Pergamon

0960-0760(94)00114-6

J. Steroid Biochem. Molec. Biol. Vol. 51, No. 1/2, pp. 89–96, 1994

0960-0760/94 $7.00 + 0.00

Androgen Metabolism in the Human Prostatic Cancer Cell Line LNCaP

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The metabolism of androgens in prostatic carcinoma has not been sufficiently studied so far, mainly because of the difficulty in obtaining human tissue specimens. The availability of LNCaP (lymph node carcinoma of the prostate) cells which retain most of the characteristics of the original carcinoma (dependence on androgens, presence of androgen receptors, production of acid phosphatase, etc.) has allowed the present *in vitro* study designed to characterize the metabolic pathways through which testosterone (T) is metabolized in malignant cells. LNCaP cells have been incubated in the presence of different labelled androgenic precursors to quantitate the formation of the respective metabolites, as indicators of the specific activities of the enzymes involved in such conversions; whenever possible, the kinetic constants (K_m and V_{max}) of the enzymes have also been calculated. It has been observed that, when [^{14}C] T is used as substrate, the cells form both dihydrotestosterone (DHT) and androst-4-en-3,17 dione (Δ_4) with the prevalence of the latter. When [^{14}C]Δ_4 is the substrate, T and 5α-androstan-3,17-dione (5α-A) are found with 5α-A representing the major product. In addition, the cells form diols and 5α-A from [^{14}C]DHT as well as androsterone (A) and DHT from [^{14}C]5α-A; there is a prevalence of diols in the former case, and of A in the latter one. The yields of the different metabolites recovered after 2 h of incubation of the cells with the appropriate labelled substrates are therefore in descending order of magnitude: 5α-A > A > diols > Δ_4 > DHT > T. These results are also confirmed by the analysis of the rate of production of the different steroids. Taken together the present results suggest that: (a) qualitatively LNCaP cells possess all the major key enzymes involved in androgen processing; (b) the metabolism of androgens in this cell line resembles quantitatively that found in prostatic cancer tissue; all the metabolic steps which contribute to DHT degradation exceed the ones leading to its accumulation; (c) 5α-reductase shows a significantly higher affinity for Δ_4 than for T; (d) LNCaP cells may represent a suitable *in vitro* model for the study of factors controlling the formation and the degradation of androgens in prostatic carcinoma, thus permitting a better understanding of the metabolic processes involved in prostatic benign or malignant (carcinoma) transformation.

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INTRODUCTION

The presence of circulating androgens, and in particular of testosterone (17β-hydroxy-androst-4-ene-3-one, T), is at least initially a growth requirement for prostatic cells undergoing either benign (benign prostatic hyperplasia, BPH) or malignant (carcinoma of the prostate) transformation. Indirect evidence suggesting that androgens are involved in these processes is provided by the results of the therapeutical approaches presently utilized for the treatment of these diseases, which are all based on their ability to suppress T production and/or action [1].

However, in the prostate, T does not act as such, but needs to be converted into its major 5α-reduced metabolites in order to be able to bind to the androgenic receptor [1]. T is irreversibly 5α-reduced to dihydrotestosterone (17β-hydroxy-5α-androstan-3-one, DHT) by the action of the enzyme 5α-reductase (5α-R). DHT is further metabolized to 3α,17β-dihydroxy-5α-androstane (3α-diol) and, to a lesser

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Received 29 Dec. 1993; accepted 14 Jun. 1994.

extent, to 3β,17β-dihydroxy-5α-androstane (3β-diol) by the action of two 3α- and 3β-hydroxysteroid-oxidoreductases (3α- and 3β-HSORs). T is also metabolized to androst-4-en-3,17-dione (Δ_4), which can subsequently be 5α-reduced to 5α-androstan-3,17-dione (5α-A) and androsterone (A). These conversions are under the control of the enzymes 17β-hydroxysteroid-oxidoreductase (17β-HSOR), 5α-R, and 3α-HSOR, respectively (see Fig. 1). The predominant androgen found in BPH tissue is DHT, because of the overwhelming potent DHT-forming ability of 5α-R, and the concomitantly low DHT-removing capacity of 3α- and 3β-HSORs [2]. In prostatic carcinoma, the metabolism of androgens has been less extensively studied [3–8].

Horoszewicz *et al.* [9, 10] have recently established a human prostatic cancer cell line, LNCaP (lymph node carcinoma of the prostate), which retains most of the characteristics of human prostatic carcinoma, like the dependence on androgens, the presence of androgen receptors, the production of acid phosphatase and of prostate specific antigen, etc. Because of this, the LNCaP cell line seems to be an attractive model for the *in vitro* studies on the biology of human prostatic carcinoma. However, although a few papers have partially described the metabolism of androgens in LNCaP cells [11–15], a complete analysis of androgen metabolism including the evaluation of the kinetic parameters of the major enzymes involved is still lacking.

The present study was designed to characterize the metabolic pathways through which T and other androgens are metabolized in LNCaP cells, and to analyse some of the properties of the enzymes involved. To this purpose, LNCaP cells were incubated in the presence of different labelled androgenic steroids to quantitate the formation of the respective metabolites, as indicators of the specific activities of the enzymes involved in such conversions.

