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COMPARISON OF TESTOSTERONE, 5α-DIHYDROTESTOSTERONE AND 5α-ANDROSTANE-3β,17β-DIOL METABOLISMS IN HUMAN NORMAL AND HYPERPLASTIC PROSTATES

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SUMMARY

Testosterone, 5α-dihydrotestosterone and 5α-androstane-3β,17β-diol metabolisms by minces of two normal and five hyperplastic human prostates were investigated in the presence and absence of NADPH. Examination of the metabolites indicated a major reductive pathway involving 17β-hydroxysteroids which was increased by NADPH supplementation to levels identical in both tissues. Unsupplemented incubations reflected the endogeneous cofactor content of the glands and comparison of metabolisms suggested that reduced cofactors were not as readily available in normal than in hyperplastic prostates.

Investigation of 5α-dihydrotestosterone metabolism by minces, homogenates, microsomes and cytosol fractions derived from normal and hyperplastic glands proved that NADPH-supplemented different tissue preparations give results which may lead into different interpretations and that neither EDTA nor $ZnCl_2$ have consistent effects on 5α-dihydrotestosterone metabolism.

Consideration of the data led to suggest that prostatic hyperplasia might only be due to 5α-dihydrotestosterone accumulation in the prostate and derived from increased 3-oxidation of 5α-androstane-3α,17β-diol and respective reductions of testosterone and 5α-androstane-3,17-dione. In contrast, formation of 5α-androstane-3β,17β-diol and of its metabolites was identical in both tissues and this finding corroborates the view that the secretory activity of the gland is maintained by this metabolite.

INTRODUCTION

Several studies have established the identity of testosterone transformation products in human prostate [1–7] and related the main pathway to 17β-hydroxy metabolites [2, 3]. Thus, 5α-reduction of testosterone was shown to be the first major enzymic step leading to 5α-dihydrotestosterone (DHT)¶ [1, 2, 3, 4, 7]. Subsequent transformation of this metabolite into epimeric androstanediols [4, 8, 9] followed by 7α-hydroxylation of the 3β-diol were also demonstrated [5, 10].

No significant differences in testosterone 5α-reduction rates were found when compared in slices of normal and hyperplastic prostates [7 11]. In contrast, the formed DHT was proved to accumulate in hyperplastic tissues [7, 11] suggesting that 3α and 3β-reductions of the 5α-reduced testosterone were less extensive in hyperplastic than in normal tissues. According to that suggestion, studies of 3α and 3β-diols content [12] and of 3α and 3β-reduction by microsomes and cytosol fractions of normal and hyperplastic prostates were carried out [8, 9] and led to conflicting conclusions.

In order to control these data and extend the scope of differences between human normal and hyperplastic prostates, we now wish to report the study of testosterone, DHT and 3β-diol transformations by minces of these tissues. Differences in DHT metabolism due to the preparation of minces, homogenates and subcellular fractions or to the presence of Zinc or EDTA in incubation media were also studied.

EXPERIMENTAL

Steroids and solvents. All solvents were of reagent grade or were distilled before use. Testosterone, DHT, 3α-diol, 3β-diol, androsterone and *iso*androsterone were used as references and purchased from Merck or Sigma. The 7α-triol was obtained as previously described [10]. Purity of steroids was checked by gas–liquid chromatography before use.

Labelled steroid substrates. [4-^{14}C]-Testosterone (50 and 57.5 mCi/mmol) was purchased from C.E.A.

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‖ Results presented in this paper are contained in a thesis to be submitted by S. Di Stéfano to the University of Brest in partial fulfillment of the requirements for the degree of Doctorat d'Université.

¶ *Abbreviations used*: DHT, 5α-dihydrotestosterone; 3α-diol, 5α-androstane-3α,17β-diol; 3β-diol, 5α-androstane-3β,17β-diol; 7α-triol, 5α-androstane-3β,7α,17β-triol; Adione, 5α-androstane-3,17-dione; *Iso*androsterone, 3β-hydroxy-5α-androstane-17-one; EDTA, Ethylene Diamine Tetra Acetate.

