Sertoli-cell prostaglandin synthesis

Effects of (follitropin) differentiation and dietary vitamin E

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The synthesis of prostanoids by the Sertoli cell was assessed as part of a study on the role of vitamin E in maintaining spermatogenesis. Analyses of eicosanoid synthesis from endogenous substrate were carried out using freshly isolated Sertoli-cell-enriched preparations from both pre-pubertal and adult rats fed purified diets with and without vitamin E, as well as cells carried in primary culture. Freshly isolated cells from both the immature and fully differentiated adult testes produced PGI_2 (prostaglandin I_2) and PGE_2 , but $\text{PGF}_{2\alpha}$ was produced only by cells of the adult vitamin E-deficient rat. Cells from adult controls synthesized $\text{PGF}_{2\alpha}$ after primary culture. In contrast with other hormone responses of this cell, which are refractory in the adult, FSH (follitropin) potentiated prostaglandin production by freshly isolated cells of both immature and adult rats. The FSH response of Sertoli cells from immature animals did not change after primary culture. Adult cells were refractory to the hormone after culture, but the total amounts of prostaglandins produced by these cells were 10-fold higher than by either freshly isolated or cells of the immature in culture. Analogues of cyclic AMP did not potentiate prostaglandin synthesis. However, mepacrine, a phospholipase inhibitor, blocked the FSH effect. The finding that Sertoli cells synthesize prostaglandins and FSH enhances prostaglandin production implicates a potential role for eicosanoids in spermatogenesis and suggests that vitamin E may affect intratesticular regulators.

INTRODUCTION

Sertoli cells, somatic cells of the testis which provide the structural framework of the seminiferous tubules and the milieu for germ-cell proliferation and differentiation, are target cells for FSH and androgen, hormones which regulate spermatogenesis. FSH-elicited events in the developing Sertoli cell include regulation of ion flux, the activity of certain enzymes, the synthesis of RNA, protein and steroids, as well as effects on cell mobility, communication and division [1–3]. These activities, stimulated by FSH in the immature rat, are refractory to the hormone in cells from the mature animal [2].

PGs are known mediators of hormonal response, cell division and differentiation. Vitamin E is required for the maintenance of spermatogenesis by a variety of animal species [4]. When weanling rats are fed diets deficient in vitamin E, spermatogenesis is irreversibly impaired [5,6]. Polyenoic fatty acids of the $n6$ family (the last double bond is six carbon atoms distant from the methyl end of the acid), potential biosynthetic precursors of prostaglandins and other eicosanoids, occur in high concentration in rat testicular tissue [7,8]. The content of arachidonic acid and polyenes derived from this fatty acid in the β -position of phospholipids increases in the rat testis during sexual maturation, and this developmental pattern is affected by vitamin E deficiency [9]. PGs are synthesized by the testis [10]. A pattern of

increased synthesis of $\text{PGF}_{2\alpha}$ by rat testis microsomes (microsomal fractions) reflects the sexual maturation of the animal [11]. The amounts of $\text{PGF}_{2\alpha}$ synthesized are decreased in vitamin E deficiency, but there is no change in the sexual-maturation-dependent profile of this eicosanoid [12,13]. During testicular maturation there are changes in the distribution of specific germ-cell populations and, in addition, the Sertoli cell becomes fully differentiated. Analyses of PG production by microsomes of testis limits evaluation of both the effect of specific cell types within the testis on the pattern observed and the cell type which is affected by vitamin E deficiency. Assessment of PG production by separated specific cell types of testis is necessary for such an evaluation.

The present study on Sertoli-cell PGs is an integral part of this assessment. The objective of the present study was to identify PGs synthesized by the Sertoli cell and to evaluate the potential effects of differentiation, dietary vitamin E and FSH on eicosanoid production. Neither the PGs produced by the Sertoli cell nor the effects of FSH on PG production by this cell have been previously reported. Analyses of PG synthesis from endogenous substrate were carried out using freshly isolated cells from both pre-pubertal and adult rats fed vitamin E-deficient or vitamin E-supplemented diets, as well on isolated cells carried in primary culture.

Abbreviations used: FSH, follicle-stimulating hormone (follitropin); $\text{C}_{20:4}$, eicosa-5,8,11,14-tetraenoic acid (arachidonic acid); $\text{C}_{22:4}$, docosa-7,10,13,16-tetraenoic acid; $\text{C}_{22:5}$, docosa-4,7,10,13,16-pentaenoic acid; PG, prostaglandin; PGH_2 , 15-hydroxy-9,11-peroxidoprostanoic acid; PGE_2 , 11,25-dihydroxy-9-oxoprostanoic acid; $\text{PGF}_{2\alpha}$, 9,11,15-trihydroxyprostan-1-oic acid; PGI_2 , prostacyclin (6,9-epoxy-11,15-dihydroxy-9-oxoprostanoic acid); LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; TX, thromboxane.

EXPERIMENTAL

Materials

FSH (NIAMDD-rat FSH-B-1 and NIAMDD-ovine FSH-14) was kindly provided as a gift by the NIAMDD Pituitary Hormone Distribution Program. Media were purchased from Gibco (Grand Island, NY, U.S.A.), buffer solutions for cell culture from Falcon (Oxnard, CA, U.S.A.), testosterone, retinol, insulin, trypsin (T-8253), deoxyribonuclease (bovine pancreatic), mepacrine, dibutyl cyclic AMP, and 8-bromo cyclic AMP from Sigma (St. Louis, MO, U.S.A.); and collagenase (4197) from Worthington (Freehold, NJ, U.S.A.). Fetal-calf serum was obtained from Flow Laboratories (McLean, VA, U.S.A.). Indomethacin was a gift from Merck and Co. (Rahway, NY, U.S.A.). PG standards were gifts from Dr. John Pike (Upjohn Co., Kalamazoo, MI, U.S.A.). Antisera to PGF_{2α} and PGB were kindly provided by Dr. Ken Kirton (Upjohn Co.) and the antiserum to 6-oxo-PGF_{1α} was a gift from Dr. Avid Hassid (Case Western Reserve University, Cleveland, OH, U.S.A.). Tritiated PGs {[5,6,8,9,11,12,14,15-³H(n)]-C_{20:4} (60 Ci/mmol) and [1-¹⁴C]C_{22:4} (40–60 mCi/mmol)} were purchased from New England Nuclear (Boston, MA, U.S.A.). Vitamin E (α-tocopherol acetate) was obtained from Eastman-Kodak Co. (Rochester, NY, U.S.A.). All other chemicals and solvents were from commercial sources.

