Steroidogenesis and Testosterone Metabolism in Cultured Principal Cells from the Ram Epididymis

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To determine if ram principal cells can synthesize or metabolize testosterone, or metabolize other steroids present in rete testis fluid, principal cells from the initial segment, central caput, and proximal corpus epididymidis were isolated and cultured in a floating collagen matrix with medium containing 20% dialyzed rete testis fluid. In the first experiment, each matrix was washed twice in testosterone-free medium on day 2.8, transferred into culture medium containing 100 nM of a tritiated steroid and incubated for 4 hours at 34 C. The tritiated steroids were pregnenolone, 5-androstene-3β,17β-diol, progesterone, 4-androstene-3,17-dione, testosterone, and dihydrotestosterone. Since testosterone was not formed from 5-androstene-3β,17β-diol or 4-androstene-3,17-dione, testosterone synthesis by ram principal cells is unlikely. Pregnenolone and 5-androstene-3β,17β-diol were not metabolized and only slight metabolism of dihydrotestosterone occurred. Progesterone, 4-androstene-3,17-dione, and testosterone were metabolized to 5α-reduced products tentatively identified as 5α-pregnane-3,20-dione and 5α-pregnan-3β-ol-20-one and/or 5α-pregnan-20α-ol-3-one; 5α-androstane-3,17-dione and 5α-androstan-3α-ol-17-one, and dihydrotestosterone, respectively. The second experiment evaluated testosterone metabolism by both cultured principal cells and minced epididymal tissue. On day 1 of culture, during 12 hours the accumulation of dihydrotestosterone in medium from cells of the central caput was 48 × and 1.1 × that in medium from cells of the initial segment and proximal corpus epididymidis. The difference ($P < 0.01$) persisted on days 3 and 6 of culture, although testosterone metabolism decreased. For 3-hour incubations of minced tissue, testosterone metabolism by tissue from the central caput was 3.6 ×, 1.3 ×, and 0.9 × that of tissues from the initial segment, distal caput, and proximal corpus, respectively. Thus, testosterone metabolism in the initial segment is low compared with more distal regions.

Key words: steroidogenesis, testosterone metabolism, epididymis, cultured principal cells, ram.

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The epididymis is an androgen-dependent organ and is the site of sperm maturation. The process of sperm maturation is not fully understood, but it is mediated by the luminal environment (Amann, 1987; Robaire and Hermo, 1987) and requires secretion of proteins and other compounds by principal cells of the epididymal epithelium. Synthesis of some critical proteins requires the availability of dihydrotestoster-

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one (DHT)* (Orgebin-Crist and Jahad, 1978). Therefore, production of testosterone (T), and especially its 5α-reduced metabolites, by principal cells lining the epididymal duct should have a pivotal role in promoting sperm maturation. Testosterone is available to the epididymal epithelium in the blood and also in the rete testis fluid entering the proximal end of the ductus epididymidis (Waites, 1977). Local production would be an alternative source of T. Frankel and Eik-Nes (1970) found no formation of androstenedione or T from acetate, cholesterol, pregnenolone, progesterone, or dehydroepiandrosterone by rabbit epididymal tissue. However, Hamilton (1971) reported that rat, rabbit, and ram epididymal tissue can synthesize T from precursor steroids, cholesterol, or acetate. Thus, the question is unresolved. The published studies relied on minced or homogenized epididymal tissue so that conclusions concerning the steroidogenic capacity of principal cells could not be made.

For rats, the activity of Δ^4-3-oxosteroid-5α-oxidoreductase (5α-reductase) is highest in homogenates of tissue or in principal cells from the proximal portion of the epididymis (Robaire et al, 1977; Pujol and Bayard, 1978; Brown et al, 1983). Regional distribution of 5α-reductase in the ram epididymis has not been reported. The 5α-reductase present in epididymal tissue (Gloyna and Wilson, 1969; de Larminat et al, 1980; Robaire et al, 1981) is mostly in the epithelium, with little activity in the stroma (Djoseland et al, 1983), and is localized in principal cells but absent in basal cells (Klinefelter and Amann, 1980).

In addition to T, rete testis fluid also contains appreciable concentrations of other steroids (Waites, 1977) that might serve as precursors for T or as alternative substrates for 5α-reductase. In bovine rete testis fluid, progesterone, dehydroepiandrosterone, and androstenedione are present at 4, 22, and 8 ng/ml, respectively, as compared to 33 ng/ml for T (Ganjam and Amann, 1976). Homogenates of rat or hamster epididymal tissue convert progesterone and androstenedione to 5α-reduced steroids (Inano et al, 1969; Setty et al, 1983; Scheer and Robaire, 1983; Tsuji et al, 1984) including 5α-androstane-3,17-dione, which could be reduced to DHT.

The objectives of these experiments were to (a) establish if ovine principal cells can synthesize T and to determine what steroidogenic enzyme systems are active in these cells, and (b) determine if there are regional differences in the metabolism of T by principal cells.

