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Steroid sulfatase activity in the rat ovary, cultured granulosa cells, and a granulosa cell line

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Abstract

Direct production of gonadal steroids from sulfated adrenal androgens may be an important alternative or complementary pathway for ovarian steroidogenesis. The conversion of sulfated adrenal androgens, present in serum at micromolar concentrations in adult women, into unconjugated androgens or estrogens requires steroid sulfatase (STS) activity. STS activity has not been characterized in the rat ovary. Substantial STS activity was present in homogenates of rat ovaries, primary cultures of rat granulosa cells, and a granulosa cell line, as determined by conversion of radiolabeled estrone sulfate (E_1S) to unconjugated estrone. The potent inhibitor estrone sulfamate eliminated the STS activity. Using E_1S as a substrate with microsomes prepared from a granulosa cell line, the K_m of STS activity was approximately 72 μM, a value in agreement with previously published data for rat STS. Therefore, ovarian cells possess STS and can remove the sulfate from adrenal androgens such as dehydroepiandrosterone sulfate (DHEA-S). Using DHEA-S as a steroidogenic substrate represents an alternative model for the production of ovarian steroids versus the "two cell, two gonadotropin" model of ovarian estrogen synthesis, whereby thecal cells produce androgens from substrate cholesterol and granulosa cells convert the androgens into estrogens. The relative contribution of STS activity to ovarian steroidogenesis remains unclear but may have important physiological and pathophysiological implications.

Keywords: Rat; Ovary; Steroid sulfatase; Steroidogenesis; Granulosa cell

1. Introduction

In the widely accepted 'two cell, two gonadotropin' model of follicular estrogen synthesis, ovarian thecal cells produce androgens and granulosa cells convert those androgens to estrogens [1]. The ovarian follicle expresses all of the enzymes necessary to produce estrogens from cholesterol, including cytochrome P450 cholesterol side-chain cleavage (CYP11A1), 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD), 17α-hydroxylase/17,20-lyase (CYP17), 17β-hydroxysteroid dehydrogenase (17β-HSD), and cytochrome P450 aromatase (CYP19). Luteinizing hormone (LH) from the anterior pituitary stimulates the thecal cells to produce androgens such as androstenedione and testosterone from cholesterol [2]. Dehydroepiandrosterone (DHEA) and androstenedione are C_{19} intermediates in this biosynthetic pathway. The androgens enter the granulosa cell compartment and are aromatized to C_{18} estrogens such as estradiol and estrone. Follicle-stimulating hormone (FSH) of anterior pituitary origin stimulates granulosa cell expression of aromatase, the enzyme responsible for aromatization of the steroidal A ring. Also localized within the granulosa cell is the microsomal enzyme 17β-HSD, which reversibly interconverts the androgens androstenedione and testosterone and the estrogens estrone and estradiol [3]. Thus, under the currently accepted model, cholesterol serves as the substrate for follicular steroidogenesis. However, it is plausible that other substrates may be available, and used, including the adrenal androgen DHEA sulfate (DHEA-S).

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Circulating DHEA-S represents a potentially significant pool of "prehormone" for an alternative or complementary pathway for ovarian steroidogenesis. DHEA-S of adrenal origin is present at micromolar concentrations in serum and is the most abundant circulating androgen in adult women [4]. Direct ovarian production of androgens or estrogens from sulfated precursor steroids was proposed over 35 years ago [5], when the "two cell, two gonadotropin" model was being established for human ovaries [6]. The conversion of sulfated steroids such as DHEA-S to unconjugated forms requires the activity of a steroid sulfatase (STS) enzyme. STS (EC 3.1.6.2; arylsulfatase C) is a microsomal enzyme that hydrolyzes sulfate esters of 3β-hydroxysteroids [7–9]. Precedents exist for the production of unconjugated, and therefore biologically active, steroids from sulfated adrenal precursors. During pregnancy, sulfated fetal adrenal androgens are converted into estrogens by the placenta [10]. Additionally, human osteoblasts remove the sulfate from estrone sulfate (E_1S), yielding estrone that can be reduced to estradiol by 17β-HSD also present in the cells [11]. Also, circulating sulfated precursors are a source of bioactive estrogens in human breast tissue, which has important clinical implications for the growth of estrogen-dependent breast cancer [12].

