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Androgen Metabolism by Human Prostatic Tumours in Organ Culture

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Abstract—*Metabolism of testosterone and 5α-dihydrotestosterone were investigated in benign and malignant human prostatic tumours maintained for* 48 hr *in organ culture. When tritiated testosterone was used as substrate there were significant differences between the metabolic pathways of the two types of tumour. Whilst the benign tumour had a predominantly reductive pathway leading to the formation of 5α-reduced metabolites of testosterone, an oxidative pathway producing androstenedione was found to be the major pathway operative in the intermediate and poorly differentiated malignant specimens studied. In contrast to these differences observed in the metabolic pathway of testosterone when tritiated 5α-dihydrotestosterone was used as substrate, no significant differences in the pattern of radiometabolites were observed.*

INTRODUCTION

TESTOSTERONE metabolism is considered to be an essential stage in the mechanism by which androgens control the growth of prostatic cells. Numerous *in vitro* studies have shown that reductive metabolism of testosterone to 5α-dihydrotestosterone (DHT) is the predominant pathway in benign prostatic hyperplasia [1–3], whereas a few comparative studies have indicated that, at least in some cases, 17-keto metabolism is more apparent in prostate cancer [3–6]. However, the majority of these studies have been carried out using tissue preparations in which the 3-dimensional architecture of the prostate is destroyed, and in most cases the cellular integrity also. In contrast to the techniques used in most biochemical studies of the prostate and other tissues, organ culture provides a method whereby structure and function can be retained *in vitro*, and consequently results obtained with this approach may give a more accurate reflection of testosterone metabolism in the patient. Recent studies by Lasnitzki [7] have confirmed the value of the method for such studies and the aim of the present investigation was to compare differences in the metabolism of testosterone by human benign and neoplastic prostatic tissue in organ culture.

MATERIALS AND METHODS

Steroids

Testosterone (Δ^4-androsten-17β-ol-3-one); 5α-dihydrotestosterone (5α-androstan-17β-ol-3-one); 5α-androstane-3α,17β-diol; 5α-androstane-3β, 17β-diol; androstenedione (Δ^4-androstene-3, 17-dione); 5α-androstane-3, 17-dione and androsterone (3α-hydroxy-5α-androstan-17-one) were obtained from the Sigma Chemical Company, U.K. Stock solutions of these steroids at a concentration of 4 mg/ml were prepared in ethanol and stored at −20°C.

(1α,2α-[^{3}H])-testosterone (sp. act. 58 Ci/mmol) and (1α,2α(*n*)-[^{3}H])-5α-dihydrostestosterone (specific activity 60 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. Purity of both compounds was >97%. For the metabolism studies the specific activity of both these radioisotopes was reduced to 1.5 Ci/mol by addition of the respective unlabelled steroid. Stock solutions having a steroid concentration of 1 mM/ml were prepared in absolute ethanol and stored at −20°C prior to use.

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Organ culture

Benign and malignant prostatic tissues were obtained routinely by transurethral resection and immediately transferred to tissue culture medium. The external surface of each sample was removed to a depth of at least 1 mm in order to exclude material damaged by the surgical procedure and the remaining tissue cut into 1 mm slices. The explants were set up in modified Trowell organ culture dishes [8], each containing 5 ml of Waymouth's MB752/1 medium supplement with 20 mM HEPES and 1% penicillin and streptomycin (Flow Laboratories, Irvine, U.K.). For each measurement of androgen metabolism six replicate dishes were prepared, each containing three explants, a total weight of 200–300 mg, together with tritiated steroid at a final concentration of 10^{-5} M. The cultures were incubated for 48 hr in a humidified atmosphere of 5% CO_2 in air. Control dishes were incubated under the same conditions in the presence of tritiated steroid but in the absence of tissue. In some experiments the medium was supplemented with 0.1 mM NADPH (Boehringer, Mannheim).

Taking five of the six replicate dishes, the cultures were terminated by the addition of excess acetone, and the tissues weighed and homogenised before bulking with the medium. The tissue from the remaining dish was fixed in formal saline and processed for routine histological examination, as had been a portion of each original sample.

The tumours were classified according to the degree of gland formation and cellular differentiation: intermediate or poorly differentiated, grades 1, 2 or 3 respectively.