Materials and Methods

Experiment 1

This study evaluated steroidogenesis in cultured principal cells from three regions of the epididymis (initial segment, central caput, and proximal corpus) using one concentration of each of six substrates. The tritiated steroids used as substrates were pregenolone, 5-androstene-3β,17β-diol, progesterone, androstenedione, T and DHT. Metabolites were identified by a combination of chromatographic systems, but since gas-liquid chromatography-mass spectroscopy was not used, identifications must be considered as tentative. The substrate concentrations providing maximum metabolism were not determined and a single time point was used. Kinetic interpretations were not desired and should not be made.

The procedure for culture of principal cells is outlined below. Within each isolation (replicate) of principal cells, duplicate matrices of cultured cells were used for each treatment for each region, except when insufficient cells were obtained from the initial segment. In that case, only one matrix per replicate was used. Incubations using cell-free matrices were included in each replicate. The culture medium contained 20% dialyzed ovine rete testis fluid.† From day 0 through day 2.8, the culture medium contained 40 μM T.

At day 2.8, each matrix was washed twice with culture medium devoid of rete testis fluid or steroid and transferred into fresh medium containing rete testis fluid plus 100 nM of tritiated steroid (10^6 dpm; added in 10 μl of 70% ethanol to the 3 ml of medium) and incubated for 4 hours at 34 C on an orbital shaker (40 rpm) in 5% CO_2 in humidified air. At the end of the 4-hour incubation, the matrix was removed from the medium and the tritiated steroids were extracted from the culture medium using an extraction column and isolated and quantified by high performance liquid chromatography (HPLC) coupled to a flow-through radioactivity detector (see below).

Experiment 2

This study tested the hypotheses that there are regional differences in accumulation of 5α-reduced metabolites of T and that these differences are not a consequence of rapid

*Trivial names used for steroids are: 3α-androstanediol = 5α-androstane-3α,17β-diol; 3β-androstanediol = 5α-androstane-3β,17β-diol; androstenedione = 4-androstene-3,17-dione; dehydroepiandrosterone = 5-androsten-3β-ol-17-one; dihydrotestosterone (DHT) = 5α-androstan-17β-ol-3-one; pregnenolone = 5-pregnen-3β-ol-20-one; progesterone = 4-pregnene-3,20-dione; and testosterone (T) = 4-androsten-17β-ol-3-one. Figure. 2, in the discussion, depicts some of the molecules and enzymes potentially involved in steroidogenesis.

†Ovine rete testis fluid, collected by indwelling catheter from several rams, was pooled and dialyzed against five changes of medium during 60 hours at 4 C.

loss of 5α-reductase activity by cultured principal cells. A 3 × 3-factorial design, replicated three times, was used to evaluate the three regions (initial segment, central caput, and proximal corpus epididymidis) and day of culture (day 1, 3, or 6). The culture medium contained 20% dialyzed rete testis fluid. Metabolism of T was measured by incubating the matrices for 12 hours in culture medium containing 5 μM [^{3}H]T for the initial segment or 20 μM [^{3}H]T for the central caput and proximal corpus epididymidis on day 1. Between days 1 and 2.5 or 3 and 5.5, matrices of cells were cultured in medium containing 20 μM T and T metabolism was measured on days 3 and 6 as described above, except that on day 6, the initial segment was incubated in medium containing 1 μM [^{3}H]T.

Experiment 2 also tested the hypothesis that the ratios of 5α-reduced metabolites to testosterone were similar in the culture medium and within the hydrated cell matrix. At the end of each 12-hour incubation for measurement of T metabolism (day 1, 3, or 6), duplicate matrices from each region-treatment were transferred from culture dishes into a 15-ml centrifuge tube containing 3 ml of new culture medium and sonicated (two 30-second intervals at 100 watts) on ice. The tubes then were centrifuged (700 × g; 20 minutes) and the tritiated steroids immediately were extracted from the supernatant. The tritiated steroids in the medium used for culture also were assayed.

To corroborate the data with cultured principal cells, aliquots of tissue from the initial segment, central caput, distal caput, and proximal corpus, each from the same epididymis, were minced into 1 to 2-mm^3 pieces immediately after castration. Minced tissue (185 to 215 mg) for each region was incubated in 3 ml of culture medium containing 20% dialyzed rete testis fluid and 40 μM [^{3}H]T in quadruplicate 25-ml flasks. Each flask was gassed with 95:5 O_2:CO_2 at the beginning and middle of the incubation (with shaking) at 34 C for 3 hours. The contents of each flask were transferred to a 15-ml centrifuge tube and the flask was rinsed with 2 ml of culture medium; the sample was frozen at −70 C. At the time of assay, the samples were thawed at 34 C, sonicated and the tritiated steroids were extracted as described below.

The overall factorial analysis of variance revealed a difference in accumulation of 5α-reduced metabolites of T associated with epididymal region and day of culture. Therefore, data for each region and day of culture were analyzed separately and treatment means were compared using Duncan's new multiple range test. In addition, a paired *t*-test was used to determine if the mean difference was zero between data for medium or cell matrix.

