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The expression of mRNAs for opioid receptors in endometrium of cyclic and early pregnant pigs; *in vitro* effects of IL-1β, IL-6 and TNFα

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ABSTRACT. The expression of *mu (μ)*, *delta (δ)* and *kappa (κ)* opioid receptors was determined *in vitro* under the basal conditions as well as in the presence of cytokines (interleukin (IL)-1β, IL-6 and tumor necrosis factor α (TNFα)) in the endometrium of gilts on days 10–11, 12–13 and 15–16 of the oestrous cycle and pregnancy. These days were chosen due to their importance for the establishment of pregnancy in pigs. During the oestrous cycle, the basal expression of μ opioid receptor mRNA in endometrial explants was greater on days 10–11 than on days 12–13 and 15–16, during pregnancy its expression was greater on days 12–13 than on days 10–11 and 15–16. The expression of δ opioid receptor mRNA did not change during the cycle, but during pregnancy it was the greatest on days 10–11. The expression of κ opioid receptors did not significantly change during studied periods. The cytokines affected the expression of μ and δ, but not κ opioid receptors in endometrial explants. The expression of μ opioid receptor mRNA was significantly decreased by IL-1β on days 10–11, but IL-6 increased and decreased it on days 12–13 and 15–16 of the cycle, respectively. During pregnancy, the expression of μ opioid receptor mRNA in endometrial explants was increased by IL-1β, IL-6 and TNFα on days 10–11, decreased by IL-1β on days 12–13, and increased by TNFα on days 15–16. During the oestrous cycle, the expression of δ opioid receptor mRNA was elevated by IL-1β on all studied days, by IL-6 on days 10–11 and 12–13, and by TNFα on days 15–16. During pregnancy, IL-1β increased the expression of δ opioid receptor transcript on days 12–13. These data suggest that opioid receptors participate in a local regulation of uterine functions in gilts during the oestrous cycle and early pregnancy and their endometrial expression can be modulated by cytokines.

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Introduction

The uterus is an essential organ for reproduction in mammals since it ensures proper environment for reception of embryos and their further development. During the oestrous cycle and pregnancy, the uterus undergoes specific remodeling and exhibits high secretory activity (Krzymowski and Stefańczyk-Krzymowska, 2008; Okrasa et al., 2014). Uterine secretory products include enzymes, growth factors, transport proteins, chemokines, cytokines, hormones and neuropeptides, such as endogenous

opioid peptides (EOPs) (Waclawik, 2011; Ziecik et al., 2011; Okrasa et al., 2014). These peptides belong to three major families: endorphins, enkephalins and dynorphins, which preferentially act through one type of opioid receptors: *mu (μ)*, *delta (δ)* or *kappa (κ)*, respectively (Simon, 1991).

The presence of EOPs and/or the expression of opioid precursor genes in the uterine tissues has been demonstrated in many studies performed with females of different species, including pigs, as discussed in previous paper (Dziekoński et al., 2015). Information concerning the presence of opioid receptors in the uterus is scarce. Specific opiate binding sites were demonstrated in the uterine membrane preparations of pregnant rats (Baraldi et al., 1985). In pregnant mouse, the expression of mRNAs for opioid receptors was demonstrated in the uteroplacental structures by *in situ* hybridization; μ receptor was identified in the nondecidualized endometrium stromal cells and in the luminal epithelium, κ receptor in the decidual basalis, both μ and κ receptors in myometrium, and δ receptor in the trophoblast giant cells (Zhu and Pintar, 1998). Moreover, the expression of mRNAs and proteins for μ and δ opioid receptors was found in the myometrium of pregnant women (Fanning et al., 2013). However, the expression of opioid receptors has not been studied in the uterus of large domestic animals yet.

