BIOLOGY OF REPRODUCTION **70,** 1292–1298 (2004)
Published online before print 7 January 2004.
DOI 10.1095/biolreprod.103.023531

The Modulatory Role of Transforming Growth Factor β1 and Androstenedione on Follicle-Stimulating Hormone-Induced Gelatinase Secretion and Steroidogenesis in Rat Granulosa Cells[1]

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ABSTRACT

To investigate the potential roles of matrix metalloproteinases (MMPs) in ovarian granulosa cell differentiation, we studied the interactive effects of FSH and local ovarian factors, transforming growth factor β1 (TGFβ1) and androstenedione, on gelatinase secretion and progesterone production in rat ovarian granulosa cells. Granulosa cells of eCG-primed immature rats were treated once with various doses of FSH and TGFβ1 and androstenedione alone or in combinations for 2 days. Conditioned media were analyzed for gelatinase activity using gelatin-zymography/densitometry and progesterone levels using enzyme immunoassay. Cell lysates were analyzed for steroidogenic acute regulatory (StAR) and cholesterol side-chain-cleavage (P450scc) enzyme protein levels. This study demonstrates for the first time that FSH dose-dependently increased the secretion of a major 63-kDa gelatinase and minor 92- and 67-kDa gelatinases. TGFβ1 also dose-dependently increased the secretion of 63-kDa gelatinase, while androstenedione alone had no effect. The 92-kDa gelatinase was identified as the pro-MMP9 that could be cleaved by aminophenylmercuric acetate into the 83-kDa active form. Importantly, we show that TGFβ1 and androgen act in an additive manner to enhance FSH stimulatory effects both on the secretion of gelatinases and the production of progesterone. We further show by immunoblotting that the enhancing effect of TGFβ1 and androstenedione on FSH-stimulated steroidogenesis is partly mediated through the increased level of StAR protein and/or P450scc enzyme. In conclusion, this study indicates that, during antral follicle development, TGFβ1 and androgen act to enhance FSH promotion of granulosa cell differentiation and that the process may involve the interplay of modulating cell-to-matrix/cell-to-cell interaction and steroidogenic activity.

cytokines, follicle-stimulating hormone, granulosa cells, ovary, progesterone

INTRODUCTION

The development of ovarian follicles that culminates at ovulation could be regarded as a two-phase process, the gonadotropin-independent early stages from primordial to preantral follicles, and the following gonadotropin-dependent stages of rapid growth from preantral to mature antral follicles [1–4]. Apart from external hormonal regulation, ovarian cells function in response to changing local environmental factors such as extracellular matrix (ECM) components [5–8] and cytokines [3, 9, 10]. Remodeling of the ECM is clearly observed during the normal ovarian cycle. The ECM has a profound effect on cellular functions and probably plays an important role in the processes of follicular development and atresia, ovulation, as well as development and regression of the corpus luteum [7, 11]. It is well established that matrix metalloproteinases (MMPs) play important roles in modulating ECM remodeling by degrading a variety of ECM components including collagens, glycoproteins (laminin, fibronectin) and proteoglycans [12, 13]. At present, 20 members of the MMP family have been identified and classified into four subclasses based on their substrate specificity and structural similarity: interstitial collagenases, gelatinases (MMP2 and MMP9, both are type IV collagenases), stromelysins and membrane type-MMPs (MT-MMPs) [13]. MMPs have been detected at all stages of follicular development. MMP2, MMP9, stromelysin-3 (MMP11), and MT1-MMP (MMP14) are expressed in most follicle types, and these MMPs are under gonadotropin regulation in vivo [14–16]. The increased MMP activity appears to be required for ovulation [15–21]. In addition, MMP2 and MMP9 are expressed in luteal cells and during luteolysis [22, 23], while collagenase 3 (MMP13) is only detected during luteal regression [23]. Increasing evidence suggests that ECM components enhance luteinization, whereas loss of ECM results in luteal cell death [11, 22].

Besides ECM, local cytokine factors including transforming growth factor β (TGFβ) have been shown to play paracrine/autocrine roles in ovarian function [9, 24]. TGFβ1 is mainly expressed in the theca cells of adult rat ovary [25]. TGFβ could act alone or synergistically with FSH in stimulating DNA synthesis in rat granulosa cells [26]. In addition, TGFβ augments gonadotropin-induced

[1]This work was supported by grants from National Science Council of Taiwan NSC 87-2314-B-010-102 and NSC 89-2320-B-010-058 (to J.-J.H.), Council of Agriculture of Taiwan 90-1.4.5-F1 (to F.-C.K.), NSC 87-2311-B-001-103 (to M.-T.L.), by awards from the Medical Research and Advancement Foundation in memory of Dr. Chi-Shuen Tsou, Taiwan (to J.-J.H.), and by grant HD17481 from the NIH and funds from the Robert A. Welch Foundation (to D.M.S.).
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Received: 23 September 2003.
First decision: 22 October 2003.
Accepted: 23 December 2003.