Animals and Sertoli-cell isolation

Male Sprague-Dawley rats, purchased from the Charles River Breeding Laboratory (Wilmington, MA, U.S.A.), were used to prepare several models of Sertoli-cell-enriched preparations.

Sertoli-cell-enriched testes induced by irradiation treatment. Pregnant animals were subjected to 125 rad of whole-body irradiation with a cobalt-60 source [14]. Male offspring were housed until weaned at 21 days with the mother, who was fed a commercial chow diet (Ralston Purina, St. Louis, MO, U.S.A.). Testes were decapsulated, gently dissociated, divided into quarters and incubated in Krebs-Ringer bicarbonate, pH 7.4, containing 1% glucose. The media were isolated and PGs quantified by radioimmunoassay.

Freshly isolated Sertoli cells. Cells used shortly after preparation were isolated from testes of pre-pubertal (21–22-day-old), adult (12 weeks and older) and hypophysectomized rats, by following a published procedure [15] modified to include deoxyribonuclease (10 μg/ml per g of tissue) in the initial digestion with trypsin. To successfully isolate cells from adult testes, incubation times with the above enzymes and with collagenase were increased. After a recovery period of 45 min at 4 °C, aliquots of the cells were removed for study. To label lipid pools, cells were preincubated for 60 min with [1-¹⁴C]C_{22:4} (0.1 μCi/10⁶ cells). Indomethacin (10–50 μg/ml of reaction system), an irreversible inhibitor of the PG synthase cyclo-oxygenase activity, was added in ethanol. Ethanol itself at the final concentration of < 1% had no effect on PG production.

Cell purity was routinely evaluated by phase-contrast microscopy on the basis of the typical size, epithelial morphology and lipid-droplet inclusions ascribed to the Sertoli cell [16]. Identifications were verified by electron

microscopy of selected samples. The primary contaminating cells were germ cells; few peritubular cells were present. The mean homogeneity of the cells from immature rats was 85%. Germ cells comprised 30–35% of the cells isolated from adults. The degree of germ-cell contamination was less when vitamin E-deficient animals were used. The recovery of Sertoli cells averaged 6 × 10⁶ cells/animal and was independent of age and diet. Cell viability as assessed by Trypan Blue (0.4%) exclusion was > 90% for all preparations.

Primary culture of Sertoli cells. Sertoli cells carried in short-term primary culture were isolated from testes of rats fed standard laboratory chow or the semi-purified diet described below. Cell isolations were performed by using the method of Welsh & Wiebe [17], with the substitution of trypsin (0.05%) for pancreatin. The purity of the preparations from immature animals was 70%, and from adults, 50–60%. The viability of the preparations was > 95%. The primary contaminating cells initially present were spermatocytes along with 3–5% peritubular cells. Sertoli cells (2 × 10⁶) were plated in a 35 mm-diameter dish in Ham's F-12 medium supplemented with bovine insulin (5 μg/ml), testosterone (0.1 μM) and retinol (0.7 μM), and cultured in a humidified incubator at 37 °C in an atmosphere of air/CO₂ (19:1). A low concentration of fetal-calf serum (1%) present during the first 3 days of culture inhibited fibroblast attachment and peritubular cell growth. The Sertoli-cell monolayers were rinsed on day 3 of culture to remove unattached germ cells and debris. Sertoli-cell enrichment was > 90% from both immature and adult testes, with a cell viability of > 95%, irrespective of animal age and dietary treatment. Fresh medium with additions as described above, but without serum, was then added and the cultures were returned to the incubator.

Cells were routinely cultured for 4–7 days. In addition to normal morphology and viability, these cells retain their functional integrity. The culture conditions used were selected as they support the synthesis of proteins by Sertoli cells of rats in primary culture [18]. We have observed that these cultured cells readily incorporate and turn over [³H]- and [¹⁴C]-C_{20:4} into both membrane phospholipids and triacylglycerol. The cells also incorporate [³²P]P_i into intracellular pools of ATP as evidenced by the incorporation and turnover of ³²P into polyphosphoinositides, phosphatidylinositol and other phospholipids [19] and phosphorylation of intracellular proteins [20]. Culture media were collected after 3 or 24 h of incubation after a media change and stored at –20 °C until analysed for PGs. The protein content of the cell pellet was determined by the method of Lowry *et al.* [21], with bovine serum albumin (Fraction V) as a standard.

Treatment of animals. Hypophysectomy was performed in 28-day-old rats maintained on the chow diet. Before Sertoli-cell isolation, the rats were treated with rat FSH (7–10 μg), intraperitoneally, twice daily for 48 h or with the vehicle control (0.1% albumin in 0.9% NaCl).

Sertoli cells were also isolated from animals fed the semipurified-vitamin E-deficient and control diets for 4–6 months. Male pups, when 5 days old, were housed in groups of six to eight with a lactating mother who was fed either the purified diet without vitamin E or the same

diet supplemented with 60 mg of α -tocopherol acetate/kg of diet. The diets, fed for the duration of the study, had the following overall composition: 20% vitamin-free casein, 62% carbohydrate (sucrose plus cornstarch), 6% fat (3% stripped lard plus 3% cod liver oil), essential trace minerals [22], ZnCO_3 (1.6 g/kg of mineral mix), vitamin mix (without vitamin E) and 8% fibre (Alphacel). This diet supports good growth in the rat and results in testicular degeneration when fed without added vitamin E [9]. All diets were fed *ad libitum*. The animals had free access to water and were housed using a 12 h light/12 h dark schedule in our fully accredited animal facility.

