Production of Tissue Inhibitor of Metalloproteinases-1 by Porcine Follicular and Luteal Cells

M. F. Smith, C. N. Kemper, G. W. Smith, T. L. Goetz, and V. L. Jarrell

Department of Animal Sciences, University of Missouri-Columbia 65211

ABSTRACT: The objectives of the first experiment were to characterize the pattern of protein secretion by 1) porcine follicles before and after the preovulatory gonadotropin surge and 2) corpora lutea on d 4 and 11 after estrus (d 0 = estrus). Gilts were ovariectomized 1) on d 20 after estrus and before the preovulatory gonadotropin surge (pre-estrus group; n = 3), 2) 18 to 36 h after the onset of estrus and after the preovulatory gonadotropin surge (estrus group; n = 3), 3) on d 4 after estrus (n = 3), and 4) on d 11 after estrus (n = 4). Changes in pattern of protein secretion were determined by densitometric scanning of fluorographs. In the pre-estrus group, the major proteins secreted by follicular shells (FS) and granulosa cells (GC) had relative molecular masses (Mr) of approximately 40,000, 46,000, and 55,000. In the estrus group (FS and GC), secretion of a Mr 40,000 protein was decreased (P < .05) and secretion of a Mr 30,000 protein was increased (P < .05) relative to the pre-estrus group. A predominant Mr 30,000 protein was also secreted by corpora lutea on d 4 and 11. The objective of Exp. 2 was to determine whether synthesis of the Mr 30,000 protein could be increased during the follicular phase by treatment with hCG, suggesting a role for the preovulatory gonadotropin surge in altering the pattern of protein secretion. Gilts were injected (i.m.) with saline (n = 3) or 500 IU of hCG (n = 3) on d 18 of the estrous cycle. Secretion of the Mr 40,000 protein was decreased (P < .07) and secretion of the Mr 30,000 protein was increased (P < .01) by preovulatory follicles treated with hCG compared to saline. The results of Exp. 3 indicated that the Mr 30,000 protein had immunological and biological properties similar to those of tissue inhibitor of metalloproteinases-1 (TIMP-1). In summary, luteinized granulosa cells and corpora lutea secreted TIMP-1, which may be involved in regulating proteolytic enzymes (metalloproteinases) preceding ovulation and in regulating cellular remodeling during the luteal phase.

Key Words: Follicles, Corpus Luteum, Protein, Proteinase Inhibitors, Metalloproteins, Proteinases

Introduction

Differences in the responsiveness of follicles within the same ovary to gonadotropins indicates that intra-ovarian regulators may have an important role in regulating ovarian function (Adashi and Rohan, 1992). The preovulatory gonadotropin surge affects steroidogenesis; however, the effect on gene expression and subsequent protein synthesis and secretion have not been thoroughly investigated. It is likely that ovarian proteins have a role in the endocrine, paracrine, and autocrine regulation of important ovarian processes including gonadotropin binding, granulosa cell proliferation, follicular rupture, angiogenesis, and luteinization (Tsafriri, 1988).

The experiments described in this paper are the result of our interest in identifying proteins that have an important role in cell signalling and tissue remodeling during the periovulatory period and also during the luteal phase. The specific objectives were 1) to characterize the pattern of protein secretion by porcine follicles before and after the preovulatory gonadotropin surge and by corpora lutea on d 4 and 11 after estrus (d 0 = estrus), 2) to determine whether production of a Mr 30,000 protein by preovulatory follicles is increased during the follicular phase following hCG treatment, and 3) to determine whether the Mr 30,000 protein has immunological and biological properties similar to a tissue inhibitor of metalloproteinases-1 (TIMP-1).
**Materials and Methods**

**Experiment 1**

Sexually mature gilts were observed for estrus twice daily and ovariectomized 1) on d 20 after estrus (d 0 = estrus) and before the preovulatory gonadotropin surge (pre-estrus group; n = 3), 2) 18 to 36 h after the onset of estrus and after the preovulatory gonadotropin surge (estrus group; n = 3), 3) d 4 after estrus (n = 3), and 4) d 11 after estrus (n = 4). Immediately following ovariectomy (pre-estrus and estrus groups), preovulatory follicles (8 to 12 mm in diameter; approximately three follicles per gilt) were dissected from ovarian stroma.

