

Long form leptin receptor mRNA expression in the hypothalamus and pituitary during early pregnancy in the pig

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Abstract

OBJECTIVE: The aim of this study was the detection and location of long form leptin receptor (OB-Rb) in different area of hypothalamus and pituitary in the pig during early pregnancy.

SETTINGS AND DESIGN: Expression of OB-Rb was examined by RT-PCR in the different area of hypothalamus: medial basal hypothalamus (MBH), preoptic area (POA), stalk median eminence (SME), as well as pituitary: the anterior (AP) and posterior (NP) lobe collected from gilts at days 14-16 (n=4) and 30-32 (n=4) of pregnancy.

RESULTS: The results showed that OB-Rb mRNA was expressed in the hypothalamus (MBH, POA and SME), pituitary (AP, NP) and adipose tissue in the pig during early pregnancy (at days 14-16 and 30-32).

CONCLUSION: These findings support the idea that leptin might play a role in the regulation of the hypothalamic-pituitary axis activity, and consequently in the control of pregnancy during critical period of embryo implantation in the pig.

Abbreviations:

AP	anterior pituitary
cDNA	complementary deoxyribonucleic acid
CRH	corticotrophin-releasing hormone
FSH	follicle-stimulating hormone
G-CSF	granulocyte colony-stimulating factor
GH	growth hormone
GnRH	gonadotrophin-releasing hormone
IL-6	interleukin-6
JAK	Janus kinase
LH	luteinizing hormone
LIF	leukemia inhibitory factor
MBH	medial basal hypothalamus
NP	posterior pituitary
NPY	neuropeptide Y
OB-R	leptin receptor
OB-Ra, c, d, f	short form of leptin receptor
OB-Rb	long form of leptin receptor
OB-Re	secreted form leptin receptor
POA	preoptic area
POMC	proopiomelanocortin
PRL	prolactin
SME	stalk-median eminence
STAT	signal transducers and activators of transcription
TSH	thyroid-stimulating hormone

Introduction

Leptin, the product of the *ob* gene, is a protein with a molecular mass of 16 kDa secreted from adipose tissue. Leptin plays an important role in regulating feed intake and energy balance [14]. Recent evidence indicated that leptin is also involved in the regulation of neuroendocrine and reproductive functions in the pig through its effect on the hypothalamic–pituitary–gonadal axis [4, 5, 34]. Leptin acts on target cells after binding to specific receptor. The leptin receptor (OB-R), is a glycoprotein consisting of a single transmembrane-spanning component and sharing a structural similarity to the class I of cytokine receptor that most resembles the gp 130 signal transducing component of the IL-6, G-CSF and LIF receptor [35, 36]. OB-R consists of six isoforms (OB-Ra, b, c, d, e, f) generated from a different splicing of *ob* gene transcript. These isoforms, with common extracellular but different intracellular domains, belong to three major classes of leptin receptor:

- 1/ the long signal–transducing isoform, OB-Rb;
- 2/ short forms with truncated intracellular domains, OB-Ra, c, d, f; and
- 3/ secreted – a soluble isoform lacking the transmembrane and intracellular domains, OB-Re [36].

All three classes of leptin receptor have been identified in the brain [21, 23, 32].

Long form of the receptor (OB-Rb) is composed of 1162 amino acids, characterized by the longest intracellular domains of about 300 amino acids and acts via the JAK (Janus kinase)-STAT pathway of signal transduction [11, 36].

So far, leptin receptor mRNA expression has been detected in hypothalamus, anterior pituitary, reproductive tissues (ovary and uterus) in prepubertal and cyclic gilts as well as in placenta [9, 19, 21, 30]. Nevertheless, there is lack of data concerning leptin receptor expression during critical period of implantation (12–18 day of pregnancy) in pigs when approximately 30–40% of embryos is lost [38]. Since during this period expression of multiple genes for variety of factors critical for embryo survival occurs, precise timing of genes activity may be crucial for this process [24]. If so, studies on the expression of leptin and its receptor genes in this critical period may significantly contribute to the improvement of reproductiveness in pigs. Recently, a key role for leptin during pregnancy has been suggested [6, 9]. In human and rat serum leptin concentration significantly increased during pregnancy and decreased at the beginning of lactation period [1, 6, 12]. Moreover, also placenta was reported to be an additional source of leptin during pregnancy [2, 9, 13] which supports an idea that leptin and its receptor not only may actively contribute to the implantation process but also may have an impact on embryo growth and development.