(France) and N.E.N. (Boston, U.S.A.), respectively. Purifications were done by thin layer chromatography and radiopurity (98.8, 99.4%) checked by crystallization of a portion to constant S.A. [4-^{14}C]-DHT and [4-^{14}C]-3β-diol (57.5 mCi/mmol) were prepared and purified as previously described [13]. Labelled steroids were stocked at 2°C in benzene–ethanol (9:1, v/v).

Prostatic tissues. Prostatic tissues with benign hyperplasia were removed by the transvesical approach from patients undergoing surgery. Two normal human prostates were obtained by the same surgical technique from 18- and 22-year old donors used for kidney transplants. As soon as they were obtained, tissues were blotted on gauze and chilled in a beaker surrounded with crushed ice before sections were taken for histopathological examination and transport to a cold room. At 4°C tissues were finely cut with scissors and forced through the 1 mm diameter holes of a sieve with an arbor tissue press (Harvard apparatus). Obtained minces were weighed in 200 mg portions for incubations. Homogenates were prepared from minces in 0.067 M phosphate buffer with a polytron homogenizer (15 s at 10,000 rev./min). Microsomes and cytosol fractions were obtained from these homogenates according to Jacobi and Wilson [9]. Quantities corresponding to 200 mg of fresh minces were used for incubation. Protein concentrations in microsomes and cytosol fractions were measured by the Biuret method.

Incubation procedures. Solutions containing 0.7 μg of the labelled steroid substrate were dried under nitrogen at the bottom of 50 ml glass-stoppered incubation tubes. Minces were placed in 200 mg portions on the inside wall of the tube and rinsed down with either 5 ml of 0.067 M phosphate buffer (pH 7.4) or 5 ml of a freshly prepared 0.5 mM solution of NADPH in the same phosphate buffer. Quantities of homogenates, microsomes and cytosol fractions equivalent to 0.2 g of tissue minces were used for incubations with 0.7 μg of labelled DHT in a total volume of 5 ml of phosphate buffer supplemented with NADPH (0.5 mM) and either 1 mM EDTA or 1 mM $ZnCl_2$ or no extra additive. Incubations were carried out at 37°C in a shaking water bath and stopped after 30 min by addition of chilled acetone (10 ml) and cooling at −20°C. Extraction with ethyl acetate (10 ml, 4 times) gave a crude extract which was concentrated under nitrogen. Recoveries of the labelled steroids (94.5–99%) were computed after measurement of radioactivity in portions of the extracts.

Thin-layer chromatography. Substrates and radiometabolites contained in crude extracts were separated by thin-layer chromatography on silica gel $HF_{254+366}$ (Merck) after two developments in benzene–95% ethanol (9:1, v/v). Radiometabolites were located on the chromatogram in regard to authentic steroid references by autoradiography. Satisfactory separation of 3α-diol from 3β-diol and of androsterone from DHT were not obtained with this system. Resolution of the eluted pairs was carried out on alumina G with two developments in benzene–95% ethanol (97:3, v/v) or two developments in dichloromethane–ether (9:1, v/v), respectively.

Radioactivity measurements. Dried portions of eluates or radiometabolite-containing gels were counted in glass vials with a Packard Tri Carb liquid scintillation spectrometer, model 3375. Quenching was corrected on the basis of external standard ratios. Only counting rates exceeding 5 times those of the background were used for computations.

RESULTS

Testosterone metabolism in minces of normal and hyperplastic prostates

Minces were used for 30 min incubations with 0.7 μg of [4-^{14}C]-testosterone (2430 pmol, 0.486 μM) in 5 ml 0.067 M phosphate buffer (pH 7.4) with or without NADPH. Quantities of separated testosterone metabolites are shown in Fig. 1.

Contamination of DHT by androsterone was proved to be minor in these cases [10] and the two metabolites were not systematically separated. Epimeric androstanediols were resolved in few instances and the 3α/3β ratios measured in digests from normal (4.6) and hyperplastic (4.5–3.0) tissues. The 7α-triol was not separated from the 5α-androstane-3β,6β,17β-triol. Previous study showed that major quantities of the 7α-triol are produced by both normal and hyperplastic prostates [10]. NADPH-supplementation of the digests resulted in a marked increase of testosterone transformation products including DHT, but no significant difference between normal and hyperplastic tissues could be found at any of the metabolites levels.

