

## Effects of Pinealectomy on the Circadian Release Pattern of Leptin in Male Rat

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### Abstract

**OBJECTIVES:** Exogenous and endogenous melatonin decrease leptin release. It is not known whether melatonin also has an effect on circadian release pattern of leptin. So, this study was planned to investigate the possible changes in the circadian release of leptin following pinealectomy.

**METHODS:** A group of rats were surgically pinealectomized while some others were exposed to sham operation. The animals were decapitated at 13.30 p.m. and 01.30 a.m. Serum leptin levels were measured by radioimmunoassay.

**RESULTS:** Serum leptin levels at 13.30 p.m. were lower than the values at 01.30 a.m. in both pinealectomized ( $P < 0.002$ ) and sham rats ( $P < 0.001$ ). Serum leptin levels measured at 13.30 p.m. and 01.30 a.m. were significantly elevated ( $P < 0.05$  and  $P < 0.01$ , respectively) in the pinealectomized rats in comparison to sham animals.

**CONCLUSION:** The circadian release of leptin does not seem to be regulated by melatonin release from the pineal gland whereas melatonin, physiologically released, may have a decreasing effect on leptin release.

## Introduction

The pineal gland and its main hormone melatonin (N-acetyl-5-methoxytryptamine) are known to be involved in a variety of physiological processes including regulation of endocrine rhythms [1], antigonadotropic effects [2, 3], neuroprotective effects [4] and stimulation of immune function [5]. There is also evidence that melatonin may regulate smooth muscle tone as well [6]. Besides these functions, it has been recently suggested that melatonin may have an effect on leptin release. It has been shown that melatonin administration suppresses plasma leptin levels in the rat [7, 8, 9]. Both melatonin and leptin are released in a circadian pattern, being higher at night times. It is not known whether the circadian release pattern of leptin is regulated by pineal gland or melatonin. So, this study was planned to investigate the possible changes in the circadian release of leptin following pinealectomy.

## Materials and methods

Adult male Wistar rats (Firat University Biomedical Unit, Elazig) were employed in this study. They were housed under controlled light (12 light and 12 dark hours) and temperature ( $21 \pm 1^\circ\text{C}$ ) conditions. The lights on was 07:00. Food and water were supplied *ad libitum*. The food contained crude matter (93.69%) consisting of fish flour, corn, wheat, barley and minerals. All the protocols in the present study were approved by the local ethics committee in the Medical School. The animals were divided into two main groups. Some rats ( $n=12$ ) were surgically pinealectomized while others ( $n=12$ ) were exposed to sham-operation. Pinealectomy was surgically performed under general anesthesia with ketamine 60 mg/kg plus xylazine (rompun) 5 mg/kg. The area of the dorsal surface of the brain around the confluence of the transverse and sagittal sinuses was exposed and the dura mater ruptured at a point just lateral and anterior to the sinus confluence. Fine forceps were then inserted beneath the confluence at an angle of  $60^\circ$  to the horizontal and withdrawn enclosing the pineal, thus rupturing the pineal stalk. A second group underwent sham operations which consisted of a similar procedure, but the forceps were kept closed during the insertion so that no tissue was removed. The rats were allowed to recover for a period of three weeks. At the end of the experiments, each group was divided into two subgroups, each consisting 6 animals. All animals were decapitated between either at 13.30 p.m. or 01.30 a.m. and trunk blood obtained into tubes. The serum samples were stored at  $-20^\circ\text{C}$  until assayed.

Serum leptin levels were determined by radioimmunoassay [10]. Rat Leptin RIA Kit (Linco Research, Inc., St. Charles, Missouri 63304 USA) was used. The antiserum was rat leptin antibody, which was produced in guinea pig. The body weights of the animals were determined at the beginning and the end of the experiment. The data were statistically analyzed by One-Way analysis of variance (ANOVA). Level of significance was set at  $P < 0.05$ . Results were expressed as ng/ml and presented as Mean  $\pm$  S.D.

## Results

Serum leptin levels are shown in Fig.1. The pinealectomized and sham-operated rats had higher ( $P < 0.002$  and  $P < 0.001$ , respectively) serum leptin levels at night, being  $3.75 \pm 0.22$ ;  $2.39 \pm 0.12$  ng/ml at 01.30 and  $1.85 \pm 0.08$ ;  $1.26 \pm 0.13$  ng/ml at 13.30, respectively. Serum leptin levels measured at 13.30 p.m. and 01.30 a.m. were significantly elevated ( $P < 0.05$  and  $P < 0.01$ , respectively) in the pinealectomized rats in comparison to sham animals.

