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Effect of orexin-A on prolactin secretion in lambs born under different photoperiod conditions – *in vitro* study

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ABSTRACT: The role of orexin-A in the regulation of prolactin secretion in lambs born in different photoperiods was determined. The experiment was conducted with 30 female lambs. Pituitary glands were collected from 40-day-old lambs in three different seasons (n = 10 per each season) and photoperiods (light (L) hours to darkness (D) hours): long (May – L 14 : D 10), intermediate (August – L 13 : D 11), and short (December – L 10 : D 14). At 40 days of age, the lambs were decapitated, and their pituitaries were dissected and cut in half along the longitudinal fissure so that each half contained both glandular and nervous parts. The *in vitro* incubation of the glands was performed for 3 h in Parker's medium at 37°C. In each season, the control pituitary glands (K1, K2, K3) were incubated in Parker's medium, while the experimental pituitary glands (Ox1, Ox2, Ox3) were incubated in medium containing 1000 ng/ml of exogenous orexin. The administration of orexin-A during the long photoperiod (May) caused a significant ($P \leq 0.01$) increase in prolactin secretion during the first hour of incubation (Ox1: 47.56 ± 8.4 ng/ml vs K1: 36.08 ± 7.8 ng/ml). During the intermediate photoperiod (August), a significantly ($P \leq 0.01$) higher concentration of prolactin was observed in the first hour of incubation in the Ox2 group than in the control group (K2). During the first hour of incubation in December, the prolactin concentrations were significantly ($P \leq 0.01$) higher in the Ox3 group than in the K3 group. Orexin-A increased prolactin secretion from pituitary explants collected from lambs born under all investigated time-points. The study results indicate that orexin-A is a stimulatory factor of prolactin secretion in sheep.

Keywords: seasonality sheep; incubation; pituitary gland

INTRODUCTION

Prolactin is synthesized mainly in lactotropic cells of the anterior pituitary. Prolactin is responsible for mammogenesis, the initiation of lactation (lactogenesis), and the maintenance of milk secretion (galactopoesis) (Shiu and Rfiesen 1980; Schams et al. 1984). Prolactin activates the expression of the milk protein gene (transcription, mRNA stabilization, translation, and posttranslational modifications of the proteins) and stimulates fatty acid synthesis due to the increased activity of lipoprotein lipase (Hooley et al. 1978, Hasiec et al. 2012). The secretion of prolactin in the sheep hypophysis is closely related to the length of day and seasonality. It has been stated that the blood concentration of this hormone is the highest in summer (long-day) and the lowest in winter (short-day) (Molik et al. 2006). Seasonal changes in prolactin secretion influence milk production in sheep and can limit milk production (Peaker and Neville 1991; Reksen et al. 1999). In recent years, increasing attention has been paid to the role of orexins in the regulation of prolactin secretion. Orexins (A and B) were discovered in the hypothalamus, a part of the brain that is crucial for the regulation of food intake and the maintenance of energy homeostasis in the body (Sakurai et al. 1998, 1999). *In vitro* studies of biological material from lactating sheep showed that orexin-A stimulated prolactin and

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growth hormone secretion (Molik et al. 2011) and that the intensity of this effect depended on the length of day. The prolactin secretion stimulated by orexin-A was more intensive in summer (long-day period) than in winter (short-day) (Molik et al. 2008). A study performed on lactating sheep showed the crucial effect of orexin-A on prolactin secretion during milk production. Therefore, the aim of this study was to determine the role of orexin-A in the regulation of prolactin secretion during mammogenesis in lambs born in different photoperiods.

MATERIAL AND METHODS

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

Thirty 40-day-old female lambs of Polish Longwool Sheep were used in the experiment carried out at the Experimental Station of the Department of Swine and Small Ruminants Breeding, Biotechnology and Genomics Laboratory of the Agricultural University in Krakow (longitude 19°57'E, latitude 50°04'N). To arrange lambing at certain times of the year, estrus in the dam was synchronized by the administration of gestagens with Chronogest sponges. The polyurethane intravaginal sponges impregnated with 40 mg of Cronolone (Intervet International, Boxmeer, the Netherlands) were inserted to the ewes for 14 days. On the day of the sponges removal the ewes were administered 500 IU of PMSG (Serogonadotropin; Biowet Drwalew S.A., Drwalew, Poland). The estrous cycle appeared 48–72 h after the administration of Cronolone. Estrus detection was performed twice daily with an adult ram equipped with an apron. Estrus was defined as the acceptance of mounting. The lambing occurred in April, July, and November, so the *in vitro* experiment was performed under different photoperiods (light (L) hours to darkness (D) hours): in May (long photoperiod – L 14 : D 10, n = 10), in August (intermediate photoperiod – L 13 : D11, n = 10), and in December (short photoperiod – L 10 : D 14, n = 10). The lambs were decapitated, and their pituitaries were dissected and cut in half along the longitudinal fissure so that each half contained both glandular and nervous parts. One of the halves was treated with medium without hormone (control, n = 10) and the second was treated with orexin-A (Ox, n = 10). The *in vitro* incubation was performed in 24-well plates (Sigma-Aldrich, St. Louis, USA) for 3 h in Parker's medium at 37°C. All tissues were first exposed to a 15-min pre-incubation in pure medium to stabilize the cell secretory function. Subsequently, one half of the pituitary was incubated in Parker's medium alone (control pituitary glands group: K1 – May, K2 – August, K3 – December), and the other half (experimental orexin pituitary glands group: Ox1 – May, Ox2 – August, Ox3 – December) was incubated in Parker's medium containing 1000 ng/ml of orexin-A (Sigma-Aldrich). The dose of orexin-A was based both on theoretical calculations and on our previous experience (Molik et al. 2008, 2011). During the subsequent 3-hour incubation, the medium was changed every 15 min, and a 1-ml sample was collected and immediately frozen at –80°C until analysis. Prolactin concentrations in the medium were determined radioimmunologically (RIA) using the method described by Kokot and Stupnicki (1985) at the Department of Endocrinology of the Kielanowski Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences in Jabłonna. The assay sensitivity for prolactin was 2 ng/ml and the intra- and inter-assay coefficients of variation were 9 and 12%, respectively.