ISSN: 0006-3363. http://www.biolreprod.org

differentiation of ovarian cells. TGFβ augments FSH-stimulated increases in progesterone production, aromatase activity, and LH receptor expression in rat granulosa cells [26–30]. TGFβ also enhances LH-induced progesterone production in rat theca-interstitial cells [31]. In addition, TGFβ may facilitate cumulus expansion in preovulatory follicles as evidenced by its ability to stimulate the secretion of hyaluronan from cumulus cells and granulosa cells [32, 33]. Together, these studies suggest that TGFβ plays important autocrine/paracrine roles in modulating ovarian cell functions, including facilitating gonadotropin-induced proliferation and differentiation of ovarian cells, and the ovulatory process. Though it is well known that TGFβ modulates ECM remodeling in a variety of tissue organs [34, 35], such effects of TGFβ in the ovary remain unclear. At present, only a single study shows that TGFβ stimulated the secreted activities of MMP2 and MMP9 from mare ovarian stromal cells [36].

To explore the potential roles that MMPs might play in ovarian granulosa cell differentiation, we studied the interactive effects of the pituitary hormone FSH and local ovarian factors, TGFβ1 and androstenedione (a major androgen derived from theca cells), on the secretion of gelatinases involved in ECM remodeling, as well as on the production of progesterone (a marker of differentiation) in rat ovarian granulosa cells. Androgen is known to enhance FSH-induced progesterone production and proliferation of granulosa cells [37, 38]. The molecular mechanism involved in the regulation of progesterone production was studied by determining the expression of the steroidogenic acute regulatory (StAR) protein and the cholesterol side-chain cleavage (P450scc) enzyme, both key players in steroidogenesis. StAR protein is responsible for the transport of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane [39, 40], where P450scc enzyme resides and catalyzes the initial step of steroidogenesis.

MATERIALS AND METHODS

Materials

Ovine FSH (oFSH-19-SIAFP, lot # AFP4117A) and eCG were kindly provided by the NHPP, NIDDK, and Dr. A. F. Parlow (Harbor-UCLA Medical Center, Torrance, CA). Human TGFβ1 and fetal bovine serum were obtained from Upstate Biotechnology Co. (Lake Placid, NY). Penicillin and streptomycin were from Atlanta Biologicals (Norcross, GA). Rabbit polyclonal antibody against human MMP9 and mouse monoclonal antibody against human MMP2 were obtained from Chemicon International Inc. (Temecula, CA). Antisera used against StAR protein was previously described [41] and P450scc enzyme [42] was kindly provided by Dr. Bon-Chu Chung (Academia Sinica, Taipei, Taiwan). Most chemicals were purchased from Sigma Chemical Co. (St. Louis, MO); sources for others are indicated individually below.

Animals

Immature Sprague Dawley-derived rats (25–27 days) were obtained from the Animal Center at National Yang-Ming University (Taipei, Taiwan). Rats were maintained under controlled temperature (20–23°C) and light conditions (14L:10D). Food (Lab Diet from PMI Feeds, Inc., St. Louis, MO) and water were available ad libitum. This study was conducted in accordance with the United States National Research Council's *Guide for the Care and Use of Laboratory Animals* and institutional guidelines.

Cell Culture and Treatment

Isolation of ovarian granulosa cells from eCG-treated immature rats was performed as previously described [21]. Granulosa cells were plated in 24-well plates (Falcon Labware, Lincoln Park, NJ) at $5–6 \times 10^5$ viable cells per well in 500 μl of DMEM/F-12 medium containing 10% fetal bovine serum, 2 μg/ml bovine insulin, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were allowed to attach and grow to confluence for 1–2 days at 37°C, 5% CO_2-95% air. Cultured cells were then washed and incubated in 500 μl of serum-free medium (DMEM/F12 containing 0.1% lactalbumin hydrolysate, 100 U/ml penicillin, and 100 μg/ml streptomycin) overnight before the beginning of treatment to avoid the interference of serum proteinase and inhibitor activities during the analysis of gelatinase activity. Cells were treated once with various doses of FSH (1–100 ng/ml) or TGFβ1 (0.1–10 ng/ml) alone, or FSH in combination with TGFβ1 (10 ng/ml) and/or androstenedione (10^{-7} M) in 500 μl of serum-free medium for 48 h. In every experiment, triplicate samples were analyzed from three independent experiments. At the end of incubation, conditioned media were collected, cleared by centrifugation, and stored at −70°C until the performance of gelatin zymography and progesterone enzyme immunoassay. Cell number was determined using the crystal violet assay as previously described [43].

