Genomic and non-genomic effects of progesterone and pregnenolone on the function of bovine endometrial cells

M.K. Kowalik, D. Slonina, J. Kotwica

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

ABSTRACT: Progesterone (P_4) decreases oxytocin (OT)-stimulated prostaglandin (PG)$F_{2\alpha}$, but not PGE_2 secretion from bovine endometrial cells and this effect is partly elicited via a non-genomic route. The aim of this study was to determine whether P_4 and pregnenolone (P_5), in the presence or absence of OT, influence: (a) the gene expression of enzymes responsible for PG synthesis: cyclooxygenase-2 (COX-2), synthase of $PGF_{2\alpha}$ (PGFS) and PGE_2 (PGES), (b) protein expression of COX-2, PGFS and PGES, and (c) P_4 receptor membrane component 1 (PGRMC1) gene expression in bovine endometrial cells. The epithelial endometrial cells (2.5×10^5/ml) from Days 14–16 of the oestrous cycle were incubated for 72–96 h to attach the cells to the bottom of a well. Next, the cells were preincubated for 30 min with P_4 and P_5 (10^{-5}M each) and incubated for 4 h and 6 h alone or with OT (10^{-7}M). Thereafter, the medium was collected for PGE_2 and PGFM determination, while cells were harvested for gene and protein expression analysis. The used steroids: (a) inhibited OT-stimulated $PGF_{2\alpha}$, but not PGE_2 secretion from endometrial cells, (b) did not affect the expression of mRNA for COX-2, PGFS, PGES and PGRMC1 in endometrial cells after 4 and 6 h, (c) they decreased OT-stimulated COX-2 mRNA expression only after 6 h incubation, and (d) did not influence COX-2, PGFS and PGES protein expression after 6 h. These results indicate that P_4 and P_5 inhibit OT-stimulated secretion/production of luteolytic $PGF_{2\alpha}$ by a transcription-independent mechanism and partly by down-regulation of COX-2 mRNA.

Keywords: non-genomic effect; progesterone; progesterone membrane receptor component 1; endometrium; bovine

Prostaglandins (PGs) produced in the uterus play an important role in the regulation of the oestrous cycle and the successful implantation of blastocysts in many species, including cattle (McCracken et al., 1999; Goff, 2004; Fortier et al., 2008). During the bovine oestrous cycle, Days 15–17 are crucial for the initiation of luteolysis or for maternal recognition of pregnancy (McCracken et al., 1999; Niswender et al., 2000). At this time, endometrial $PGF_{2\alpha}$ is released in a pulsatile manner in the first case (McCracken et al., 1999), while PGE_2 acts as luteotropic and antiluteolitic factor preventing luteolysis during early pregnancy (Goff, 2004). Both these PGs are synthesized in endometrial cells from arachidonic acid, which is converted into prostaglandin H_2 (PGH_2) by cyclooxygenase (COX-1 and COX-2). Next, PGH_2 is metabolized to PGF_α and PGE_2 by PGF synthase (PGFS) and PGE synthase (PGES), respectively (Goff, 2004; Fortier et al., 2008). In the bovine endometrium, a change in COX-1 mRNA levels and protein product has not been detected. However, a significant in-

Supported by the Ministry of Science and Higher Education (Grant No. 2P06K 038 29) and by the Polish Academy of Sciences.

crease in COX-2 mRNA and protein expression has been detected on Days 13–21 of the estrous cycle (Arosh et al., 2002). Moreover, it was shown that oxytocin (OT) stimulated COX-2 mRNA and protein expression, but did not affect COX-1 mRNA expression in cows (Asselin et al., 1997; Parent et al., 2003). Thus, it is suggested that COX-2 is essential for OT influence on $PGF_{2\alpha}$ and PGE_2 synthesis (Asselin et al., 1997). It has been discovered that the PGFS, involved in the production of $PGF_{2\alpha}$ in bovine endometrium at the time of luteolysis, is aldoketoreductase 1B5 (AKR1B5) (Madore et al., 2003; Fortier et al., 2008). Moreover, it was found that microsomal PGES-1 (m-PGES1) is highly correlated with COX-2 (Murakami et al., 2002) and that this PGES is responsible for PGE_2 synthesis in the bovine endometrium (Arosh et al., 2002; Parent et al., 2002).

Progesterone (P_4) and oestrogens may directly affect the basal $PGF_{2\alpha}$ secretion by the endometrium (Xiao et al., 1998; Goff, 2004). Moreover, these steroids can regulate endometrial responsiveness to OT and to other regulatory factors, and OT further stimulates $PGF_{2\alpha}$ and PGE_2 secretion from endometrial cells of cows (Kim and Fortier, 1995). It has been demonstrated that P_4 inhibits OT-stimulated $PGF_{2\alpha}$ secretion from bovine endometrial cells via genomic (Skarzynski et al., 1999) and non-genomic mechanisms (Grazzini et al., 1998; Bogacki et al., 2002; Rekawiecki and Kotwica, 2007; Rekawiecki et al., 2008).

Recently, we showed that P_4, its precursor – pregnenolone (P_5) and metabolite – 17β-hydroxyprogesterone impaired OT-stimulated $PGF_{2\alpha}$, but not PGE_2 secretion from endometrial cells on Days 14–16 of the oestrous cycle (Duras et al., 2005; Kowalik and Kotwica, 2007). This inhibitory effect appeared after short-term culture (4 h), so the effect of steroids could not be mediated through genomic mechanisms. It is possible that steroids indirectly suppress the transcription of specific genes, e.g., the OT receptor and/or PG synthase (Salomonsen et al., 1990; Raw et al., 1996). Steroids used in our studies also decrease intracellular Ca^{2+} mobilization, which plays an important role in mediating the stimulatory effect of OT on $PGF_{2\alpha}$ secretion (Duras et al., 2005). This data suggests that different cellular mechanisms exist for steroids affecting secretion of both prostaglandins from endometrial epithelial cells. There is no clear explanation for this phenomenon, but one possibility is that the reduction in the Ca^{2+} response may affect enzymes that control the ratio of PGE_2 to $PGF_{2\alpha}$ (Duras et al., 2005; Kowalik and Kotwica, 2007). It is also possible that the decrease of Ca^{2+} mobilization in endometrial cells is evoked by interaction of P_4 with its membrane receptor – progesterone membrane receptor component 1 (PGRMC1) (Peluso, 2006).

