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Developmental Regulation of Sertoli Cell Aromatase Activity and Plasminogen Activator Production by Hormones, Retinoids and the Testicular Paracrine Factor, PModS[1]

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ABSTRACT

Testicular peritubular cells produce a paracrine factor termed PModS that has dramatic effects on Sertoli cell function in vitro. The current study was designed to examine the actions of PModS and hormones on Sertoli cell aromatase activity and plasminogen activator production at various stages of pubertal development. Sertoli cells were isolated from 10-, 20-, and 35-day-old rats (ages correspond to prepubertal, midpubertal, and late-pubertal stages of development). Aromatase activity was found to be high and hormone-responsive in prepubertal Sertoli cells and to decline and be nonresponsive to hormones in late-pubertal Sertoli cells. FSH was the only hormone found to influence aromatase activity and estrogen production. PModS alone was not found to affect aromatase activity at any of the developmental stages examined. Interestingly, PModS was found to suppress the ability of FSH to stimulate aromatase activity and estrogen production in midpubertal Sertoli cells. Results imply that PModS may promote Sertoli cell differentiation to a more adult stage of development that is less responsive to FSH in stimulating aromatase activity. In contrast to aromatase activity, plasminogen activator production was found to increase during pubertal development. Production of Sertoli cell tissue-type plasminogen activator (tPa) was stimulated by FSH at each of the developmental stages examined, whereas production of urokinase-type plasminogen activator (uPa) was influenced by FSH only in prepubertal Sertoli cells. Insulin also stimulated uPa and tPa production by prepubertal Sertoli cells, and retinol significantly suppressed uPa production and the ability of FSH to stimulate tPa production by midpubertal Sertoli cells. Purified PModS was found to have no effect on plasminogen activator production. Results imply that PModS may not influence Sertoli cell functions that appear in part independent of cellular differentiation such as plasminogen activator production. Combined results support the proposal that PModS may act as a differentiation factor to promote and maintain Sertoli cell differentiation during pubertal development. In addition, data indicate that the Sertoli cell hormone responsiveness and the action of hormones vary as the cell differentiates during pubertal development.

INTRODUCTION

Sertoli cells play a fundamental role in the maintenance of spermatogenesis by creating the proper cytoarchitectural support and unique microenvironment for developing germinal cells. Peritubular cells surround the seminiferous tubule and interact with Sertoli cells through the complex extracellular matrix between the cells. In addition, peritubular cells and Sertoli cells interact through the production of local paracrine factors [1]. Peritubular cells produce a factor termed PModS [2] that has dramatic effects on Sertoli cell function in vitro [3, 4]. PModS has been purified and exists in two functionally related forms, PModS(A) and PModS(B) [3]. The production of PModS appears to be under the control of androgens [2, 5]. The hypothesis has developed that the gonadotropin LH stimulates Leydig cell androgen production, which acts on peritubular cells to increase the secretion of PModS, which in turn modulates Sertoli cell functions important for the process of spermatogenesis [2, 3, 6]. The purified paracrine factor PModS stimulates midpubertal Sertoli cell production of transferrin and androgen-binding protein (ABP) to a greater extent than any single regulatory agent previously identified, including FSH [3, 4, 7]. The actions of purified PModS on additional Sertoli cell functions at various stages of pubertal development has not been thoroughly investigated and is the focus of the present study.

A cellular function primarily attributed to early pubertal Sertoli cells is the aromatization of androgens to estrogen by the P450-associated enzyme aromatase [8–10]. FSH has been shown to stimulate aromatase activity in prepubertal Sertoli cells [8–11]; however, the ability of FSH to regulate aromatase activity decreases in more adult stages of development [9]. Therefore, aromatase activity is high and responsive to hormones in the early pubertal stages but decreases to low levels as the Sertoli cell differentiates to a more adult stage of development. Previously, a crude mixture of peritubular cell-secreted proteins (PSP) was shown to decrease aromatase activity in midpubertal Sertoli cells [12]. The possibility that this inhibitory activity is due to PModS is addressed in the current study.

Plasminogen activator (Pa) is a Sertoli cell product that is postulated to be important for the proteolytic activity needed for tissue remodeling of the seminiferous tubule, particularly during the translocation of spermatogenic cells across the blood-testis barrier [13, 14]. Through increasing the levels of cAMP, FSH stimulates Pa production by Sertoli

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cells [13, 15]. Although the developmental regulation of Pa production has not been thoroughly examined, Pa production appears to be somewhat independent of Sertoli cell differentiation and primarily requires elevation of cAMP. Previously a crude preparation of PSP that contained PModS was not found to influence Pa production by Sertoli cells [16]. Peritubular cells, however, have been shown to produce an inhibitor of Pa [14] that may have masked the effects of PModS in crude secreted protein preparations [16]. In the study reported here, we examined the actions of purified PModS on Pa production.

MATERIALS AND METHODS

Cell Preparation and Culture

Sertoli cells and peritubular cells were isolated from the testis of rats 10, 20, and 35 days of age. Sertoli cells were isolated by sequential enzymatic digestion [17], including trypsin, collagenase, and hyaluronidase, with a modified procedure previously described [18]. With 10-day-old rats, short, very low-speed centrifugations (20 × *g*) were required to sediment Sertoli cells between enzymatic treatments and washes. Cells were plated at approximately 5×10^5 cells per well. Peritubular cells were obtained from the collagenase digestion supernatant of the testicular cell preparation as previously described [3]. The cells were collected by low-speed centrifugation, resuspended, and plated at approximately 1×10^5 cells per (1.7 cm^2) 1-ml well of a 24-multi-well plate. Cells were maintained at 32°C in a 5% CO_2 atmosphere with medium changes every 48 h. Sertoli cells were maintained in Ham's F-12 medium supplemented with 0.01% BSA, and peritubular cells were maintained in medium with 10% newborn calf serum. In some experiments, the cells were cultured with one or more regulatory agents, including FSH (National Pituitary Agency, Baltimore, MD), 100 ng/ml; insulin, 5 μg/ml; retinol, 0.35 μM; and testosterone 1 μM. Where indicated, cells were treated with PSP at a concentration of 50 μg protein/ml, or PModS(A) or PModS(B) at a minimal concentration of 25 ng protein/ml. Treatments were initiated at the time of plating and maintained until the times stated in *Results*.

