Ontogeny of the long form of leptin receptor gene expression in the porcine ovarian follicles

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Abstract

Leptin is a polypeptide hormone produced predominantly in adipocytes. It has been found to be implicated in the regulation of satiety and energy homeostasis. A role for leptin in reproduction was later suggested by findings that this hormone may be involved in the regulation of the hypothalamic-pituitary-gonadal axis via endocrine, paracrine and/or autocrine pathways. The objective of the study was to investigate the ontogeny of the long isoform of leptin receptor (OB-Rb) gene in porcine ovarian follicles. The expression of OB-Rb gene was detected in porcine primordial, primary, secondary and antral follicles by in situ hybridization. In summary, our data suggest that leptin might have a direct effect on porcine follicles and plays an important role in the follicular development.

Key words: gene expression, ISH, leptin receptor, ontogeny, ovarian follicles, pigs

Introduction

Leptin is a 167-amino acid protein secreted by adipocytes. It has been recognized as an important hormone in appetite reduction and energy expenditure. Recent studies have revealed that leptin also plays a major role in reproduction (Zhang et al. 1994, Moschos et al. 2002). Leptin activities are mediated through the leptin receptor, a member of the class I cytokine receptor superfamily. The leptin receptor has been shown to have at least six splice variants. The isoforms can be classified into three types: long form (OB-Rb), short form (OB-Ra, -Rc, -Rd, -Rf), and secreted form (OB-Re). Only the OB-Rb isoform is thought to be capable of full intracellular signal transduction (Tartaglia et al. 1995, Bjorbaek et al. 1997).

It has been reported that leptin receptors are present in human and rat theca and granulosa cells (Cioffi et al. 1997, Ryan et al. 2003) and in theca and granulosa cells harvested from antral follicles of prepubertal gilts, in porcine corpora lutea (CL) and ovarian stroma (OS) (Ruiz-Cortes et al. 2000, Smolinska et al. 2007). These results suggest that leptin may have a direct effect on ovarian function. The identification of leptin mRNA and protein in human granulosa and theca cells, follicular fluid, oocytes and CL (Antczak and Van Blerkom 1997, Cioffi et al. 1997, Welt et al. 2003), in murine theca and granulosa cells, oocytes, CL and OS (Antczak and...
Van Blerkom 1997, Ryan et al. 2002, 2003, Archanco et al. 2003) as well as in porcine CL and OS (Smolinska et al. 2010) suggests that leptin is a product of the ovary. To date, ontogeny of the expression of the long form of leptin receptor gene in porcine ovarian follicles has not been previously examined. Therefore, the aim of the present study was to identify the localization of OB-Rb mRNA in porcine ovarian primordial, primary, secondary and antral follicles by in situ hybridization.

Materials and Methods

Experimental animals

The investigations were carried out in accordance with the principles and procedures of the Animal Ethics Committee at the University of Warmia and Mazury in Olsztyn (Poland). Cycling gilts (Large White x Polish Landrace; n=3), 7-8 months old, weighing 120-130 kg, were obtained from private breeders. The pigs were last fed in the afternoon of the day before their slaughter. Within a few minutes after slaughter, ovaries were collected. All the samples were frozen in liquid nitrogen and maintained at -80°C until in situ hybridization was performed.

In situ hybridization

Hybridization was carried out according to Smolinska et al. (2007). Briefly, paraformaldehyde-fixed sections (6 μm) from porcine ovarian samples were acetylated for 10 min in 0.25% acetic anhydride (Fluka, USA) in 0.1 M triethanolamine/0.9% NaCl and then dehydrated with ethanol series. The antisense oligonucleotide probes (5’ – TTG GGA TGC TGA TCT GAT AA – 3’) were labeled with [35S]-αdATP (Perkin Elmer, USA) at the 3'-end using terminal deoxynucleotidyl transferase (Roche, France). The sense oligonucleotide probes (5’ – AAC CCT ACG ACT AGA CTA TT – 3’) were used as a negative control for hybridization specificity. Tissue sections were air-dried and incubated for 22 h with 100 μl of hybridization solution containing 50% formamide, 10% dextran sulfate, 1×Denhardt’s solution, 4×SSC (saline sodium citrate), 0.5 mg/ml salmon sperm DNA, 0.25 mg/ml transfer RNA and appropriate probes (10⁷ cpm/ml) under parafilm coverslips at 42°C in humidified chambers. After hybridization, the slides were subjected to several washes in 1×SSC for 10 min, 2×SSC/50% formamide at 42-45°C for 15 min, 1×SSC for 15 min, and finally in distilled water for 1 min. The slides were then serially dehydrated in ethanol and air-dried. The [35S]-α dATP-labeled sections were dipped in LM-1 emulsion (Amersham Biosciences, UK), exposed for 9 days at 4°C, developed in D-19 (4 min), and fixed in Fixer (5 min; Eastman Kodak, USA). In addition, the sections were stained with hematoxylin/cosin, dehydrated in grade series of ethanol, and coverslipped with Entellan (Merck, Germany). All sections were observed under the CH30/CH40 microscope (equipped for bright-field and dark-field microscopy) and photographed by the C-5060 WZ digital camera (Olympus, Japan).

