Expression of peroxisome proliferator activated receptor (PPAR) genes in porcine endometrium exposed in vitro to IL-6 and INFγ

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Received: 9 Jan 2012; accepted: 15 May 2012

SUMMARY

The aim of the present study was to examine the effects of interferon gamma (INFγ) or interleukin 6 (IL-6) on gene expression of PPARs in the porcine endometrium on day 14 of the estrous cycle and pregnancy. Endometrial tissue (200–210 mg), after 18 h of pre-incubation, was incubated for 6 or 12 h in the presence of INFγ (5 or 50 ng/ml) or IL-6 (1 or 10 ng/ml). Gene expression was analyzed by quantitative real-time RT-PCR.

During the estrous cycle, neither INFγ nor IL-6 affected PPARα and PPARβ/δ transcript levels in the endometrium of the cyclic pigs incubated for 6 or 12 h. The presence of INFγ (5 ng/ml) significantly
(p<0.05) increased PPARγ1 gene expression in the tissue incubated for 12 h. During pregnancy, INFγ (50 ng/ml) significantly (p<0.05) enhanced PPARα and PPARβ/δ mRNA levels in the endometrium incubated for 6 h, whereas IL-6 (1 or 10 ng/ml) did not change their expression at any incubation time. The effect of both cytokines on PPARγ1 transcript level differed and was dependent on the incubation time. We observed an inhibitory (after 6 h of incubation, p<0.0001) and a stimulatory (after 12 h of incubation, p<0.05) effect of INFγ (5 ng/ml) or IL-6 (10 ng/ml) on PPARγ1 gene expression. The present study indicates that INFγ and IL-6 modulate PPARs gene expression in the porcine endometrium during the estrous cycle and pregnancy. The effect depends on the reproductive status of animals and the length of in vitro incubation of endometrial tissue with the treatments. Reproductive Biology 2012 12 2: 157–170.

Key words: PPAR alpha, PPAR beta, PPAR gamma, implantation, reproduction

INTRODUCTION

Successful implantation requires the precise synchronization of the fetus development and the uterus receptivity. The highest mortality of fetuses was found during the peri-implantation period [19]. Implantation in mammals necessitates significant changes in secretion of various hormones, growth factors, cytokines, enzymes and adhesion molecules crucial for the embryo-maternal interaction [for review see: 2, 8, 24]. Cytokines are primarily known for their immunological significance, however they are also involved in the regulation of reproductive processes [20, 23]. For instance, the presence of some cytokines in the uterus is essential for pregnancy maintenance [32]. A transient increase in IL-6 expression in the porcine conceptus and a higher IL-6 activity in uterine lumen fluid found during early pregnancy (days 11–21) imply its role during the implantation [2, 31]. It has been reported that IL-6 regulates ovarian steroidogenesis [20, 23] as well as enhances endometrial cell
proliferation, blood flow and vessel permeability [31]. Moreover, elevated level of IL-6 was found to accompany many reproductive disorders including endometriosis [36, 42].

Embryonic interferons (including INFγ) were initially associated with the ability to protect cells from viral infections, but afterward their involvement in the regulation of reproductive functions has been frequently reported [10, 33]. During early pregnancy INFγ is secreted in large amounts by the porcine trophoblast, and this is probably linked to the endometrial defense against viral antigens [35]. High antiviral activity has been observed during blastocyst implantation [14]. In addition, INFγ plays an important role in the remodeling of uterine endometrial epithelium and in promoting cell adherence during the implantation [11, 33].

Peroxisome proliferator activated receptors (PPARs) are nuclear receptors which are predominantly known for their involvement in glucose and lipid metabolism. Recently, their role in the regulation of female reproduction has also been emphasized. PPARβ/δ and PPARγ are required for normal fertility, proper placenta development and maternal-fetal nutrient exchange [for review see: 17, 40]. The absence of the PPARγ gene interferes with the implantation process and leads to embryo death during early pregnancy in rodents. All PPAR isoforms have been found in many reproductive tissues including the ovary, uterus and placenta [for review see: 30, 41], and they were reported to regulate steroidogenesis, angiogenesis, prostaglandin production and/or tissue remodeling [for review see: 28].