The ability of the ovary to utilize adrenal androgens such as DHEA-S as prehormones is dependent upon the presence of STS. Ovarian STS activity has been reported in several mammalian species, including humans [13–15], monkeys [16], hamsters [17], and rabbits [18]; however, it has not been described in the rat ovary, which is a widely employed model system of follicular development and ovarian function [19].

In this paper, we have determined the STS activity of crude ovarian homogenates from immature female rats treated with gonadotropin in vivo [20] and of primary cultures of rat granulosa cells matured in vitro with FSH and testosterone [21]. Furthermore, we have evaluated STS activity in two immortalized rat granulosa cell lines that are known to express genes characteristic of granulosa cells in vivo [22]. Moreover, we have extended previous reports of ovarian sulfatase activity by using a highly potent inhibitor of STS, EMATE [23,24], in these studies.

2. Materials and methods

2.1. Chemicals and reagents

^{3}H-E_1S (ammonium salt, [6,7-^{3}H(N)]-; 49 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Radioinert steroids were obtained from Sigma Chemical Co. (St. Louis, MO). Estrone sulfamate was synthesized as previously described [25]. Cell culture reagents and supplies were purchased from Gibco-BRL (Long Island, NY) and Sigma (St. Louis, MO).

2.2. Animals

Immature female Sprague-Dawley rats were obtained from Zivic-Miller (Zelionople, PA) at 21–23 days of age, housed in standard caging, and provided food and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee, Duquesne University and were in accordance with the NIH guidelines for the care and use of animals in research. Starting on day 22, some animals were injected twice daily with 0.1 IU human chorionic gonadotropin (hCG) (in 0.2 ml PBS; CG-B; Sigma Chemical Co., St. Louis, MO) for up to 48 h to induce follicular development. In another series of experiments, animals were injected with 1.5 mg 17β-estradiol (in 0.2 ml propylene glycol) once daily for three days.

At selected time points following initial hormonal treatments, animals were euthanized by inhalation of Halothane. Ovaries were aseptically removed to DMEM/F12 tissue culture medium (Gibco-BRL, Long Island, NY) containing 20 mM HEPES, 0.24% (w/v) sodium bicarbonate, and 1% (v/v) penicillin–streptomycin (Sigma Chemical Co., St. Louis, MO). The ovaries were quickly trimmed of surrounding adipose tissue, bursa, and connective tissue. Ovaries were either flash frozen in liquid nitrogen and then stored at −80°C, or placed in tissue culture medium for granulosa cell culture as described below.

2.3. Cell culture

Immature female rats treated with estradiol, as described above, were euthanized 24 h after the last estradiol injection and ovaries were quickly excised, and trimmed free of surrounding tissues. Primary cultures of rat granulosa cells were established as previously described [26]. Briefly, granulosa cells were physically expressed with 26 ga needles into tissue culture medium and cultured at approximately 1 million cells in a 35 mm^2 tissue culture dish. Cells were maintained at 37°C in a water saturated 5% CO_2 environment in medium containing ovine follicle stimulating hormone (oFSH-20; 5.71 ng/ml) and testosterone (10 ng/ml) for up to 48 h. STS activity was assayed after cells were cultured for approximately 30 h as described below.

Two immortalized rat granulosa cell lines, POGS5 and POGRS1 [27], were cultured in DMEM-F12 containing 5% fetal bovine serum (Sigma T-2441) until approximately 70% confluent and then used for assays of STS activity as described below. These experiments were repeated five times for each cell line.