The previous studies have demonstrated that expression/activity of endogenous opioid system is controlled by many factors, including cytokines. The proopiomelanocortin (*POMC*) gene expression in the rat anterior pituitary was increased *in vivo* by interleukin (IL)-1β (Parsadaniantz et al., 1997) as well as in the mouse corticotroph tumor cell line AtT20 by IL-1β and tumor necrosis factor α (TNFα) (Ruzicka and Akil, 1995; Takayasu et al., 2010). In turn, an inhibitory effect of IL-1β on proenkephalin (*PENK*) mRNA expression was found in astrocytes derived from hippocampus of the rat brain (Ruzicka and Akil, 1997). The expression of prodynorphin (*PDYN*) gene was inhibited by IL-6 and TNFα in the human macrophage U-937 line (Sun et al., 2006), however, IL-1 was not effective in this study. In our recent study, the effects of selected cytokines (IL-1β, IL-6 and TNFα) on the expression of genes encoding opioid precursors in the porcine endometrial explants on days 10–11, 12–13, 15–16 of the oestrous cycle and early pregnancy were observed (Dziekoński et al., 2015). The strongest influence was exerted by IL-6 on the *PENK* gene expression during all studied days of the cycle as well as on days 10–11 and 12–13 of pregnancy. Furthermore, TNFα increased the *PENK* gene expression on days 10–11, but IL-1β decreased it on days 15–16 of pregnancy. These data indicate a differentiated potential of proinflammatory cytokines to affect the expression of genes for opioid precursors depending on species and physiological status of experimental animals as well as studied type of cells/tissues.

Cytokines have also been found to affect the expression of genes for opioid receptors in different cells and tissues. IL-1β increased the level of μ opioid receptor mRNA and reduced κ opioid receptor, but did not affect the δ opioid receptor mRNA level in astrocytes-enriched cultures derived from chosen rat brain regions (Ruzicka and Akil, 1997). In turn, IL-4 and TNFα induced the expression of μ opioid receptor in human primary blood cells (T cells, polymorphonuclear leukocytes), immune cell lines (Raji, U-937 and HMEC-1) and dendritic cells (Kraus et al., 2001, 2003). IL-6 induced the μ opioid receptor mRNA in the human nueroblastoma cell line SH SY5Y, but did not affect the δ opioid receptor mRNA level (Börner et al., 2004). Moreover, in the brain of IL-6-knockout mice, significantly lower level of μ opioid receptor was found than in wild-type animals (Bianchi et al., 1999). In rats, the κ opioid receptor mRNA level was reduced by IL-6 in primary Sertoli cells (Jenab and Morris, 2000). It is known that cytokines may affect different processes in the uterus, such as secretory activity, adaptation to the early pregnancy or immunomodulation (Dimitriadis et al., 2005; Waclawik, 2011; Ziecik et al., 2011; Franczak et al., 2012, 2013; Okrasa et al., 2014), however their effect on the expression of genes encoding opioid receptors in this organ has not been tested yet.

This study was undertaken to verify hypothesis that genes coding for opioid receptors are expressed in the porcine endometrium and this process is modulated by proinflammatory cytokines. Therefore, the objective of this study was to determine: 1 - the endometrial expression of genes coding for the opioid receptors (μ, δ and κ) at mRNA level on days 10–11, 12–13 and 15–16 of the oestrous cycle and early pregnancy and 2 - the effect of selected cytokines (IL-1β, IL-6 and TNFα) on the expression of these genes during studied periods in the pig. The above mentioned periods were selected since in pregnant pigs they are important for: 1 - migration of the embryos to and within the uterus (days 10–11), 2 - maternal recognition of pregnancy (days 12–13) and 3 - corpus luteum (CL) protection against luteolysis and the onset of implantation (days 15–16) (Geisert et al., 1982). The same days of the oestrous cycle were considered for comparative purposes.

Material and methods

Animals

All experiments were performed in accordance with the principles and procedures of the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn (Poland). Tissues were harvested from mature cross-bred (Large White × Polish Landrance) gilts, weighting 90–110 kg, during the oestrous cycle and early pregnancy, on days 10–11, 12–13, 15–16 (n = 5 for each group). Oestrus behaviour was determined by using intact boar. The onset of the second oestrus was designated as day 0 of the oestrous cycle. Half of experimental gilts were randomly naturally bred on the second day of oestrus. The animals were slaughtered on appropriate day of the cycle or pregnancy in a local abbatoir. The stage of the oestrous cycle was verified by testing morphology of the ovaries (Dziekoński et al., 2015), while pregnancy was confirmed by the presence of embryos in the uterine flushings. Uterus was placed in ice-cold phosphate buffered saline (PBS) supplemented with 100 IU · ml^{-1} of penicillin and 100 µg · ml^{-1} of streptomycin and transported to the laboratory for endometrial tissue isolation.