Zymography Analysis

Gelatin zymography was performed as previously described [44]. In brief, medium samples were electrophoresed on 7.5% SDS-polyacrylamide gels (14 cm × 10 cm) containing 0.1% gelatin obtained from porcine skin. The volume of each medium sample analyzed was normalized based on cell number. Electrophoresis was performed in 192 mM glycine, 25 mM Tris (pH 8.0), and 0.1% SDS at 80 V/gel during the stacking period and at 120 V/gel during the separation period. At the end of electrophoresis, gels were washed in 2.5% Triton X-100 for approximately 45 min with a change of solution, and in reaction buffer (50 mM Tris-HCl, pH 8.0, containing 5 mM $CaCl_2$, 0.02% NaN_3) for 30 min. Gels were incubated in reaction buffer at 37°C for about 20 h, then stained with 0.25% Coomassie brilliant blue R-250 in 10% acetic acid-30% ethanol, and destained in the same solution without dye. Quantification of gelatinases was achieved by computerized image analysis using two-dimensional laser scanning densitometry (Molecular Dynamics, Sunnyvale, CA). To further characterize the gelatinases secreted by granulosa cells, after electrophoresis and Triton-wash, the gels were incubated in a reaction buffer containing 5 mM 1,10-phenanthroline, a general metalloproteinase inhibitor as previously described [21]. In addition, latent gelatinases were activated by incubation of the medium sample with 1.5 mM aminophenylmercuric acetate (APMA) for 60 min at 37°C before gelatin zymography analysis [45].

Immunoblot Analysis of MMP9, MMP2, StAR Protein, and P450scc

Granulosa cells were cultured in 60-mm culture dishes and incubated in the presence of 100 ng/ml FSH alone or in combination with 10 ng/ml TGFβ1 and/or 10^{-7} M androstenedione in serum-free medium for 48 h. Each treatment group in one experiment included a single culture, and this was performed for three independent experiments. Conditioned media were collected, concentrated, and desalted using a microconcentrator (mol wt cut-off, 10 kDa; Millipore Corporation, Bedford, MA), lyophilized, resuspended in Laemmli SDS sample buffer, and analyzed for the presence of MMP9 or MMP2. The cells were washed with ice-cold PBS and then extracted with lysis buffer (RIPA buffer containing a protease inhibitor cocktail and 1 mM of the phosphatase inhibitors Na_3VO_4 and NaF). Cell lysates were analyzed for the presence of 30-kDa StAR protein, P450scc enzyme with β-actin used as an internal control. Concentrated conditioned medium samples (~10 ml equivalent each) and cell lysates (30 μg protein each) were analyzed by SDS-PAGE and electroblotting as previously described [21, 41, 42]. Specific signals were detected using an enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech UK Limited, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's protocol. Relative quantitation of ECL signals on x-ray film were analyzed using two-dimensional laser scanning densitometry (Molecular Dynamics).

Enzyme Immunoassay of Steroids

Progesterone levels in conditioned media were measured using enzyme immunoassay. Progesterone standard, progesterone-horseradish peroxidase conjugate, and enzyme substrate 2,2′-azino-bis(3-ethylbenzthiazoline 6-sulfonic acid) diammonium were purchased from Sigma Chemical Co. Progesterone antibody was produced and characterized as previously described [46]. The protocol followed was that furnished in a commercial progesterone assay kit (Diagnostic Systems Laboratory, Webster, TX). The absorbance of reaction products was measured at 410 nm using an ELISA reader (Dynatech MR5000, Worthing, West Sussex, UK). The sensitivity

