Androgen metabolism and actions in rat ventral prostate epithelial and stromal cell cultures

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The rat ventral prostate requires androgens for normal development, growth, and function. To investigate the relationship between androgen metabolism and its effects in the prostate and to examine differences between the epithelial and stromal cells, we have established a system of primary cell cultures of immature rat ventral prostate cells. Cultures of both cell types after reaching confluency (6–7 days) actively metabolized ^{3}H-labelled testosterone (T), 5α-dihydrotestosterone (5α-DHT), 5α-androstane-3α,17β-diol, and 5α-androstane-3β,17β-diol. The epithelial cells actively reduced T to 5α-DHT and formed significant amounts of 5α-androstane-3,17-dione from T, 5α-DHT, and 5α-androstane-3α,17β-diol. All substrates were converted to significant amounts of $C_{19}O_3$ metabolites. The stromal cells also metabolized all substrates, but very little 5α-androstane-3,17-dione was formed. The metabolism studies indicate that both cell types have Δ^4-5α-reductase, 3α- and 3β-hydroxysteroid oxidoreductase and hydroxylase activities. The epithelial cells have significant 17β-hydroxysteroid oxidoreductase activity. The epithelial cells cultures grown in the presence of T have higher acid phosphatase (AP) contents (demonstrated histochemically and by biochemical assay). Tartrate inhibition studies indicate that the epithelial cells grown in the presence of T are making secretory AP. Stromal cell AP is not influenced by T. The results indicate that the cultured cells maintain differentiated prostatic functions: ability to metabolize androgens and, in the case of the epithelial cells, synthesize secretory AP.

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La prostate ventrale des rats requiert des androgènes pour se développer, croître et fonctionner normalement. Pour rechercher la relation entre le métabolisme des androgènes et leurs effets dans la prostate et pour examiner les différences entre les cellules épithéliales et les cellules du stroma, nous avons établi un système de cultures cellulaires primaires des cellules prostatiques ventrales de rats immatures. Après atteinte de la confluence (6–7 jours), les cultures des deux types de cellules métabolisent activement la [^{3}H]testostérone (T), la 5α-dihydrotestostérone (5α-DHT), 5α-androstane-3α,17β-diol et le 5α-androstane-3β,17β-diol. Les cellules épithéliales réduisent activement la T en 5α-DHT et forment des quantités importantes de 5α-androstane-3,17-dione à partir de la T, de la 5α-DHT et du 5α-androstane-3α,17β-diol. Tous les substrats sont convertis en quantités importantes de métabolites $C_{19}O_3$. Les cellules du stroma métabolisent aussi tous les substrats, mais la formation de 5α-androstane-3,17-dione est minime. Les études du métabolisme montrent que les deux types de cellules possèdent des activités Δ^4-5α-réductasique, 3α, et 3β-hydroxystéroïde oxydoréductasique et hydroxylasique. Les cellules épithéliales ont une activité 17β-hydroxystéroïde oxydoréductasique importante. Les cultures des cellules épithéliales croissant en présence de T ont des teneurs plus élevées de phosphatase acide (AP) (essais histochimique et biochimique). Les études d'inhibition par le tartrate montrent que les cellules épithéliales croissant en présence de T fabriquent une AP sécrétoire. L'AP des cellules du stroma n'est pas influencée par la T. Les résultats démontrent que les cellules cultivées maintiennent des fonctions prostatiques différenciées : pouvoir de métaboliser les androgènes et, dans le cas des cellules épithéliales, de synthétiser une AP sécrétoire.

[Traduit par la revue]

ABBREVIATIONS: T, testosterone; 5α-DHT, 5α-dihydrotestosterone; AP, acid phosphatase; 5α-R, Δ^4-3-ketosteriod-5α-reductase (EC 1.3.1.4); 3α-adiol, 5α-androstane-3α,17β-diol; 3β-adiol, 5α-androstane-3β,17β-diol; 5α-A, 5α-androstanedione; An, androsterone (5α-androstan-3α-ol-17-one); LAP, lysosomal AP; SAP, secretary AP; BPH, benign prostatic hyperplasia; HBSS, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) – sodium hydroxide buffered saline solution; HPLC, high pressure liquid chromatography; 17β-HSOR, 17β-hydroxysteriod oxidoreductase (EC 1.1.1.63); 3α-HSOR, 3α-hydroxysteriod oxidoreductase (EC 1.1.1.50); 3β-HSOR, 3β-hydroxysteroid oxidoreductase (EC 1.1.1.51); A, 4-androstene-3,17-dione; isoAn, isoandrosterone (5α-androstan-3β-ol-17-one); 6α-atriol, 5α-androstane-3β,6α,17β-triol; 7α-atriol, 5α-androstane-3β,7α,17β-triol; 6β-atriol, 5α-androstane-3β,6β,17β-triol; 6α-H, 6α-hydroxylase; 7α-H, 7α-hydroxylase; 6β-H, 6β-hydroxylase; *p*NPP, *p*-nitrophenol phosphate.

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Introduction

It is well established in a number of species that the development, growth, and function of the male reproductive tract ogans such as the prostate are under androgenic control. Associated with the actions of androgens in the prostate is extensive androgen metabolism (for review see Ref. 1 and Fig. 1 of this paper). While T is the principal circulating androgen, it is irreversibly reduced (2, 3) in the prostate through the actions of 5α-R to 5α-DHT. It is 5α-DHT which accumulates (4) in the prostate cell nuclei and exerts androgenic effects. The prostate can convert 5α-DHT to a number of other metabolites and these reactions can potentially play a role in controlling the intracellular concentration of 5α-DHT or terminate its actions. 5α-DHT can be reduced to either 3α- or 3β-adiol (2). 3α-Adiol and 5α-DHT are interconvertible (5). 3β-Adiol is converted back to 5α-DHT to a small extent (6, 7). 3β-Adiol is more efficiently hydroxylated at the 6 and 7 positions (8, 9); these reactions are irreversible and can end the expression of 5α-DHT actions. Both 5α-DHT and 3α-adiol can be oxidized at C17 giving 5α-A and An, respectively.

