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Comparison of Testosterone Metabolism in Benign Prostatic Hyperplasia and Human Prostate Cancer Cell Lines *In Vitro*

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Pathways of testosterone metabolism in tissue slices and cell suspensions of human benign hyperplastic prostate (BPH) tissue and human prostate cancer cell lines (DU145, HPC-36M, PC-3/MA2 and LNCaP) were investigated. Thin layer chromatography analysis was used to identify the following tritiated metabolites: testosterone, 5α-dihydrotestosterone (DHT), 5α-androstane-3α/3β-17β-diol (androstanediols), 4-androstene-3,17-dione (androstenedione) and 5α-androstanedione. The predominant pathway for testosterone metabolism in BPH was via 5α-reductase producing 5α-dihydrotestosterone (71% and 75% total metabolites in slices and suspensions incubated for 24 h, respectively). The cancer cell lines DU145 and HPC-36M resembled BPH by metabolizing testosterone predominantly to DHT (68% and 82% total metabolites, respectively), although the rate of metabolism was much lower in the cell lines (0.099 and 0.05 pmol testosterone/mg protein/h in DU145 and HPC-36M) compared to the BPH cell suspensions (6.4 pmol testosterone/mg protein/h). In contrast, PC-3/MA2 contained high 17β-HSD activity forming large amounts of 4-androstene-3,17-dione (84% total metabolites), converting testosterone at a rate faster (12.8 pmol testosterone/mg protein/h) than the BPH cell suspensions. LNCaP rapidly converted testosterone exclusively to a glucuronide conjugate (7.4 pmol testosterone/mg protein/h), although after incubation with [^{3}H]-4-androstene-3,17-dione, 5α-reductase activity was demonstrated. LNCaP was the only cell line whose growth and colony-forming ability was stimulated by testosterone and DHT. BPH and all the cell lines tested had 5α-reductase activity, but only the prostate tissue and the cell lines DU145 and HPC-36M converted testosterone predominantly to DHT.

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INTRODUCTION

Human prostate cells depend on androgens for growth and maintenance of normal function [1]. Androgens are also implicated in the aetiology of the two most common disorders of the prostate; namely, benign prostatic hyperplasia (BPH) and prostate cancer [2, 3]. Testosterone is the major circulating androgen in man, but in the prostate it is converted to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase. The observation that 5α-reductase deficient males (pseudo-hermaphrodites) have small atrophic prostates and do not develop BPH or prostate cancer provides evidence that DHT is the active androgen modulating prostate growth in man [4]. Inhibition of 5α-reductase in adult males might mimic the effects of 5α-reductase deficiency and thereby provide treatment for androgen-dependent disorders of the prostate [5]. Finasteride, the first 5α-reductase inhibitor to be tested in man, has been shown to cause a substantial reduction in plasma and prostatic DHT levels, a reduction in prostate volume and a small improvement in urinary flow [6].

Prostate cancer relapses following anti-androgenic therapy because there is usually a progression from androgen-dependent to androgen-independent growth. However, the observation that prostate cancer does not develop in men castrated prior to puberty [3] and is not reported in 5α-reductase deficient males [7] suggests that suppression of DHT levels with 5α-reductase inhibitors might be useful in preventing the development of prostate cancer in high risk groups [8] and in

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the treatment of prostate cancer which remains androgen-dependent [9–11]. Growth of human prostate tumours implanted into rats is inhibited when the major source of androgen is removed by castration. These tumours also show decreased growth when the animals are treated with 5α-reductase inhibitors [12–14], indicating that prostate cancer probably depends on DHT and not testosterone for its hormonal support.

We have compared testosterone metabolism in BPH tissue slices and cell suspensions with that in the human prostate cancer cell lines DU145, HPC-36M, PC-3/MA2 and LNCaP. The aim of this work was to establish whether these cell lines possess 5α-reductase activity and to determine if testosterone was metabolized differently in hormone-sensitive cells (LNCaP) compared to hormone-insensitive prostate cancer cells (DU145, HPC-36M and PC-3/MA2). The effects of testosterone and DHT on the growth of the cell lines was also investigated using colony-forming assays and MTT assays to determine if the effects on growth were related to the pattern of testosterone metabolism.