Despite many evidence supporting the importance of cytokines and PPARs in the regulation of reproductive processes in females, no information on a relationship between these hormones and receptors is available. Our unpublished data suggest that factors produced by the embryo (e.g. cytokines) locally affect PPAR endometrial gene expression [7]. Therefore, in this study we examined the effects of IL-6 or INFγ on gene expression of three PPAR isoforms in the endometrium collected on day 14 of the porcine estrous cycle and pregnancy.
MATERIALS AND METHODS

Animals

The experiment was carried out on crossbred sows of Polish Landrace and Pietrain originating from a commercial farm. Animals were maintained under ambient photoperiod and temperature (18–20°C) with free access to water and food. Prepubertal gilts (100 kg, 7 month-old) were synchronized and superovulated by a single intramuscular (im) injection of 750 I.U. PMSG (Folligon, Intervet, Netherlands) followed by 500 I.U. hCG (im; Chorulon, Intervet, Netherlands) administered 72 h later [6, 26]. The animals were divided into two groups: cyclic (n=4) or pregnant (n=4) group. The gilts from the latter group were inseminated two times: 24 h and 36 h after hCG treatment. Pregnancy was confirmed by the embryo presence in uterine flushings. Animals were slaughtered on day 14 of the estrous cycle or pregnancy. The uterine horns were dissected and transported to the laboratory on ice in PBS with antibiotics. Uterine horn was selected randomly and cut longitudinally. The endometrium was collected from several randomly selected spots in the middle portion of the mesometrial side of the uterine horn. All procedures were approved by the Local Animal Ethics Committee and the study was conducted in accordance with the national guidelines for animal care.

In vitro experiment

Endometrial tissue collection and incubation of endometrial tissue was described previously [18]. Briefly, the endometrium was separated from the myometrium by scraping with scalpel blade, minced into small pieces (200–210 mg) and washed twice with PBS [18]. Each tissue piece was individually placed in a sterile culture vials with 2 ml medium 199 supplemented with 0.1% BSA, gentamycin (40 mg/ml) and nystatin (120 IU/ml). The endometrial pieces were pre-incubated in water bath for 18 h in an atmosphere of 95% O₂ and 5% CO₂ and then treated with INFγ (5 or 50 ng/ml, Biomol, GMBH, Germany) or IL-6 (1 and 10 ng/ml, Biomol, GMBH,
Germany) for 6 h or 12 h. The cytokine concentrations and incubation times were selected according to earlier reports (IL6: [2, 18, 31]; INFγ: [3, 27]) and results of a preliminary study. After incubation, the endometrial explants were washed with cold PBS and were snap frozen in -80°C for total RNA isolation and quantitative real-time RT-PCR analysis.

**RNA extraction and real-time RT-PCR**

Total RNA was isolated with ‘Total RNA’ kit (A&A Biotechnology, Poland) and quantified spectrophotometrically. The integrity of the product was confirmed on 1.5% agarose gel. The sequences of primers and Taqman probes for PPARα (GenBank acc no. NM_001044526), PPARβ/δ (GenBank acc no. NM_214152), PPARγ1 (GenBank acc no. AJ006756) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GenBank acc no. U48832) were designed using Primer Express 3 Software (Applied Biosystems, CA, USA), and were synthesized by Applied Biosystems. The sequences, access numbers in GenBank and lengths of the products were reported previously [6].

Quantitative real-time RT-PCRs were performed using TaqMan®RNA-to-CTTM 1-Step Kit (Applied Biosystems, CA, USA; [6]). The concentrations of the PCR primers were 300–600 nM (depending on the gene). TaqMan fluorogenic probes (200 nM) were labeled with 6-FAM,6-carboxyfluorescein (FAM) dye. Real-time RT-PCR was carried out in an ABI PRISM 7300 sequence detector (Applied Biosystems, CA, USA) using the following parameters: one cycle at 48°C for 30 min, then one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and one cycle at 60°C for 1 min. All expression data were normalized to the amount of GAPDH mRNA expression level.