A number of biochemical markers have been utilized to assess the effects of androgens in the prostate (10). These include total protein and DNA concentrations, as well as specific proteins such as prostatic binding protein (11), spermine binding proteins (12), and AP (13). Our laboratory has demonstrated that prostatic AP is a useful biochemical marker of the androgen status in the rat (10, 13–17). In the rat ventral prostate the are two forms of AP: lysosomal (LAP) and secretory (SAP) (18–21). LAP is found in all tissues, while SAP is found only in the prostate. L(+)-Tartrate, an AP inhibitor, has aided in the characterization of the two forms (18–22). LAP is inhibited markedly by L(+)-tartrate, while SAP is relatively resistant. Castration of the adult rat leads to a marked decrease in SAP as seen by polyacrylamide gel electrophoresis (13, 14, 23) or isoelectric focusing (16, 21, 24) patterns. The number of LAP bands on isoelectric focusing gels increases after castration (16). The daily adminstration of pharmacological doses of androgen starting immediately following castration maintains both prostate size and SAP activity (14, 16, 23).

In recent years there has been considerable evidence from *in vivo* (25, 26) and *in vitro* (27, 28) tissue recombinant experiments demonstrating an important role for epithelial–stromal cellular interactions for the embryonic development of the prostate. These studies indicate that in the fetal rodent the presence of the stroma is required for the development and maintenance of the urogenital sinus epithelium. The stroma requires the presence of androgens for this inductive effect. Post embryonically, it is less certain whether epithelial–stromal cell interactions are as important, although there is some evidence of such (29). Recently McNeal (30) has speculated that human BPH may result from the "reawakening" of the inductive potential of the stroma. Earlier studies by Franks et al. (31) showed that epithelium mechanically separated from human BPH could not be maintained in culture independently of the stroma. Such studies suggested that the epithelium was dependent on factors released from the stroma. Some experiments on human BPH tissue have suggested that 5α-DHT production occurs primarily in the stroma (32–34), but this is less clear from more recent studies (35) which indicate that the epithelial tissue is quite capable of reducing T to 5α-DHT.

Our laboratory has been developing rat prostate epithelial and stromal cell cultures to aid in the investigation of androgenic control of prostatic cell proliferation and secretory function (SAP production). Such culture systems could yield information on the interrelationships between the two cell types. This paper presents results on the metabolism of androgens by epithelial and stromal cell cultures and the response of both cell types to androgens in terms of AP characteristics.

Materials and methods

Establishment of primary cultures of epithelial and stromal cells

Ventral prostates were aseptically removed from immature rats (21–22 days) and minced in HBSS (36). Twenty-four rats were used for each experiment. The minced tissue was dissociated into cells with a mixture of 0.1% collagenase, 0.1% trypsin, and 1% chicken serum (37). After a 20-min digestion period, the undissociated tissue was allowed to sediment, the supernatant containing the dissociated cells was removed, and the incubation was repeated. After several such digestions, the supernatants were combined and the cells were obtained by centrifugation and resuspended in a small volume of HBSS.

The epithelial and stromal cells were separated on preformed, continuous isopycnic Percoll gradients as previously described (37) with the following modifications. Percoll (density, 1.055 g/mL) was spun at 20 000 × *g* for 20 min in a Beckman Ti60 fixed-angle rotor prior to cell loading. The cells were layered on top in a small volume of HBSS. The preparation was centrifuged in swing-out buckets at 1000 × *g* for 30 min in a Sorval model RC-3B centrifuge. The epithelial-enriched cell fraction (85–90% of cells by light microscopy) sedimented at the gradient region corresponding to 1.040–1.055 g/mL. The stromal-enriched cell fraction sedimented at densities greater than 1.060 g/mL (90–95% nonepithelial cells). The cell fractions were individually removed and resuspended in F12/DME culture medium.

Cells were seeded onto 60-mm dishes and cultured in F12/DME medium containing 10% fetal bovine serum, 20 m*M* Hepes, 10 m*M* $NaHCO_3$, insulin (5 μg/mL), transferrin (5 μg/mL), 50 n*M* testosterone, penicillin (100 U/mL), streptomycin (100 μg/mL), and fungizone (1 μg/mL). As well, the medium for the epithelial cells contained 50 n*M* dexamethasone and 500 n*M* retinoic acid. Following 24–36 h