MATERIALS AND METHODS

Chemicals

Radioactive steroids [1α,2α-^{3}H]testosterone (51 Ci/mmol) and [1β,2β-^{3}H]androst-4-ene-3,17-dione (48.1 Ci/mmol) were obtained from Du Pont, U.K. Testosterone, 5α-dihydrotestosterone (DHT), 5α-androstane-3α-17β-diol, 5α-androstane-3β-17β-diol (androstanediols), 4-androstene-3,17-dione (androstenedione), 5α-androstanedione, 5α-androstan-3α-ol-17-one (androsterone), 5α-androstan-3β-ol-17-one (isoandrosterone), collagenase type 1A, hyaluronidase, β-glucuronidase (type B10) and the tetrazolium salt MTT [3,-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazoliumbromide) were purchased from Sigma, Poole, U.K. Stock solutions of 2×10^{-2} M testosterone and DHT dissolved in absolute ethanol were stored at −20°C, and diluted in RPMI 1640 before each experiment to produce a final concentration of ethanol of 0.5% or less.

Cells and cell culture

DU145 [15], LNCaP [16] and PC-3/MA2 [17] were given to us by Dr J. Fogh, Dr S. Horoszewicz and Dr M. E. Kaighn, respectively. HPC-36M is a subline of the HPC line [18] (a gift from Dr D. M. Lubaroff) re-established in culture following passage through nude mice [19]. All cell lines were routinely maintained in RPMI 1640 supplemented with 5% foetal calf serum (FCS) and 2 mM L-glutamine under standard tissue culture conditions. Steroid-depleted FCS (DCC–FCS) was prepared by treating the same batch of serum with dextran-coated charcoal [20] and cell lines were passaged 3–4 times in 5% DCC–FCS supplemented RPMI 1640 prior to use in growth assays. Levels of testosterone and DHT in FCS were determined by radioimmunoassay (Amersham RIA Kit) and the efficiency of steroid stripping was evaluated by following the removal of labelled testosterone, DHT and oestradiol from FCS and were found to be 98%, 97% and 99% respectively. Total testosterone plus DHT concentration in 5% FCS supplemented RPMI 1640 medium was approx. 4×10^{-11} M, while the concentration of DHT in FCS and total testosterone plus DHT in DCC–FCS were all lower than the detection limit of the RIA.

Preparation of BPH slices and cell suspensions

Human BPH tissue was obtained at transurethral resection (TUR) and transported to the laboratory in ice-cold phenol-red free RPMI 1640 containing penicillin (100 units/ml) and streptomycin (100 μg/ml). The external surface of each tissue sample was removed to exclude material damaged by the surgical procedure prior to cutting into slices of 3 × 2 × 1 mm or cubes of 2 mm^3. Cell suspensions were prepared by incubating the cubed tissue at 37°C for 12 h in RPMI 1640 supplemented with 5% FCS containing collagenase type 1A (225 units/ml) and hyaluronidase (125 units/ml) and antibiotics. Dissociated cells were centrifuged for 10 min (250 *g*) and the pellet was resuspended in serum-free RPMI 1640 and passed through cotton gauze to remove any undigested pieces of tissue. This was repeated three times to wash out the collagenase. Finally the pellet was resuspended in 4–5 vol RPMI 1640 for every 1 ml of PBS displaced by the tissue before digestion. Protein levels were measured by the method of Lowry *et al.* [21].

Testosterone metabolism studies

The cell lines were grown to near confluency in 5% FCS supplemented RPMI 1640 in 25 cm^2 flasks and 24 h prior to the experiment the medium was replaced with 5 ml serum-free and steroid-free RPMI 1640. The monolayers were washed in PBS and then incubated at 37°C for 3 h in 5 ml of RPMI 1640 containing 1 μCi of [^{3}H]testosterone. For studies with BPH cell suspensions, 1 ml samples were incubated for 1 h with 1 μCi of [^{3}H]testosterone. Tissue slices were set up in 5 cm petri dishes containing 3 ml of RPMI 1640 with 1 μCi [^{3}H]testosterone using a modified Trowell technique [22] and were incubated for 24 h at 37°C. In all experiments final testosterone concentration was adjusted to give 2×10^{-8} M, which is reported to be the mean plasma level in man [23].

Metabolites were extracted from the medium around tissue slices and from cells using 2 vol of ethylacetate containing 10 μg/ml of trace steroids (testosterone, DHT, androstenedione, androstanediols, androstanedione, androsterone). For BPH cell digests, 2 ml of ethylacetate was added to the tubes which were vortex mixed and then centrifuged for 10 min (1000 *g*). The ethylacetate layer was removed from the aqueous phase and evaporated to dryness under N_2, the residue was

redissolved in 70 μl ethanol and spotted onto a silica gel TLC plate (Merck $60F_{254}$).