Preparation and incubation of endometrial explants

Endometrial tissue was separated from the myometrium by careful scraping with a scalpel blade from the middle part of uterine horn of each gilt. Next, the tissue was cut into 200–210 mg slices and washed twice with PBS. Slices from each pig were placed in culture vials containing 2 ml of Medium 199 (Sigma-Aldrich; St. Louis, MO, USA) supplemented with 0.1% bovine serum albumin (BSA) fraction V (Roth; Karlsruhe, Germany) and 20 µg of gentamycin (Sigma-Aldrich; St. Louis, MO, USA). Endometrial explants were preincubated for 18 h at 37 °C under the atmosphere of 95% O_2 and 5% CO_2, and then incubated in fresh media for 12 h without or with the addition of IL-1β (10 ng·ml^{-1}), IL-6 (10 ng·ml^{-1}) or TNFα (10 ng·ml^{-1}) under the same conditions. All cytokines have been purchased from Biomol GmbH (Hamburg, Germany). The concentrations of studied factors were selected on the basis of the previous studies by Franczak et al. (2012). After incubation, the tissue explants were recovered, washed with PBS and stored at –80 °C for further analysis.

RNA isolation and reverse transcription

Isolation of total RNA was conducted with Qiagen-RNeasy columns (Qiagen; Hilden, Germany) according to the manufacturer's protocol. The quantity and purity of RNA were determined spectrophotometrically (TECAN; Männedorf, Switzerland), then randomly selected samples were additionally tested by the electrophoresis in 1.5% agarose gel. Reverse transcription (RT) was performed using QuantiTect Reverse Transcription Kit (Qiagen; Hilden, Germany) in the way described before (Dziekoński et al., 2015). Obtained cDNAs were frozen at –20 °C and stored for Real-Time PCR analysis.

Real-Time PCR and sequencing

The expression of genes coding for opioid receptors was determined by Real-Time PCR method (7500 Real-Time PCR System, Applied Biosystems; Waltham, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used as a normalization control. Primers for this gene (Table 1) were used according to Bogacka et al. (2006). Primers for genes encoding opioid receptors (Table 1) were designed using Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/). Each sample prepared for Real-Time PCR analysis contained: SYBR Green MIX (12.5 µl) (Applied Biosystems; Waltham, MA, USA), specific primers at the concentration 10 nM (1 µl for each studied gene), cDNA (2 µl) and filled up with RNase free water to the final volume (25 µl). The PCR programme was started with an initial denaturation step (10 min at 95 °C) and then comprised 40 cycles. Each cycle consisted of the following steps: denaturation (95 °C) for 15 sec, primers annealing at a specific temperatures (Table 1) for 1 min and elongation (72 °C) for 1 min. The last PCR cycle included a final extension

Table 1. Primers specific for genes encoding opioid receptors and GAPDH used for Real-Time PCR

Gene	Forward primer	Reverse primer	T_A°C	Product size	NCBI
GAPDH	CCTTCATTGACCTCCACTACATGGT	CCACAACATACGTAGCACCACGAT	59	183	U48832
MOR	CCTGTGATGTTCATGGCAAC	GATTCTTCGCAGGTTCCTATC	60	234	NM_001001538.1
DOR	AACATCTGCATCTGGGTCCT	AGCATAAGGCCGTAGCACAC	63	205	XM_003356260.3
KOR	TCGAGTGCTCCTTGCAGTTC	ATGCGACGGAGATTGCGATC	60	193	XM_003355059.3

GAPDH – glyceraldehyde-3-phosphate dehydrogenase, *MOR* – *mu* opioid receptor, *DOR* – *delta* opioid receptor, *KOR* – *kappa* opioid receptor, NCBI – mRNA sequence accession number, T_A – the temperature of annealing

step (10 min at 72 °C). All runs included negative controls which were performed without RT products. After Real-Time PCR reaction, randomly selected samples were sequenced (Genomed; Warsaw, Poland) to confirm the specificity of amplicons for studied genes.

Statistical analysis

All data were normalized using the comparative Ct method (ΔΔCt method) and expressed as means ± standard error of the mean (SEM; Livak and Schmittgen, 2001). Statistical analysis was performed using Statistica 10 software (StatSoft Inc., 2011). The obtained results, after logarithmical transformation, were submitted to one-way analysis of variance (ANOVA) followed by NIR Fisher's post-hoc test to determine significant differences among mean values. Differences with $P < 0.05$ were considered to be statistically significant.

