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Molecular characterization of a disintegrin and metalloprotease-17 (ADAM17) in granulosa cells of bovine preovulatory follicles

Khampoun Sayasith *, Jean Sirois

Centre de Recherche en Reproduction Animale, Département de Biomédecine Vétérinaire, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec J2S 7C6, Canada

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ABSTRACT

A disintegrin and metalloprotease-17 (ADAM17) is thought to play a key role in the release of soluble and active epiregulin (*EREG*) and amphiregulin (*AREG*) in ovarian follicles but its transcriptional regulation in follicular cells remains largely unknown. The objectives of this study were to characterize the regulation of *ADAM17* transcripts in bovine follicles prior to ovulation and to investigate its transcriptional control in bovine granulosa cells. To study the regulation of *ADAM17* transcripts, RT-PCR analyses were performed using total RNA extracted from bovine follicles collected between 0 h and 24 h post-hCG. Results showed that levels of *ADAM17* mRNA were low prior to hCG (0 h), markedly and transiently increased 6–12 h post-hCG ($P < 0.05$), and returned to low baseline levels at 24 h post-hCG in granulosa and theca interna cells of preovulatory follicles. To determine the transcriptional control of *ADAM17* expression, primary cultures of bovine granulosa cells were used. Forskolin (FSK) stimulation induced a pattern of *ADAM17* mRNA up-regulation in vitro similar to that observed by hCG in vivo. 5′-Deletion mutagenesis studies identified a minimal region of the bovine *ADAM17* promoter containing basal and FSK-inducible activities, which were dependent on the presence of a consensus AP1 *cis*-element. Electrophoretic mobility shift assays revealed an interaction between AP1 and the *trans*-acting factor Fra2. Chromatin immunoprecipitation assays confirmed an endogenous interaction between Fra2 and the *ADAM17* promoter in granulosa cell cultures. FSK-inducible *ADAM17* promoter activity and mRNA expression were suppressed by PKA and ERK1/2 inhibitors but not by a p38MAPK inhibitor, pointing to the importance of PKA and ERK1/2 signaling pathways in the up-regulation of bovine *ADAM17* mRNA. Collectively, these findings describe the gonadotropin/FSK-dependent up-regulation of *ADAM17* transcripts in bovine preovulatory follicles and unravel for the first time some of the molecular mechanisms involved in *ADAM17* gene expression in granulosa cells of a monoovulatory species.

1. Introduction

A disintegrin and metalloproteinase-17 (ADAM17), also called TNF-alpha converting enzyme (TACE), belongs to the family of ADAM proteins (Mizui et al., 1999), which are Zn-dependent transmembrane metalloproteases known to be expressed in several tissues, including testis and ovary (Hernandez-Gonzalez et al., 2006; Mizui et al., 1999; Sayasith et al., 2013a; Yamashita et al., 2007). ADAM17 is composed of multiple domains, including a pro-domain, a catalytic domain, a disintegrin domain, a membrane proximal domain, a transmembrane domain and a cytoplasmic domain. Its biological role involves the release of cell adhesion proteins, cytokines, epidermal growth factor receptors (EGFR) and EGFR ligands from cellular membranes, affecting downstream signaling and cellular responses (Menghini et al., 2013; Rose-Jonh, 2013; Scheller et al., 2011). Previous studies have shown that mice deficient in *Adam17* gene died soon after birth (Jackson et al., 2003), precluding analyses of fertility. However, targeted *Adam17* gene disruption in mice provided a protection from septic shock, and caused a profound inhibition of EGFR activation (Chalaris et al., 2010; Horiuchi et al., 2009; Mohler et al., 1994). EGFR activation, through its binding with specific ligand, is known play a key role in the progression of cancers and inflammatory reactions (Rose-Jonh, 2013; Scheller et al., 2011).

Ovulation is a physiological process that shares several classic landmarks of an acute inflammatory reaction, including hyperemia,

Abbreviations: LH, luteinizing hormone; hCG, human chorionic gonadotropin; COC, cumulus-oocyte complex; PTGS2, prostaglandin synthase 2; TNFAIP6, tumor necrosis factor-alpha-induced protein 6; EGFR, epidermal growth factor receptor; EREG, epiregulin; AREG, amphiregulin; ADAM17, a disintegrin and metalloprotease-17; TACE, TNF-alpha converting enzyme; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PKA, protein kinase A; PKI, PKA inhibitor peptide; ERK1/2, extracellular signal-regulated kinases 1 and 2.