Although the rapid, non-genomic effect of P_4 on the function of endometrial cells has been well documented (Bogacki et al., 2002; Dunlap and Stormshak, 2004; Duras et al., 2005; Bishop and Stormshak, 2006; Franczak et al., 2006) the molecular mechanism of its action on the secretion of $PGE_2/PGF_{2\alpha}$ in the bovine endometrial cells is poorly understood. The present study was undertaken to determine whether this inhibitory effect of P_4 and P_5 on OT-stimulated secretion $PGF_{2\alpha}$ involved genomic or non-genomic mechanisms. Therefore, we studied whether P_4 and P_5, with and without OT, have effects on: (a) mRNA expression of enzymes responsible for PG biosynthesis, COX-2, PGFS and PGES, (b) protein expression of COX-2, PGFS and PGES and (c) PGRMC1 mRNA expression in bovine endometrial cells from Days 14–16 of the estrous cycle.

MATERIAL AND METHODS

Tissue collection

Uteri were collected from a commercial slaughterhouse from mature heifers and nonpregnant cows on Days 14–16 of the estrous cycle and transported within 1 h to the laboratory in ice-cold phosphate buffered saline (PBS; 137mM NaCl; 27mM KCl; 10mM Na_2HPO_4; 2mM KH_2PO_4; pH 7.4). Days of the oestrous cycle were assessed by morphological observations of the reproductive tract (Fields and Fields, 1996). Uteri from 4 animals were collected for each experiment. All materials used in these studies were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

Isolation of endometrial epithelial cells

Endometrial epithelial cells were isolated using a procedure described previously (Duras et al., 2005). The number of cells and cell viability were determined by means of 0.04% trypan blue exclusion, and only the cells with a viability of above 85% were used. The cells were suspended (2.5 ×

10^5 cell/ml medium) in DMEM/Ham's-F12 medium with 10% FCS and 20 µg/ml gentamycin added into each of 6-well plates (Nunc GmbH&Co. KG, Wiesbaden, Germany), and incubated (Heraeus BB-6060, Hanau, Germany) at 38°C in a humidified atmosphere of 95% O_2 and 5% CO_2. Medium was changed after 48 and 72 h and after 96 h if necessary for the attachment of cells to the bottom of the well. Cells were then incubated in DMEM/Ham's-F12 medium containing 0.1% BSA and 20 µg/ml gentamycin for 24 h.

Experiment 1. To study the mRNA expression of COX-2, PGFS and PGES, endometrial epithelial cells were preincubated for 30 min with: P_4 (10^{-5}M) or P_5 (10^{-5}M; Serva, Germany), and then the medium was supplemented with OT (10^{-7}M). Cells incubated without hormones served as controls. After a 4 or 6 h incubation the medium was collected into tubes containing 10 µl of solution (30 µM EDTA, 1% acetylosalicylic acid), and stored (at –20°C) until analysis of PGE_2 and $PGF_{2\alpha}$ metabolite – PGFM concentrations. PGFM concentrations reflects 50% of the $PGF_{2\alpha}$ secretion of the endometrium as reported earlier (Skarzynski et al., 1999) and both these terms are used in this paper. The cells were stored at –80°C until RNA isolation.

Experiment 2. To establish the protein level for COX-2, PGFS and PGES in the endometrial epithelial cells, these were preincubated for 30 min with: P_4 (10^{-5}M; Serva, Germany), and then the medium was supplemented with OT (10^{-7}M) or OT with P_4. Cells incubated without hormones served as the control. After 6 h incubation, the medium was collected for determination of PGFM and PGE_2 concentration. Thereafter, 200 µl of lysis buffer (0.25M sacharose, 10mM Tris, 0.1mM EDTA, pH 7.4) with Protease Inhibitor Cocktail was added into each well. The cells were scraped off, transferred into tubes and homogenized on ice with an ultrasound homogenizer (Sonic&Materials Inc, USA). The concentration of protein was determined by the Bradford method (1976) and the protein was used for Western blot analysis.

Experiment 3. To investigate mRNA expression for PGRMC1, the endometrial epithelial cells were incubated in the same way as in Experiment 1, but arachidonic acid (AA) was used as positive control for the cellular response as measured by prostaglandin secretion. After 4 or 6 h the medium was collected for PGFM and PGE_2 determination, while the cells were stored at –80°C for RNA isolation and semi-quantitative PCR.

EIA of prostaglandins

Concentrations of PGFM were determined directly in medium by enzyme immunoassay (EIA) using horseradish peroxidase-labelled PGFM as a tracer (1 : 40 000; final dilution) and anti-PGFM serum (1 : 80 000) described previously (Homonics and Silvia, 1988). The standard curve ranged from 62.5 to 32 000 pg/ml. The intra- and inter-assay coefficients of variation were 13.4% and 16.4%, respectively.

PGE_2 concentrations were determined also by EIA using horseradish peroxidase-labelled PGE_2 (1 : 30 000; final dilution) and anti-PGE_2 serum (diluted 1 : 80 000). The standard curve ranged from 78 to 20 000 pg/ml. The intra- and inter-assay coefficients of variation were 5.75% and 11.3%, respectively.