Results

Basal expression of opioid receptor genes in the porcine endometrial explants

***Mu* opioid receptor.** The basal expression of μ opioid receptor mRNA in the endometrial explants was the greatest on days 10–11 and significantly different ($P < 0.05$) from those on days 12–13 and 15–16 of the oestrous cycle (Figure 1A). During pregnancy, the expression of μ opioid receptor mRNA was greater ($P < 0.05$) on days 12–13 than on days 10–11 and 15–16. Comparison between parallel days of studied periods has demonstrated significantly greater values during the cycle – on days 10–11 ($P < 0.05$) and during pregnancy – on days 12–13 ($P < 0.05$).

***Delta* opioid receptor.** The expression of δ opioid receptor mRNA in the porcine endometrial explants under the basal conditions did not significantly change in the oestrous cycle (Figure 1B). During pregnancy, the expression of this gene was the greatest on days 10–11 and significantly different ($P < 0.05$) from those on days 12–13 and 15–16. Comparison between parallel days of the oestrous cycle and pregnancy has shown significant difference in the expresion of δ opioid receptors only on days 15–16 ($P<0.05$), which appeared to be greater in cyclic gilts.

***Kappa* opioid receptor.** The basal expression of κ opioid receptor mRNA in the endometrial explants did not significantly differ between studied days of the oestrous cycle or pregnancy (Figure 1C). However, significantly greater ($P < 0.05$) expression of κ opioid receptor gene was noted on days 15–16 of the cycle than on the respective days of pregnancy.

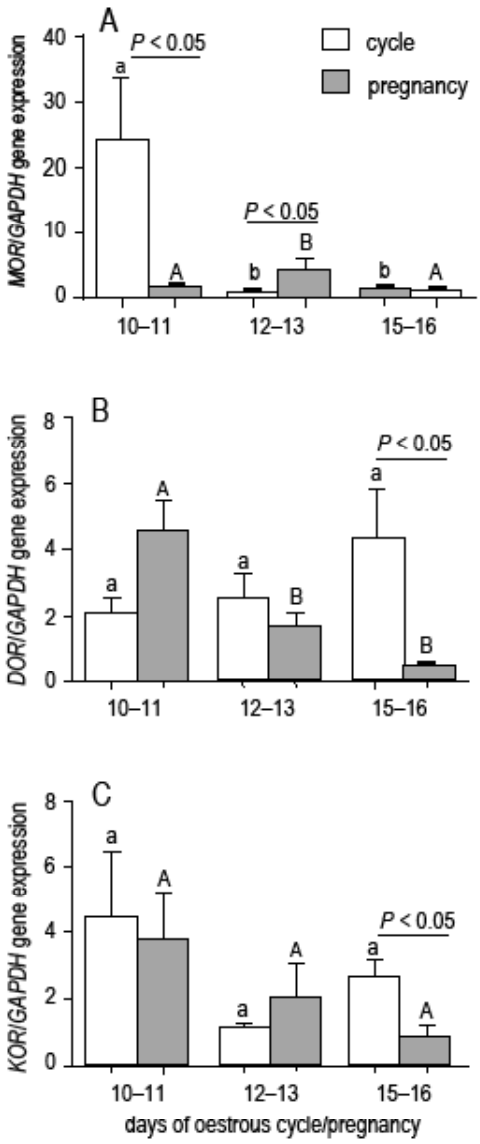

Figure 1. Changes in basal expression of A – *mu* opioid receptor (*MOR*), B – *delta* opioid receptor (*DOR*) and C – *kappa* opioid receptor (*KOR*) genes in porcine endometrium during oestrous cycle and pregnancy (days 10–11, 12–13, 15–16). Various letters indicate significant differences between different days ($P < 0.05$) of oestrous cycle (lower case letters) or pregnancy (upper case letters). Significant differences between comparable days of cycle and pregnancy are marked with horizontal lines and *P* values

The influence of IL-1β, IL-6 and TNFα on the expression of opioid receptor genes in the porcine endometrial explants

***Mu* opioid receptor.** During the oestrous cycle (Figure 2A), IL-1β significantly decreased ($P < 0.05$) the expression of μ opioid receptor mRNA in the endometrial explants on days 10–11. IL-6 significantly increased ($P < 0.05$) this receptor mRNA on days 12–13, but decreased it ($P <0.01$) on days 15–16. TNFα tended to decrease ($P = 0.055$) the expression of μ opioid receptor mRNA on days 10–11. During pregnancy (Figure 2B), the expression