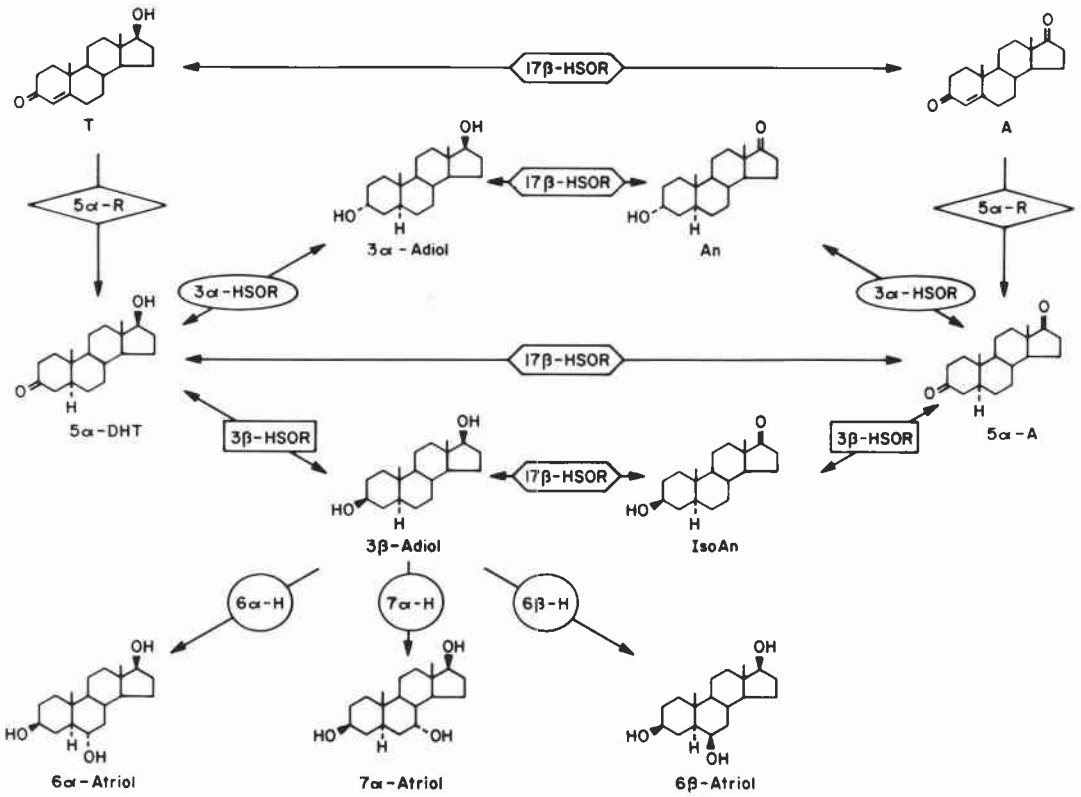

FIG. 1. Overview of the principal pathways of androgen metabolism in the prostate. Refer to the section Abbreviations for the definitions of the acronyms used.

culture the epithelial cells, which were still in suspension, were collected, resuspended in fresh medium, and cultured. This allowed for further cell enrichment as any contaminating fibroblasts or stromal cells had attached to the flask surface (38, 39). Primary cultures of stromal cells were grown to confluency and used immediately or following 5–7 days in culture. They were removed from the culture dish surface with 0.05% trypsin and 0.02% EDTA, washed, and plated in fresh culture dishes for subsequent experiments. No differences were observed between primary cultures of stromal cells or cells after the first subculture. Cultures were maintained in a humidified incubation at 37°C in 98% air – 2% carbon dioxide.

Cultures of both cell types were monitored daily using a Leitz (Wetzlar) inverted, phase-contrast light microscope.

Studies on androgen metabolism

Androgen metabolism was studied in cultures of cells after they reached confluency (6–7 days). The culture medium was removed and replaced with serum-free and steroid-free medium for 24 h. This medium was then replaced with fresh F12/DME medium containing ^{3}H-labelled androgens. Following a further 24-h incubation, medium and cells were extracted with methylene chloride. ^{14}C-labelled steroids were added prior to extraction to monitor losses. The extracts were filtered through anhydrous sodium sulphate and evaporated to dryness under nitrogen.

The labelled steroids in the extracts were analyzed utilizing HPLC. They were chromatographed on a reverse-phase Beckman C-18 with 5-μm spherical packing and an ultrasphere-ODS column, using an isocratic mobile phase of acetonitrile–methanol–water (1:3:3) (J. Orlowski and A. F. Clark, in preparation). ^{3}H and ^{14}C in the effluent was monitored with a radioactivity flow detector.

Acid phosphatase assays

AP contents of the cultured cells were assayed following cell lysis and sonication with *p*-nitrophenol phosphate as the substrate (13). Tartrate inhibition studies were performed with 14 m*M* L(+)-tartrate. Histochemical demonstration of AP was performed utilizing naphthol AS-BI phosphate and fast garnet GBC salt.

Protein concentrations were measured by the method of Bradford (40) using the Bio-Rad kit. DNA was measured by the method of Labarca and Paigen (41) utilizing the enhancement of fluorescence of bisbenzimide (Hoechst 33258) stain.

Results and discussion

Cell culture characteristics

Rat ventral prostate epithelial cells in culture reached confluency in 7 days. As shown in Fig. 2, when the cells were examined by light microscopy they were polygonal in shape and closely associated with each other, which is characteristic of epithelial cells in culture. The stromal