Extraction of radiolabelled steroids

Acetone was removed from the bulked explants by rotary evaporation at 75°C. The residual tissue homogenate and medium were extracted three times with 2 vol. of diethyl ether and the pooled ether extracts evaporated to dryness. The residues were reconstituted in 500 μl dichloromethane and stored at -35°C prior to analysis. Recoveries were estimated by measuring the radioactivity in a 50-μl aliquot of the reconstituted residue and were found to range from 84 to 92% of the added radioactivity.

Separation of radiometabolites

Thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) were carried out according to the procedure described previously [9]. The results obtained by GLC were comparable to those obtained by TLC, but additionally permitted the resolution of androsterone from 5α-dihydrotestosterone. However, this steroid metabolite contributed less than 2% to the total value of the 5α-dihydrotestosterone zone in all samples studies and, therefore, was not routinely separated.

Mathematical treatment of results

Values in count/min obtained for each steroid zone from TLC were expressed as a percentage of the total radioactivity recovered for each sample. The results from control incubations (steroid alone) were then subtracted. The percentage of each radiometabolite was then calculated by taking the total radiometabolites to be 100%. Overall substrate metabolism was expressed as a percentage of substrate metabolized per 100 mg tissue. Statistical analysis of the results were performed by using Student's t test.

RESULTS

Tissue culture

All cultures were well maintained during the 2-day period. The only histological differences between the cultured and fresh tissue were the presence of a small number of necrotic cells in the alveolar lumen and, particularly in the specimens of BPH, resurfacing of the cut edges of the explants by cells continuous with those of the lining alveoli. No major differences were observed between tissue cultured in the presence and absence of radioactive steroids for 48 hr, although in a few control cultures the epithelium appeared in some alveoli to be 2- to 3-layered in contrast to the 1–2 layers usually observed.

Effect of cofactor supplementation on benign and malignant explants

When five benign hypertrophied prostate tissues were cultured with 10^{-5} M [^{3}H]-testosterone in the presence or absence of 0.1 mM NADPH supplementation there were no substantial differences in the patterns of radiometabolites recovered, nor in the total amount of [^{3}H]-testosterone metabolised by 100 mg of tissue (Table 1). When two untreated and two treated intermediate grade malignant tumours were cultured under the same experimental conditions there was a consistent increase in the percentage contribution made by androstenedione to the overall metabolite with a proportionate decrease in the combined contribution of 5α-dihydrotestosterone and the 5α-androstanediols with supplementation. However, with the exception of androstenedione formation, which was significantly raised ($P < 0.05$) in the supplemented malignant specimens, no other significant differences were obtained.

Table 1. Effect of cofactor supplementation on metabolism of [^{3}H]-testosterone by benign and malignant prostatic explants

Radiometabolites	Tissue (No.) Benign hypertrophied ($n = 5$) No supplementation	+NADPH(0.1 mM)	Malignant ($n = 4$) No supplementation	+NADPH(0.1 mM)
Unidentified	2.6 ± 0.7	2.0 ± 0.5	1.8 ± 1.1	2.1 ± 1.2
5α-Androstanediols*	53.4 ± 4.4	51.7 ± 4.6	24.1 ± 5.8	15.9 ± 5.2
5α-Dihydrotestosterone	39.3 ± 4.1	39.8 ± 4.6	20.3 ± 7.0	16.1 ± 4.0
Δ^4-Androstenedione	1.9 ± 0.6	2.6 ± 0.8	46.8 ± 10.3	59.2 ± 7.9†
5α-Androstanedione	2.8 ± 0.9	3.1 ± 0.5	6.4 ± 2.2	7.3 ± 0.3
% substrate metabolized per 100 mg tissue	11.8 ± 2.0	11.9 ± 1.9	6.5 ± 1.3	6.0 ± 1.3

Values are mean ± S.E.M. expressed as percentage of radiometabolites recovered.
*Combined 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol.
†Significant difference between supplemented and unsupplemented levels ($P < 0.05$).

Table 2. Radiometabolites obtained from benign and malignant human prostate explants following culture with [^{3}H]-testosterone for 48 hr

Radiometabolite	Benign hypertrophied ($n = 10$)	Malignant ($n = 12$)	Significance (value for P)
Unidentified	4.1 ± 1.0	4.8 ± 1.1	n.s.*
5α-Androstanediols†	52.7 ± 3.1	20.4 ± 4.9	<0.001
5α-Dihydrotestosterone	34.4 ± 2.9	17.3 ± 3.4	<0.01
Δ^4-Androstenedione	4.7 ± 1.9	50.8 ± 6.9	<0.001
5α-Androstanedione	4.1 ± 1.6	6.7 ± 1.3	n.s.
% substrate metabolized per 100 mg tissue	11.5 ± 1.3	8.4 ± 1.8	n.s.