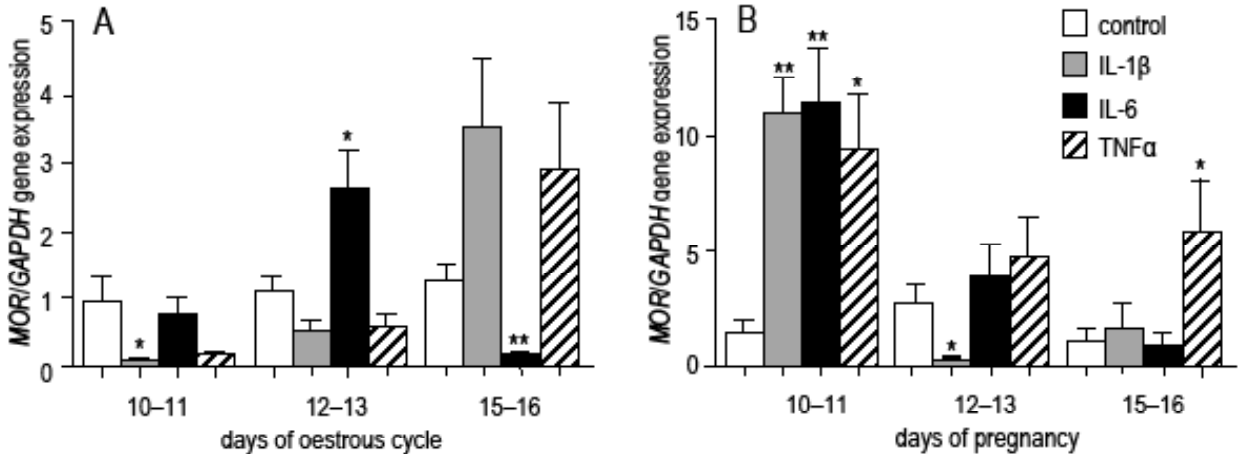

Figure 2. Influence of IL-1β (10 ng · ml^{-1}), IL-6 (10 ng · ml^{-1}) or TNFα (10 ng · ml^{-1}) on *mu* opioid receptor (*MOR*) gene expression in porcine endometrium during A – oestrous cycle and B – pregnancy (days 10–11, 12–13, 15–16) after 12 h incubation (n = 5). Significant differences in comparison to respective control value are marked with asterisks (* $P < 0.05$; ** $P < 0.01$)

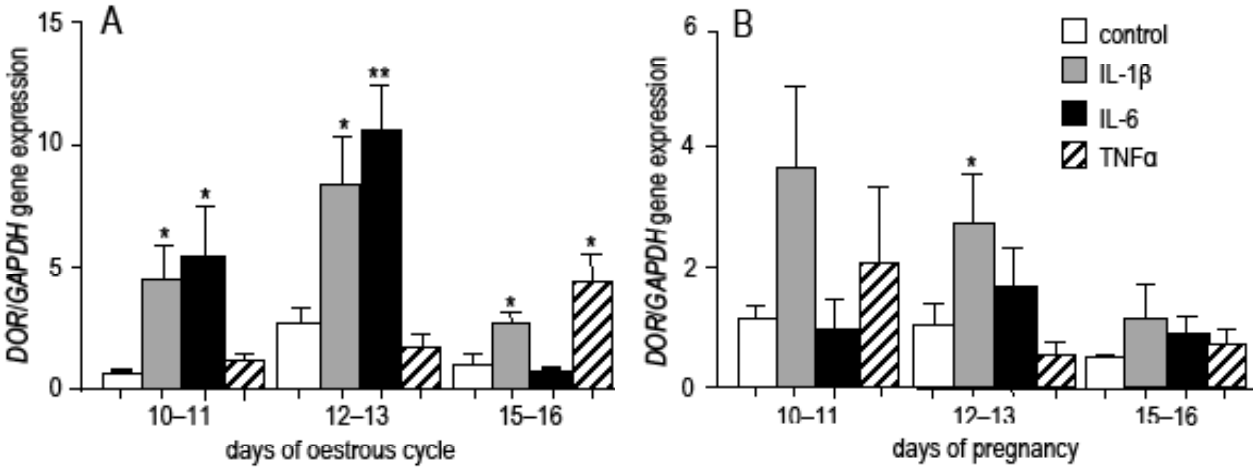

Figure 3. Influence of IL-1β (10 ng · ml^{-1}), IL-6 (10 ng · ml^{-1}) or TNFα (10 ng · ml^{-1}) on *delta* opioid receptor (*DOR*) gene expression in porcine endometrium during A – oestrous cycle and B – pregnancy (days 10–11, 12–13, 15–16) after 12 h incubation (n = 5). Significant differences in comparison to respective control value are marked with asterisks (* $P < 0.05$; ** $P < 0.01$)