EXPERIMENTAL

Labelled steroids

[4-^{14}C]T (sp. act. 54.4 mCi/mmol, Amersham, England), [4-^{14}C]Δ_4 (sp. act. 51.4 mCi/mmol, NEN, Research Product, DuPont Co., Boston, MA), [4-^{14}C]DHT (sp. act. 58.3 mCi/mmol, NEN) and [4-^{14}C]5α-A (sp. act. 58.3 mCi/mmol) were used as labelled substrates in the various experiments. Carbon labelled 5α-A was synthesized by oxidation of [4-

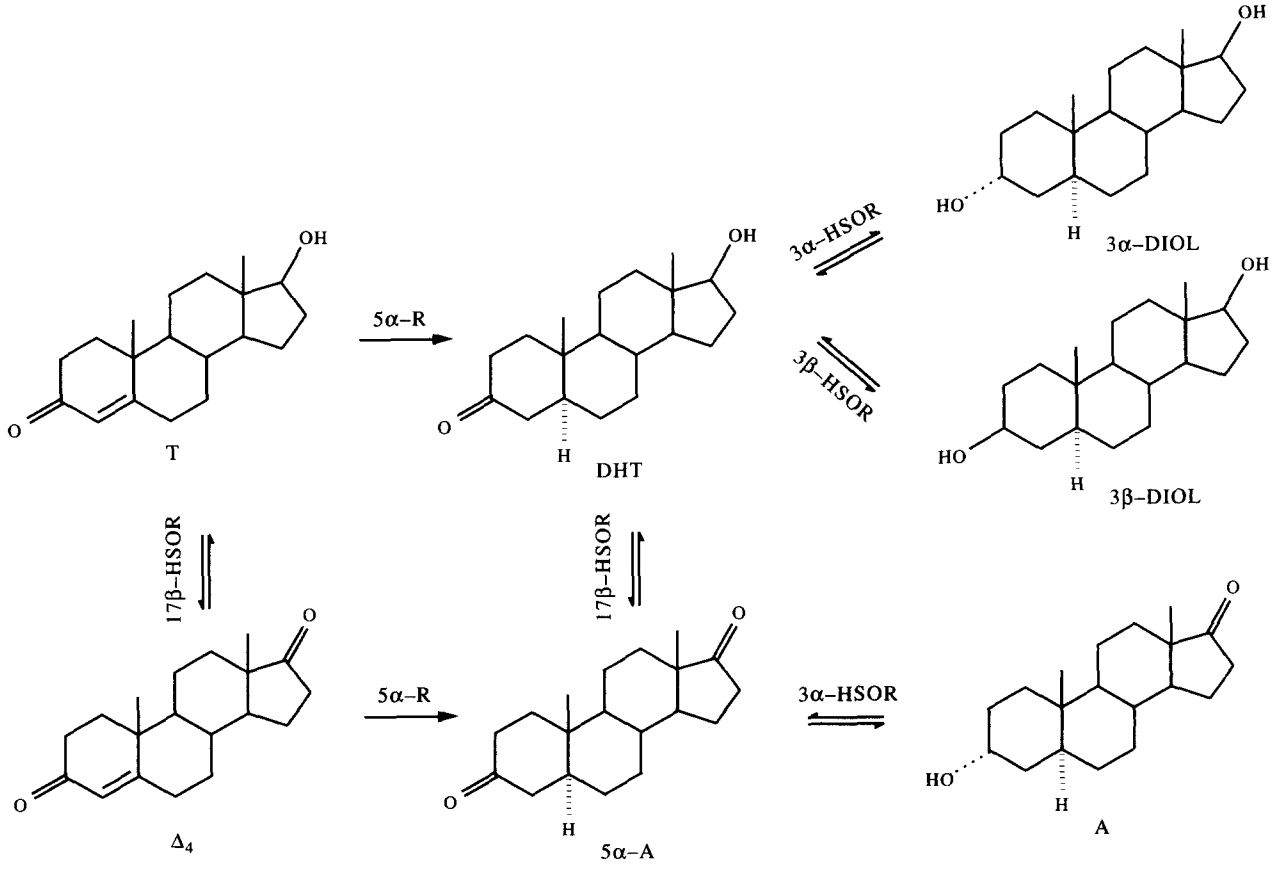

Fig. 1. Diagrammatic pathways of androgen metabolism in the prostate. List of abbreviations: T, testosterone; DHT, 17β-hydroxy-5α-androstan-3-one, dihydrotestosterone; 3α-diol, 3α,17β-dihydroxy-5α-androstane; 3β-diol, 3β, 17β-dihydroxy-5α-androstane; Δ_4, androst-4-en-3,17-dione; 5α-A, 5α-androstane-3,17-dione; A, 3α-hydroxy-5α-androstan-17-one, androsterone; 5α-R, 5α-reductase; 17β-HSOR, 17β-hydroxysteroid-oxidoreductase; 3α-HSOR, 3α-hydroxysteroid-oxidoreductase; 3β-HSOR, 3β-hydroxysteroid-oxidoreductase.

^{14}C]DHT according to Jones' procedure (see Ref. [16] for methodological details). Tritiated T, DHT, Δ_4 and A were purchased from NEN; [^{3}H]5α-A was synthesized from [^{3}H]DHT as quoted above [16]. The tritated steroids were utilized for the calculation of the recoveries. All the radiolabelled steroids were routinely purified by thin layer chromatography (TLC) before every experimental procedure.