To determine whether follicles had been exposed to a gonadotropin surge, 1) follicular fluid was collected from each follicle for RIA of progesterone (Keisler and Keisler, 1989) and estradiol (Kessler et al., 1977), 2) the cumulus/oocyte complex removed from each follicle was examined to determine whether expansion of the cumulus cells induced by the preovulatory gonadotropin surge had occurred, and 3) a section of each follicular wall was fixed in 10% neutral buffered formalin for histological analysis (examination of the follicular wall for evidence of dissociation of granulosa cells). Fixed tissue was dehydrated through a series of increasing concentrations of ethanol, fats were extracted into xylene, and tissue was embedded in paraffin. Sections (5 μm) were rehydrated through a series of decreasing concentrations of ethanol and stained with hematoxylin and eosin.

Each follicle was divided into two follicular shells, and each shell contained granulosa and theca cells. Granulosa cells were collected from one of the shells by gentle scraping with a pair of forceps. Intact follicular shells and scraped granulosa cells were explanted into four-well plates in preparation for radiolabeling. Corpora lutea collected on d 4 or 11 were dissected free of ovarian stroma, sliced into 1-mm pieces, and washed in serum-free Dulbecco's Modified Eagles Medium (DMEM)/Hams F-12 (F-12; 1 vol/1 vol) medium.

Follicular shells, granulosa cells, and luteal explants were incubated for 6 h with .1 mCi of L-[35S]methionine/mL (specific activity > 1,000 Ci/mmol) in methionine-free Eagles Minimum Essential Medium (MEM). After radiolabeling, the medium was collected and centrifuged at low speed to pellet any suspended cells, and the supernatant was collected for subsequent analysis of secreted proteins.

Radiolabeled proteins in media were separated by one-dimensional SDS-PAGE. Incorporation of L-[35S]methionine into trichloroacetic acid-precipitable protein was determined, and an equal number of counts was applied to each lane of a 12.5% polyacrylamide gel. Labeled proteins were visualized with fluorography. The intensity of autoradiographic signals obtained from fluorographs was determined by densitometry with a BioRad (Richmond, CA) model 620 video densitometer and the accompanying one-dimensional analysis and integration software. Signal intensity is expressed as peak height in optical density (O.D.) units.

**Experiment 2**

Sexually mature gilts were observed for estrus twice daily. On d 18 of the estrous cycle, gilts were injected i.m. with either saline (n = 3) or 500 IU of hCG (n = 3) and ovariectomized 24 h later. No gilts were detected in estrus before injection. Immediately following ovariectomy, the largest follicles (4 to 11 mm; n = 2 per gilt) were dissected from ovarian stroma and partitioned into follicular shells containing granulosa and theca layers or granulosa cells as described for Exp. 1. Intact follicular shells or granulosa cells were explanted into four-well plates in preparation for radiolabeling as described for Exp. 1. Separation and visualization of radiolabeled secreted proteins was the same as described for Exp. 1. Densitometric analysis of signal intensity was conducted as described for Exp. 1.

**Experiment 3**

Ovaries from prepuberal gilts were collected at an abattoir and follicles (> 3 mm) were dissected from ovarian tissue. Granulosa cells were collected, explanted into flasks, and cultured in a 5% CO2 incubator at 38°C in DMEM/F-12 supplemented with 10% fetal bovine serum. After confluence was attained, the granulosa cells were washed three times with serum-free medium. Granulosa cells were incubated with serum-free DMEM/F-12, which was collected daily for 6 d.

