Photoperiodic Regulation of Leptin Synthesis in Anterior Pituitary Gland of Rats

Sıçan Ön Hipofiz Bezinde Leptin Sentezinin Fotoperiyodik Regülasyonu

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Abstract
Purpose: The goal of this study was to perform an immunohistochemical evaluation, investigate the effects of photoperiod on leptin synthesis in the rat anterior pituitary cells.
Material and Methods: For this purpose, 21 male Wistar rats were used. Animals were divided into three equal groups. Control rats in group I were kept under 12 hrs light: 12 hrs dark conditions (12L: 12D) for 10 weeks. Animals in group II were exposed to long photoperiods (18L: 6D), while rats in group III were exposed to short photoperiods (6L: 18D) for 10 weeks. At the end of experimental period, all animals were killed by decapitation. The pituitary glands of all rats were removed and processed for semi-quantitative evaluation of immunohistochemical leptin staining. Intensity of immunostaining was determined on a scale between 0 (no staining) and 5 (heavy staining). Statistical analysis was performed using the Kruskal-Wallis and Dunn's tests.
Results: Immunostaining of leptin was moderate (3+) in control rats, strong (4+) in rats exposed to long photoperiods and minimal (1+) in animals exposed to short photoperiods, respectively. The present immunohistochemical findings suggest that leptin production in anterior pituitary cells increase after exposure to long photoperiods and decrease after exposure to short photoperiods.
Conclusion: In view of the present findings, photoperiod seems to have effects on leptin synthesis in the anterior pituitary gland of rats.

Key Words: **Leptin; Photoperiod; Pituitary gland; Rat.**

Özet
Amaç: Bu çalışmada, fotoperiyodun ön hipofizdeki leptin sentezi üzerine olan etkilerinin immunohistokimyasal olarak incelenmesi amaçlandı.
Gereç ve Yöntem: Bu amaçla, 21 adet Wistar-Albino cinsi erkek sıçan kullanıldı. Hayvanlar üç eşit gruba ayrıldı. I. gruptaki kontrol sıçanlar, günün 12 saati ışık ve 12 saati karanlık ortamda tutuldu. II. gruptaki hayvanlar uzun süreli fotoperiyoda maruz bırakılırken (18 saat ışık - 6 saat karanlık), III gruptaki sıçanlar ise kısa süreli fotoperiyoda tabi tutuldu (6 saat ışık - 18 saat karanlık). 10 haftalık deney süresi sonunda bütün sıçanlar dekapitasyon yöntemiyle öldürüldü. Daha sonra sıçanların hipofiz bezleri çıkartılarak yarı-kantitatif değerlendirme amaçlı immunohistokimyasal leptin boyaması işlemlerinden geçirildi. Boyanma şiddeti 0 (boyanma yok) ile 5 (aşırı boyanma) değerleri arasında derecelendirildi. İstatistiksel analiz için Kruskal-Wallis ve Dunn testleri kullanıldı.
Bulgular: Kontrol grubuna ait sıçanların ön hipofiz bezinde orta yoğunlukta (3+) leptin boyanması tespit edilirken, uzun süreli ışığa maruz kalan sıçanlarda boyanmanın yoğun (4+) olduğu, kısa süreli ışığa maruz kalan sıçanlarda ise minimal seviyelerde (1+) olduğu belirlendi. Elde ettiğimiz bu immunohistokimyasal bulgular; ön hipofiz bezindeki leptin sentezinin uzun süreli fotoperiyod sonrası arttığını, kısa süreli fotoperiyod sonrası ise azalmış olduğunu gösterdi.
Sonuç: Bu bulgular ışığında, fotoperiyodun sıçan ön hipofiz bezindeki leptin sentezini etkilediği görülmektedir.

Anahtar Kelimeler: **Leptin; Fotoperiyod; Hipofiz bezi; Sıçan.**

Introduction

Leptin is a 167-aminoacid protein transcribed from obese gene (ob) and produced primarily by adipose tissue (1). It is believed to play an important role in the regulation of metabolic efficiency, energy expenditure and food intake (2, 3). Besides its well-known role in body weight homeostasis, increasing evidence suggests that leptin may also play a significant role in the regulation of metabolism (4), sexual development (5), reproduction (6), immunity (7), gastrointestinal functions (8), sympathetic activation (9) and angiogenesis (10).

Leptin is secreted mainly by the white adipose tissue. However, it has been reported that there is leptin synthesis in anterior pituitary cells (11, 12). This hormone may act as the critical link between adipose tissue and the reproductive system, indicating whether adequate energy reserves are present for normal reproductive function (13). Leptin induces pituitary cells to synthesize and secrete both LH and follicle – stimulating hormone (FSH) bringing about the onset of puberty (14). It also has a direct effect on the pituitary to enhance growth hormone-releasing hormone (GHRH) -induced growth hormone (GH) secretion (15). In the rat anterior pituitary gland, there are paracrine relationships between leptin-producing cells and cells with leptin receptor (leptin-R) that may regulate the function of GH cells (16). Leptin has an acute stimulatory effect on TSH release in vivo, acting probably at the hypothalamus. However, the direct pituitary effect of leptin is inhibitory and data also provide evidence that leptin may act as an autocrine/paracrine inhibitor of TSH release in the rat pituitary (17).