Results are mean ± S.E.M. expressed as percentage of radiometabolites recovered.
*n.s. = no significance.
†Combined 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol.

Metabolism of [^{3}H]-testosterone by benign and malignant prostate tumour explants

Samples from ten benign hypertrophied human prostates were cultured in the presence of 10^{-5} M [^{3}H]-testosterone, and the radiometabolite profiles are shown in Table 2. The predominant metabolites were the 17β-hydroxysteroids (5α-dihydrotestosterone and the 5α-androstanediols), with the 17-oxosteroids (androstenedione and androstanedione) contributing less than 9% to the total.

In contrast, when twelve carcinomatous tissues were cultured under these conditions the mean metabolite pattern revealed a predominantly oxidative pathway, with the 17-oxosteroids forming over 58% of the radiometabolites. The contribution made by the 17-hydroxysteroids in malignant tumours was significantly lower than that obtained in benign tissues (Table 2). Additionally, the overall metabolism was lower in carcinomatous tissue than in benign tumours. A substantial variation in metabolites was observed between the individual carcinomatous tissues (Table 3).

Metabolism of [^{3}H]-5α-dihydrotestosterone

For this study four benign hypertrophied and four malignant untreated tumours were cultured with 10^{-5} M [^{3}H]-5α-dihydrotestosterone. Both types of tissue yielded a similar radiometabolite profile, with the major product comprising the 5α-androstanediols (Table 4), and no significant differences were obtained when compared with an unpaired t test.

DISCUSSION

The histological observations are in general agreement with previous findings [10, 11] in that little or no change was observed other than the resurfacing of explants by epithelial cells. In cultures of BPH maintained in organ culture for a longer period (up to 6 days) Lasnitzki noted epithelial hyperplasia in control cultures which, to a large extent, was inhibited by the addition of testosterone or DHT to the medium [7]. No consistent histological changes were observed in the prostate cancer samples as a result of culture or steroid supplementation. Histological examination is a useful method for assessing viability and

Table 3. Radiometabolites obtained from explants of malignant human prostates cultured with [^{3}H]-testosterone for 48 hr

Tumour No.:	1	2	3	4	5	6	7	8	9	10	11	12
Tumour grade:		Grade 2				Grade 3		Grade 2		Grade 3	Grade 2	Grade 2
Patient treatment:		Untreated				Untreated		Orchiectomy/ stilboestrol		Orchiectomy/ stilboestrol	Cyproterone acetate	Ethinyl oestradiol
Radiometabolite	% of radiometabolites recovered											
Unidentified	3.7	n.d.*	8.3	8.2	6.6	8.5	8.8	n.d.	3.6	0.4	n.d.	9.8
5α-Androstanediols†	23.7	31.1	18.3	54.3	12.9	5.9	42.5	33.8	8.0	n.d.	12.5	2.0
5α-Dihydrotestosterone	27.7	36.1	8.3	25.9	23.4	n.d.	25.6	5.4	12.4	20.8	21.9	n.d.
Δ^4-Androstenedione	36.3	23.0	58.3	9.0	49.6	80.5	18.8	62.2	65.7	61.0	60.9	82.4
5α-Androstanedione	8.1	7.4	6.7	2.6	7.4	5.1	4.4	n.d.	10.2	17.8	4.7	5.9

Tumours are either grade 2 (intermediate differentiated) or grade 3 (poorly differentiated) carcinomas.
*n.d. = not detectable.
†Combined 5α-androstane-3α, 17β-diol and 5α-androstane-3β, 17β-diol.

structural maintenance in organ cultures, but a relatively insensitive means for determining hormone response, particularly over such a short culture period as 2 days.

The addition of 0.1 mM NADPH to benign tumour explants was found to provide no enhancement of the 5α-reduction of testosterone and, in fact, reduced to some extent the reductive metabolites in malignant tumours. However, this reduction did not constitute a major shift and was equivalent to a minor proportion (<1%) of the available substrate. Although NADPH has been shown to be essential for optimum enzyme activity in cell-free studies [12], the lack of effect in this study using cultured explants is more indicative of the tissue's integrity in maintaining its own cofactor requirements. Thus it was not included for the whole study.