Separation of metabolites was carried out by thin layer chromatography (TLC) using dichloromethane:acetone (12.3:1 v/v) [24]. This system does not completely resolve DHT, androsterone and epiandrosterone and in order to achieve separation of these metabolites the appropriate area on the plate was scraped off, eluted in 2 × 3 ml dichloromethane:acetone (2:1) and rechromatographed using aluminium oxide plates (Merck F_{254} Type E) and a solvent system of toluene:diethylether (1:1) [modified from 24].

After the first chromatographic separation radioactive metabolites were quantified using a Raytest radio-TLC plate reader (Fig. 1). Metabolites were identified by co-chromatography with authentic standards which were located by UV light absorption and by development in iodine vapour. Following the second TLC separation the areas of silica gel corresponding to DHT, androsterone and epiandrosterone were located, scraped off and counted by liquid scintillation counting.

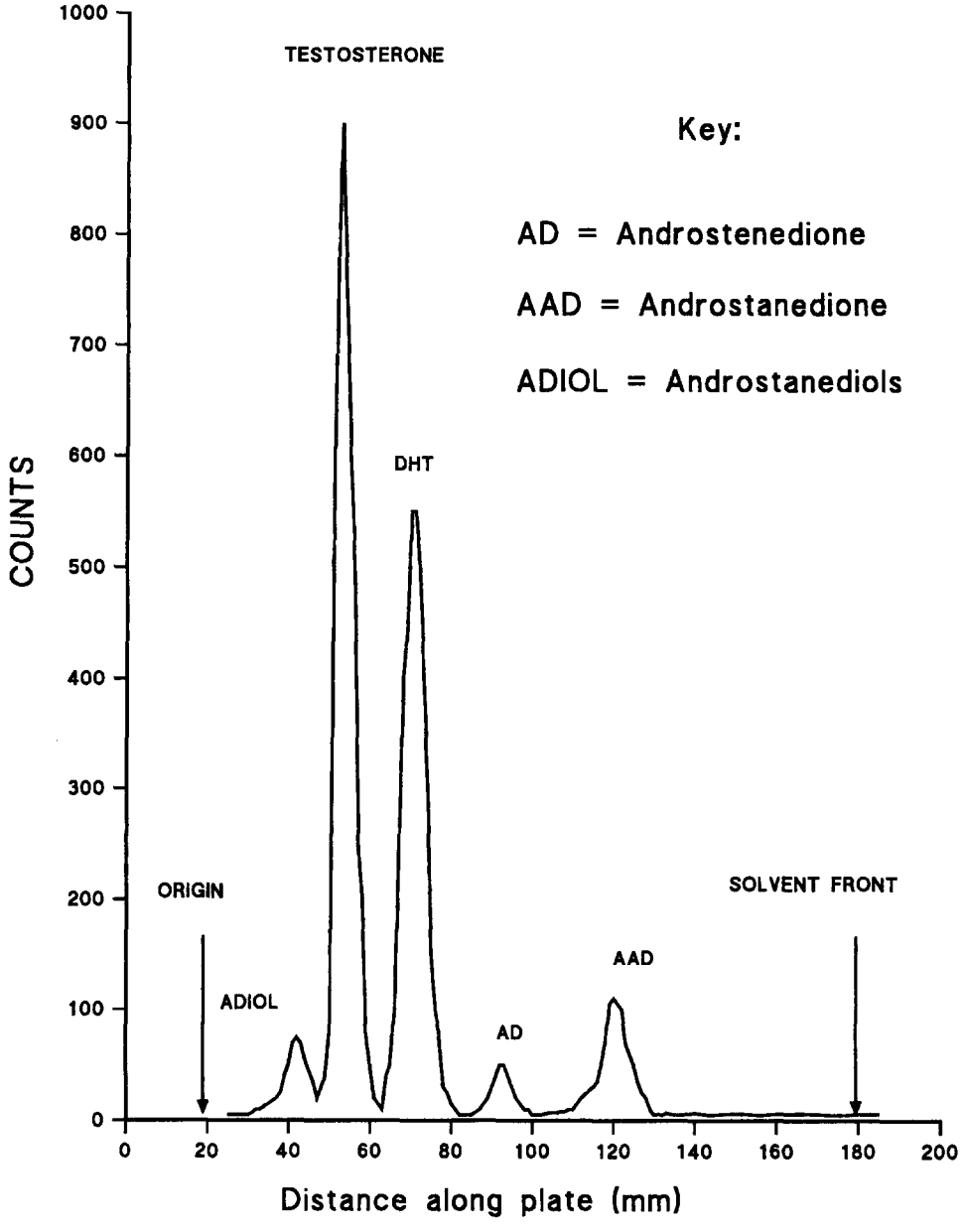

Fig. 1. Radio-TLC analyser scan of a thin layer chromatographic separation of [^{3}H]testosterone radio-metabolites produced by human BPH cells. Suspensions of human BPH cells (protein content 2–4 mg/ml) were incubated with [^{3}H]testosterone (20 nM, 1 μCi) for 1 h. Metabolites were extracted with ethylacetate and separated by TLC using a solvent system of dichloromethane:acetone (12.3:1 v/v). The metabolites were identified by co-chromatography with authentic standards and UV absorption characteristics.

