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Effects of orexigenic peptides and leptin on melatonin secretion during different photoperiods in seasonal breeding ewes: An in vitro study

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Abstract

The pineal gland (PG) acts as a neuroendocrine transducer of daily and seasonal time through the nocturnal release of melatonin. Here, we examined the interaction of season, orexin, ghrelin, and leptin on melatonin secretion by pineal explants in short-term culture. Glands were collected after sunset from 12 ewes during long days (LD; April and May) and from an additional 12 ewes during short days (SD; October and November). Glands were transected sagittally into strips, with each equilibrated in 2.5 mL of Dulbecco's modified Eagle's medium for 60 min, followed by a 2-h incubation in control medium or medium containing orexin B (10 and 100 ng/mL), ghrelin (10 and 100 ng/mL), or 50 ng/mL of leptin. After a 3-h incubation, some PG explants treated previously with lower doses of orexin or ghrelin were challenged with 50 ng/mL of leptin and those treated with both doses of orexin were challenged with 300 nM of the β-agonist isoproterenol. One milliliter of medium was harvested and replaced from each well every 30 min. Treatment with the low dose of orexin during LD increased melatonin secretion about 110% ($P < 0.01$); treatment with a high dose increased melatonin secretion about 47% ($P < 0.001$). During the SD period, leptin stimulated ($P < 0.05$) melatonin secretion slightly compared with mean melatonin concentration in controls. However, together, orexin and leptin depressed ($P < 0.01$) melatonin secretion. Both doses of ghrelin reduced ($P < 0.01$) melatonin concentration during the SD season compared with control culture. Addition of ghrelin and leptin to culture medium increased ($P < 0.01$) melatonin concentration compared with ghrelin-treated culture and decreased melatonin concentration ($P < 0.01$) compared with leptin-treated culture during SD. Isoproterenol stimulated ($P < 0.01$) melatonin secretion compared with values observed during the pretreatment period. We conclude that orexigenic peptides (orexin B and ghrelin) and an anorectic peptide (leptin) affect PG directly. The responses of PG to those hormones depend on day length. Moreover, secretion of melatonin from the ovine PG is under an adrenergic regulation.

Keywords: Melatonin; Sheep; Photoperiod; Orexin; Ghrelin; Leptin; Isoproterenol

1. Introduction

The mammalian pineal gland (PG) converts external signals (principally light) to an endocrine message: melatonin, produced mainly during the dark phase. In seasonally reproductive animals, especially sheep, the duration of melatonin secretion informs the animal of

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the day length [1]. Light stimuli are received by the suprachiasmatic nucleus (SCN) directly through the retinohypothalamic pathway and active SCN neurons, thus transmitting the light signal to hypothalamic target areas. Via a multisynaptic pathway, including the paraventricular nucleus (PVN) of the hypothalamus, neurons of the SCN project to the interomediolateral cell column of the spinal cord [2].

The regulation of melatonin secretion in sheep is not well understood; however, the β-adrenergic system appears to be involved [3]. Ovine PG is the target of several (neuro)transmitters and (neuro)peptides of various origins. Recently, mRNA for the long form of the leptin receptor was detected in the SCN and PG of a ruminant species [4], suggesting an interaction among photoperiod, melatonin, and leptin [5]. Our laboratory showed that leptin modulates melatonin release by the ovine PG and demonstrated that the ability of leptin to create this effect is seasonally dependent [6]. Furthermore, Archer et al [7] demonstrated that orexin gene expression in the lateral hypothalamus of sheep is under the influence of photoperiod. Orexin directly affects melatonin synthesis in rats [8], and orexin-positive fibers innervate rat PG through a central pathway. Another orexigenic hormone, ghrelin, has been shown to decrease melatonin secretion from PG in rats [9] and to inhibit serotonin, the precursor of melatonin synthesis [10]. Cowley et al [11] demonstrated that ghrelin is expressed in a previously uncharacterized group of neurons in the hypothalamus that lie in the space between the lateral hypothalamus, arcuate (ARC), ventromedial (VMN), and PVN nuclei. These areas overlap with the projection from the SCN, which may allow the production of ghrelin to be directly modulated by the circadian clock.

As we demonstrated previously, the interaction between melatonin and leptin signaling exists in sheep. Furthermore, the hormones leptin, orexin, and ghrelin are strongly engaged in the regulation of energy homeostasis, food intake, and reproduction, processes that are under the influence of photoperiod in sheep. Thus, we sought to investigate the possible melatonin-orexin and melatonin-ghrelin interactions in connection to the effects of leptin on PG melatonin secretion.

2. Materials and methods

All animal-related procedures used in these studies were approved by the Local Agricultural Animal Care and Use Committee of Krakow.

The study was carried out at the Experiment Station in the Department of Swine and Small Ruminant Breeding and in the Laboratory of Genomics and Biotechnology at the Agricultural University of Krakow (longitude, 19° 57′ E; latitude, 50° 04′ N). Twenty-four Polish Longwool ewes, a breed that exhibits strongly seasonal reproduction, were used. Ewes were 2 to 3 yr of age, weighed 60 ± 5 kg, and were kept under natural photoperiodic and thermoperiodic conditions in individual boxes. Ewes were in good body condition (BCS = 3; 1–5 scale) and were fed once daily at 0700 h with a diet formulated to provide 100% of the National Research Institute of Animal Production recommendations for maintenance [12]. Water was available ad libitum.

PGs were removed aseptically from ewes within 10–15 min of death. Glands were collected after sunset from 12 ewes selected randomly during the long-day season (LD, the end of April and the beginning of May, n = 6 per month) and from an additional 12 ewes during the short-day season (SD, the end of October and the beginning of November, n = 6 per month). Glands were placed into Hank's balanced salt solution (Laboratory of Vaccines, Lublin, Poland) and transported to the laboratory. All subsequent procedures were performed under sterile conditions.

2.1. Pineal gland explant culture

Isolation, processing, and culture of PG explants were performed as previously described [6]. Briefly, PGs were cut sagittally into approximately 0.5 × 2 mm strips and incubated separately in a gas-liquid interface. Each explant was placed on lens paper supported on a stainless-steel grid [13] that was immersed in 2.5 mL of Dulbecco's modified Eagle's medium (Laboratory of Vaccines) with 10% fetal calf serum (Sigma Chemical Co, St Louis, MO, USA). Cultures were carried out in a 6-well tissue culture dish (Corning Glass Works, New York, NY, USA) under 95% humidified air and 5% CO_2 at 37 °C for 5.0 h, including a 60-min period of equilibration and a 120-min period of treatment with 0 (control), 10, or 100 ng/mL of rat orexin B (NeoMPS, PolyPeptide Laboratories, Strasbourg, France), 10 or 100 ng/mL ovine ghrelin (NeoMPS, PolyPeptide Laboratories), or 50 ng/mL of recombinant ovine leptin (Ray Biotech, Inc, Norcross, GA, USA). After 180 min of culture, some PG explants treated previously with lower doses of orexin or ghrelin were challenged with 50 ng/mL of recombinant ovine leptin and others treated with orexin were challenged with 300 nM of the β-agonist isoproterenol (Sigma Chemical Co). The doses of leptin, orexin, and ghrelin were chosen based