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Angiotensin II, progesterone, and prostaglandins are sequential steps in the pathway to bovine oocyte nuclear maturation

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Abstract

Oocyte meiotic resumption is triggered by the ovulatory gonadotropin surge; in cattle, angiotensin II (AngII) and prostaglandins (PG) are key mediators of this gonadotropin-induced event. Here, we tested the hypothesis that progesterone (P_4) is also involved in oocyte meiotic resumption induced by the gonadotropin surge. In Experiment I, P_4 induced nuclear maturation in a dose-dependent manner using a coculture of follicular hemisections and cumulus-oocyte complexes. In the second experiment, using an *in vivo* model, an injection of mifepristone (MIFE; P_4 receptor antagonist) at the antrum of preovulatory follicles prevented GnRH-induced oocyte meiotic resumption *in vivo*. In Experiment III (coculture system similar to that of Experiment I), MIFE prevented stimulatory effects of AngII on resumption of meiosis, but saralasin (AngII receptor antagonist) did not inhibit P_4 actions. In Experiments IV and V, fibroblast growth Factor 10 (FGF10; known to suppress steroidogenesis in granulosa cells), blocked AngII-but not P_4-induced oocyte meiotic resumption. Therefore, we inferred that AngII is upstream to P_4 in a cascade to induce meiotic resumption. Previously, we had reported that AngII acted throughout the PGs pathway to modulate nuclear progression. In Experiment V, indomethacin inhibited resumption of meiosis induced by P_4, providing further support to the AngII-P_4 sequential effect on meiotic resumption. In conclusion, we inferred that AngII, P_4 and PGs are sequential steps in the same pathway that culminates with bovine oocyte maturation.

Keywords: Ovulation; Meiotic resumption; Angiotensin; Steroid; Eicosanoid

1. Introduction

The preovulatory gonadotropin surge triggers a cascade of events that culminates with ovulation and nuclear oocyte maturation. Recently, angiotensin II (AngII) has been recognized as one of the earliest mediators of gonadotropin-induced ovulation and oocyte maturation [1–3]. The positive effect of AngII in these processes is mediated through a Type 2 receptor [1]. Furthermore, the concentration of AngII and expression of its receptors (AT2) within the follicle increased during the interval between the gonadotropin surge and ovulation (Siqueira, et al, unpublished data). Other studies provided additional evidence that AngII regulated secretion of progesterone (P_4) and prostaglandins (Pg), hormones involved in ovulation [4,5]. In granulosa cell culture, AngII upregulated expression of cyclooxygenase 2 (COX-2), the rate-limiting enzyme for PG production [3].

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During follicle development, bovine oocytes remain arrested at prophase of the first meiotic division, and resume meiosis after the preovulatory LH surge [6], or after removal from the follicular environment [7]. The presence of follicular wall fragments in a coculture system with cumulus-oocyte complexes (COCs) prevents meiotic resumption [8]. This coculture system is a good model to study the role of factors that act through follicular cells on oocyte nuclear maturation [9,10]. Using this coculture system, we reported that AngII acted through a PG pathway to mediate gonadotropin-induced oocyte meiotic resumption [2].

The cyclooxygenase pathway is a classical mediator of LH-induced ovulation and nuclear oocyte maturation in cattle [11–15]. Progesterone is another key element in the ovulatory cascade and oocyte maturation [13,14,16]. Indeed, there are indications that PGs are downstream factors to this steroid; in that regard, a gonadotropin surge stimulates an increase in intrafollicular P_4, which acts by binding to its nuclear receptor and increasing abundance of mRNA for COX2 [14]. The role of P_4 on oocyte nuclear maturation in cattle remains controversial. Nuclear and membrane progesterone receptors are present in bovine COCs, and regulated during *in vitro* maturation in the presence of FSH and LH [16]. Although Sirotkin [17] reported a stimulatory effect on oocyte meiotic resumption, more recent studies concluded that P_4 was not necessary to promote nuclear maturation, cumulus expansion, and early embryo development [18,19].

Follicular cells secrete factors that prevent oocyte meiotic resumption before the LH surge. The family of fibroblast growth factors (FGFs) is composed of more than 20 factors, largely studied for their roles in embryogenesis and oogenesis. Buratini, et al [20] reported that the bovine theca cells and oocytes expressed FGF10. Expression of FGF10 receptor (FGFR2IIIb) was identified in theca [21], granulosa [20], and cumulus cells [22]. Furthermore, FGF10 in the granulosa cell culture inhibited steroidogenesis [20] and AT2 expression [23]. Activation of FGF receptors (FGFRs) appeared to be involved in inhibition of germinal vesicle breakdown (GVBD) in mice [24]. Conversely, Zhang, et al [25] reported that FGF10 improved bovine oocyte maturation, cumulus expansion and subsequently embryo development in medium containing estradiol and in the absence of follicular cells.

The information summarized above provided an impetus to investigate interactions between FGF10 and factors involved in triggering bovine oocyte meiotic resumption. In the present study, a combination of *in vivo* and *in vitro* experiments were conducted to test the hypothesis that P_4 plays a role in regulation of oocyte meiotic progression induced by gonadotropin surge in concert with AngII and PGs. In an *in vitro* experiment, interactions of P_4 and AngII with FGF10 (an antisteroidogenic factor recently described as an important regulator of follicular development) were studied, with regards to their roles in resumption of meiosis.

2. Materials and methods

All experimental procedures were reviewed and approved by the Federal University of Santa Maria Animal Care and Use Committee (23081.004717/2010–53 CCR/UFSM). All chemicals used were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless otherwise indicated in the text.

2.1. Preparation of follicular hemisections, oocyte recovery and nuclear maturation

Bovine ovaries at various stages of the estrous cycle were obtained from an abattoir and transported to the laboratory in saline solution (0.9% NaCl) at 30 °C containing 100 IU ml^{-1} penicillin and 50 μg ml^{-1} streptomycin sulfate. Procedures for follicle dissection and culture procedures were previously validated in our laboratory [2,9,10]. Briefly, transparent follicles, 2 to 5 mm in diameter, were selected and dissected from ovarian stromal tissue, and sectioned into halves. Follicular hemi-sections were washed in TCM 199 containing 0.4% bovine serum albumin (BSA) and randomly distributed into four-well culture dishes (Nunc, Roskilde, Denmark) containing culture medium with the desired treatment. There were eight follicular halves per 200 μl of medium. Dishes were incubated for 2 h before adding COCs.

The COCs were aspirated from follicles 3 to 8 mm in diameter, recovered under a stereomicroscope, and selected according to Leibfred and First [26]. Grades 1 and 2 COCs (n = 10 to 30) were randomly distributed into treatments and cultured in an incubator at 39 °C in a saturated humidity atmosphere containing 5% CO_2 in air and 95% air, for either 7, 15, or 24 h, depending on the experiment. The culture medium used was TCM 199 containing Earle's salts and L-glutamine (Gibco BRL, Grand Island, NY, USA) supplemented with 25 mM HEPES, 0.2 mM pyruvic acid, 2.2 mg ml^{-1} sodium bicarbonate, 5.0 μg/ml LH (lutropin-V, Bioniche, ON, Canada), 0.5 μg/ml FSH (Folltropin-V, Bioniche), 0.4% fatty acid-free BSA, 100 IU ml^{-1} penicillin, and 50 μg ml^{-1} streptomycin sulfate. At the end of the