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Prostaglandins & other Lipid Mediators 79 (2006) 218–229

PROSTAGLANDINS & other LIPID MEDIATORS

Role of intraluteal prostaglandin $F_{2\alpha}$, progesterone and oxytocin in basal and pulsatile progesterone release from developing bovine corpus luteum

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Received 22 November 2005; received in revised form 16 January 2006; accepted 23 January 2006
Available online 21 February 2006

Abstract

The present study examined the role of intra-luteal prostaglandin (PG) $F_{2\alpha}$, progesterone (P4) and oxytocin (OT) on the corpus luteum function by using specific hormone antagonists. Luteal cells from the developing CL (days 5–7 of the estrous cycle) were exposed to P4 antagonist (onapristone, OP, 10^{-4} M), OT antagonist (atosiban, AT; 10^{-6} M) or indomethacin (INDO; 10^{-4} M), for 12 h and then stimulated with $PGF_{2\alpha}$ (10^{-8} M) for 4 h. Pre-treatment of the cells with OP, AT or INDO resulted in an increase in P4 secretion in response to $PGF_{2\alpha}$. To examine the temporal effects of P4, OT and PGs on P4 secretion, dispersed luteal cells were pre-exposed to OP, AT or INDO for 1, 2, 4, 6 or 12 h. Prostaglandin $F_{2\alpha}$ stimulated P4 secretion ($P < 0.05$) after 2 h of pre-exposition. In the microdyalisis study, the spontaneous release of P4 from developing CL tissue was of pulsatile nature with irregular peaks at 1–2 h intervals. Treatment with OP increased the number of P4 peaks ($P < 0.05$), whereas AT and INDO significantly reduced the number of P4 peaks detected ($P < 0.05$). Interestingly, INDO completely blocked the pulsatile nature in the release of P4, but it secretion remained stable troughout the experimental period. These results demonstrate that luteal $PGF_{2\alpha}$, OT, and P4 are components of an autocrine/paracrine intra-ovarian regulatory system responsible for the episodic (pulsatile) release of P4 from the bovine CL during the early luteal phase.

Keywords: Corpus luteum; $PGF_{2\alpha}$; Oxytocin; Progesterone; Cattle

1. Introduction

It is well documented that injection of prostaglandin (PG) $F_{2\alpha}$ at the mid luteal stage induces functional (decrease in progesterone; P4) and structural regression of the corpus luteum (CL) in ruminants in vivo [1]. However, treatment of the luteal cells with $PGF_{2\alpha}$ resulted in an increase of P4 production in vitro. The mechanisms involved in this opposite actions of $PGF_{2\alpha}$ on P4 remains not fully understood.

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doi:10.1016/j.prostaglandins.2006.01.002

Although temporal pattern of pulsatile P4 secretion is well established in cow [2], the factors regulating such pulsatile nature of P4 secretion are incompletely understood. Early observations [2,3] showed that LH induces pulsatile release of P4 from the bovine ovary. Based on this finding, a close functional and temporal coupling of the CL function and pituitary LH inputs has been proposed. However, it has been also demonstrated the pulsatile P4 secretion by bovine CL in vitro is independent of LH influence [4–6]. This autonomous, episodic P4 release from the CL is indicative of an intra-luteal generating system, which is independent of gonadotropin stimulation and may be regulated by local factors.

The bovine CL has been shown to be the site of P4, PG and oxytocin (OT) [7,8] production. Moreover, in addition to the presence of $PGF_{2\alpha}$ receptors [9], high affinity binding sites for OT [10] and P4 [11] have also been demonstrated in bovine CL. The intraluteal concentration of $PGF_{2\alpha}$ is higher during the early luteal stage compared with mid- and late-luteal stages. This profile of $PGF_{2\alpha}$ contrats with that of P4 concentration, which is highest at the mid-luteal phase [12]. These changes in $PGF_{2\alpha}$ and P4 together with OT [13] throughout the luteal phases suggest that these hormones are directly involved in the regulation of bovine CL function.

Recently, we have demonstrated that $PGF_{2\alpha}$ has the capacity to increase OT secretion from luteal cells only when a tridimentional cell-to-cell contact is established in vitro [14]. Miyamoto and Schams showed that OT stimulates P4 release from microdialyzed bovine CL in vitro [13]. This stimulatory effect of OT on P4 secretion was highest on days 5–7 of the estrous cycle and declined from days 8–12 to days 15–18 of the cycle. These findings let us to hypothesize that ovarian $PGF_{2\alpha}$ and OT control the frequency and amplitude of the autonomous, pulsatile P4 secretion from bovine CL during the developing stage. Therefore, this study was aimed to determine the role of intra-luteal P4, $PGF_{2\alpha}$ and OT in the release of P4 from developing CL using specific P4, $PGF_{2\alpha}$ and OT antagonists in a cell culture as well as in a tissue culture system in vitro.

2. Materials and methods

2.1. Collection of CL

Healthy, normally cycling Holstein/Polish Black and White (75/25%; respectively) cows (4–6 lactation) were used for collection of the ovaries with CL. The animals were eliminated by the owner (Spółka Rolna,Wroblik" Sp. z o.o, Lidzbark Warminski, Poland) from the dairy cows hard because of lower milk production. The estrus was synchronized using implants of a progesterone analogue (Crestar, Intervet, Holland) with additional injection of an analogue of $PGF_{2\alpha}$ (cloprostenol; Bioestrophan, Biowet, Gorzow Wielkopolski, Poland), as recommended by the manufacturer for the estrus synchronization of multiparous cows. One day before slaughter, the animals were transported to the local abattoir (Ubojnia zwierząt rzeźnych Gucin, Łukta, Poland). Ovaries with CL were collected within 10–15 min after slaughter, placed in ice-cold saline and transported to the laboratory. For in vitro MDS study, the CL were washed several times with a saline solution and suspended in Medium 199 (Sigma, Deisenhofen, FRG) containing 10 mmol Na HCO_3, Earle's salts, L-glutamine, 25 mmol HEPES/L, 5 g BSA/L, 60 mg penicillin/L, 100 mg streptomycin/L, 2 mg amphotericin/L and 56 mg ascorbic acid/L at pH 7.5 at 38 °C.

2.2. Cell isolation

Dissociation of the luteal tissue and the culture of luteal cells were performed as previously described [10]. The cells were counted with a hemocytometer. Cell viability was higher than 85% as assessed by trypan blue exclusion. The cell suspension contained about 5% endothelial cells or fibroblast and no erythrocytes, with about 10% of large luteal cells and 80% and small luteal cells. The cell concentration was adjusted to 1×10^5 viable cells/ml of cultured medium: Dulbecco's Modified Eagle's medium and F-12 Ham's medium (DMEM/Ham's F-12; 1:1 [v/v]; Sigma; #D-8900) supplemented with 5% calf serum (CS; Sigma; # C-6278), 2 mM L-glutamine and 15 mM HEPES, and containing 1.2 g/ml sodium bicarbonate and 20 μg/ml gentamicin (Gibco Laboratories; # 600–5750AD). The cells were cultured for 24 h in 48-well culture plates (Costar, Cambridge, MA, USA) in humidified incubator at 37.5 °C in a 5% CO_2/air atmosphere.

After the cells attachment (18–24 h), the culture media were replaced by fresh serum semi-free culture medium, DMEM/F-12 containing 0.1% BSA, 5 ng/ml sodium selenite, 0.5 mM ascorbic acid, 5 μg/ml transferrin and 20 μg/ml