In LNCaP, 95% of the radioactivity was recovered in the aqueous phase. In order to determine if the aqueous fraction contained steroid glucuronides it was removed, adjusted to pH 4.5 with acetic acid and treated with β-glucuronidase (400 units/ml) for 20 h at 37°C to release free steroids [25]. These were extracted with ethylacetate and analyzed by TLC as described above.

Clonogenic assays

A single cell suspension of exponentially growing cells was plated into 5 cm petri dishes containing either 5 ml of 5% FCS or 5% DCC–FCS supplemented RPMI 1640 so as to produce approx. 150 colonies after 14 days of growth. After incubation at 37°C for 24 h, testosterone or DHT was added to give final concentrations ranging from 10^{-12}–10^{-5} M (3 dishes/concentration). The plates were incubated at 37°C for a further 14 days and then the cells were fixed in 70% methanol and stained with Giemsa's stain. Colonies comprising more than 50 cells were counted and results expressed as a percentage of control values.

Determination of viable cell number

To determine the effect of the androgens on cell number we used the MTT assay. This assay is based on the ability of mitochondrial enzymes in viable cells to metabolize a tetrazolium salt (MTT) to a coloured formazan product [26]. LNCaP were plated into 96 well plates at a density of 3000 cells/well in 150 μl medium containing either 5% FCS or 5% DCC–FCS and left overnight to attach. The medium was then replaced with 200 μl medium containing testosterone or DHT (10^{-12}–10^{-5} M) with 8 replicates/concentration and the cells left at 37°C for 7 days. Viable cell number was determined by adding 50 μl of MTT solution (5 mg/ml) to each well followed by 3 h incubation at 37°C. The medium was removed and 100 μl of DMSO added to each well to solubilize the formazan product and absorbance was measured at 540 nm using a Flow Titertek Multiskan plate reader. The first column of 8 wells did not contain cells and provided a blank. The second column of 8 wells contained control untreated cells to which solvent only was added. Results are expressed as a percentage of control absorbance in the second column.

RESULTS

Validation of extraction procedure for testosterone and its metabolites

The recoveries of 1 μCi [^{3}H]testosterone obtained by ethylacetate extraction of 1 ml and 5 ml RPMI 1640 medium after 1 and 3 h incubations, respectively, at 37°C were 90–95%. TLC analysis of this extracted radioactivity demonstrated that it was 99.5% testosterone, showing that there was no significant conversion of testosterone in medium alone. In each experiment blank incubations (no cells or tissue) were performed and analysed in the same way as the samples containing cells. The amount of radioactivity detected in the blanks (by the TLC plate analyser) associated with each individual metabolite was subtracted from that detected in the samples. Individual metabolites detected in blank incubations never exceeded 0.1% of the total radioactivity recovered.

Table 1. Rates of testosterone metabolism in human BPH cell suspensions and in human prostate cancer cell lines

In vitro system	Rate of testosterone conversion (pmol/mg protein/h)
BPH cells	6.4 ± 0.2
HPC-36M	0.05 ± 0.003
DU145	0.099 ± 0.02
PC-3/MA2	12.8 ± 1.2
LNCaP	7.4 ± 1.5

Rates ± SD were estimated using data from 3 h incubation experiments in PC-3/MA2 and LNCaP (data from one time-course experiment during the first hour while the rate of conversion was linear), from 1 h incubation experiments in BPH cell suspensions (n = 6) and from various experiments in HPC-36M and DU145 (n = 4). Testosterone concentration in all experiments was 20 nM (1 μCi). The rate of metabolism in tissue slices was not determined.

Testosterone metabolism in human BPH slices and cell suspensions

Human BPH tissue slices and cell suspensions formed predominantly 5α-reduced metabolites from testosterone (Tables 1–3). DHT accounted for 88% and 75% of the total metabolites in BPH cell suspensions (1 h and 24 h incubations, respectively) and 71% in BPH tissue slices. The formation of metabolites of DHT (androstanedione and androstanediol), increased in the tissue slices and cell suspensions incubated for 24 h compared to the cell suspensions incubated for 1 h. Only low levels of androstenedione (less than 2%) were present in both preparations due to its further conversion to 5α-reduced metabolites. The mean rate of metabolism in six cell suspensions at 1 h was 6.4 pmol testosterone/mg protein/h (Table 1). The rate of metabolism was linear for the first 2–3 h, peaked by 4–5 h and then plateaued for the remaining period up to 24 h (data not shown). The rate of metabolism in the tissue slices was not calculated.