Cell culture conditions

The cell line LNCaP-FGC (lymph node carcinoma of the prostate-fast growing colony) was obtained from American Type Culture Collection (Rockville, MD U.S.A.) and used at passages 25–35. LNCaP cells were routinely grown in RPMI-1640 (Seromed, Biochrom KG, Berlin, Germany), supplemented with 10% foetal calf serum (FCS, Gibco, Life Technologies Ltd, Paisley, Scotland), 1 mM of glutamine (Seromed, Biochrom KG, Berlin, Germany), and antibiotics (penicillin G K-salt, Squibb, 100 IU/ml and streptomycin sulphate, Squibb, 100 μg/ml). Cells were incubated at 37°C in a humidified atmosphere (5% CO_2–95% air), changing the medium every 3 days. All the experiments were performed on exponentially growing LNCaP cells; to this purpose, cells were plated at a density of 2×10^4 cells/cm^2 in 35-mm Petri dishes. Cells were allowed to attach and grow for 4 days; 24 h before the beginning of each experiment, the seeding media were replaced with the experimental media consisting of 1.5 ml of RPMI-1640 supplemented with glutamine, antibiotics and 10% charcoal-dextrane-treated FCS, i.e. deprived of steroids; this in order to avoid possible interferences with the metabolism of the added steroids.

Incubation conditions

At the time of incubation, ^{14}C-steroids were added, dissolved in 5 μl of ethanol, to each dish containing LNCaP cells. The final ethanol concentration (0.3%) has been shown in preliminary experiments not to be toxic for the cells. The dishes were then incubated for the selected times without shaking at 37°C in a thermostatic room. Within each experiment, dishes containing the medium and the appropriate labelled substrate (see below) but devoid of cells were processed as blanks. In order to achieve an accurate evaluation of each enzymatic activity, the metabolites of the various androgens were assayed starting from different substrates as follows: DHT and Δ_4 from [^{14}C]T; 5α-A and T from [^{14}C]Δ_4; diols and 5α-A from [^{14}C]DHT; A and DHT from [^{14}C]5α-A. The specific conditions, in terms of substrate concentrations and incubation times, are detailed in each table and figure. At the selected times, the incubations were stopped by removing the media, which were separately transferred to 20 ml glass vials; the vials were then stored at −20°C until extraction and determination of the androgen metabolites formed. LNCaP cells were harvested from each dish with 500 + 500 μl of phosphate-buffered saline (PBS) without calcium, and sonicated for 5 s; protein content (about 0.2–0.4 mg/well) was determined by the Bradford method [17] in an aliquot of each suspension.

Detection of formed metabolites

The labelled metabolites formed by LNCaP cells and present in the media were evaluated with an *in vitro* radiometric method routinely used in the authors' laboratory and described in detail elsewhere [18, 19]. In summary, the samples were extracted with diethyl ether, evaporated to dryness and applied on silica gel TLC plates which were then developed in dichloromethane–diethyl ether (11 : 1, v/v) twice at 4°C. The steroids were identified on the plates with iodine vapours; each identified spot was scraped off, and the radioactivity present was counted in a Packard Tri-carb 1600CA liquid scintillation analyser. Quench corrected dpm of the two isotopes were obtained by a calibration standard curve. The ng of steroid formed/mg of proteins were calculated utilizing a specific program and a Macintosh LC II computer. The chromatographic solvent system (dichloromethane–diethyl ether, 11 : 1, v/v) used in the present experiments does not allow a good separation between 3α- and 3β-diol; therefore the two isomers were assayed together, and referred to, in tables and figures, as diols, while 3α/β HSOR refers to the activity of both the 3α- and 3β-HSORs.

The identity and purity of each metabolite formed during the incubation with the different substrates was confirmed in a separate set of experiments by three subsequent recrystallizations to constant ^{3}H/^{14}C ratio (Table 1). Because of the reason mentioned above, it was not possible to perform the recrystallization to constant ^{3}H/^{14}C ratio of 3α- and 3β-diol. The increased ratio observed in the 2° and 3° crystallization of A does not seem to be linked to the presence of impurities, but likely due to a spill-over problem between tritiated and carbon labelled materials in the double labelling counting system. This may be deduced also from the observation that the ratio of the 1° crystallization is practically identical to that of the starting solution.

Table 1. *Recrystallization of the different androgen metabolites to a constant $^3H/^{14}C$ ratio*

	^{3}H/^{14}C Ratio				
	T	DHT	Δ_4	5α-A	A
Starting solution	4.49	3.74	2.01	2.99	1.76
I crystallization	4.58	3.62	1.84	2.82	1.70
II crystallization	4.79	3.53	1.89	2.76	2.20
III crystallization	4.45	3.52	1.75	2.69	2.96

The various metabolites were obtained from different substrates according to the protocol adopted in the experiments (see Experimental). The following three pairs of solvents were used: absolute ethanol–water, acetone–water, diethyl-ether–*n*-hexane. See Fig. 1 for steriod abbreviations.

Statistics

The regression curves were evaluated according to the linear regression method, using a computer statistical package. The kinetic parameters of the various enzymes (K_m and V_{max}) were calculated using the Mackintosh version of a specific non-linear least-squares curve fitting program (ENZYME, [20]). On the basis of the statistical model suggested by Munson and Rodbard [21], the computer program MacELLIPSE [22] was then used to generate the 95% joint confidence ellipses for the two parameters of the 5α-R, which catalyses the reduction of T and Δ_4 to DHT and 5α-A, respectively.