There were no significant differences between the average values of the body weights of the groups, being  $235.58 \pm 13.00$  and  $231.40 \pm 12.10$  g at the beginning of the experiment and  $261.50 \pm 15.25$  and  $253.60 \pm 13.20$  g at the end of the experiment in the pinealectomized and sham-operated groups, respectively. The weight gains were 9.7% and 11.20% in the pinealectomized and sham groups, respectively.

## Discussion

Melatonin [11] and leptin [12] are known to be secreted in a circadian pattern, being higher at night and lower during the day. Biological clock control of the sympathetic input to the adipocyte [13] and the pineal gland [14] are essential for regulation of the daily rhythm in leptin and melatonin release, respectively. Melatonin not only informs the brain about the environmental lighting and darkness but may be part of an integrative system to coordinate reproductive, immunologic, and other physiological processes to cope successfully with energetic stressors during winter [15]. Leptin gives signals the brain about the energy status of the body. Leptin also signals nutritional status to several other physiological systems and modulates their function [16].

The previous studies show that melatonin may affect leptin release. Pinealectomy has also been showed to affect leptin release. Our hypothesis was that the circadian release pattern of leptin might be regulated by the pineal gland. So, serum leptin levels were measured during the day and at night following pinealectomy. Our results show that pinealectomy does not change circadian release pattern of leptin.

But, serum leptin levels were higher in the pinealectomised rats than in the sham group during the day and at night.

A similar leptin-decreasing effect of melatonin was observed in one study [Rasmussen *et al.*, 1999] in which only intact rats were used. So, the present experiment provides indirect evidence that melatonin has an inhibitory role in the release of leptin. The mechanism by which pinealectomy increases decreases leptin release remains to be determined in the rat.

One question to be answered is why the pinealectomized rats had higher leptin levels compared to sham operated ones during the day? It has been shown that the pineal gland is the main melatonin source in the body. After pinealectomy, melatonin is usually reduced to undetectable level in the serum. So, melatonin is not expected to affect leptin release following pinealectomy during the day. The other pineal factors may contribute melatonin's leptin-decreasing effect. Thus, the pineal gland itself may have an inhibitory role in the leptin release.

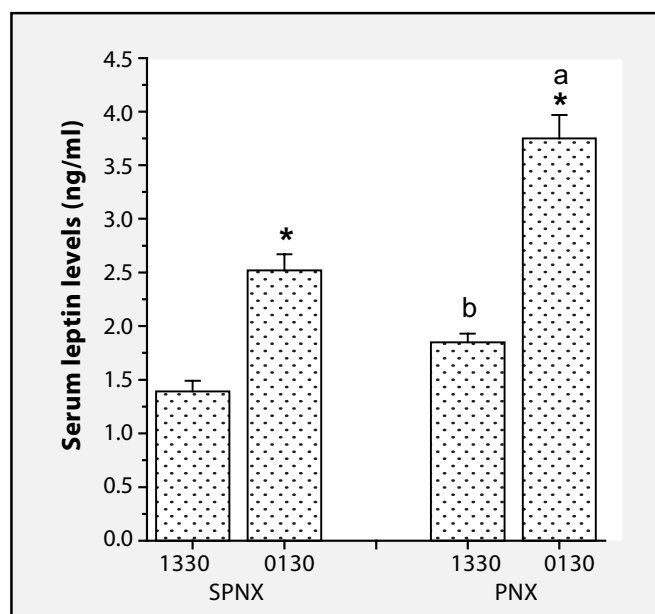
The average values of the body weights of the pinealectomized and sham operated rats did not show any significant changes. Therefore, the differences in the leptin release may not be attributed to the changes in the body weights or fatness.

There may be a functional antagonism between melatonin and leptin. Leptin is known to reduce appetite and causes hypophagia [17]. In contrast, melatonin has been shown to have an hyperphagic effect [18]. Melatonin may exert its hyperphagic effect by reducing leptin release. The fact that night eaters had lower nocturnal rise in both melatonin and leptin levels show there may be a different interaction between human being and rodents. To make sure, melatonin levels need measuring in this kind of studies. Thus, the real interaction between melatonin and leptin levels may be determined.

In conclusion, this is the first report that the circadian release of leptin is not seen to be directly controlled by the pineal gland although there is an increase in the leptin release following pinealectomy. Further studies are needed to determine the interaction between the pineal gland and leptin release.

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**Fig. 1.** Serum leptin levels in pinealectomised (PNX) and sham-operated (SPNX) animals during the day (1330h) and night (0130h). \*  $P < 0.002$  and  $P < 0.001$  as compared to 1330h values in PNX and SPNX groups, a  $P < 0.05$  and b  $P < 0.01$  vs. SPNX values.

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