Corpora lutea were collected from sexually mature gilts during the mid-luteal phase of the estrous cycle. Luteal tissue was dissected free of ovarian stroma and enzymatically dispersed with type IV collagenase according to previously published procedures (Smith et al., 1993). Upon dissociation, cells were washed with serum-free DMEM/F-12, explanted into culture flasks (106 cells/mL of medium), and cultured overnight in DMEM/F12 supplemented with 10% fetal bovine serum to allow attachment. Monolayers were washed three times in serum-free DMEM/F-12 and subsequently cultured for 6 d in serum-free DMEM/F-12. Luteal-cell conditioned medium was collected daily for 6 d.

After collection of granulosa- or luteal-cell conditioned media, the media (containing secreted proteins) were pooled and dialyzed, and the concentration of protein was determined (Smith et al., 1985). Bovine serum albumin was used as the protein standard.

**Gelatin Zymographic Analysis**. Gelatin zymographic analysis, which allows for detection of metalloproteinase inhibitor activity, was conducted according to standard procedures (DeClerk et al., 1989).
Proteins secreted by granulosa cells (25 to 200 μg/ lane) were separated with one-dimensional SDS PAGE containing 1% (wt/vol) porcine skin gelatin. Following electrophoresis, gels were incubated in 2.5% (vol/vol) Triton X-100 and then rinsed in water. The gels were incubated overnight in phorbol ester-stimulated, aminophenylmercuriacetate-activated, uterine fibroblast conditioned medium, which has metalloproteinase activity. After staining gels with Coomassie Brilliant Blue and destaining, metalloproteinase inhibitor activity was evident as dark bands of undigested gelatin. Metalloproteinase (gelatinase) activity was observed as clear bands of digested gelatin.

Northern Blot Analysis. Total cellular RNA was collected by the guanidium thiocyanate/cesium chloride method (Chirgwin et al., 1979). The RNA pools were made from luteal tissue collected on d 2, 4, 6, and 11 after estrus (n = 3 per group) and subjected to electrophoresis (7.5 μg/lane) through agarose-formaldehyde gels. The RNA was capillary-transferred to nylon membranes (Sambrook et al., 1989). An ovine TIMP-1 cDNA (Smith et al., 1994) was random prime-labeled (Sambrook et al., 1989) to a specific activity of 6 × 10^8 cpm/μg DNA with α-[32P]dCTP. Filters were prehybridized at 42°C for 16 h in 5x SSC (1x: .3 M NaCl, .03 M Na3 citrate, pH 7.0), 5x Denhardt’s (1x: .02% wt/vol ficoll, .02% wt/vol polyvinyl-pyrrolidone, .02% wt/vol BSA), .1% SDS, .05 M NaPO4, 50% formamide, 150 pg/mL of herring sperm DNA, and 50 μg/mL of yeast tRNA. Filters were hybridized for 18 h at 42°C in fresh hybridization buffer containing 1.5 × 10^6 cpm/mL of 32P-labeled ovine TIMP-1 cDNA. Filters were subsequently washed in 2x SSC/1% SDS for 30 min at 42°C, followed by .1x SSC/1% SDS at 65°C for 20 min. Filters were exposed to XAR-5 film at -80°C. The size of mRNA transcripts detected was determined based on relative migration of RNA molecular weight markers.

Western Blot Analysis. Luteal-cell secreted proteins (100 μg/lane) were reduced with β-mercaptoethanol, separated on one-dimensional SDS-PAGE, and electrophoretically transferred to nitrocellulose paper. Nonspecific binding sites were blocked by incubating nitrocellulose strips (~5 mm) in Tris-buffered saline (TBS; pH 7.5) containing 3% BSA for 1 h. Nitrocellulose strips were washed in TBS containing .05% (vol/vol) Tween-20 and incubated in normal rabbit serum (control; 1:1,000 dilution in TBS-Tween) or in rabbit anti-human TIMP-1 serum (1:3,600 dilution in 1% BSA/TBS-Tween; donated by I. M. Clarke, Cambridge, U.K, and funded by SmithKline Beecham Pharmaceuticals, Philadelphia, PA). Details of the primary antibody have been published (Clark et al., 1991). Strips were washed in TBS-Tween and incubated with goat anti-rabbit immunoglobulin conjugated to alkaline phosphatase at a 1:3,000 dilution in 1% BSA/TBS-Tween. Immunoreactive proteins were detected by incubation in alkaline phosphatase buffer (10 mM Tris, 100 mM NaCl, 5 mM MgCl2, pH 9.5/mL) containing nitroblue tetrazolium (80 μg/mL) and 5-bromo-4-chloro-indoyl phosphate (185 μg/mL).

