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The expression of progesterone receptor coregulators mRNA and protein in corpus luteum and endometrium of cows during the estrous cycle

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ABSTRACT

The aim of this study was to examine whether changes in the mRNA and protein expression of the progesterone receptor (PGR) coactivator P300/CBP-associated factor (PCAF) and the corepressor Nuclear Receptor Corepressor 1 (NCOR1) may participate in the regulation of PGR function during the estrous cycle in corpus luteum (CL) and endometrium and thus modulate the effect of progesterone (P4) within the reproductive system. The experimental material included CL and endometrial tissues from cows on days 2–5, 6–10, 11–16, and 17–20 of the estrous cycle. The mRNA expression of PCAF and NCOR1 was determined by means of real-time PCR, and protein levels were determined using western blotting. The highest mRNA and protein expression for PCAF ($P < 0.01$) and NCOR1 ($P < 0.01$) was found on days 6–16 in CL, whereas mRNA and protein expression for PCAF in endometrium was the highest on days 2–10 ($P < 0.05$), but for NCOR1 it was the highest on days 2–5 ($P < 0.05$) and decreased thereafter. Significant correlations were found between PCAF and NCOR1 mRNA and protein in CL and endometrium, between PCAF mRNA or protein and P4 levels only in CL, and between NCOR1 protein and P4 levels in endometrium only. Correlations between PCAF and NCOR1 mRNA and PCAF and NCOR1 protein were also found. These data suggest that the variable expression of these coregulators in CL and endometrium during the estrous cycle may depend on the influence of P4, and in these tissues both coregulators may compete for binding to the PGR.

1. Introduction

Progesterone (P4) is a steroid sex hormone produced mainly by the corpus luteum (CL), follicle and uterus during the estrous cycle (Niswender et al., 2000). It is a key regulator of reproductive processes in females of many species of mammals, including cows. The physiological effect of P4 is carried out through a genomic mechanism via specific nuclear progesterone receptors (PGRs) and through a non-genomic mechanism by interaction with a specific membrane progesterone receptor. The nuclear PGR occurs in two main isoforms: isoform A (PGRA) and isoform B (PGRB). Both isoforms are transcribed from the same gene but under the influence of two different promoters (Mulac-Jericevic and Conneely, 2004). Isoform PGRB is longer than PGRA by approximately 164 nucleotides in humans; in other species, this difference varies from 128 to 165 amino acids (Mulac-Jericevic and Conneely, 2004). It has been established that PGRB acts mainly as an activator of progesterone-responsive genes, but when both receptors are expressed, PGRA acts as a repressor of PGRB activity (Wen et al., 1994; Pieber et al., 2001). The inactive form of the PGR receptor is associated with a complex of chaperone proteins (Cheung and Smith, 2000). Connection of the hormone to the receptor causes dissociation of the

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protein complex and translocation of the receptor to the cell nucleus. Inside the nucleus, the receptors dimerize and bind to hormone response elements (HREs) located in the promoters of target genes. The last step in PGR activation is attachment to the receptor of additional transcriptional regulatory elements known as coregulators, a group of proteins that interact with the receptor complex without binding to the DNA of the target gene sequence (Glass and Rosenfeld, 2000). These proteins are characteristic regulators of any member of the nuclear receptor superfamily while there is no data on their involvement in regulation of membrane receptors (Lonard and O'Malley, 2012). The coregulators consist of two groups of proteins: coactivators, proteins that enhance the transcription of target genes, and corepressors, proteins that inhibit the transcription of such genes (Xu et al., 1999). Coactivators have an intrinsic histone acetyltransferase (HAT) activity that, by acetylation of histones, loosens chromatin and causes greater availability of transcription factors and polymerase at the appropriate gene sequence (Tyler and Kadonaga, 1999). Corepressor proteins cooperate with histone deacetylases (HDACs) and remove acetyl groups from histones, thereby increasing chromatin condensation, and transcription of the target gene is not initialized (Lazar, 2003). Both coactivators and corepressors may exhibit variable expression in the estrous cycle, as shown in the human endometrium (Shiozawa et al., 2003). Therefore, it is possible that these changes may affect P4 action or functions of PGR in cows. Thus, the aim of these studies was to evaluate expression of mRNA and protein of the coactivator P300/CBP-associated factor (PCAF) and the corepressor Nuclear Receptor Corepressor 1 (NCOR1) in luteal and endometrial tissue during the estrous cycle in cows.

2. Material and methods

2.1. Tissue collection

Corpora lutea ($n = 5$) and endometrial tissue ($n = 5$) from non-pregnant cows and mature heifers were harvested at a commercial slaughterhouse on days 2–5, 6–10, 11–16, and 17–20 of the estrous cycle. Days of the estrous cycle were determined by criteria reported by Ireland et al. (1980) and Fields and Fields (1996), respectively. Immediately after collection, the whole CLs and 1–2 prepared strips of each endometrium, weighing approximately 5 g were frozen in liquid nitrogen, transported to the laboratory and stored at −80 °C until further use. The deeply frozen tissues were homogenized with a Retsch MM-2 vibratory mill (Retsch GmbH, Haan, Germany). Tissue powder was divided into individual portions for isolation of RNA and protein level determination.

2.2. Progesterone concentrations

Progesterone concentrations was determined by EIA as described by (Prakash et al., 1987) using a reader plate (Multiscan EX, Labsystem, Helsinki, Finland) for the measurement of absorbance at 450 nm. Progesterone was extracted from CL and endometrial tissue using petroleum ether (Tsang et al., 1990). Recovery of this hormone averaged 91% and data were corrected for procedural losses. Progesterone labelled with horse radish peroxidase was used at final dilution of 1:40,000 and P4 antiserum (IFP4) used at a final dilution of 1:60,000 was characterized earlier (Kotwica et al., 1994). The range of the standard curve was 0.1–25 ng/mL and the sensitivity of the procedure was 0.15 ng/mL. Intra- and inter-assay coefficients of variation for the control samples were on average 6.1 and 7.8%, respectively. The relationship between the added and measured amounts of hormone ($n = 7$) was significant ($r = 0.96$). The values of steroids shown on the graph were calculated per gram of CL and endometrial tissue.

2.3. RNA isolation and reverse transcription

Total RNA was extracted from homogenized tissue by means of a Universal RNA Purification Kit (EURx, Gdansk, Poland) following the manufacturer's instructions using a method described previously (Chomczynski and Sacchi, 1987). Isolated RNA was stored at −80 °C until further analysis. The purity and concentration of the RNA were determined by measuring the absorbance at 260 nm and 280 nm wavelengths using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). DNase treated RNA (1 μg) was reverse transcribed using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) according the manufacturer's instructions.

2.4. Real-time PCR

Real-time PCR was performed by means of the Applied Biosystems 7900 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The oligonucleotide primers used for PCR amplification and the expected product sizes of PCAF, NCOR1 (one of the most often coregulators used in the studies on the other species) and TBP (a housekeeping gene) are shown in Table 1. The real-time PCR reaction mixture (20 μL) consisted of 100 ng cDNA, 10 μL of Master Mix, and 0.2 mM of PCR primers for each gene of interest. The PCR protocol had an initial denaturation step (10 min at 95 °C), followed by 40 cycles of denaturation (15 s at 95 °C) and annealing and extension (1 min at 60 °C). All reactions ($n = 5$) were performed in duplicate.

2.5. Western blot analysis

Radioimmunoprecipitation assay buffer (RIPA) with protease inhibitors was used for protein sample preparation (25 mM Tris–HCl, pH 7.6; 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS)). Proteins