In all benign tissues studied, the principal metabolites of testosterone were 5α-dihydrotestosterone and the 5α-androstanediols, with the oxidative metabolites only contributing a minor portion to the overall profiles. The result compares well with previous reports by other groups [1, 4, 5, 12, 13] and indicates that this characteristic reductive pathway is easily demonstrable and independent of the technique employed. However, in the malignant explant the mean metabolite values revealed a significantly ($P < 0.01$) restricted ability for 5α-reduction of testosterone. Although the overall metabolism of substrate was markedly reduced in all but one of the malignant tissues, the oxidative metabolites were significantly raised ($P < 0.001$). In only one tissue from 12 carcinoma specimens was a metabolite profile comparable to BPH tissues obtained, and this exception may highlight the problems associated with the selection of representative sections of the tumour when studying malignant prostate tissues [14]. A similar shift towards oxidative metabolism in carcinomatous tissues has been noted previously [5, 15, 16]. In this study by Morfin *et al.* [5] using a short-term tissue biopsy incubation procedure the oxidative pathway was dominant in only two of the eight poorly differentiated carcinomas examined. Habib *et al.* [16] only demonstrated this finding in a single poorly differentiated specimen studied using short-term supernatant incubation, whilst Jenkins and McCaffery [4] and Bard and Lasnitzki [13] did not find this to be the dominant pathway. Our present findings indicate that the shift to an oxidative metabolism is a prime feature of both the intermediate and poorly differentiated untreated carcinoma.

In addition, our results with tissues obtained from five patients following endocrine therapy indicated a possible further decrease in 5α-

Table 4. Radiometabolites obtained from benign and malignant human prostate explants following culture with [^{3}H]-dihydrotestosterone for 48 hr

Radiometabolite	Tissue (No.) Benign hypertrophied ($n = 4$)	Malignant ($n = 4$)
Unidentified	7.5 ± 4.4	3.2 ± 1.9
5α-Androstanediols*	84.0 ± 7.2	86.1 ± 4.8
Testosterone	1.7 ± 0.9	n.d.†
Δ^4-Androstenedione	1.0 ± 0.7	0.1 ± 0.1
5α-Androstanedione	5.6 ± 2.6	10.3 ± 3.9
% substrate metabolized per 100 mg tissue	10.1 ± 0.5	11.9 ± 1.1

Results are mean ± S.E.M. expressed as percentage of radiometabolites recovered.
*Combined 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol.
†n.d. = not detectable.

reductase activity in those patients receiving stilboestrol/orchiectomy treatment; however, this was not statistically different from the untreated tissues. Jenkins and McCaffery [4] found distinct enhancement of the oxidative pathway in three poor–intermediate-grade carcinomas from patients receiving oestrogen therapy, but did not find the oxidative pathway to be dominant in untreated poorly differentiated tumours. Bard and Lasnitzki [13] also noted androstenedione to be the major metabolite in one carcinoma from a patient treated with stilboestrol prior to surgery. It is unlikely that this apparent alteration in metabolism is caused by the direct effect of stilboestrol in the explants, as *in vitro* studies [4, 13] have shown that inhibition is only obtained with very high levels of oestrogens which would not be present in this explant technique. However, prolonged oestrogen therapy and orchiectomy do cause a change in histological characteristics of the gland [17] which could be associated with this finding.

The metabolism of 5α-dihydrotestosterone by benign and carcinomatous tissues revealed a slightly lower, but not significantly different, level of substrate usage in the benign specimens. Hudson [18] has reported a lower activity of 3α-hydroxysteroid dehydrogenase in benign prostate cytosols than in either malignant or normal specimens, whilst in contrast, Krieg *et al.* [6] reported this activity to be lowest in malignant specimens. Although the main product of the 5α-androstanediols was similar with both types of tissue, there was a slight increase in the proportion of 5α-androstanedione in the malignant tissues, which reflects the oxidative activity in the tissue. This finding has also been noted by Morfin *et al.* [5], who found substantial conversion of 5α-dihydrotestosterone to 5α-androstanedione in three specimens of poorly differentiated carcinoma.

The present study clearly demonstrates that there is a distinct difference between the metabolic pathways in benign hypertrophied and both intermediate and poorly differentiated malignant human prostates. The main feature in the malignant tumours was a major oxidative metabolic pathway, whilst in the benign tumours the reductive route was predominant. It is interesting to note that in well-differentiated carcinomatous tissues the oxidative route is not as predominant as in either intermediate or poor grades of tumours [5, 13]. The present study also highlights the value of organ culture techniques in which the integrity and function of the tissue can be maintained and differences in the metabolic pathways revealed. These differences are particularly relevant to the understanding of the mechanism by which androgens exert their biological action on the prostate.

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