Domestic Animal Endocrinology 48 (2014) 69–76

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Influence of season and nutritional status on the direct effects of leptin, orexin-A and ghrelin on luteinizing hormone and growth hormone secretion in the ovine pituitary explant model

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ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form 19 February 2014
Accepted 20 February 2014

Keywords:
Sheep
Ghrelin
Leptin
Orexin-A
Pituitary gland
Nutritional status

ABSTRACT

The aim of this study was to examine whether leptin (anorexigenic peptide), orexin-A, and ghrelin (orexigenic peptides) could directly (ie, independently of hypothalamic influences) affect the secretion of luteinizing hormone (LH) and growth hormone (GH) by adenohypophyseal (AP) explants obtained from normally fed or fasted (48 h) ewes during the breeding and nonbreeding seasons. In addition, a specific ovine super leptin antagonist (SLAN-3) was used to assess the interactions between leptin and ghrelin and/or orexin-A. Pituitary glands from 16 ovariectomized Polish Longwool ewes that had received estradiol-releasing subcutaneous implants were collected in the breeding (November; n = 8) and nonbreeding (May; n = 8) seasons. The AP explants were incubated for 240 min in a gas-liquid interface and treated with leptin (50 ng/mL), ghrelin (100 ng/mL), orexin-A (100 ng/mL), and SLAN-3 (500 ng/mL) with orexin-A or ghrelin. Treatments with leptin and SLAN-3 + orexin-A increased ($P < 0.05$) LH concentrations in the cultures of AP explants from fasted animals in the breeding season. Orexin-A increased ($P < 0.05$) LH secretion by AP explants from both fasted and fed animals in the breeding season. Ghrelin stimulated ($P < 0.05$) GH secretion by AP explants collected from fasted animals in nonbreeding season and from normally fed ewes in both seasons. Leptin decreased ($P < 0.05$) GH secretion by AP explants collected from fasted ewes in both seasons and from nonfasted ewes in the breeding season. However, the treatment with SLAN-3 + ghrelin resulted in greater ($P < 0.05$) GH concentrations compared with leptin treatment of AP explants from fasted ewes in the breeding season and from normally fed ewes in nonbreeding season. In summary, leptin, orexin-A, and ghrelin exerted direct effects on AP secretory function in an *ex situ* model and both the reproductive season and nutritional status of the animals impinged on the direct effects of the peptides on LH and GH release. Specifically, orexin-A was more potent than leptin in directly stimulating LH secretion in cycling ewes, whereas ghrelin and leptin generally had opposing effects on the secretory function of somatotrophs in sheep.

1. Introduction

Seasonally polyestrous animals exhibit circannual fluctuations in the secretory patterns and bioavailability of gonadotropic hormones, growth hormone (GH), and certain neuropeptides and hormones involved in the maintenance

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http://dx.doi.org/10.1016/j.domaniend.2014.02.005

of energy homeostasis [1–3]. These fluctuations reflect not only annual changes in the activity of the hypothalamo-pituitary-ovarian axis but also variations in feed intake and body fat composition [4,5]. The mechanisms whereby the central nervous system integrates the coordination of energy balance with the secretion of GH and luteinizing hormone (LH) are not fully understood.

Metabolic energy deficiency may hamper regeneration and growth of body organs and tissues, and disturb the reproductive function [4]. A number of endogenous peptides governing the control of energy balance can be involved in the regulation of the somatotropic and gonadotropic axes [6]. The key regulatory agents include peptides with orexigenic properties such as ghrelin and orexin-A as well as peptides with anorexigenic properties (eg, leptin) that jointly "inform" the central nervous system about the current nutritional status [7–9]. Reduction in the pulsatile secretion of LH caused by nutritional restrictions can be reversed by leptin administration in sheep and cattle [10,11], and by orexin-A in female rats [12]. Leptin and ghrelin regulate GH secretion in both the negative and the positive energy balance states in sheep [10,13]. The expression of leptin receptors has been found not only in the hypothalamic nuclei responsible for the regulation of feed intake, including the mediobasal hypothalamus area and median eminence, but also in the anterior pituitary of the ewe [14,15]. Studies have demonstrated that ghrelin and orexin-A receptors are expressed in the ovine pituitary gland [16,17]; moreover, in vivo study performed by our laboratory proved that the photoperiod was able to influence the secretion of leptin, ghrelin, and orexin in seasonally breeding sheep [1], but the direct effects of orexigenic and anorexigenic peptides on the secretory activity of the adenohypophysis (AP) in sheep have yet to be elucidated.

