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Preliminary Evidence That Pharmacologic Melatonin Treatment Decreases Rat Ghrelin Levels

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Ghrelin is a signal peptide isolated from rat stomach antagonistic to actions of leptin. Ghrelin stimulates the secretion of growth hormone (GH) and increases food intake, body mass, and adiposity in rodents. Photoperiod and melatonin regulate leptin secretion of mammals. The aim of the study was to investigate possible melatonin-ghrelin interactions in weight regulation by studying the effects of continuous pharmacologic melatonin treatment and constant light on plasma ghrelin, leptin, and GH levels in rats. Plasma ghrelin concentrations were significantly reduced by exogenous melatonin. Ghrelin levels correlated negatively with plasma leptin levels in control rats kept in 12 h of light/12 h of dark but not in the melatonin-treated animals. The inverse ghrelin-leptin relationship was also disrupted by constant illumination. The circulating ghrelin and GH levels may not be interrelated in all metabolic situations. The results suggest new interplay between the pineal gland and energy metabolism as well as reenforce the hypothesis that ghrelin is antagonistic to leptin.

Key Words: Ghrelin; growth hormone; leptin; melatonin; rat.

Introduction

Ghrelin is a peptide hormone of 28 amino acids isolated recently from rat stomach *(1)*. It is secreted by the gastrointestinal tract and the hypothalamus *(1,2)*. Ghrelin is an endogenous ligand for growth hormone secretagogue (GHS) receptors expressed, e.g., in the hypothalamus and pituitary gland *(3)*. GHSs are synthetic compounds stimulating the release of growth hormone (GH) in the pituitary *(4)*. Exogenous ghrelin increases circulating GH concentrations in rats *(5)* and humans *(6)*. Administration of ghrelin causes a dose-dependent increase in food intake and body mass gain in rodents *(7)*. Circulating ghrelin levels in the rat are increased by fasting and reduced by refeeding or administration of glucose. Ghrelin concentrations are suppressed in human obesity *(8)*.

Leptin is a peptide hormone secreted principally by the white adipose tissue of mammals *(9)*. Plasma leptin levels in humans and rodents correlate positively with body mass index (BMI) *(10)*. Leptin treatment causes weight reduction in genetically obese *ob*/*ob* mice *(11)*. Leptin is involved in the neuroendocrine response to fasting via neuropeptide Y (NPY), a potent activator of food intake. Decreasing leptin levels during periods of inadequate nutrition disinhibit the production of NPY *(12)*. High leptin levels, on the other hand, inhibit NPY production. Exogenous leptin increases the fasting-suppressed levels of rat GH by preventing the inhibitory action of NPY on GH secretion *(13)*.

Ghrelin antagonizes leptin action in the hypothalamus by activating the NPY pathway *(14,15)*. The decrease in food intake caused by exogenous leptin can be reversed by ghrelin treatment. This suggests that the decrease in leptin levels seen in fasting and the simultaneous increase in the circulating ghrelin concentrations both contribute to the increase in NPY production and food intake.

Melatonin is a hormone secreted mainly by the pineal gland in darkness *(16)* affecting, e.g., energy metabolism of mammals *(17)*. Melatonin decreases GH secretion of the rat pituitary in vitro *(18)*. Exogenous melatonin also suppresses circulating leptin levels in rats *(19)*. In the garden dormouse (*Eliomys quercinus*) *(20)* and in the mink (*Mustela vison*) *(21)*, however, melatonin increases leptin levels in adipose tissue and plasma, suggesting species-specific effects of melatonin on weight regulation of mammals. We have recently demonstrated interactions among melatonin, leptin, ghrelin, and GH in the raccoon dog (*Nyctereutes procyonoides*) *(22)*.

The aim of the present study was to investigate possible melatonin-ghrelin interactions in weight regulation by studying the effects of continuous melatonin treatment and constant light on the plasma ghrelin levels of the rat (*Rattus norvegicus*). The effects exerted by exogenous melatonin on leptin levels of mammals and the newly discovered interactions among melatonin, ghrelin, leptin, and GH suggest an interplay of these hormones in the regulation of energy homeostasis.

