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Expression of membrane progestin receptors (mPRs) in the bovine corpus luteum during the estrous cycle and first trimester of pregnancy

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ABSTRACT

Progesterone (P4) affects luteal cell function through nuclear P4 receptors and via nongenomic mechanisms, presumably involving membrane P4 receptors. There are 2 types of these receptors: progesterone receptor membrane component (PGRMC) and membrane progestin receptor (mPR), including mPR alpha (mPRα), beta (mPRβ), and gamma (mPRγ), which belong to the progestin and adipoQ receptor family (PAQR 7, 8, and 5, respectively). The aim of this study was to evaluate mRNA expression, protein expression, and localization of mPRα, mPRβ, and mPRγ in the bovine corpus luteum (CL) on days 2–5, 6–10, 11–16, and 17–20 of the estrous cycle as well as on weeks 3–5, 6–8, and 9–12 of pregnancy (n = 5/each period). The highest *mPRα* mRNA expression was found on days 11–16 ($P < 0.05$) and 17–20 ($P < 0.001$) of the estrous cycle compared with other stages of the estrous cycle and pregnancy. The *mPRβ* mRNA level was highest ($P < 0.01$) on days 11–20 of the estrous cycle and in all stages of pregnancy. *mPRγ* mRNA expression was highest ($P < 0.001$) on days 17–20 of the estrous cycle and also during weeks 9–12 of pregnancy compared with the other stages of the estrous cycle and pregnancy. Only the mPRα protein was changed during the estrous cycle; there were no significant differences in protein expression of mPRβ and mPRγ during the estrous cycle and pregnancy. Immunostaining for the mPRα, mPRβ, and mPRγ proteins was detectable in the CL sections at all stages of the estrous cycle and pregnancy. Strong positive staining was observed in small luteal cells; this reaction was less evident in large luteal cells. All proteins were also localized in endothelial cells of blood vessels. The obtained data indicate variable expression of mPRα, mPRβ, and mPRγ in bovine CL during the estrous cycle and first trimester of pregnancy and suggest that P4 may be involved in the regulation of CL function via these membrane receptors during both the estrous cycle and pregnancy.

1. Introduction

The corpus luteum (CL) is a transient endocrine gland that is composed of several cell types, including large and small luteal cells, endothelial cells, fibroblasts, and immune cells [1,2]. The main function of the CL is the production of progesterone (P4), a steroid hormone, which regulates the duration of the estrous cycle and establishes and maintains pregnancy in many species, including cows [1]. Progesterone acts on luteal cells through specific nuclear receptors (PGR) and via a nongenomic mechanism. PGR acts as a ligand-activated transcription factor and has 2 main isoforms: PGR-A and PGR-B, which arise from a single gene but are under the control of different promotors [3]. These 2 isoforms are expressed in bovine CL during the estrous cycle and pregnancy [4,5]. However, there are reports of nongenomic action of P4 in the CL of different species [6–9], including cow [10–13]. This effect of P4 is mediated by 2

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different types of membrane receptors: (1) progesterone receptor membrane component (PGRMC) 1 and 2, which belong to the membrane-associated progesterone receptor protein family [14,15]; (2) membrane P4 receptors (mPRs), which belong to the class II progestin and adipoQ receptor (PAQR) family [16,17].

Our earlier studies have reported variable expression of mRNA and/or protein for PGRMC1 and PGRMC2 in bovine CL [12,13] during the estrous cycle and first trimester of pregnancy. Moreover, we suggested that PGRMC proteins are involved in the regulation of luteal cell function in cow [13]. It is also possible that mPRs are involved in this process, but data on expression of this receptor in bovine CL are limited.

Membrane progesterone receptors are 7-transmembrane proteins with high-affinity binding of P4 at the cell membrane [18]. To date, 3 major isoforms of the receptor encoded by different genes, including mPRα (PAQR7), mPRβ (PAQR8), and mPRγ (PAQR5), have been identified in humans and other vertebrates [14,16]. Moreover, 2 additional isoforms, mPRδ (PAQR6) and mPRε (PAQR9), were found in different regions of the human brain [19]. The mPRs participate in rapid P4 actions in target cells by regulating different intracellular signaling pathways. It was found that these receptors, after binding P4, can suppress adenylyl cyclase activity and cyclic adenosine monophosphate (cAMP) production [14,20] or activate mitogen-activated protein kinase signaling and intracellular Ca^{2+} mobilization [8,18,21,22]. It was suggested that these pathways are responsible for blood vessel relaxation as well as gap junction communication in the nervous and cardiovascular systems [23,24], or the development of cancer [25,26].