Histochemical analysis of the cultured cells from each age group was performed to establish the purity of the cell populations utilized. Germinal cell contamination was determined with the method of Berg [19], and peritubular cell contamination was determined with the immunohistochemical localization of desmin [20] and α-smooth muscle isoactin [21] as previously described. The germ cell contamination after 5 days of culture was not detectable in Sertoli cell preparations from 10-day-old rats and was 4% and 9% of the cell populations from 20- and 35-day-old rats, respectively. The peritubular cell contamination was less than 2% in the Sertoli cell cultures from 20- and 35-day-old rats and was 40% in cell preparations from 10-day-old rats.

PModS Preparation

PModS was purified from concentrated peritubular cell serum-free conditioned medium as previously described [3]. Briefly, freshly collected peritubular cell serum-free conditioned medium was concentrated 100-fold by ultrafiltration with an Amicon system (Amicon Corp., Lexington, MA) using a membrane with a 3 000 M_r exclusion limit. An ammonium sulfate precipitate of this concentrated conditioned medium was applied to a size-exclusion HPLC column. The active peak, determined by bioassay of Sertoli cell transferrin production, was collected and applied to a heparin-sepharose affinity column. Bound proteins were eluted with a high-salt buffer and applied to two successive C4 reverse-phase columns and eluted with acetonitrile. Purified forms of PModS were stored at −70°C before use, in the presence of 1 mg/ml BSA. The purity of PModS preparations was determined chromatographically and electrophoretically as previously described [3]. The mass of PModS used was determined with combined procedures including absorbance at 214 nm, biochemical protein assays, and amino acid analysis.

Aromatase Activity

Aromatase activity was assessed by the release of ^3H_2O from aromatization of [1α-^{3}H]androst-4-ene-3,17-dione or [1,2α-^{3}H]testosterone (New England Nuclear, Boston, MA) as previously described [22] with the following modification: 500 μl medium was removed from the 1-ml cell culture on the day of the assay and replaced with 500 μl medium containing 1 μCi/ml radiolabel. After a 17-h incubation at 32°C in a 5% CO_2 atmosphere, the cultures were sonicated and an aliquot was removed for DNA analysis. The remaining sample was transferred to a tube and incubated with dextran-coated charcoal (0.2% [wt/vol] 70K dextran [Sigma Chemical Co., St. Louis, MO], activated 2% [wt/vol] with Norit-A charcoal, 10 mM Tris, and 1 mM EDTA, pH 7.5) for 2 h at 4°C, and then centrifuged at 13 000 × *g* for 15 min. Aliquots of the supernatant containing ^3H_2O were then counted. Blank values were established from identical incubations in the absence of cells. Aromatase activity (^3H_2O released) was expressed as counts per minute (cpm) and was normalized per micrograms of DNA. To confirm the production of estrogen by the cells, an estrogen RIA was used with a direct assay of Sertoli cell-conditioned medium.

Pa Assay

Pa activity was measured with an indirect spectrophotometric assay [23]. A 100-μl sample of Sertoli cell-conditioned medium was incubated at 37°C in 400 μl Tris 0.10 M, pH 7.5, containing 0.13 μM plasminogen (bovine plasma, Sigma); 0.01% (v/v) Tween 80; and 0.30 mM S-2251 (D-Val L-Leu L-Lys, *p*-nitroanilide, Sigma). When required, 0.06 mg/ml fibrinogen fragment was added for the activation of the tissue-type (tPa) form of the enzyme. The absorbance change

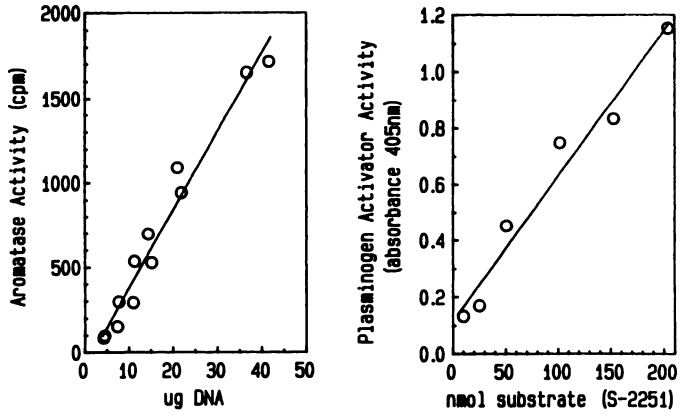

FIG. 1. Linearity of aromatase activity and Pa activity assays. Aromatase activity in Sertoli cells isolated from 20-day-old rats, with freshly isolated cells plated at increasing cell number (μg DNA/ml) in the presence of 0.5 μCi/ml ^{3}H-andro-4-ene-3,17-dione after a 17-h incubation. Pa activity was assayed with increasing concentrations of hydrolyzed substrate (nmol substrate, S-2251) as detected with an absorbance at 405 nm.

after a 6-h incubation was measured against blanks without conditioned media. The Pa-independent protease was detected by omitting plasminogen from the assay mixture. The results of the activity assay were calculated in regard to the nanomole converted substrate (S-2251) and normalized to the micrograms of DNA present in the culture well. A standard curve of the amount of reacted substrate was obtained by hydrolyzing the substrate S-2251 non-enzymatically with 10 M NaOH.

For the preparation of the fibrinogen fragment, 10 mg of fibrinogen (human plasma, Sigma) was treated with 13 mg of cyanogen bromide in 1 ml 70% formic acid overnight at room temperature. The solution was diluted 1:10 with water and dialyzed against water overnight. The fibrinogen fragment was lyophilized and stored at −20°C. The protein concentration was measured according to the method of Bradford [24].