RNA isolation and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells with the Total RNA Prep Plus Kit (A&A Biotechnology, Gdansk, Poland) according to the manufacturer instructions. Concentration and quality of RNA were determined spectrophotometrically (BioRad, Hercules, CA, USA). Total RNA (1 µg) was treated with DNAse and reverse transcribed into cDNA in a total reaction volume of 20 µl containing: 50mM Tris-HCl (pH 8.3), 75mM KCl, 3mM $MgCl_2$, 5 mM dithiotreitol, 10 mM dNTP mix, 1 µg oligo(dT)23 primers (Fermentas, Vilnius, Lithuania), 200 IU of reverse transcriptase RevertAidTM M-MuLV (Fermentas, Vilnius, Lithuania). RNA was denaturated at 70°C for 10 min. The subsequent RT reaction was carried out at 42°C for 60 min and terminated by heating for 10 min at 70°C. Based on the gene sequences available in GenBank, (NCBI), primers were designed using primer 3 software (http://frodo.wi.mit.edu/primer3/input.htm); for PGRMC1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA); for: COX-2, PGFS, PGES and 18 sRNA. These primers were synthesized by TIB MolBiol (Poznan, Poland). The sequences of the primers, the expected PCR product length and the number of cycles are shown in Table 1. Complementary DNA was amplified by a PCR reaction carried out in total volume of 25 µl containing 12.5 µl REDTaq ReadyMix

Table 1. Forward and reverse primer sequences, expected amplicon length and number of cycles used in PCR. Every primer set is designed according to the accession number in the The Entrez Nucleotide database

Primer	Sequence (forward/reverse)	Amplicon length (bp)	Number of cycles	Accession number
COX-2	5' GATCCCCAGGGCACAAATCT 3' 5' GGTGAAGTGCTGGGCAAAGA 3'	50	27	NM_174445
mPGES	5' TTCCTGGCAACTGGCTGAG 3' 5' AAACACACACAGGCCCCCT 3'	54	29	NM_174443
PGFS	5' CAATCGACCTTGGGTACCGT 3' 5' TCATTCTGGTACACGTGGGC 3'	51	28	S54973
PGRMC1	5' GCCTTTGCATCTTTCTGCTC 3' 5' TGGCTCCTCCTTGTCTGAGT 3'	448	25	NM_001075133
18sRNA	5' GTTCGATTCCGGAGAGGGA 3' 5' CCTTCCTTGGATGTGGTAGCC 3'	51	21	AF176811
GAPDH	5' TGTTCCAGTATGATTCCACCC 3' 5' TCCACCACCCTGTTGCTGTA 3'	850	21	NM_001034034

PCR Reaction Mix, 2 µl cDNA, and 0.5 µl (50 µM) of both PCR primers for each studied gene. The optimal number of cycles ensuring the termination of amplification for studied genes in the lag phase was determined in a series of preliminary studies. The PCR profiles consisted of initial denaturation at 94°C for 3 min, an appropriate number of cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR reaction products were separated by electrophoresis on a 2% agarose (Eurogentec, Seraing, Belgium) gel (PGRMC1 and GAPGH) or 15% polyacrylamide gel (COX-2, PGFS, PGES and 18sRNA) together with molecular mass standards (DNA, Gdansk, Poland). The intensity of each band was quantified using Kodak EDAS 290 Software (Rochester, New York, USA). The relative amounts of PCR products were estimated by normalizing the signal intensities of the gene products in relation to housekeeping gene products.

Western Blots

Total cellular protein obtained from the endometrial cells (20 µg) was dissolved in SDS gel-loading buffer (50mM Tris-HCl, pH 6.8; 4% SDS, 20% glycerol, 2% β-mercaptoethanol), heated to 95°C for 5 min and separated on 10% (for COX-2 and PGFS) and 15% (for PGES) SDS-PAGE. Separated proteins were electroblotted onto Immobilon PVDF membrane (Millipore, USA) in transfer buffer (25mM Tris-HCl, pH 8.3; 192mM glycine; 20% methanol; 0.05% SDS). The nonspecific binding sites were blocked with 5% non-fat dry milk in TBS buffer (50mM Tris-HCl; 0.9% NaCl) containing 0.05% Tween-20, for 1.5 h at room temperature. Thereafter, the membrane was incubated overnight at 4°C with the primary polyclonal antibodies, diluted as follows: 1/250 rabbit COX-2, 1/250 rabbit PGES (Cayman Chemicals, Ann Arbor, MI), 1/750 rabbit PGFS (Kindly provided by Professor M.A. Fortier, Ontogeny and Reproduction Unit CHUQ-CHUL, Quebec, Canada). Next, the membrane was washed three times for 10 min in TBS-T buffer and incubated with secondary antibody (alkaline phosphatase-conjugated goat anti-rabbit IgG) for 1.5 h at room temperature at a dilution of 1/30 000. Immunoreactive bands were detected using a mixture of 5-bromo-4-chloro-3- indolyl phosphate and nitrotetrazolium blue chloride, as visualisation stock solution (NBC/BCIP with alkaline phosphatase assay buffer 0.1 M NaCl, 5 mM MgCl2, 100 mM Tris–HCl, pH 9.5). Western blots were quantitated using Kodak EDAS 290 Software (Eastman Kodak, Rochester, New York, USA).

Statistical analysis

All data were expressed as means ± (S.E.M.). Prostaglandins concentrations were presented as percentages and compared to control values accepted as 100%. Due to the heterogeneity of the data they were transformed into logarithms (ln) before ANOVA analysis for repeated measures and

followed by the Newman- Keuls post test (PRISM, Graph Pad Software, San Diego, CA, USA).