Testosterone metabolism in human prostate cancer cell lines

HPC-36M and DU145 formed predominantly 5α-reduced metabolites with DHT accounting for 82% of the total in HPC-36M and 68% in DU145 (Tables 2 and 3). Androstenedione, androstanediols and androstanedione accounted for the remaining 18% of metabolites in HPC-36M. In contrast, DU145 produced greater amounts of androstanediols and

Table 2. Metabolites of testosterone formed by BPH slices, BPH cells and human prostate cancer cell lines

In vitro system and exposure period	%Total metabolites						
	ADIOLS	DHT	AD	AAD	A	T-G	%T Met
BPH slices (24 h)	11 ± 6	71 ± 7	2 ± 3	16 ± 5	0	ND	47 ± 9
BPH cells (24 h)	8 ± 1.2	75 ± 3	5 ± 2.4	12 ± 2.4	0	ND	89 ± 7
BPH cells (1 h)	4 ± 0.4	88 ± 1	2 ± 0.2	6 ± 0.9	0	ND	59 ± 10
HPC-36M (3 h)	8 ± 1.4	82 ± 2	8 ± 3	2 ± 1	0	ND	5.9 ± 3.0
DU145 (3 h)	23 ± 4	68 ± 5	9 ± 4	0	0	ND	11.9 ± 4.4
PC-3/MA2 (3 h)	0	0	84 ± 2	15 ± 1	1 ± 0.2	ND	95 ± 2
LNCaP (3 h)	0	0	0	0	0	100	98 ± 1.8

BPH slices ($n = 10$ independent experiments), BPH cells (protein 2–4 mg in 1 ml RPMI 1640, 1 h and 24 h incubation, $n = 11$ independent experiments) and prostate cancer cell lines (monolayers in 25 cm^2 flasks, $n = 4$ independent experiments). After incubation with [^{3}H]testosterone (2×10^{-8} M, 1 μCi) metabolites in solvent extracts were separated by TLC in dichloromethane : acetone (12.3 : 1) and quantified using a radio-TLC plate reader. The identity of the metabolites was confirmed by co-chromatography with authentic standards and UV absorption (testosterone and androstenedione). Results are expressed as percentage total metabolites (mean ± SD). ADIOLS, androstanediols; DHT, 5α-dihydrotestosterone; AD, androstenedione; AAD, androstanedione; A, androsterone; T-G, testosterone-glucuronide; 0, not detected; ND, not done.

androstanedione could not be detected. A single time-course study of testosterone metabolism in these two cell lines over 3 h indicated that metabolism was linear throughout this period (data not shown). On the basis of four experiments (including the time-course) the estimated rates of testosterone metabolism by DU145 and HPC-36M were 0.081 and 0.042 pmol testosterone/mg protein/h, respectively (Table 1).

The predominant metabolites of testosterone formed by PC-3/MA2 were androstenedione and androstanedione which accounted for 84% and 15% of total metabolites, respectively. Androsterone was also present (1% total metabolites), but DHT could not be detected. PC-3/MA2 converted over 90% of the added testosterone to metabolites during a 3 h incubation, with an estimated rate during the first hour while there was a linear increase of 12.8 pm/mg protein/h (Table 1).

After 3 h incubation of LNCaP monolayers with [^{3}H]testosterone only 2% of the added radioactivity was recovered from the medium in the ethylacetate fraction (see Table 4), in comparison to over 85% in the other cell lines. TLC analysis demonstrated that only unchanged substrate was present in the ethylacetate fraction. A time course experiment demonstrated the rapid loss of ethylacetate-extractable radioactivity from the medium and a concomitant increase in radioactivity in the aqueous phase (data not shown). After 30 min it was possible to recover only 25% of the radioactivity in the non-polar phase of the medium. Treatment of the polar phase with β-glucuronidase resulted in approx. 50% of the radioactivity being converted to an ethylacetate-extractable form, which again on TLC analysis was found to consist entirely of free testosterone. This result showed that in LNCaP approximately half the testosterone is rapidly converted to a glucuronide conjugate. The other 50% might be in the form of sulphate. When LNCaP was incubated with [^{3}H]androstenedione (2×10^{-8} M), which is not a direct substrate for conjugation, 90% of the radioactivity was recovered in the ethylacetate fraction as unmetabolized androstenedione with the 5α-reduced metabolite androstanedione accounting for the remaining 10%.