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Table 1
Mean Body Weights at Beginning and End of Study,
Cumulative Food Intake and Plasma Melatonin, Ghrelin, Leptin and GH Concentrations of Rats (Mean ± SE) [a]

	Control 12L:12D	Control 24L	Melatonin 12L:12D	Melatonin 24L
Weight at beginning of study (g)				
Males	262 ± 9.2[b]	265 ± 7.8[b]	264 ± 7.4[b]	265 ± 5.5[b]
Females	187 ± 4.4[a]	186 ± 3.8[a]	188 ± 4.5[a]	183 ± 5.7[a]
Weight at end of study (g)				
Males	333 ± 13.7[b]	337 ± 9.8[b]	338 ± 11.4[b]	333 ± 10.8[b]
Females	221 ± 6.6[a]	208 ± 4.4[a]	217 ± 5.6[a]	209 ± 5.1[a]
Cumulative food intake (g)				
Males	552 ± 28.7[b]	560 ± 11.1[b]	560 ± 22.7[b]	529 ± 19.7[b]
Females	404 ± 16.6[a]	396 ± 7.6[a]	382 ± 8.4[a]	394 ± 2.8[a]
Melatonin (pg/mL)	3.5 ± 0.46	4.6 ± 0.53	356.6 ± 22.78[b]	409.2 ± 35.19[b]
Ghrelin (ng/mL)	1.6 ± 0.14[b]	1.6 ± 0.06[b]	1.1 ± 0.12[a]	0.9 ± 0.08[a]
Leptin (ng/mL)	3.0 ± 0.44	2.8 ± 0.37	3.4 ± 0.62	2.4 ± 0.24
GH (ng/mL)	2.4 ± 0.36	2.8 ± 0.54	2.8 ± 0.30	2.8 ± 0.51

[a] When there is sexual dimorphism within a variable the data are presented separately for the males and females. Values with no common superscript symbol differ from each other (one-way ANOVA, post hoc Duncan's test, $p < 0.05$).
[b] Significant difference between the control and the melatonin-treated groups (Mann-Whitney U test, $p < 0.05$).

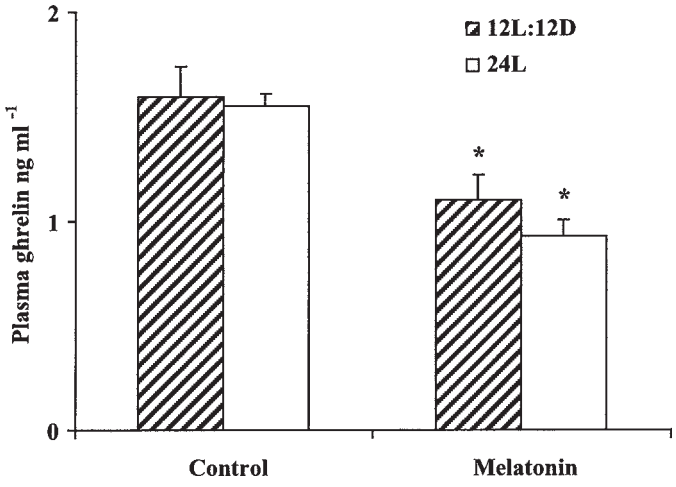

Fig. 1. The plasma ghrelin concentrations (ng/mL^{-1}) of the control and the melatonin-treated rats in 12L:12D or in 24L, all study groups consisted of 10 rats, *differs statistically from the both control groups ($p < 0.05$; ANOVA, post hoc Duncan's test).

Results

Plasma melatonin concentrations were higher in the melatonin-treated rats compared with the controls (Mann-Whitney U test, $p < 0.0004$; Table 1). Lighting condition had no effect on the daytime melatonin levels. Plasma ghrelin concentrations were suppressed by exogenous melatonin treatment (analysis of variance [ANOVA]; $p < 0.05$) while continuous light did not have any effect on the results (Fig. 1, Table 1). Plasma leptin and GH concentrations were not influenced by exogenous melatonin or continuous light (Table 1), nor were body weights, food intake, or BMIs.

Plasma ghrelin concentrations of the 12 h light/12 h dark (12L:12D)-maintained control rats correlated negatively with plasma leptin levels ($n = 10$, $r_s = –0.893$, $p < 0.01$), but there was no relation between these parameters in the melatonin-treated rats kept in 12L:12D ($n = 10$, $r_s = –0.231$, NS) or kept in 24L ($n = 10$, $r_s = –0.050$, NS) (Fig. 2). The correlation was also absent in the 24L-maintained control rats ($n = 10$, $r_s = –0.048$, NS). The leptin levels of the 24L-maintained rats ($n = 20$, $r_s = 0.529$, $p < 0.05$) but not of the 12L:12D-kept animals ($n = 20$, $r_s = –0.038$, NS) correlated positively with BMIs. Plasma ghrelin levels did not correlate with plasma GH concentrations or body adiposity.

