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European Journal of Pharmacology 428 (2001) 145–148

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Short communication

Effects of pinealectomy and exogenous melatonin on serum leptin levels in male rat

Sinan Canpolat, Suleyman Sandal, Bayram Yilmaz, Abdullah Yasar, Selim Kutlu, Giyasettin Baydas, Haluk Kelestimur *

Department of Physiology, Medical School, Firat University, 23119 Elazig, Turkey

Received 9 May 2001; received in revised form 17 July 2001; accepted 20 July 2001

Abstract

The effects of pinealectomy and exogenous melatonin (*N*-acetyl-5-methoxytryptamine) on serum leptin levels were investigated in rats. Exogenous administration of melatonin to intact rats resulted in significant decreases in serum leptin levels ($P < 0.05$) compared to those of the intact control group. Serum leptin levels were significantly elevated in the pinealectomised rats in comparison to the sham-pinealectomised animals ($P < 0.001$) and were significantly suppressed by exogenous administration of melatonin compared to those of non-treated pinealectomised rats ($P < 0.001$). Hormone concentrations in the melatonin-treated pinealectomised group were found to be similar to those seen in the sham-pinealectomised group. These results suggest that pineal gland has an effect on leptin release.

Keywords: Leptin; Melatonin; Pinealectomy; (Rat)

1. 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 (Forsling et al., 1993), antigonadotropic effects (Yilmaz et al., 2000; Kus et al., 2000), neuroprotective effects (Kilic et al., 1999) and stimulation of immune function (Guerrero and Reiter, 1992). There is also evidence that melatonin may regulate smooth muscle tone as well (Ayar et al., 2000). Besides these functions, it has been recently suggested that melatonin may have an effect on leptin release. A few studies have yielded contradictory results on the possible relationship between melatonin and leptin release. It has been shown that daily melatonin administration suppresses plasma leptin levels in the rat (Rasmussen et al., 1999; Wolden-Hanson et al., 2000). In one study (Mastronardi et al., 2000), melatonin (1 mg/rat) was found to lower leptin levels at night (11:00 p.m.) but not significantly, and to have no effect on leptin release in late morning (11:00 a.m.) in the rat. In contrast to these studies, in another study, melatonin has been shown to elevate plasma leptin concentrations in the mink, the reproduction of which strongly depends on seasonality and photoperiodism, unlike laboratory rodents or humans (Mustonen et al., 2000).

The present study was designed to determine whether or not pinealectomy and exogenous melatonin administration to pinealectomised rats have an effect on serum leptin levels.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 250–300 g (Firat University Biomedical Unit, Elazig) were used in this study. They were housed under controlled light (12-h light and 12-h dark) and temperature (21 ± 1 °C) conditions. 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

* Corresponding author. Tel.: +90-424-237-0000x3286; fax: +90-424-237-9141.

E-mail address: hkelestimur@firat.edu.tr (H. Kelestimur).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01230-4

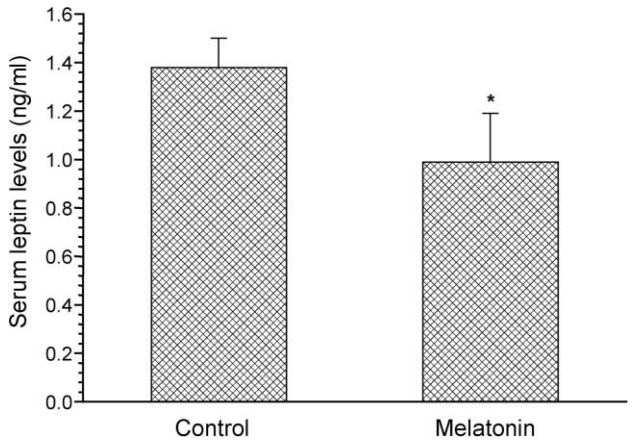

Fig. 1. Serum leptin levels (mean ± S.D.) in intact control and melatonin-administered male rats. * $P < 0.05$ compared to the control group, one-way ANOVA.

study were approved by the local ethics committee of the Medical School.

2.2. Study design

The animals were divided into five groups. Intact controls (group I, $n = 10$) received saline alone (1 ml/kg). Another group of intact animals (group II, $n = 8$) was subcutaneously (s.c.) injected with daily melatonin (0.5 mg/kg/day) for 7 days. In the second experimental model, sham pinealectomy was performed in rats (group III, $n = 8$). Sixteen rats were pinealectomised and allowed to recover for a period of 2 months or longer. Half of these animals were assigned to the pinealectomised group (group IV, $n = 8$), and the remaining pinealectomised rats (group V, $n = 8$) received daily injections of melatonin (0.5 mg/kg/day, s.c.) for 7 days.

At the end of the experiments, all animals were decapitated between 09:00 and 10.00 a.m. and trunk blood collected into tubes. The serum samples were stored at −20 °C until assayed.

2.3. Pinealectomy and melatonin administration

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 was 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° to the horizontal and withdrawn enclosing the pineal gland, thus rupturing the pineal stalk. A second group underwent a sham operation, which consisted of a similar procedure, but the forceps were kept closed during the insertion so that no tissue was removed.

Melatonin (Sigma, St. Louis, MO, USA) was dissolved in ethanol and further diluted in saline to give a final ethanol concentration of 5%. Melatonin injection was performed between 9:00 and 10:00 a.m.