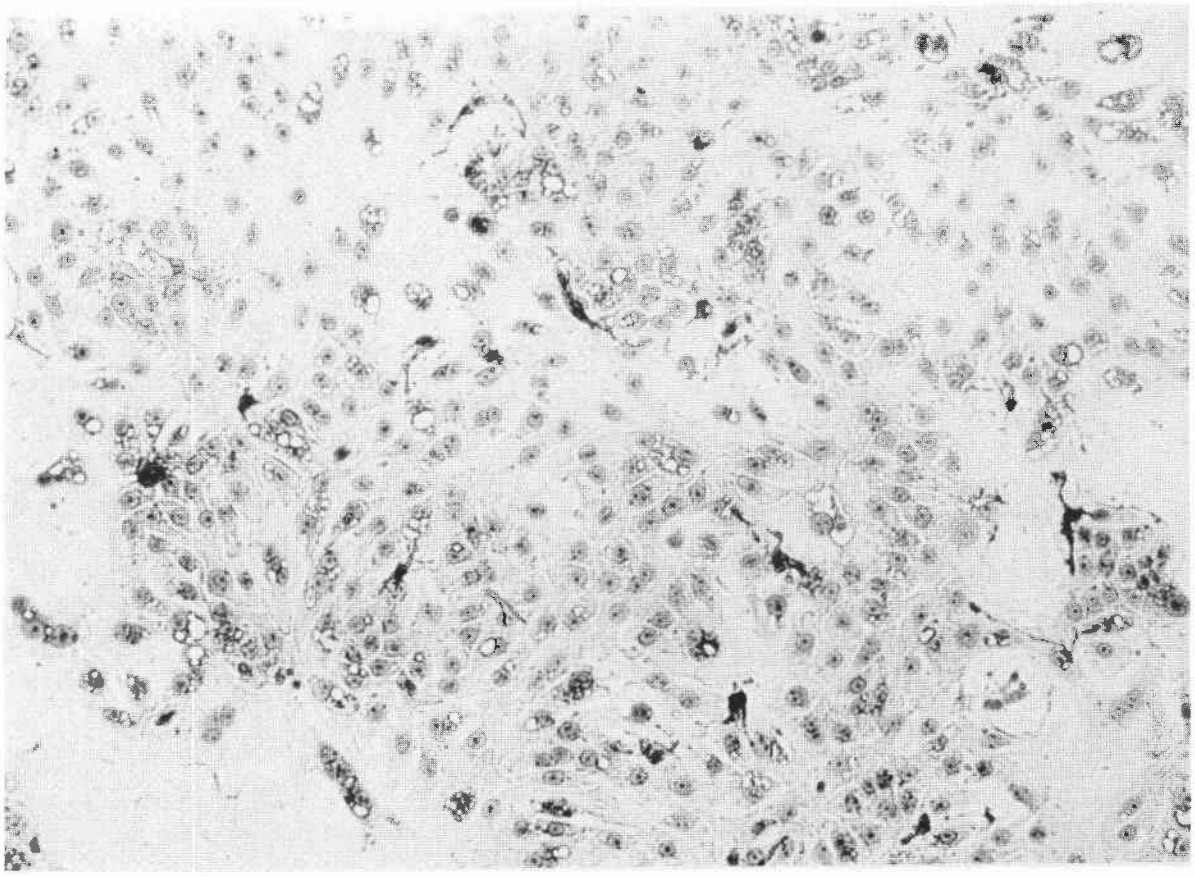

FIG. 2. Light-microscope photomicrograph of a 7-day primary culture of rat ventral prostate epithelial cells. Cells were fixed with 100% methanol and stained with Geimsa stain. Magnification, ×80.

cells (Fig. 3), which were also confluent after 7 days, were elongated, spindle-shaped, and loosely associated with each other. This is characteristic of fibroblastic-type cells in culture. The appearance of both cell types was similar whether they were grown in the absence or presence of 50 n*M* testosterone.

Androgen metabolism by cultures

To examine the ability of the two cell types to metabolize androgens, confluent cell cultures were incubated with ^{3}H-labelled androgens (T, 5α-DHT, 3α-adiol, and 3β-adiol) for 24 h in serum-free and steroid-free medium. Table 1 summarizes the results for the epithelial cell cultures. The epithelial cells metabolized the ^{3}H-labelled androgens by pathways previously established in incubations with tissue homogenates, organ cultures, and subcellular fractions (for review, see Ref. 1). It can be seen that all substrates were extensively metabolized during this time period. The small amount of T substrate remaining after the incubation period indicated high 5α-R activity. Similar amounts of 5α-DHT (~10%) were present after the 24-h incubations with T, 5α-DHT, and 3α-adiol. The other major metabolites for these same three substrates were 5α-A and the $C_{19}O_3$ metabolites (i.e., hydroxylated $C_{19}O_2$ steroids). The formation of significant amounts of 5α-A indicated significant 17β-HSOR activity. 17β-HSOR can form 5α-A directly from 5α-DHT (see Fig. 1) or indirectly from T via A which would be converted to 5α-A by 5α-R. 3α-Adiol can be oxidized to An by 17β-HSOR, which in turn can be converted to 5α-A through the oxidative reaction of 3α-HSOR. Alternatively, 3α-adiol can be converted to 5α-A via 5α-DHT. Furthermore, the large formation of $C_{19}O_3$ metabolites following metabolism of T, 5α-DHT, and 3α-adiol indicated significant activity by the 3β-HSOR–hydroxylase pathway. High hydroxylase activity for 3β-adiol is indicated by the fact that only the $C_{19}O_3$ metabolites were formed in significant amounts when it was the labelled substrate.

With the stromal cell cultures, less T and 5α-DHT were metabolized (Table 2). This is probably related in