Eicosanoid production by Sertoli cells

Freshly isolated cells $[(0.5\text{--}2.0) \times 10^6]$ were incubated in 2 ml of Krebs–Ringer bicarbonate with 0.1% glucose, pH 7.4, then acidified to pH 3.5 with citric acid (0.1 ml, 1.5 M). The cells were pelleted and 1000 c.p.m. of $[\text{^3H}]\text{PGF}_{1\alpha}$ added to monitor recovery. For cultured cells, the media were decanted and assayed directly for $\text{PGF}_{2\alpha}$ and the stable breakdown product of PGI_2 , 6-oxo- $\text{PGF}_{1\alpha}$. PGE_2 was quantified as PGB_2 after extraction and alkaline isomerization. PGH_2 metabolism was studied by using cell homogenates which were either preincubated for 5 min at 34 °C or heat-inactivated before the addition of 12000 c.p.m. of $[\text{^1-}^{14}\text{C}]\text{PGH}_2$. After citrate acidification, PGs were extracted with ethyl acetate. The extract was washed until neutral and the organic phase dried under N_2 and applied to a small silicic acid (0.5 g) column. PGs were eluted with benzene/ethyl acetate/methanol (3:2:1 and 30:20:1, by vol.) [23]. Routine recoveries were 75%.

PG quantification

PGs were quantified by radioimmunoassay using conditions for each antiserum giving a linear standard curve in the 25–500 pg range. The values represent two different volumes of sample assayed in duplicate and yielding a dose response in the range of the standard curve. The values agreed within 2 s.d., and the within- and between-assay coefficients of variation were < 5% and < 10% respectively.

The specificity of the PG antisera were as follows: antiserum to $\text{PGF}_{2\alpha}$ cross-reacted 30% with $\text{PGF}_{1\alpha}$, 15% with 6-oxo- PGE_1 , 30% with 13,14-dihydro- $\text{PGF}_{2\alpha}$ and 1% with $\text{PGF}_{2\beta}$, PGA_1 , PGB_2 , PGB_1 , PGE_2 , PGE_1 , 6-oxo- PGE_1 , and related 13,14-dihydro and 15-oxo metabolites and 12-HETE. PGB_2 antisera cross-reacted 100% with PGA_1 , 1.1% with PGE_2 , 6.25% with 13,14-dihydro- PGE_2 and less than 0.1% with 6-oxo- $\text{PGF}_{1\alpha}$, PGD_2 , $\text{PGF}_{2\alpha}$, $\text{PGF}_{2\beta}$, 12-HETE, $\text{C}_{20:4}$ and all other related metabolites. Antisera to 6-oxo- $\text{PGF}_{1\alpha}$ cross-reacted 100% with 6-oxo- $\text{PGE}_{1\alpha}$, 17.5% with $\text{PGF}_{2\alpha}$, 18% with $\text{PGF}_{1\alpha}$, 2.5% with PGE_1 , 13% with 13,14-dihydro-6-oxo- $\text{PGF}_{1\alpha}$ and less than 1% with all other metabolites.

Antisera were diluted to give 30–35% binding and 2000 c.p.m. of $[\text{^3H}]\text{ligand}$ (> 40 Ci/mM) added in assay buffer (0.05 M- $\text{Na}_2\text{HPO}_4/0.15$ M- $\text{NaCl}/1\%$ gelatin, pH 8.2). Standard curves were constructed by using media or buffer. PGE_2 was added to controls to monitor recovery of PGB_2 (routinely 95%). Antiserum-bound radioactivity were separated after a 1 h incubation at 4 °C with dextran-coated charcoal [100 ml of water, 187 mg of Dextran T-70 (Pharmacia) and 1.5 g of Norit A (Baker)]. Supernatants were counted for radioactivity

for 10 min in EP Ready Solv (Beckman) in a liquid-scintillation counter at 40% efficiency.

Lipoxygenase products were quantified by radioimmunoassay in the laboratory of Dr. Lawrence Levine (Brandeis University, Waltham, MA, U.S.A.) with antisera for HETEs [24] and for sulphidopeptide-containing LTs [25].

PGs synthesized from $[\text{^3H}]\text{C}_{20:4}$ or $[\text{^1-}^{14}\text{C}]\text{PGH}_2$ were extracted, dried under N_2 and separated on a C_{18} μ Bondapak reverse-phase column (Waters Associates) in an isocratic system [26] with a flow rate of 2 ml/min for 60 min. The radioactivity of the fractions eluted was determined by liquid-scintillation counting. Elution patterns of the products correlated well with those of corresponding tritiated standards and published retention times. PGH_2 was prepared by incubating microsomes isolated from ram seminal vesicles with $[\text{^1-}^{14}\text{C}]\text{C}_{20:4}$ (50 Ci/mmol) [27]. The products were extracted into cold diethyl ether/methanol/0.28 M-citric acid (30:40:1, by vol.), and separated on silicic acid columns at 4 °C. $[\text{^1-}^{14}\text{C}]\text{PGH}_2$ was verified by t.l.c. and by h.p.l.c. after reduction, with triphenylphosphine, to $\text{PGF}_{2\alpha}$. PGs were separated by t.l.c. on silica-gel G plates (Analtech) using ethyl acetate/iso-octane/acetic acid/water (11:5:2:10, by vol.). Recovery standards were also chromatographed. Zones of interest were scraped off, eluted with acidic methanol and assayed by radioimmunologically. Amounts of PGs produced are reported as ng/ 10^6 cells or as ng/mg of protein.

RESULTS

PG production by freshly isolated Sertoli-cell-enriched preparations

Sertoli cells of immature rats. Sertoli cells of the immature rat synthesize both PGE_2 and PGI_2 (Table 1). Small basal amounts of both PGE_2 and of 6-oxo- $\text{PGF}_{1\alpha}$, the stable hydrolysis product of PGI_2 , are present in the media of freshly prepared, non-incubated, Sertoli-cell-enriched preparations. PG synthesis is linear with respect to time under the following conditions: $(0.5\text{--}1.2) \times 10^6$ cells; 0.063–0.188 mg of protein/2 ml of reaction system; 60 min incubation. The amounts of PGE_2 and PGI_2 produced are similar, and PG synthesis is inhibited by indomethacin. Incubation of the cells with exogenous substrate, $\text{C}_{20:4}$ (7.4 $\mu\text{g/ml}$), evokes a modest stimulation, about 45%, in the amounts of PGE_2 and PGI_2 compared with synthesis from endogenous substrate. Neither $\text{PGF}_{2\alpha}$ nor TXA_2 was detectable.