The objective of this study was to detect the expression and location of the long (OB-Rb) form leptin receptor mRNA in the discrete areas of the hypothalamus and pituitary in the pig during early pregnancy.

Material and methods

Experimental animals

The studies 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). Pregnant gilts, at 9–10 month of age, and 90–110 kg b.w. were used in this study. The gilts were killed at days 14–16 (n=4) and 30–32 (n=4) of gestation. Pregnancy was confirmed through washing out and ascertainment of an embryo/foetus presence. Immediately after slaughter discrete areas of brain tissue encompassing the hypothalamus, the pituitary gland and the adipose tissue from each animal were excised. Each hypothalamic area was divided into medial basal hypothalamus (MBH), preoptic area (POA) and stalk median eminence (SME) as described by Sesti and Britt [31] while pituitary was separated on the anterior pituitary (AP) and posterior pituitary (NP) lobes. Tissue samples were frozen in liquid nitrogen and kept at –80°C until RNA isolation.

RNA Isolation and Purification

RNA was isolated from all separated tissues (MBH, POA, SME; AP, NP and adipose tissues) using RNA isolation kit (Qiagen, Maryland, USA). RNA concentration was determined on spectrometer (Lambda Bio 10, Perkin Elmer, USA), its purity was estimated from the wavelength ratio of 260/280 nm and fluctuated from 1.6 to 2.0. For each tissue sample, 2 µg of RNA

aliquot was electrophoresed in 1.5% (w/v) agarose gel to verify RNA integrity.

RT-PCR

Complementary DNA (cDNA) was generated, using from 0.5 to 2 µg of total RNA, by the Omniscript RT Kit (Qiagen, Maryland, USA). For RT-PCR leptin mRNA expression analysis, PCR amplification (GeneAmp PCR System 2400, Perkin Elmer, USA) was performed in a total volume of 50 µl containing: 40 pmol of leptin receptor primers (F: 5' TCG GAA GAT ATC AGT GTT GA 3'; R: 5' TTG GGA TGC TGA TCT GAT AA 3') and 10 pmol of internal control GAPDH primers (F: 5' CTG GCA AAG TGG ACA TTG TCG CC 3'; R: 5' CTT GGC AGC GCC GGT AGA AGC 3'), 25 µl HotStartTaq Master Mix (2.5 U HotStartTaq DNA Polymerase, 1 x PCR buffer containing 1.5 mM MgCl₂ and 200 µM of each dNTP, and 5 µl of first strand cDNA. After an initial denaturation at 95°C for 15 min, the samples were amplified for 38 cycles with

leptin receptor primers and 28 cycles with GAPDH primers (because of its higher expression level) by denaturing at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. Leptin receptor primers (access no: AF0 036908) were complementary to positions 7–26 (F) and 370–389 (R) of the leptin receptor gene sequence while GAPDH primers (access no: SSU 48832) encompassed positions 28–50 (F) and 579–599 (R) of the GAPDH gene sequence. The reaction was completed by a final extension at 72°C for 10 min. As a result of PCR amplification, bands of 382 and 571 bp representing, respectively, porcine leptin receptor and GAPDH gene fragments were obtained. PCR-amplified DNA was electrophoresed on 1.5% agarose in Tris-borate buffer, visualized by ethidium bromide staining and pictures were saved as a tif. files by FOTO/Analist Archiver software (Fotodyne, USA).