Statistical Analysis. Differences in concentrations of estrogen and progesterone in follicular fluid between the pre-estrus and estrus groups were analyzed with Student’s t test (Steel and Torrie, 1960). Differences in signal intensity were also analyzed with Student’s t-test.

Results

Experiment 1

The first experiment was conducted to characterize the pattern of protein secretion by porcine follicles and corpora lutea at different stages of the estrous cycle. Concentrations of estrogen and progesterone in follicular fluid, cumulus cell expansion, and morphology of the follicular wall (marked dissociation of granulosa cells) verified that ovaries collected from gilts in the estrus group had been exposed to a preovulatory gonadotropin surge, whereas ovaries collected from gilts in the pre-estrus group had not been exposed to a gonadotropin surge (Table 1).

A number of proteins were secreted by follicles and corpora lutea collected at different stages of the estrous cycle. In general, the predominant secreted proteins by follicular shells (Figure 1) or granulosa cells (Figure 2) had Mr of 40,000, 46,000, and 55,000 in the pre-estrus group and Mr of 30,000, 46,000, and 70,000 in the estrus group. In general, the predominant proteins secreted by luteal tissue (Figure 3) on d

<table>
<thead>
<tr>
<th>Steroid concentration, ng/mL</th>
<th>Cumulus oocyte complex</th>
<th>Follicular wall (granulosa cells)</th>
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<tbody>
<tr>
<td>Estradiol</td>
<td>Progesterone</td>
<td></td>
</tr>
<tr>
<td>Pre-estrus</td>
<td>130 ± 21a</td>
<td>Compact</td>
</tr>
<tr>
<td>Estrus</td>
<td>22 ± 12b</td>
<td>Expanded</td>
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Table 1. Comparisons of concentrations of steroids in follicular fluid, expansion of cumulus/oocyte complexes, and dispersion of granulosa cells between follicles in the pre-estrus and estrus groups

^a,b^ Means (± SE) within a column having different superscripts differ (P < .05).
Pre-Estrus

Figure 1. Fluorograph of a one-dimensional SDS-polyacrylamide gel showing changes in patterns of protein secretion by follicular shells from the pre-estrus and estrus groups (each lane represents follicular shells from different gilts); $M_r$ 30,000 protein [large arrow]; $M_r$ 40,000 protein [small arrow].

4 and d 11 had $M_r$ of 30,000, 46,000, 70,000, and 200,000. The pattern of protein secretion by follicular shells and granulosa cells differed between the pre-estrus and estrus groups. In the estrus group, the production of the $M_r$ 40,000 protein by follicular shells and granulosa cells was decreased ($P < .05$) and production of the $M_r$ 30,000 protein was increased ($P < .05$) compared to the pre-estrus group (Figures 1 and 2; Table 2). In the estrus group, $M_r$ 30,000 protein was the major secretory product and was also produced by luteal tissue on d 4 and 11 after estrus (Figure 3). Signal intensity (O.D. units) of the $M_r$ 30,000 protein produced by luteal tissue was greater ($P = .05$) on d 4 ($\bar{x} \pm SE$; 1.70 ± .16) than on d 11 (.45 ± .16).