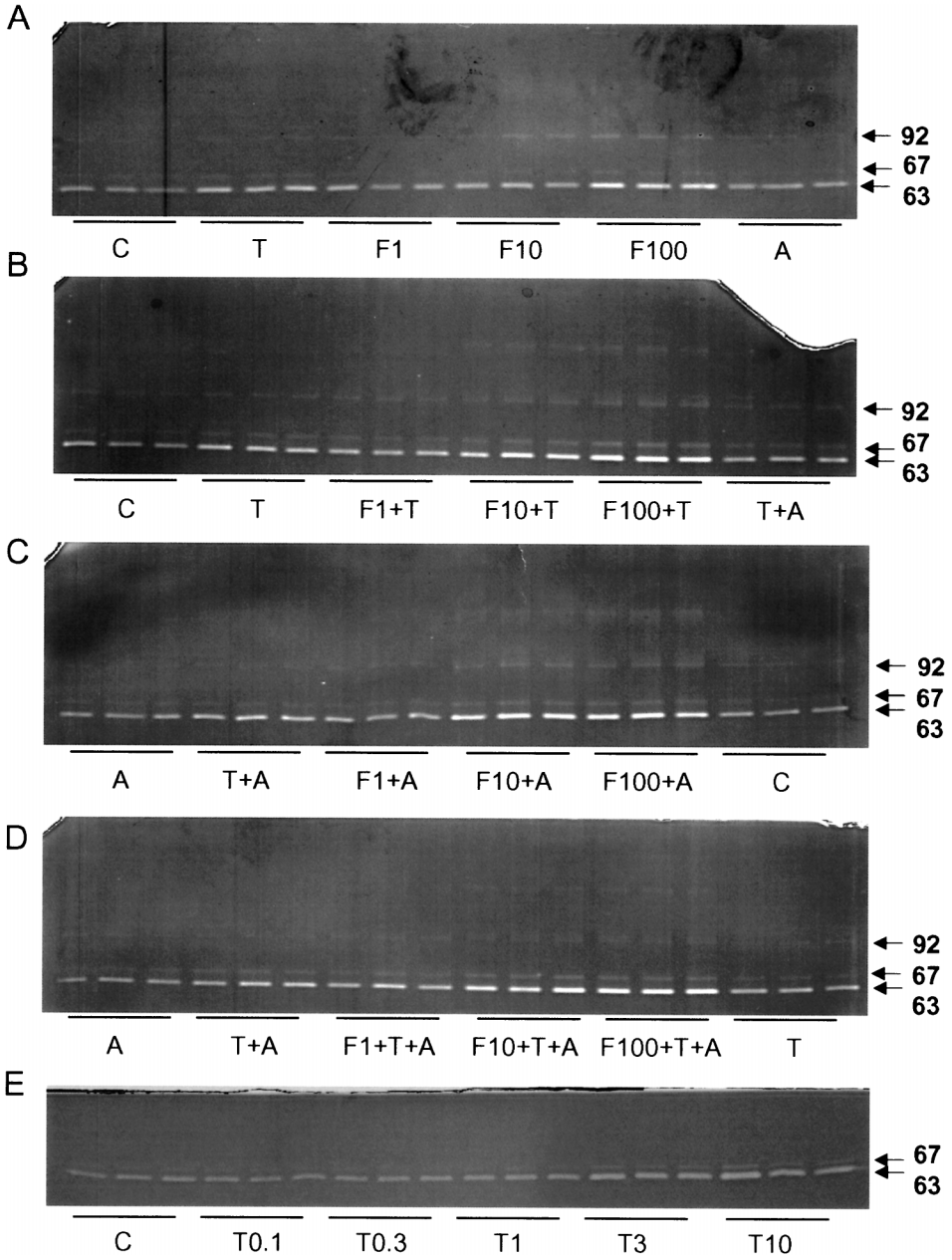

FIG. 1. Effect of FSH, TGFβ1, and androstenedione on gelatinase secretion in rat granulosa cells. Representative gelatin zymograms of conditioned media from cells treated with (**A**) various doses of FSH, (**B**) FSH plus 10 ng/ml of TGFβ1, (**C**) FSH plus 10^{-7} M androstenedione, (**D**) FSH plus TGFβ1 and androstenedione, or (**E**) various doses of TGFβ1 for 48-h culture. Each treatment group within one experiment was performed in triplicate. A, Androstenedione; C, control; F1 to F100, FSH 1 ng/ml to 100 ng/ml; T, TGFβ1.

of the assay was 5 pg per well and the intra- and interassay coefficients of variation were 4.3% (n = 3) and 8.2% (n = 3), respectively.

Statistics

Data are presented as the mean ± SEM and were analyzed by analysis of variance and Duncan multiple range test at a significance level of 0.05 using the general linear model of the SAS program (SAS Institute Inc., Cary, NC) [47].

RESULTS

ECM remodeling is believed to play an important role in ovarian follicle development, and MMPs are key enzymes involved in this event. To investigate the potential roles of MMPs in ovarian granulosa cell differentiation, we studied the interactive effects of the pituitary hormone FSH and the local ovarian factors, TGFβ1 and androstenedione (a major androgen derived from theca cells), on the secretion of gelatinases as well as on the production of progesterone (a marker of differentiation) in rat ovarian granulosa cells.