2.4. Steroid sulfatase activity assays

Whole ovaries: Ovaries stored at −80°C were quickly weighed without thawing, placed in ice-cold 0.01 M Tris buffer (pH 7.4; 10 mg:1 ml) and homogenized for one minute on ice with a Tissue-Tearor (BioSpec Products, Bartlesville, OK). In the first experiment, ovaries were used from animals ($n = 2$) that had received 0.1 IU hCG for 48 h to induce follicular development. Aliquots (100 μl) were assayed in duplicate for STS activity. Some aliquots of the ovarian homogenate were heated for 5 min at 100°C (Boiled). Ovarian homogenate in the absence or presence of a specific STS inhibitor (EMATE; 1 μM), heat-denatured homogenate (boiled) and buffer alone (no homogenate) were incubated at 37°C with ^{3}H-E_1S (E_1S; ~240 000 dpm). After 2 h, unconjugated steroids were extracted from the samples using 3 ml of toluene. Samples were vortexed for 1 min followed by centrifugation at 1000 *g* for 3 min to separate the aqueous and organic phases. One milliliter aliquots of the organic phase were removed and placed into scintillation vials and 4 ml of scintillation cocktail (Ultima Gold, Packard, Meriden, CT) was added. Radioactivity was counted in a Packard Tri-Carb scintillation counter with 50% efficiency for ^{3}H. Total toluene-extractable dpm per ovary was calculated. In another experiment, using ^{3}H-E_1S with ovarian homogenates following periods of exposure to hCG in vivo, unlabeled cold E_1S was added to a final concentration of 1 μM. Protein concentration of the ovarian homogenates was determined by BCA assay (Pierce Chemical Co., Rockford, IL).

Primary cultures of granulosa cells in vitro: Following approximately 30 h of incubation of rat granulosa cells with the FSH and testosterone (as described above), DMEM-F12 tissue culture medium was removed from the wells by aspiration. The media was replaced with medium containing ^{3}H-E_1S (E_1S; ~300 000 dpm) and 1 μM radioinert E_1S, in the presence or absence of 1 μM estrone sulfamate, a specific inhibitor of STS [28]. In addition, wells containing media, but no cells, were included in each experiment to control for spontaneous hydrolysis of the ^{3}H-E_1S. All plates were returned to the tissue culture incubator.

After 18 h, plates were removed from the incubator, cooled to room temperature, and then duplicate 0.5 ml aliquots of media were removed to test tubes. Unconjugated steroids were extracted as described above. Results are expressed as the average pmol STS activity per well ($n = 3$ wells) and are representative of two independent experiments. The average of the dpm ($n = 3$) in the wells without cells was subtracted from the dpm of all other wells before further analysis to control for spontaneous hydrolysis.

Granulosa cell lines in vitro: The POGS5 and POGRS1 cell lines were cultured as described above and STS activity assayed from quadruplicate wells as described above. In addition, the number of cells per well were counted by hematocytometer in parallel wells following trypsinization. Data are expressed as average pmol STS activity per million cells and are the average of five independent experiments.

Granulosa cell line microsomal preparations: The rat granulosa cell line POGS5 was cultured in 100 mm^2 tissue culture dishes, removed by scraping in phosphate-buffered saline (137 mM NaCl/2.7 mM KCl/4.3 mM $Na_2HPO4{\cdot}7H_2O$/1.4 mM K_2HPO4; pH 7.3) and pelleted at 500 *g* for 5 min at 4°C. Cell pellets were resuspended in ice-cold Tris–sucrose buffer (50 mM Tris–HCl, 25 mM sucrose, pH 7.5) and homogenized with a Tissue Tearor using three 30 s bursts. The nuclear fraction was pelleted by centrifugation at 1500 × g for 15 min at 4°C. The supernatant was decanted and centrifuged at 10 000 *g* for 30 min at 4°C to pellet the mitochondrial fraction. The resulting supernatant was removed and centrifuged at 100 000 *g* at 4°C to yield the microsomal fraction. The pellet was resuspended in 50 mM Tris–HCl buffer (1:5 original wt:volume) and protein content of the microsomal suspension was determined by the BCA assay (Pierce Chemical Co., Rockford, IL). ^{3}H-E_1S was diluted in 50 mM Tris–HCl buffer and 50 μl (140 000 dpm) were added to all assay tubes. Radioinert E_1S was dissolved in ethanol and then diluted into 50 mM Tris–HCl buffer. To achieve final concentrations of E_1S, 100 μl of desired concentrations were added to the assay tubes. EMATE was dissolved in ethanol and then diluted in 50 mM Tris–HCl buffer. Tubes not having experimental compounds contained 50 μl of an equal concentration of ethanol and 50 mM Tris–HCl buffer. POGS5 microsomes were diluted with Tris–HCl buffer to 25 μg per 300 μl buffer. The microsomes in assay tubes containing steroids were preincubated for 5 min at 37°C in a water bath. The assay was initiated by addition of microsomes (300 μl) in 15 s intervals to the tubes containing tritiated steroid, radioinert steroid, and in some cases, EMATE. Some control samples with EMATE received microsomes while other control samples with no membranes did not. After 20 min of incubation at 37°C, 500 μl of 0.1 N NaOH were added to all tubes in 15 s intervals to quench the assay. Extraction of radiolabeled estrone was accomplished by adding 3 ml of toluene to each tube. The quenched samples were vortexed for 1 min and centrifuged at 1500 *g* for 5 min. Two 500 μl aliquots of the organic phase were removed from each sample with the addition of 4 ml of scintillation cocktail. All aliquots were placed in the Packard Tri-Carb scintillation counter for determination of product formation. Each sample was run in duplicate. K_m was determined by linear regression of the reciprocal of the velocity in micromole per minute versus the reciprocal of the substrate concentra-