In marked contrast, unsupplemented digests showed a more extensive formation of DHT in hyperplastic than in normal tissue minces and a larger 17-oxidation of testosterone in normal than in hyperplastic tissues. Due to the low number of cases, statistics were only tentatively applied to the data and these differences were found to be significant ($P = 0.05$ and $P = 0.025$, respectively). Since such differences were not found in NADPH-supplemented digests of the same tissues, it is suggested that minces from normal prostates possess a 5α-reducing capability identical to that of hyperplastic prostate but are depleted in the reduced cofactor.

DHT metabolism in minces of normal and hyperplastic human prostates

Minces were incubated for 30 min with [4-^{14}C]-DHT (2415 pmol, 0.483 μM) in 5 ml 0.067 M phosphate buffer (pH 7.4) with or without NADPH. The obtained DHT metabolites are presented in Fig. 2. Epimeric androstanediols resulting from DHT transformations were separated and the 3α/3β ratios measured in normal prostates (3.0, 3.9) and in few instances of hyperplastic tissues incubations (2.4–3.2).

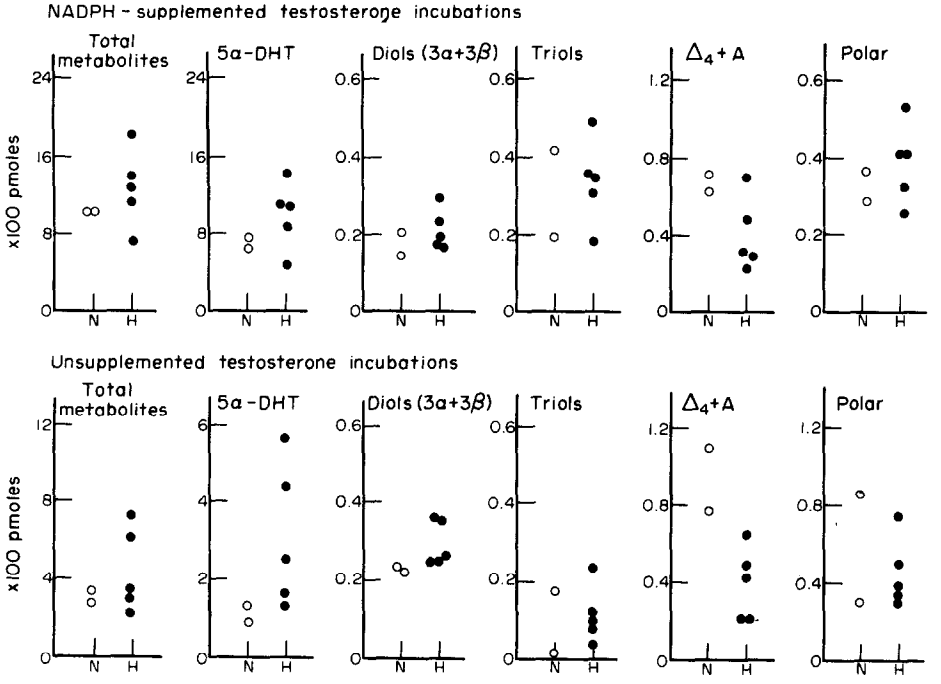

Fig. 1. [4-^{14}C]-Testosterone metabolites formed by minces of 2 normal and 5 hyperplastic human prostates in the presence or absence of NADPH. Data result from incubations for 30 min at 37°C of 0.2 g minces with 2430 pmol of [4-^{14}C]-testosterone (0.486 μM) in 5 ml 0.067 M phosphate buffer (pH 7.4) in the presence or absence of 0.5 mM NADPH. Diols = 3α-diol + 3β-diol, Triols = 7α-triol + 5α-androstane-3β,6β,17β-triol, Δ^4 + A = 4-androstene-3,17-dione + Adione, Polar = non identified polar metabolites, N = normal prostate minces, H = hyperplastic prostate minces.