Interactive Effects of TGFβ1 and Androstenedione on FSH-Stimulated Secretion of Gelatinases from Rat Granulosa Cells

Representative gelatin zymograms are shown in Figure 1. We demonstrate that FSH alone dose-dependently (1–100 ng/ml) increased the secretion of a major 63-kDa gelatinase and minor 92- and 67-kDa gelatinases in rat granulosa cells (Figs. 1A and 2A). TGFβ1 also dose-dependently (0.1–10 ng/ml) increased the secretion of 63-kDa gelatinase (Figs. 1E and 2B); furthermore, TGFβ1 exerted an additive effect with FSH (1–100 ng/ml) on gelatinase secretion (Figs. 1B and 2A). On the other hand, androstenedione (10^{-7} M) alone exhibited no effect on gelatinase secretion, yet it augmented FSH-stimulated secretion of 63-kDa gelatinase at doses of 1 and 10 ng/ml (Figs. 1C and

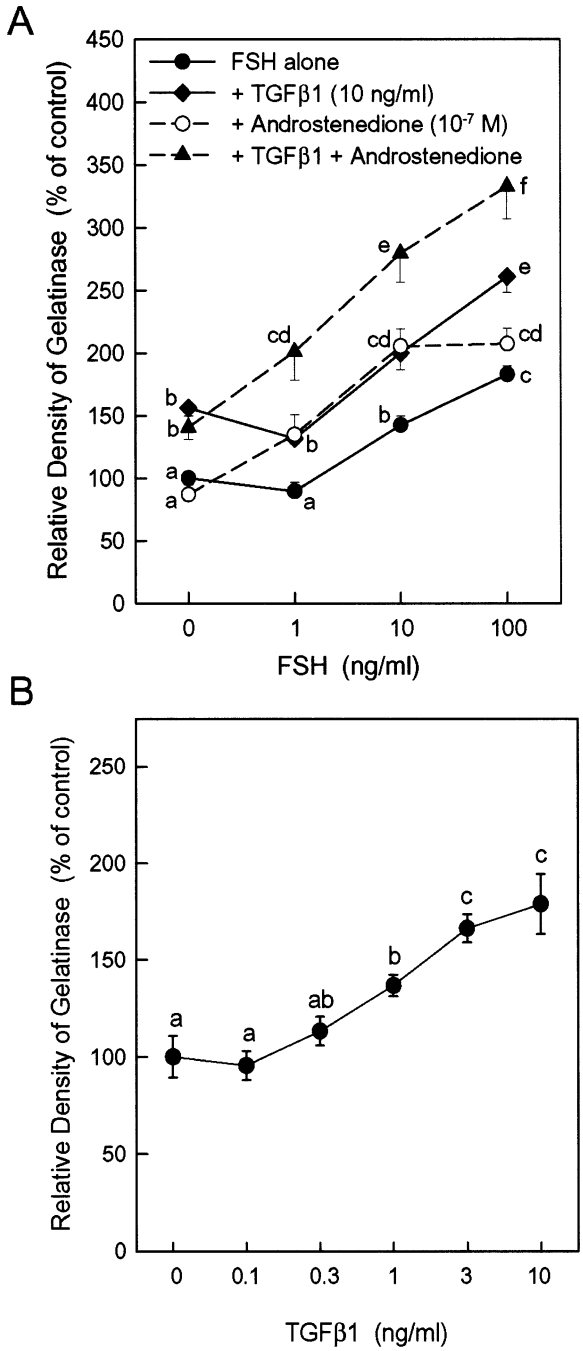

FIG. 2. Effect of TGFβ1 and androstenedione on FSH-stimulated secretion of 63-kDa gelatinase in rat granulosa cells. Cells were treated as described in Figure 1. Conditioned media were collected and analyzed for gelatinase activity using gelatin zymography and scanning densitometry. Each point represents the mean (± SEM) of mean percentage density from triplicate samples of three independent experiments. Percentage of density was calculated setting the mean density of the control group as 100%. Different lowercase letters indicate significant differences among all treatment groups ($P < 0.05$).

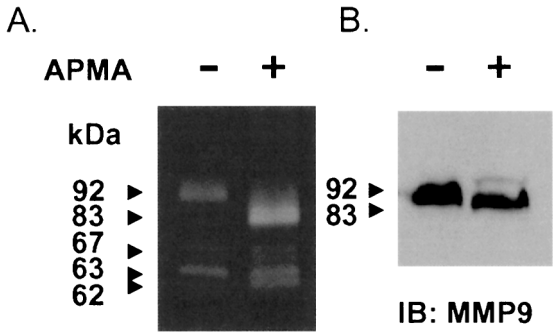

FIG. 3. Characterization of FSH-stimulated secretion of gelatinases in cultured rat granulosa cells. Conditioned media from FSH + TGFβ1, FSH + androstenedione, and FSH + TGFβ1 + androstenedione treatment groups were pooled and concentrated (using a microconcentrator with mol wt cut-off of 10 kDa), treated with or without 1.5 mM of APMA for 60 min to allow the activation of gelatinases, and then analyzed by (**A**) gelatin zymography and (**B**) immunoblotting using an antibody that recognizes both precursor and active forms of MMP9.