Isolation and Culture of Principal Cells

Principal cells were isolated by enzymatic dissociation and elutriation from 2.0 to 2.5 g of tissue representing the initial segment, central caput, or proximal corpus epididymidis (Wagley et al, 1984) of mature rams castrated under local anesthesia during a light-induced (Exp. 1) or natural (Exp. 2) breeding season. Isolated principal cells were aliquoted to six to 12 culture dishes per region and were mixed with a collagen solution that was allowed to gel; cell-containing matrices (2.5 × 10^6 principal cells/35-mm matrix) resulted. Each matrix was cultured at 34 C, free-floating, in an individual 60-mm tissue culture dish as described by Wagley et al (1984), except that bovine serum albumin was deleted from the culture medium. The culture medium (Brown et al, 1983) consisted of Eagle's MEM (Gibco #410-1500) plus 2 μg/ml gentamycin sulfate (Sigma G-3632), 1 mM sodium pyruvate (Sigma P-2256), 1 mM L-carnitine (Sigma C-7393), 5 μg/ml transferrin (Sigma T-2252), 1 μg/ml retinol (Sigma R-2750), 100 nM insulin (Sigma I-5500), 400 nM hydrocortisone (Sigma H-4001) and 20% dialyzed ovine rete testis fluid.

Measurement of Testosterone Metabolism

Preliminary experiments tested the linearity of T metabolism over time and concentration effects. Matrices of principal cells from the distal caput and proximal corpus epididymidis (three replicates each) were cultured with 5 μM [^{3}H]T on day 2.5 to 3.0 for 1.5, 3, 6, 9, 12, or 15 hours. Since the accumulation of 5α-reduced metabolites of T was linear through 15 hours (≤ 70% of substrate was metabolized), a 12-hour incubation was used for all measurements of T metabolism. With cultured principal cells, the concentration of T available to the intracellular 5α-reductase is regulated by cellular transport mechanisms, and not solely by substrate concentration in the medium, as would be true for an extract or homogenate. Nevertheless, in a preliminary experiment we established that the concentration of [^{3}H]T (10, 15, or 25 μM) in the culture medium during a 12-hour assay had little effect (≤ 20% difference; N = 4) on accumulation of 5α-reduced metabolites with cultures of cells from the central caput epididymidis, provided at least 20% of the substrate remained.

Metabolism of [^{3}H]T was evaluated by measuring accumulation during 12 hours of [^{3}H]DHT and [^{3}H]3α-androstanediol (the only 5α-reduced metabolites detected) in the culture medium. Concentration of substrate T was adjusted for region and day of culture so that 40 to 95% of the initial mass of T remained after 12 hours, but in each case, 10^6 dpm of [^{3}H]T was in the 3 ml of culture medium. The [^{3}H]T and its metabolites were extracted from the culture medium and quantified by HPLC as described below. Of the ^{3}H placed in the culture dish, about 75% was recovered in the 200 μl of extract used for HPLC and most of the remaining steroid (about 20% of the total) could be recovered from the hydrated cell matrix by 10 minutes of further incubation in steroid-free medium followed by extraction of this medium (see Exp. 2). Both of these extracts were of similar composition. Extraction and isolation of tritiated steroids from duplicate cultures of principal cells gave intraassay coefficients of variation of 8.7 and 15.3% for day 3 or 6 of culture.

Steroid Extraction and Quantification

The extraction procedure used a 1-ml octadecylsilane C_{18} extraction column (J.T. Baker) fitted with a 2.5-ml glass syringe. The column was activated by successive applications of 3 ml absolute acetonitrile and 3 ml water. The < 3 ml of culture medium followed by 3 ml water and

1 ml of 15% acetonitrile in water (to remove polar compounds including the phenol red from the culture medium) were passed through the column and steroids were eluted using 1.0 ml of 80% acetonitrile in water. The 1-ml eluate was evaporated to dryness under air; the steroids were dissolved in 200 μl of 80% acetonitrile in water and stored at 5 C until assayed. Since the culture medium, water and 15% acetonitrile eluted from the extraction columns were not routinely checked for ^3H, the possible formation of highly polar steroids (not retained by the column) cannot be excluded. A vacuum manifold (J.T. Baker) was used to process up to 10 samples simultaneously. Extraction columns were reused up to five times by washing with 3 ml absolute acetonitrile and 3 ml water; no change in recovery of a known mass of [^3H]T occurred.

Recoveries of seven tritiated steroids from culture medium were measured in two preliminary experiments (quadruplicate extractions for four or five independent replicates) and were 72.3 ± 0.8% for pregnenolone, 92.2 ± 0.6% for 5-androstene-3β,17β-diol, 83.6 ± 1.8% for progesterone, 104.8 ± 2.0% for androstenedione, 103.0± 1.8 or 100.7 ± 0.9% for T, 94.5 ± 0.7 or 99.2 ± 0.9% for DHT, 97.5 ± 2.0% for 3α-androstanediol, and 90.8 ± 1.5% for estradiol. Pooled across steroids, the intra- and interreplicate coefficients of variation for the recovery of steroids through the extraction were 2.8 and 5.2%. Since recovery data were not available for all potential metabolites, data were not corrected for differences in recovery during the extraction process.