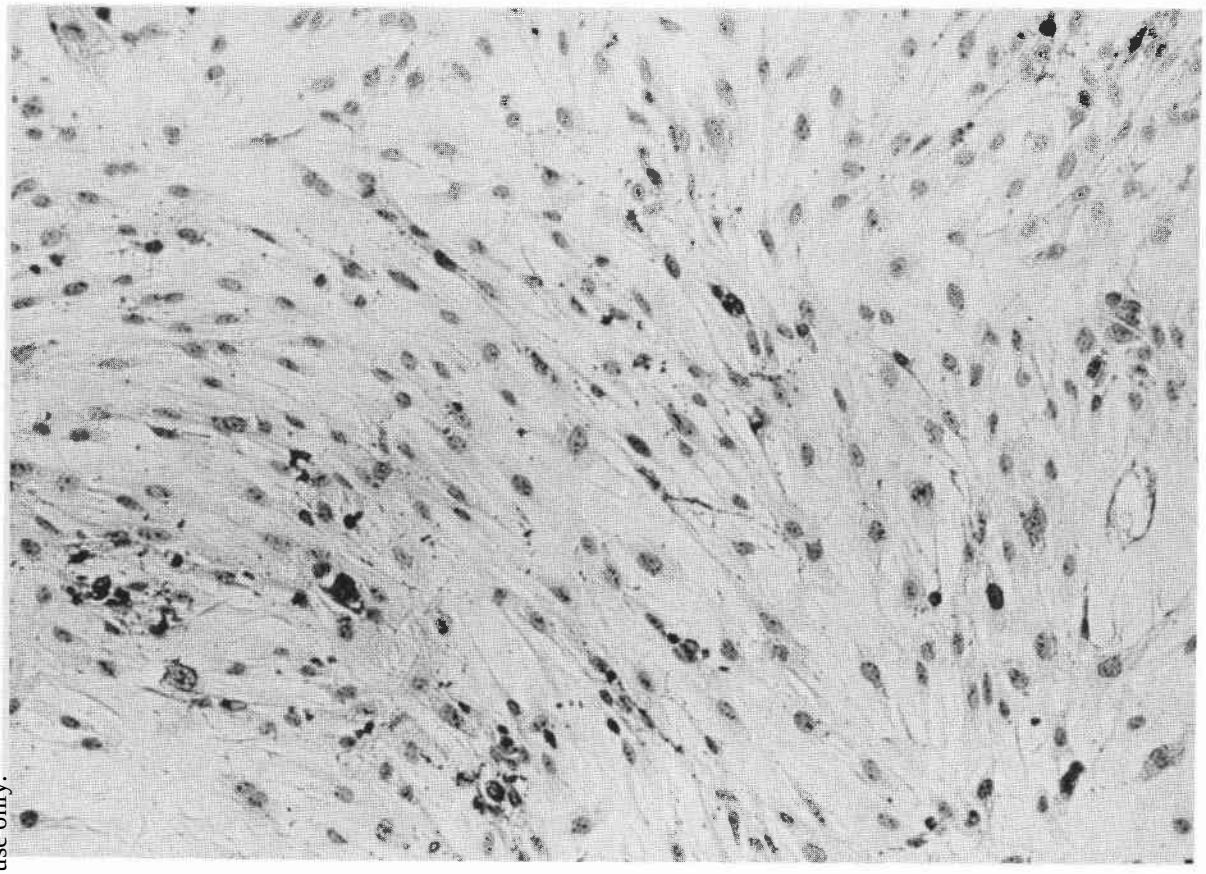

FIG. 3. Light-microscope photomicrograph of a 7-day primary culture of rat ventral prostate stromal cells. Cells were fixed with 100% methanol and stained with Geimsa stain. Magnification, ×80.

part to fewer stromal cells than epithelial cells per plate (8.7 versus 21 μg DNA/plate); stromal cells are not closely associated with each other so that confluency is attained with fewer cells. 5α-R is, in part, rate limiting as only 25% of the added T was metabolized. The major metabolites were 5α-DHT and the $C_{19}O_3$ metabolites. For 5α-DHT, the prominent metabolites were the $C_{19}O_3$ metabolites. Reduction of 5α-DHT to 3α-adiol by the 3α-HSOR pathway was negligible. 3α-HSOR activity was efficient in the oxidative direction in that approximately 50% of 3α-adiol was metabolized to 5α-DHT. The formation of the 17-oxo-$C_{19}O_2$ metabolites (i.e., A, 5α-A, An, isoAn) was greatly reduced compared with the epithelial cells, indicating very low 17β-HSOR activity. Again $C_{19}O_3$ metabolites from T, 5α-DHT, and 3α-adiol were found in significant amounts (~40%). The formation of $C_{19}O_3$ metabolites from 3β-adiol was very efficient in the stromal cells.

Further time course studies are being completed to delineate the relative contribution and rates of activity of each of the enzymes involved in androgen metabolism.

Comparing the HPLC profiles of the $C_{19}O_3$ metabolites formed from 3β-adiol with the epithelial and stromal cell cultures indicated some differences (Fig. 4, Table 3). The results indicated that the epithelial cells hydroxylated the 3β-adiol to form three atriols (6α, 7α, and 6β). This is in agreement with a recent report (42) which examined the hydroxylation of 3β-adiol using isolated rat ventral prostrate microsomes. This contrasts with previous studies using rat ventral prostrate homogenates (8, 9) or adult rat prostate epithelial cell cultures (43) which reported the identification of only the 6α- and 7α-atriols. Unlike the epithelial cells, the stromal cells formed only the 6α- and 7α-atriols. In both instances, the 6α-hydroxylated metabolite is present in the greatest quantity (66–68%), in accordance with other studies (8, 9, 43). Kinetic studies (9, 42) have suggested that one and the same enzyme may be responsible for prostatic 6α-, 7α-, and 6β-hydroxylation of 3β-adiol and has been identified as belonging to the NADPH-dependent cytochrome P-450 family of enzymes (42). However, further purification and characterization of the enzyme(s) is required to confirm this observation.

Analysis of the HPLC profiles of the $C_{19}O_3$ metabo-

TABLE 1. Metabolism of 50 n*M* radiolabelled androgens by primary epithelial cell cultures of rat ventral prostate

Substrates	μg DNA/ culture dish	μg protein/ culture dish	% substrate recovered	% metabolites								
				A	5α-DHT	3α-Adiol	3β-Adiol	5α-A	An	IsoAn	$C_{19}O_3$	Aqueous metabolites
T	21±2	402±42	2.4±0.9	1.6±0.3	10.3±1.8	2.8±0.6	6.6±3.0	24.7±2.6	1.4±0.4	3.6±1.3	43.3±3.2	5.8±0.9
5α-DHT	21±2	402±42	9.0±1.3			1.9±0.9	2.7±0.9	27.5±4.8	1.5±0.5	5.5±2.6	44.6±4.1	4.2±0.8
3α-Adiol	21±2	402±42	2.2±0.9		9.1±1.2		1.1±0.8	26.1±3.1	1.4±0.5	3.3±1.3	53.2±6.7	4.9±0.6
3β-Adiol	21±2	402±42	2.6±1.8		2.3±0.9	0.3±0.1		3.2±1.0	0.4±0.1	0.9±0.5	76.3±5.6	16.5±1.3

NOTE: Epithelial cells were grown in culture until they reached a confluent monlayer (6–7 days). Following a 24-h culture period in serum-free, steroid-free medium, the cells were cultured in serum-free medium with ^{3}H-radiolabelled androgen substrate (50 n*M*) for 24 h. The reaction was terminated by adding the medium plus cells to methylene chloride. The extracted metabolites were analyzed by HPLC (see text). Results are expressed as means ± SD (n = 5).

TABLE 2. Metabolism of 50 n*M* radiolabelled androgens by primary stromal cell cultures of rat ventral prostate

Substrates	μg DNA/ culture dish	μg protein/ culture dish	% substrate recovered	% metabolites							
				A	5α-DHT	3α-Adiol	3β-Adiol	5α-A	An	$C_{19}O_3$	Aqueous metabolites
T	8.7±0.1	471±49	74.7±0.8	1.7±0.1	7.0±1.0	1.0±0.4	0.6±0.1	0.7±0.4		5.9±1.5	1.1±0.1
5α-DHT	8.7±0.1	471±49	36.9±1.4			1.8±0.6	0.9±0.5	3.3±0.3		49.6±1.3	4.4±0.6
3α-Adiol	8.7±0.1	471±49	2.9±0.6		49.5±4.9	2.9±0.6	0.9±0.6	2.8±1.6	0.2±0.1	40.8±1.1	4.0±0.6
3β-Adiol	21±2	471±49	1.0±0.1		1.0±0.4	0.2±0.1				72.4±2.3	23.7±2.1

NOTE: Stromal cells were grown in culture until they reached a confluent monlayer (6–7 days). Following a 24-h culture period in serum-free, steroid-free medium, the cells were cultured in serum-free medium with ^{3}H-radiolabelled androgen substrate (50 n*M*) for 24 h. The reaction was terminated by adding the medium plus cells to methylene chloride. The extracted metabolites were analyzed by HPLC (see text). Results are expressed as means ± SD (n = 3).

