Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 141-145. Pergamon Press. Printed in Great Britain

METABOLISM OF 5α-DIHYDROTESTOSTERONE IN HUMAN BENIGN HYPERPLASTIC PROSTATE

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(*Received* 6 *June* 1976)

SUMMARY

Quantitative aspects of the metabolism of 5α dihydrotestosterone (DHT) by hyperplastic human prostate were studied *in vitro* by superfusion of tissue slices with mixtures of labeled DHT, 3α, 17β androstanediol (Adiol), 3β, 17β androstanediol (3β Adiol), and androstanedione (Adione) in Krebs-Ringer phosphate buffer. The relative values of the rate constants of conversion of DHT to Adiol, Adione, and 3β Adiol were found to be about 10:5:1. Most of the intracellular DHT appeared in the medium unchanged rather than metabolized.

The significant interconversion observed to occur between DHT and Adiol favored the formation of DHT, as indicated by the ratio of rate constants corresponding to the forward and backward reactions. Most of the intracellular Adiol was found to be metabolized by direct conversion to DHT and androsterone; a smaller fraction left the cells as Adiol.

Comparison of isotope ratios revealed that the conversion of DHT to androsterone proceeded almost exclusively via Adiol.

INTRODUCTION

Dihydrotestosterone (DHT)*, the compound directly responsible for androgenic stimulation in prostatic tissue [1-5], is present in higher concentrations in hyperplastic than in normal prostates [6]. Due to the physiologic importance of DHT and its relation to benign prostatic hyperplasia (BPH), the formation of this compound by reduction of testosterone in target tissues has been extensively studied, both in normal and hyperplastic human prostates [7-10].

The purpose of this investigation was to examine the quantitative aspects of the metabolism of DHT in hyperplastic prostates. It was considered that the concentration of the hormone in tissue is influenced as much by the rate at which it appears *de novo* in the tissue as by its rate of intracellular clearance.

Several investigators[11-15] have studied the metabolism of DHT either by incubating prostatic tissue with labeled testosterone (T) or DHT, or by injecting the radioactive precursors before prostatectomy. In these experiments, the metabolites were identified and the distribution of radioactivity among them was determined at specified times of exposure to the labeled substrates. It was found that DHT can act as a substrate for 3α, 3β and 17β dehydrogenases to produce 3α, 17β androstanediol (Adiol), 3β, 17β androstanediol (3β Adiol) and 3, 17 androstanedione (Adione), products which can be further metabolized to androsterone and isoandrosterone. The metabolite of [^{3}H]-DHT consistently reported to appear in higher concentration is Adiol.

The present study was based on the application of an *in vitro* tracer superfusion method, described elsewhere [16,17], which involves incubation of tissue slices in buffer flowing at a constant rate without recycle. The medium contained mixtures of labeled DHT and either Adiol, 3β Adiol, or Adione, labeled with another isotope. Concentrations of radioactive compounds in the tissue and the superfusate were measured at the isotopic steady state. The data allowed the calculation of rate constants of conversion of DHT to the metabolites and of release of DHT to the medium, parameters which cannot be estimated from currently available information on distribution of radioactivity among metabolites of DHT. The reversibility of these metabolic reactions, e.g. the extent of conversion of Adiol to DHT, was evaluated in the same experiments.

MATERIALS AND METHODS

Tissue. Benign hyperplastic prostatic tissue was obtained from patients undergoing suprapubic prostectomy. Samples were collected immediately after surgical removal and brought to the laboratory in ice-cold saline. Experiments 3 and 4 involved two different portions of the same tissue specimen.

Steroids. [1,2-^{3}H]-Adiol (44 Ci/mmol), [4-^{14}C]-DHT (50.6 mCi/mmol), [1,2^{3}H]-DHT (44 Ci/mmol) were obtained from New England Nuclear and [1,2-^{3}H]-3β Adiol (45 Ci/mmol) was purchased from Amersham-Searle. The radioactive steroids were purified by t.l.c. before use. [^{3}H]-Adiol and [^{3}H]-3β Adiol

* Abbreviations used: DHT, 5α dihydrotestosterone; Adiol, 5α androstane 3α, 17β diol; 3β Adiol, 5α androstane, 3β, 17β diol; Adione, 5α androstane, 3, 17 dione.

were further purified by high pressure liquid chromatography. Crystalline steroids were obtained from Steraloids (Pauling, N.Y.). [^{14}C]-Adione was prepared [18] by chromic acid oxidation of [4-^{14}C]-DHT (50.6 mCi/mmol) and purified by t.l.c.