The tritiated steroids were isolated and quantified by HPLC (Dupont Model 870 pump, Model 860 column compartment equipped with a Supelcosil LC-18® column at 40 C) with on-line measurement of ^3H using a Flo-one HP radioactivity detector (Radiomatic Instruments Co., Tampa, FL). The isocratic elution used 1.5 ml/min of 7:3 (v/v) methanol and water. The elution time of each unknown tritiated steroid was compared with those of authentic tritiated steroids (Table 1). There was no evidence of tritiated steroids eluting early or late. Based on duplicate, independent HPLC isolations of 82 extracts of culture medium, the coefficient of variation for analytic error was 3.2%. When calculated from triplicate injections of a standard mixture of tritiated steroids each day HPLC analyses were made, the intra- and interassay coefficients of variation for analytic error were 2.2 and 5.5% for T, 3.0 and 8.0% for DHT and 1.4 and 4.3% for progesterone, respectively.

The relative amount(s) of each tritiated metabolite (M_1 or M_2; e.g., DHT and 3α-androstanediol) formed from a given tritiated substrate (S; e.g., T) was calculated as the ratio (expressed as percent) of cpm for M_1 to the total cpm for $S + M_1 + M_2$ detected in the HPLC eluate. Values reported may be slight underestimates since, under some conditions (see Exp. 2), the hydrated matrix contained a greater concentration of metabolites than the assayed culture medium. Nevertheless, since the medium routinely assayed contained about 75% of the total tritium, and an additional 20% could be washed from the hydrated matrix, it is unlikely that major metabolites went undetected. Under the conditions of Exp. 1, 1% conversion of a tritiated substrate to a tritiated metabolite should have been detected as > 400 counts of tritium above background.

TABLE 1. Elution Times of Tritiated Steroid Standards and of Tritiated Substrates and Tritiated Metabolites Isolated from Culture Medium

Substrate and Potential Metabolites	Elution Time (min)*	
	Standards	Extract
Pregnenolone	10.7	10.6 ± 0.2
Dehydroepiandrosterone	5.8	ND
17α-ol-5-pregnen-3β-ol-20-one	NS	ND
Progesterone	8.4	ND
Progesterone	8.4	8.4 ± 0.1
4-pregnen-20α-ol-3-one	8.2	ND
17α-ol-4-pregnene-3,20-dione	5.4	ND
5α-pregnane-3,20-dione	NS	12.7 ± 0.2
5α-pregnan-20α-ol-3-one	NS	12.7 ± 0.2
5α-pregnan-3α-ol-20-one	NS	ND
5α-pregnan-3β-ol-20-one	NS	12.7 ± 0.2
5-androstene-3β,17β-diol	5.3	5.3 ± 0.1
Testosterone	5.2	ND
Androstenedione	4.8	4.7 ± 0.2
Testosterone	5.2	ND
5α-androstane-3,17-dione	NS	6.4 ± 0.1
Dihydrotestosterone	6.9	ND
5α-androstan-3α-ol-17-one	8.8	8.9 ± 0.1
Testosterone	5.2	5.2 ± 0.1
5α-androstane-3β,17β-diol	6.1	ND
Dihydrotestosterone	6.9	6.9 ± 0.1
5α-androstane-3α,17β-diol	8.2	ND
Dihydrotestosterone	6.9	6.9 ± 0.1
5α-androstane-3β,17β-diol	6.1	ND
5α-androstane-3α,17β-diol	8.2	8.2 ± 0.2

*Elution time to the tritium detector when using a Supelcosil LC-18 column (4.5 × 125 mm) operated at 40 C with 7:3 methanol:water at 1.5 ml/min. Several potential 5α-reduced metabolites were unavailable as a tritiated steroid (NS) and could not be detected by UV absorbance. Potential metabolites not detected in the extracts of culture medium are designated ND. At least two metabolites of progesterone comigrated under these conditions; by other systems, one was tentatively identified as 5α-pregnane-3,20-dione and the other(s) as 5α-pregnan-20α-ol-3-one and/or 5α-pregnan-3β-ol-20-one.