Sertoli cells of adult rats. The differentiation of the Sertoli cell is reflected by age-dependent changes in the regulation of intracellular events [2]. Sertoli cells from both normal adults and those from vitamin E-deficient testes produce PGs (Table 1). Sertoli cells from the adult vitamin E-deficient testis produce $\text{PGF}_{2\alpha}$, in direct contrast with cells from gonads of either the immature or adult rat control. Concentrations of PGE_2 are similar to those from the immature rats on the basis of cell number, but only about one-third on the basis of cell protein. There are conflicting reports on Sertoli-cell protein/cell-number changes which accompany differentiation [16,28]. Our analyses show that the number of cells/mg of protein is higher for the immature (8.38 ± 0.69 , $n = 7$) than for the adult (3.24 ± 0.52 , $n = 12$) and that the amount of protein (mg) per 10^6 cells is thus lower in the

Table 1. Prostaglandin PG production by freshly isolated Sertoli cells from immature and adult rats

Freshly isolated Sertoli cells (1×10^6 cells) from 21-day-old rats and from adult rats fed the semi-purified diets either supplemented with vitamin E [adult control (14–42 weeks)], or without vitamin E [adult, vitamin E-deficient (16–20 weeks)] were incubated at 34 °C in 2 ml of Krebs–Ringer bicarbonate/1% glucose, pH 7.4, without additions or in the presence of indomethacin (50 µg/ml for 21-day-old and 10 µg/ml for adult). Incubation media were extracted, partitioned on silicic acid columns, and PGs of appropriate eluate fractions quantified by radioimmunoassay. Results are means $\pm$ S.E.M. for preparations from the numbers of animals indicated in parentheses. ND indicates that the product was not detected and NA that analyses for the product were not done. Protein content/ 10^6 cells was 0.125 mg for the cells isolated from the immature, and 0.30 mg for those from adult, rats.

Source of Sertoli cells	Additions	Incubation time (min)	PG production (ng/ 10^6 cells)		
			PGI ₂	PGE ₂	PGF _{2α}
Immature	None	30	2.19 $\pm$ 0.41 (6)	1.79 $\pm$ 0.17 (4)	ND (6)
	None	60	5.60 $\pm$ 0.30 (3)	2.50 $\pm$ 0.11 (4)	ND (4)
	Indomethacin	30	1.74 $\pm$ 0.28 (4)	0.70 $\pm$ 0.19 (2)	ND (4)
	Indomethacin	60	2.06 $\pm$ 0.65 (3)	0.90 $\pm$ 0.06 (2)	ND (3)
Adult, control	None	30	NA	2.72 $\pm$ 0.64 (4)	ND (4)
	Indomethacin	30	NA	0.75 $\pm$ 0.63 (3)	ND (3)
Adult, vitamin E-deficient	None	30	NA	2.80 $\pm$ 0.17 (4)	2.00 $\pm$ 0.53 (4)
	Indomethacin	30	NA	0.81 $\pm$ 0.17 (2)	0.68 $\pm$ 0.18 (3)

immature (0.13) than in the adult (0.32). When corrected for heterogeneity, cells from the adult control produce about 50% more PGE₂ than do those from the degenerate vitamin E-deficient testes. PGI₂ was not assessed in the preparations from adult rats.

Effect of FSH on freshly isolated cells. Peptide hormones stimulate PG synthesis in a variety of systems. FSH enhances PG synthesis in the granulosa cell, an ovarian cell with considerable embryological and functional analogy to the Sertoli cell [29]. Addition of 5 µg of rat FSH/ml enhances PG synthesis by cells from both immature and adult rats, but higher concentrations appear to inhibit (Table 2). The FSH response by cell preparations from adults, a 2–4-fold increase in PGE₂, is similar to that of cells from immature animals. PGF_{2 α} synthesis by Sertoli cells of vitamin E-deficient testes is also enhanced. In view of the known non-responsiveness

of the cells of the adult to FSH, this stimulation is noteworthy.

PG production by Sertoli cells in primary culture

Cells carried in primary culture were used to further characterize Sertoli-cell PGs and the FSH effect. Scanning and transmission electron micrographs of the primary cultures of Sertoli cells revealed the typical cytoplasmic processes, lipid droplets, nuclear-membrane folds, prominent nucleolus and other features characteristic of Sertoli-cell morphology [15]. The biochemical responses of the cultured cells also appear to be normal. Treatment of immature cells with ovine FSH (0.1 µg/ml) results in a 10-fold increase in intracellular cyclic AMP and stimulation of both aromatase and of plasminogen-activator activity. The number of FSH receptors on cells from adults measured by using ¹²⁵I-FSH is in good agreement with published values [28]. The value, mg of

Table 2. FSH stimulation of prostaglandin production of freshly isolated Sertoli cells

Freshly isolated Sertoli cells from animals fed standard laboratory chow (21–30 days, immature) or the semi-purified diets with vitamin E (adult, control, 19–42 weeks old) or without vitamin E (adult, vitamin E-deficient, 16–20 weeks old) were incubated in systems of 1×10^6 cells/2 ml of Krebs–Ringer bicarbonate/1% glucose, pH 7.4, with rat FSH (1–5 µg/ml) at 34 °C for 60 min. PGs were extracted and quantified by radioimmunoassay. Values are expressed as percentage increases (FSH-treated/untreated $\times$ 100) $\pm$ S.E.M. Abbreviations used: ND, not detectable by radioimmunoassay; NA, not analysed. The numbers of different preparations analysed are indicated in parentheses.