Statistical analysis. Non-parametric statistics, the Kruskal-Wallis test, and then the Mann-Whitney *U*-test were used to determine the significance of differences in prolactin and orexin levels in the pituitary gland tissue between control and orexin groups. All data were expressed as means ± SEM. The results obtained were analyzed statistically by one-way analysis of variance and Scheffé's test using the SAS software (Version 9.1, 2007).

RESULTS

During the long photoperiod (May), prolactin concentrations in the media were significantly ($P < 0.01$) higher (almost 32%) during the first hour of incubation in the Ox1 group than in the K1 group. Both in the second and third hour of incubation, the prolactin concentrations in the Ox1 group were significantly ($P < 0.05$) higher than in the K1 group (Figures 1A and 3).

In August (intermediate photoperiod), in the first hour of incubation, the concentration of prolactin in group Ox2 was by 90% higher than in the control group (K2). Significant differences were also found in prolactin concentrations between the Ox2 group

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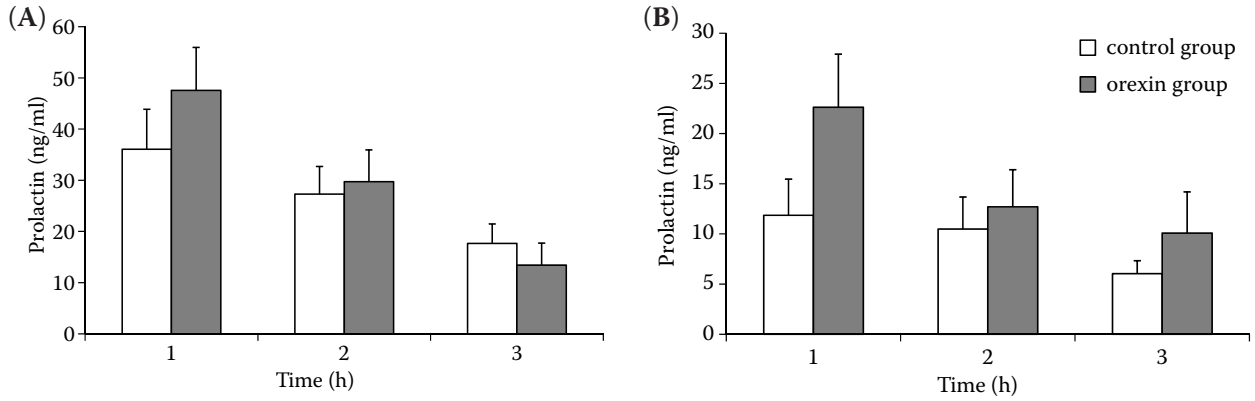

Figure 1. Mean concentrations (± SEM) of prolactin in media collected during 3-hour experiments carried out on pituitary explants collected during (**A**) long day (May) and (**B**) intermediate photoperiod (August). Pituitary explants were incubated with medium alone (control group) or medium containing orexin-A (orexin group)

differences between groups: (**A**) $^{*}P < 0.05$, $^{**}P < 0.01$ and (**B**) $^{**}P < 0.01$

and the K2 group ($P < 0.01$). Both in the second and third hour of incubation, the concentrations of prolactin were significantly ($P < 0.05$) higher in the Ox2 group than in the K2 group (Figures 1B and 3).

During the short photoperiod (December), the concentrations of prolactin decreased equally both in the control group and the treatment group. In the first hour of incubation, prolactin concentrations in group Ox3 were by 65% higher ($P < 0.01$) than in group K3. In the second hour of incubation, prolactin concentrations in group Ox3 were still significantly ($P < 0.05$) higher than in group K3. In the third hour of incubation, prolactin concentrations decreased, though they remained significantly ($P < 0.05$) higher in group Ox3 than in group K3 (Figures 2 and 3).