Identification of Steroids

Based on elution volumes during the initial HPLC analyses (Table 1), it was suspected that progesterone had been metabolized to 5α-pregnane-3,17-dione and possibly other 5α-reduced metabolites, and that androstenedione had been metabolized primarily to 5α-androstane-3,17-dione. Identification of the latter metabolite was supported by data from thin-layer chromatography (see below) and HPLC using 1:1 acetonitrile:water (for superior resolution of androstenedione and T) before and after differential reduction using $NaBH_4$. Aliquots of an extract containing both substrate [^3H]androstenedione and tri-

tiated metabolite(s) (2×10^5 dpm) were mixed with 3 nmol of androstenedione and dried in a tube. Within a few minutes, 25 μl of fresh, ice-cold $NaBH_4$ (1.0 mg/ml) were added to the tube, which was vortexed, and after 4, 10, or 60 seconds the reaction was stopped by adding 3 drops of glacial acetic acid followed by 0.5 ml of water. The reaction mixture was vortexed and returned to the ice bath. After 5 to 10 minutes, 2 ml of culture medium were added to the tube, the *p*H was adjusted to neutrality, and the steroids were extracted and analyzed by HPLC as described above. Under these conditions (Ganjam, 1976; Ganjam and Amann, unpublished data), androstenedione is converted almost entirely to T (plus small amounts of 3β-androstanediol). It was anticipated that if the unknown steroid was 5α-androstane-3,17-dione, it should be reduced to DHT (detectable at 4 seconds), which, in turn, would be reduced to 3β-androstanediol (predominant at 10 or 60 seconds). Similarly, 5α-androstan-3α-ol-17-one would be reduced to 3α-androstanediol. Two different samples of the unknown metabolite were analyzed in this manner and the expected steroids were detected. At 0, 4, 10, and 60 seconds, tritiated metabolites with elution times identical to those of 5α-androstane-3,17-dione represented 65, 0, 0, and 0% of the mixture, respectively; DHT 0, 32, 18, and 0%; 3β-androstanediol 0, 50, 65, and 65%; 5α-androstan-3α-ol-17-one 9, 9, 9, and 0%; and 3α-androstanediol 0, 6, 6, and 12%, respectively. At 0 seconds, [^{3}H]androstenedione represented 18% of the radioactivity and was reduced to T within 10 seconds.

Other aliquots of the extracts containing [^{3}H]progesterone and metabolites were chromatographed on a Zorbax ODS column using 53.5:47.5 acetonitrile:water. The relative elution time of putative [^{3}H]5α-pregnane-3,20-dione was similar to that reported for authentic standard using the same column (Francis and Kinsella, 1984), although a peak thought to be [^{3}H]5α-pregnan-20α-ol-3-one or [^{3}H]5α-pregnan-3β-ol-20-one also was detected.

To substantiate the tentative identity of tritiated metabolites, the mixtures, as well as individual standards, were subjected to thin-layer chromatography using Gelman ITLC SA plates pre-treated with propylene glycol:acetone (1:4) and carbon tetrachloride:cyclohexane (9:1) as the mobile phase (Goldman and Klingele, 1974; Hammerstedt and Amann, 1976). Aliquots of the extracts of incubations with [^{3}H]progesterone and [^{3}H]androstenedione, which had not been subjected to HPLC, were mixed with authentic 5α-pregnane-3,20-dione, 5α-pregnan-3α-ol-20-one, 5α-pregnan-3β-ol-20-one, 5α-pregnan-20α-ol-3-one and 17α-ol-4-pregnene-3,20-dione or T, 5α-androstane-3,17-dione and 5α-androstan-3α-ol-17-one. Similarly, tritiated metabolites of [^{3}H]progesterone isolated by HPLC using the Zorbax column were subjected to thin layer chromatography. Androgens were treated with primuline and visualized under ultraviolet light while progestins were exposed to iodine vapor. Spots of carrier steroids and all other portions of each lane were cut from the fiberglass plates and extracted with scintillation fluid; each extract was assayed for radioactivity. The tritiated steroids from the incubation media comigrated with the expected authentic steroids, but putative [^{3}H]5α-pregnan-3β-ol-20-one and/or [^{3}H]5α-pregnan-20α-ol-3-one were not identified unequivocally.

Results

Steroidogenesis

Metabolism of pregnenolone and 5-androstene-3β,17β-diol was not detected (Table 1). Progesterone and androstenedione were not metabolized to 17α-ol-progesterone or T, respectively (Table 1). Consequently, 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase probably are not present in principal cells of the ram epididymis or, if present, did not metabolize the substrates tested under the conditions used. Furthermore, since pregnenolone and 5-androstene-3β,17β-diol were not metabolized to progesterone and T, the 3β-hydroxysteroid dehydrogenase plus isomerase complex probably is absent in ovine principal cells. Consequently, we concluded that principal cells from the initial segment, central caput or proximal corpus epididymidis of the ram cannot synthesize T from the four substrates tested.

Metabolism of progesterone to 5α-pregnane-3,20-dione, androstenedione to 5α-androstan-3,17-dione, and T to DHT by ovine principal cells was evident (Tables 1 and 2). Thus, 5α-reductase must be present in all three regions studied.