**Statistical analysis**

Data are expressed as means±SEM. Statistical analysis was performed using Statistica (version 8.0, StatSoft Inc, USA). Significant differences were established by two-way Anova followed by least significant differences (LSD) post hoc test. Two-way Anova was performed to determine the effect
of the tested factors, incubation time and interaction between the factors and time. Different letters designate significant statistical differences (p≤0.05).

RESULTS

PPARα and PPARβ/δ gene expression

The expressions of PPARα (fig. 1) and PPARβ/δ (fig. 2) mRNA in the porcine endometrium incubated with INFγ or IL-6 displayed a similar pattern. During the estrous cycle, INFγ or IL-6 did not affect PPARα (fig. 1A and 1B) and PPARβ/δ (fig. 2A and 2B) transcript level in the endometrium incubated for 6 or 12 h. During pregnancy, the higher dose of INFγ significantly (p<0.05) increased mRNA level of both PPARs in the endometrial explants incubated for 6 h, but not in the explants incubated for 12 h (fig. 1/2C and 1/2D). IL-6 did not affect PPARα and PPARβ/δ mRNA expression in endometrium of pregnant gilts (fig. 1/2 C and D). Two-way Anova showed a significant impact of treatment (F=2.912; p<0.05) on the PPARβ/δ mRNA expression during pregnancy.

PPARγ1 gene expression

During the estrous cycle, the lower dose of INFγ significantly (p<0.05) increased PPARγ1 gene expression in the endometrium incubated for 12 h (fig. 3B). Two-way Anova showed a significant impact of interaction between treatment and incubation time (F=3.711; p<0.05) on PPARγ1 mRNA expression.

During pregnancy, the effect of both cytokines on PPARγ1 transcript level differed and was dependent on the length of incubation time. We observed an inhibitory (after 6 h, p<0.0001, fig. 3C) and a stimulatory (after 12 h, p<0.05, fig. 3D) effect of INFγ (5 ng/ml) and IL-6 (10 ng/ml) on PPARγ1 gene expression. There was a significant impact of the incubation time (F=20.558; p<0.0001) and the interaction between treatment and incubation time (F=11.130; p<0.0001) on PPARγ1 mRNA expression.
Figure 1. The effects of interferon gamma (INFγ; 5 or 50 ng/ml) and interleukin 6 (IL-6; 1 or 10 ng/ml) on PPARα mRNA expression in the endometrial tissue obtained from gilts on day 14 of the estrous cycle (A and B) and day 14 of pregnancy (C and D). Tissue explants were incubated with treatments for 6 (A and C) or 12 (B and D) hours. The expression of mRNA was determined by quantitative real-time RT-PCR. Different letters designate significant statistical differences (p<0.05).

DISCUSSION

The present study demonstrated the modulatory effect of IL-6 and INFγ on PPARs gene expression in the porcine endometrium. This effect was dependent on the reproductive status of pigs and the time of tissue exposure to the treatments. The influence of cytokines was very weak on
Figure 2. The effects of interferon gamma (INFγ; 5 or 50 ng/ml) and interleukin 6 (IL-6; 1 or 10 ng/ml) on PPARβ/δ mRNA expression the endometrial tissue obtained from gilts on day 14 of the estrous cycle (A and B) or day 14 of pregnancy (C and D). Tissue explants were incubated with treatments for 6 (A and C) and 12 (B and D) hours. The expression of mRNA was determined by quantitative real-time RT-PCR. Different letters designate significant statistical differences (p<0.05).

day 14 of the estrous cycle, but clearly evident on day 14 of pregnancy. IL-6 did not change transcript levels of any of the three PPAR isoforms in the endometrium of cyclic pigs, but it significantly affected (decreased or stimulated) PPARγ1 mRNA level in pregnant gilts. INFγ did not affect the expression of PPARα or PPARβ/δ in the endometrium of cyclic gilts in any of the incubation periods, but significantly increased PPARγ1 mRNA
level after 12 h of incubation. In pregnant sows, INFγ affected (decreased or increased) the levels of all PPARs mRNA.