Discussion

Plasma ghrelin concentrations of the rats were reduced by exogenous melatonin (Fig. 1). Continuous light, which abolishes the endogenous melatonin secretion rhythm *(23)*, did not have any effect on the results. This implies that circulating ghrelin concentrations and the effects of exogenous melatonin on them can be independent of the prevailing photoperiod. Previously, melatonin treatment has affected plasma ghrelin concentrations of the raccoon dog *(22)*. The principal site of ghrelin synthesis is the stomach *(1)*, which is also one of the locations of melatonin production and binding *(24)*. Another site of ghrelin synthesis, the hypothalamic arcuate nucleus *(1)*, exhibits melatonin-binding sites as well *(25)*. It remains unknown whether melatonin affects ghrelin secretion peripherally, centrally, or by both mechanisms. In our experiment, high peripheral melatonin levels resulted in a relatively small reduction (about 35%) in plasma ghrelin levels. The physiologic significance and mechanism of this interaction are unknown and will require future studies to be revealed.

Ghrelin has been reported to antagonize leptin action in the hypothalamus by activating the NPY pathway *(14,15)*.

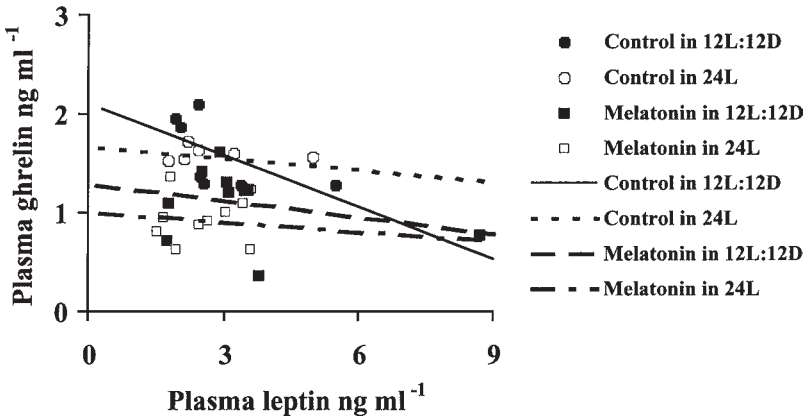

Fig. 2. The relation between the plasma ghrelin (ng/mL^{-1}) and leptin concentrations (ng/mL^{-1}) in 12L:12D kept control rats (●) (r_s = –0.893, $p < 0.01$), the 24L kept control rats (○) (r_s = –0.048, NS), the 12L:12D kept melatonin-treated rats (■) (r_s = –0.231, NS), and the 24L kept melatonin-treated rats (□) (r_s = –0.050, NS).

This is in concordance with our results revealing that the plasma ghrelin and leptin concentrations of the 12L:12D-maintained control rats correlated negatively with each other (Fig. 2). Exogenous melatonin treatment disrupted this relationship. The dissociation was probably caused by suppression of the ghrelin levels by exogenous melatonin, which did not affect plasma leptin concentrations. Continuous illumination also masked the relationship between the two hormones although it did not influence plasma ghrelin or leptin concentrations *per se*. In general, our results reenforce the hypothesis that ghrelin is antagonistic to leptin *(14,15)*.

Although the plasma ghrelin concentrations were decreased by exogenous melatonin, the levels of leptin and GH were not affected by the treatment. Exogenous melatonin reduces rat GH secretion in vitro *(18)*, and ghrelin treatment stimulates the release of GH from the rat anterior pituitary in vitro as well as in vivo *(1)*. Our results propose that changes in circulating ghrelin concentrations do not automatically cause a similar change in plasma GH levels (see also ref. *22*). Tschöp et al. *(7)* have also documented in GH-deficient dwarf rats that the metabolic changes induced by ghrelin can be independent of GH.

Rodent leptin levels have been reported to correlate with body adiposity *(10)*. In our experiment, only the leptin levels of the 24L-kept rats correlated with BMIs. Dissociation between leptin concentrations and body adiposity has, however, been observed in other species *(26)*. Melatonin has been reported to increase leptin gene expression in adipose tissue of the garden dormouse *(20)* and autumnal leptin levels of the mink *(21)*. Rasmussen et al. *(19)* have reported suppressed body mass and plasma leptin concentrations in rats with chronic administration of melatonin. The results of the present study indicate that exogenous melatonin does not subacutely affect circulating leptin levels or body adiposity of young rats. Melatonin probably has more pronounced effects on leptin levels of seasonal mammalian species owing to the importance of the pineal gland and melatonin in their weight regulation *(21,22)*.