2.4. Leptin assay

Serum leptin levels were determined by radioimmunoassay (Ma et al., 1996), using the Rat Leptin RIA Kit (Linco Research, St. Charles, MO 63304, USA). The antiserum was rat leptin antibody, which was produced in guinea pigs.

2.5. Statistics

The data were statistically analysed by one-way analysis of variance (ANOVA). Level of significance was set at $P < 0.05$. Results are expressed as ng/ml and presented as means ± S.D.

3. Results

Serum leptin levels are shown in Figs. 1 and 2. Exogenous administration of melatonin to intact rats resulted in significant decreases (0.98 ± 0.22 ng/ml) in plasma leptin levels ($P < 0.05$) compared to those of the intact control group (1.38 ± 0.12 ng/ml).

Serum leptin levels were significantly elevated ($P < 0.001$) in the pinealectomised rats (2.70 ± 0.3) in comparison to the sham-pinealectomised animals (1.48 ± 0.3). They were significantly ($P < 0.001$) suppressed by exogenous administration of melatonin (1.05 ± 0.33) compared to those of non-treated pinealectomised rats. Hormone

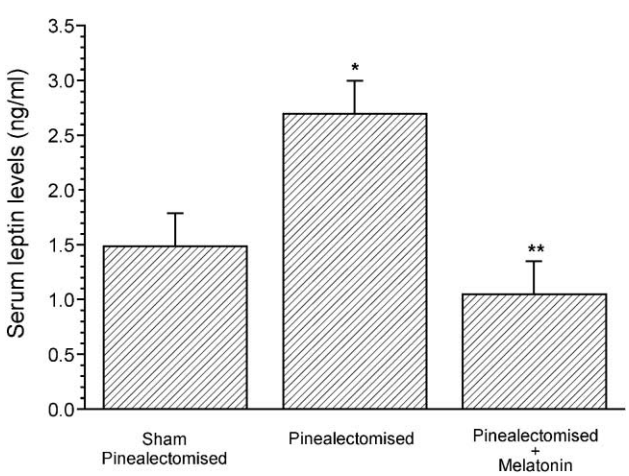

Fig. 2. Serum leptin levels (mean ± S.D.) in sham-pinealectomised, pinealectomised and pinealectomised + melatonin-treated male rats. * $P < 0.001$ compared to the sham-pinealectomised group; * * $P < 0.001$ compared to pinealectomised group, one-way ANOVA.

concentrations in the melatonin-treated pinealectomised group were found to be similar to those seen in the sham-pinealectomised group.

4. Discussion

Our results show that pinealectomy increases leptin release. The administration of exogenous melatonin to pinealectomised rats reversed this effect. Exogenous melatonin decreased the serum levels of leptin in both pinealectomised and intact rats. A similar leptin-decreasing effect of melatonin was observed in a study (Rasmussen et al., 1999) in which only intact rats were used. So, the present experiment provides further evidence that melatonin has an inhibitory role on the release of leptin. The mechanism by which pinealectomy increases and exogenous melatonin decreases leptin release remains to be determined in the rat. A recent preliminary study (Blask et al., 1999) reported that melatonin may have a direct effect on adipocytes. In that study, physiological levels of melatonin were reported to block fatty acid transport via a melatonin receptor-mediated mechanism. Leptin is known to be produced in adipocytes in response to nutrient cycling (Wang et al., 1998).

Thus, melatonin may decrease in the release of leptin into the circulation by blocking fatty acid transport into the adipocytes. After pinealectomy, leptin release into the circulation may increase due to an increased fatty acid transport into adipocytes. It has been shown that melatonin given in drinking water for 12 weeks suppresses plasma leptin levels independent of total body fat (Wolden-Hanson et al., 2000). In our study, melatonin was given s.c. for only 7 days. During this time, a change in the amount of total body fat was not expected. So, both acute and chronic application of melatonin can be said to decrease leptin release without affecting total body fat.

It has been reported that exogenous melatonin elevates leptin levels in the mink, the reproductive function of which strongly depends on seasonality and photoperiodism (Mustonen et al., 2000). The best-known function of melatonin is to provide photoperiodic information. Melatonin may have a role in regulating the energy balance and fat distribution of species in which reproductive, immunologic and other physiological adaptations are mediated by changes in the photoperiod (Nelson and Demas, 1997). The relationship between melatonin and leptin release seems to be different in seasonally and annually breeding animals.

There seems to be a functional inverse relationship between melatonin and leptin. It has been reported that melatonin inhibits gonadotropin release at the hypothalamic level (Yilmaz et al., 2000). In contrast, leptin is suggested to stimulate gonadotropin secretion and to induce puberty (McCann et al., 1998). The failure of the pineal gland to produce sufficient melatonin causes precocious puberty (Waldhauser et al., 1991), whereas insufficient leptin release may result in pubertal delay (Wauters et al., 2000). Thus, sexual maturation seems to be signaled by a decrease and an increase in melatonin and leptin levels, respectively. There is also an inverse interaction in terms of the effect of ageing on the levels of both hormones (Matsumoto et al., 2000). The release of melatonin declines with ageing while leptin release increases with ageing.

In conclusion, pinealectomy and exogenous melatonin affect leptin release. Further studies are needed to determine the interaction between melatonin and leptin release.

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