RESULTS

Experiment 1

Oxytocin increased PGFM and PGE_2 concentration in culture medium after 4 h and 6 h ($P < 0.05 – P < 0.001$) of incubation of endometrial cells (Figure 1). This indicates that the cells responded to physiological stimulation. Any of the used steroids affected the basal secretion of $PGF_{2\alpha}$ (Figure 1a, b). However, both P_4 and P_5 decreased ($P < 0.05$) the effect of OT on $PGF_{2\alpha}$ secretion after 4 h (Figure 1a) and 6 h (Figure 1b). Only P_5 (Figure 1d) increased ($P < 0.05$) the basal secretion of PGE_2 after 6 h of incubation. There was no observed inhibitory effect of the used steroids on OT-stimulated PGE_2 secretion ($P > 0.05$) after 4 h (Figure 1c) and 6 h of incubation (Figure 1d). All of the used treatments affected the expression of mRNA for COX-2 (Figure 2a), PGFS and PGES (data not shown) in bovine endometrial cells after 4 h of incubation. After 6 h of incubation OT only increased ($P < 0.05$) the expression of COX-2, while applied steroids decreased ($P < 0.05$) the effect of OT on COX-2 mRNA level in these cells (Figure 2b). All used treatments had an impact on PGFS and PGES expression after 6 h incubation of the cells (data not shown).

Experiment 2

Since P_5 impaired the effect of OT on gene expression with an intensity similar to P_4, only the latter was used in this experiment. Oxytocin increased ($P < 0.05$) PGFM and PGE_2 concentration in medium after 6 h of incubation (Figure 3). Progesterone did not affect basal PGFM and PGE_2 concentrations, but it decreased the effect of OT on the secretion of $PGF_{2\alpha}$

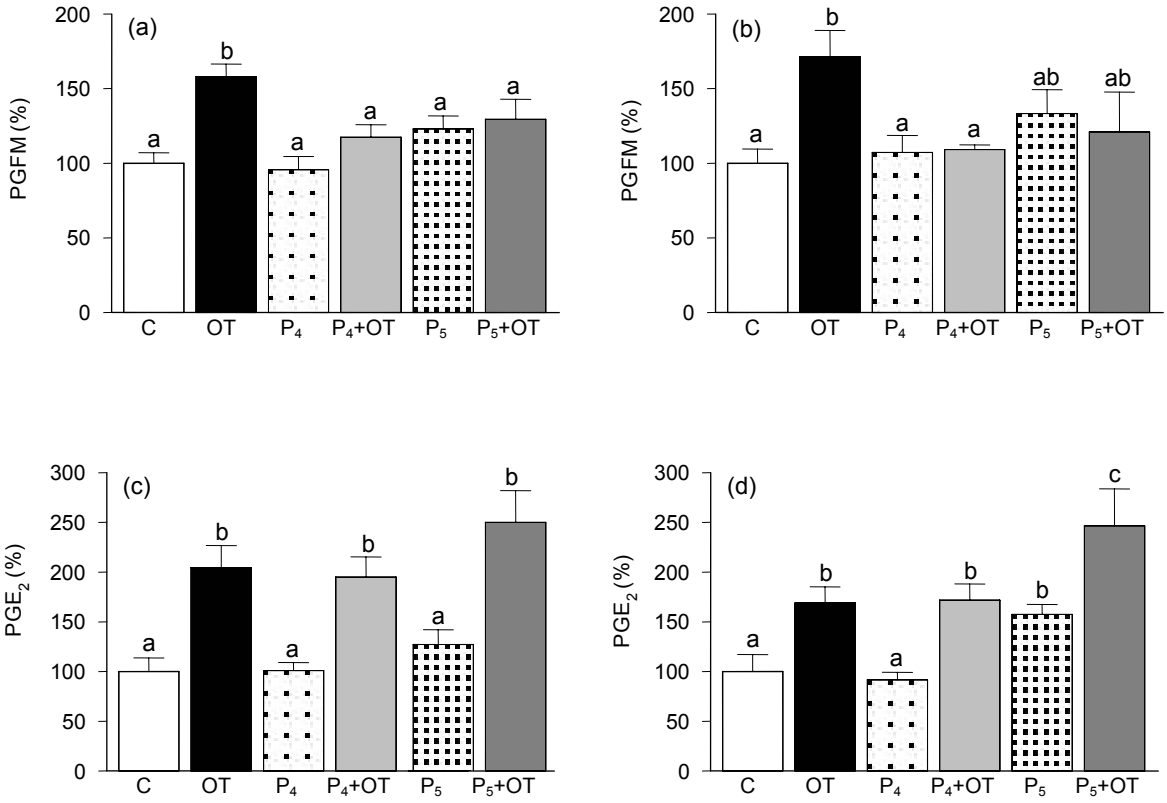

Figure 1. Mean (±SEM) concentration of PGFM (a), (b) and PGE2 (c), (d) in medium incubated with endometrial cells on Days 14–16 of the oestrous cycle of the cows ($n = 4$). The cells were pre-incubated for 30 min with progesterone (P_4; 10^{-5}M) and pregnenolone (P_5; 10^{-5}M), and next incubated 4 h (a),(c) or 6 h (b), (d) with oxytocin (OT; 10^{-7}M), and OT together with each steroid. Values were compared to controls (100%). Bars with different superscripts are significantly different ($P < 0.05$)