RESULTS

Figure 2 shows the *in vitro* metabolism of androgens in LNCaP cells after 2 h incubation. When [^{14}C]T is used as the substrate, the cells have the ability to convert T both into DHT and Δ_4; the formation of Δ_4 exceeds that of DHT of about 3 times [Fig. 2(a)]. When [^{14}C]Δ_4 is used as the substrate, T and 5α-A are formed; the formation of 5α-A is 10 times higher than that of T [Fig. 2(b)]. In addition, the formation of 5α-A from [^{14}C]Δ_4 [Fig. 2(b)] is 8 times higher than the production of DHT from [^{14}C]T [Fig 2(a)] (note the difference in the scales); the formation of T from [^{14}C]Δ_4 [Fig. 2(b)] is 4 times lower than the production of Δ_4 from [^{14}C]T [Fig. 2(a)]. Figure 2 also illustrates the formation of the metabolites derived from [^{14}C]DHT [Fig. 2(c)] and from [^{14}C]5α-A [Fig. 2(d)]. It is evident that the diols as well as 5α-A are formed from [^{14}C]DHT; the formation of the diols is twice as high as that of 5α-A [Fig. 2(c)]. When the cells are incubated with [^{14}C]5α-A, the formation of A predominates 5 times over that of DHT [Fig. 2(d)]. Unfortunately, due to the unavailability of carbon-labelled 3α-, 3β-diols and A, the formation of DHT and 5α-A from the respective precursors, and consequently the oxidative pathway of the 3α/β-HSOR, could not be examined. Although the figure illustrates the formation of one pair of steroids for each substrate, it must be reminded that each steroid can be

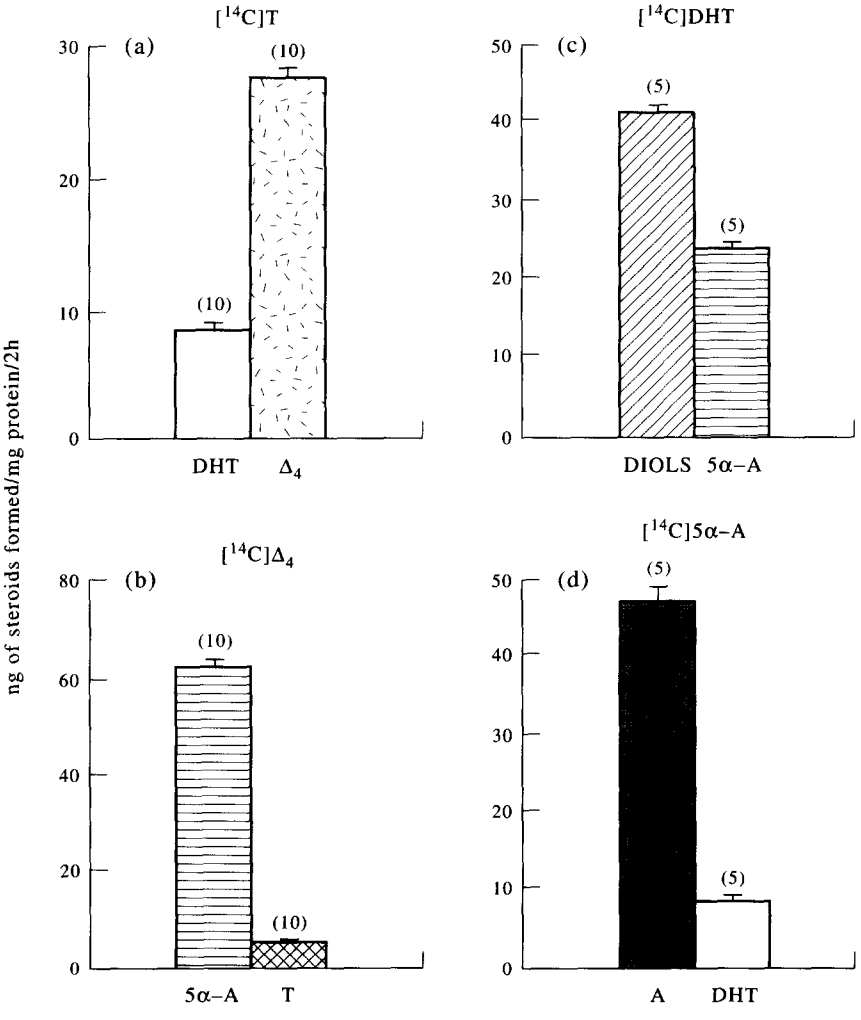

Fig. 2. Amounts of various androgen metabolites in LNCaP cells recovered after 2 h of incubation with different substrates added at the dose of 1.2 μM. Results expressed as mean ± SE (in parentheses number of replicates). See Fig. 1 for steroid abbreviations, DIOLS, sum of 3α- and 3β-diol.