Superfusion media. Krebs–Ringer phosphate (pH 7.5) containing 0.9 mg glucose per ml was used. Oxygen was bubbled through the medium prior to use. The following mixtures of tracers were used in the media:

10^5 c.p.m./ml of [^{3}H]-Adiol (1 ng/ml) with 2×10^4 c.p.m./ml of [^{14}C]-DHT (85 ng/ml);

4×10^3 c.p.m./ml of [^{3}H]-3β Adiol (0.034 ng/ml) with 2.5×10^4 c.p.m./ml of [^{14}C]-DHT (107 ng/ml);

10^5 c.p.m./ml of [^{3}H]-DHT (0.85 ng/ml) with 2×10^4 c.p.m./ml of [^{14}C]-Adione (85 ng/ml).

Superfusion. Details of the superfusion technique have been described previously [19]. Minced prostatic tissue (300 mg) was placed in the superfusion chamber and the medium, kept at 37 C, was forced to flow through it at a rate of 20 ml/h during the 100 min of superfusion. Twenty minute fractions of the superfusate were collected separately in chilled test tubes. At the end of the superfusion, the tissue was rapidly washed with ice-cold buffer and homogenized with 5 ml of methanol containing the following amounts of carriers: 500 μg DHT, 500 μg Adiol, 100 μg Adione, 100 μg androsterone and 100 μg 3β Adiol. Five ml of each superfusate fraction was extracted with an equal vol. of ethyl acetate containing the same amounts of carriers.

Thin layer chromatography. The extracts were dried and purified using Silica gel glass plates (Analtech GF 254) developed with chloroform:acetone:hexane, 4:1:3. (by vol.) The various steroid bands, located by exposing a reference plate to iodine vapor, were scraped and eluted with ethyl acetate. Testosterone, DHT, Adiol and Adione were well separated from each other but 3β Adiol moved along with Adiol, and androsterone with DHT.

High pressure liquid chromatography. The eluted steroids were dried and further purified by high pressure liquid chromatography, using the Waters Associate system (model 6000). A column (0.7 cm × 30 cm) packed with microporacil (small diameter porous silica) and a cloroform–isooctane (6:4 v/v) solvent mixture yielded satisfactory resolution of Adione, DHT, androsterone, Adiol and 3β Adiol. The elution of the steroid was monitored with an on-line refractometer (Waters Associate, Differential Refractometer R401) connected to a recorder (Houston Instrument) and the material under each peak was collected as it emerged from the column. The flow rates used were 3 ml/min for the elution of Adione, DHT, and androsterone, and 4 ml/min for the elution of Adiol and 3β Adiol. Androsterone and 3β Adiol were passed through the same column twice to ensure purity. DHT and Adiol were rechromatographed in another column of the same size, packed with microbondapak C_{18} (organosilane bonded to silica) using the system acetonitrile–water (45:55 v/v). These steroids appeared as sharp peaks in the early fractions in this 'reverse phase' column.

Quantitation of the mass of eluted steroids, necessary for the estimation of losses during the isolation procedures, was achieved by measuring the areas under the peaks in the high pressure liquid chromatograms. A standard curve was obtained by plotting areas against known amounts of steroids.

Radioactivity measurements. Eluates from the column corresponding to each whole peak area were collected in vials and dried. Radioactivity of the residues was measured with a liquid scintillation counter (Nuclear Chicago, Isocap 300), using 10 ml of Scintiverse (Fisher Scientific).

Calculation of concentration of labeled steroids. Corrections for losses of steroids during the isolation procedures were made on the basis of the amounts of carrier recovered after high pressure liquid chromatography. Protein content of the precipitate obtained by treatment of the superfused tissue with methanol was measured by the method of Lowry *et al.* [20]. The amount of protein present in 1 g of tissue (60–80 mg) was similarly determined. These measurements allowed the estimation of the concentration of labeled steroids in superfusate (c.p.m./ml) and in tissue (c.p.m./mg protein or c.p.m./g tissue).