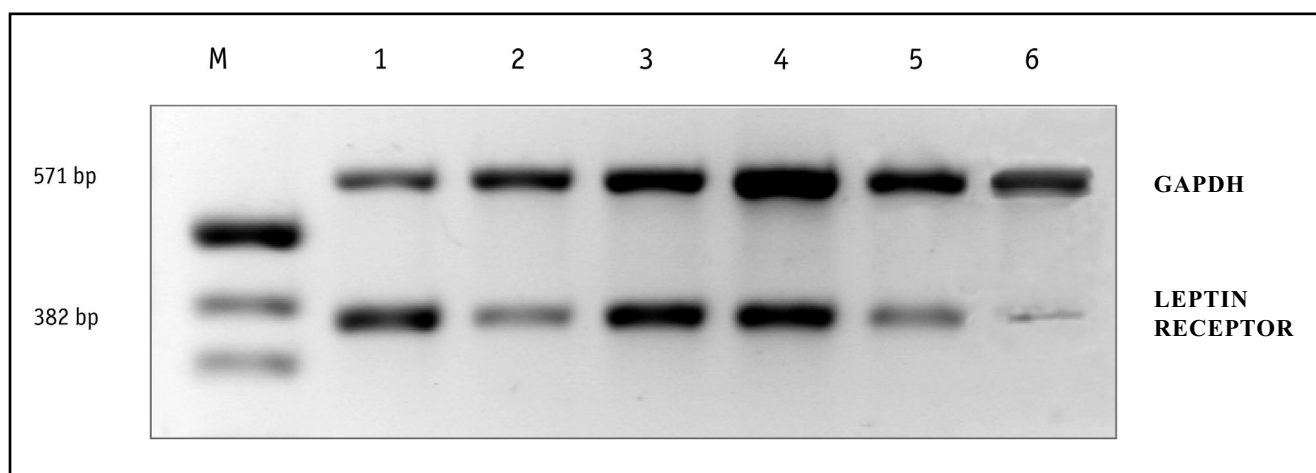


Figure 1. RT-PCR analysis for the expression of the leptin receptor (OB-Rb) in various tissues of the pig at day 14–16 of gestation. Expression levels were present in: 1/ medial basal hypothalamus (MBH); 2/ preoptic area (POA); 3/ stalk-median eminence (SME); 4/ anterior pituitary (AP); 5/ posterior pituitary (NP) and 6/ adipose tissue. GAPDH was used to check the quality of the mRNA.

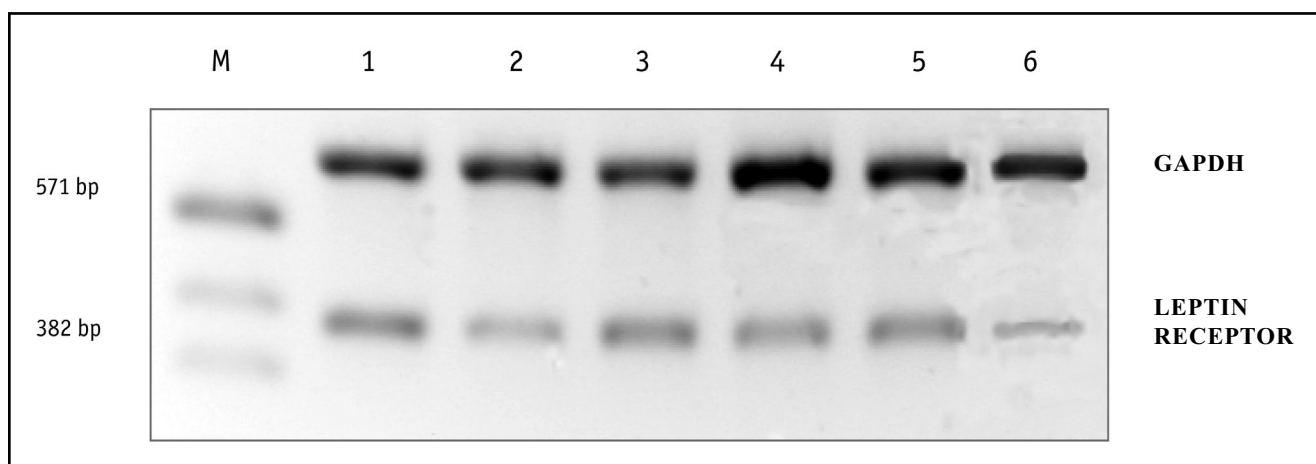


Figure 2. RT-PCR analysis for the expression of the leptin receptor (OB-Rb) in various tissues of the pig at day 30–32 of gestation. Expression levels were present in: 1/ medial basal hypothalamus (MBH); 2/ preoptic area (POA); 3/ stalk-median eminence (SME); 4/ anterior pituitary (AP); 5/ posterior pituitary (NP) and 6/ adipose tissue. GAPDH was used to check the quality of the mRNA

Sequence Analysis

RNA was reverse transcribed and PCR-amplified as described above. PCR-amplified DNA was electrophoresed on 1.5% agarose in Tris-acetate buffer. After isolation from gel DNA was sequenced (ABI Prism™ BigDye™ Terminator Cycle Sequencing kit, ABI Prism 3777 DNA sequencer, CA, USA) in both directions at the Institute of Biochemistry and Biophysics of Polish Academy of Sciences (Warsaw, Poland).

