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Bovine ovarian cells have (pro)renin receptors and prorenin induces resumption of meiosis in vitro☆

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ABSTRACT

The discovery of a receptor that binds prorenin and renin in human endothelial and mesangial cells highlights the possible effect of renin-independent prorenin in the resumption of meiosis in oocytes that was postulated in the 1980s.This study aimed to identify the (pro)renin receptor in the ovary and to assess the effect of prorenin on meiotic resumption. The (pro)renin receptor protein was detected in bovine cumulus-oocyte complexes, theca cells, granulosa cells, and in the corpus luteum. Abundant (pro)renin receptor messenger ribonucleic acid (mRNA) was detected in the oocytes and cumulus cells, while prorenin mRNA was identified in the cumulus cells only. Prorenin at concentrations of 10^{-10}, 10^{-9}, and 10^{-8} M incubated with oocytes co-cultured with follicular hemisections for 15 h caused the resumption of oocyte meiosis. Aliskiren, which inhibits free renin and receptor-bound renin/prorenin, at concentrations of 10^{-7}, 10^{-5}, and 10^{-3} M blocked this effect ($P<0.05$). To determine the involvement of angiotensin II in prorenin-induced meiosis resumption, cumulus-oocyte complexes and follicular hemisections were treated with prorenin and with angiotensin II or saralasin (angiotensin II antagonist). Prorenin induced the resumption of meiosis independently of angiotensin II. Furthermore, cumulus-oocyte complexes cultured with forskolin (200 μM) and treated with prorenin and aliskiren did not exhibit a prorenin-induced resumption of meiosis ($P<0.05$). Only the oocytes' cyclic adenosine monophosphate levels seemed to be regulated by prorenin and/or forskolin treatment after incubation for 6 h. To the best of our knowledge, this is the first study to identify the (pro)renin receptor in ovarian cells and to demonstrate the independent role of prorenin in the resumption of oocyte meiosis in cattle.

1. Introduction

Evidence is accumulating that supports possible roles for the (pro)renin receptor ([P]RR)-dependent system in the development of insulin resistance and hypertension. Interestingly, women with diabetes and hypertension often present with infertility problems [3,28,33]. Likewise, delays in oocyte maturation have been documented in animal models of diabetes [12,14]. Findings from other studies carried out in the 1980s have suggested that prorenin has a role in the resumption of oocyte meiosis [29], but this remains unclear.

Oocytes are arrested during the first meiotic division (prophase I) by the follicular environment. The preovulatory luteinizing hormone (LH) surge induces the closure of gap junctions and a reduction in the inhibitory cyclic guanosine monophosphate (cGMP) signal from the cumulus cells to the oocyte in vivo. The low level of cGMP generated by the granulosa cells results in phosphodiesterase 3 activation, cyclic adenosine monophosphate (cAMP) hydrolysis, and the subsequent resumption of meiosis in the oocyte [15,27,38,40,45,55]. The oocyte also resumes nuclear maturation when the cumulus-oocyte complex (COC) is removed from the

Abbreviations: (P)RR, (prorenin)receptor; RAS, renin-angiotensin system; AngII, angiotensin II; AngI, angiotensin I; GVBD, germinal vesicle breakdown; MI, metaphase I; IBMX, 3-isobutyl-1-methylxanthine; P450, cytochrome aromatase; CYP17A1, 17a-hydroxylase; Sar, saralasin; FSK, forskolin.

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follicular environment [42]. We have used forskolin (FSK) to maintain the high concentration of cAMP and to delay meiosis in an in vitro model [6,13,52]. Furthermore, follicular hemisections have been used to mimic the follicular environment and to delay the resumption of meiosis [4,22,43,48,51]. We have used these in vitro and in other in vivo models to study the role of angiotensin II (AngII) in the resumption of meiosis in cattle [1,49,4,22].

The classic concept of the renin-angiotensin system (RAS) portrays prorenin as an enzymatically inactive precursor of renin, which is an aspartyl protease, and as being dependent on AngII to trigger the activation of intracellular signaling pathways. In fact, plasma prorenin levels, but not plasma renin levels, increase after the LH surge [46]. Similarly, prorenin levels in the follicular fluid increase to about 12-times the concentrations detected in women's plasma after LH stimulation during in vitro fertilization procedures, and approximately 99% of the total renin identified in ovarian follicular fluid is prorenin [23].

The (P)RR was the first receptor identified that binds to an aspartyl protease [37]. The (P)RR acts within an extracellular-signal-regulated kinase (ERK1/2) pathway [54] that seems to be essential for the resumption of meiosis in mammals [16]. The (P)RR also stimulates the AngII pathway by binding to renin and prorenin, which promotes the cleavage of angiotensinogen to angiotensin I (AngI) [37,54]. The presence of (P)RR messenger ribonucleic acid (mRNA) has been demonstrated in the bovine theca and granulosa cells during follicular dominance [18]. However, the presence of the (P)RR protein in ovarian follicular cells is yet to be confirmed, and the role of prorenin in the resumption of meiosis in oocytes is unknown. The aim of this study was to characterize the (P)RR in the cumulus-oocyte complex (COC), theca cells, granulosa cells, and corpus luteum (CL). Moreover, we evaluated the role of prorenin in the induction of oocyte meiotic resumption in cattle.