Source of Sertoli cells	[Rat FSH] (µg/ml)	FSH stimulation (% of control)		
		PGE ₂	PGI ₂	PGF _{2α}
Immature	1.25	160	210	ND
	2.50	220	240	ND
	5.00	460 $\pm$ 90 (4)	294 $\pm$ 41 (3)	ND
	10.00	120	0	ND
Adult				
Control	5.00	380 $\pm$ 140 (4)	NA	ND
Vitamin E-deficient	5.00	230 (2)	NA	310 (2)

Table 3. Prostaglandin production by Sertoli cells in primary cultures derived from immature and adult rats

Sertoli cells isolated from 21-day-old rats fed the standard laboratory chow, and 4-month-old adult rats fed the semi-purified diets, either supplemented with vitamin E (adult control) or not (adult, vitamin E-deficient), were carried in primary culture as described in the Experimental section. There were 2.5×10^6 cells (0.25 mg of protein)/dish in the cultures of the immature and 1×10^6 cells (0.2 mg of protein) in those of the adults. Indomethacin (10 $\mu\text{g}/\text{ml}$) and ovine FSH (oFSH) (0.1 μg or 0.5 $\mu\text{g}/\text{ml}$) were added simultaneously to the cultures. Media were decanted after 24 h and PGs assayed by radioimmunoassay. Values for adults represent the mean $\pm$ S.E.M. for triplicate dishes from a representative experiment; * indicates the mean for duplicate dishes. The values in parentheses are the numbers of dishes from immature rats assayed from three different cultures and the data are given as means $\pm$ S.E.M. ND indicates no detectable PGs. 'Total PG' is the sum of the amounts of $\text{PGI}_2 + \text{PGE}_2 + \text{PGF}_{2\alpha}$. Significance levels by Student's *t* test are denoted as follows: **P* < 0.005 compared with basal value; ***P* < 0.005 compared with 0.5 μg of ovine FSH; ****P* < 0.001 from basal value.

Source of Sertoli cells	Additions	Prostaglandin production (ng/mg of cell protein)			
		PGI_2	PGE_2	$\text{PGF}_{2\alpha}$	Total PG
Immature	None	0.73 ± 0.11 (4)	2.42 ± 0.35 (4)	ND	3.15
	oFSH (0.1 μg)	0.56 ± 0.11 (3)	5.82 ± 0.55 (4)*	ND	6.38
	oFSH (0.5 μg)	2.59 ± 0.28 (4)***	2.28 ± 0.55 (4)	ND	4.87
	Indomethacin	1.11 ± 0.14 (4)**	2.51 ± 0.35 (3)	ND	3.62
	+ oFSH (0.5 μg)				
Adult, control	None	14.78 ± 2.67	30.19 ± 4.28	8.90 ± 0.57	53.87
	oFSH (0.1 μg)	22.09 ± 3.82	35.17 ± 2.84	9.68 ± 1.32	66.84
	oFSH (0.5 μg)	14.55 ± 4.75	21.65 ± 8.03	4.73 ± 1.64	41.00
	Indomethacin	6.40 ± 0.39	9.55 ± 0.67	3.45 ± 0.20	19.41
	+ oFSH (0.5 μg)				
Adult, vitamin E-deficient	None	21.54 ± 5.72	10.10 ± 1.01	20.76 ± 1.75	52.36
	oFSH (0.1 μg)	15.86 ± 2.60	14.74 ^a	14.26 ± 5.11	44.87
	oFSH (0.5 μg)	19.07 ± 3.46	10.18 ± 1.88	16.01 ^a	45.26
	Indomethacin	11.65 ± 1.15	19.33 ^a	23.62 ± 2.17	54.33
	+ oFSH (0.5 μg)				

protein/ 10^6 cells, for the immature rat (0.14, $n = 5$) is not affected by culture, but is decreased after culture in adult cells (0.26, $n = 3$). No differences reflecting dietary vitamin E are apparent. Phagocytosis of germ cells may enhance the protein content of the Sertoli cell from adult testes.

Sertoli cells of immature rats in culture. Both PGI_2 and PGE_2 are produced from endogenous precursor by cultured cells from immature rats, but $\text{PGF}_{2\alpha}$ is not detectable (Table 3). PG production is linear with respect to time for protein concentrations up to 0.5 mg (4.4×10^6 cells) per reaction system. FSH addition enhances the amounts of both PGI_2 and PGE_2 , but the threshold appears to be lower for PGE_2 (100 ng) than for PGI_2 (500 ng) (Table 3). Although there is a 2–5-fold stimulation, enhancement as high as 30-fold was occasionally observed. Such variability in responsiveness to FSH has been reported and may be attributable to Sertoli cells from tubules in different stages of spermatogenesis [30]. Whereas indomethacin inhibits FSH stimulation of PGI_2 and PGE_2 by apparently 'optimal' amounts of hormone, higher concentrations of FSH inhibit PG synthesis. The amounts of FSH used for routine assessment of the effect of hormone were selected on the basis of results of trial dose–response studies. Comparison of the ratio of FSH stimulated to basal PGs in a representative study utilizing 50 ng, 100 ng, 500 ng, 1 μg and 10 μg of ovine FSH, gave the following fold increases for PGE_2 : 6.2, 10.1, 2.8, 1.5 and 1.0; the values for PGI_2 were 2.8, 3.0, 4.1, 1.5 and 1.0 respectively.

Sertoli cells of adult rats in culture. After primary culture there are marked differences in PG synthesis

between cells of pre-pubertal and adult rats, which reflect both animal age and dietary treatment. Not only do cells of adults from both dietary groups synthesize PGE_2 and PGI_2 but also, in contrast with freshly isolated cells, adult control cells synthesize $\text{PGF}_{2\alpha}$ after culture (Table 3). Cultured cells from adults produce at least 10-fold larger amounts of PGs than do cultured cells from the immature animal. Total PGs ($\text{PGE}_2 + \text{PGI}_2 + \text{PGF}_{2\alpha}$) are similar for cells from control and vitamin E-deficient rats, but their relative distribution is different. PGE_2 represents half of the PGs detected in the adult control, but only 20% of the total in cells from the adult, vitamin E-deficient, animals in which the major products appear to be PGI_2 (40%) and $\text{PGF}_{2\alpha}$ (40%). FSH has no effect on either the total amounts or their relative distribution. Indomethacin, tested in the presence of FSH, inhibits PGI_2 , PGE_2 and $\text{PGF}_{2\alpha}$ in cells from the control, but only PGI_2 in those from the vitamin E-deficient rat.