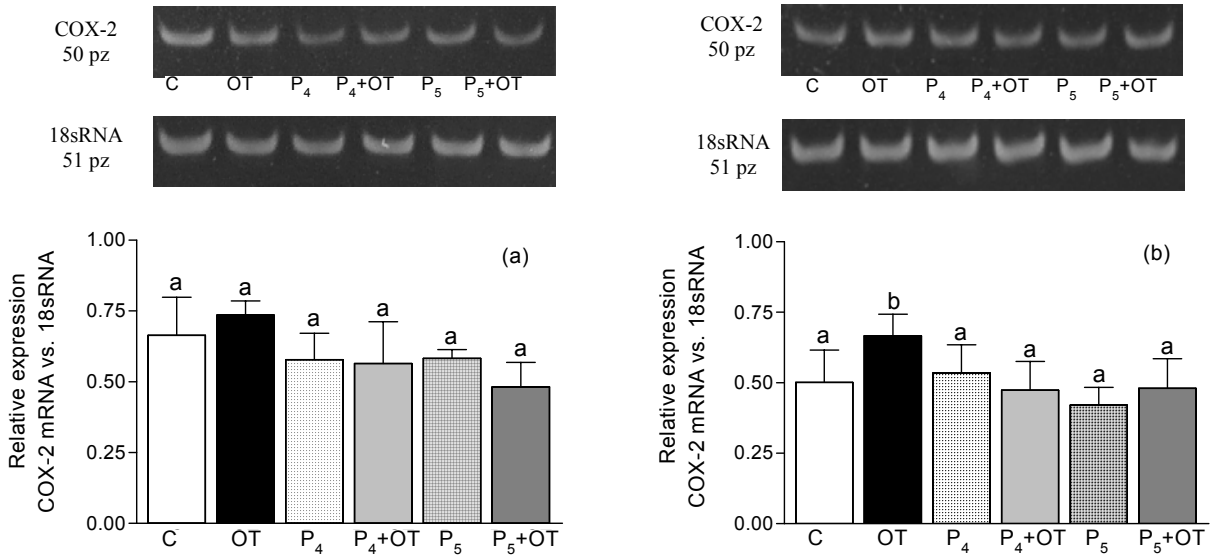

Figure 2. Mean (± SEM) expression of COX-2 mRNA levels in bovine endometrial cells after 4 h (a) and 6 h (b) incubation of cells with control medium (C), oxytocin (OT; 10^{-7}M), progesterone (P_4; 10^{-5}M), pregneneolone (P_5; 10^{-5}M) and OT together with each steroids. All values are expressed as a ratio of COX-2 to 18sRNA (n = 4). Different superscripts indicate significant differences ($P < 0.05$)

(Figure 3). However, none of the used treatments changed the levels of COX-2, PGFS and PGES protein in bovine endometrial cells from Days 14–16 of the estrous cycle after 6 h of incubation (Figure 4).

Experiment 3

The expression of PGRMC1 mRNA was observed in the bovine endometrial cells from Days 14–16 of the estrous cycle (Figure 5). However, neither OT nor the used steroids separately or jointly with OT had an effect on the expression of PGRMC1 mRNA in endometrial cells after 4 h and 6 h incubation of the cells (Figure 5).

DISCUSSION

The treatment of bovine endometrial cells on Days 14–16 of the estrous cycle with OT increased $PGF_{2\alpha}$ and PGE_2 secretion after both 4 h and 6 h stimulation and this was the main criterion used to determine the usefulness of endometrial cells

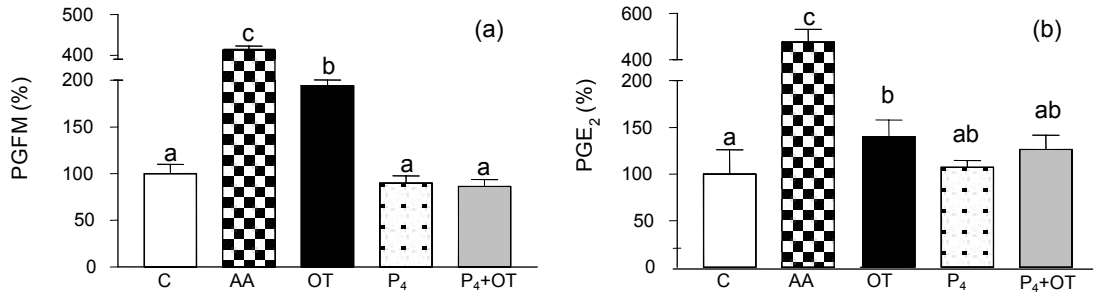

Figure 3. Concentration (mean ± SEM) of PGFM (a) and PGE_2 (b) in medium incubated with endometrial cells on Days 14–16 of the oestrous cycle of the cows (n = 4). The cells were pre-incubated for 30 min with progesterone (P_4; 10^{-5}M), and next incubated 6 h with arachidonic acid (AA; 10^{-5}M), oxytocin (OT; 10^{-7}M), and OT together with P_4. Values were compared to controls (100%). Bars with different superscripts are different ($P < 0.05$). Different superscripts indicate significant differences ($P < 0.05$)