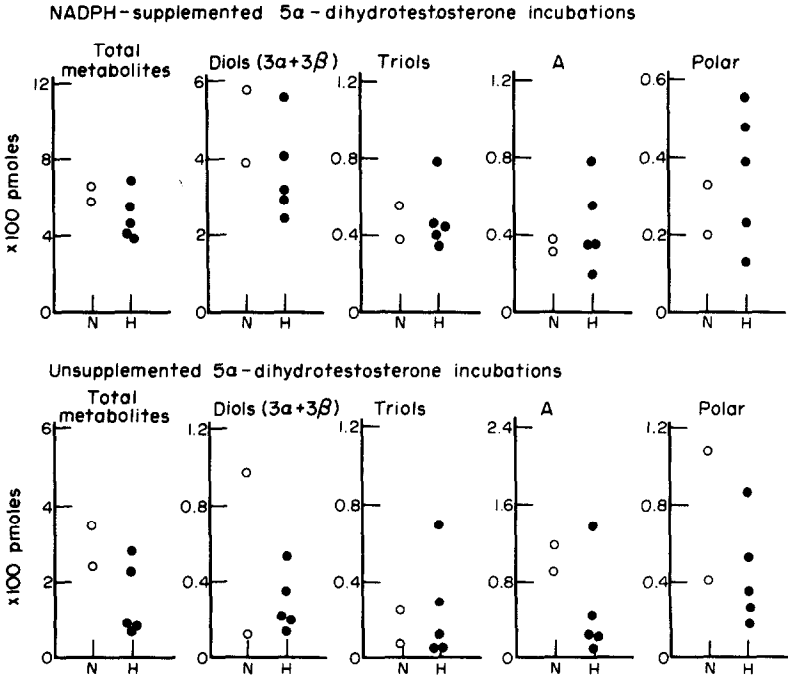

Fig. 2. [4-^{14}C]-DHT metabolites formed by minces of 2 normal and 5 hyperplastic human prostates in the presence or absence of NADPH. Data result from incubations for 30 min at 37°C of 0.2 g minces with 2415 pmol of [4-^{14}C]-DHT (0.483 μM) in 5 ml 0.067 M phosphate buffer (pH 7.4) in the presence or not of 0.5 mM NADPH. Diols = 3α-diol + 3β-diol, Triols = 7α-triol + 5α-androstane-3β,6β,17β-triol, A = Adione, Polar = non identified polar metabolites, N = normal prostate minces, H = hyperplastic prostate minces.