DNA Assay

DNA was measured fluorometrically with ethidium bromide [25] as previously described [3]. An aliquot of the sonicated cell suspension was added to an equal volume of ethidium bromide solution and allowed to incubate at room temperature for 30 min. Fluorescent emission at 585 nm with 350 nM excitation was then monitored. A standard curve with calf thymus DNA was used to quantify DNA levels in the culture wells. This assay has a sensitivity of approximately 0.1 μg DNA.

RESULTS

For the validation of the activity assays, the linearity of both the aromatase and Pa assays are shown in Figure 1. The aromatase activity assay was based on the release of ^3H_2O from ^{3}H-androgen upon aromatization to estrogen [22].

TABLE 1. Pa activity (nmol converted substrate).*

Plasminogren	−	+	+
Fibrinogren	−	−	+
uPa (10^{-6} IU)	ND†	76.4	76.4
tPa (0.12 IU)	ND	ND	54.4
SSP (10 μg)	ND	246 ± 46	492 ± 46

*Standard preparations (IU) of tPa (Boehringer, Indianapolis, IN) and uPa (Sigma, St. Louis, MO) were compared to Sertoli cell-secreted proteins (SSP) in the Pa enzymatic assay in the absence or presence of plasminogen and fibrinogen fragment.
†ND denotes nondetectable levels of Pa activity.

Sertoli cells from midpubertal animals were plated at various cell densities and then incubated with ^{3}H-androgen to assess aromatase activity. The aromatase activity (release of ^3H_2O) was found to be linear to the micrograms of DNA present in the culture well (Fig. 1). The cell densities generally used were between 10 and 20 μg DNA/well and are within the linear range of the assay. The Pa assay was based on the hydrolysis of a chemically reactive peptide (S-2251) that can be detected in a colorometric assay [23]. This Pa spectrophotometric assay is linear over a broad range of substrate (S-2251) concentrations with regard to the absorbance at 405 nm generated from the hydrolyzed product (Fig. 1). The absence of plasminogen in the assay is used to determine the background non-Pa proteolytic activity, which was negligible. Fibrinogen fragments have previously been shown to specifically activate tPa activity [23]. The presence of plasminogen and fibrinogen fragment was used to specifically detect tPa. The presence of plasminogen and the absence of fibrinogen fragments was used to detect urokinase-type plasminogen activator (uPa). The use of tPa and uPa standards in the assay is shown in Table 1

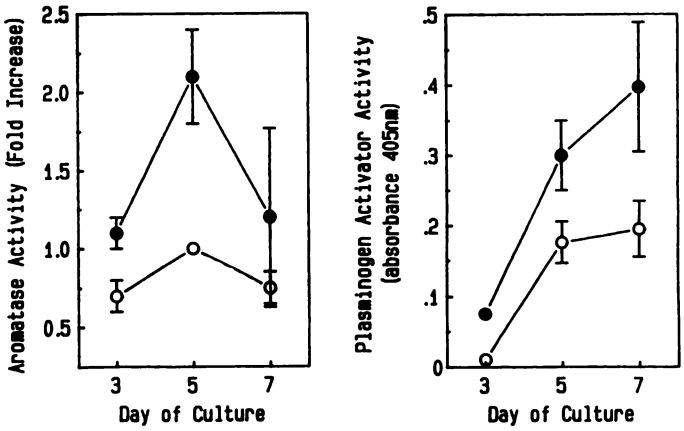

FIG. 2. Time course of aromatase activity and Pa production in Sertoli cells from 20-day-old rats with samples/cells collected on Days 3, 5, and 7 of culture. Cells were incubated in the absence (○) or presence (●) of 100 ng/ml FSH at the time of plating. The results are presented for aromatase activity as fold increase of activity (cpm/μg DNA) present in control cells on Day 5 of culture. The results are presented for Pa activity as the level of converted substrate as assessed with absorbance at 405 nm. The mean ± SEM is presented from three different experiments done in triplicate.

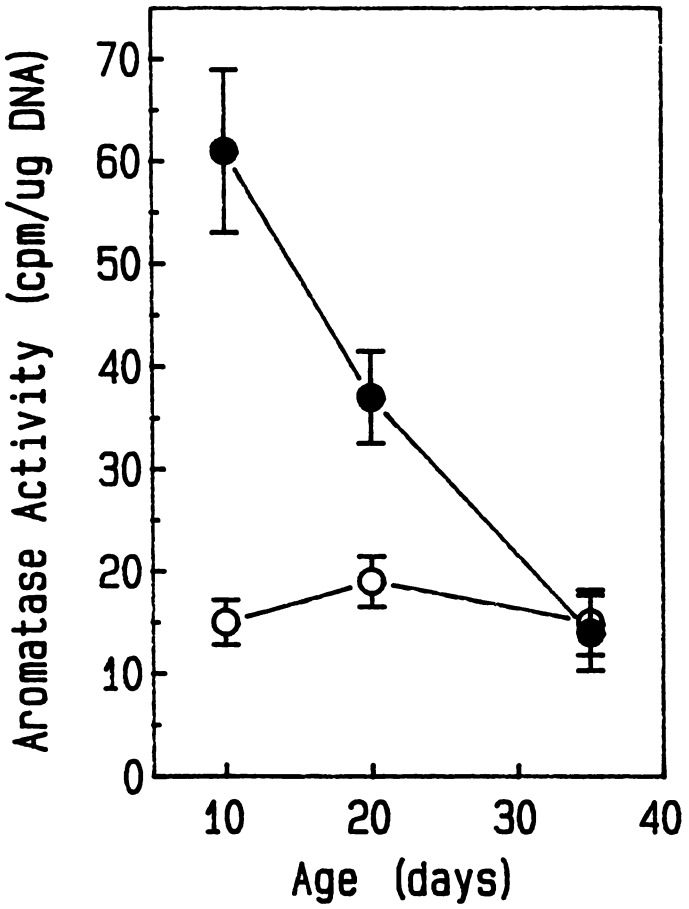

FIG. 3. Aromatase activity in Sertoli cells from various stages of pubertal development. Sertoli cells were isolated from 10-, 20-, and 35-day-old rats and cultured for 5 days in the absence (○) or presence (●) of 100 ng/ml FSH. Sertoli cells were incubated in the presence of 0.5 μCi/ml ^{3}H-andro-4-ene-3,17-dione for 17 h and subsequently were sonicated; the amount of ^3H_2O release (cpm) was detected with the charcoal assay. Results are presented as cpm/μg DNA and are the mean ± SEM from a minimum of three different experiments done in triplicate.