tion (μM) using the Lineweaver-Burke calculations contained within the Prism analysis software (GraphPad Software, San Diego, CA). The assay was independently performed three times using previously published methods [29].

Statistical analysis: STS activity data were analyzed by one-way ANOVA using InStat for Macintosh (GraphPad Software, San Diego, CA), followed by a post-hoc Student–Newman–Kuel's test to determine whether significant differences ($P < 0.05$) existed between means. In some experiments, a Welch's unpaired t-test was used to determine whether significant differences exist between means ($P < 0.05$).

3. Results

3.1. Steroid sulfatase activity in ovarian homogenates

Ovarian homogenates prepared from immature female rats ($n = 2$) treated with hCG for 48 h exhibited STS activity, as indicated by the significantly higher conversion of ^{3}H-E$_1$S to unconjugated forms by the control homogenates (Fig. 1, upper panel) compared to the negative control tubes (buffer alone). In addition, boiling of the homogenates prior to assay significantly reduced the conversion to the level of buffer alone. Furthermore, the highly specific inhibitor of STS EMATE (1 μM) significantly reduced conversion of ^{3}H-E$_1$S.

In another experiment, STS activities of rat ovarian homogenates from animals after 0, 24, or 48 h hCG treatment were also assayed as described. Ovaries from all three time points exhibited substantial STS activity. There were no significant differences in STS activity between the three time points following initial hCG administration (Fig. 1, lower panel).

3.2. Steroid sulfatase activity of rat primary granulosa cells

Primary cultures of rat granulosa cells exhibited STS activity (Fig. 2), as indicated by the conversion of ^{3}H-E$_1$S to unconjugated forms. The rate of ^{3}H-E$_1$S conversion was 15.0 ± 2.51 pmol/well over an 18 h time period in vitro. EMATE (1 μM) significantly inhibited conversion of ^{3}H-E$_1$S to levels not significantly different from wells containing no cells.

3.3. Steroid sulfatase activity of rat granulosa cell lines

POGS5 rat granulosa cells exhibited STS activity (Fig. 3), as indicated by the conversion of ^{3}H-E$_1$S to unconjugated forms. The rate of ^{3}H-E$_1$S conversion was 106 ± 28.7 pmol/million cells over an 18 h time period in vitro. EMATE (1 μM) significantly inhibited conversion of ^{3}H-E$_1$S to levels similar to wells containing media alone (data not shown).