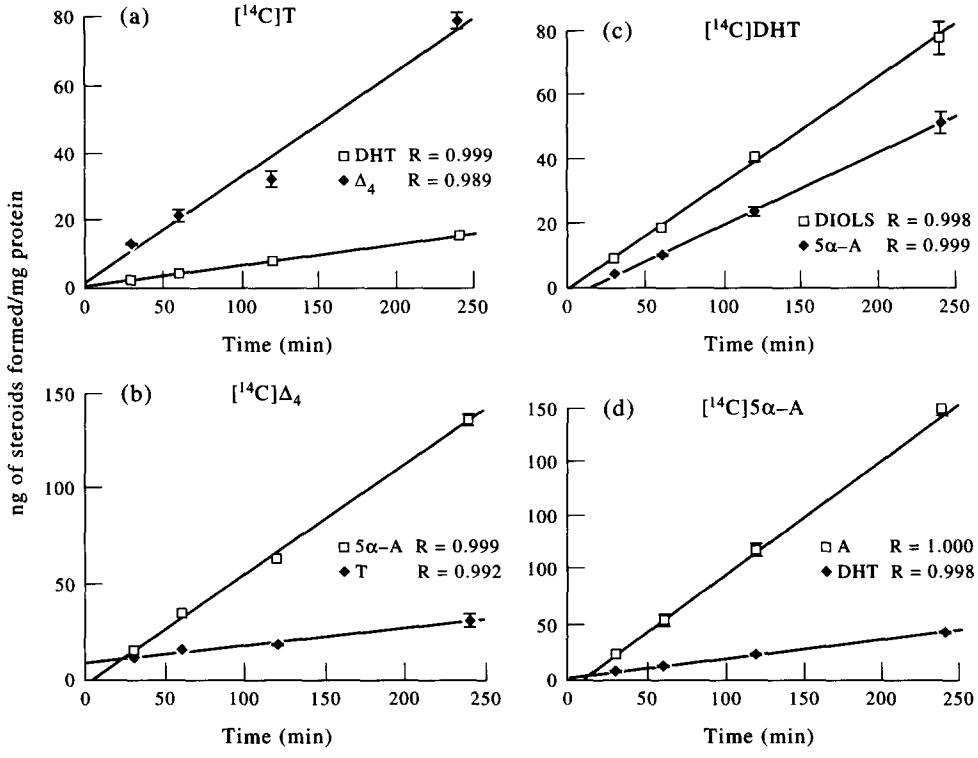

Fig. 3. Time course of the formation of the various androgen metabolites in LNCaP cells incubated with different substrates at the dose of 1.2 μM. Results expressed as mean ± SE (at least 5 replicates/time). See Fig. 1 for steroid abbreviations, DIOLS, sum of 3α- and 3β-diol.

further transformed into other metabolites; this may lead to an underestimation of the real quantity of product formed by each enzyme.

The amounts of the various metabolites formed from the different substrates as a function of the incubation time is shown in Fig. 3. It appears that, independently of the substrate used, the production of all metabolites is directly related to the incubation period up to 4 h (R ranging from 0.989 to 1.000). Moreover, utilizing [^{14}C]T as substrate [Fig. 3(a)] the rate of formation of Δ_4 exceeds that of DHT, while, using [^{14}C]Δ_4 as substrate [Fig. 3(b)], the rate of formation of 5α-A is higher than that of T. When [^{14}C]DHT is used as precursor [Fig. 3(c)], the rate of formation of the diols exceeds that of 5α-A; when [^{14}C]5α-A is the substrate [Fig. 3(d)], the rate of formation of A is much higher than that of DHT.

Table 2 shows the kinetic parameters (K_m and V_{max}) of the enzymes involved in the metabolism of androgens in LNCaP cells as well as the V_{max}/K_m ratio (expressed as picomoles of product formed/mg of protein/h over molar concentration) which represents an indirect index of the metabolic activity of each enzyme. It must be pointed out that all constants are given as "apparent", because the kinetic properties of the enzymes have not been established with pure preparations. In the experimental conditions adopted, the apparent K_m values calculated for 5α-R have been shown to be higher for T than Δ_4 (4-fold difference); since this parameter is an index of the affinity of the substrate for the enzyme, it seems that Δ_4 shows a greater affinity for 5α-R than T. When the V_{max} values (which provide an index of the concentration of the enzyme) are calculated for 5α-R, the V_{max} of the

Table 2. Kinetic parameters of some enzymes involved in androgen metabolism in LNCaP cells

Enzyme	Substrate	Product	K_m (μM)	V_{max} (ng/mg/h)	V_{max}/K_m[a]
5α-R	T	DHT	12.7 ± 1.2	35.1 ± 1.5	9,4
	Δ_4	5α-A	3.5 ± 0.3	71.6 ± 3.3	71,4
17β-HSOR (red)	Δ_4	T	110 ± 16.9	248.6 ± 23.1	7,8
	5α-A	DHT	235 ± 28	1115 ± 94	16,6
3α-HSOR (red)	5α-A	A	298 ± 37	9225 ± 855	108,5

The kinetic experiments were performed incubating LNCaP cells (at least 4 replicates per concentration) for 1 h with the different substrates (3–200 μM) according to the procedure detailed in Experimental. The values are expressed as mean ± SE of the parameters. See Fig. 1 for steroid and enzyme abbreviations.

[a]The V_{max}/K_m ratio is expressed as pmol/mg protein/h over molar concentration.

formation of 5α-A appears to be twice as high as that of DHT. The lower K_m value, associated with the greater V_{max} value, results in a V_{max}/K_m ratio 8 times higher for the conversion of Δ_4 to 5α-A than for that of T to DHT. It will be noted that no K_m and V_{max} values are given for 17β-HSOR for either substrates studied (T and DHT); this depends on the insolubility of the substrates, and consequently on the lack of saturation.

As stated in the Experimental section, the impossibility to separate 3α- from 3β-diol did not allow the determination of the kinetic parameters of the enzymes responsible for the formation of the two isomers.