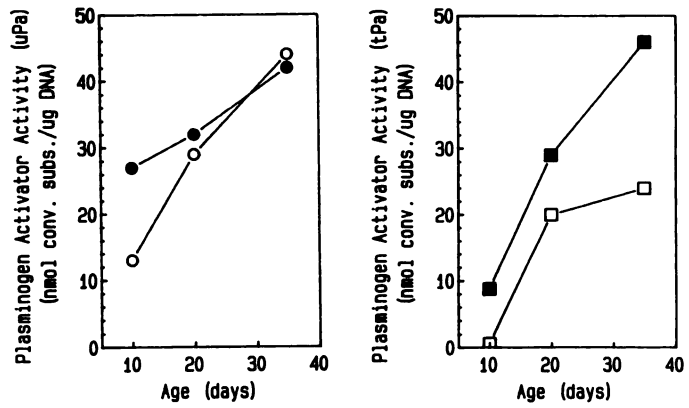

FIG. 4. Pa production by Sertoli cells from various stages of pubertal development. Sertoli cells were isolated from 10-, 20-, and 35-day-old rats, and a 72-h-medium collection was obtained on Day 5 of culture with cells cultured in the absence (○) or presence (●) of 100 ng/ml FSH. Both uPa and tPa activity was examined. Results are presented as nmol converted substrate/μg Sertoli DNA and are the mean of a representative experiment.

to demonstrate the specificity of the Pa assay. Hereafter, "Pa activity" will denote tPa and uPa activity combined.

The aromatase activity in midpubertal Sertoli cells and Pa activity present in conditioned medium at various durations of cell culture are shown in Figure 2. Cells were incubated in the absence or presence of FSH for 3, 5, and 7 days of culture. Basal levels of aromatase were found to remain relatively constant, and optimal FSH stimulation was observed on Day 5 of the culture (Fig. 2). Basal levels of Pa production were found to increase during culture, and FSH stimulation was similar at each time point examined (Fig. 2). A more thorough analysis with statistics is shown in subsequent figures. Data indicated that optimal analysis of aromatase activity was on Day 5 of culture and for Pa production on either Day 5 or Day 7 of culture. Preliminary analysis indicated that similar data were obtained with prepubertal and late-pubertal Sertoli cells. For subsequent experiments, data were obtained on Day 5 of Sertoli cell culture.

The basal and FSH-stimulated levels of aromatase and Pa production by Sertoli cells from 10-, 20-, and 35-day-old rats are shown in Figures 3 and 4. For aromatase activity, the basal levels remained relatively constant between the prepubertal, midpubertal, and late-pubertal Sertoli cells (Fig. 3). FSH-stimulated aromatase activity was highest with prepubertal Sertoli cells, reduced with midpubertal cells, and absent with late-pubertal cells. Therefore, as the Sertoli cells reached a more adult stage of differentiation, aromatase activity became nonresponsive to FSH. The Pa production was separated into uPa and tPa activities (Fig. 4). With pubertal development, uPa was found to increase slightly, and it was responsive to FSH only at the prepubertal stage of development. Basal levels of tPa production were lower than those of uPa and increased slightly with pubertal development. FSH was found to stimulate tPa production by prepubertal, midpubertal, and late-pubertal Sertoli cells. As previously shown [26], tPa is the form of Pa that is hormone responsive, whereas uPa is nonresponsive to FSH in midpubertal and late pubertal Sertoli cells. The hormone stimulation of tPa was found to be independent of the differentiated stage of Sertoli cells.

A more detailed analysis of the regulation of Pa production is shown in Figures 5 and 6. Both midpubertal and late-pubertal Sertoli cells were nonresponsive to FSH in regard to uPa production; interestingly, however, FSH significantly stimulated uPa production by prepubertal Sertoli cells (Fig. 5). As indicated above, FSH stimulated tPa production from Sertoli cells at all developmental stages examined (Fig. 6). Insulin at high concentrations (5 μg/ml/ml), probably acting at the insulin-like growth factor-I (IGF-I) receptor, stimulated both uPa and tPa production from prepubertal Sertoli cells. This was in contrast to the lack of insulin effects observed with midpubertal and late-pubertal Sertoli cells. Retinol alone had no effect on prepubertal uPa production, but it did significantly inhibit basal uPa production