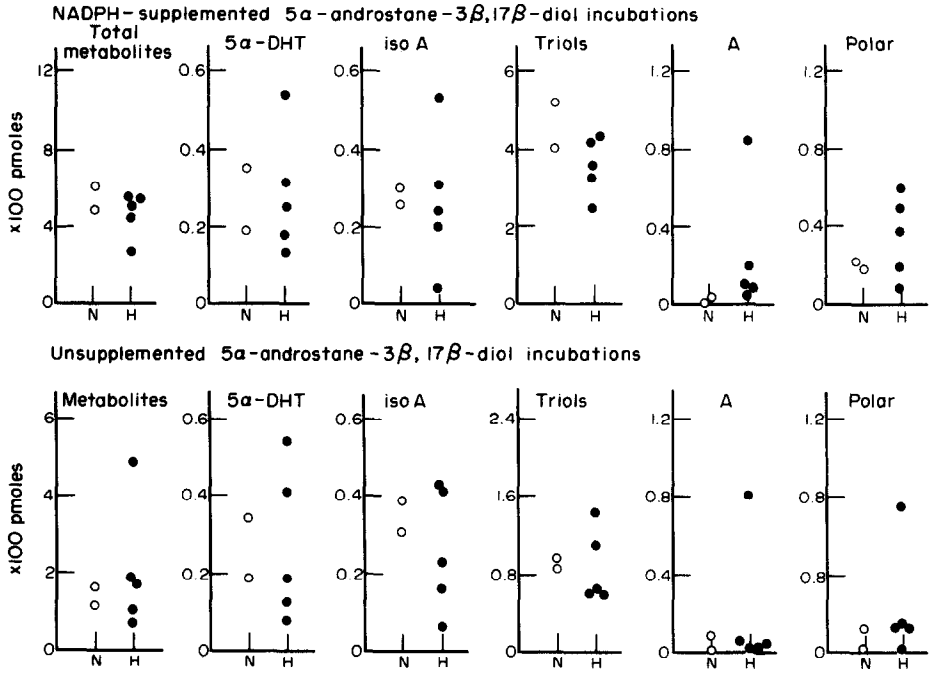

Fig. 3. [4-^{14}C]-3β-Diol metabolites formed by minces of 2 normal and 5 hyperplastic human prostates in the presence or absence of NADPH. Data result from incubations for 30 min at 37°C of 0.2 g minces with 2400 pmol of [4-^{14}C]-3β-diol (0.48 μM) in 5 ml 0.067 M phosphate buffer (pH 7.4) in the presence or absence of NADPH (0.5 mM). *Iso*A = 3β-hydroxy-5α-androstane-17-one, Triols = 7α-triol + 5α-androstane-3β,6β,17β-triol, A = Adione, Polar = non identified polar metabolites, N = normal prostate minces, H = hyperplastic prostate minces.

Both 7α-triol and 5α-androstane-3β,6β,17β-triol were contained after chromatography in a single radioactive zone. Previous study indicated the 7α-triol to be the major component of the mixture [10] and radioactivity was measured without separation of the two metabolites.

NADPH-supplementation of the digests resulted in a dramatic increase in epimeric androstanediols formation and overall metabolism. No significant differences between normal and hyperplastic tissues could be found at any of the metabolite levels. Similarly, no significant difference could be found with unsupplemented digests of normal and hyperplastic prostates, except for Adione formation which was significantly increased in normal ($P = 0.05$) when compared with hyperplastic tissues. This finding suggests that minces from normal prostatic tissues contain larger quantities of oxidized cofactor than those of the hyperplastic tissue preparations.

3β-Diol metabolism in minces of normal and hyperplastic human prostates

Thirty min incubations of the tissue minces were carried out in 5 ml 0.067 M phosphate buffer (pH 7.4) with 0.7 μg of [4-^{14}C]-3β-diol (2400 pmol, 0.480 μM) in the presence or not of NADPH (0.5 mM). Metabolites formed are shown in Fig. 3. Previous study showed the 7α-triol to be the major metabolite formed from 3β-diol by prostatic tissue minces [10].

NADPH supplementation of the digests determined a 4 to 5 fold increase in formation of the triols. Production of other metabolites was not significantly affected by the reduced cofactor. Nevertheless, no significant differences in metabolites formation could be found between normal and hyperplastic tissue preparations in NADPH-supplemented or unsupplemented digests.

DHT metabolism in minces, homogenates, microsomes and cytosol fractions from normal and hyperplastic human prostates

Experiments were carried out with one of the normal and two extra hyperplastic prostates. Thirty min incubations of the tissue preparations were carried out in 5 ml 0.067 M phosphate buffer (pH 7.4) with 0.7 μg [4-^{14}C]-DHT (2415 pmol, 0.483 μM). All incubations were done in triplicate, one set containing only NADPH (0.5 mM) the other two sets containing either NADPH (0.5 mM) and EDTA (1 mM) or NADPH (0.5 mM) and $ZnCl_2$ (1 mM).

In all cases quantities equivalent to 0.2 g of prostatic minces were used. DHT metabolites from minces and homogenate digests were computed in pmol/0.2 g tissue (Fig. 4).