Since putative 5α-pregnane-3,20-dione or 5α-androstane-3,17-dione and authentic DHT were metabolized (Table 2), 3α-hydroxysteroid dehydrogenase(s) acting on these substrates was in principal cells. The ratio of 5α-pregnane-3,20-dione to 5α-pregnan-20α-ol-3-one (or 5α-pregnan-3β-20-one) was not determined for all samples because of comigration on the Supelcosil column used for initial analyses. However, the Zorbax column separated 5α-pregnane-3,20-dione from the other metabolite(s) and ≥95% of the radioactivity associated with 5α-pregnanes eluted as a peak later isolated and identified by thin layer chromatography as [^{3}H]5α-pregnane-3,20-dione. The tritiated metabolites in the other fraction isolated by HPLC comigrated with both 5α-pregnane-20α-ol-3-one and 5α-pregnan-3β-ol-20-one in thin layer chromatograms. For two pools of metabolite(s) produced by cultures of principal cells from the central caput epididymidis, isolated by HPLC and subjected to thin layer chromatography, the ratios of 5α-pregnane-20α-ol-3-one to 5α-pregnan-3β-ol-20-one were 2.2:1 and 5.2:1. It was concluded that 5α-pregnane-20α-ol-3-one was produced from progesterone. Thus, 20α-hydroxysteroid dehydrogenase was detected and 20β-hydroxysteroid dehydrogenase also may be present.

Although 5α-androstan-3α-ol-17-one was produced from 5α-androstane-3,17-dione (formed from

TABLE 2. Metabolites of 100 nM Tritiated Steroids Secreted by Cultured Principal Cells from Three Regions of the Epididymis*

Substrate and Metabolites†	Principal Cells From Initial Segment	Central Caput	Proximal Corpus
Progesterone	91 ± 3	51 ± 2	78 ± 2
5α-pregnanes‡	9 ± 3	48 ± 2	22 ± 2
Androstenedione	29 ± 2	29 ± 1	11 ± 1
5α-androstane-3,17-dione	71 ± 2	71 ± 1	89 ± 1
5α-androstan-3α-ol-17-one	2 ± 1	7 ± 1	4 ± 1
Testosterone	82 ± 4	45 ± 2	38 ± 2
Dihydrotestosterone	18 ± 4	55 ± 2	62 ± 2
3α-androstanediol	-	4 ± 1	-
Dihydrotestosterone	97 ± 1	94 ± 1	99 ± 1
3α-androstanediol	3 ± 1	6 ± 1	1 ± 1

*Least squares mean (± SEM) percentage of recovered tritiated steroids based on HPLC; steroids identified by elution time before or after $NaBH_4$ reduction, use of alternative HPLC conditions, and thin-layer chromatography.

†Metabolism of pregnenolone and 5-androstene-3β,17β-diol was not detected (< 1%).

‡5α-pregnane-3,20-dione plus 5α-pregnan-3β-ol-20-one and/or 5α-pregnan-20α-ol-3-one were identified, but not quantified for all replicates, by HPLC in alternative systems and by thin-layer chromatography.

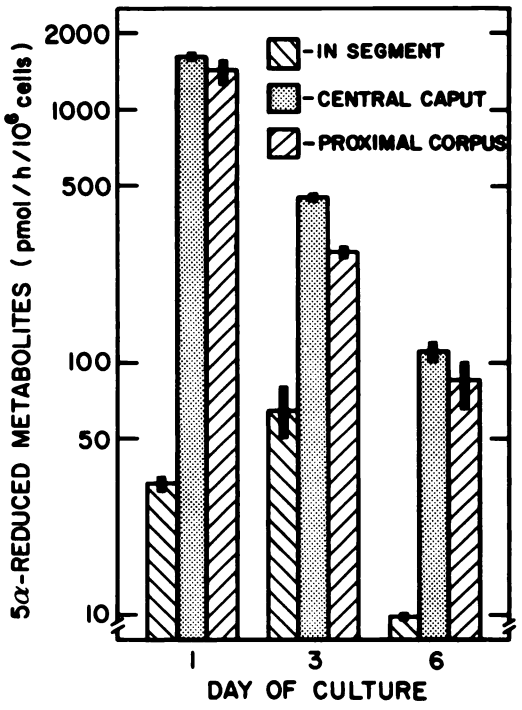

Fig. 1. Effects of region of origin for principal cells (initial segment, central corpus, or proximal corpus epididymidis) and day of culture on accumulation of 5α-reduced metabolites of [^{3}H]T. The medium contained 20% dialyzed rete testis fluid. Means ± SEM for three replicates; note log scale.

androstenedione) and 5α-androstane-3α,17β-diol was produced from DHT, these apparently were relatively minor metabolites (< 10% of the metabolites recovered; Table 2). Thus, 3α-hydroxysteroid dehydrogenase was detected, but there was no evidence for a 3β-hydroxysteroid dehydrogenase acting on androgens (Table 1).

Regional Differences in Testosterone Metabolism

The ancillary data demonstrated that it was valid to analyze only the medium for tritiated steroids since the ratios of substrate T to 5α-reduced metabolites in the culture medium and in the hydrated cell matrix were not different ($P > 0.05$) for cells from each of the three regions on both days 3 and 6 of culture. On day 1, however, for the central caput and proximal corpus epididymidis, the apparent rate of accumulation of T metabolites (primarily DHT) was greater ($P < 0.05$) if the cell matrix (cells plus retained medium) was analyzed rather than the culture medium (1754 vs. 1632 and 1576 vs. 1428 pmol/h/10^6 principal cells for the central caput and proximal corpus, respectively).