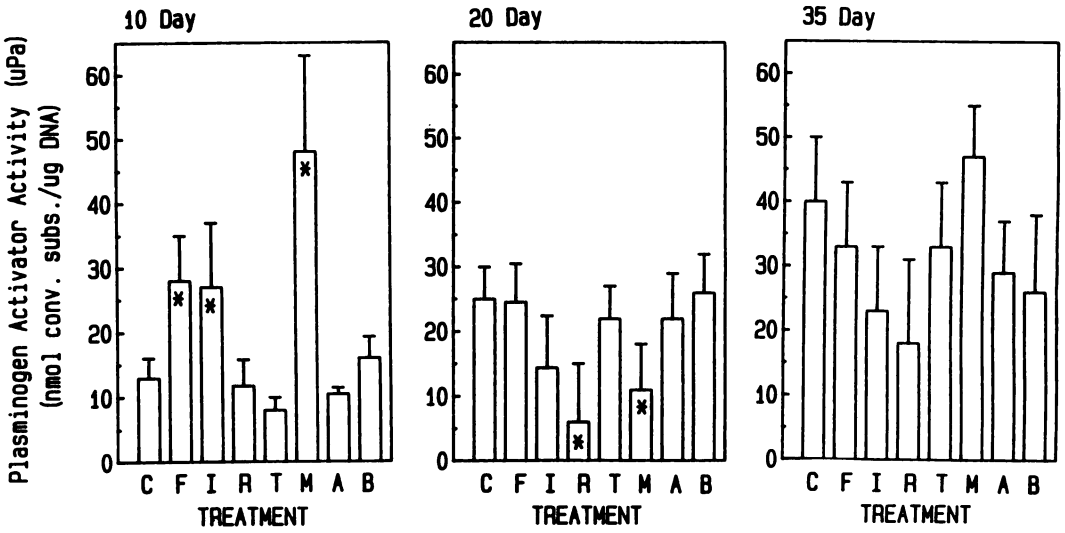

FIG. 5. Effect of regulatory agents on uPa production by Sertoli cells isolated from 10-, 20-, and 35-day-old rats. Cells were cultured in the absence (Control, C) or presence of 100 ng/ml FSH (F); 5 μg/ml insulin (I), 0.35 μM retinol (R); 1 μM testosterone (T); a mixture of FSH, insulin, retinol, and testosterone (M); and 25 ng/ml PModS(A) (A) or PModS(B) (B). Medium collected on Day 5 of culture was assessed for activity and expressed as nmol converted substrate/μg Sertoli DNA. Results are presented as the mean ± SEM from a minimum of three different experiments done in triplicate. *Statistically different from control, with $p < 0.05$ using Student's t-test.

by midpubertal Sertoli cells and tended to reduce uPa production by late-pubertal cells, although the reduction was not statistically significant (Fig. 5). Retinol alone had no effect on basal tPa production, but inhibited the ability of FSH to stimulate tPa production by midpubertal Sertoli cells (Fig. 6). Therefore, retinol inhibited basal levels of uPa production and FSH-stimulated tPa production by midpubertal Sertoli cells. The minimum and maximum effective retinol concentrations for this inhibitory effect were 100 nM and 1 μM, respectively (Fig. 7). The IC_{50} for the inhibitory effects of retinol on midpubertal Sertoli cell Pa production was approximately 250 nM. Testosterone was not found to influence Sertoli cell Pa production at any of the developmental stages examined (Figs. 5 and 6). The purified forms of PModS, PModS(A) and PModS(B), were not found to influence Pa production by prepubertal, midpubertal, or late-pubertal Sertoli cells (Figs. 5 and 6). PModS also failed to influence the actions of FSH on Sertoli cell Pa production (data not shown).

A detailed analysis of the regulation of aromatase activity is shown in Figure 8. FSH was the only regulatory agent found to stimulate prepubertal and midpubertal Sertoli cell

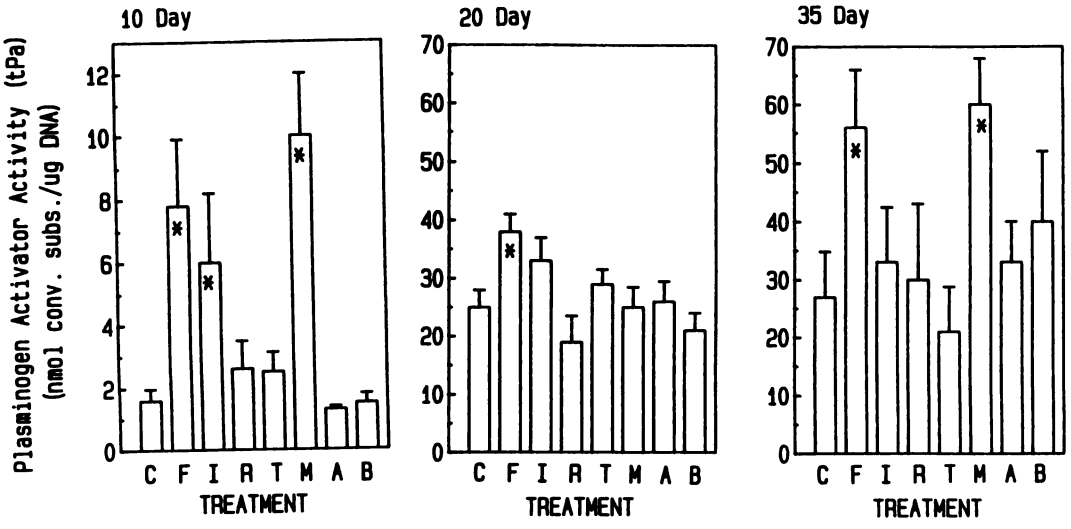

FIG. 6. Effect of regulatory agents on tPa production by Sertoli cells isolated from 10-, 20-, and 35-day-old rats. Cells were cultured and treated and the data are presented as described in the legend to Figure 5.