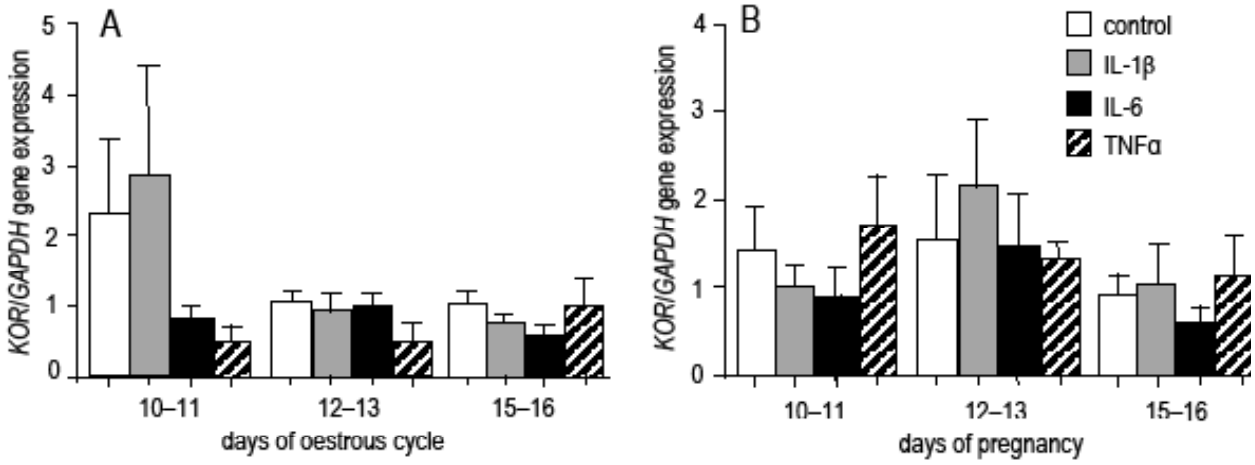

Figure 4. Influence of IL-1β (10 ng · ml^{-1}), IL-6 (10 ng · ml^{-1}) or TNFα (10 ng · ml^{-1}) on *kappa* opioid receptor (*KOR*) gene expression in porcine endometrium during A – oestrous cycle and B – pregnancy (days 10–11, 12–13, 15–16) after 12 h incubation (n = 5)

of μ opioid receptor mRNA in endometrial explants was increased by all studied cytokines on days 10–11, decreased ($P < 0.05$) by IL-1β on days 12–13, and increased ($P < 0.05$) by TNFα on days 15–16.

***Delta* opioid receptor.** During the oestrous cycle (Figure 3A), the expression of δ opioid receptor mRNA in the endometrial explants was increased ($P < 0.05$) by IL-1β during all studied days, IL-6 – on days 10–11 ($P < 0.05$) and 12–13 ($P < 0.01$), and TNFα – on days 15–16 ($P < 0.05$). During pregnancy (Figure 3B), only IL-1β significantly increased ($P < 0.05$) the expression of this gene on days 12–13.

***Kappa* opioid receptor.** In response to tested cytokines, no significant changes in the expression of κ opioid receptor mRNA in the endometrial explants were observed during both, the oestrous cycle (Figure 4A) and early pregnancy (Figure 4B).

Discussion

The uterine expression of opioid precursor genes and/or opioid peptides in different species, including the pig, has been demonstrated in numerous studies (Dziekoński et al., 2015), but data concerning the presence and regulation of opioid receptors in the uterus are limited (Baraldi et al., 1985; Zhu and Pintar, 1998; Fanning et al., 2013). The present study demonstrated *in vitro* changes in the expression of mRNAs for opioid receptors in the porcine endometrial explants under the basal conditions as well as in the presence of proinflammatory cytokines (IL-1β, IL-6 and TNFα) during the oestrous cycle and early pregnancy.