FSH stimulation of PG synthesis

Possible mechanism(s) of FSH were evaluated by using several models. The testis of the Sertoli-cell-enriched model is relatively germ-free, does not require exposure to proteolytic enzymes and appears to have intact FSH-receptor function [31]. The PG profile of the Sertoli-cell-enriched testis is similar to that of freshly isolated pre-pubertal Sertoli cells. Neither addition of dibutyryl cyclic AMP (2 mM) nor of 8-bromo cyclic AMP (1 mM) affects PG synthesis, suggesting the hormone effect is not mediated via adenylate cyclase. Mepacrine, a phospholipase inhibitor, at concentrations of 1 mM and higher, inhibits basal PG production, but this high concentration also results in cell toxicity and death. At lower concentrations of mepacrine (0.3 mM), the stimu-

Table 4. Prostaglandin production by Sertoli cells from hypophysectomized rats

Sertoli cells were isolated from 68-day-old rats that had been hypophysectomized at 28 days and either treated, before being killed, intraperitoneally with FSH for 2 days (10 µg, twice daily) ('FSH-treated'), or with 0.1% bovine serum albumin ('untreated control'). Incubations were carried out for 60 min at 34 °C using 0.5×10^6 freshly isolated cells (0.125 µg of protein), with additions of rat FSH (rFSH), indomethacin (50 µg/ml) or mepacrine (0.3 mM). Media were extracted and PGs assayed by radioimmunoassay. Values represent means $\pm$ S.E.M. for two aliquots of sample (analysed in duplicate) from a representative experiment. ND indicates no detectable PG. Significance: ^aStatistically significant ($P < 0.01$) from reaction system with no additions; ^bstatistically significant ($P < 0.01$) from reaction system with addition of 0.5 µg of FSH; ^cstatistically significant ($P < 0.01$) from reaction system with addition of 5 µg of FSH; ^duntreated control compared with FSH-treated, $P < 0.05$.

Additions	Prostaglandin production (ng/mg of protein)			
	Untreated control		FSH-treated	
	PGE ₂	PGI ₂	PGE ₂	PGI ₂
None	0.65 $\pm$ 0.05	ND	1.16 $\pm$ 0.25	0.72 $\pm$ 0.22
rFSH(0.5 µg/ml)	1.10 $\pm$ 0.11 ^{a,d}	ND	2.50 $\pm$ 0.24 ^{a,d}	2.70 $\pm$ 0.12 ^a
rFSH(5 µg/ml)	2.65 $\pm$ 0.15 ^{a,b}	ND	2.13 $\pm$ 0.20	1.52 $\pm$ 0.15 ^{a,b}
rFSH(5 µg/ml) + indomethacin	0.67 $\pm$ 0.04 ^{b,c}	ND	0.50 $\pm$ 0.05 ^{b,c}	0.74 $\pm$ 0.09 ^{b,c}
rFSH(5 µg/ml) + mepacrine	1.14 $\pm$ 0.15 ^{a,c}	ND	0.33 $\pm$ 0.04 ^{b,c}	1.03 $\pm$ 0.04 ^{b,c}

lating effect of FSH is blocked and cell viability and basal PG production are normal.

The effect of FSH *in vivo* tested with cells from hypophysectomized rats suggests that Sertoli cells from rats treated with FSH before they were killed may have a lower threshold for FSH *in vitro* than do those from animals not treated with FSH (Table 4). PGE₂ production by cells of the control is enhanced by FSH in a dose-dependent manner. In contrast, cells from the FSH-treated animals have an apparent maximum response to FSH at the low dose, and PGI₂ values are lower at 5.0 µg than at 0.5 µg of FSH. PGI₂ is not detectable in cells from hypophysectomized rats not subjected to hormone therapy. The FSH response by both types of cells is inhibited by indomethacin or mepacrine.

Analyses of PG synthesis were performed on cells cultured for 24 h, a common procedure for cultured cells. Many of the effects of FSH on Sertoli-cell function are discernible by 1 h [2]. Although PGs are detectable in the media of freshly isolated Sertoli cells after an 1 h incubation and are affected by FSH, the studies were not reproducible. PGs can readily be detected after a 3 h reaction time (Table 5). Amounts of PGs after 3 h are similar to those after 24 h, suggesting that PGs are released shortly after media changes. FSH addition enhances PGE₂ and PGI₂ 7–8-fold, but dibutyryl cyclic AMP does not elicit this effect. Mepacrine partially blocks (30–46%) the FSH effect. Addition of Ca²⁺ ionophore A23187 results in larger amounts of PGs than observed with 100 ng of ovine FSH.

Confirmation of PGs produced

The immunoresponsiveness of products to relatively specific antisera and their inhibition by indomethacin confirm that Sertoli cells synthesize PGs. Additional evidence for the identity of PGs include, for cells from the immature rats, an 86% recovery of PGE₂ as PGB₂ after alkaline isomerization or as PGF_{2α} after reduction. Moreover, reverse-phase h.p.l.c. of the media of cells

prelabelled with [³H]C_{20:4} reveals peaks coincident to [³H]6-oxo-PGF_{1α} and [³H]PGE₂. The presence of PGI₂ synthase and PGE₂ isomerase activities are further documented when Sertoli-cell homogenates are incubated with [¹⁴C]PGH₂. Reverse-phase h.p.l.c. partition of the products shows a large peak of [¹⁴C]6-oxo-PGF_{1α} and a smaller one of [¹⁴C]PGE₂. The Sertoli cell may be the major cell of the seminiferous tubule to synthesize prostacyclin. Other testicular cells, spermatocytes, round spermatids and Leydig cells, each have a characteristic PGH₂-converting-enzyme activity profile, but none of these cells produce PGI₂ [32].

Validation of PGE₂ produced by adult control cells is also nearly quantitative, but is not for cells from the

Table 5. Effects of FSH and other additions on prostaglandin synthesis by primary cultures of Sertoli cells after short-term incubation

Sertoli cells in culture from 21-day-old rats (2.5×10^6 cells, 0.25 mg of protein) were incubated in culture media for 3 h at 34 °C with the following additions: ovine FSH (0.1 µg/ml); dibutyryl cyclic AMP (2 mM); mepacrine (0.2 mM); or Ca²⁺ ionophore A23187 (5 µM). PGs in the media were analysed directly by radioimmunoassay. Tubes containing cyclic AMP were extracted before determination of 6-oxo-PGF_{1α} owing to interference of the nucleotide with the assay.