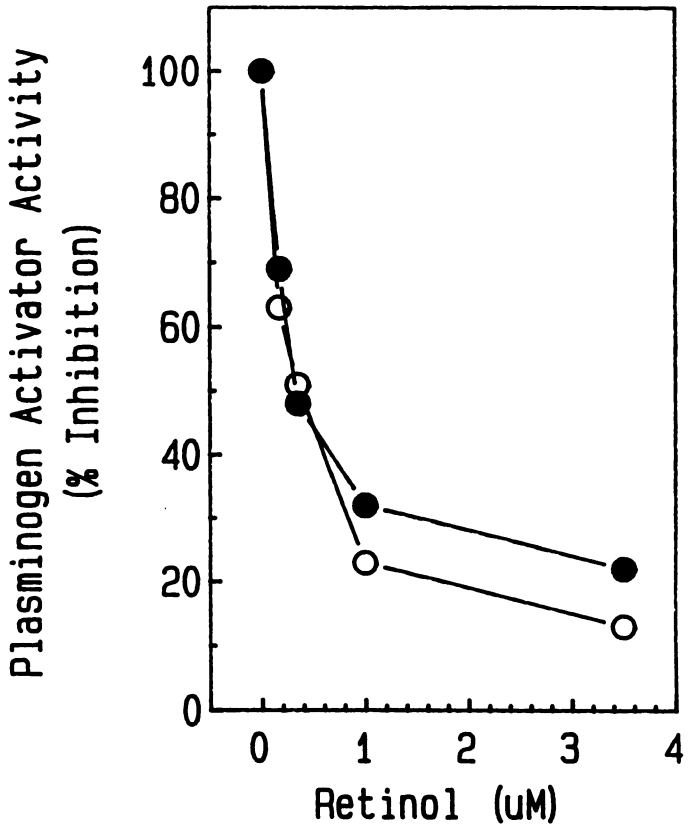

FIG. 7. Inhibitory effects of retinol on Pa production by Sertoli cells isolated from 20-day-old rats. Cells were incubated in the presence of FSH and increasing concentrations of retinol (μM), and a 72-h-medium collection was obtained on Day 5 of culture to determine Pa activity for both uPa (○) and tPa (●). Results are presented as the percentage of inhibition and are the mean from a representative experiment. All treatments were found to be statistically different ($p < 0.05$) from control non-retinol treated cells with Students *t*-test.

aromatase activity. Insulin, retinol, and testosterone had no effect on aromatase activity at any of the developmental stages examined (Fig. 8). A combination of FSH, insulin, retinol, and testosterone had similar effects as FSH alone. None of the regulatory agents including FSH had any effect on aromatase activity in late-pubertal Sertoli cells. The concentrations of regulatory agents used have previously been shown to be optimal for the regulation of a number of Sertoli cell functions [27].

Analysis of the actions of PModS on aromatase activity is also shown in Figure 8. Neither PModS(A) nor PModS(B) alone was found to affect basal levels of aromatase activity in prepubertal, midpubertal, or late-pubertal Sertoli cells. The same preparations of PModS at the same concentrations were found to optimally stimulate transferrin production by midpubertal Sertoli cells (data not shown). To extend the analysis of PModS actions on aromatase activity, cells were treated with FSH in the absence or presence of PModS (Fig. 9). Neither of the forms of PModS was found to influence the actions of FSH on prepubertal or late-pubertal Sertoli cells. Interestingly, PModS(B), but not PModS(A), inhibited the actions of FSH on midpubertal Sertoli cells (Fig. 9). Similar effects were observed with 20- and 50-ng/ml concentrations of PModS(A) and PModS(B). To confirm this inhibitory activity of PModS(B), ^{3}H-testosterone was also used as a substrate in the aromatase activity assay (Fig. 10). A similar inhibition of FSH-stimulated aromatase activity was observed with PModS(B). Estrogen accumulation was assessed in midpubertal Sertoli cell-conditioned medium from Days 2–5 of culture with the same treatment conditions (Fig. 10). PModS(B) also inhibited the ability of FSH to stimulate estrogen production by midpubertal Sertoli cells. PModS(A) had no statistically significant

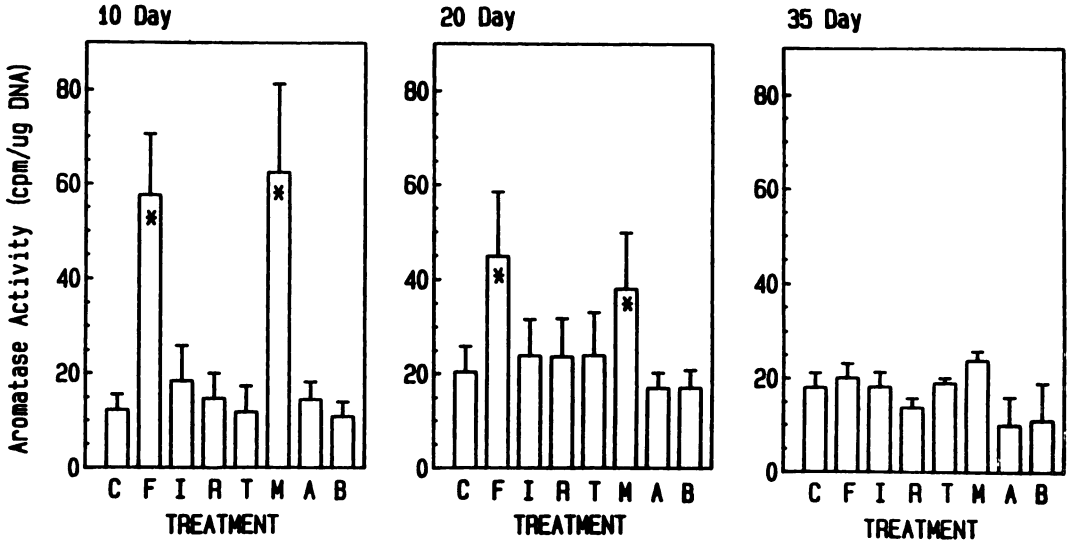

FIG. 8. Effect of regulatory agents on aromatase activity in Sertoli cells isolated from 10-, 20-, and 35-day-old rats. Aromatase activity was determined on Day 5 of culture after a 17-h incubation with ^{3}H-andro-4-ene-3,17-dione and presented as cpm/μg Sertoli DNA. Cells were cultured and treated and the data are presented as described in the legend to Figure 5.

