Brain Research 1678 (2018) 278–287

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

# Obestatin stimulates the somatotrophic axis activity in sheep

Anna Wójcik-Gładysz, Michał Szlis *, Anna Misztal, Bartosz Jarosław Przybył, Jolanta Polkowska

*Department of Animal Physiology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland*

ARTICLE INFO

*Article history:*
Received 27 April 2017
Received in revised form 25 September 2017
Accepted 31 October 2017
Available online 6 November 2017

*Keywords:*
Hypothalamus
Pituitary
Somatostatin
Growth hormone
Sheep
Obestatin

ABSTRACT

The effects of obestatin (an anorexigenic peripheral peptide) on somatotrophic axis activity in ruminants have not yet been determined. The aim of this study was to investigate the consequence of intracerebroventricular infusions of obestatin on the activity of the somatotrophic axis in peripubertal female sheep. Animals were randomly divided into two groups: control group received intracerebroventricular infusions of the vehicle, and the obestatin group was infused with obestatin (25 μg/120 μL $h^{-1}$). The series of four hourly infusions on three consecutive days were performed. The blood samples were collected on day 0 and on day 3. Immediately after the end of experiment sheep were slaughtered. Parts of the brains were fixed *in situ* for further immunohistochemical analysis, while the remaining brains were frozen for Real Time RT-qPCR analysis.

Substantial changes in the activity of the somatotrophic axis were observed in obestatin-infused sheep. In those animals obestatin evoked an increase in growth hormone-releasing hormone (GHRH) mRNA expression and a decrease in somatostatin mRNA expression in the anterior hypothalamic area. Moreover, a decrease in somatostatin immunoreactivity in the periventricular nucleus and an increase in somatostatin immunopositive fibers in the median eminence were noted. Changes in the GHRH and somatostatin activity are associated with an increase in growth hormone (GH) gene expression and in the amount of GH immunoreactive material stored in the somatotrophic pituitary cells. Consequently, an increase in GH concentration in the peripheral blood, due to an increase in the number of pulses was observed.

It was revealed that obestatin affects the somatostatin/GHRH/GH system at the level of protein synthesis, accumulation and release. It is suggested that obestatin participates in the mechanism modulating somatotrophic axis activity at the central level by stimulating GH release through suppression of somatostatin output. Thereby, it can be concluded that obestatin may be involved in the modulation of growth processes in sheep.



## 1. Introduction

Mechanisms controlling growth processes have been extensively studied in recent years. It may seem that the growth control issue is already well understood; nevertheless, the existing knowledge is still insufficient. Information on newly discovered compounds (hormones and neurotransmitters) continuously appears, and these compounds frequently turn out to be extremely important for the mechanisms of somatotrophic axis activity. Among such compounds is obestatin, a peripheral hormone synthetized mainly in the gastrointestinal tract, derived from post-translational cleavage of the same precursor as ghrelin (Zhang et al., 2005).

Obestatin is generally considered as a ligand for the orphan receptor GPR39 and/or glucagon-like peptide 1 receptor (GLP-1R), the expression of which was found in hypothalamic structures. Nevertheless, the studies evaluating this issue performed on rodents are controversial (Chartrel et al., 2007; Granata et al., 2008; Tremblay et al., 2007; Van Dijck et al., 2009). However, recent studies using nuclear magnetic resonance analysis clearly showed that obestatin contains a domain necessary for the GPR39 activation (Alén et al., 2012). Moreover, *in vitro* studies conducted on mouse C2C12 myoblasts provided evidence that obestatin could indeed act *via* the GPR39 receptor (Gurriarán-Rodríguez et al., 2015).

*Abbreviations:* AHA, anterior hypothalamus area; ARC, arcuate nucleus; EU, European Union; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GH, growth hormone; GHRH, growth hormone-releasing hormone; IR, immunoreactive; MBH, mediobasal hypothalamus; ME, median eminence; PBS, phosphate-buffered saline; PEV, periventricular nucleus; SST1/5, somatostatin receptor 1 and 5.

* Corresponding author.
*E-mail address:* m.szlis@ifzz.pl (M. Szlis).

https://doi.org/10.1016/j.brainres.2017.10.036
0006-8993/

It has been shown that obestatin participates in the regulation of metabolic functions at both central and peripheral levels (food intake, pancreatic activity or/and proliferation of adipocytes) (Granata et al., 2008; Li et al., 2011). It is involved in the complex gut-brain neurohormonal networking whereby hormones from the gastrointestinal tract signal the hypothalamus about the feeling of satiety and hunger (Lacquaniti et al., 2011). The first studies on the effects of obestatin in rats showed that it causes appetite- and weight-losses, and delays the gastric emptying (Zhang et al., 2005). Obestatin evokes an attenuation of hunger and so impedes food intake in male rats (Bresciani et al., 2006). Moreover, obestatin inhibits also exogenous ghrelin action of food intake in mice (Zizzari et al., 2007). On the other hand, in comprehensive studies performed on rodents, no significant effect of peripherally or centrally administered obestatin on food intake, energy expenditure or body weight with either acute or chronic treatment was observed (Gourcerol et al., 2007; Holst et al., 2007; Mondel et al., 2008).