In the present study, the expression of mRNAs for three major types of opioid receptors (μ, δ and κ) in the pig endometrial explants was confirmed under the basal conditions during the oestrous cycle and early pregnancy. The presence of the opioid binding sites has been revealed in the uterus of rats during different stages of pregnancy with the use of ^{3}H-naloxone (Baraldi et al., 1985). Zhu and Pintar (1998) have found μ and κ opioid receptors in the endometrium and myometrium of pregnant mice. Furthermore, μ and δ opioid receptors were identified in woman myometrium (Fanning et al., 2013). The present study demonstrated *in vitro* the time-depended changes in the basal expression of genes for opioid receptors in the porcine endometrial explants. During the oestrous cycle, the expression of μ opioid receptor mRNA was evidently the greatest on days 10–11. Interestingly, in previous studies, decreased expression of *POMC* mRNA in the porcine endometrium was found on the same days of the cycle (Dziekoński et al., 2015). Conversely, on days 10–11 of pregnancy, the expression of μ opioid receptor mRNA was low, but that of *POMC* appeared to be increased in our former studies (Dziekoński et al., 2015). It should be mentioned that POMC is a precursor of β-endorphin, which preferentially binds to μ opioid receptors (Simon, 1991). Thus, the opposite relation between expression of μ opioid receptor and *POMC* mRNAs may result from the ligand-cognate receptor interaction since the μ opioid receptor down-regulation by specific agonists has been found in the brain of mice under *in vivo* conditions (Stafford et al., 2001). Moreover, the expression of μ opioid receptor mRNA was relatively low during pregnancy, except small, but significant, its increase on days 12–13 (the time of maternal recognition of pregnancy) in comparison to other studied days during this period. In turn, the endometrial expression of δ opioid receptor mRNA did not change during the oestrous cycle, but during pregnancy was greater on days 10–11 than on other studied days. It is noteworthy that, in the present study, the expression of this receptor gene was significantly lower on days 15–16 of pregnancy in comparison to the respective days of the cycle what coincides with the CL protection against luteolysis and the beginning of implantation (Geisert et al., 1982). The expression of κ opioid receptors in the endometrial explants did not markedly change during studied periods. Generally, changes in the endometrial expression of genes coding for opioid precursors (Dziekoński et al., 2015) and receptors, recorded in our present study, suggest that the opioid system plays a role in the local regulation of uterine functions during the oestrous cycle and early pregnancy in the pig.

In previous studies, it has been demonstrated that EOPs may be implicated in the regulation of several processes in the uterus such as: its contractility, secretory activity, local immunomodulation, cell proliferation and apoptosis (Petit et al., 1993; Cemerikic et al., 1994; Adjroud, 1995; Környei et al., 1997; Zoumakis et al., 1997; Chatzaki et al., 2001; Fanning et al., 2013). Variable data concerning the modulation of uterine muscle contractility by opioids have been reported for different species and reproductive stages. For example, Met-enkephaline analogue (DAMEA) and enkaphalinase inhibitors stimulated the duration and amplitude of spontaneous uterine contractions in rats during late pregnancy and these excitatory effects were blocked by naloxone (Adjroud, 1995). Conversely, DAMEA caused the utero-relaxant effect in terms of human non-labouring myometrium *in vitro*, reversible by naloxone, which coincided with the

presence of μ and δ opioid receptors in the tissue (Fanning et al., 2013). In turn, there was no significant effect of β-endorphin or naloxone on uterine motility in cows during oestrus and dioestrus (Ko et al., 1989). DAMEA inhibited the basal rate of uterine cell proliferation in cultures prepared from immature rats (7-day-old). However, DAMEA did not affect the basal proliferation of uterine cells derived from adult rats, but decreased the oestradiol-induced proliferation of these cells. It was suggested that this opioid effect is mediated mainly by μ opioid receptors (Környei et al., 1997). In turn, κ opioid receptors seem to be involved in the modulation of endometrial stromal cell apoptosis in humans since their highly specific agonist (U69593) markedly increased apoptosis of these cells. This effect was accompanied by a rapid, but transient, up-regulation of Fas protein as well as the stimulation of anti-apoptotic proteins of the Bcl-2 family (Bcl-2 and Bcl-X_L) as a parallel rescue response (Chatzaki et al., 2001). In humans, an involvement of κ opioid receptors in stimulation of human chorionic gonadotropin (hCG) and placental lactogen secretion by trophoblast has been shown (Petit et al., 1993; Cemerikic et al., 1994). Ligands of κ opioid receptor stimulated hCG secretion by trophoblast tissue affecting gonadotropin-releasing hormone (GnRH) release from this tissue (Cemerikic et al., 1994). Previous studies have demonstrated the presence of GnRH in the porcine uterus (Li et al., 1993; Okrasa et al., 2003) and possibly the uterine production of GnRH in pigs is also influenced by EOPs. The involvement of endometrial opioids in a local modulation of immunological processes has been postulated in humans (Zoumakis et al., 1997). Overall, it might be stated that the role of opioid system and particular types of opioid receptors in the regulation of intrauterine processes taking place during the oestrous cycle and early pregnancy is still not fully delineated.