2A). However, the stimulatory effect of FSH at 100 ng/ml was not significantly different in the absence or presence of androstenedione (Fig. 2A). We further noticed that TGFβ1 (10 ng/ml) and androstenedione (10^{-7} M) exerted an additive modulatory effect on FSH-stimulated secretion of 63-kDa gelatinase in cultured rat granulosa cells as compared with TGFβ1 plus FSH-treated and androstenedione plus FSH-treated groups (Figs. 1D and 2A). We also observed similar changes in the secretion of 67- and 92-kDa gelatinases in response to FSH alone and FSH plus TGFβ1 (Fig. 1); however, quantification analyses of these gelatinase bands were not performed due to the weak activities in several groups that were beyond accurate determination.

These gelatinases, secreted by cultured rat granulosa cells, were characterized as metalloproteinases because their activities were completely inhibited by 5 mM 1,10-phenanthroline (data not shown) as reported in an earlier study [21]. In addition, we used aminophenylmercuric acetate (APMA) to activate latent gelatinases. When the concentrated conditioned medium of granulosa cells (approximately equivalent to 2 ml) was treated with 1.5 mM APMA, there were a decrease in the activity of 92-kDa gelatinase and increases in 83- and 62-kDa gelatinases (Fig. 3A). Due to the size of these gelatinases, we then determined whether they could be the pro- and active forms of MMP9 or MMP2 by immunoblotting using monoclonal antibodies that recognize the precursor and active forms of MMP2 or MMP9. Immunoblotting analysis of concentrated granulosa cell conditioned media (approximately equivalent to 10 ml) shows that the 92-kDa band is the pro form of MMP9, and that it could be cleaved and activated by APMA to the 83-kDa form of MMP9 (Fig. 3B). In contrast, the 67-, 63-, and 62-kDa gelatinases were not recognized by either the MMP9 or the MMP2 antibodies (data not shown).

Interactive Effects of TGFβ1 and Androstenedione on FSH-Stimulated Steroidogenesis in Rat Granulosa Cells

To address the correlation between the interactive effects of the pituitary hormone FSH and the local ovarian factors, TGFβ1 and androstenedione, on the secretion of gelatinases and the differentiation of rat granulosa cells, we examined their regulatory effects on progesterone production. In rat granulosa cells cultured for 48 h, FSH alone at doses of 10 and 100 ng/ml exerted a weak but significant stimulation on progesterone production as compared with the control (Fig. 4). In contrast with the stimulatory effect on 63-kDa gelatinase secretion (Fig. 2), TGFβ1 (0.1–10 ng/ml) alone had no effect on progesterone production (data not shown). Consistent with previous studies [26, 27], TGFβ1 (10 ng/ml) dramatically enhanced FSH-stimulated progesterone secretion in rat granulosa cells (Fig. 4). Also consistent with previous studies [37, 38], androstenedione (10^{-7} M) en-

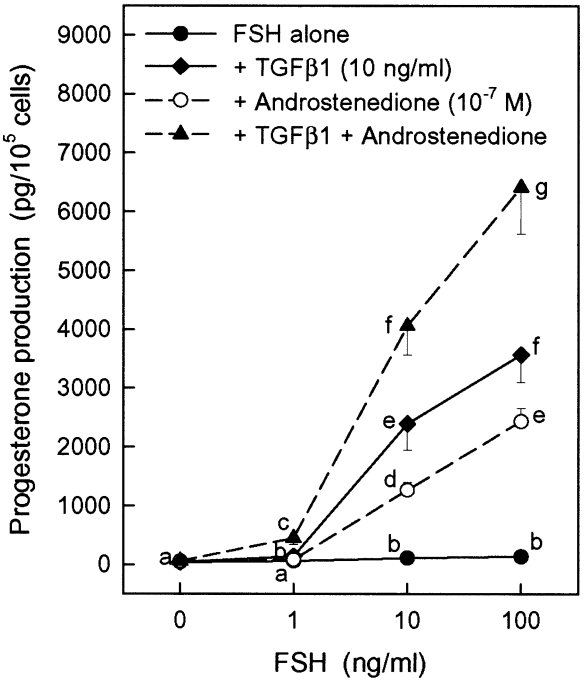

FIG. 4. Effect of TGFβ1 and androstenedione on FSH-induced progesterone production in rat granulosa cells. Cells were treated with various doses of FSH, FSH plus 10 ng/ml of TGFβ1, FSH plus 10^{-7} M of androstenedione, or FSH plus TGFβ1 and androstenedione for 48 h in culture. Progesterone levels in conditioned media were analyzed using enzyme immunoassay. Each point represents the mean (± SEM) of mean progesterone levels from triplicate samples of three independent experiments. Different lowercase letters indicate significant differences among all treatment groups ($P < 0.05$).