Calculations. In order to evaluate quantitatively the metabolism of DHT in slices of prostatic tissue, it is necessary to estimate first what fraction of the labeled compound in the superfusion medium enters the cells. The ratio of steady state concentrations of labeled DHT in the outflow and inflow of the superfusion chamber reflects the net-uptake of the exogenous compound, i.e., the difference between the fraction of superfused labeled DHT entering (α) and leaving (β) the cells:

$$\alpha_{DHT} - \beta_{DHT} = 1 - \frac{c^{14C}_{DHT} \text{ in superfusate}}{c^{14C}_{DHT} \text{ in superfusion medium}}$$

Intracellular labeling of DHT with ^{3}H derived from superfused Adiol allows the estimation of β and therefore of α. Thus,

$$\beta_{DHT} = \frac{(^{14}C/^{3}H)_{DHT} \text{ in tissue} \times c^{3H}_{DHT} \text{ in superfusate}}{c^{14C}_{DHT} \text{ in superfusion medium}}$$

These formulas and others that are listed below have been extensively discussed elsewhere [21]. For the purpose of illustrating the calculations, the formulas corresponding to parameters defining the metabolism of DHT during superfusions of mixtures of [^{14}C]-DHT and [^{3}H]-Adiol are shown here. Since α_{DHT} was found to equal α_{Adiol} (see Results), the formulas are simplified in accordance to this finding.

—Fraction of superfused DHT released to the medium as Adiol

$$\gamma_{DHT \to DHT} = c^{14C}_{Adiol} \text{ in superfusate}/c^{14C}_{DHT} \text{ in medium.}$$

—Ratio of rate constants of interconversion

between DHT and Adiol[22]

$$\frac{k_{\text{Adiol}\rightarrow\text{DHT}}}{k_{\text{DHT}\rightarrow\text{Adiol}}} = \frac{(c^{3H}_{DHT}/c^{14C}_{Adiol}) \text{ in tissue}}{(^3H/^{14}C)_{\text{superfused}}}$$

This parameter reveals the "preferred direction" in the reversible conversion of DHT to Adiol. A rate constant is defined as the ratio of the corresponding rate (μmol/g × h) and the concentration of the compound in tissue (μmol/g).

—Fraction of DHT converted to Adiol (conversion factor):

$$\rho_{\text{DHT}\rightarrow\text{Adiol}} = (^3H/^{14}C)_{\text{superfused}}/(^3H/^{14}C)_{\text{Adiol}} \text{ in tissue}$$

—Uptake of DHT, i.e., ratio of concentrations of superfused DHT in tissue and in medium, at the steady state:

$$(T/M)_{DHT} = c^{14C}_{DHT} \text{ in tissue}/c^{14C}_{DHT} \text{ in medium}$$

—Intracellular clearance of DHT, a parameter that denotes the ratio between the rate at which the hormone appears "*de novo*" intracellulary per g of tissue (its production rate, PR_{DHT}/W, μmol/g × h) and the resulting concentration of DHT in tissue (c_{DHT}, μmol/g):

$$IC_{DHT} = \frac{\phi\,\alpha_{DHT}}{W} \Big/ \left(\frac{T}{M}\right)$$

where ϕ is the flow rate and W is the weight of the superfused tissue. Note the following relations:

$$\frac{IC_{Adiol}}{IC_{DHT}} = \frac{(T/M)_{DHT}}{(T/M)_{Adiol}}$$

$$\frac{IC_{Adiol}}{IC_{DHT}} = \frac{k_{\text{Adiol}\rightarrow\text{DHT}}}{k_{\text{DHT}\rightarrow\text{Adiol}}} \times \frac{\rho_{\text{DHT}\rightarrow\text{Adiol}}}{\rho_{\text{Adiol}\rightarrow\text{DHT}}}$$

—The rate of conversion of DHT to Adiol in the superfused tissue can be estimated in terms of $\rho_{\text{DHT}\rightarrow\text{Adiol}}$, $\rho_{\text{Adiol}\rightarrow\text{DHT}}$ and the rates of intracellular production of these compounds. The formula for the calculation of the rate constants of conversion of DHT to Adiol follows immediately:

$$k_{\text{DHT}\rightarrow\text{Adiol}} = \frac{\rho_{\text{DHT}\rightarrow\text{Adiol}}}{1 - \rho_{\text{DHT}\rightarrow\text{Adiol}}\ \rho_{\text{Adiol}\rightarrow\text{DHT}}} IC_{DHT}$$

and

$$k_{\text{DHT(in)}\rightarrow\text{(out)}} = \frac{\beta_{DHT}}{\alpha_{DHT}} IC_{DHT}$$

Similar formulas are applicable to data obtained with the other superfused compounds.