Experiment 2

In general, changes in the pattern of protein secretion by follicular shells and granulosa cells were

Table 2. Least squares means for signal intensity ($M_r$ 30,000 and 40,000 proteins) determined by densitometry of autoradiographs

| Item     | Exp. 1 |           |           |           |           |           |           |           |
|----------|--------|-----------|-----------|-----------|-----------|-----------|-----------|
|          | Follicular shells | Granulosa cells | Follicular shells | Granulosa cells | Follicular shells | Granulosa cells | Follicular shells | Granulosa cells |
|          | Pre-estrus | Estrus   | Pre-estrus | Estrus   | Pre-estrus | Estrus   | Pre-estrus | Estrus   |
| $M_r$ 30,000 | .58c   | .29d     | .06c   | .17d   | .20c   | .112d   | .23c     | 1.56d     |
| SEb      |      .21 |      .21 |       .15 |       .15 |       .20 |       .20 |       .24 |       .24 |
| $M_r$ 40,000 | 1.51c | .43d     | .89c   | .23d   | .94c   | .26d   | 1.17e     | .25f     |
| SEb      |      .21 |      .21 |       .15 |       .15 |       .20 |       .20 |       .24 |       .24 |

- Signal intensity is expressed as mean peak height in optical density units.
- SE = standard error.
- Least squares means in a row, within experiment (1 or 2) and tissue (follicular shells or granulosa cells), with a different superscript differ ($P < .05$).
- Least squares means in a row, within experiment (1 or 2) and tissue (follicular shells or granulosa cells), with a different superscript differ ($P < .05$).
Figure 2. Fluorograph of a one-dimensional SDS-polyacrylamide gel showing changes in patterns of protein secretion by granulosa cells from the pre-estrus and estrus groups (each lane represents granulosa cells from different follicles; two follicles per gilt); $M_r$ 30,000 protein [large arrow]; $M_r$ 40,000 protein [small arrow].

Similar to that observed in Exp. 1. Follicles from gilts injected with hCG had decreased ($P < .07$) production of the $M_r$ 40,000 protein by follicular shells and granulosa cells and increased ($P < .01$) production of the $M_r$ 30,000 protein by follicular shells and granulosa cells (Figure 4; Table 2).

Experiment 3

In previous studies, we identified a $M_r$ 30,000 major secretory product of ovine granulosa and luteal cells as TIMP-1 (Smith and Moor, 1991; Smith et al., 1993). Therefore, the overall objective of this experiment was to determine whether the $M_r$ 30,000 product of porcine follicular and luteal tissue was TIMP-1.

Gelatin Zymographic Analysis

The objectives were to determine whether luteinized granulosa cells produced proteins that have metalloproteinase inhibitor activity and to determine whether these correspond to the $M_r$ 30,000 protein. Cultured granulosa cells produced a $M_r$ 30,000 protein that had metalloproteinase inhibitor activity as evidenced by dark bands of undigested gelatin (Figure 5). Granulosa cells also secreted proteins (in vitro; predominantly $M_r$ 46,000 to 100,000) that had metal-
Northern Blot Analysis

The objective was to determine whether TIMP-1 mRNA was expressed in porcine luteal tissue. Northern hybridizations demonstrated that TIMP-1 mRNA was expressed by corpora lutea throughout the luteal phase (d 2 to 11 after estrus). An ovine TIMP-1 cDNA probe hybridized specifically to a transcript approximately 900 bases in length within each of the luteal RNA samples (Figure 6). The size of the TIMP mRNA-1 detected was similar to that reported for cattle (Freudenstein et al., 1990), humans (Rapp et al., 1990), sheep (Smith et al., 1993), and pigs (Tanka et al., 1992). Specific hybridization to bovine liver RNA was not detectable (data not shown).

Western Blot Analysis

The objective was to determine whether the $M_r$ 30,000 protein produced by luteal cells would crossreact with an anti-human TIMP-1 antibody. The anti-human TIMP-1 antibody specifically recognized a protein of $M_r$ 30,000 secreted by luteal cells (Figure 7).