Table 3. Metabolites of testosterone formed by BPH slices, BPH cells and human prostate cancer cell lines

In vitro system and exposure period	pmol Testosterone converted/mg protein						
	ADIOLS	DHT	AD	AAD	A	T-G	%T Met
BPH slices (24 h)	0.02 ± 0.01	0.139 ± 0.014	0.004 ± 0.005	0.03 ± 0.01	0	ND	47 ± 9
BPH cells (24 h)	0.70 ± 0.1	4.54 ± 0.01	0.31 ± 0.19	1.0 ± 0.32	0	ND	89 ± 7
BPH cells (1 h)	0.26 ± 0.03	5.63 ± 0.06	0.13 ± 0.013	0.38 ± 0.06	0	ND	59 ± 10
HPC-36M (3 h)	0.011 ± 0.002	0.11 ± 0.004	0.011 ± 0.004	0.003 ± 0.001	0	ND	5.9 ± 3.0
DU145 (3 h)	0.061 ± 0.011	0.132 ± 0.013	0.024 ± 0.011	0	0	ND	11.9 ± 4.4
PC-3/MA2 (3 h)	0	0	1.78 ± 0.04	0.32 ± 0.02	0.02 ± 0.004	ND	95 ± 2
LNCaP (3 h)	0	0	0	0	0	2.18 ± 0.04	98 ± 1.8

Results are expressed as pmol testosterone converted per mg protein (mean ± SD). ADIOLS, androstanediols; DHT, 5α-dihydrotestosterone; AD, androstenedione; AAD, androstanedione; A, androsterone; T-G, testosterone-glucuronide; 0, not detected; ND, not done.

Table 4. Recovery of radioactivity from LNCaP monolayers before and after treatment with β-glucuronidase

	% Radioactivity recovered from	
	Cells	Medium
Pre-hydrolysis		
Aqueous fraction	6 ± 1	76 ± 8
Ethylacetate fraction	4 ± 0.6	2 ± 0.8
Post-hydrolysis		
Aqueous fraction	<0.5	33 ± 20
Ethylacetate fraction	4 ± 1	26 ± 10

Monolayers of LNCaP (25 cm^2 flasks) were incubated with [^{3}H]testosterone (100 nM, 1 μCi) for 3 h. After ethylacetate extraction of cells and medium the recovery of radioactivity was determined before and after treatment with β-glucuronidase (400 units/ml, 20 h, pH 4.5) by liquid scintillation counting. Results expressed as % total radioactivity recovered from the medium (mean ± SD, 4 replicates).

The effect of testosterone and DHT on the growth of prostate cell lines using clonogenic assays

Addition of testosterone and DHT to LNCaP grown in medium containing 5% DCC–FCS produced stimulation of colony-formation with a maximum stimulation at 10^{-10} M testosterone or 10^{-11} M DHT (Fig. 2). Concentrations of 10^{-9} M and greater resulted in inhibition of colony-formation by LNCaP. No stimulation was observed in medium containing 5% FCS and colony-formation was inhibited by testosterone and DHT at concentrations greater than 10^{-10} M (Fig. 3). Colony-formation by HPC-36M was completely inhibited when the concentration of testosterone or DHT reached 10^{-5} M, while there was still approx. 50% and 100% colony-formation by DU145 and PC-3/MA2 respectively (Figs 1, 2). The effect of testosterone and DHT on colony-formation by DU145, HPC-36M and PC-3/MA2 was not affected by replacing FCS supplemented medium with DCC–FCS medium (Fig. 3).

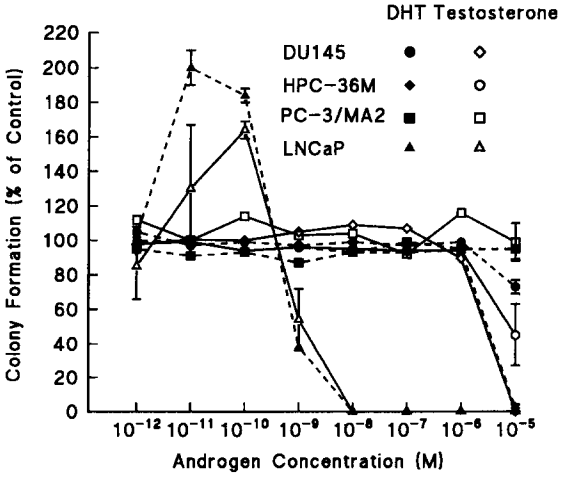

Fig. 2. The effects of androgens on colony-formation in prostate cancer cell lines grown in 5% DCC–FCS-supplemented medium. Testosterone and DHT (10^{-12}–10^{-5} M) were added to human prostate cancer cell lines in 5% DCC–FCS supplemented medium and colonies were counted after 14 days. Results are expressed as percentage of colony formation in controls (mean ± SD, 3 independent experiments).