TABLE 3. Relative formation of the different hydroxylated metabolites of 3β-adiol following its metabolism by primary cultures of rat ventral prostate epithelial and stromal cells

	Epithelial		Stromal	
Hydroxylated metabolite	K^a	%	K	%
6α-Atriol	0.67±0.02	66.4±5.2	0.65±0.02	68.3±3.3
7α-Atriol	0.93±0.03	20.3±5.6	0.94±0.03	31.7±3.3
6β-Atriol	1.32±0.03	13.3±0.9		

[a] K is the capacity factor which is determined by subtracting the void volume of the column from the elution volume of the particular steroid and dividing this difference by the void volume. Results are expressed as means ± SD (n = 3).

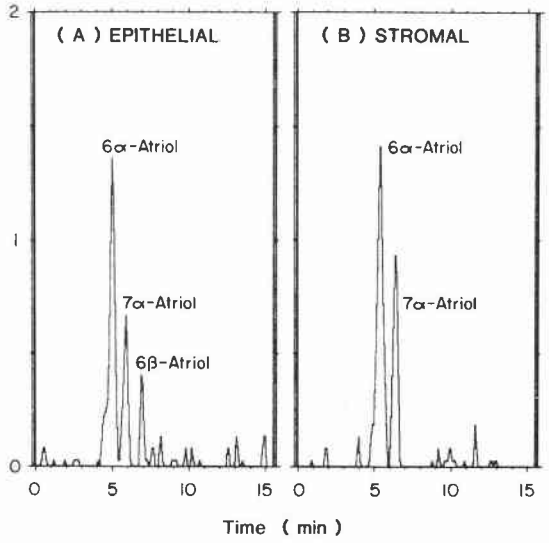

FIG. 4. HPLC radiochromatogram showing the separation of the hydroxylated metabolites of 3β-adiol in rat ventral prostate epithelial (A) and stromal (B) cells in primary monolayer culture. The metabolites are 6α-, 7α-, and 6β-atriol. 3β-Atriol eluted from the HPLC column between 25 and 26 min (peak not shown).

olites following the 24-h incubations of T, 5α-DHT, and 3α-adiol by epithelial and stromal cells have indicated that the $C_{19}O_3$ steroids contain the atriols, as well as other $C_{19}O_3$ steroids (J. Orlowski and A. F. Clark, in preparation). The relative amounts of the $C_{19}O_3$ metabolites formed in the two cell types also differs and suggests differential pathways for controlling the removal of biologically active intracellular androgens. We have not examined the nature of the aqueous metabolites. They could represent atriols that are not extracted; we originally optimized our procedures for the $C_{19}O_2$ metabolites and the more polar $C_{19}O_3$ steroids may not be efficiently extracted. They could also represent glucuronide conjugates of 3α-adiol or other conjugated metabolites, as their formation by the prostate has been reported previously (44).

Our results on androgen metabolism differ somewhat from the results of Ofner et al. (43). In the epithelial cells, we had more extensive metabolism and greater formation of 5α-A. In both the epithelial and stromal cells there was greater formation of $C_{19}O_3$ steroids except when 3β-adiol was the substrate, where they also had extensive formation of $C_{19}O_3$ steroids. There were some significant differences in experimental conditions between their studies and ours. They utilized adult rats, while we utilized immature rats, and we utilized 50 nM substrate concentrations, while they utilized 3 and 300 nM concentrations. Furthermore, Ofner et al. (43) initiated their metabolism studies on semiconfluent monolayers of both cell types. Our studies were performed using confluent monolayers. Terracio et al. (45) has shown that, using canine prostate epithelial cell cultures at low cell protein per culture dish, T metabolism proceeded mainly to the 5α-reduced $C_{19}O_2$ metabolites, which included similar amounts of 17β-hydroxy-$C_{19}O_2$ and 17-oxo-$C_{19}O_2$ metabolites and minimal formation of $C_{19}O_3$ metabolites. In contrast, at high cell protein levels, metabolism proceeded mainly by oxidative pathways to predominantly 5α-reduced 17-oxo-$C_{19}O_2$ and $C_{19}O_3$ metabolites. In support of this finding, recent results in our laboratory using semiconfluent monolayers of rat prostate epithelial cells has indicated a lower rate of formation of the 17-oxo-$C_{19}O_2$ and $C_{19}O_3$ metabolites (J. Orlowski and A. F. Clark, unpublished observation). As well, there were differences in the additions to the culture medium.

The epithelial cell cultures responded to the presence of androgens by synthesizing more AP. In the culture grown in the absence of T (Fig. 5A) there were only a few areas of cells which stained for AP activity. The serum in the culture medium was not charcoal stripped to remove endogenous androgens. The culture medium including serum contained T and 5α-DHT at concentra-