Results

The results of the RT-PCR analysis for the expression of the leptin receptor mRNA in examined tissues of pregnant pigs on two stages of early pregnancy (14–16 and 30–32 days) are presented on Fig.1 and Fig. 2. In pigs at days 14–16 and 30–32 of gestation a product of expected size (382 bp) was detected both in the discrete areas of hypothalamus (MBH, POA and SME), AP and NP lobes as well as in adipose tissue. These results were highly reproducible in all animals (in both stages of pregnancy) tested. Sequencing analysis of obtained 382 bp PCR product revealed a 100% homology with the known gene sequence for pig leptin receptor.

Discussion

The present study indicates for the first time that the long form of leptin receptor mRNA is expressed in the discrete areas of the hypothalamus (MBH, POA, SME) and pituitary (AP, NP) during early pregnancy in the pig. Gene expression for leptin receptor within the hypothalamus, pituitary and adipose tissues has been previously observed in several species including rat [27, 40], mouse [18], sheep [8], pig [19] and human [15]. In pigs, OB-Rb was detected in hypothalamus in 106 day-old foetuses, in 7-day-old female piglets and in 3.5 mo-old and 6 mo-old prepubertal females [19]. Moreover, OB-Rb was shown to be age-dependent since in pigs it increased between final period of foetal life and 3.5 mo of age and remaining elevated at 6 mo of age. Additionally, distribution of neurons containing leptin receptors in the hypothalamus in prepubertal gilts were examined by immunohistochemical staining methods [7] and leptin receptor immunoreactivity was detected both in the anterior and preoptic hypothalamic areas while no immunoreactive structures were found in the median eminence. Interestingly, in our study we observed leptin receptor mRNA expression in SME what suggests that leptin receptor might be also expressed within median eminence. So far, it has not been cleared to what extent different physiological status of animals can affect the expression of functional leptin receptor and whether the disturbances of translation process within the median eminence exist. OB-Rb mRNA was expressed in the anterior pituitary in 3.5 mo-old gilts [21] and anterior pituitary cells in 6–6.5 mo-old gilts [20]. Jin et al. localised expression of leptin receptor in several pituitary cell types in human [15], mouse and rat [17]. OB-Rb was detected in

normal rat pituitary and in various rat and mouse pituitary cell lines (FS, GH3 and α T3-1 cells) as well as in normal and neoplastic human pituitary. Leptin receptors have been localised almost exclusively to pituitary GH-secreting cells [33] and OB-Rb levels are regulated by GRF [20]. Though all anterior pituitary cell types have been suggested to express the leptin receptor [22], studies by Sone et al. [33] indicate that leptin-positive secretory cells do not possess leptin receptors. Moreover, also folliculostellate cells have been reported to express leptin receptors [16]. Additionally, leptin receptor immunoreactivity was detected in the anterior and intermediate lobes, but not in the posterior lobe of the pituitary gland in neonatal rats [27]. Further studies are necessary to explain detailed mechanisms responsible for pituitary engagement in the modulation of leptin action.

The presence of leptin receptors both at the level of the hypothalamus and pituitary indicates that these structures are target organs for leptin which not only might have direct effects on their function but also, through the modulation of hypothalamic-pituitary axis activity, could regulate different endocrine glands. It is well established that hypothalamus is the primary site for leptin action and complex interaction between leptin and NPY, GnRH, CRF, orexin, somatostatin and POMC have been clearly documented. Hakansson et al. [10] reported leptin receptor immunoreactivity in different peptidergic hypothalamic neurons, also a colocalisation of leptin receptor mRNA with NPY, CRF, POMC and orexin mRNAs was shown [19, 26]. In rats, GnRH neurons express functional leptin receptor and nitric oxide action is suggested to be involved in mediating stimulatory effect of leptin on GnRH release [39]. Moreover, it has been suggested that centrally synthesized leptin may regulate the induction of GnRH-LH axis maturation [28].

Leptin is also implicated in an anterior pituitary function as a factor regulating growth and differentiation of pituitary cells. High leptin concentration was reported to inhibit cell proliferation in human and rat pituitary cell lines [15] and *in vitro* studies indicated that leptin stimulated GH [3], LH, FSH, PRL [4, 25, 37] secretion but inhibited TSH secretion [29].

In summary, our results demonstrate the expression of the long form of the leptin receptor mRNA in the hypothalamus (MBH, POA and SME), anterior (AP), posterior (NP) pituitary and adipose tissues in pig at days 14–16 and 30–32 of gestation. These findings support the idea that leptin might play a role in regulation of the hypothalamic-pituitary axis activity and, consequently, in the control of pregnancy during critical period of implantation in the pig.

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