It has been frequently reported that cytokines and PPARs play an important role during gestation, especially at the implantation stage [for review see: 8, 10, 11, 25]. Most researchers described PPARs as important immunomodulators in various types of cells, including vascular smooth
muscle cells, macrophages, T lymphocytes, airway epithelial cells and endothelial cells [5, 21, 24, 38]. The activation of PPARs has different effects dependent on the type of cell or tissue. PPARγ activators have been shown to have a stimulating effect on IL-6 expression and secretion in human endometrial cells [30, 39], whereas an inhibitory effect has been observed in immune and vascular wall cells [13]. Lappas et al. [29] reported that PPARγ ligands inhibited lipopolysaccharide-stimulated release of IL-6 by human placental, amnion and chorionic tissues.

There are limited data concerning the involvement of PPARs in the production of proinflammatory cytokines in female reproductive tissues [27]. An opposite effect has not been reported, though such association has been observed in other tissues. The inhibitory effect of IL-6 on PPARα or PPARβ/δ expression has been demonstrated in fetal rat hepatocytes incubated in vitro [34] and in human HepG2 cells (hepatocytes cell line; [1, 22]). IL-6 has been shown to have an opposite effect on PPARs expression in human skeletal muscle cells [1, 22]. Furthermore, treatment of the type 2 diabetic patients with IL-6 decreased PPARα, PPARγ and PPARβ/δ mRNA levels in skeletal muscles, but increased the levels in adipose tissue [1, 9]. Thus, the effect of IL-6 on PPARs expression is evident, but it depends on the type of tissue.

In the present experiment, IL-6 did not have a significant impact on PPARα and PPARβ/δ transcript levels in the porcine endometrium during the estrous cycle or pregnancy, but the cytokine had a marked effect on PPARγ1 transcript level. The observed effect varied dependent on incubation time. The inhibitory action of IL-6 after 6 h of incubation and its stimulatory effect after 12 h of incubation are difficult to explain. The intracellular effects of IL-6 are typically mediated by the activation of the Janus kinase (Jak)/signal transducer and the activator of transcription (STAT) pathway [15]. The involvement of other signaling pathways cannot be ruled out [15]. It has been reported that the exposure of primary human skeletal muscle cells to IL-6 increased the phosphorylation of STAT-3, ERK1/2 and AMP-activated protein kinase (AMPK) with a concomitant augmentation of PPARs gene expression [1]. A different mechanism involving CCAAT/enhancer-binding protein isoforms has also been proposed for IL-6-mediated inhibition
of PPARα gene expression in human hepatocytes [12]. In our experiment, the diverse effects of IL-6 on PPARs gene expression could be linked to the activation of various signaling pathways in porcine endometrial tissue. Further work is required to investigate the above possibility.

There is limited information regarding possible association between PPARs and INFγ. It has been demonstrated that the activation of macrophages by INFγ enhanced the expression of several nuclear receptors, including PPARγ [4]. However in adipocytes, INFγ inhibited PPARγ synthesis and increased the degradation of PPARγ in proteasomes [16, 37]. The presence of such relationships in the reproductive system has not been described. Our results indicate that INFγ, to greater extent than IL-6, regulates gene expression of PPARs in endometrial tissue during the estrous cycle.

To conclude, our study demonstrated that INFγ and IL-6 modulate PPARs gene expression in the porcine endometrium on day 14 of the estrous cycle and pregnancy. INFγ affected transcript levels of all PPAR isoforms, whereas IL-6 modulated only the expression of the PPARγ1 gene. In addition, our results demonstrated that the length of incubation time significantly affected gene expression. A clear relationship between the cytokine presence and PPARs gene expression on day 14 of pregnancy suggests that IL-6 and INFγ are involved in the regulation of embryo implantation.

ACKNOWLEDGMENTS

This research was supported by the State Committee for Scientific Research (Project N N311 360235). We sincerely appreciate Dr. Katarzyna Kamińska and Michal Blitek for their technical assistance in the experiment.

REFERENCES


28. Komar C 2005 Peroxisome proliferator-activated receptors (PPARs) and ovarian function-implications for regulating steroidogenesis, differentiation, and tissue remodeling. *Reproductive Biology and Endocrinology* 3 41.