POGRS1 rat granulosa cells were cultured under identical conditions as POGS5 cells and assayed for sulfatase activity. In five independent experiments, STS

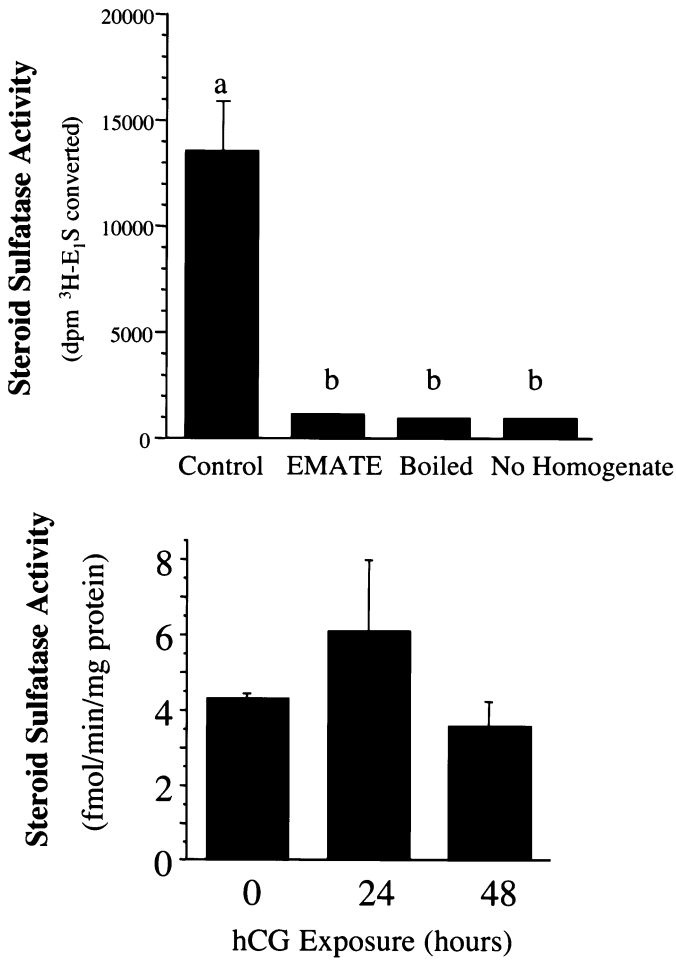

Fig. 1. *Upper panel:* STS activity of rat ovarian homogenates. Ovaries were removed from immature female rats treated with 0.1 IU hCG twice daily for two days and then homogenized in assay buffer. Homogenate was incubated with ^{3}H-E$_1$S (E$_1$-S; ~240 000 dpm) for 2 h at 37°C, either alone (Control), after heating for 5 min at 95°C (Boiled), or in the presence of EMATE (1 μM). Unconjugated ^{3}H-steroids were extracted in duplicate aliquots from the assay tubes and measured by liquid scintillation counting (Section 2). Buffer alone (No homogenate) served as a negative control for spontaneous hydrolysis of labeled steroid. Results are expressed as mean STS activity ± 1 standard deviation ($n = 2$ ovaries with quadruplicate determinations). Statistical significance was determined by one-way ANOVA ($F = 114.9$; df = 3,4; $P < 0.001$), followed by a post hoc Student–Newman–Keuls test. Significant differences between means are indicated by different letters ($P < 0.05$). *Lower panel:* Ovaries were removed from immature female rats treated with 0.1 IU hCG for either 24 or 48 h as above or were obtained at the time of the first hCG injections (0 h). Ovaries were treated as described above and were then assayed for STS activity, except in the presence of 1 μM unlabeled E$_1$S. Protein concentrations were determined and used to normalize the data. There were no significant differences in STS activity between any time points following initial hCG administration ($F = 1.222$; df = 2,3; $P > 0.01$).