DISCUSSION

The present data demonstrate that LNCaP cells possess all the major key enzymes involved in the metabolism of T which are also found in the normal prostate, in BPH and in prostatic cancer. The DHT promoting enzyme, 5α-R, and the DHT removing enzymes, 3α/β- and 17β-HSORs are all present in this cell line. The data obtained in the various sets of experiments (formation of the metabolites after 2 h of incubation, rate of their production, kinetic characteristics of the enzymes involved) are all consistent and allow to draw the following general conclusions (see Fig. 4): (a) 5α-R, which converts T and Δ_4 to the corresponding 5α-reduced compounds (DHT and 5α-A, respectively), appears to be more active on the latter steroid, suggesting a preference for the substrate Δ_4 over T; (b) the activity of 3α-HSOR (the enzyme catalysing the formation of A from [^{14}C]5α-A) is rather elevated in its reductive direction; the reductive activity of 3α/β-HSOR seems to be relevant also in the formation of the diols, even if their relative activity has not been estimated (see Experimental section); (c) the activity of 17β-HSOR (the enzyme which catalyses the interconversion between T and Δ_4, and between DHT and 5α-A) is mainly directed towards the oxidative (formation of Δ_4 and 5α-A) rather than the reductive pathway (formation of T and DHT); (d) 3α- and 17β-HSOR enzymes are much more concentrated (see

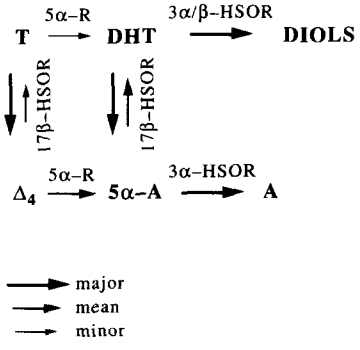

Fig. 4. Diagram illustrating the relative rates of activity of the androgen metabolizing enzymes occurring in LNCaP cells. See Fig. 1 for steroid and enzyme abbreviations, DIOLS, sum of 3α- and 3β-diol.

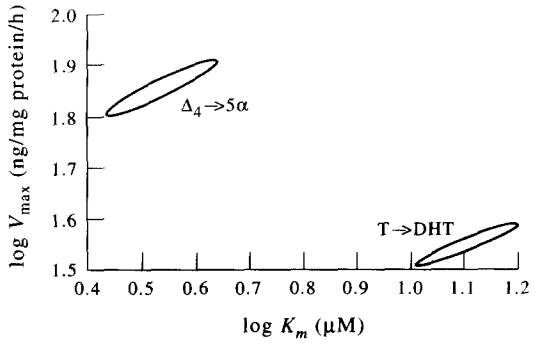

Fig. 5. Graphical representation of joint 95% confidence regions for K_m and V_{max} of 5α-R converting T and Δ_4 to DHT and 5α-A, respectively. The log-values of the parameters obtained from the experiments shown in Table 2 have been plotted and the confidence ellipses have been generated by the MacEllipse program. No overlapping ellipses represent a significant difference in the kinetic characteristics of 5α-R metabolizing the two substrates.

the corresponding V_{max} values), but less specific (see the corresponding K_m values) for androgens than 5α-R.

The finding that 5α-R is more active in reducing Δ_4 than T is confirmed by the statistical co-analysis of the K_m and V_{max} of the two metabolic reactions performed with a specific computer program for the simultaneous analysis of correlated parameters (Fig. 5). This analysis indicates that, from a kinetic point of view, the 5α-R activity converting T to DHT is significantly different from that metabolizing Δ_4 to 5α-A. These results suggest that LNCaP cells might express preferentially one 5α-R isoenzyme which shows a greater affinity for Δ_4 than for T. It is known that 5α-R isoenzymes may be present in different tissues (prostate, epididymis, etc.) [18, 19, 23], and that two 5α-Rs have been recently cloned [24, 25]. Moreover, with regard to the V_{max}/K_m ratio for the 5α-R, Hudson and Wherrett [3] have found, in agreement with the present findings, that in the epithelial cells obtained from a prostatic carcinoma specimen, this parameter is 10 times higher for the 5α-R bringing to the formation of 5α-A than for the same enzyme involved in the formation of DHT. The low activity of 5α-R in forming DHT might not be the only factor responsible for the low amounts of this metabolite recovered in the present experiments; another reason may be found in the concomitant rapid degradation of DHT. The present results indeed show a rapid conversion of DHT to diols, as well as a fast oxidation of DHT to 5α-A; this as a consequence of the high activities of 3α/β- and 17β-HSORs acting respectively in the reductive and in the oxidative direction (see Fig. 4). This is apparently not the case when considering the formation of 5α-A, since the quantities of this steroid formed are elevated, even if its transformation to A is high. The low formation of DHT observed in the present experiments might also be due to the fact that 5α-R is prevalently localized in the prostatic stroma, as shown by the higher V_{max} for the enzyme found in the stroma than in the epithelium of normal,

hyperplastic and carcinomatous human prostatic tissue [2, 26]; LNCaP cells originate from the epithelial component of a human prostate carcinoma [10, 27].

LNCaP cells possess a significant 3α/β-HSOR activity as judged by the elevated rate of production of diols from DHT and of A from 5α-A (see Fig. 4). The 3α- and 3β-HSORs are known to be important enzymes involved in the equilibrium between DHT and diols and between 5α-A and A; however, the high rate of formation of diols and of A (as indicated by the high activity of the 3α/β-HSOR in their reductive directions), coupled with the elevated rate of transformation of DHT to 5α-A, may suggest that LNCaP cells possess more DHT-removing than DHT-forming enzymatic activities. It must be remembered that both diols and A show an extremely low affinity for the androgen receptor [28]. The great DHT-removing capacity of 3α/β-HSOR is probably justified by the epithelial origin of LNCaP cells. It is known that 3α-HSOR is primarily localized in the prostatic epithelium, as shown by the higher V_{max} values obtained with prostatic epithelial tissue as opposed to prostatic stroma [29].