Accumulation of 5α-reduced metabolites of T in the culture medium on day 1 for cells from the central caput was 48 × that for cells from the initial segment and 1.1 × that for cells from the proximal corpus (Fig. 1). The difference ($P < 0.01$) between values for the initial segment and the two distal regions persisted on days 3 and 6. There was no effect ($P > 0.05$) of day of culture on T metabolism in principal cells from the initial segment. For the central caput and proximal corpus, however, T metabolism was greater ($P < 0.05$) in principal cells on day 1 than on day 3 and on day 3 than day 6 (Fig. 1). Between day 1 and 3 of culture, T metabolism decreased by 72% for the central caput and 81% for the proximal corpus. Between days 3 and 6 of culture, T metabolism decreased by a mean of 38% across all three regions. Values for cells from the central caput and proximal corpus were different ($P < 0.05$) only on day 3.

For minced epididymal tissue, the accumulation of 5α-reduced metabolites of T over 3 hours with tissue from the central caput was 3.6 × that for tissue from the initial segment, 1.3 × that for tissue from the distal caput and 0.9 × that for tissue from the prox-

imal corpus epididymidis (133 ± 3, 37 ± 5, 100 ± 3, and 152 ± 4 pmol/h·mg^{-1} of tissue). Thus, the low rate of T metabolism by cultured cells from the initial segment was not an artifact induced by cell isolation and culture.

Discussion

By use of cultured principal cells, confounding influences from spermatozoa, smooth muscle cells or fibroblasts are eliminated and conclusions concerning steroidogenesis in principal cells, the major component of the epithelium, can be made. This study demonstrated that cultured principal cells from three regions of the ovine epididymis cannot synthesize T by the classical pathways (Fig. 2). Consequently, the substrate T used for production of DHT by the epididymal epithelium probably is taken from rete testis fluid or blood.

Based on studies with minced epididymal tissues from several species including the ram, there are conflicting data concerning T synthesis (Inano et al, 1969; Frankel and Eik-Nes, 1970; Hamilton and Fawcett, 1970; Hamilton, 1971). Also, homogenates of rat epididymal tissue have been reported to metabolize pregnenolone to several metabolites (Inano et al, 1969; Frankel and Eik-Nes, 1970). The apparent difference between our results and data reported by others may reflect species differences, analytic approach, or the fact that we studied intact principal cells rather than minced or homogenized tissue.

Although considerable 5α-androstane-3,17-dione was produced from androstenedione, neither T nor DHT was detected. Thus, a 17β-hydroxysteroid dehydrogenase active on androstenedione or 5α-androstane-3,17-dione probably is not present in principal cells. It is unlikely that intracellular NADPH was deficient since this cofactor also is necessary for 5α-reductase, which clearly was active. Consequently, the 5α-androstane-3,17-dione produced by principal cells from the proximal epididymis cannot be converted directly to DHT in these regions. Therefore, androstenedione probably is not an alternative to T as a source for the DHT necessary for sperm maturation.

Metabolism of T by cells from the central caput was 6 × greater than by cells from the initial segment of the ram epididymis (Fig. 1), which contrasts markedly to data based on cultured principal cells