Results mainly concerned the 3-oxo reduction of the DHT substrate through the prostatic 3α-hydroxysteroid oxidoreductase (3α-diol formation) and through the 3β-hydroxysteroid oxidoreductase (3β-diol formation). Since the 7α-triol is only derived from 3β-diol, activity of the 3β-hydroxysteroid oxi-

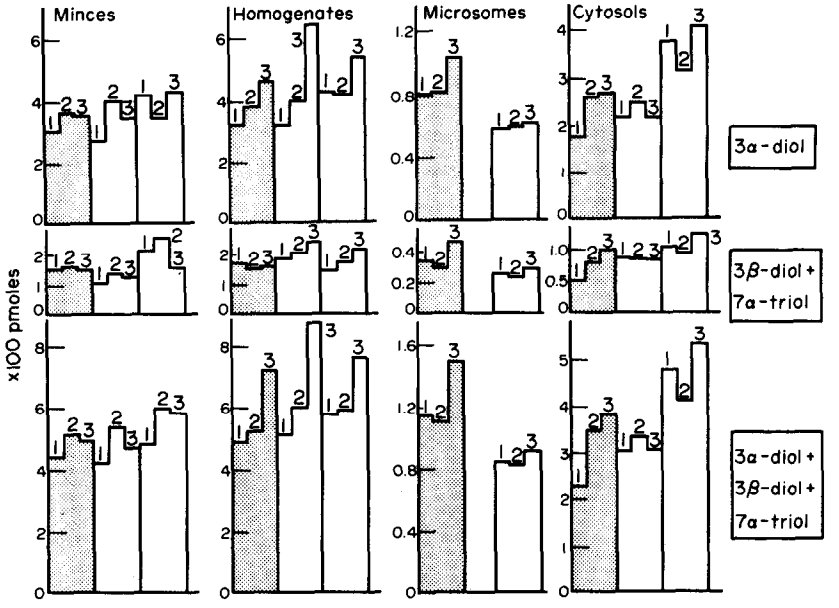

Fig. 4. [4-^{14}C]-DHT metabolites formed by minces, homogenates, microsomes and cytosol fractions derived from 1 normal and 2 hyperplastic prostates. Data result from incubations for 30 min at 37°C of 0.2 g or 0.2 g-equivalent of tissue preparation with 2415 pmol of [4-^{14}C]-DHT (0.483 μM) in 5 ml 0.067 M phosphate buffer (pH 7.4) in the presence of 0.5 mM NADPH. ■ normal prostate, □ hyperplastic prostates, 1. = Incubation medium containing 0.5 mM NADPH. 2. = Incubation medium containing 0.5 mM NADPH and 1 mM EDTA. 3. = Incubation medium containing 0.5 mM NADPH and 1 mM $ZnCl_2$. 7α-triol = 7α-triol + 5α-androstane-3β,6β,17β-triol.

doreductase was computed from 3β-diol and 7α-triol quantities. Results are presented in Fig. 4.

No significant difference was observed in 3α or 3β-reduction of DHT by minces of normal and hyperplastic prostates and modifications brought by the presence of EDTA or $ZnCl_2$ were not consistent. Homogenates gave interesting results, although no significant difference was found between normal and hyperplastic tissues. Presence of EDTA and $ZnCl_2$ consistently increased rates of 3α-reduction and to lesser extent those of 3β-reduction. $ZnCl_2$ was the most effective for such increments. In marked contrast, microsomes from normal prostate (2.76 mg protein/0.2 g tissue) produced significantly more 3α and 3β-hydroxy metabolites than those derived from hyperplastic tissues (5.56 mg protein/0.2 g tissue). This difference is better shown when reduction rates are expressed per mg of microsomal protein instead of per 0.2 g equivalent of microsomes. Effects of EDTA and $ZnCl_2$ on these enzymic activities were not consistent. Cytosol derived from normal prostate (7.05 mg protein/0.2 g tissue was markedly less active in 3α and 3β-reduction of DHT than those from hyperplastic prostates (6.31 and 9.12 mg protein/0.2 g tissue) when only supplemented with NADPH. This difference was maintained when incubation rates were expressed per mg of microsomal protein, but addition of EDTA or $ZnCl_2$ erased this difference, the changes in reduction rates being neither consistent nor significant.