RESULTS

The results obtained from the four superfusions of prostate tissue with mixtures of labeled DHT and Adiol are shown in Table 1. The values listed were estimated by applying the formulas presented in the section on Calculations. The data on concentrations of labeled compounds in superfusate used for these calculations corresponded to the average of the values in the last two fractions collected in each experiment (60–80, and 80–100 min). Achievement of an isotopic steady state was evident. The fractions of superfused tracers entering the cells ranged from 20–40%; no significant differences between α_{Adiol} and α_{DHT} were found in any of these experiments.

The data in Table 1 can be summarized as follows. About 66% of the DHT taken up by the tissue is returned to the medium as DHT, 8% is converted to Adiol and eliminated as Adiol or as a direct metabolite of this compound, e.g., androsterone, and the rest (26%) is converted to products other than Adiol, e.g., Adione and 3β Adiol. About 18% of the Adiol

Table 1. Parameters calculated from isotopic data obtained from superfusion of slices of human hypertrophic prostates with [^{3}H]-Adiol and [^{14}C]-DHT

Parameter	Symbol	Unit	Experiment No. 1	2	3	4	Average
Interconversion							
Rate constant Adiol → DHT / Rate constant DHT → Adiol	$\frac{k_{\text{Adiol}\rightarrow\text{DHT}}}{k_{\text{DHT}\rightarrow\text{Adiol}}}$	—	4	4	3	3	3.5
Conversion factor Adiol → DHT	$\rho_{\text{Adiol}\rightarrow\text{DHT}}$	%	15	10	23	23	18
Conversion factor DHT → Adiol	$\rho_{\text{DHT}\rightarrow\text{Adiol}}$	%	7	5	11	10	8
Distribution							
c^{3H}_{Adiol} in tissue/c^{3H}_{Adiol} in medium	$(T/M)_{Adiol}$	—	4	4	6	7	5
c^{14C}_{DHT} in tissue/c^{14C}_{DHT} in medium	$(T/M)_{DHT}$	—	9	7	9	10	9
c^{3H}_{Adiol} in tissue/c^{3H}_{DHT} in tissue	$\left(\frac{Adiol}{DHT}\right)$ from Adiol	—	4	5	3	3	4
c^{14C}_{DHT} in tissue/c^{14C}_{Adiol} in tissue	$\left(\frac{DHT}{Adiol}\right)$ from DHT	—	25	33	14	14	21
Release to medium							
Adiol in tissue → Adiol in medium	$(\beta/\alpha)_{Adiol}$	%	17	15	21	23	18
DHT in tissue → DHT in medium	$(\beta/\alpha)_{DHT}$	%	95	60	42	82	66
Adiol in tissue → DHT in medium	$\gamma_{\text{Adiol}\rightarrow\text{DHT}}/\alpha_{Adiol}$	%	13	7	11	14	11
DHT in tissue → Adiol in medium	$\gamma_{\text{DHT}\rightarrow\text{Adiol}}/\alpha_{DHT}$	%	1	0.6	2	3	1.6
Intracellular clearance							
Adiol	IC_{Adiol}	h^{-1}	3.4	6.8	2.5	1.9	3.3
DHT	IC_{DHT}	h^{-1}	1.5	4.7	1.9	1.1	2.1

Table 2. ($^3H/^{14}C$) Ratio in metabolites isolated from human hypertrophic prostates superfused with [^{3}H]-Adiol and [^{14}C]-DHT

Compound	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Adiol	78	110	48	51
DHT	0.8	0.6	1.2	1.2
3βAdiol	—	—	2.7	2.7
Adione	2.2	2.1	3.0	—
Androsterone	16	33	12	12

Superfusion ratios (c^{3H}Adiol/^{14C}DHT, c.p.m./c.p.m., in superfusion medium): 5.4, 5.3, 5.1, and 5.1 in Experiments 1, 2, 3, and 4, respectively.

entering the tissue is converted to DHT, 18% leaves as Adiol and the rest (64%) is converted to other metabolites, mainly androsterone.