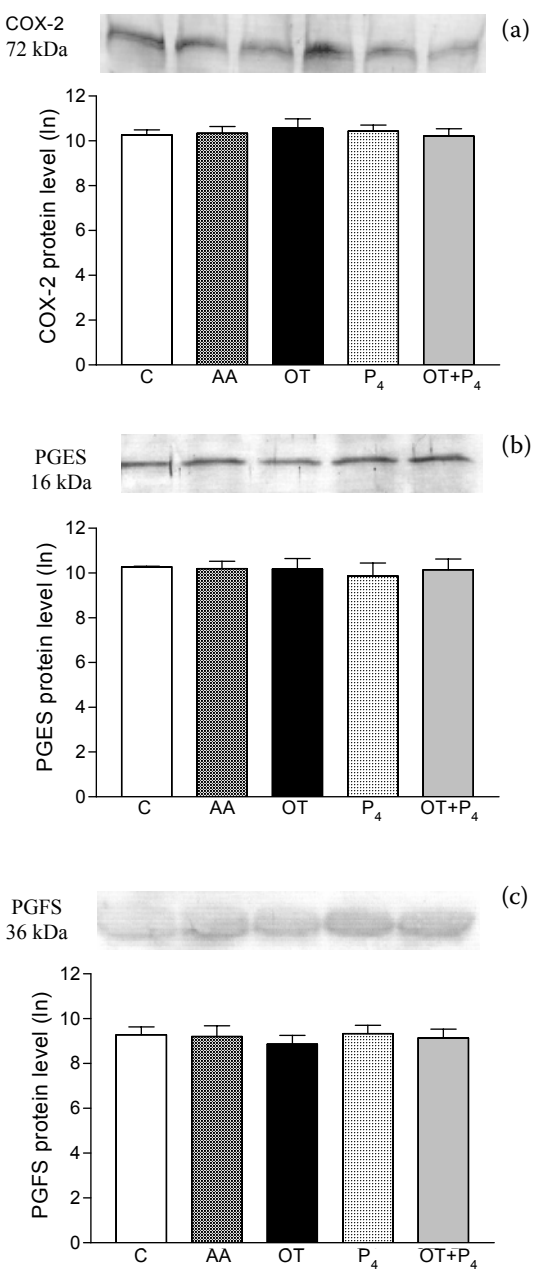

Figure 4. COX-2 (a), PGES (b) and PGFS (c) protein level in bovine endometrial cells (n = 4) after 6 h incubation of cells with control medium (C), arachidonic acid (AA; 10^{-5}M), oxytocin (OT; 10^{-7}M), progesterone (P_4; 10^{-5}M), and OT together with each steroids. Upper panels: representative blots of COX-2, PGES and PGFS antibodies. Lower panel: densitometric analyses of COX-2, PGES and PGFS

for the further experiments. As shown previously (Bogacki et al., 2002; Duras et al., 2005; Kowalik and Kotwica, 2007), P_4 and P_5 do not affect the basal $PGF_{2\alpha}$ secretion, but both steroids suppressed OT-stimulated $PGF_{2\alpha}$ secretion from endometrial cells. This inhibitory effect of steroids was observed after both 4 h and 6 h of cell culture, therefore it is assumed that both transcription-independent and transcription-dependent mechanisms may be involved in this process. A non-genomic effect of P_4 has been found in a number of tissues from the female reproductive tract (Grazzini et al., 1998; Bogacki et al., 2002; Duras et al., 2005; Bishop and Stormshak, 2006, 2008; Franczak et al., 2006), but the nature of this rapid action has not been fully understood. It was found that P_4 impaired binding of OT to its membrane receptor (Grazzini et al., 1998; Bogacki et al., 2002; Dunlap and Stormshak, 2004; Bishop and Stormshak, 2008). However, this effect was not confirmed by other authors (Burger et al., 1999; Ivell et al., 2000). It was also suggested that P_4 may influence the concentration of cholesterol in the cell membrane causing conformational changes in the OT receptor and in this way to reduce the effect of OT on cells (Gimpl and Fahrenholz, 2001). Moreover, P_4 can influence cell function via its membrane receptors (Bramley, 2003; Peluso, 2006; Bishop and Stormshak, 2008). It was also found that P_4 directly decreases the expression of the OT receptor and prostaglandin synthases (Salomonsen et al., 1990; Raw et al., 1996). Our earlier studies (Duras et al., 2005; Kowalik and Kotwica, 2007) suggested that P_4 and other steroids inhibit PGFS mRNA expression. These steroids did not affect expression of mRNA for PGES but they blocked OT-stimulated $PGF_{2\alpha}$ secretion. The present study demonstrated that P_4 and P_5 did not affect COX-2, PGFS and PGES mRNA expression in endometrial cells after 4 h and 6 h of incubation. Moreover, we did not observe changes in genes expression after 4 h incubation of cells supplemented with OT, so it can be assumed that the steroids in question act on PG secretion via a transcription-independent mechanism. On the other hand, COX-2 mRNA expression was stimulated by OT after 6 h, but this effect was not observed in cells pretreated with P_4 and P_5. These results indicate that the used steroids may inhibit OT-stimulated $PGF_{2\alpha}$ secretion/production by down-regulation of the transcription of the COX-2 gene.

Moreover, we studied the influence of P_4 with and without OT on the expression of COX-2, PGFS and PGES protein after 6 h of incubation with the endometrial cells. None of the treatments influenced the expression of the protein level for the studied enzymes. It should be noted that OT stimulated the expression of both the COX-2 mRNA and protein in

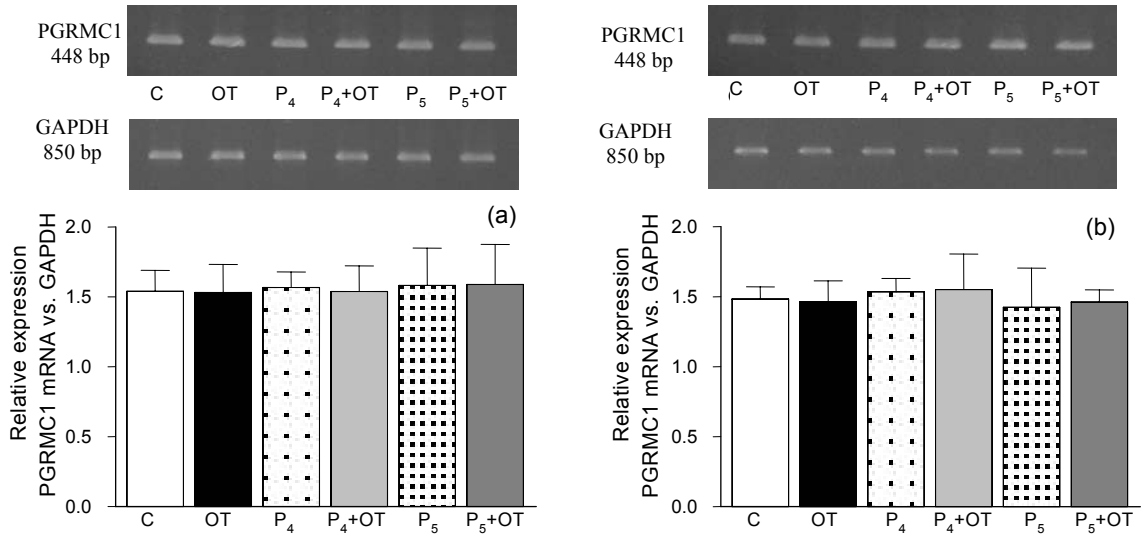

Figure 5. Means (± SEM) expression of PGRMC1 (progesterone receptor membrane component 1) mRNA determined by semi-quantitative PCR in bovine endometrial cells (n = 4) after 4 h (a) and 6 h (b) incubation of cells with control medium (C), oxytocin (OT; 10^{-7}M), progesterone (P_4; 10^{-5}M), pregnenolone (P_5; 10^{-5}M) and OT together with each steroids. All values are expressed as a ratio of PGRMC1 to GAPDH. Upper panel: representative images of agarose gels; lower panel: densitometric analyses of PGRMC1 mRNA relative to GAPDH mRNA