The main goal of this study was therefore to determine whether leptin, orexin-A, and ghrelin could directly influence the secretion of LH and GH by the anterior pituitary *ex situ*. At the level of the hypothalamus, leptin and ghrelin and/or orexin-A are thought to have opposing effects [1], but similar interactions in the pituitary gland of the ewe have not been examined. Hence, a specific ovine super leptin antagonist (SLAN-3) was used to assess the interactions between leptin and orexin-A in the control of LH secretion and between leptin and ghrelin in the control of GH secretion by AP explants obtained from normally fed or fasted ewes. Because the primary biological effects of orexin-A and ghrelin revolve around the control of gonadotropic and somatotropic functions, respectively, the effects of orexin-A on GH release and of ghrelin on LH release have not been addressed in this study. The present experiment was carried out in the breeding (November) and nonbreeding (May) seasons.

2. Materials and methods

2.1. Animals and experimental procedures

All procedures performed on animals in this study were approved by the Local Agricultural Animal Care and Use Committee in Kraków, Poland. The present study was conducted in the field research station of the Department of Swine and Small Ruminant Breeding, Agricultural University of Kraków, Poland (longitude, 19°57′E and latitude, 50°04′N). Sixteen Polish Longwool ewes (a breed with a pronounced reproductive seasonality; 2–3 y of age and a mean body weight of 60 ± 5 kg) were used in this experiment: 8 ewes in May (nonbreeding season) and 8 animals in November (breeding season). The animals were housed in a pen under natural conditions of photoperiod and ambient temperature. All ewes were in good body condition (body condition score = 3 on a scale from 1 to 5; [18]). As in our earlier studies [12,19], the ewes had been ovariectomized ∼2 m before the collection of pituitary glands and received estradiol-releasing subcutaneous implants that maintained circulating concentrations of estradiol from 2 to 4 pg/mL. This is a widely accepted neuroendocrine experimental model devised to provide a constant physiological level of estrogen feedback and avoid the confounding effects of the ovarian cycle on hormonal interactions [11]. The collection and preparation of pituitary glands during both reproductive seasons (nonbreeding season day length equal to ∼15 h and breeding season day length equal to ∼10 h) began consistently at dusk.

2.2. Experimental design

In both seasons, the ewes were randomly allocated to 1 of the 2 experimental groups (n = 4 per group). In the first group, the ewes were fasted for 48 h before the beginning of the experiment, whereas animals in the second group received twice daily (7 AM and 2 PM) a diet formulated to provide 100% of the National Research Institute of Animal Production's recommendation for maintenance [20]. Water was available ad libitum to animals in both groups.

All ewes were killed by exsanguination following captive bolt stunning and decapitation. Diencephalons were removed after disconnecting the infundibuli from the APs. APs were then removed from the *sella turcica* and kept on ice until tissue processing. The APs collected from each ewe were dissected and sliced in a saggital plane into 0.5- to 2-mm thick strips. Four strips from each AP were selected randomly, placed on a stainless steel grid covered with lens paper, and incubated in a gas-liquid interface in 2.5 mL of Medium 199 (Biomed, Lublin, Poland). Initially, the explants were incubated in a 6-well tissue culture dish (Corning Glass Works, New York, NY) under 95% humidified air and 5% CO_2 at 37°C for a 30-min period (equilibration) to stabilize the tissue's secretory function. After equilibration, the explants were further incubated for 210 min; 50 ng/mL of recombinant ovine leptin (Ray Biotech, Inc, Norcross, GA), 100 ng/mL of rat orexin-A (NeoMPS; PolyPeptide Laboratories, Strasbourg, France), 100 ng/mL of ovine ghrelin (NeoMPS; PolyPeptide Laboratories) (Fig. 1A), and 500 ng/mL of the ovine SLAN-3 (PLR Ltd, Rehovot, Israel) (Fig. 1B) were added to incubation media at 0, 30, and 60 min after equilibration. Subsequently, the AP explants pretreated with SLAN-3 were treated with 100 ng/mL of orexin-A or 100 ng/mL of ghrelin at 120, 150, and 180 min after equilibration (Fig. 1B). The doses of leptin, orexin-A, ghrelin, and SLAN-3 were based on previous studies [21–23]. Media samples (1 mL) were collected at 30-min intervals and stored at −20°C until hormone assays at a later date. The weight of individual AP