Theriogenology 86 (2016) 1615–1621

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Ghrelin suppresses the GnRH-induced preovulatory gonadotropin surge in dairy heifers

T.M. Chouzouris [a], E. Dovolou [a], K. Dafopoulos [b], P. Georgoulias [c], N.G. Vasileiou [a], G.C. Fthenakis [a], G. Anifandis [b], G.S. Amiridis [a,*]

[a] Department of Obstetrics and Reproduction, Veterinary Faculty, University of Thessaly, Karditsa, Greece
[b] Department of Obstetrics and Gynecology, Faculty of Medicine, University of Thessaly, Larissa, Greece
[c] Department of Nuclear Medicine, Faculty of Medicine, University of Thessaly, Larissa, Greece

ARTICLE INFO

Article history:
Received 12 March 2016
Received in revised form 19 May 2016
Accepted 19 May 2016

Keywords:
Ghrelin
Growth hormone
Luteinizing hormone
Follicle stimulating hormone
Progesterone
Cattle

ABSTRACT

Ghrelin, a known growth hormone (GH) secretagogue, alters gonadotropin secretion in many species. Our objectives were to study the effects of ghrelin, on GH, LH, FSH secretion, and on luteal function of the ensuing estrous cycle in cattle. The estrous cycles of eight heifers were synchronized with progesteron releasing intravaginal device, and ovulation was induced with GnRH. Eight animals were treated with 1.5 μg kg^{-1} bovine ghrelin (group Ghr, n = 4) or saline (group C, n = 4). Starting with the first ghrelin injection, 13 blood samples were collected over a 4-hour period for the determination of ghrelin, GH, LH, and FSH concentration. Progesterone levels were measured in samples collected every other day after estrus expression. Data were analyzed by repeated measures of ANOVA followed by Bonferroni post hoc testing and *t* test. In group Ghr, ghrelin concentration increased significantly 15 minutes after the first injection and remained in elevated levels until the 90th minute after the last injection. At the time of third ghrelin injection, GH was significantly higher in the Ghr group compared with C (17.1 ± 1.3 vs. 2.6 ± 0.3 ng mL^{-1}, $P < 0.0001$). Similar differences were found for the next three samples collected 15, 30, and 60 minutes later; no difference was evident after 90 minutes. In group Ghr, the area under the curve for LH and FSH were significantly reduced compared with the ones of group C (266 ± 10.3 vs. 331.9 ± 7.3, $P = 0.007$ and 102.3 ± 2.0 vs. 134.9 ± 5.5, $P < 0.005$ for LH and FSH respectively). At particular time points the concentration of the two gonadotrophins in group Ghr was significantly lower than those of group C (15, 30, 45, 75, and 90 and 60, 75, 90, 120, and 150 minutes after GnRH administration for LH and FSH respectively). The duration of the following estrous cycle was shorter ($P = 0.004$) in group Ghr (19.0 ± 0.4 days) compared with C (21.8 ± 0.5 days). In days 4, 6, 8, 10, and 14, progesterone concentration was lower ($P < 0.05$) in group Ghr compared with C; similarly the progesterone area under the curve for group Ghr (113.1 ± 4.8) was suppressed ($P = 0.007$) compared with that of C (141 ± 4.8). These results imply that ghrelin acts on pituitary causing impaired response to the GnRH stimulus, and it is likely to affect luteinization of the cellular compartment of the preovulatory follicle, and/or to suppress steroidogenetic activity of the luteal cells.

1. Introduction

In the last 2 decades, cumulative evidence suggests that ghrelin is an important neuroendocrine regulator, possessing prominent metabolic role; however, it has been

* Corresponding author. Tel.: +30 24410 66073; fax: +302441066041.
E-mail address: gsamir@vet.uth.gr (G.S. Amiridis).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.05.022

documented that the hormone has a widespread pattern of expression and multifaceted biological actions. Ghrelin is composed of 28 amino acids, and it is modified by the addition of an n-octanoyl ester believed to drive the stimulatory effects of ghrelin on somatotropin secretion [1,2]. However, recent evidence suggests that some of the endocrine properties of the hormone are exerted by the unacylated isoform [3,4]. The hormone is mainly secreted by enteroendocrine cells and A-like cells in the oxyntic glands of the stomach [2,5,6]. Smaller amounts of ghrelin are secreted in various organs [7]. The endocrine properties of the hormone are expressed after its binding to a specific receptor (Growth hormone secretagogue receptor 1a–GHSR-1a). The receptor is a transmembrane receptor belonging to the G-coupled domain receptors, and it has considerable distribution in several structures of the brain, but it is mainly expressed in the hypothalamus and pituitary [8,9].