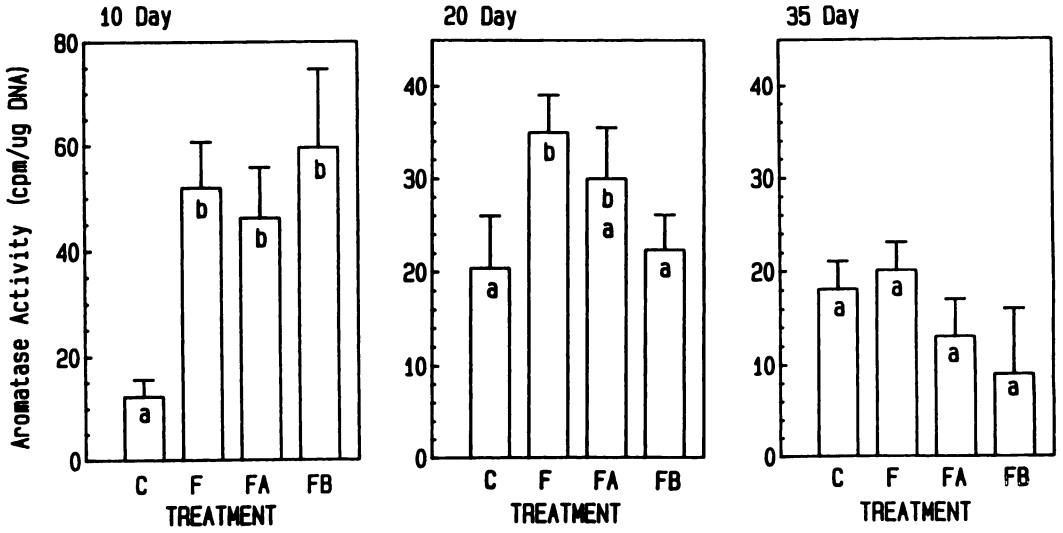

FIG. 9. Effect of PModS on FSH regulation of aromatase activity in Sertoli cells. Cells were isolated form 10-, 20-, and 35-day-old rats and cultured for 5 days in the absence (control, C) or presence of FSH (F); FSH plus PModS(A) (FA); or FSH plus PModS(B) (FB). Aromatase activity was determined after a 17-h incubation with ^{3}H-andro-4-ene-3,17-dione and presented as cpm/μg Sertoli DNA. Results are presented as the mean ± SEM from a minimum of three different experiments done in triplicate. The letter inset within bars denotes the results of a statistical analysis of variance, with a difference in letter indicating a statistical difference ($p < 0.05$).

effect on FSH actions with aromatase activity or estrogen production, but did have slight inhibitory actions (Figs. 9 and 10B). Data indicate that PModS(B), but not PModS(A), can reduce the ability of FSH to stimulate aromatase activity and estrogen production by midpubertal Sertoli cells.

DISCUSSION

The testicular paracrine factor PModS has been shown to have dramatic effects on Sertoli cell transferrin and ABP production in vitro [3, 4, 7]. The actions of PModS on other Sertoli cell functions have not been examined and were investigated in the study reported here to help elucidate the potential physiological role of PModS. Two previously identified Sertoli cell functions are the ability of the cells to aromatize androgen to estrogen with the P450 aromatase enzyme complex [8, 9] and the production of Pa [13]. The procedures for assessing the levels of aromatase and Pa activities were established and validated as previously described, and optimal culture conditions for examining these activities were determined. An experimental variable to consider, however, is the purity of the cell populations used. Peritubular cells produce PModS and are the primary somatic cell contaminant of Sertoli cell preparations. As previously described [7], both midpubertal and late-pubertal Sertoli cell cultures contain a low level (less than 2%) of peritubular contaminant. However, prepubertal Sertoli cells from 10-day-old rats contain a higher level of contaminant (approximately 40%), which must be considered [7]. The presence of peritubular cells interferes with data normalization and has been shown to influence Sertoli cell function and hormone responsiveness [2, 28, 29]. The potential production of PModS by this peritubular cell contaminant in the prepubertal Sertoli cell preparation may mask the effects of added PModS. In addition, the effects of extracellular matrix derived from peritubular cells can also influence Sertoli cell hormone responsiveness [30]. Therefore, this experimental limitation needs to be considered in subsequent analysis of data on prepubertal Sertoli cell preparations.

Sertoli cells have been shown to produce both uPa and tPa [14]. The proteolytic activity associated with Pa and its ability to activate plasminogen are postulated to be important for the continuous tissue remodeling required for spermatogenesis. Therefore, the expression of Pa is regarded as a requirement throughout pubertal development and in the adult for processes such as the translocation of spermatogenic cells across the blood-testis barrier [13]. The primary regulatory agent controlling production of Pa activity is FSH through the actions of cAMP [13, 15, 26]. The form of Pa activity influenced by FSH is tPa, whereas uPa has been shown to be relatively nonresponsive to FSH [26]. Results presented in the current study, however, indicate that FSH can significantly stimulate uPa production by the prepubertal Sertoli cell preparation. Therefore, as the Sertoli cell becomes differentiated, the ability of FSH to regulate uPa expression is lost; in contrast, tPa is regulated by FSH throughout pubertal development. Previous reports have indicated that FSH is the primary regulator of Pa production [13, 15]; however, results of the current study indicate that insulin can stimulate the production of both uPa and tPa by prepubertal Sertoli cells to the same degree as FSH. These actions of insulin, probably acting at the IGF-I re-

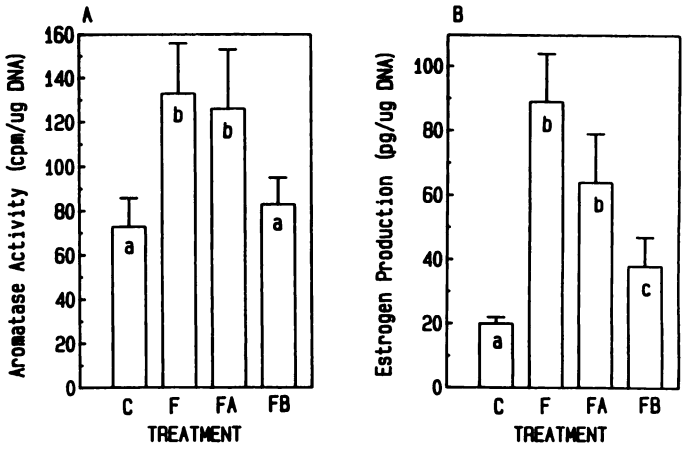

FIG. 10. Effect of PModS on FSH regulation of (A) aromatase activity and (B) estrogen production by Sertoli cells isolated from 20-day-old rats. Cells were cultured in the absence (Control, C) or presence of FSH (F); FSH plus PModS(A) (FA); or FSH plus PModS(B) (FB). Aromatase activity was determined on Day 5 of culture after a 17-h incubation with ^{3}H-testosterone and data are presented as cpm/μg Sertoli DNA. Estrogen production was assessed with a 72-h-medium collection on Day 5 of culture and is expressed as pg/μg Sertoli DNA. Results are presented as the mean ± SEM from a minimum of three different experiments. The letter inset within the bars denotes the results of an analysis of variance, with a difference in letter indicating a statistical difference ($p < 0.05$).

ceptor, are lost as the Sertoli cells are differentiated to the midpubertal and late-pubertal stages of development. Results imply that the regulation of Pa production by prepubertal Sertoli cells is distinct from the predominant FSH regulation of tPa production observed at later stages of pubertal development. A limitation of this interpretation of data, however, is the presence of peritubular cells in the prepubertal Sertoli cell preparation. Although the actions of insulin on peritubular cells have not been documented, the possibility that insulin may mediate its effects indirectly through the peritubular cell requires further investigation.