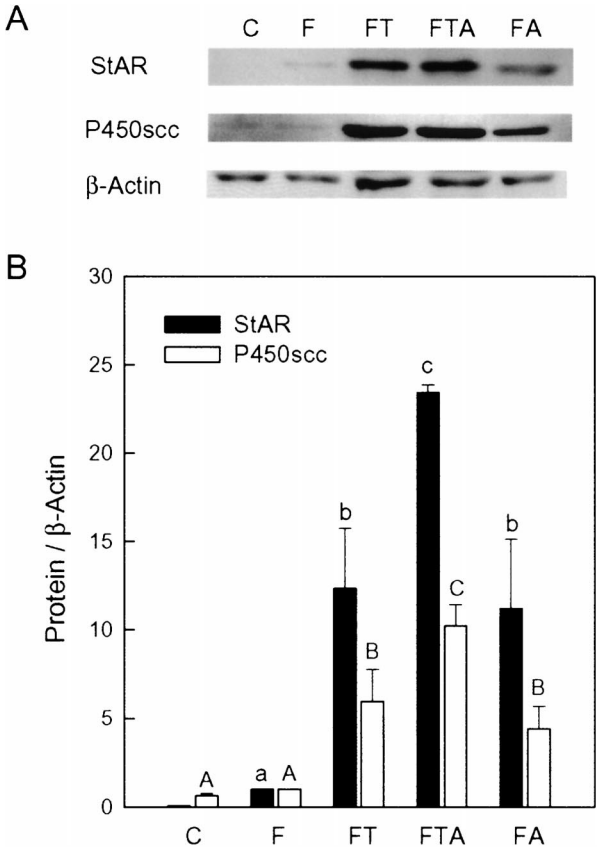

FIG. 5. Effect of FSH, TGFβ1 and androstenedione on the level of StAR protein and P450scc enzyme in rat granulosa cells. Cells were treated with vehicle control, 100 ng/ml of FSH, FSH plus 10 ng/ml of TGFβ1, FSH plus 10^{-7} M of androstenedione, or FSH plus TGFβ1 and androstenedione for a 48-h culture period. **A**) Cell lysates were analyzed by immunoblotting for StAR protein, P450scc enzyme, and β-actin used as an internal control. **B**) Quantitative analysis of StAR and P450scc in reference to β-actin was performed using two-dimensional scanning densitometry. Each point represents the mean (± SEM) relative density from single sample of three independent experiments. Relative density was calculated setting the mean density of the FSH-treated group as one. Different lowercase (or uppercase) letters indicate significant differences among all treatment groups ($P < 0.05$). A, Androstenedione; C, control; F, FSH; T, TGFβ1.

hanced FSH-stimulated progesterone secretion in rat granulosa cells, while androstenedione alone had no effect (Fig. 4). This study further demonstrates that TGFβ1 (10 ng/ml) and androstenedione (10^{-7} M) exert an additive modulatory effect on FSH-stimulated progesterone production in cultured rat granulosa cells when compared with TGFβ1 plus FSH-treated and androstenedione plus FSH-treated groups (Fig. 4).

We further examined whether the effect of TGFβ1 and androstenedione on FSH-stimulated progesterone production is mediated through modulation of the level of the StAR protein and/or the P450scc enzyme. Representative immunoblots are shown in Figure 5A. We demonstrate that treatment of FSH (100 ng/ml) in combination with TGFβ1 (10 ng/ml) and/or androstenedione (10^{-7} M) increased the level of 30-kDa StAR protein and P450scc enzyme protein, whereas FSH alone moderately stimulated the StAR protein level but had no significant effect on P450scc enzyme level (Fig. 5B). Also, the stimulatory effect of FSH plus TGFβ1 plus androstenedione is stronger than that of FSH plus TGFβ1 or FSH plus androstenedione (Fig. 5B). The progesterone levels in the conditioned media of these samples were also determined, and the results are similar to those described above (FSH + TGFβ1 + androgen > FSH + TGFβ1 > FSH + androgen > FSH > control).

DISCUSSION

It has been reported that rat ovarian theca cells express TGFβ1 and produce androgen during antral follicle development and that TGFβ1 first appears primarily in the granulosa cells of preovulatory follicles [25]. Earlier studies [37, 38] and the present study suggest that TGFβ and androgen play important autocrine/paracrine roles in modulating ovarian cell functions, most importantly in facilitating gonadotropin-induced proliferation and differentiation of ovarian cells. This study demonstrates for the first time that TGFβ1 and androgen act in an additive manner in augmenting FSH-stimulated increases in the production of progesterone and the secretion of gelatinases in rat ovarian granulosa cells. This indicates that TGFβ and androgen may act partly through the interplay of modulating cell-to-matrix and/or cell-to-cell interactions and steroidogenic activity in facilitating FSH-induced cell differentiation during antral follicle development.