* Corresponding author. Centre de Recherche en Reproduction Animale and the Département de Biomédecine Vétérinaire, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec J2S 7C6, Canada. Tel.: +450 773 8521 Ext. 8428; fax: +450 778 8103.
E-mail address: k.sayasith@umontreal.ca (K. Sayasith).

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edema, leukocytic infiltration, and induction of proteolytic and collagenolytic activities (Espey, 1980; Richards et al., 2002). Its induction triggered by the luteinizing hormone (LH) surge activates a number of signaling pathways, leading to the induction of ovulatory genes, including prostaglandin endoperoxide synthase 2 (*PTGS2*) (Davis et al., 1999; Matsumoto et al., 2001; Sirois, 1994; Sirois and Doré, 1997), prostaglandin E2 receptor (*PTGER2*) (Espey and Richards, 2002; Hizaki et al., 1999; Sayasith et al., 2009), tumor necrosis factor alpha-induced protein 6 (*TNFAIP6*) (Fulop et al., 2003; Ochsner et al., 2003; Sayasith et al., 2008), a disintegrin and metalloproteinase with thrombospondin motif 1 (*ADAMTS1*) (Brown et al., 2010; Sayasith et al., 2013a; Shindo et al., 2000), progesterone receptor (*PGR*) (Conneely et al., 2000; Lydon et al., 1995; Robker et al., 2000) and CCAAT/enhancer-binding protein β (*C/EBPβ*) (Fan et al., 2009). Because the expression of LH receptors is present on the surface of mural granulosa and theca cells but not that of cumulus cells and oocytes of a number of species, including rodent, cattle and mare (Eppig et al., 1997; Goudet et al., 1999; Robert et al., 2003), which do not response to direct exposure to LH (Peng et al., 1991), the molecular and biological mechanisms by which LH exerts its effect on the cumulus–oocyte complex (COC) are thought to depend on factors secreted by mural granulosa and theca cells (Eppig et al., 1997; Goudet et al., 1999; Lawrence et al., 1980; Robert et al., 2003). Indeed, amphiregulin (*AREG*) and epiregulin (*EREG*) are two major EGFR ligands that are structurally and functionally related (Harris et al., 2003). The expression of both genes is rapidly induced by LH or human chorionic gonadotropin (hCG) in mural granulosa cells of preovulatory follicles (Ashkenazi et al., 2005; Park et al., 2004; Sayasith et al., 2013b). As cumulus cells express EGFR, and its activation by binding with AREG and EREG was required for successful ovulation (Ashkenazi et al., 2005; Orly et al., 1994; Panigone et al., 2008; Park et al., 2004), LH-induced AREG and EREG in mural granulosa cells are thought to act as endogenous messengers to diffuse signals throughout the follicles prior to ovulation (Conti et al., 2006; Panigone et al., 2008). In addition, cultures of intact COCs with AREG and EREG showed the induction of cumulus expansion and oocyte meiotic resumption (Panigone et al., 2008; Park et al., 2004).

AREG and EREG are synthesized as transmembrane precursors containing a single bioactive EGF-like sequence in the ectodomain, and the proteolytic cleavage of the ectodomain plays a key role in shedding soluble and active AREG and EREG (Lee et al., 2003; Massagué and Pandiella, 1993). Previous studies have revealed that ADAM17 was involved in shedding bioactive AREG and EREG in rodent embryonic cells (Sahin et al., 2004). In the ovary, *ADAM17* transcripts were shown to be expressed in preovulatory follicles of the mouse and the cow (Hernandez-Gonzalez et al., 2006; Li et al., 2009), whereas studies using follicular cell cultures have provided evidence for the expression of *ADAM17* mRNA in bovine granulosa cells after the co-treatment with LH/angiotensin II (Portela et al., 2011), and in bovine and porcine COCs after the stimulation with BMP15 (Caixeta et al., 2013) and FSH/LH, respectively (Yamashita et al., 2007). However, the molecular control of *ADAM17* expression in preovulatory follicles remains largely unknown. Therefore, the objectives of the present study were to characterize the gonadotropin-dependent regulation of *ADAM17* transcripts in bovine follicles prior to ovulation, and to document some of the molecular mechanisms involved in *ADAM17* gene expression in granulosa cells of bovine preovulatory follicles.