Finally, in LNCaP cells the activity of 17β-HSOR is preferentially directed in the oxidative rather than in the reductive direction, as indicated by the higher formation of Δ_4 and 5α-A from T and DHT, respectively (see Fig. 4). A high 17β-HSOR oxidase activity seems to be peculiar of malignant tissues, in the case of both the human prostate [4] and the human breast cancer [30].

The results presented show that, from a quantitative point of view, the steroids formed by LNCaP cells from the appropriate labelled substrates under the influence of the specific enzymes are in order of magnitude: 5α-A > A > diols > Δ_4 > DHT > T. There are only few data in the literature regarding the metabolism of androgens in LNCaP cells which can be compared to the present results. Berns *et al.* [11] have found that approx. 60% of the labelled DHT incubated with LNCaP cells is converted to unidentified polar steroids; Damassa *et al.* [12] have identified the polar steroids as being primarily 3α-diol. Furthermore, Lacoste *et al.* [13] and Belanger *et al.* [14] have reported the formation of Δ_4 and T from dehydroepiandrosterone, of 3α-diol from DHT and of T and DHT from Δ_4 in LNCaP cells. Contrary to the results presented in this paper, Hasenson *et al.* [15] have not been able to find any significant formation of labelled metabolites after the addition of [^{3}H]T to LNCaP cell cultures. This difference is probably linked to the timing of the assay adopted by Hasenson's group (48 h of incubation vs the 2–4 h adopted in the present experiments). The lack of a significant presence of any free metabolite of T may be due to the fact that, after 24 h of incubation, more than 50% of the labelled substrates added to LNCaP cells and of their metabolites are present as conjugated derivatives [12–14]. Furthermore, the cell clone used by Hasenson *et al.* [15], at variance with the one used in the present experiments, does not seem to respond, or responds poorly, to androgen administration; consequently, these "androgen resistant" cells might not have been able to express the enzymatic machinery involved in the metabolism of T and observed in the androgen-dependent LNCaP cells used in the present experiments.

The pattern of androgen metabolism observed in LNCaP cells appears to be similar to that found in human prostatic carcinoma [3–8]. In particular in both cases, when T is the substrate, the formation of Δ_4 predominates over that of DHT; this is due to a decreased activity of 5α-R forming DHT, coupled with an increased oxidative activity of 17β-HSOR. This is at variance with BPH, where DHT constitutes the major metabolite formed [2, 31]. Because of the similarity of androgen metabolism in LNCaP cells and in human prostatic carcinoma tissue, this cell line represents a suitable model to facilitate our understanding of the role played by the different pathways metabolizing androgens in the regulation of tumoural prostatic cell growth. This cell line might also be of help in analysing the factors involved in the control of androgen metabolism and degradation in prostatic carcinoma and in developing future compounds able to interfere with the activity of the various enzymes (e.g. new selective 5α-R inhibitors, etc.).

Acknowledgements—This study was supported by A.I.R.C., by Consiglio Nazionale delle Ricerche through the special projects "A.C.R.O." (contract No. 93.02197.PF39), "F.A.T.M.A." (contract No. 93.00700.PF41), "Aging" (contract No. 93.00438.PF40) and by MURST. The authors are deeply grateful to Dr P. Ferraboschi (Dept of Chemistry and Medical Biochemistry, via Saldini 50, Milano), who has synthesized [^{14}C]- and [^{3}H]5α-A.

REFERENCES

1. Motta M. and Serio M.: *Hormonal Therapy of Prostatic Disease: Basic and Clinical Aspects.* Medicom, The Netherlands (1988).
2. Tunn S., Hochstrate H., Grunwald I., Fluchter S. H. and Krieg M.: Effect of aging on kinetic parameters of 5α-reductase in epithelium and stroma of normal and hyperplastic prostate. *J. Clin. Endocr. Metab.* **67** (1988) 979–985.
3. Hudson R. W. and Wherrett D.: Comparison of the nuclear 5α-reduction of testosterone and androstenedione in human prostatic carcinoma and benign prostatic hyperplasia. *J. Steroid Biochem.* **35** (1990) 231–236.
4. Smith C. B., Master J. R. W. and Ghanadian R.: Differential androgen metabolism in prostatic tumors. *J. Endocr.* **85** (1980) 5P–6P.
5. Krieg M., Bartsch W., Jassen W. and Voigt K. D.: A comparative study of binding, metabolism and endogeneous levels of androgens in normal, hyperplastic and carcinomatous human prostate. *J. Steroid Biochem.* **11** (1979) 615–624.
6. Habib F. K., Busuttil A., Robinson R. A. and Chisholm G. D.: 5α-Reductase activity in human prostate cancer is related to the histological differentiation of the tumor. *Clin. Endocr.* **23** (1985) 431–438.
7. Hudson R. W.: Comparison of nuclear 5α-reductase activities in the stromal and epithelial fractions of human prostatic tissue. *J. Steroid Biochem.* **26** (1987) 349–353.
8. Klein H., Bressel M., Kastendieck H. and Voigt K. D.: Quantitative assessment of endogenous testicular and adrenal sex steroids and of steroid metabolizing enzymes in untreated human prostatic cancerous tissue. *J. Steroid Biochem.* **30** (1988) 119–130.