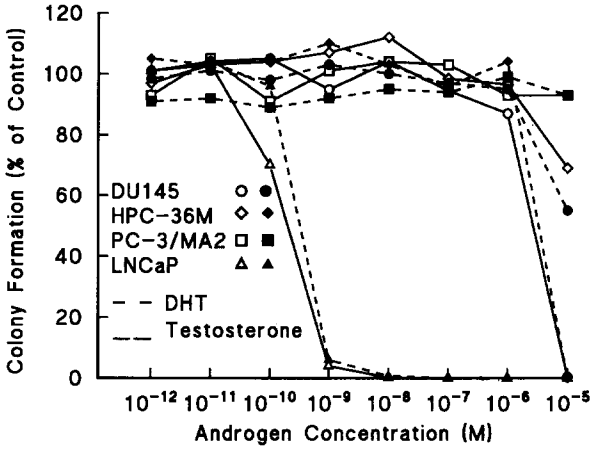

Fig. 3. The effects of androgens on colony-formation in prostate cancer cell lines grown in 5% FCS-supplemented medium. Testosterone and DHT (10^{-12}–10^{-5} M) were added to human prostate cancer cell lines in 5% DCC–FCS supplemented medium and colonies were counted after 14 days. Results are expressed as percentage of colony formation in controls (means ± SD, 3 independent experiments).

The effect of testosterone and DHT on the growth of LNCaP using MTT assays

In order to assess the effects of androgens on the viable cell number of LNCaP, as opposed to colony-formation, we used the MTT assay. In 5% FCS supplemented medium, testosterone or DHT produced a dose-dependent inhibition of LNCaP cell proliferation, whereas in steroid-depleted medium there was a biphasic response with maximal stimulation of growth at 10^{-10} M testosterone or DHT and inhibition at 10^{-8} M and above (Fig. 4). The results therefore reflect those obtained from colony-forming assays. MTT assays were not performed on DU145, HPC-36M and PC-3/MA2 as no stimulation of growth was seen in the colony-forming assays.

DISCUSSION

We have demonstrated that 5α-reductase activity is present in the androgen-sensitive LNCaP prostate cancer cell line and also in the androgen insensitive lines DU145, HPC-36M and PC-3/MA2. Steroid 5α-reductase activity has previously been reported in DU145, PC-3/MA2 and LNCaP [27, 28]. The cell lines retain the complement of androgen metabolizing enzyme activities that are expressed in slices and cell suspensions of BPH tissue (see Table 5). However, the

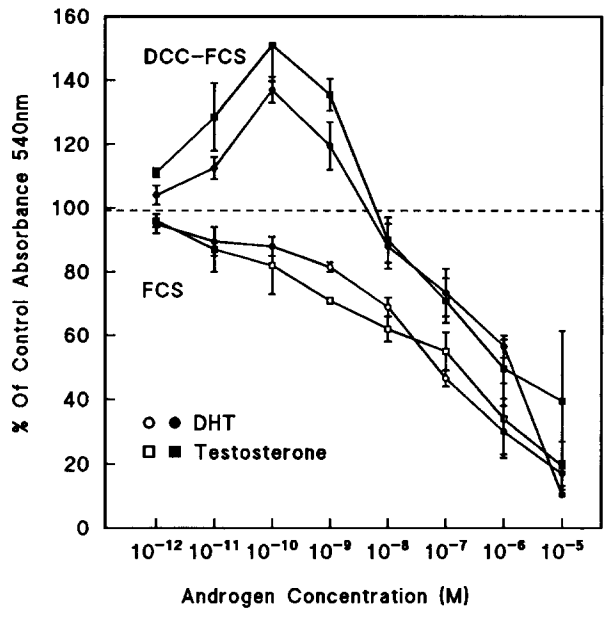

Fig. 4. Effects of androgens on cell proliferation in LNCaP. LNCaP cells were plated (3000/well) in 5% FCS or 5% DCC-FCS supplemented medium. After 24 h incubation medium containing testosterone or DHT (10^{-12}–10^{-5} M) was added and the plates incubated at 37°C for a further 6 days. MTT was added to the cells for 3 h and then the absorbance of DMSO-solubilized formazan measured at 540 nm. Results were expressed as a percentage of control absorbance (mean ± SD, 3 independent experiments).

overall profiles of metabolism are quite distinct due to differences in the relative rates and levels of each activity, and although 5α-reductase is present in all the cell lines, DHT is the predominant metabolite of testosterone in only two of the four prostate cancer cell lines.