The present study demonstrates that either FSH or TGFβ1 alone can stimulate the secretion of a major 63-kDa gelatinase and minor 67-kDa and 92-kDa gelatinases (MMPs) in rat granulosa cells of ovarian antral follicles and that concomitant treatment with TGFβ1 and FSH stimulated gelatinase secretion in an additive manner. This suggests that TGFβ1 and FSH may act through different signaling pathways in stimulating gelatinase secretion. FSH may signal through the cAMP pathway because cAMP alone stim-

ulated gelatinase secretion in rat granulosa cells as reported in our earlier study [44]. The 92-kDa gelatinase increased by FSH was identified as MMP9 (a type IV collagenase), suggesting that FSH could induce the remodeling of the basement membrane where granulosa cells reside. The identities of the 63- and 67-kDa gelatinases remain to be determined. Also consistent with earlier studies [26, 27], TGFβ1 greatly enhances FSH-stimulated progesterone production in rat granulosa cells cultured for 48 h, while TGFβ1 alone had no effect. We further demonstrate that TGFβ1 together with FSH may act partly by increasing the protein levels of both StAR protein and P450scc enzyme, enhancing FSH-induced progesterone production during 48 h in culture. Particularly, TGFβ1 appears to play a permissive role for FSH in increasing the P450scc enzyme level that contributes to progesterone production. Though a recent study shows that TGFβ pretreatment but not cotreatment increased the acute (2-h) effect of FSH-induced StAR mRNA level [48], it is possible that TGFβ cotreatment with FSH for culture longer than 2 h might also have the effect as evidenced in this study. The mechanism(s) whereby TGFβ augments FSH effects on ovarian cell function may involve the regulation of receptor levels and/or cross-talking of receptor signaling. The former is supported by earlier studies showing that TGFβ attenuated FSH-induced down-regulation of FSH receptors and increased the expression of FSH receptors in rat granulosa cells [29, 49]. However, TGFβ enhancement of the FSH-induced effect may not be acting through cAMP because a recent study showed that TGFβ either alone or in combination with FSH did not affect intracellular cAMP levels in rat granulosa cells [30]. TGFβ is currently known to signal predominantly through Smad proteins [50], yet it remains unclear whether TGFβ cross-talk with FSH in regulating ovarian granulosa cell functions involves Smad proteins.

During antral follicle development, FSH stimulates aromatase activity in granulosa cells, which promotes estrogen production from androgens produced by theca cells [51]. This study demonstrates that androgen enhanced the FSH-induced secretion of gelatinase in rat granulosa cells of antral follicles, while alone it had no effect. Also consistent with earlier studies [37, 38], this study shows that androgen enhanced the FSH-stimulated production of progesterone in rat granulosa cells, while alone it had no effect. These results suggest that, besides being a precursor for estrogen synthesis, androgen also plays an important role in modulating gonadotropin-induced differentiation of granulosa cells and that this process may involve the interplay of modulating cell-to-matrix and/or cell-to-cell interactions and steroidogenic activity. Furthermore, we demonstrate that TGFβ1 and androgen exert an additive effect on FSH-stimulated secretion of gelatinase and progesterone production in rat granulosa cells when compared with the TGFβ1 plus FSH- and androgen plus FSH-treated groups. And their effect on progesterone production is attributed partly by their enhancement on the levels of StAR protein and P450scc enzyme. This study supports the hypothesis that both TGFβ and androgen play important autocrine/paracrine roles in modulating ovarian cell function and that both may act through different signaling mechanisms in promoting FSH-induced granulosa cell differentiation. In addition, while TGFβ potentiates FSH- and LH-induced progesterone production in rat granulosa cells and theca-interstitial cells, respectively [26, 27, 31], TGFβ inhibits LH-induced androgen production in rat theca-interstitial cells [31]. Also, androgen was shown to inhibit FSH-stimulated expression of the LH receptor in rat granulosa cells [52]. These studies suggest that, in a physiological context, TGFβ may play an important role in coordinating FSH-induced granulosa cell differentiation and LH-induced theca cell differentiation.

In summary, this study indicates that, during antral follicle development, gonadotropin may interact with the local ovarian factors, TGFβ and androgen, in promoting granulosa cell differentiation and that the process involves the interplay of modulating cell-matrix/cell-cell interactions and steroidogenic activity.

ACKNOWLEDGMENTS

The NHPP, NIDDK, and Dr. A. F. Parlow are gratefully acknowledged for providing ovine FSH and eCG. Antisera against P450scc enzyme was kindly provided by Dr. Bon-Chu Chung (Academia Sinica, Taipei, Taiwan). We also thank Mr. Yi-Jen Hsueh for his technical assistance in graphic preparation.

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