2. Materials and methods

2.1. Materials and reagents

Lipofectamine Plus reagent, TRIzol total RNA isolation reagent, 1-kb DNA ladder, synthetic oligonucleotides, culture medium and fetal bovine serum were obtained from Invitrogen Life Technologies (Burlington, Ontario, Canada). [^{32}P]dCTP was purchased from Perkin Elmer Life Sciences (Woodbridge, Ontario, Canada). The dual-luciferase reporter assay system and plasmids pGEM-T Easy, pGL3-basic and pRL.SV40 were obtained from Promega Corp. (Madison, Wisconsin). Restriction enzymes were purchased from Amersham Pharmacia Biotech (Baie D'Urfé, Québec, Canada). JunB, JunD, cJun, Fra1, Fra2, FosB and cFos polyclonal antibodies and protein A/G-Sepharose beads were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, California). Expand high-fidelity PCR system was obtained from Roche Applied Science (Laval, Québec, Canada). Proteinase K, OneStep RT-PCR system, and DNeasy tissue kit were purchased from QIAGEN Inc. (Mississauga, Ontario, Canada). Forskolin (FSK) and signaling pathway inhibitors were purchased from Calbiochem (San Diego, California). QuikChange site-directed mutagenesis kit was obtained from Stratagene (La Jolla, California).

2.2. Animal tissues and RNA extraction

Holstein heifers (2–3 yr old) exhibiting normal estrous cycles were used as previously described (Sirois, 1994). Briefly, luteolysis was induced on day 7 of the estrous cycle (day 0 = day of estrus) with 25 mg PGF_{2a} (Lutalyse, Upjohn, Kalamazoo, Michigan). An ovulatory dose of hCG (3000 IU) was administered 36 h after the induction of luteolysis, and the ovary bearing the preovulatory follicle was isolated by ovariectomy (via colpotomy) from individual heifers 0–24 h post-hCG (n = 4 cows/time point). The interval of time from hCG administration to ovulation is 26–28 h in this animal model. Follicles were dissected from the ovary with a scalpel, and follicular wall preparation (theca interna with attached granulosa cells) and isolated preparations of granulosa and theca interna cells were obtained as described (Sirois, 1994). Total RNAs were extracted with TRIzol reagent, according to manufacturer's instructions using a Kinematica PT 1200C Polytron Homogenizer (Fisher Scientific, Montréal, Canada). All animal procedures were approved by the Institutional Animal Care and Use Committee of the Université de Montréal and were consistent with the Guidelines of the Canadian Council of Animal Care.

2.3. Semiquantitative RT-PCR

The semiquantitative analysis of *ADAM17* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNAs levels in follicular cells was performed using total RNA extracts (100 ng), the OneStep RT-PCR kit as directed by the supplier, and specific sense and antisense primers, as previously described (Sayasith et al., 2008). Primers used for amplifying cDNAs were designed from sequences published in GenBank (Table 1; accession XM_002691486 for bovine *ADAM17* [generating a 576-bp DNA fragment], and accession number NM_001034034 for bovine *GAPDH* [producing an 850-bp DNA fragment]). Cycling conditions were one cycle 50 °C for 30 min and 95 °C for 15 min followed by 30 (*ADAM17*) or 25 (*GAPDH*) cycles of 94 °C for 30 s, 59 °C for 1 min, and 72 °C for 2 min. The number of PCR cycles used was optimized for each gene to fall within the linear range of PCR amplification. PCR products were resolved on 2% Tris–acetate/EDTA–agarose gels containing ethidium bromide (0.5 μg/ml), and the intensity of the band was quantified by densitometry using the ImageQuant software (Amersham Pharmacia Biotech, Baie D'Urfé, Québec, Canada). PCR products were subcloned into pGEM-T Easy vector, and sequenced to confirm their identity.

2.4. Bovine ADAM17 promoter constructs

A fragment of the bovine *ADAM17* promoter was obtained by PCR amplification using specific primers (oligonucleotides 1 [sense] and 2 [antisense]; Table 1), an extract of bovine genomic DNA, and