9. Horoszewicz J. S., Leong S. S., Chu T. M., Wajsman Z. L., Friedman M., Papsidero L., Kim J., Chai L. S., Kakati S., Arya S. K. and Sandberg A. A.: The LNCaP cell line—a new model for studies on human prostatic carcinoma. In *Models for Prostate Cancer* (Edited by G. P. Murphy). Alan R. Liss Inc., New York (1980) pp. 115–132.
10. Horoszewicz J. S., Leong S. S., Kawinsky E., Karr J. P., Rosenthal H., Chu T. M., Mirand E. A. and Murphy G. P.: LNCaP model for human prostatic carcinoma. *Cancer Res.* **43** (1983) 1809–1818.
11. Berns E. M. J. J., De boer W. and Mulder E.: Androgen-dependent growth regulation and release of specific protein by the androgen containing human prostate tumor cell line LNCaP. *Prostate* **9** (1986) 247–259.
12. Damassa D. A., Lin T. M. and Soto A. M.: Biological effects of sex hormone-binding globulin on androgen-induced proliferation and androgen metabolism in LNCaP prostate cells. *Endocrinology* **129** (1991) 75–84.
13. Lacoste D., Bélanger A. and Labrie F.: Steroid metabolism in LNCaP human prostatic cancer. *8th International Congress of Endocrinology*, Kyoto, Japan (1988) Abstr. 16-19-112.
14. Bélanger A., Brochu M., Lacoste D., Noel C., Labrie F., Dupont A., Cusan L., Caron S. and Couture J.: Steroid glucuronides: human circulatory levels and formation by LNCaP cells. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 593–598.
15. Hasenson M., Hartley-Asp B., Kihlfors C., Lundin A., Gustafsson J. A. and Pousette A.: Effect of hormones on growth and ATP content of a human prostatic carcinoma cell line, LNCaP-2. *Prostate* 7 (1985) 183–194.
16. Fieser L. F. and Fieser M.: *Reagents for Organic Synthesis*. Wiley & Sons, New York, Vol. 1 (1967) pp. 142–144.
17. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analyt. Biochem.* 72 (1976) 248–254.
18. Motta M., Zoppi S., Brodie A. M. and Martini L.: Effect of 1,4,6-androstatriene-3,17-dione (ATD), 4-hydroxy-4-androstene-3,17-dione (4-OH-A) and 4-acetoxy-4-androstene-3,17-dione (4-Ac-A) on the 5α-reduction of androgens in the rat prostate. *J. Steroid Biochem.* **25** (1986) 593–600.
19. Zoppi S., Lechuga M. and Motta M.: Selective inhibition of the 5α-reductase of the rat epididymis. *J. Steroid Biochem. Molec. Biol.* **42** (1992) 509–514.
20. Lutz R. A., Bull C. and Rodbard D.: Computer analysis of enzyme-substrate-inhibitor kinetic data with automatic model selection using IBM-PC compatible microcomputer. *Enzyme* **36** (1986) 197–206.
21. Munson P. J. and Rodbard D.: Computerized analysis of ligand binding data: basic principles and recent development. In *Computers in Endocrinology* (Edited by D. Rodbard and G. Forti). Raven Press, New York (1984) pp. 117–145.
22. Maggi R. and Rovati G. E.: MacEllipse: A graphical aid to the problem of the joint confidence region for correlated parameters. *Pharmac. Res.* **28** (1993) 351–358.
23. Rennie P. S., Bruchovsky N., Mcloughlin M. G., Batzold F. H. and Dunstal-Adams E. E.: Kinetic analysis of 5α-reductase isoenzymes in benign prostatic hyperplasia (BPH). *J. Steroid Biochem.* **19** (1983) 169–173.
24. Andersson S. and Russel D. W.: Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 3640–3644.
25. Labrie F., Sugimoto Y., Luu-The V., Simard J., Lachance Y., Bachvaron D., Leblanc G., Durocher F. and Paquet N.: Structure of human type II 5α-reductase gene. *Endocrinology* **131** (1992) 1571–1573.
26. Bruchovsky N., Rennie P. S., Batzold F. H., Goldenberg S. L., Fletcher T. and Mclouglin M. G.: Kinetic parameters of 5α-reductase activity in stroma and epithelium of normal, hyperplastic and carcinomatous human prostate. *J. Clin. Endocr. Metab.* **67** (1988) 806–816.
27. Eaton C. L., Davies P., Harper M., France T., Rushmere N. and Griffith K.: Steroids and the prostate. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 175–183.
28. Saartok T., Dahlberg E. and Gustafsson J. A.: Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in the prostrate, as well as to sex-hormone binding globulin. *Endocrinology* **114** (1984) 2100–2106.
29. Tunn S., Haumann R., Hey J., Fluchter S. H. and Krieg M.: Effect of aging on kinetic parameters of 3α(β)-hydroxysteroid oxidoreductases in epithelium and stroma of human normal and hyperplastic prostate. *J. Clin. Endocr. Metab.* **71** (1990) 732–739.
30. Mann V. Z., Newton C. J. and Tait G. H.: 17β-hydroxysteroid dehydrogenase in human breast tissues: purification and characterization of soluble enzymes and the distribution of particulate and soluble forms in adipose, non-adipose and tumor tissues. *J. Molec. Endocr.* 7 (1991) 45–55.
31. Isaacs J. T., Brendler C. B. and Walsh P. C.: Changes in the metabolism of dihydrotestosterone in the hyperplastic human prostate. *J. Clin. Endocr. Metab.* **56** (1983) 139–146.