rats.

4. Discussion

Data in the present study confirm the findings of previous studies about the role of leptin in the initiation of puberty. Leptin signaling appears to be more important in the onset of puberty in the female, since more energy consumption is required for the reproductive functions in the female. It informs the brain about whether there are sufficient energy stores to initiate reproductive functions (38). Increases

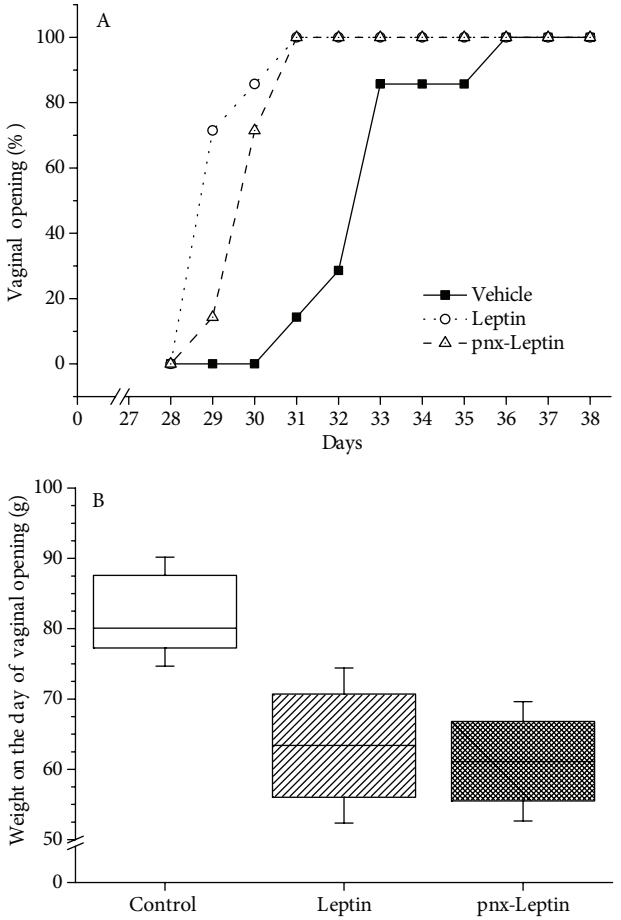

Figure 1. Leptin advances onset of puberty in sham and pinealectomized rats. A) Cumulative percentage of animals showing vaginal opening. B) Median body weights on the day of vaginal opening in control (n = 7) and leptin-treated sham (n = 7) and pinealectomized (n = 7) rats. *: P < 0.01 vs. control-treated controls.