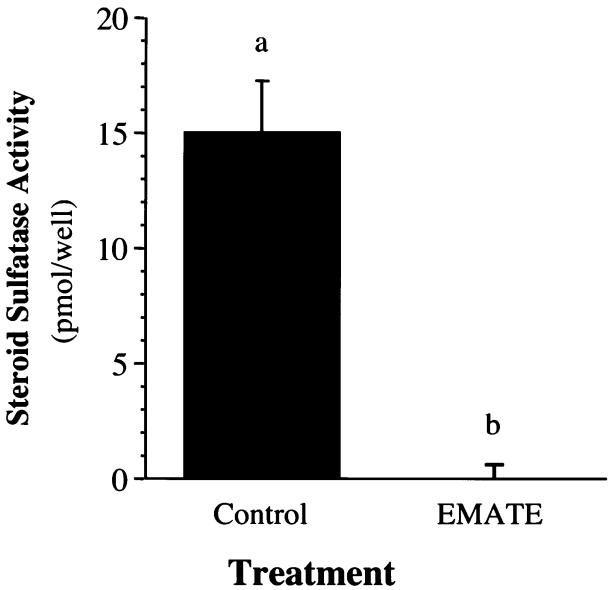

Fig. 2. STS activity of rat granulosa cells. Primary cultures of rat granulosa cells ($\sim 1 \times 10^6$) were cultured in serum-free medium as described in Section 2. After 30 h in vitro, medium was replaced with medium containing ^{3}H-E_1-S ($\sim 205\,000$ dpm), and 1 μM E_1-S in the absence (Control) or presence of EMATE (1 μM). Unconjugated ^{3}H-steroids were extracted in duplicate aliquots of the media from each well and measured by liquid scintillation counting. Results are expressed as the mean sulfatase activity ± 1 standard error of the mean ($n = 3$ wells with quadruplicate determinations per well). Statistical significance was determined by Welch's unpaired t-test ($t = 10.322$; df = 2). Significant differences between means are indicated by different letters ($P < 0.05$). These data represent one of two independent experiments.

activity was assayed by the ^{3}H-E_1S conversion assay. Conversion rates were never significantly different from media alone (data not shown). POGRS1 cells have no detectable STS activity.

The K_m of STS activity in POGS5 microsomes (Fig. 4), using ^{3}H-E_1S as substrate, was determined by the Lineweaver–Burke method to be 71.31 ± 0.66 μM (mean $\pm$ standard error of the mean) in three independent assays.

4. Discussion

The currently accepted model of ovarian steroidogenesis involves production of estrogens by granulosa cells, using precursor androgens synthesized from cholesterol by theca cells [1]. However, evidence is accumulating that this "two cell, two gonadotropin" model may not be the only mechanism to produce ovarian steroids [30]. One alternative pathway for the production of ovarian steroids is the use of the circulating DHEA-S of adrenal origin as a precursor. In order for the ovary to utilize DHEA-S as a steroidogenic precursor, it must possess the STS enzyme [9]. Thus, it is important to determine that the ovary has STS activity. In this paper, we have characterized STS activity in the rat ovary at several different organizational levels, including ovarian homogenates, cultured granulosa cells and granulosa cell lines.

We first used crude ovarian homogenates to determine if STS activity was present in rat ovaries. We used E_1S rather than DHEA-S as a substrate to measure ovarian STS activity because of our extensive experience in using this substrate for studies of STS activity in breast cancer cells [31–34]. Previous studies, using a human STS cDNA expression vector transfected into COS-1 cells, found that STS removes the sulfate group from either E_1S or DHEA-S [7]. Furthermore, in some studies, E_1S was shown to be converted at a faster rate than DHEA-S [7,35]. The STS activity observed in rat ovarian homogenates was completely eliminated by heat denaturation, supporting the involvement of a protein rather than non-specific hydrolysis. These studies were conducted in Tris buffer at a pH of 7.5, which is suitable for measurement of STS activity but not for other mammalian arylsulfatases. Other arylsulfatases are lysosomal in origin and therefore have very low activity at neutral pH and optimal activity at low pH