The catalysis of testosterone reduction by 5α-reductase is dependent on NADPH. Our finding that BPH cell suspensions prepared by collagenase digestion display a high capacity for conversion of testosterone to DHT in the absence of exogenously added NADPH suggests that the cells are intact and metabolically active. Furthermore, our observations that the profile of testosterone metabolites produced by these cells is similar to that in prostate tissue slices and also resembles the profile of androgen metabolites reported for BPH tissue [29], indicate that the cell suspension system is representative of the prostate *in vivo*.

Despite the high level of DHT formation in cells from BPH tissue, the rate of further metabolism is relatively low, indicating that the tissue's capacity for producing DHT far exceeds that for removing it. This is in contrast to the situation in DU145 and HPC-36M where overall conversion of testosterone was low but androstanediols (formed by 3α/β-HSD) and androstenedione (formed by 17β-HSD) were detectable. The capacity for removal of potent androgens (testosterone and DHT) was greatest in PC-3/MA2 which appears to over-express 17β-HSD activity, producing high levels of androstenedione, and is in contrast to DU145, HPC-36M and BPH tissue where DHT is the major metabolite of testosterone. However, PC-3/MA2 also possesses 5α-reductase activity as indicated by androstanedione formation which may arise from DHT or androstenedione by the action of 3α/β-HSD and 5α-reductase, respectively. It is not clear whether 17β-HSD activity in the prostate is due to a single form of enzyme possessing oxidative and reductive activities, or form(s) having only oxidative activity similar to those reported in human breast tissue [30]. Our data indicates that PC-3/MA2 may express only the oxidative form since the ratio of androstenedione:testosterone after incubation with testosterone exceeded 9:1. The PC-3/MA2 line, therefore, provides a useful system in which to further study 17β-HSD.

We have demonstrated that LNCaP forms testosterone-glucuronide, while previous reports have shown that LNCaP also forms DHT-glucuronide [31] and androsterone-glucuronide [32]. This shows that in LNCaP conjugation may occur via either the 17-position (with DHT) or the 3-position (with andros-

Table 5. Summary of testosterone metabolizing enzyme activities in human BPH tissue and human prostate cancer cell lines

	5α-reductase	17β-HSD	3α/β-HSD	Glucuronyl-transferase
Prostate *in vivo**	+ + +	+ +	+ +	ND
BPH cells	+ + +	+ +	+ +	ND
BPH slices	+ + +	+	+	ND
HPC36M	+	+	+	ND
DU145	+	+	+	ND
PC-3/MA2	+ +	+ + +	+	ND
LNCaP	+	(−)	(−)	+ + +

Key: +, less than 2% of radioactivity recovered; + +, 2–20% total radioactivity recovered; + + +, more than 20% total radioactivity recovered. The relative activities were assessed as follows: 5α-reductase, from the sum of the 5α-reduced metabolites formed; 17β-HSD, from the sum of androstenedione and androstanedione formed; 3α/β-HSD, from the amount of androstanediols formed. Abbreviations: 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3α/β, 3α/β-hydroxysteroid dehydrogenase, (−), not detected; ND, not determined. * from [34].

terone), whereas *in vivo* the predominant glucuronide in plasma is androsterone-glucuronide [32]. The glucuronidation of testosterone by LNCaP is an interesting finding when considered in relation to the colony-forming assays which have shown that concentrations of testosterone and DHT exceeding 10^{-9} M are inhibitory. Stimulation of growth of LNCaP occurred only when testosterone and DHT over the range 10^{-12}–10^{-10} M was added to medium containing steroid-depleted serum, suggesting that androgen levels in FCS are sufficient to maintain maximum cell proliferation. Thus, LNCaP is stimulated by low concentrations of androgens under certain conditions and is also inhibited by androgen concentrations which fall within the range found in prostate and plasma [23].

This study has shown that the ability of prostate cancer cell lines to form DHT is much lower than that of BPH tissue. Furthermore, the two cell lines forming DHT as the predominant metabolite, HPC-36M and DU145, had much lower rates of metabolism than BPH tissue. However, the low rate of metabolism was not characteristic of all the cell lines. LNCaP metabolized testosterone at a similar rate and PC-3/MA2 at twice the rate of BPH tissue. However, these two cell lines did not produce detectable DHT. In the light of the recent reports describing two forms of 5α-reductase in the prostate [33] it is our aim to determine which form is expressed in the prostate cancer cell lines, in particular DU145 and HPC-36M.

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