Discussion

The potential physiological role of ovarian proteins in the endocrine, paracrine, and/or autocrine regulation of ovarian processes has become an important area of research that may increase our understanding of mechanisms associated with folliculogenesis, ovulation, and/or luteal function. Although there has been considerable effort toward identifying proteins in porcine follicular fluid (Tsafriri, 1988), there is very little information available on the effect of the preovulatory gonadotropin surge on the pattern of follicular protein secretion or on the secretion of luteal proteins. Three general findings have emerged from our experiments. First, follicular cells produced a number of proteins, and the electrophoretic pattern of protein secretion ($M_r$ 30,000 and 40,000 proteins) was altered by injection of hCG and presumably by the preovulatory gonadotropin surge. Second, luteal tissue secretes a variety of proteins, and the pattern of protein secretion differs between the early (d 4) and mid- (d 11) luteal phase. With the exception of oxytocin and relaxin (Schams, 1987, 1989), the physiological role of luteal proteins has remained unexplored. Third, luteinized granulosa cells and luteal cells produce a $M_r$ 30,000 protein that was identified as TIMP-1.

Gonadotropins acting by transcriptional mechanisms can alter the pattern of protein secretion in sheep granulosa cells (Moor and Crosby, 1987). This alteration was characterized by a decrease in the secretion of $M_r$ 46,000 to 60,000 proteins and stimulation of secretion of a $M_r$ 30,000 protein after exposure of ovine follicles to gonadotropins. Subsequently, Smith and Moor (1991) demonstrated that the $M_r$ 30,000 protein was a major secretory product of the ovine corpus luteum, and Smith et al. (1993) identified the $M_r$ 30,000 protein as TIMP-1. The results in sheep are similar to the results of the present study in which administration of hCG or the preovulatory gonadotropin surge was followed by a decrease in the $M_r$ 40,000 protein and the appearance of a $M_r$ 30,000 protein.
Granulosa Cell Secreted Protein (μg)

Figure 5. Gelatin zymograph of proteins secreted by porcine granulosa cells (25 to 200 μg of protein/lane). Metalloproteinase inhibitor activity is visualized as a dark band of undigested gelatin at Mr 30,000 (arrow head). Areas of metalloproteinase (gelatinase) activity are visualized as clear zones of digested gelatin ranging from approximately Mr 46,000 to 100,000.

The identity of the Mr 40,000 protein remains unknown; however, the Mr 30,000 protein was identified as TIMP-1. The results of the present study demonstrate that the Mr 30,000 protein and TIMP-1 have a similar relative molecular mass, biological activity, and immunological properties. Evidence for the production of TIMP-1 by granulosa and luteal cells came from gelatin zymographic analysis and Western blot analysis. These experiments demonstrated that porcine granulosa (gelatin zymographic analysis) and luteal (Western blot analysis) cells produced proteins having metalloproteinase inhibitor activity or immunoreactivity similar to TIMP-1. The presence of metalloproteinase inhibitor activity has been reported for human follicular fluid (Curry et al., 1988) and rat granulosa cell-conditioned medium (Mann et al., 1991).

Although changes in TIMP-1 secretion or amounts of mRNA were not directly measured in the present study, it is likely that changes in secretion of the Mr 30,000 protein as determined by one-dimensional SDS-PAGE, fluorography, and densitometry reflect changes in TIMP-1 production. In sheep, the expression of TIMP-1 mRNA increased within sheep follicles following a preovulatory gonadotropin surge, and the increased expression was primarily localized to the granulosa cell layer (Smith et al., 1994). In the present study, signal intensity of the Mr 30,000 protein produced by porcine granulosa cells was increased following hCG treatment. Our results also demonstrate that TIMP-1 mRNA was expressed by porcine luteal tissue on d 2, 4, 6, and 11. Expression of TIMP-1 mRNA has been reported for murine (Nomura et al., 1989), bovine (Freudenstein et al., 1990), ovine (Smith et al., 1993, 1994), and porcine (Tanka et al., 1992) luteal tissue.