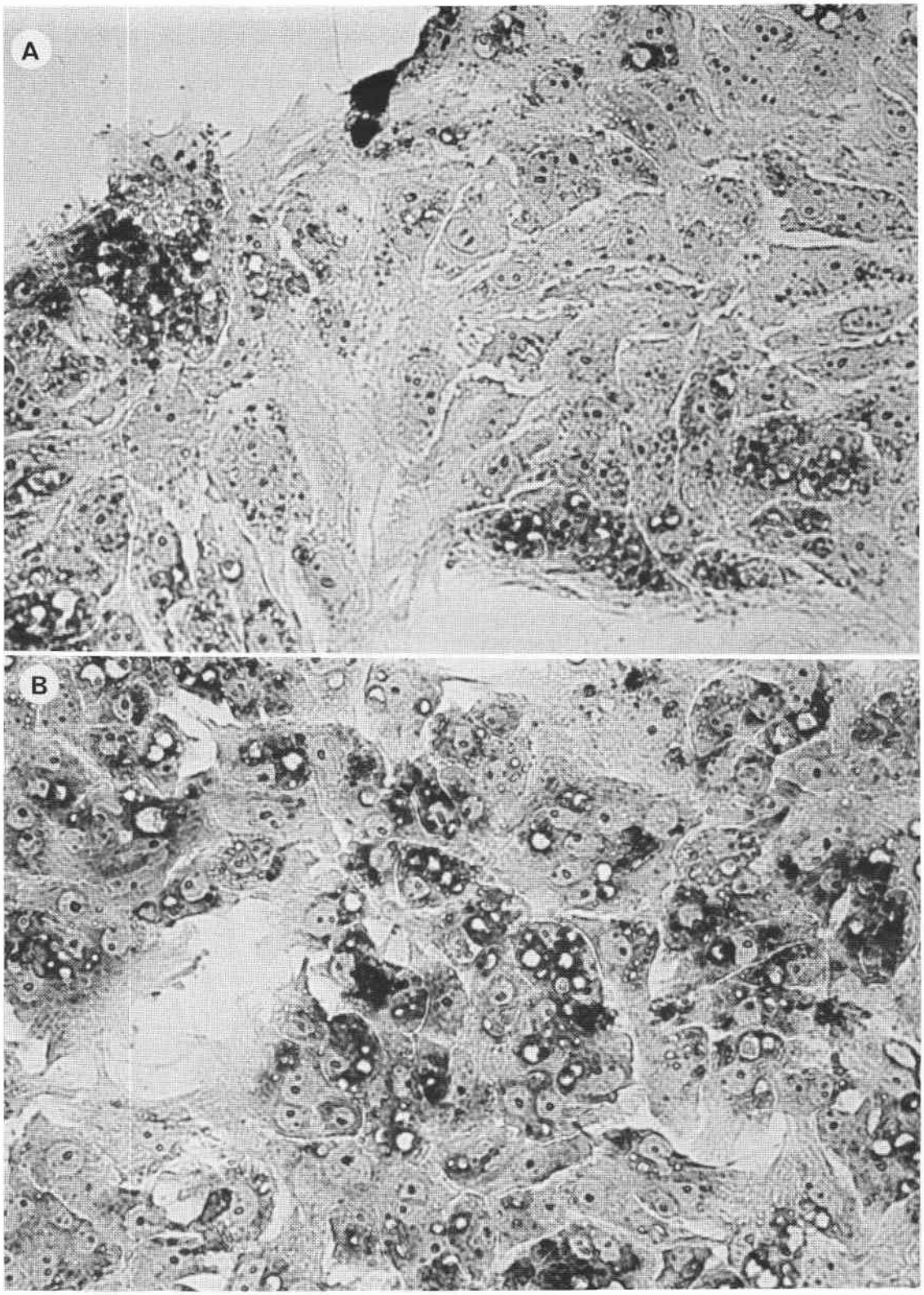

FIG. 5. Light-microscope photomicrograph of a primary culture of rat ventral prostate epithelial cells stained for AP. Cells were cultured for 7 days in the (A) absence or (B) presence of T (50 n*M*). Magnification, ×200.