The "preferred direction" of the extensive interconversion between DHT and Adiol is towards the formation of DHT, as indicated by the ratio of rate constants of conversion of Adiol to DHT and of DHT to Adiol (Table 1) and by the value of the conversion factors.

Both DHT and Adiol are concentrated by the tissue with respect to the medium but the uptake of DHT is 1.5 to 2 times greater than the uptake of Adiol, as estimated from the T/M values of these compounds, as shown in Table 1. The clearance of Adiol from the tissue is therefore greater than the clearance of DHT; Adiol is mostly converted to other products and DHT is mostly returned to the medium.

Table 2 shows the steady state isotopic ratios (c.p.m./c.p.m.) in Adiol, DHT and various metabolites isolated from the superfused tissue. Comparison of these ratios indicates that 3β Adiol and Adione are directly formed from DHT, as a result of a single enzymatic reaction. In contrast, the $^3H/^{14}C$ ratio in androsterone is intermediate between the isotopic ratios in DHT and Adiol. It can be estimated from these data that 25% of the androsterone derived from DHT is formed via Adiol, the rest may be formed via Adione.

Table 3 shows results obtained from superfusion of BPH tissue with mixtures of labeled DHT and Adione, as well as of DHT and 3β Adiol. The conversion of DHT to Adione and 3β Adiol was lower than the conversion of DHT to Adiol. Some formation of DHT from Adione and 3β Adiol was noted, but the conversions were too small to allow reliable measurements in these experiments.

DISCUSSION

The superfusion system used in these studies allows estimation of the extent to which a steroid is released from the cell to the medium, as well as the extent to which it is metabolized. In spite of the variability to the fraction of unchanged DHT which leaves the cells in the specimens studied, its high value indicates a main route of disposition of DHT by the prostatic tissue under the *in vitro* conditions. In contrast, under similar conditions, most of the intracellular Adiol (Table 1) and testosterone [9], is metabolized to other products rather than released to the medium. Consistent with these findings is the report by Giorgi *et al.* [7] indicating that more DHT than testosterone leaves superfused slices of human hyperplastic prostates, even at physiological intracellular levels of these two compounds. That significant amounts of DHT may be released by the prostate *in vivo* is evident from the findings of Mahadeau *et al.*[23], who reported higher endogenous levels of DHT (and higher DHT/testosterone concentration ratios) in prostatic venous blood than in peripheral blood of patients with BPH.

A limitation of the present study is imposed by the S.A. of [^{14}C]-DHT superfused, which leads to intracellular concentrations about 100 times higher than those present endogenously. Although the metabolism of DHT may be influenced by the concentration of the steroid in the tissue, the average intracellular clearance values found in these experiments were similar to those of Giorgi *et al.*, conducted at near physiologic concentrations [7]. The intracellular concentrations of Adiol, although possibly unphysiologic, were about 20-fold lower than the concentrations of DHT.

A striking observation in this study is the extensive conversion of Adiol to DHT, larger than expected from previous kinetic studies with tissue homogenates [15]. The androgenic effect of exogenously administered Adiol on the prostate should then be expected [24]. However, the physiologic importance of this compound for prostatic stimulation may not be quantitatively significant since the plasma concentration of Adiol in normal adult men is much lower than the concentration of testosterone, with similar binding of both compounds to the sex steroid binding globulin [25], and most of the Adiol in circulation derives from secreted testosterone [25, 26].

Table 3. Conversion of DHT to Adione and 3β Adiol

	($^3H/^{14}C$ ratios (c.p.m./c.p.m.) Tissue				
	Superfusion medium	Adione	3β Adiol	Conversion factors (%) ρDHT → Adione	ρDHT → 3β Adiol
[^{3}H]-DHT + [^{14}C]-Adione	6.4	0.35	—	5	—
	5.1	0.14	—	3	—
[^{3}H]-3β Adiol + [^{14}C]-DHT	0.16	—	32	—	0.5

The values reported here may be of interest for further studies on the metabolism of DHT by prostatic tissue under normal and pathologic conditions and during hormonal treatment.

Acknowledgements—We are grateful to the physicians in the Department of Urology for their cooperation. This work was supported by grant HD 07197, from the National Institutes of Health.

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