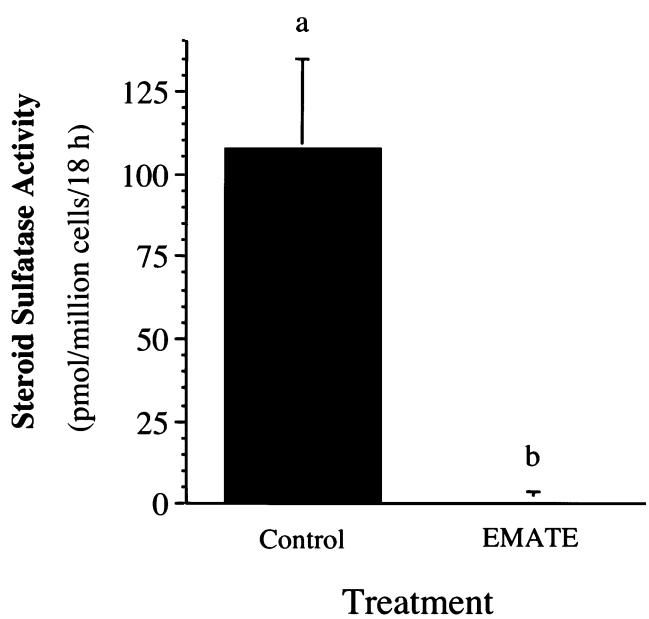

Fig. 3. STS activity of POGS5 rat granulosa cells. The rat granulosa cell line POGS5 was plated at approximately 1×10^6 cells/well in medium containing 5% fetal bovine serum. After cells were at least 70% confluent, medium was replaced with medium containing ^{3}H-E_1-S ($\sim 205\,000$ dpm), and 100 μM E_1-S in the absence (Control) or presence of EMATE (1 μM). Unconjugated ^{3}H-steroids were extracted in duplicate aliquots of the medium from each well and measured by liquid scintillation counting. Results are expressed as the mean sulfatase activity ± 1 standard error of the mean ($n = 5$ independent experiments with quadruplicate determinations of activity in 3 wells/experiment). Statistical significance was determined by Welch's unpaired t-test ($t = 8.3246$; df = 4). Significant differences between means are indicated by different letters ($P < 0.05$).

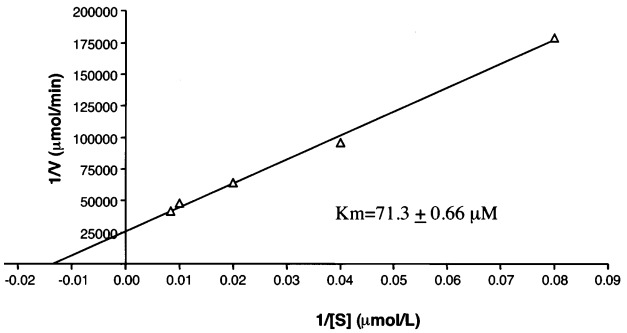

Fig. 4. Representative Lineweaver–Burke plot of STS activity in POGS5 rat granulosa cell microsomes. In three independent experiments, the reciprocal plots were linear ($r \geq 0.99$). Microsomal preparations containing 25 μg protein in Tris–HCl buffer were incubated with ^{3}H-E_1S (53 Ci/mmol) and various amounts of radioinert E_1S to achieve final concentrations of 12.5–120 μM E_1S. Following a 20 min incubation at 37°C, the assay was quenched with 1 ml 0.1 N NaOH and unconjugated ^{3}H-steroids were extracted with 3 ml toluene from replicate assay tubes. Duplicate aliquots (0.5 ml) of the organic phase from each assay tube were analyzed by liquid scintillation counting. K_m for each independent experiment was determined by using Lineweaver-Burke analysis.

[9]. Thus, it is likely that the activity reported here is that of STS (EC 3.1.6.2; arylsulfatase C) and not other arylsulfatases.

Ovarian STS may be either widespread throughout the ovary or localized to only one or a few cell types. Previous reports have characterized ovarian sulfatase activity in human follicles, stroma, and corpora lutea, but not in specific ovarian cell types [13]. To address the question of whether granulosa cells express sulfatase activity, we employed cultures of rat granulosa cells in vitro. Primary cultures of rat granulosa cells are a well-established model system to study the regulation of ovarian function [19], including the expression of steroidogenic enzymes [26] and steroid receptors [36]. The method we used was culture of granulosa cells with gonadotropin and androgens in vitro. Under these conditions, rat granulosa cells express aromatase and 17β-HSD, enzymes necessary for estradiol synthesis from androgenic precursors [1]. In our hands, estradiol production by these cultures is dependent upon the presence of FSH (to induce aromatase expression) and androgen (to serve as aromatase substrate) (unpublished data). This suggests that our cultures do not contain appreciable thecal cell contamination because they require exogenous androgen for estrogen synthesis. To our knowledge, the present study is the first to report STS activity in isolated and cultured granulosa cells of any species. The results presented here suggest that rat granulosa cells are capable of utilizing sulfate esters of 3β-hydroxysteroids as substrates for steroid biosynthesis. However, these results do not rule out the presence of STS in other ovarian cell types.