Photoperiod (day length) is a potent environmental stimulus that affects mammalian endocrine system, clearly visible in secretion of hormonal products of the pituitary and adrenal glands, but most prominent in the function of the pineal gland. Photoperiod has also been suggested to have a role in leptin synthesis. It has been shown that long photoperiods increase and short photoperiods decrease plasma leptin levels (18-21). However, to the best of our knowledge, there is no experimental study concerning the effects of photoperiod on the leptin production in pituitary cells.

Therefore, in the present study, we have immunohistochemically investigated the effects of photoperiod on the leptin synthesis in anterior pituitary gland.

Materials and Methods

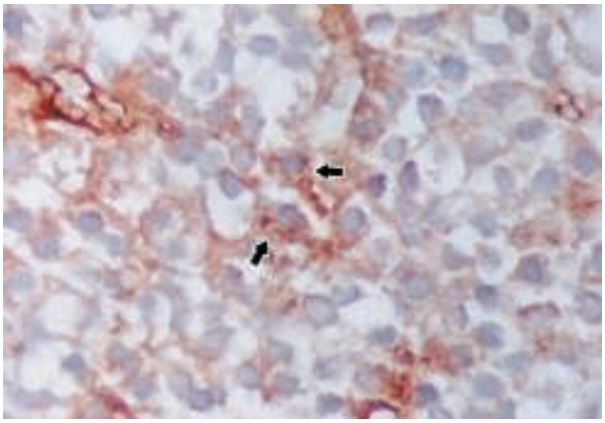
Animals and Experiment Design. Adult male Wistar rats (weighing 180-200 g, n = 21) were used in this study. The animals were randomly divided into three groups, each including seven rats. Control rats in Group I were kept under 12 hrs light: 12 hrs dark conditions (12L:12D) for 10 weeks. Animals in Group II were exposed to long photoperiods (18 hrs light: 6 hrs dark conditions, 18L:6D), while rats in Group III were exposed to short photoperiods (6 hrs light: 18 hrs dark conditions, 6L:18D) for 10 weeks. All animals received humane care in compliance with the European Community Guidelines on the care and use of laboratory animals (86/609/EEC). During the whole experiment the animals were kept at a constant temp (21±1 ^{o}C). Food (standard pellet diet) and tap water were supplied ad libitum.

The animals were killed by decapitation at the end of the experiments. The pituitary glands of all rats were removed and fixed in Bouin's solution. The specimens were embedded in paraffin and serially sectioned (thickness, 5m).

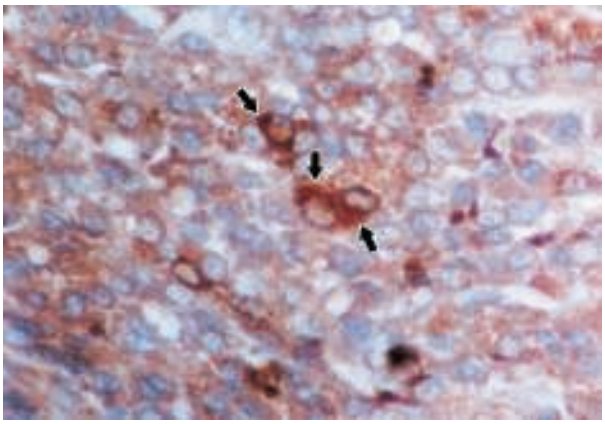
Immunohistochemical Procedure. Avidin-biotin-peroxidase technique was used for determination of leptin protein expression in this study. Five µm paraffin sections were dewaxed in xylene, treated with 0.1% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase, blocked with 10% normal goat serum in PBS for 20 minutes and incubated overnigth 4 ºC with Leptin Ob (A-20) Rabbit polyclonal IgG antibody (Santa Cruz, California). Sections were then incubated with biotinylated goat anti-rabbit IgG for 30 minutes, followed by avidine-peroxidase for 30 minutes and treated with 0.5 mg/ml diaminobenzidine with 0.1% hydrogen peroxide until the brown reaction product was obtained. Finally sections were counterstained with hematoxylin, dehydrated in alcohol, cleared in xylol and mounted. Sections were viewed and photographed with BH2 Olympus photomicroscope.

Immunohistochemical leptin staining of the cytoplasm of anterior pituitary cells was evaluated semi-quantitatively in all sections by an experienced observer unaware of the study design. Intensity of immunostaining was scored as follows: no staining (0), minimal (1+), mild (2+), moderate (3+), strong (4+), heavy (5+).

