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Protein and messenger RNA expression of interleukin 1 system members in bovine ovarian follicles and effects of interleukin 1β on primordial follicle activation and survival in vitro

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ABSTRACT

This study aimed to investigate the expression of interleukin 1 (IL-1) system members (proteins and messenger RNA of ligands and receptors) and its distribution in ovarian follicles of cyclic cows and to evaluate the effects of IL-1β on the survival and activation of primordial follicles in vitro. The ovaries were processed for localization of IL-1 system in preantral and antral follicles by immunohistochemical, real-time polymerase chain reaction, and Western blot analysis. For in vitro studies, ovarian fragments were cultured in α-MEM$^+$ supplemented with IL-1β (0, 1, 10, 50, or 100 ng/mL), and after 6 d, the cultured tissues were processed for histologic analysis. Immunohistochemical results showed that the IL-1 system proteins IL-1β, IL-1RA, IL-1RI, and IL-1RII were detected in the cytoplasm of oocytes and granulosa cells from all follicular categories and theca cells of antral follicles. Variable levels of messenger RNA for the IL-1 system members were observed at different stages of development. After 6 d of culture, the presence of IL-1β (10 or 50 ng/mL) was effective in maintaining the percentage of normal follicles and in promoting primordial follicle activation. In conclusion, IL-1 system members are differentially expressed in ovarian follicles according to their stage of development. Moreover, IL-1β promotes the development of primordial follicles. These results indicate an important role of the IL-1 system in the regulation of bovine folliculogenesis.

1. Introduction

Ovarian follicular development is regulated by several substances, such as hormones, growth factors, and cytokines [1]. Interleukins (ILs) are proinflammatory cytokines that have been reported to be involved in both endometriosis and ovarian carcinogenesis [2,3] and actively participate in the process of ovulation [4,5]. The IL system 1 consists of 2 bioactive ligands, IL-1α and IL-1β [6], 2 receptors, IL-1RI and IL-1RII [7], and a receptor antagonist (IL-1RA) that regulates the biological activity of IL-1 in a competitive action on the receptor [8]. IL-1RI is a glycoprotein of 80 kDa [9], and IL-1RII has a molecular weight of 60 to 65 kDa [10,11]. Both receptors, IL-1RI and IL-1RII, can bind IL-1; however, only IL-1RI can mediate the IL-1 signal [12–14]. The IL-1RII cannot mediate the signal because of

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its shorter cytoplasmic domain tail of 29 amino acids, compared with 215 amino acids for IL-RI [7]. When IL-1 binds to IL-1RI, a conformational change occurs in the receptor, which allows the association of IL-1RA to IL-1–IL-1RI complex [15–17]. The association of IL-1RA to the IL-1–IL-1RI complex is essential for signaling [18–20]. All these receptors and ligands are expressed and are effective in a wide variety of tissues, including rat ovarian cells [21,22].

It is known that components of IL system are expressed in oocytes, cumulus, and mural granulosa cells of rat antral follicles [21]. Not long ago, Sirotkin [23] reported that the ovary is a site for action and production of ILs in various mammalian species. Previously, it was shown that IL-1 modulates rat [24] and bovine [25] granulosa cell proliferation in vitro, dependent on the follicle size, and inhibits steroidogenesis in bovine undifferentiated follicles but stimulates the release of progesterone in differentiated follicles [25,26]. In addition, IL-1 promoted the proliferation of ovarian cells, suppression of apoptosis, and follicular growth [27]. These authors also reported that IL-1β acts on granulosa and theca cells to regulate steroidogenesis and ovulation in rats [28]. This cytokine also promotes germinal vesicle breakdown in rabbit oocytes [29] and stimulates the resumption of meiosis and nuclear oocyte maturation in mares [30,31]. However, there is no information about the messenger RNA (mRNA) expression of the IL-1 system proteins IL-1α, IL-1β, IL-1RA, IL-1RI, and IL-1RII and the protein localization of these ligands and their receptors in bovine ovarian follicles. In addition, the effects of IL-1β on early follicular development in bovine species are still not known.

The present study was carried out to examine the existence of an intrafollicular IL-1 system (ligands and receptors) and its distribution in ovarian follicles of cyclic cows and to find evidence of a role of this system in early folliculogenesis. Therefore, mRNA expression for IL-1 system was investigated by real-time polymerase chain reaction (PCR), whereas the presence and cellular localization of the corresponding proteins were analyzed by immunohistochemistry and Western blot. The effects of IL-1β on primordial follicle activation and survival in vitro were also investigated.