We next used the oncogene-transformed granulosa cell lines to further characterize STS activity. The POGRS1 and POGS5 cell lines are stable in passage, have responses similar to those of primary cultures of granulosa cells to activators of signal transduction systems (protein kinases A and C), and express enzymes and proteins characteristic of granulosa cells in vivo, such as follistatin and cytochrome P450 cholesterol side-chain cleavage (CYP11A) [21]. For these reasons, they represent a convenient and highly reproducible model of granulosa cell function. The POGRS1 and POGS5 granulosa cell lines were derived from cultures of preovulatory and pre-antral cells, respectively [26]. Interestingly, STS activity was not detectable in the POGRS1 cells derived from granulosa cells of pre-ovulatory follicles but was substantial in the pre-antral cells derived from the granulosa cells of an earlier stage of follicular development, the pre-antral follicle. After demonstrating substantial STS activity in the intact POGS5 cells in vitro, microsomal preparations were used for K_m determinations using E_1S as a substrate. Our results for the K_m determination of ovarian and granulosa cell STS using E_1S are similar to those previously reported for rat liver using identical methodology [28], which further supports our conclusion that granulosa cells express STS activity.

We used the highly potent inhibitor of STS activity, EMATE, to characterize sulfatase activity in experiments with either the crude homogenate or intact cells [26]. EMATE has been shown to completely inhibit STS activity in a variety of assay systems [22,30–33] and this activity is believed to be specific for STS and not other mammalian arylsulfatases. In both ovarian homogenates and intact cells, the STS activity was completely inhibited by EMATE (1 μM). The inhibition of the observed STS activity by EMATE further supports the presence of substantial STS activity in the rat ovary and granulosa cells.

Given the presence of ovarian STS, it is likely that rat follicles in vivo possess significant ability to use circulating adrenal androgens as prehormones for ovarian steroidogenesis. The presence of significant STS in the rat ovary and in various tissues of the human ovary [5,13], along with the observation that follicular fluid testosterone is in large part derived from DHEA-S of adrenal origin [37], suggests that adrenal androgens may be important substrates for ovarian steroidogenesis. Changes in ovarian STS activity could lead to altered ovarian androgen production even if circulating adrenal androgen levels are unchanged.

Ovarian STS activity permits adrenal androgens present at micromolar concentrations in human serum [38] and at nanomolar concentrations [39] in rat serum to be converted into androgens within ovarian cells. However, the physiological and pathophysiological significance of ovarian STS activity is at present unclear.

Androgens as intraovarian regulators, rather than substrates for estrogen production, have been proposed to play a role in follicular growth, atresia, and anovulation [40]. The central role of androgens in the pathophysiology of polycystic ovarian syndrome (PCOS) is well recognized, yet the pathogenesis of PCOS is as yet undetermined [41]. PCOS is defined by hyperandrogenemia of ovarian origin and chronic oligomenorrhea or amenhorrea and is frequently associated with hirsutism, chronic anovulation, supernormal numbers of small follicles (cysts), insulin resistance, and hyperinsulinemia [42]. Changes in circulating DHEA-S or DHEA levels do not appear to be necessary for development of PCOS [43]. Alterations in ovarian sulfatase activity may represent one component in the development of PCOS. Functional hyperandrogenemia associated with the pathology of PCOS could result from an increased conversion of the circulating DHEA-S into active androgens by ovarian tissue.

In conclusion, we have shown that the rat ovary, cultured granulosa cells, and a granulosa cell line all possess significant STS activity. Thus, ovarian cells possess the enzymatic machinery to remove the sulfate from adrenal androgens such as DHEA-S. Using DHEA-S rather than cholesterol as a steroidogenic substrate represents an alternative pathway to the "two cell, two gonadotropin" model for the production of ovarian steroid hormones. The relative contributions of STS activity to ovarian steroidogenesis remains unclear, but this pathway may have important physiological and pathophysiological implications.

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