Metalloproteinases (collagenase, gelatinase, and stromelysin) are metal-dependent enzymes that degrade proteinaceous components of the extracellular matrix, including collagen, fibronectin, laminin, and proteoglycans (Alexander and Werb, 1989; Docherty and Murphy, 1990; Matrisian, 1990). The preceding extracellular matrix components may affect cellular processes such as cell motility, cell proliferation, cell differentiation, and gene expression (Alexander and Werb, 1989; McDonald, 1989; Getzenberg et al., 1990, Zetter and Brightman, 1990; Liotta et al., 1991). Extracellular matrix remodeling within reproductive tissues occurs during ovulation, angiogenesis, luteinization, luteolysis, and follicular atresia.

In the present study, porcine granulosa cells produced gelatinolytic enzymes in vitro. Although identification of the specific metalloproteinases...
secreted by follicular and luteal cells was beyond the scope of the present study, the predominant gelatinolytic activity was evident between $M_r$ 72,000 and 92,500, a range corresponding to the relative molecular mass of gelatinases (Docherty and Murphy, 1990). Concomitant increases in production of metalloproteinases and TIMP-1 has been reported for osteoblast-like cells (Otsuka et al., 1984), fibroblasts (Murphy et al., 1985), and ovarian homogenates (LeMaire, 1989).

The preovulatory gonadotropin surge initiates a series of morphological and biochemical changes resulting in follicular rupture (Murdoch and Cavender, 1987; Lipner, 1988). Proteolytic degradation of connective tissue within the follicular wall is required for stigma formation and release of the oocyte (Espey, 1980; Lipner, 1988). In rats, degradation of connective tissue within the follicular wall is thought to be due to increased ovarian collagenase (Reich et al., 1985; Curry et al., 1986), gelatinase (Curry et al., 1992), and proteoglycanase (Curry et al., 1992) activity. Evidence for an increase in collagenase activity within ovine preovulatory follicles has also been reported (Murdoch et al., 1986). Increased production of TIMP-1 following the preovulatory gonadotropin surge may provide a mechanism for controlling metalloproteinase activity and consequently collagenolysis within the preovulatory follicular wall.

Following ovulation, TIMP-1 may have an important role in tissue remodeling during luteal development. Formation of corpora lutea is accompanied by breakdown of the basement membrane (Pedersen, 1951; O'Shea et al., 1980; Reynolds et al., 1992), migration of endothelial cells during neovascularization (Bassett, 1943; Reynolds et al., 1992), infiltration of fibroblasts (Pedersen, 1951), and movement of steroidogenic cells (O'Shea et al., 1980). The coordinated secretion of metalloproteinases and their inhibitors may be important regulators of the preceding processes.

Tissue inhibitor of metalloproteinases-1 may have biological properties in addition to metalloproteinase inhibitor activity. The nucleotide sequence of a hematopoietic growth factor (erythroid potentiating activity) was shown to be identical to that of human fibroblast TIMP-1 (Docherty et al., 1985). Recently TIMP-1 has been shown to cause proliferation of both erythroid (Hayakawa et al., 1990) and nonerythroid (Hayakawa et al., 1992) cell types. Consequently, TIMP-1 may also act locally as a growth factor within follicular and/or luteal tissue.

Implications

Exposure of preovulatory follicles to gonadotropin increased the production of a $M_r$ 30,000 protein, which continued to be secreted until d 11 of the luteal phase. Based on immunological and biological properties, the $M_r$ 30,000 protein was identified as tissue inhibitor of metalloproteinases-1. This protein is known to regulate metalloproteinases and may participate in the cellular remodeling that occurs at ovulation and corpus luteum formation. In addition, tissue inhibitor of metalloproteinases-1 may also act as a paracrine or autocrine growth factor within the ovary.

Literature Cited


Polyclonal and monoclonal antibodies against human tissue inhibitor of metalloproteinases (TIMP) and the design of an enzyme-linked immunosorbent assay to measure TIMP. Matrix 11:76.