2. Materials and methods

All experimental procedures using cattle were reviewed and approved by the Federal University of Santa Maria Care and Use Committee (no. 003/2012).

2.1. Chemicals

All of the chemicals used were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA) unless otherwise indicated.

2.2. Collection of the ovaries

Bovine ovaries at different stages of the estrous cycle were obtained from a local abattoir and transported to the laboratory in phosphate-buffered saline (PBS) at 4 °C for mRNA and protein analyses [8,9], or in a 0.9% NaCl solution containing penicillin (100 IU/ml) and streptomycin sulfate (50 μg/ml) at 30 °C for the COC culture experiments [4].

2.3. Western blotting

Proteins from the COCs, theca cells, granulosa cells, and CL were extracted using radioimmunoprecipitation assay buffer. The proteins were boiled at 95 °C for 5 min, subjected to 12% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, and the proteins were transferred onto nitrocellulose membranes. After blocking the membranes for 3 h using 5% skimmed milk in Tris-buffered saline (TBS) containing 0.1% Tween® 20 (TBS-T), the blots were incubated overnight with an antibody to the (P)RR (anti-ATP6IP2; diluted 1:1000; ab40790; Abcam plc, Cambridge, UK) at 4 °C while being agitated. Subsequently, the blots were washed three times for 5 min each time in TBS-T. The blots were then incubated with a goat anti-rabbit secondary antibody (diluted 1:2000; IgG-HRP; sc-2004; Santa Cruz Biotechnology, Inc., Dallas TX, USA) for 1 h while being agitated, which was followed by three washes for 5 min each in TBS-T. The immunoreactivity was detected using the Clarity™ Western ECL Substrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions, and the images were visualized using the ChemiDoc™ XRS+ imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The blots were incubated in a western blot stripping buffer, which comprised β-mercaptoethanol, 20% SDS, and 1 M Tris-HCl, at pH 6.8, for 1 h at 50 °C. Then, the membranes were washed three times with TBS-T with each wash lasting 20 min, and the membranes were re-blotted with an anti-beta actin antibody (diluted 1:5000; control; ab8227; Abcam plc, Cambridge, UK).

2.4. Ribonucleic acid extraction, reverse transcription, and the quantitative polymerase chain reaction

Total ribonucleic acid (RNA) was extracted using Trizol® (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. A NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to quantify the RNA and verify lack of contamination. Only RNAs with purity values of more than 1.8, based on the ratios of the absorbance at 260 and 280 nm, were used in the experiments. The integrity was verified in a 1.2% agarose gel that visualized the ribosomal RNA (rRNA). To generate the complimentary deoxyribonucleic acid (cDNA), the RNA (1 μg) was first treated with 0.2 U of deoxyribonuclease I (DNase I, Amplification Grade, Invitrogen Life Technologies, Waltham, MA, USA) and it was heated at 37 °C for 5 min, then at 65 °C for 10 min. Subsequently, the reverse transcription was performed using a QuantiTect Reverse Transcription Kit® (Qiagen, Venlo, Limburg, Netherlands) in accordance with the manufacturer's instructions.

The quantitative real-time polymerase chain reaction (RT-PCR) was conducted in a Step One Plus® instrument (Applied Biosystems, Foster, CA, USA) using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA) and primers that were specific for bovine prorenin and the (P)RR [18]. After an initial denaturation step at 95 °C for 3 min, 40 cycles at 95 °C for 15 s were carried out, followed by 30 s at 60 °C and 30 s at 72 °C to amplify each transcript. The reaction was performed in duplicate, and the melting-curve was analyzed to determine the product's identity. The target mRNA concentration was normalized to the amplification of the constitutional gene GAPDH, which was the housekeeping gene [18]. The calculation of the relative expression was performed as described by Pfaffl [41]. All of the primers were designed using Primer Express Software, version 3 (Life Technologies, Carlsbad, CA, USA), and the primers were synthesized by Invitrogen (Waltham, Massachusetts, USA). The primers used in the experiments were as follows: prorenin (F-GGGTGCCGTCCACCAA and R-TCCGTCCCATTCTCCACATAG), (P)RR (F-TGATGGTGAAAGGAGTGGACAA and R-TTTGCCACGCTGTCAAGACT) [18], and GAPDH (F-GATTGTCAGCAATGCCTCCT and R-GGTCATAAGTCCCTCCACGA) [18].

2.5. Preparation of the follicular hemisections

The follicular hemisections were obtained from transparent follicles that ranged in diameter from 2 to 5 mm. These follicles were isolated from the ovaries and halved as described by Richard and Sirard [43], washed in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered tissue culture medium (TCM)-199 (Gibco Labs, Waltham, MA, USA), and incubated for 2 h before the COCs were added. Eight follicular