POSSIBLE ROLE OF INTERLEUKIN-1 IN BOVINE CORPUS LUTEUM

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Introduction

Interleukin-1 (IL-1) was mainly produced by activated macrophages, and its primary role is to stimulate proliferation and maturation of lymphocytes. The presence of IL-1[1] and its multi-hormonal effects have been demonstrated in the corpora lutea (CL) in a variety of species[2]. In bovine CL, IL-1 is known as a potent stimulator of prostaglandin (PG) production, and luteal PGs seem to play a role in the regulation of progesterone (P4) production[3]. On the other hand, many of the cell types known to produce IL-1, such as macrophages, endothelial cells and fibroblasts, are present within the CL[2,4]. These findings suggest that IL-1 plays some roles as a luteotropic factor in bovine CL throughout the estrous cycle by stimulating the local synthesis of PGs. Therefore, if IL-1 plays roles in the regulation of luteal function in bovine CL, bovine CL must have IL-1 receptors.

The present study was carried out to determine the presence of IL-1 receptor (IL-1R) mRNA in bovine CL throughout the estrous cycle. The effects of IL-1 on $PGF_{2\alpha}$ and PGE_2 secretion by cultured bovine mid-luteal cells were also examined.

Methods and Materials

Collection of Bovine CL

Bovine CL were collected and stored as described previously[5].

RT-PCR

Total RNA was prepared from CL and cultured luteal cells using Isogen according to the directions of the manufacturer (Nippon Gene, Toyama, Japan). Levels of IL-1R and β-actin mRNAs were measured by RT-PCR. The sequences of the IL-1R primers were 5'-CAC TCT GCT GGA CTC TAA GGA G-3'and 5'-CCT AAA TCT GTC TAT AGA TGG TG-3'. The primers for β-actin were 5'-GAG GAT CTT CAT GAG GTA GTC TGT CAG GTC-3' and 5'-CAA CTG GGA CGA CAT GGA GAA GAT CTG GCA-3'. The conditions for the PCRs were as described previously[6] and 21 (β-actin) or 30 (IL-1R) cycles of reactions were used for the denaturation for the amplification.

Preparation of Luteal Cells

Luteal cells were prepared and cells were cultured as described previously[5].

Cell Culture and Experiments

The dispersed luteal cells were seeded at 2.0 x 10^5 viable cells in 0.5 ml, in 48-well cluster dishes (Costar, Cambridge, MA, 3524). After 12 h of culture, the medium was replaced by fresh medium. The cells were then exposed to varying concentrations of IL-1β (0.3-30 ng/ml) for 24 h. The conditioned media from the last 24 h of culture were collected and stored at –30 °C until assayed for $PGF_{2\alpha}$ and PGE_2.

$PGF_{2\alpha}$ Determination

Concentrations of $PGF_{2\alpha}$ were determined directly from the cell culture media with an enzyme immunoassay as described previously[5]. The standard curve ranged from 0.016 to 4 ng/ml, and the effective dose of the assay for 50% inhibition (ED_{50}) was 0.33 ng/ml. The intra- and inter-assay coefficients of variation were 3.7% and 13.2%, respectively.

PGE_2 Determination

Concentrations of PGE_2 were determined directly from the cell culture media with an enzyme immunoassay as described previously[4]. The standard curve ranged from 0.11 to 28.20 ng/ml, and the effective dose of the assay for 50% inhibition (ED_{50}) was 6.5 ng/ml. The intra- and inter-assay coefficients of variation were 4.1% and 14.3%, respectively.

Statistical Analysis

Experimental data are shown as the mean ± SEM. The data on the effects of IL-1β on $PGF_{2\alpha}$ and PGE_2 are shown as percentages of the control. The statistical significance of differences in each experiment was assessed by ANOVA followed by Fisher's protected least-significant difference procedure (PLSD) as a multiple comparison test.

Results and Discussion

The present study demonstrated the expression of IL-1R mRNA in the bovine CL throughout the estrous cycle (Fig. 1). Moreover, IL-1R mRNA levels are higher in the early luteal stage than in the other luteal stages (Fig. 1). These findings suggest that IL-1 plays roles in regulating luteal function in bovine CL throughout the estrous cycle, especially at the early luteal stage of bovine CL. In the present study, IL-1β stimulated both $PGF_{2\alpha}$ and PGE_2 secretion by bovine luteal cells (Fig. 2). It has been demonstrated that PG production in bovine CL is greatest during the first several days after ovulation[7], and that inhibition of in vivo PG synthesis during this period results in a reduced luteal life span[8]. In addition, the number of macrophages, which appear to be the primary source of IL-1 in the bovine CL[5], increased during luteal development in cows[9]. Therefore, we speculate that macrophage-derived IL-1 plays a role as a modulator of luteal development stimulating the synthesis of luteal PGs. On the other hand, it has been demonstrated that IL-1 was secreted by endothelial cells and fibroblasts within the CL[2,4], and the presence of IL-1 was observed throughout the estrous cycle[1]. Since the expression of IL-1R mRNA was observed in the bovine CL throughout the estrous cycle (Fig. 1), we assume that endothelial cell- and fibroblast-derived IL-1 may also play a local role in regulating bovine CL function throughout the estrous cycle. In addition, IL-1β stimulated $PGF_{2\alpha}$ secretion as well as PGE_2 secretion in bovine mid-luteal cells (Fig. 2). Since PGE_2 and $PGF_{2\alpha}$ were shown to stimulate P4 secretion by bovine luteal cells[3] in vitro, it is generally accepted that luteal $PGF_{2\alpha}$ and PGE_2 are luteotropic agents in bovine CL. Therefore, it is possible that IL-1 secreted by luteal endothelial cells and fibroblasts plays a role as a luteotropic agent by stimulating luteal $PGF_{2\alpha}$ and PGE_2 secretion in bovine CL.

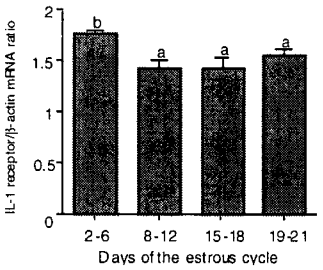

Fig. 1. Relative levels of IL-1R mRNA based on the results of RT-PCR (arbitrary units) in bovine CL during the estrous cycle. All values represent mean ± SEM from 4CL/stage of the densitmetric analysis of IL-1R mRNA levels in CL (relative to β-actin mRNA levels). Different superscript letters indicate significant difference (P<0.05).

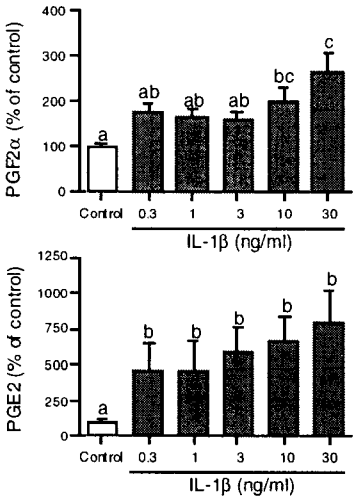

Fig. 2. Effects of IL-1β on PGF2α and PGE2 secretion by bovine mid-luteal cells. Different superscript letters indicate significant difference (P<0.05).

In conclusion, the overall results lead us to hypothesize that IL-1 plays physiological roles in regulating bovine CL function throughout the estrous cycle, especially at the early stage of the estrous cycle.

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