bovine endometrial cells after 9 h and 24 h (Asselin et al., 1997; Parent et al., 2003), while P_4 did not affect COX-2 mRNA expression (Xiao et al., 1998). Moreover, neither OT (Xiao et al., 1998), nor P_4 (Asselin and Fortier, 2000) had an effect on PGFS mRNA in the bovine endometrium. In our study, OT and P_4 did not influence COX-2, PGFS and PGES protein expression after 6 h, however, they affected the secretion of $PGF_{2\alpha}$ and PGE_2. These results strengthen the idea that P_4 may affect PG secretion via a non-genomic mechanism.

It is possible that the non-genomic effect of P_4 observed in endometrial cells is evoked partly via progesterone membrane receptor component 1 (PGRMC1) (Peluso, 2006). This protein may be involved in the regulation of cholesterol and steroid synthesis (Losel et al., 2008) and this latter stabilizes the membrane OT receptor and improves its affinity for the ligand (Klein et al., 1995). P_4, on the other hand, inhibits the signal transduction of G-protein-coupled receptors and the intracellular transport of cholesterol (Gimpl and Fahrenholz, 2001). Therefore, it is possible that P_4 action via PGRMC1 can influence the concentration of cholesterol in the cell membrane and change the effect of OT on the target cell. The results presented in this study show PGRMC1 mRNA expression in bovine epithelial endometrial cells from Days 14–16 of the estrous cycle. However, no effect of OT and steroids on PGRMC1 gene expression was observed in this study. Nevertheless, the obtained results did not exclude PGRMC1 participation in the non-genomic activity of P_4 in endometrial cells, since expression of PGRMC1 mRNA has been demonstrated during the development of *corpus luteum* in cows (Kowalik and Kotwica, 2008).

In conclusion, this study indicated differential effects of P_4 and P_5 on the regulation of $PGF_{2\alpha}$ and PGE_2 secretion stimulated by OT in cultured bovine endometrial cells. The results show that the studied steroids inhibit OT-stimulated secretion/ production of $PGF_{2\alpha}$ in endometrial cells in a transcription-independent manner and partly by down-regulation of COX-2 mRNA. This inhibitory effect of steroids on $PGF_{2\alpha}$ secretion may be crucial for the lifespan of the *corpus luteum* and for the establismant of early pregnancy.

Acknowledgments

We thank Professor W.W. Thatcher (University of Florida, Gainesville, FL) and Professor W.J. Silvia (University of Kentucky, USA) for PGE_2 and PGFM

antisera, respectively. Prostaglandin $F_{2\alpha}$ synthase antibodies (AKR1B5) were kindly provided by Professor M.A. Fortier of the Ontogeny and Reproduction Unit CHUQ-CHUL, Quebec, Canada.

REFERENCES

Arosh J.A., Parent J., Chapdelain P., Sirois J., Fortier M.A. (2002): Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the oestrus cycle. Biology of Reproduction, 67, 161–169.

Asselin E., Fortier M.A. (2000): Detection and regulation of the messenger for a putative bovine endometrial 9-keto-prostaglandin E_2 reductase: effect of oxytocin and interferon-tau. Biology of Reproduction, 62, 125–131.

Asselin E., Drolet P., Fortier M.A. (1997): Cellular mechanisms involved during oxytocin-induced prostaglandin $F_{2\alpha}$ production in endometrial epithelial cells *in vitro*: role of cyclooxygenase-2. Endocrinology, 138, 4798–4805.

Bishop C.V., Stormshak F. (2006): Nongenomic action of progesterone inhibits oxytocin-induced phosphoinositide hydrolysis and prostaglandin $F_{2\alpha}$ secretion in the ovine endometrium. Endocrinology, 147, 937–942.

Bishop C.V., Stormshak F. (2008): Non-genomic actions of progesterone and estrogens in regulating reproductive events in domestic animals. The Veterinary Journal, 176, 270–280.

Bogacki M., Silvia W.J., Rękawiecki R., Kotwica J. (2002): Direct inhibitory effect of progesterone on oxytocin-induced secretion of prostaglandin $F_{2\alpha}$ from bovine endometrial tissue. Biology of Reproduction, 67, 184–188.

Bramley T. (2003): Non-genomic progesterone receptors in the mammalian ovary: some unresolved issues. Reproduction, 125, 3–15.

Burger K., Fahrenholz F., Gimpl G. (1999): Non-genomic effects of progesterone on the signaling function of G protein – coupled receptors. FEBS Letters, 464, 25–29.

Dunlap K.A., Stormshak F. (2004): Nongenomic inhibition of oxytocin binding by progesterone in the ovine uterus. Biology of Reproduction, 70, 65–69.

Duras M., Mlynarczuk J., Kotwica J. (2005): Non-genomic effect of steroids on oxytocin-stimulated intracellular mobilization of calcium and on prostaglandin F_2 and E_2 secretion from bovine endometrial cells. Prostaglandins and Other Lipid Mediators, 76, 105–116.

Fields M.J., Fields P.A. (1996): Morphological characteristics of the bovine *corpus luteum* during the estrous cycle and pregnancy. Theriogenology, 45, 1295–1325.

Fortier M.A., Krishnaswamy K., Danyod G., Boucher-Kovalik S., Chapdelaine J.A. (2008): A postgenomic integratred view of prostaglandins in reproduction: implications for other body systems. Journal of Physiology and Pharmacology, 59 (Suppl. 1), 65–89.