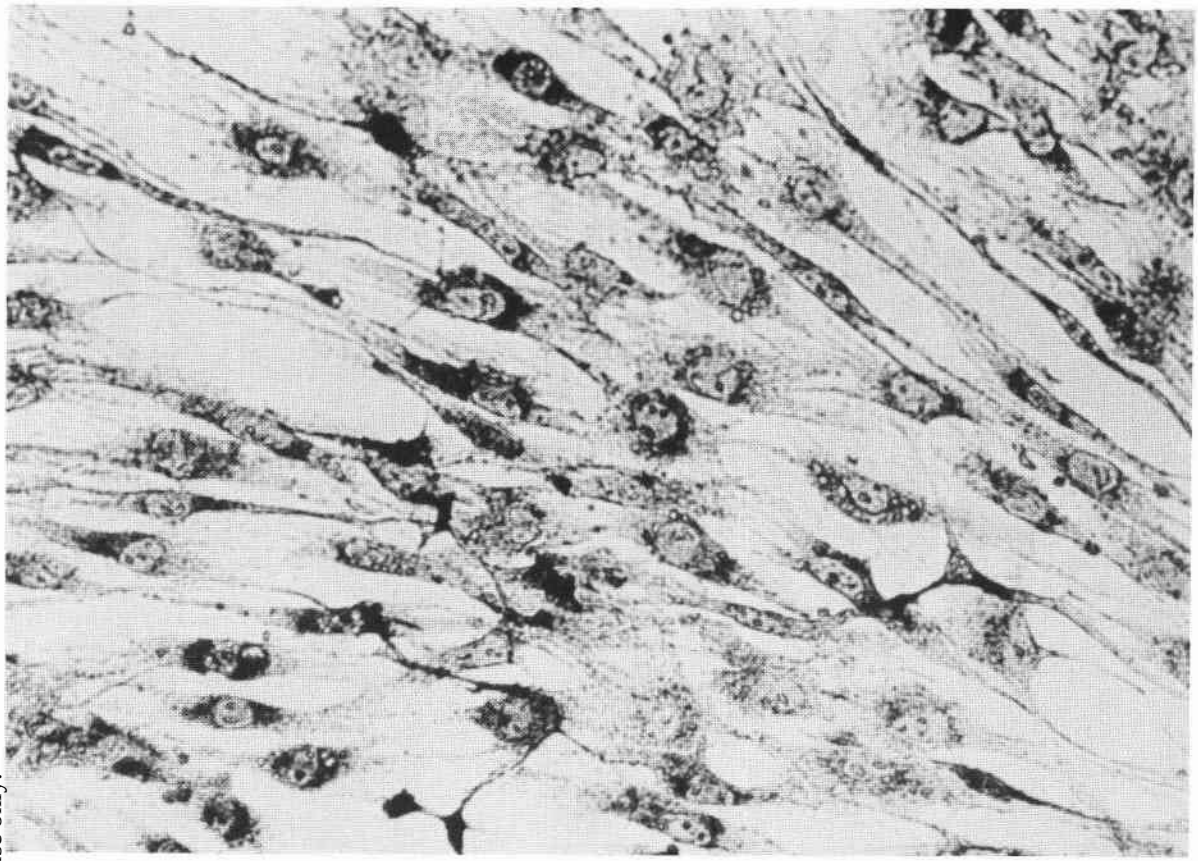

FIG. 6. Light-microscope photomicrograph of a primary culture of rat ventral prostate stromal cells stained for AP. Cells were cultured for 7 days in the absence of T. Magnification, ×200.

tions of 62.5 and 27.0 p*M*, respectively (determined by radioimmunoassay; data not shown). In contrast, when the cells were grown in the presence of 50 n*M* T most of the cells (Fig. 5B) stained for AP activity. The stromal cell cultures stained for AP in only a few areas surrounding the perinuclear region of the cell, whether grown in the absence or presence of T (culture grown in absence of T is shown in Fig. 6).

Figure 7 indicates the cell content of AP for both cell types grown in the absence and presence of T (50 n*M*) for 5 and 10 days. The only significant effect was seen for epithelial cells grown in the presence of T after 10 days in culture. Our results to date indicate that AP synthesis is stimulated only after the epithelial cells have reached confluency (i.e., 6–7 days). This is similar to what occurs *in vivo* when androgens are administered to rats castrated 7 days, previously; in this situation the ventral prostate undergoes rapid cell proliferation and returns to normal size before SAP appears (16, 23, 46).

The extent of inhibition of AP by L(+)-tartrate (14 n*M*) for whole prostates and for cultures of both cell types grown in the absence and presence of T is given in Table 4. Similar to previously reported results (14), tartrate inhibition for adult rats was 41.2%. For adult rats castrated 7 days previously the value was 55.4%. The increase is related to the loss of the androgen-dependent SAP which is tartrate resistant, leaving LAP to account for the remaining activity (14, 16). The result for immature rats was 51.1% which is similar, but significantly lower ($p < 0.01$), to adult castrated rats. This is related to the presence of very low levels of SAP and normal levels of LAP (J. Orlowski, C. E. Bird, and A. F. Clark, unpublished results). For the epithelial cells in culture, the percent inhibition of AP by tartrate after 5 days was high in the absence or presence of T (50 n*M*). After 10 days the extent of inhibition had decreased. This was more pronounced for the cells grown in the presence of T. This concurs with the appearance of AP forms similar to SAP on isoelectric focusing gels (J. Orlowski and A. F. Clark, unpublished results). Stromal cells after 5 and 10 days in culture in the absence or presence of T showed results similar to prostrate homogenates from adult rats castrated 7 days previously. This suggested the presence of only LAP. This

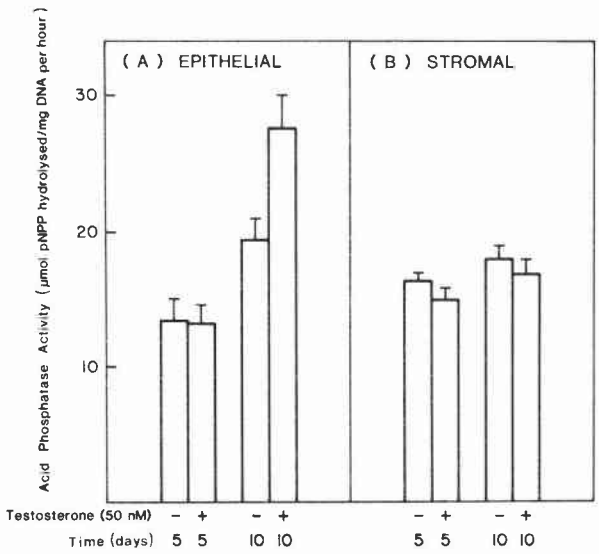

FIG. 7. Effect of T on AP content in primary cultures of rat ventral prostate epithelial (A) and stromal (B) cells. Both cell types were cultured in the absence (−) or presence (+) of T (50 n*M*). AP activity was measured in cell extracts on days 5 and 10 of culture. The cells reached a confluent monolayer on day 7. Results are expressed as the mean ± SD (n = 3).

TABLE 4. Tartrate inhibition of AP activity from rat ventral prostate

	% inhibition
Whole prostate homogenate	
Adult	41.2±0.7
Adult (7-day castrate)	55.4±1.0[a]
Immature (21 days old)	51.1±0.9[a,b]
Epithelial cells in culture	
Day 5, −T	52.3±1.4
Day 5, +T	55.5±2.3
Day 10, −T	47.9±1.6[c]
Day 10, +T	44.2±1.1[d,e]
Stromal cells in culture	
Day 5, −T	56.7±1.1
Day 5, +T	55.5±1.7
Day 10, −T	54.9±2.1
Day 10, +T	59.1±3.2

NOTE: Results are expressed as means ± SD (n = 3).
[a]Statistical significant difference compared with adult, $p < 0.01$.
[b]Statistical significant difference compared with adult (7-day castrate), $p < 0.01$.
[c]Statistical significant difference compared with day 5, $p < 0.05$.
[d]Statistical significant difference compared with day 5, $p < 0.01$.
[e]Statistical significant difference compared with day 10 without T, $p < 0.05$.

has been supported by localizaion of only LAP on isoelectric focusing gels (J. Orlowski and A. F. Clark, unpublished results).

In conclusion, the ability of the primary epithelial and stromal cultures to metabolize androgens and synthesize SAP and (or) LAP is a strong indication that the prostate cells have retained differentiated function. The primary cultures should provide a useful model for the study of hormonal regulation of prostate growth and function.

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