Addition	Prostaglandin production (ng/mg of cell protein)	
	PHI ₂	PGE ₂
None	0.71	1.36
Ovine FSH	5.15	10.90
Dibutyryl cyclic AMP	1.80	0.26
Mepacrine	0.60	0.38
Ovine FSH + mepacrine	3.66	5.93
Ionophore A23187	11.70	10.42

vitamin E-deficient rat (40–50%), in which the PGE_2 fraction has compounds more polar than PGs. Significantly more $\text{PGF}_{2\alpha}$ is recovered from the media of vitamin E-deficient cultures than from the control. The PGF -silicic acid column effluents of adult cells contain products which partition on t.l.c. with R_F values similar to those of lipoxygenase products.

These products, more polar than PGs, include materials which are immunoreactive with LT and HETE antisera. LT values are increased 78–100% and HETE values 56–79% after addition of Ca^{2+} ionophore A23187 (10 μM), but addition of indomethacin (10 $\mu\text{g/ml}$) has little effect. The enhancement of the LT values by Ca^{2+} ionophore 23187 is a typical response of LT-producing cells and has been attributed to activation of 5-lipoxygenase.

DISCUSSION

Synthesis of PGs from endogenous substrate by the Sertoli cell is enhanced by FSH and affected by the differentiation of the cell and by dietary vitamin E. The PG profile of a cell reflects both substrate availability and the activity of eicosanoid-metabolizing enzymes. Potential biosynthetic precursors of eicosanoids, $\text{C}_{20:4}$ and C_{22} polyenes, comprise almost 30% of the total fatty acids of the Sertoli cell [33,34]. Evidence that $\text{C}_{20:4}$ is a precursor for PGs in the Sertoli cell includes PG synthesis by cells prelabelled with $[^3\text{H}]\text{C}_{20:4}$ and from PGH_2 . Whether C_{22} eicosanoids are also formed and/or affect oxygenation of $\text{C}_{20:4}$ is unknown. C_{22} polyenes are converted into eicosanoids by some [35,36], but not all, tissues [35,37]. PG synthesis by the Sertoli cell reflects active phospholipases, PG synthase and PGH_2 -converting enzymes (PGI_2 synthase, PGE isomerase and PG reductase).

$\text{PGF}_{2\alpha}$ is solely a product of the adult Sertoli cell. Differentiation could affect expression of a reductase. Both PGE 9-oxoreductase [38] and PGH_2 - PGD_2 11-oxoreductase [39] synthesize $\text{PGF}_{2\alpha}$. Rat testis has a very active PGE 9-oxoreductase, with maximal activity in the pre-pubertal testis [40], but $\text{PGF}_{2\alpha}$ is not produced by Sertoli cells from immature animals. Testis is not known to have an 11-oxoreductase.

$\text{PGF}_{2\alpha}$ synthesis and stimulation by FSH is provocative. The eicosanoid profile usually does not change unless a cell undergoes differentiation or transformation. Freshly isolated Sertoli cells are contaminated by germ cells. Both spermatocytes and round spermatids synthesize $\text{PGF}_{2\alpha}$, but this requires much larger numbers of these cells [32] than are present in Sertoli-cell preparations. Contact between Sertoli cells and germ cells may affect $\text{PGF}_{2\alpha}$ synthesis. During spermatogenesis, RNA, cytoplasm and residual bodies are shed and, along with degenerating germ cells, phagocytosed by the Sertoli cell. This results in increased lipid, believed to be a precursor of hormones controlling spermatogenesis [41].

$\text{PGF}_{2\alpha}$ production by adult cells may not be directly related to residual body ingestion, as the latter also occurs in cells of the adult control. There is an apparent correlation with exposure to degenerating germ cells. That cells cultured from vitamin E-deficient animals produce more $\text{PGF}_{2\alpha}$ than those of controls supports this hypothesis. Sertoli cells of vitamin E-deficient rats are exposed to larger numbers of degenerating germ cells *in vivo* than are the adult controls. Although both freshly

isolated and cultured cells from immature animals have similar PG profiles, cells from adult controls acquire the capability to synthesize $\text{PGF}_{2\alpha}$ after primary culture. Phagocytosis and differential activation of eicosanoid synthesis are linked in both mouse macrophages [42] and human monocytes [43]. Sertoli-cell-germ-cell interactions may be facilitated in a milieu of degenerating germ cells. Co-culture of germ cells with Sertoli cells stimulates DNA and RNA synthesis [44] and leucine metabolism [45]. Co-culture with peritubular cells results in transfer of RNA to the Sertoli cell [46]. Cell-cell contact, transmission of chemical messengers and phagocytosis may stimulate $\text{PGF}_{2\alpha}$ synthesis.

The effect of dietary tocopherol on Sertoli-cell PG synthesis appears as a shift in the species of products synthesized. Observed dietary effects of vitamin E deficiency on eicosanoid production include both decreased [12,47,48] and increased [49] synthesis as compared with controls. *In vitro*, vitamin E prolongs the viability of cultured Leydig cells and decreases the release of PGE_2 and $\text{PGF}_{2\alpha}$ [50]. The mechanisms by which vitamin E effects these changes are not clear. Vitamin E is a membrane component believed to stabilize membranes, decrease their fluidity and serve as an intracellular antioxidant. Several steps in the pathways of $\text{C}_{20:4}$ oxygenation are potential sites for antioxidant action. Dioxygenation and cyclization of $\text{C}_{20:4}$ to the endoperoxide, PGG_2 , proceeds by a free-radical process, as does the subsequent peroxidase conversion into PGH_2 . Lipid hydroperoxides are implicated in the regulation of PG synthase, PGI_2 synthase, and lipoxygenases [51]. Differences in the PG profiles of cells from control and vitamin E-deficient rats may indicate an effect of vitamin E on intracellular peroxides and peroxide tone rather than PG synthase, as the total amounts of PGs produced are similar.