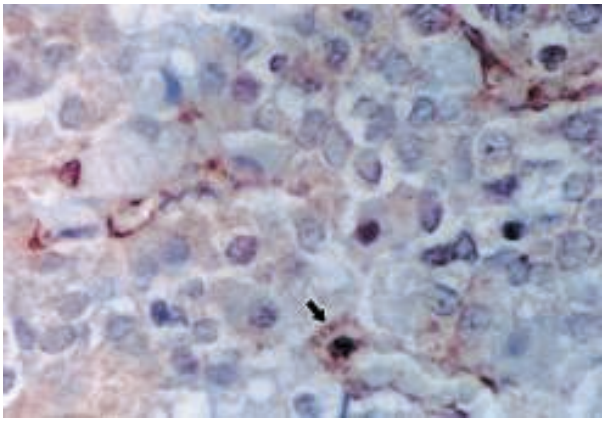
Statistical Analysis. All values are expressed as median (minimum–maximum). Differences between groups were



Picture 1. Immunohistochemical staining of leptin protein in pituitary gland of control rats, showing moderate levels of leptin in the cytoplasm of anterior pituitary cells (arrows). (X40).



Picture 2. Immunostaining of leptin in anterior pituitary gland of rats exposed to long photoperiods, showing strong leptin staining in the cytoplasm of anterior pituitary cells (arrows). (X40).



Picture 3. In the pituitary gland of rats exposed to short photoperiods, minimal leptin staining was seen in the cytoplasm of anterior pituitary cells (arrow). (X40).

assessed by Kruskal-Wallis test, followed by Dunn's multiple-comparison post hoc analysis. Statistical analyses were performed by SPSS software for Windows (version 15.0; SPSS Inc, Chicago, Illinois, USA). P values of <0.05 were considered statistically significant.

Results

In the process of immunohistochemical staining that was performed in order to have the leptin protein visible in anterior pituitary cells and to investigate it, an evaluation was made according to the density of observed staining. The more leptin antigen (leptin protein) present in the cell, the more binding will occur and as a result, the darker staining will be seen.

Immunostaining of leptin protein was moderate (3+) in control rats (Picture 1), strong (4+) in rats exposed to long photoperiods (Picture 2) and minimal (1+) in animals exposed to short photoperiods (Picture 3), respectively. By statistical analysis, there was a remarkable difference in leptin protein expression among the groups. All p values were significant (<0.001). The median (min-max) scores in controls and rats exposed to long and short photoperiods were respectively 3 (2-5), 1 (0-3) and 4 (2-5).

In sum, leptin synthesis in anterior pituitary cells increased after exposure to long photoperiods and decreased after exposure to short photoperiods.

Discussion

Leptin is secreted by white adipose tissue, and a rising level of leptin as triglyceride stores increase is proposed to serve as a negative feedback signal to the brain, resulting in decreased food intake, increased energy expenditure and resistance to obesity (22-24). In addition to this primary role, circulating leptin appears to play an important role in the neuroendocrine axis (25), including regulation of reproductive functions (26, 27).

The effects of photoperiod on plasma leptin levels have been shown in various mammalian species. Bocquier et al. (18) reported that leptin hormone is regulated by daylight. While they found an increase in leptin synthesis and lipogenic activity in long photoperiods, they observed low leptin levels during the short days in their investigation. Similarly, Klingenspor et al. (19, 20) showed that leptin gene expression and hormone release in white adipose tissue were diminished during winter in Syrian hamsters. In the same studies by Klingenspor et al. (19, 20), a decrease in leptin production after exposure of artificial short

photoperiods was determined. The changes of leptin levels in circulation are related to long and short photoperiods due to melatonin secretion by the pineal gland. Because, melatonin is released during the dark phase of the day and melatonin inhibits leptin production by the white adipose tissue (28-31).

On the other hand, although leptin is produced mainly by adipose tissue, it has been reported that there is leptin production in human (11), rat and mouse anterior pituitary cells (12). The results of the present study have confirmed leptin production in the rat anterior pituitary, as previously reported by Jin et al. (12). Leptin is synthesized and stored within the pituitary gland and suggested to modulate secretion of other pituitary hormones. Pituitary leptin has been therefore suggested to be a novel paracrine regulator of pituitary function (32). Colocalization studies with leptin and anterior pituitary cells showed that 70% of ACTH cells are positive for leptin, 21% of GH cells, 29% of LH cells, 33% of FSH cells, 32% of TSH cells, 64% folliculo-stellate cells whereas very few PRL cells (3%) were positive (33). Leptin is also expressed in TSH cells of rat anterior pituitary (12).

Leptin has been shown to have many functions in the anterior pituitary. It directly influences GH regulation at the pituitary level (34). Leptin has also a stimulatory effect on LH release in the pituitary both in vivo and in vitro (35, 36). All of the anterior pituitary cell types express the leptin receptor. However, leptin has been localized in specific subtypes of anterior pituitary cells indicating cell type-specific production of leptin in the anterior pituitary (37). To the best of our knowledge, there is no experimental study concerning the photoperiodic regulation of leptin synthesis in anterior pituitary cells. Thus, we found that long photoperiods increased and short photoperiods decreased leptin production in anterior pituitary cells of rats.

In conclusion, according to our immunohistochemical data, it is suggested that photoperiod (day length) may have an important role in the regulation of leptin synthesis in anterior pituitary.

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