2. Materials and methods

2.1. Experiment 1: protein localization and mRNA quantification for IL-1 system members in bovine ovarian follicles

2.1.1. Collection of ovaries

Ovaries from adult mixed breed cows (*Bos taurus*) were collected at a slaughterhouse. After collection, the ovaries were washed (for approximately 10 s) in 70% alcohol, then washed twice in 0.9% saline solution, and transferred to minimum essential medium alpha (α-MEM; Sigma-Aldrich, Saint-Louis, MO, USA) supplemented with 100-mg/mL penicillin and 100-mg/mL streptomycin. Subsequently, ovarian pairs from each cow were transported to the laboratory within 1 h at 4°C [32].

2.1.2. Real-time PCR

Ovaries (n = 20) from adult cows were collected as described previously. To isolate secondary follicles, the ovaries were carefully dissected and placed immediately in warmed α-MEM. Briefly, ovarian cortical slices (1- to 2-mm thick) were cut from the ovarian surface, and follicles were visualized under a stereomicroscope (SMZ645; Nikon, Tokyo, Japan) and manually microdissected from strips of ovarian cortex using 26-gauge needles. After isolation, the absence of antral cavity was confirmed by using an inverted microscopy (NIKON, Eclipse, TS100), and the follicles were washed and 3 groups of 10 follicles were collected and stored at −80°C until RNA extraction. This procedure was completed within 2 h.

A second group of ovaries (n = 20) was collected as described previously, to isolate oocyte and cells from antral follicles. In the laboratory, the cumulus-oocyte complexes (COCs) were aspirated from small (<3 mm) and large (>3 mm) antral follicles with an 18-gauge needle. From the follicle content so collected, compact COCs were selected as described by Van Tol and Bevers [33]. Thereafter, the cumulus was separated from the oocyte by a combination of vortexing and aspiration via a narrow-bore Pasteur pipette. Three groups of 10 denuded oocytes and 3 groups of cumulus cells from 10 COCs were stored at −80°C until RNA extraction. Then, the ovaries were also used to obtain follicular walls. To this end, follicles were isolated from the ovaries and dissected free of stromal tissue [34], and transparent follicles measuring 1 to 3 mm and >3 mm in diameter were selected. The follicles were sectioned into equal halves with a scalpel, and the oocytes were removed and discarded.

To evaluate gene expression for IL-1 system (IL-1β, IL-1RI, IL-1RII, and IL-1RA), total RNA extraction was performed using Trizol purification kit (Invitrogen, São Paulo, SP, Brazil). According to the manufacturer's instructions, 800 μL of Trizol solution was added to each frozen samples, and the lysate was aspirated through a 20-gauge needle before centrifugation at 10,000*g* for 3 min at room temperature. Thereafter, all lysates were diluted 1:1 with 70% ethanol and subjected to a minicolumn. After binding of the RNA to the column, DNA digestion was performed using RNAse-free DNAse (340 Kunitz units/mL) for 15 min at room temperature. After washing the column 3 times, the RNA was eluted with 30-μL RNAse-free water. The RNA concentration was estimated by reading the absorbance at 260 nm and was checked for purity at 280 nm in a spectrophotometer (Amersham Biosciences, Cambridge, England), and 1-μg of total RNA was used for reverse transcription. Before the reverse transcription reaction, samples of RNA were incubated for 5 min at 70°C and then cooled in ice.

Reverse transcription was performed in a total volume of 20 μL, which was composed of 10 μL of sample RNA, 4 μL of 5X reverse transcriptase buffer (Invitrogen), 8 units RNAse out, 150 units Superscript III reverse transcriptase, 0.036 U random primers (Invitrogen), 10 mM of dithiothreitol, and 0.5 mM of each deoxiribonucleotide triphosphates. The mixture was incubated for 1 h at 42°C, for 5 min at 80°C, and then, stored at −20°C. Negative controls were prepared under the same conditions but without the inclusion of the reverse transcriptase. Quantification of mRNA was performed using GoTaq qPCR Master Mix. PCR reactions were composed of 1-μL complementary DNA as a template in 7.5 μL of GoTaq qPCR Master Mix (Promega