Several studies have revealed that mPRs have important roles in reproduction and are expressed in many reproductive tissues, including the uterus, placenta, ovaries, and oviduct of several species [6,16,17,20–22,27–30]. These receptors are involved in the regulation of maturation [27], transport of oocytes [28], preparation of the uterus for implantation [14,21,22,27], pregnancy [17], labor [20], and development of the placenta [29].

The expression of mPRs mRNA was detected recently in the bovine CL [31]. These findings suggest that mPRs may participate in P4 signaling in bovine CL, but there are limited data describing the expression and cellular localization of mPRs throughout the estrous cycle and pregnancy. Therefore, the aim of the present study was to determine the (1) cellular localization, (2) mRNA expression, and (3) protein expression of mPRα, mPRβ, and mPRγ in bovine CL during the estrous cycle and first trimester of pregnancy.

2. Materials and methods

2.1. Corpora lutea collection

All experiments were performed in accordance with principles and procedures approved by the Local Animal Ethics Committee, Olsztyn, Poland.

Corpora lutea (CLs) from healthy mature cows were collected at a local slaughterhouse within 20 min of slaughter. CLs were assigned to the following stages: days 2–5 (early), 6–10 (mid), 11–16 (late), and 17–20 (regressed luteal stage) of the estrous cycle and weeks 3–5, 6–8, and 9–12 of pregnancy (n = 5–6 CL/stage). The estrous cycle stages were defined by macroscopic observation of the ovaries (CL and follicles) and uterus as previously described [32,33]. Pregnancy was confirmed by the presence of embryos in the uterus, and the stages of pregnancy were estimated according to Jainudeen and Hafez [34]. Moreover, the luteal P4 concentrations in CL from each cow were determined to confirm the correct evaluation of the estrous cycle and pregnancy stage. The CL was removed from the ovaries and divided into 2 sections after collection. One section was immediately snap frozen in liquid nitrogen and stored at 80°C until further use. Deep-frozen luteal tissues were homogenized with a Retsch MM-2 vibratory mill (Retsch GmbH, Hann, Germany). Tissue powder was divided into individual portions for determination of P4 and RNA as well as protein isolation. The other section was used for immunohistochemistry; it was first fixed in 4% paraformaldehyde in 0.1-M PBS (pH 7.4) for 24 h, washed with distilled water, dehydrated in an ethanol gradient, and subsequently embedded in paraffin.

All materials used in these studies were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

2.2. P4 determination

The P4 concentrations were determined by EIA as described by [35] using a plate reader (Multiscan EX, Labsystem, Helsinki, Finland) for the measurement of absorbance at 450 nm. Progesterone was extracted from CL tissues using petroleum ether (efficiency above 85%) [35]. Progesterone labeled with horseradish peroxidase was used at final dilution of 1:60,000, and P4 antiserum (IFP4) was used at a final dilution of 1:60,000 and was characterized earlier [36]. The range of the standard curve was 0.1–25 ng/mL. The sensitivity of the procedure was 0.15 ng/mL. The intra-assay and interassay coefficients of variation were 9.6% and 12.7%, respectively.

2.3. Immunohistochemistry of mPRs

For immunohistochemical localization of the mPRs protein in bovine luteal tissue, sections that were 6-μm thick were cut from paraffin-embedded samples and mounted on Super Frost Plus microscope slides (Superfrost Plus, Menzel-Gläser, Braunschweig, Germany). The sections were deparaffinized in xylene and rehydrated in a series of ethanol dilutions (100%, 96%, 70%, H_2O) as previously described [13,30]. Antigen retrieval was performed by pressure cooking in 0.01-M sodium citrate with 0.05% Tween 20 (pH 6.0). After blocking of endogenous peroxidase activity with 10% hydrogen peroxide in H_2O for 30 min, the sections were incubated with 10% Normal Goat Serum (NGS; for mPRα) or Normal Rabbit Serum (NRS; for mPRβ and mPRγ) in a blocking buffer containing 0.1 M PBS, 0.1% BSA, and 0.05% thimerosal to block nonspecific binding sites (1 h at room temperature). Next, the sections were incubated overnight at 4°C with primary antibodies at dilutions of 1:80 for the rabbit polyclonal anti-mPRα (Sigma), 1:15 for the goat polyclonal anti-mPRβ (Santa Cruz Biotechnology, Dallas, TX), and 1:10 for the goat polyclonal anti-mPRγ (Santa Cruz