Franczak A., Kurowicka B., Oponowicz A., Petroff B.K., Kotwica G. (2006): The effect of progesterone on oxytocin-stimulated intracellular mobilization of Ca^{2+} and prostaglandin E_2 and $F_{2\alpha}$ secretion from porcine myometrial cells. Prostaglandins and Other Lipid Mediators, 81, 37–44.

Gimpl G., Fahrenholz F. (2001) The oxytocin receptor system: structure, function, and regulation. Physiological Review, 81, 629–683.

Goff A.K. (2004): Steroid hormone modulation of prostaglandin secretion in the ruminant endometrium during the estrous cycle. Biology of Reproduction, 71, 11–16.

Grazzini E., Guillon G., Mouilac B., Zingg H.H. (1998): Inhibition of oxytocin receptor function by direct binding of progesterone. Nature, 392, 509–512.

Homonics G.E., Silvia W.J. (1988): Effects of progesterone and estradiol-17β on uterine secretion of prostaglandin $F_{2\alpha}$ in response to oxytocin in ovariectomized ewes. Biology of Reproduction, 38, 723–728.

Ivell R., Fuchs A.R., Bathgate R., Tillmann G., Kimura T. (2000): Regulation of the oxytocin receptor in bovine reproductive tissues and the role of steroids. Reproduction in Domestic Animals, 35, 134–141.

Kim J.J., Fortier M.A. (1995): Cell type specificity and protein kinase C dependency on the stimulation of prostaglandin E_2 and prostaglandin F production by oxytocin and platelet-activating factor in bovine endometrial cells. Journal of Reproduction and Fertility, 103, 239–247.

Klein U., Gimpl G., Fahrenholz F. (1995): Alteration of the myometrial plasma membrane cholesterol content with β-cyclodextrin modulates the binding affinity of the oxytocin receptor. Biochemistry, 34, 13784–13793.

Kowalik M., Kotwica J. (2007): Non-genomic effect of ovarian steroids on oxytocin-stimulated prostaglandin (PG)$F_{2\alpha}$ and E_2 secretion from bovine endometrial cells. Bulletin of the Veterinary Institute in Pulawy, 51, 37–42.

Kowalik M.K., Kotwica J. (2008): Progesterone receptor membrane component 1 (PGRMC1) gene expression in *corpus luteum* during estrous cycle in cows. Reproductive Biology, 8, 291–297.

Losel R.M., Besong D., Peluso J.J., Wehling M. (2008): Progesterone receptor membrane component 1 – Many tasks for a versatile protein. Steroids, 73, 929–934.

Madore E., Harvey N., Parent J., Chapdelaine P., Arosh J.A., Fortier M.A. (2003): An aldose reductase with 20α-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin $F_{2\alpha}$ in the bovine endometrium. Journal of Biological Chemistry, 278, 11205–11212.

McCracken J.A., Custer E.E., Lamsa J.C. (1999): Luteolysis: a nuroendocrine mediated event. Physiological Review, 79, 263–323.

Murakami M., Nakatani Y., Tanioka T., Kudo I. (2002): Prostaglandin E synthase. Prostaglandins and Other Lipid Mediators, 68–69, 383–399.

Niswender G.D., Juengel J.L., Silvia P.J., Rollyson M.K., McIntush E.W. (2000): Mechanism controlling the function and life span of the *corpus luteum*. Physiological Review, 80, 1–29.

Parent J., Chapdelaine P., Sirois J., Fortier M.A. (2002): Expression of microsomal prostaglandin E synthase in bovine endometrium: coexpression with cyclooxygenase type 2 and regulation by interferon-τ. Endocrinology, 143, 2936–2943.

Parent J., Villeneuve C., Fortier M.A. (2003): Evaluation of the contribution of cyclooxygenase 1 and cyclooxygenase 2 to the production of PGE_2 and $PGF_{2\alpha}$ in epithelial cells from bovine endometrium. Reproduction, 126, 539–547.

Peluso J.J. (2006): Multiplicity of progesterone's actions and receptors in the mammalian ovary. Biology of Reproductive, 75, 2–8.

Raw R.E., Silvia W.J., Curry T.E. (1996): Effects of progesterone and estradiol on prostaglandin endoperoxide synthase in ovine endometrial tissue. Animal Reproduction Science, 40, 17–30.

Rekawiecki R., Kotwica J. (2007): Molecular regulation of progesterone synthesis in the bovine *corpus luteum*. Veterinarni Medicina, 52, 405–412.

Rekawiecki R., Kowalik M.K., Slonina D., Kotwica J. (2008): Regulation of progesterone synthesis and action in bovine *corpus luteum*. Journal of Physiology and Pharmacology, 59 (Suppl. 9), 75–89.

Salomonsen L.A., Hampton A.L., Clemens J.A., Findlay J.K. (1990): Regulation of gene expression and cellular localization of prostaglandin synthase by oestrogen and progesterone in ovine uterus. Journal of Reproduction and Fertility, 92, 393–406.

Skarzynski D.J., Bogacki M., Kotwica J. (1999): Involvement of ovarian steroids in basal and oxytocin-stimulated prostaglandin (PG)F_2 secretion by the bovine endometrium *in vitro*. Theriogenology, 52, 385–397.

Xiao C.W., Liu J.M., Sirois J., Goff A.K. (1998): Regulation of cyclooxygenase-2 and prostaglandin F synthase gene expression by steroid hormones and interferon-t in bovine uterine endometrial cells. Endocrinology, 139, 2293–2299.

Received: 2009–03–31
Accepted after corrections: 2009–05–18

Corresponding Author:

Jan Kotwica, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima Street 10, 10-747 Olsztyn, Poland

Tel. +48 89 539 31 15, Fax +48 89 524 03 47, E-mail: janko@pan.olsztyn.pl