As metabolic hormone, ghrelin has strong orexigenic effects that have been reported in many species; during the preprandial period, blood concentrations of ghrelin increase in a surge like fashion, and sharply decrease after feeding [10], whereas, exogenously administered ghrelin acts as an appetite promoter, found, in rodents and humans, to increase food consumption [11,12].

Beyond its metabolic role, ghrelin has been shown to be an important regulator in tuning functionality of the reproductive system, contributing to the regulation of the onset of puberty [13], gonadotropin secretion [14–18], oocyte maturation [19], and embryo development [20]; in all these functions, ghrelin plays a rather suppressive role.

Studies in sheep and rodents have provided evidence that centrally (intracerebroventricularly) administered ghrelin caused suppression of LH secretion [11,18,21], possibly due to the partial inhibition of GnRH release; these findings support the notion for a major central effect at the hypothalamic level [22]. It has also been recently shown that ghrelin can also suppress GnRH-induced gonadotropin surge in superovulated ewes; those results provided some evidence for a direct action of ghrelin on pituitary [14]. In humans, bolus injections of ghrelin-attenuated pulsatility of LH secretion and reduced basal LH and FSH blood concentrations [16,17,23]. A direct effect of ghrelin on pituitary level has been shown in rats, after inhibition of GnRH-induced LH surge [24]. A large body of empirical evidence suggests that ghrelin may act as metabolic modulator of the gonadotrophic axis, in line with its role as a signal for negative energy balance; hence, the role of ghrelin on reproductive physiology is exerted under energy restriction or energy expenditure conditions [13,15].

Reduced fertility is probably the most important problem of the global dairy cattle industry, which should be inevitably attributed to the historic adherence to selection for increased milk production [25]. As fertility of dairy cows is associated to energy reserves and metabolic responses to nutrition [26], involvement of signaling molecules, and hormones that control partitioning of energy and/or nutrients and, at the same time, are expressed in the hypothalamus or the pituitary should be carefully examined as potent fertility regulators. A number of reports provide persuasive evidence for the role of ghrelin as feed intake stimulant [27], insulin [28,29] or GH secretion promoter [29–31], and fat oxidation suppressor [32]. However, to the best of our knowledge, its role in regulating gonadotropin secretion in cattle has not been examined. Consequently, objective of this study was to investigate the role of ghrelin action on stimulated gonadotrophin secretion in dairy cattle and on characteristics of the ensuing estrous cycle.

2. Materials and methods

All procedures described herein were performed in compliance with the principles and guidelines set by the EU regulations and were approved by the Ethical Committee of Animal Welfare of the University of Thessaly.

2.1. Animals and treatments

Eight Holstein heifers, aged 11 to 11.5 months, in which at least two estruses had been previously recorded, were included into the study. Mean (±standard deviation of the mean) bodyweight of animals was 281 ± 28.1 kg. All animals were fitted with electronic pedometres. Animals were randomly allocated into two groups (Ghr and C, each n = 4).

One month before the scheduled initiation date of the experiment, the heifers were moved to a separate barn, where they kept tethered for at least 8 hours per day. During that period animals were provided, twice daily, a total mixed ration consisting of maize silage, wheat straw, corn, soy bean meal, wheat brans, and a vitamin/mineral premix, formulated according to the NRC recommendations; water was offered *ad libitum*.

The estrous cycles of the animals were synchronized using a progesteron releasing intravaginal device (PRID) Delta (CEVA, Santé Animal, Libourne, France) containing 1.5 g of progesterone, that stayed *in situ* for 8 days. The day before PRID removal, a luteolytic dose of prostaglandin F2α (600 μg, cloprostenol; Estrumate MSD, Fryesoythe, Germany) was intramuscularly administered.

Thirty hours after PRID removal and 45 minutes after morning feeding, animals into Ghr group received an intravenous injection (by means of an indwelling jugular vein catheter), which had been checked for patency and filled with heparinized saline (200 units mL^{-1}) of acylated bovine ghrelin (Anaspec, Fermont, USA) at a dose of 1.5 μg kg^{-1}, whereas animals into C group received an injection of normal saline. Then, 15 minutes later, the treatment was repeated and animals also received a GnRH analogue (0.25 μg kg^{-1}, buserelin; Receptal, MSD, Boxmeer, the Netherlands) by intramuscular injection, scheduled to evoke a timed preovulatory gonadotrophin surge. Finally, animals received two further ghrelin treatments as above, 15 and 30 minutes after the second (i.e., the regime was completed within 1 hour).

Estrus detection was performed by combining physical activity data collected from the pedometres and the findings from visual and clinical observations that were carried out on twice daily basis (AM/PM); animals showing