Results

The localization of OB-Rb mRNA was found in porcine oocytes and flattened/cuboidal granulosa cells of primordial follicles, in granulosa cells of primary follicles and in granulosa cells and theca cells of secondary ones. In antral stages, OB-Rb gene expression was detected in all follicular compartments, i.e., the oocyte, cumulus cells, granulosa cells and theca cells. When the sense probes were used, no stain was detected in porcine ovarian follicles (Fig. 1).

Discussion

In this study we have shown that OB-Rb mRNA is present in porcine oocytes and flattened/cuboidal granulosa cells of primordial follicles, in granulosa cells of primary follicles and in granulosa cells and theca cells of secondary ones. In antral stages, OB-Rb gene expression was detected in all follicular compartments, i.e., the oocyte, cumulus cells, granulosa cells and theca cells. When the sense probes were used, no stain was detected in porcine ovarian follicles (Fig. 1).
Fig. 1. Localization of OB-Rb mRNA determined by *in situ* hybridization (dark-field images) in porcine primordial and primary (a), secondary (b) and antral (c-g) follicles. Corresponding bright-field images depict hematoxylin/eosin staining. NC – Negative controls with the sense sequence (h). O – oocyte, G – granulosa cells, T – theca cells, CC – cumulus cells. Bar = 20 μm.

Ryan et al. 2003) as well as in porcine granulosa and theca cells, CL and OS (Ruiz-Cortes et al. 2000, 2003, Smolinska et al. 2007). This study coupled with our previous findings and results obtained in other species support the conclusion that the ovary is an important source of leptin and the hormone may act within the gland as an autocrine/paracrine factor directly modulating ovarian function.

In leptin deficient mice the number of primordial follicles and the total number of ovarian follicles have been reduced. In these animals apoptosis of GC and follicular atresia have been increased (Hamm et al. 2004). Moreover, leptin administration increased the number of primary follicles and the total number of ovarian follicles in leptin deficient mice (Barash et al. 1996). In immature rats, leptin accelerated the onset of puberty by attenuation follicular atresia and enhancement of follicular development (Almog et al. 2001). These results suggest that leptin might play a role in initiation of primordial follicle growth and the early stages of folliculogenesis, and in survival of primordial follicles.

It is conceivable that the effect of leptin on folliculogenesis is obtained through control of steroid hormones’ production. However, the action of leptin on ovarian steroidogenesis is controversial. It has been found that leptin affects the secretion of steroids in a dose-dependent and species-dependent manner. In cultured human granulosa cells, low doses of leptin increased both basal oestradiol (E2) and progesterone (P4) secretion, but high doses inhibited the release of these steroids (Tsai et al. 2002, Karamouti et al. 2009). Similarly, in porcine granulosa cells, leptin at physiological doses increased the activity of steroidogenic acute regulatory protein and the accumulation of P4, while a high dose inhibited them (Ruiz-Cortes et al. 2003). On the other hand, in mice, leptin at a high concentration significantly stimulated follicular E2, P4, and testosterone production (Swain et al. 2004). Moreover, it has been demonstrated that leptin inhibited the insulin-induced secretion of E2 and P4 by bovine granulosa cells (Spicer and Francisco 1997). Consistent with these findings, it has been reported that leptin attenuates LH-stimulated E2 production in human granulosa cells (Karlsson et al. 1997). Agarwal et al. (1999) reported an inhibitory effect of this hormone on combined IGF-I- and FSH-stimulated E2 production by human granulosa cells, and IGF-I (insulin-like growth factor I)- and LH-induced androstenedione secretion by cultured...
theca cells. In addition, in porcine preovulatory follicles, leptin impaired basal and IGF-I- and GH-stimulated E2 production (Gregoraszczuk et al. 2003, 2004). However, other studies revealed that leptin increased oestrogen secretion in cultured human granulosa cells (Kitawaki et al. 1999), and basal and IGF-I- and GH-stimulated P4 release by porcine preovulatory follicles (Gregoraszczuk et al. 2003, 2004). Therefore, the effect of leptin on ovarian follicle steroidogenesis needs to be further elucidated.

In conclusion, our results indicate that OB-Rb transcripts are present in porcine primordial, primary, secondary and antral ovarian follicles. These findings suggest that leptin might directly affect porcine follicles and plays an important role in folliculogenesis.

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References