Collective analysis of the data showed that prolactin concentrations were higher in experimental groups (Ox1–Ox3) than in control groups (K1–K3) in all photoperiods – long (May), intermediate (August), and short (December).

DISCUSSION

The results of the *in vivo* studies carried out by Russel et al. 2000 showed that the administration of orexin-A decreased prolactin secretion in rats. On the other hand, the results of *in vitro* experiments carried out in pedigree-bred immature rat females showed significant increases in prolactin secretion depending on the dose and time of pituitary incubation (Martynska et al. 2006). A study carried out on

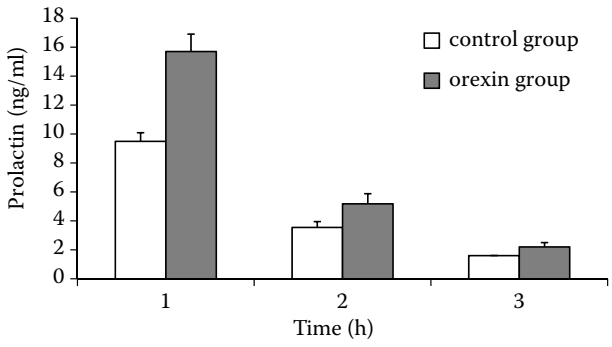

Figure 2. Mean concentrations (± SEM) of prolactin in media collected during 3-hour experiments carried out on pituitary explants collected during short day (December). Pituitary explants were incubated with medium alone (control group) or medium containing orexin-A (orexin group)

differences between groups: $^{*}P < 0.05$, $^{**}P < 0.01$

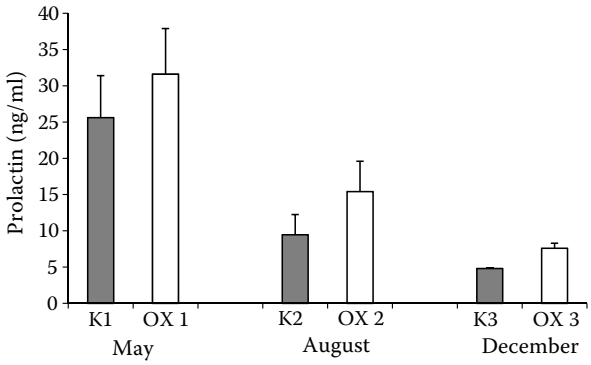

Figure 3. Mean concentrations (± SEM) of prolactin in control and orexin A-treated pituitary explant cultures during long-day (May), decreasing (August), and short (December) photoperiod

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lactating sheep showed that orexin has a stimulating effect on prolactin secretion (Molik et al. 2008), and this effect is conditioned by the day length; a stronger stimulating effect is exerted in summer than in winter (Lincoln and Clarke 2000). The response of pituitary tissue to orexin during the short days was weaker than on long days, most likely because of lactotropic resistance to orexin. The reaction of endocrine cells to orexin can be explained by the existence of a seasonal rhythmic secretion of orexin in sheep regulated throughout the photoperiod. It has been shown that orexin gene precursor has higher expression during the shorter than longer days in the ruminant (Archer et al. 2002). Iqbal et al. (2003) indicated that an increase in preproorexin concentration in the winter season was a result of the higher orexin gene expression. It is likely that the level of saturation of orexin receptors on lactotropic cells by endogenous ligands is so high that adding exogenous orexin-A does not initiate as strong reaction as it would be on longer days. The results of this study showed that prolactin concentrations in 40-day-old lambs varied by season despite of the fact that the pituitaries were collected on days varying in length. Studies on the initiation of lactation in different photoperiods carried out previously in sheep showed that prolactin was secreted in a seasonal rhythmic manner characterized by higher concentrations during periods of longer days and lower concentrations during periods of shorter days (Molik et al. 2007, 2009). Analyzing the changes in prolactin concentration during a 3-hour incubation period confirmed that lactotropic pituitary cells displayed the highest secretion activity in the first hour of the experiment in both the control and the experimental group. *In vitro* studies conducted with the biological material from lactating sheep also showed that cell secretion was the highest in the first hour of incubation. Prolactin secretion was pronounced for periods of short days in the control group. A study also showed that the introduction of exogenous orexin-A stimulates prolactin secretion in sheep initiating lactation in different photoperiods (Molik et al. 2008).

CONCLUSION

In seasonal animals such as sheep, orexin-A stimulates the secretion of lactotropic hormones. The mechanism of orexin activity, however, is not clear. The initiation and maintenance of lactation in sheep requires the presence of several hormones and the participation of orexin. In conclusion, our study of pituitary explants demonstrated that the pituitary tissue of sheep was sensitive to the photoperiod and orexin-A.

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