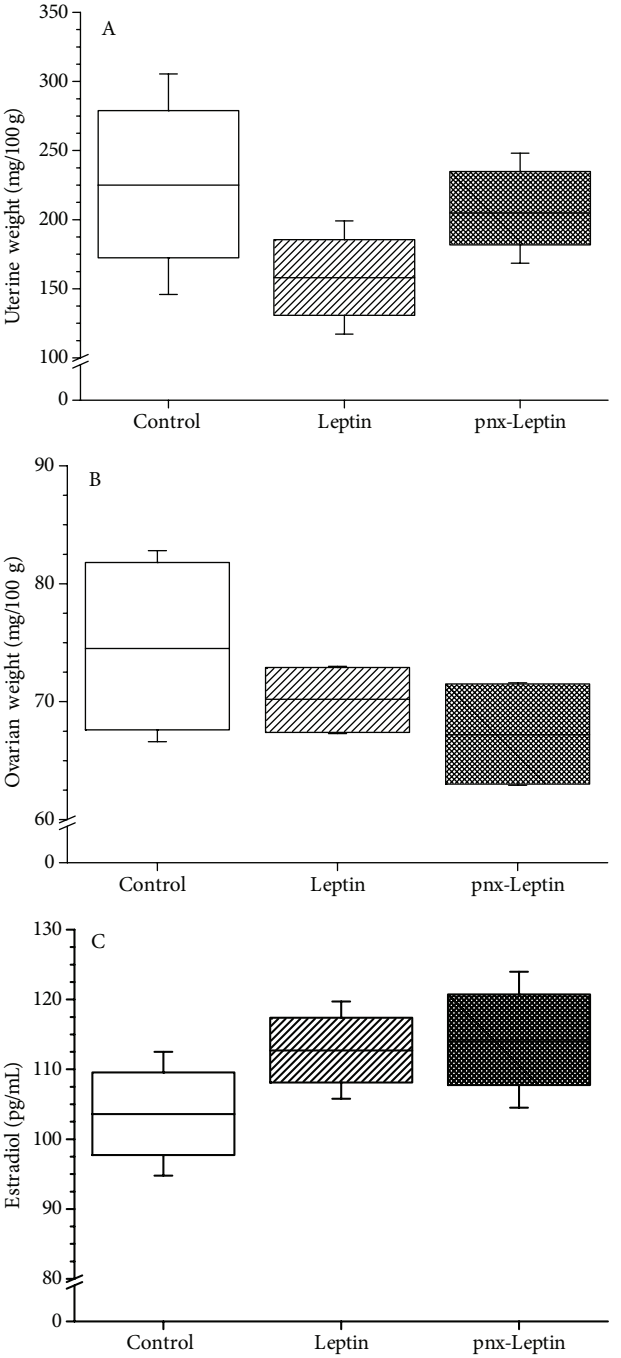

Figure 2. Leptin did not affect the postpubertal reproductive parameters studied. A) Uterine weight in control and leptin-treated sham and pinealectomized rats. B) Ovarian weights in control and leptin-treated sham and pinealectomized rats. C) Estradiol levels in control (n = 7) and leptin-treated sham and pinealectomized rats (n = 7 for all groups).

in serum leptin levels have been observed around the beginning of the menarche (39) or the estrous cycle (40). Therefore, it is generally accepted that leptin is essential in the induction of puberty in the female.

It has been suggested that intracerebroventricular infusion of leptin into the brain accelerated the onset of puberty; however, peripheral administration of this hormone only prevented delayed puberty caused by insufficient feeding in rats (41). It has been reported that leptin caused early onset of puberty in mice fed with a normal diet (14). In view of these findings, it is thought that leptin may directly initiate the onset of puberty as well as acting as a permissive metabolic signal in this process.

In the present study, leptin administration was started before weaning and animals were continuously given leptin for 28 days. In order to examine melatonin modulation of leptin's effects on pubertal maturation, pinealectomies were performed on a group of rats to minimize melatonin secretion in the present study. Thus, increased release of leptin and reduced secretion of melatonin were induced, similar to the physiological profiles of these hormones

around the onset of puberty. VO, one of the morphological signs of puberty, was observed early in rats receiving leptin, with or without pinealectomy. We have thus shown that peripheral administration of leptin may accelerate the onset of puberty in young female rats with access to normal unrestricted food intake.

The onset of puberty was determined to be on days 29 and 30 in the sham leptin and PNX leptin groups, respectively, and on day 33 in the intact control group. Body weight in the sham leptin and PNX leptin groups was lower than in the intact control group when vaginal opening was observed. Exogenous leptin infusion was presumed by the brain to show that the adipose tissue was developed enough to provide the energy needed to initiate puberty, although body weight, and thus fat tissue, was not at a level critical enough to release leptin in these animals. Another interesting finding observed in this study was that removal of the pineal gland did not cause any significant change in leptin's action. The onset of puberty already occurred in a physiologically early phase; therefore, it would be biologically difficult to expect an even earlier onset of puberty as a result of removal of the pineal gland. In addition, melatonin is known to be secreted by extrapineal tissues (42), such that melatonin may still be able to prevent early onset of puberty despite the pinealectomy.

In our study, serum estradiol values and uterine and ovarian weights did not significantly change among the groups. Since all animals were sacrificed on the day of estrus, no change would be expected in terms of serum estradiol levels. It has been reported by others (14) that leptin did not significantly affect the weights of the uterus or ovaries.

In view of the present results, we suggest that: 1) peripheral leptin administration before weaning causes early induction of puberty, 2) leptin infusion may cause sexual maturation even though body weight has not reached normal pubertal weight, and 3) removal of the pineal gland appears not to affect leptin's action on the onset of puberty.

Acknowledgments

This work was supported by a grant from TÜBİTAK (Project No. 107T825).

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