PG synthesis is effectively inhibited by indomethacin in cells of the control; this is not the case for the cells from the adult, vitamin E-deficient, testes. Products occur which do not conform with PG standards, including LT and other lipoxygenase metabolites. The identity of these products is uncertain, as the LT antiserum used is not selective for the various 6-sulphidopeptide LTs [25], and the 12-HETE antiserum reacts (3–5%) with other HETEs and LTB_4 [24]. Both Sertoli-cell LTs and HETEs are enhanced by addition of ionophore A23187; 5-lipoxygenase, a cytosolic Ca^{2+} -dependent enzyme, requires a low level of peroxide for activity and is inactivated at higher peroxide concentration [52]. LT synthesis may reflect the activity of a testicular microsomal lipoxygenase [53] and the high γ -glutamyl transpeptidase activity of the Sertoli cell [54]. Both 15-lipoxygenase [55] and 5-lipoxygenase [56] are inhibited by addition of vitamin E *in vitro*. Lipoxygenase activity has been implicated in the sperm acrosome reaction and lutropin-stimulated steroidogenesis in rat Leydig cells [57]. Both rat tumour and testicular Leydig cells produce LTB_4 [58]. LTs, products characteristic of cells of the immune system, are produced by both the Leydig and Sertoli cells of the testis.

The more-than-10-fold increase in the total amounts of PGs produced by cells from adults than by either freshly isolated adult cells or cells cultured from immature rats may reflect enhanced phospholipase activity, changes in membrane physical properties or increased activities of eicosanoid-synthesizing enzymes.

A critical rate-limiting step for PG synthesis is the availability of substrate. Insulin, retinol and testosterone in the culture medium [59] could affect phospholipases of adult cells differently from those of immature cells. Changes in membrane physical properties during differentiation and membrane vitamin E could affect accessibility of target membrane phospholipids to phospholipase action. Lipids from the phagocytosed residual bodies may influence lipid pools and their availability. Altered membrane structure may increase or decrease the activity of membrane-bound enzymes and the turnover and oxygenation of $C_{20:4}$.

FSH-induced potentiation of PGs is one of the first events reported to respond to this hormone in Sertoli cells from adults. Prior exposure to steroid may facilitate a FSH response. Enhanced FSH responses have been shown in oestrogen-primed human Sertoli cells [60] and oestrogen-treated mature rats [61]. FSH effects on spermatogenesis are postulated to be mediated via stimulation of adenylate cyclase, cyclic AMP-dependent protein kinase and other events before these activities become constitutive in the adult Sertoli cell [2]. The PG response of immature cells to FSH is not affected by culture, is discernible after relatively short-term exposure to FSH and is observed in cells from hypophysectomized rats, in apparent agreement with FSH responses correlated with cyclic AMP. In contrast, neither of the analogues of cyclic AMP (8-bromo cyclic AMP or dibutyryl cyclic AMP) affect PG synthesis.

The FSH response is, however, blocked by mepacrine, which inhibits both phospholipases A_2 [62,63], and C [64]. By interacting with membrane phospholipid, mepacrine may perturb membrane structure [65]. Mepacrine has little effect on basal PG synthesis, but does inhibit FSH-stimulated synthesis. Activation of specific hormone-sensitive pools of $C_{20:4}$ [66,67] may explain the differences in FSH concentrations which enhance PGE_2 and PGI_2 . Higher doses of FSH are required to augment cyclic AMP than for aromatase activity or androgen-binding protein secretion by Sertoli cells [29]. Agonist-induced eicosanoid synthesis is linked to inositol trisphosphate and diacylglycerol as second messengers [68]. The latter mobilizes intracellular Ca^{2+} , and diacylglycerol activates protein kinase C. Protein kinase C activity has been described for freshly isolated Sertoli cells [69] as well as in primary cultures from adult animals [20]. Polyphosphatidylinositol turnover, release of Ca^{2+} and diacylglycerol and activation of calmodulin and phospholipid-dependent protein kinases may occur in Sertoli cells of both immature and adult animals in response to FSH stimulation.

The loss of FSH responsiveness of adult cultured cells appears to reflect primary culture conditions. Lack of response to FSH in adult Sertoli cells is not due to a decrease in the number of receptors. A variety of receptor agonists stimulate membrane turnover of phosphatidylinositols, but have little effect on cyclic AMP. FSH may be acting via an α -adrenergic response which remains functionally intact in the differentiated Sertoli cell, in contrast with the β -receptor-like response coupled with adenylate cyclase, which is refractory to FSH in the adult cell [2]. β -Adrenergic receptors have been identified in immature Sertoli cells [70]. That immature Sertoli cells may acquire such FSH-responsive β -receptors during culture, and that these receptors are not detectable in adult seminiferous tubules [71], has

recently been reported. Changes in the physical properties of membranes as a result of differentiation, membrane-bound vitamin E and cell-culture conditions may affect phospholipases and systems which are either membrane-bound or utilize lipid. Alterations in the activity of phospholipases would affect the availability of $C_{20:4}$ for oxygenation. Such changes could have profound effects on cell function, as suggested by a recent study showing that α - and β -adrenergic receptor coupling is regulated in an inverse manner and reciprocal manner by changes in phospholipase activity and is dependent on synthesis of a prostanoid [72]. FSH-stimulated PG production suggests activation of phospholipases. The lack of effect of FSH in the adult Sertoli cell after culture is accompanied by enhanced PG synthesis, which may represent fully activated phospholipase no longer responsive to the hormone.

The finding that Sertoli cells synthesize PGs and FSH potentiates PG production implicates a role for eicosanoids in spermatogenesis. PGE_2 and PGI_2 are potent activators of adenylate cyclase. PGs stimulate Sertoli-cell cyclic AMP production [73]. Since PGs function proximal to the site of synthesis, they may influence adenylate cyclase of neighbouring germ cells. Activators of germ-cell adenylate cyclase have not been characterized [74]. Sertoli-cell production of $PGF_{2\alpha}$ may be part of a feedback loop to signal germ-cell development. $PGF_{2\alpha}$ has been implicated in stimulating cell proliferation [75] and differentiation [76]. The effect of vitamin E on spermatogenesis may reflect changes in membrane organization, peroxide tone and the generation of eicosanoids, potential intratesticular regulators of gametogenesis.

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