Information on the participation of obestatin in the regulation of key hormone activity in the somatotrophic axis is available mainly from the experiments on rodents. The initial studies were focused on understanding the potential effects of obestatin on growth hormone (GH) secretion from somatotrophic pituitary cells. The data obtained from both *in vitro* and *in vivo* experiments on GH release in rat pituitary or tumor cell lines are quite limited and fragmentary (Bresciani et al., 2006; Yamamoto et al., 2007; Zhang et al., 2005; Zizzari et al., 2007). No effect of obestatin on either spontaneous or ghrelin-induced GH release has been demonstrated by *in vivo* studies in rats (Bresciani et al., 2006; Yamamoto et al., 2007). On the contrary, *in vivo* studies performed by Zizzari et al. (2007) revealed that obestatin is able to antagonize ghrelin-dependent release of GH. In recent years, the information about the possibility that obestatin could act at the level of the hypothalamus appeared in the literature. Studies conducted on explants isolated from mouse hypothalamus showed that obestatin inhibited ghrelin-dependent release of growth hormone-releasing hormone (GHRH), although it had no influence on activity of somatostatin neurons (Feng et al., 2011; Hassouna et al., 2012).

As mentioned previously, data concerning the role of obestatin in growth processes is derived mainly from studies conducted on rodents – monogastric animals with different eating habits and distinct physiology of the gastrointestinal tract than ruminants. Moreover, sheep as ruminant with seasonal reproductive strategy, constitutes a diametrically different research model from rodents which are animals with sesonal independent model of reproduction. On that account, neurohormonal mechanisms regulating the food intake-depended processes in these groups of animals are not the same. It should be emphasized that in ruminants the GH secretion is sensitive to perturbations in nutritional status, and growth process modulation mechanisms are different than those in rodents (Gładysz et al., 2001). Food restriction in rodents causes a decrease in the secretory activity of somatotrophic pituitary cells and a significant decrease in the concentration of GH in the peripheral blood (Casanueva and Dieguez, 2005). Conversely, an increase in the synthesis and release of GH has been observed in ruminants under the same conditions (Gładysz et al., 2001; Wójcik-Gładysz et al., 2010). Furthermore, these changes in ruminants are generally related to the attenuation of the inhibitory effect caused by somatostatin (Gładysz et al., 2001; Thomas et al., 1991; Wójcik-Gładysz et al., 2010). In ovariectomised sheep, long term dietary restrictions result in a decreased release of somatostatin into hypophyseal portal vessels without any effect on GHRH (Thomas et al., 1991). Having in mind the fact that hypothalamic somatostatin constitutes the principal inhibitor of pulsatile GH secretion (Giustina and Veldhuis, 1998), a question arises: is somatostatin withdrawal necessary or sufficient to mediate obestatin's stimulation of GH secretion?

In sheep, several development periods during the postnatal ontogenesis has been described: infantile (5–9 weeks of age), juvenile (9–16 weeks of age), prepubertal (16–31 weeks of age), peripubertal (32–33 weeks of age) and pubertal (after ovulation) (Wańkowska and Polkowska, 2010). In this experiment, model of female peripubertal sheep was chosen. Peripubertal period seems to be extremely important for the interaction between the hypothalamo-gonadotrophic and hypothalamo-somatotrophic axes (Polkowska et al., 2008a, 2008a). In this transitional ontogenetic period dramatic neuroendocrine changes occurring in the activity of those axes leads to the first ovulation. Our previous study demonstrated that in female sheep during peripubertal period the activity of somatostatin-containing neurons in the periventricular nucleus (PEV) are known for its involvement in the control of GH secretion (Polkowska et al., 2008b; Tillet et al., 2010). Moreover, this particular population of somatostatin neurons is the most sensitive to the action of the orexigenic (ghrelin) or anorexigenic (leptin) peripheral hormones (Polkowska et al., 2011; Wójcik-Gładysz et al., 2010).

So, it can be assumed that in sheep, obestatin may be involved in the modulation of somatotrophic axis activity, as another regulating peptide engaged in the complex neurohormonal network modulating the axis function. The aim of this study was to verify the hypothesis whether obestatin acts as a hormonal signal affecting the activity of the main components involved in the growth processes regulation in peripubertal sheep, i.e., hypothalamic GHRH, somatostatin neurons, and pituitary somatotrophic cells.

The experiment was performed on 32-weeks old Polish Merino sheep (n = 28), randomly divided into two groups. The sheep in the control group received intracerebroventricular (icv) infusions of the Ringer-Lock solution, obestatin group were infused with obestatin (25 μg/120 μL $h^{-1}$). The series of four hourly infusions on three consecutive days were performed. The blood samples were collected on day 0 (before the infusion) and on day 3. Immediately after the end of experiment all sheep were slaughtered. Selected brain regions were dissected and fixed *in situ* or frozen according to protocols for further analyses. The processes of the synthesis, storage, and release of these hormones were examined using Real Time RT-qPCR and immunohistochemical techniques. GH pulsatile profiles in the blood plasma were investigated using radioimmunological methods.

## 2. Results

### 2.1. Hypothalamic somatostatin and GHRH gene expression

Real Time qPCR analyses revealed that somatostatin mRNA transcript was present in all examined areas of the hypothalamus: anterior hypothalamic area (AHA), mediobasal hypothalamus (MBH) and median eminence (ME). In the obestatin group somatostatin gene expression was significantly ($P < .05$) lower in the AHA than in the same area in the control group (0.61-fold; Fig. 1A). No changes were observed in the somatostatin mRNA level in the MBH in both group of animals (Fig. 1B). In the ME, the somatostatin mRNA level was significantly ($P < .05$) lower in the obestatin group than in the control one (Fig. 1C). GHRH gene expression in the MBH was significantly ($P < .05$) higher (1.56-fold) in the obestatin group than in control group of sheep (Fig. 1D).

### 2.2. Somatostatin immunoreactivity in the hypothalamus

Microscopic observations revealed that the localization of immunoreactive (IR) somatostatin neurons in the hypothalamus was similar in both groups of sheep. Immunoreactive somatostatin perikarya created a distinct region, located in the periventricular