In conclusion, our in vivo studies indicate that exogenous melatonin suppresses plasma ghrelin levels in the rat. The inverse ghrelin-leptin relationship is disrupted by melatonin treatment and constant light. The circulating ghrelin and GH levels are not interrelated in all metabolic situations. Further studies are needed to clarify the role of the melatonin-ghrelin interaction in the regulation of energy homeostasis.

Materials and Methods

Animals and Procedure

Barrier-bred Wistar rats (Kuo:WH, 20 males and 20 females) were purchased from the National Laboratory Animal Center of the University of Kuopio (Kuopio, Finland). The animals were conventionally maintained in a dark room with artificial illumination from 6:00 AM to 6:00 PM (12L:12D) at 20 ± 1ºC. They were housed singly in solid-bottomed plastic cages (Makrolon; 42 × 22 × 15 cm) with wood shavings for bedding and had free access to tap water and a pelleted commercial diet (Avelsfoder för råtta och mus R36; 18.5% raw protein, 4.0% raw fat, 1 260 kJ metabolizable energy 100 g^{-1}; Lactamin, Stockholm, Sweden). All procedures were in accordance with the institutional guidelines of the University of Joensuu and with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

The rats were 9 wk of age and weighed 163–203 (females) and 240–287 g (males) at the beginning of the experiment. On d 1, half the animals were maintained in the photoperiod of 12L:12D, and the randomly assigned other half was moved to continuous light (24L) to suppress melatonin secretion of the animals *(23)*. The rats of both lighting conditions were further divided into two randomly assigned groups. Half received sc melatonin implants, and the other half was sham operated. PRIME-X® melatonin implants containing 12 mg of melatonin in a silastic matrix manufactured by Wildlife Pharmaceuticals (Fort Collins, CO) were used. The capsules were administered with a sterile

syringe into the interscapular sc tissue. The release of melatonin from the implant was known to be without any 24-h rhythm. It has been documented in ewes that constant-release melatonin implants provide animals with a short-day signal without causing a functional pinealectomy *(27)*. We chose to use continuous-release implants to study the pharmacologic effects of melatonin without interference of the diurnal melatonin secretion rhythm.

The randomly assigned study groups were as follows: group 1: control rats in 12L:12D, group 2: control rats in 24L; group 3: melatonin-treated rats in 12L:12D; group 4: melatonin-treated rats in 24L. All the study groups consisted of five male and five female rats. A 4-wk study period was considered to be long enough to be able to observe possible changes in body wt and food intake induced by the treatments. The body wt and food intake of the rats were recorded every fifth day at 10:00 AM to 12:00 PM throughout the study. BMIs were calculated as follows: weight (kg) length^3 (m)$^{-1}$. At the end of the experiment, the rats were sacrificed at 10:00 AM to 2:00 PM by diethyl ether. Blood samples were obtained by cardiac puncture with aseptic needles into test tubes containing EDTA and centrifuged at 1000*g* to obtain plasma. The samples were immediately frozen in liquid nitrogen and stored at –40ºC.

Plasma ghrelin concentrations were measured with the Ghrelin (Human) RIA kit from Phoenix Pharmaceuticals (Belmont, CA). Human ghrelin is homologous to rat ghrelin apart from two amino acids *(1)*, and the crossreactivity of the human ghrelin kit to rat ghrelin is 100%. For measurement of plasma leptin concentrations, we used the Multi-species Leptin RIA kit developed by Linco Research (St. Charles, MO). Although the Multi-species Leptin RIA kit is not specific for the leptin of the rat, the crossreactivity to rat leptin is about 61%. The multi-species kit was chosen instead of the rat kit because leptin levels of several other animal species were analyzed at the same measurement. Plasma GH levels were measured with the Double Antibody Human Growth Hormone (hGH) kit from DPC (Los Angeles, CA) and plasma melatonin concentrations with the Melatonin RIA kit manufactured by DLD Diagnostika GmbH (Hamburg, Germany).

Statistical Analyses

Homogeneity of variances and normality of distribution were tested with the Levene test and Kolmogorov-Smirnov test, respectively. Multiple comparisons were performed with the SPSS program using one-way ANOVA followed by post hoc Duncan's test. For paired comparisons, we used the Student's *t*-test. For nonparametric data, the Mann-Whitney U test was performed. Correlations were tested with the Spearman correlation coefficient (r_s). The p value <0.05 was considered statistically significant.

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