The present study demonstrated the effects of cytokines (IL-1β, IL-6 and TNFα) on the expression of genes encoding μ and δ opioid receptors (but not κ) in endometrial explants of cyclic and pregnant gilts. The modulation of expression of gene coding for opioid receptors by cytokines (e.g., IL-1β, IL-4, IL-6 or TNFα) has been observed in different cell types including: rat astrocytes (Ruzicka and Akil, 1997), human blood cells, dendritic cells, immune cell lines (Kraus et al., 2001, 2003) and neuroblastoma cell line SH SY5Y (Börner et al., 2004) as well as rat Sertoli cells (Jenab and Morris, 2000), but this aspect of cytokine action has not been studied in the uterus yet. In the present study, the effect of tested cytokines on the expression of μ and δ opioid receptor mRNAs was differentiated during studied periods. The expression of μ opioid receptor mRNA was significantly decreased by IL-1β and IL-6 on days 10–11 and 15–16 of the cycle, respectively. During pregnancy, all studied cytokines resulted in a transient increase of the μ receptor mRNA expression on days 10–11. Interestingly, on days 12–13 of pregnancy, IL-1β inhibited this receptor expression in endometrial explants, but other cytokines (IL-6 and TNFα) were ineffective. On days 15–16 of pregnancy, TNFα increased the expression of μ receptor mRNA. The most intriguing of the above observations is unanimous increase of the μ opioid receptor expression in the endometrial explants on days 10–11 of pregnancy, when migration of the embryos to and within the uterus occurs in the pig (Geisert et al., 1982). This suggests that EOPs, acting through μ opioid receptors, in cooperation with cytokines may play an important role in the regulation of this process. Nevertheless, a connection of the relationship between cytokines and the μ opioid receptor system with other processes occurring during early pregnancy, such as the maternal recognition of pregnancy (days 12–13) and/or the protection of CL from luteolysis and embryo implantation (days 15–16), cannot be excluded. Previous studies performed with neuronal or immune cells have indicated that transcription of the gene encoding μ opioid receptor may be regulated by cytokine signal directly transduced to its promotor, e.g., for IL-6 *via* Signal Transducers and Activators of Transcription (STAT) 1 and STAT3 (Börner et al., 2004), IL-4 *via* STAT6 (Kraus et al., 2001) and TNFα *via* NFκB (nuclear factor *kappa*-light-chain-enhancer of activated B cells; Kraus et al., 2003). In our study, during the oestrous cycle, the mRNA expression of δ opioid receptor in endometrial explants was elevated by IL-1β during all studied days, IL-6 – on days 10–11 and 12–13, and TNFα – on days 15–16. During pregnancy, only IL-1β significantly increased the expression of δ opioid receptor transcript on days 12–13. Thus, these data indicate that endometrial modulation of the δ opioid receptor expression by cytokines is mainly functioning during the oestrous cycle and may be a part of mechanism controlling the course of secretory phase in the pig uterus. Generally, this study implies that μ and δ opioid receptors are responsible, at least partially, for biological effects induced by cytokines in the porcine uterus during the oestrous cycle and/or pregnancy. Nevertheless, further investigations concerning the regulation of the opioid receptors expression by cytokines and other factors in connection with processes taking place in the uterus are required.

Conclusions

This study indicates decreased a potencial of porcine endometrium for the expression of genes encoding opioid receptors: *mu (μ)*, *delta (δ)* or *kappa (κ)* during chosen days (10–11, 12–13, 15–16) of the oestrous cycle and early pregnancy as well as susceptibility of this process to cytokine action. The research has demonstrated time-depended *in vitro* changes in the expression of genes encoding opioid receptors (μ, δ and κ) in endometrial explants under the basal conditions as well as the effects of proinflammatory cytokines (interleukin (IL)-1β, IL-6 and tumor necrosis factor α (TNFα)) on the mRNA expression for μ and δ opioid receptors in this tissue during studied periods. Generally, these data indicate that opioid receptors are involved in a local, paracrine and/or autocrine, regulation of uterine functions in pigs, including processes responsible for maternal adaptation of the uterus to pregnancy.

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