As previously reported, androgens do not influence Pa production with the culture procedures utilized; however, retinol inhibited basal uPa production and FSH-stimulated tPa production by midpubertal Sertoli cells. The actions of retinoids on other Sertoli cell functions have previously been demonstrated [27], but this study appears to be the first to report inhibitory activity associated with retinol. The inverse actions of FSH and retinol provide a potentially efficient mechanism for regulating Pa production. Further investigation of the actions of retinoids on Pa production is required to address questions such as why the inhibitory response was primarily isolated to midpubertal Sertoli cells. Pa production was found to increase slightly with the more advanced developmental stages of cultured Sertoli cells, and FSH increased tPa production by prepubertal, midpubertal, and late-pubertal Sertoli cells. Therefore the production and regulation of Pa production appears somewhat independent of the differentiation stage of the Sertoli cells.

Previously, a crude preparation of PModS was found to have no influence on Pa production by midpubertal Sertoli cells [16]. Recently, however, peritubular cells have been shown to produce a Pa inhibitor [14] that may mask the actions of PModS in the crude preparation of PSP [16]. The current study demonstrated that the purified forms of PModS, PModS(A) and PModS(B), had no effect on Pa production by cultured Sertoli cells from any of the developmental stages examined. This is the first Sertoli cell function observed that is not influenced by the actions of PModS. The lack of Pa regulation by PModS suggests that PModS is not a general activator of all Sertoli cell functions, but instead may be primarily involved in influencing the differentiation of the cell. This possibility is more directly addressed with the other Sertoli cell function examined, aromatase activity.

The ability of freshly isolated [10] and cultured [8, 9] Sertoli cells to synthesize estrogen has been shown to be due to the aromatase enzyme complex associated with the endoplasmic reticulum of the cell. The initial function proposed for estrogen production by Sertoli cells was regulation of Leydig cell steroidogenesis [8–11]; however, Leydig cells have also been shown to produce estrogen [11, 31]. The physiological role for estrogen production by Sertoli cells remains to be elucidated. FSH has been shown to be the primary regulator of estrogen production and aromatase activity [8–10, 22]. Estrogen production is a Sertoli cell function that is predominant at the early stages of puberty [9]. Aromatase activity was found to be high and hormone responsive in prepubertal Sertoli cells but declined and was not responsive to hormones at the late-pubertal stages of development. Therefore, aromatase activity is suppressed as the Sertoli cell differentiates. The purified forms of PModS, PModS(A) and PModS(B), alone were found to have no influence on aromatase activity at any of the developmental stages examined. A limitation of this interpretation of the data, however, is the presence of peritubular cells in the prepubertal Sertoli cell preparation that produced PModS. Although the levels of PModS present in unconcentrated conditioned media from these preparations were negligible, the possibility that endogenous PModS may mask effects of exogenous PModS remains to be investigated. The inability of PModS to directly influence aromatase activity probably correlates with the lack of effect of PModS on cAMP levels. PModS has been shown to have no effect on cAMP levels, but instead stimulates cGMP levels [4]. Aromatase activity in several cell types, including Sertoli [9, 22], Leydig [10, 31], and human adipose cells [32], has been shown to be regulated by alterations in cAMP levels and cAMP-dependent protein kinase activity. Since PModS has no effect on cAMP, PModS alone may not have the ability to directly influence aromatase activity. Examination of the combined effects of PModS and FSH demonstrated that PModS(B) inhibited the ability of FSH to stimulate aromatase activity in midpubertal Sertoli cells. PModS(A) caused a suppression that was not statistically significant. Optimal concentrations of both PModS(A) and PModS(B) from various preparations were used in the analysis, as previously demonstrated [3].

This difference in the actions of PModS(B) on aromatase is the first functional difference observed between the two forms of PModS. Further characterization of the two forms is required to understand the significance of this difference in bioactivity. The ability of PModS(B) to suppress the actions of FSH on midpubertal cells suggests an alteration in Sertoli cell differentiation to a more adult stage of development. Results imply that PModS may promote Sertoli cell differentiation to a late-stage pubertal state that is less hormone responsive. Previously a crude preparation of PSP was shown to inhibit the actions of FSH on aromatase activity with midpubertal Sertoli cells [12], and it was suggested that this inhibitory activity may be due to the presence of PModS. The actions of purified PModS(B) observed in the current study support this previous speculation.

The current study provides a thorough analysis of the hormonal regulation of aromatase activity and Pa production at various stages of pubertal development. Pa production appears in part to be independent of Sertoli cell differentiation whereas aromatase activity is an early pubertal cell function that decreases in late-pubertal Sertoli cells. Both activities appear to be primarily regulated by FSH. Analysis of PModS actions suggests that a potential function of PModS may be to promote and maintain Sertoli cell differentiation. This is supported by the observation that PModS had no effect on Pa production and suppressed aromatase activity in response to FSH. Characterization and molecular cloning of PModS are required to further our understanding of the functions and actions of this testicular paracrine factor. In addition, the observations made with PModS in the current study utilized an in vitro analysis of Sertoli function; elucidation of the physiological importance and function of PModS will ultimately require in vivo physiological studies.

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