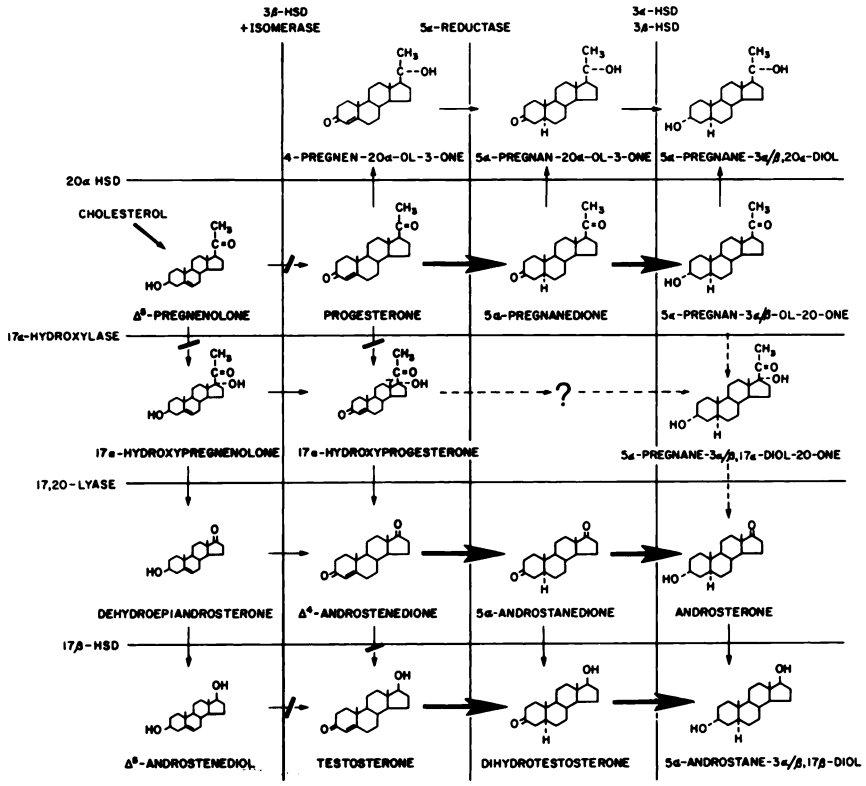

Fig. 2. Potential steroidogenic pathways for the production of T and for the formation of 5α-reduced metabolites of progesterone, androstenedione, and T. Based on evidence presented, conversions occurring in ram principal cells are shown with a heavy arrow; other reactions cannot be excluded. Metabolism of pregnenolone or 5-androstene-3β,17β-diol was not detected nor was formation of 17α-ol-progesterone from progesterone.

from the proximal or distal portions of the rat caput epididymitis (Brown et al, 1983) or homogenates of rat tissue (Robaire et al, 1981), but is consistent with data for minced bovine epididymal tissue (Hammerstedt and Amann, 1976). Incubation of minced ovine epididymal tissue with [^{3}H]T confirmed the data for cultured principal cells, showing that metabolism of T is at a low level in the initial segment of the ram epididymis as compared with the central caput, distal caput, or proximal corpus epididymidis.

It is unlikely that principal cells in the initial segment of the ram epididymis require DHT for their function, since there is little DHT in blood or rete testis fluid (Waites, 1977) and these principal cells do not rapidly metabolize T to DHT. Thus, as conceptualized by Glover and Nicander (1971) and discussed by Amann (1987), the specific localization of function along the length of the epididymis must be different in rats and rams. Nevertheless, in all species studied, the metabolism of T to 5α-reduced androgens is greater in the caput epididymidis than in more distal regions. Therefore, secretion of 5α-reduced metabolites into the luminal fluid in the caput epididymidis may provide the DHT essential for epithelial function in more distal regions of the epididymis.

The conditions of culture in Exp. 2 (30 or 120 nmol T in 3 ml of medium containing $<$ 15 nmol albumin and 19 pmol ABP from 20% rete testis fluid) dictated that most of the steroid in the medium was free rather than bound. This probably is the opposite of the situation *in vivo* (Turner et al, 1984) and may have influenced T metabolism (Lasnitzki and Franklin, 1972; Mercier-Bodard et al, 1976). However, use of lower concentrations of T in the culture medium resulted in virtually complete metabolism to DHT. Product inhibition of 5α-reductase by extracellular accumulation of DHT apparently does not occur *in vivo*, did not occur with our cultures, and also did not occur with monkey epididymal tissue (de Larminat and Blaquier, 1978).

The conversion of progesterone, androstenedione, and T to 5α-reduced metabolites by cultured principal cells is consistent with previous reports concerning activity of 5α-reductase in principal cells (Klinefelter and Amann, 1980; Brown and Amann, 1984) and in minced or homogenized epididymal tissue (Inano et al, 1969; Frankel and Eik-Nes, 1970; Setty et al, 1983; Scheer and Robaire, 1983; Tsuji et al, 1984). The 5α-pregnane-3,17-dione or 5α-androstane-3,17-dione produced by principal cells might have a role in controlling cellular function within the epididymis as has been shown for the former steroid in the case of neural tissue (Zanisi et al, 1984).

It is established that both progesterone and androstenedione are competitive, or mixed, inhibitors that compete with T for sites on 5α-reductase. In addition to inhibition studies using homogenates of rat epididymal tissue (Scheer and Robaire, 1983), Brown and Amann (1984) demonstrated that progesterone is inhibitory to T metabolism in cultured rat principal cells. They speculated that progesterone present in rete testis fluid or blood might modulate metabolism of T by the epididymal epithelium and have a role in control of the localized production of DHT within the epididymis. The suppression of epididymal 5α-reductase is a possible approach for male contraception, and may be involved in the anti-epididymal effect of progesterone (Lubicz-Nawrocki, 1973; Lubicz-Nawrocki and Chang, 1973).

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Message from the Awards Committee

Beginning in 1988, the Distinguished Andrologist and Young Andrologist Awards will be announced and presented at the annual meeting. Previously the recipients were announced a year in advance of the award presentations. Because awardees have been selected by the 1987 committee for recognition in 1988, nominations for these awards will not be solicited this year.

Student awards to be presented at the 1988 meeting include The New Investigator Award ($500) and Student Travel Awards (five $100 awards). All students, both members and nonmembers of the Society, are eligible to compete for these merit awards provided they are still in training and are within four years of having received a final degree. These awards are given for the best original laboratory or clinical research reports presented at the Annual Meeting. To be considered for these awards, students should check the appropriate box on the 1988 abstract form and secure the signature of their advisor to verify their student status.