DISCUSSION

The comparison that we achieved between normal and hyperplastic human prostates is based on two normal glands obtained over a period of 3 years. Nevertheless, use of these normal prostates which did not undergo ischemia from young "live" transplant donors has never been reported and we consider these tissues as good models, since they were collected and processed exactly as the hyperplastic glands obtained from surgery. We trust our results to be significant but an extensive study involving more cases will certainly be needed to further confirm our conclusions.

Our work and published data now permit an extensive comparison of testosterone metabolic pathways in minces of normal and hyperplastic human prostates. Hence, results from other workers [9, 10] and from this study indicate that minces from normal and hyperplastic prostates metabolize testosterone by the same pathways involving identical enzymes (Fig. 5). Thus a reductive pathway related with testosterone 17β-hydroxymetabolites may be opposed to an oxidative pathway concerned with testosterone 17-oxo metabolites. Two steps may be distinguished in the reductive pathway: one leading to DHT through the NADPH-dependent 5α-reduction of testosterone, the other involving the 3-oxo reduction of DHT into epimeric androstanediols and subsequent metabolites.

NADPH-supplementation of the minces digests increased the reductive pathway in both tissues but did

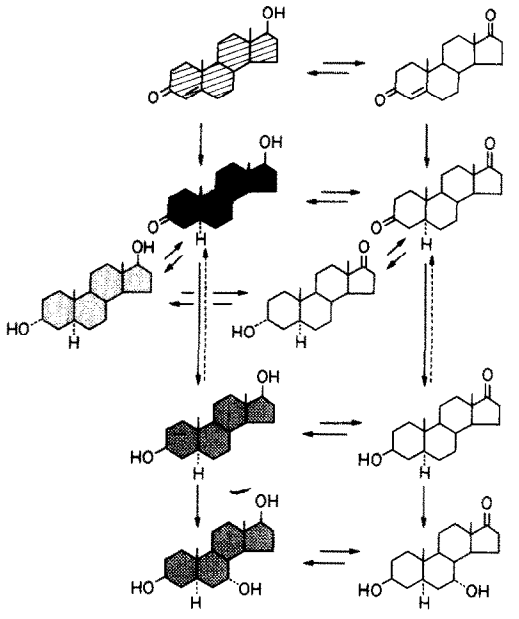

Fig. 5. Pathways of testosterone, DHT and 3β-diol metabolism in human prostate. Shades on the 17β-hydroxysteroids are tentatively proportional with suggested potencies to trigger hyperplasia or secretion in the prostate gland.

not lead to any significant differences between those of normal and those of hyperplastic tissue minces. In contrast, unsupplemented incubations reflecting the endogeneous cofactor content of the minces showed the first step of the reductive pathway to be decreased in normal tissue minces while oxidative pathway appeared to be significantly increased. The second step of the reductive pathway was not noticeably different in normal and hyperplastic tissues but the oxidative pathway, as shown by Adione formation from DHT substrate, was more effective with normal than with hyperplastic tissue minces. Thus, if increased formation of DHT and decreased formation of 4-androstene-3,17-dione in hyperplastic tissue minces leads to DHT accumulation, its transformation into epimeric androstanediols remains identical in both tissues. This sole difference points toward the first step of the reductive pathway and DHT as being involved in prostatic hyperplasia and agrees with the findings of Siiteri and Wilson [7] who showed DHT to accumulate in human hyperplastic prostates and with the works of Robel *et al.* [14] who showed DHT to trigger epithelial cells growth and hyperplasia in rat prostate tissue cultures.

In marked contrast, the finding that 3-oxo reduction of DHT remains unchanged in normal and hyperplastic prostates suggests that the second step of the reductive pathway is not involved in hyperplasia and that related metabolites may only be active in the triggering of prostatic secretion. Support for this suggestion is provided by the work of Robel *et al.* [14] who showed the 3β-diol to maintain epithelial cells height and secretion in rat prostate tissue cultures and the fact that no significant difference in prostatic secretion is known between normal and hyperplastic tissues.

In summary, our findings and comparison of testosterone metabolic pathways in normal and hyperplastic prostates suggest that the DHT accumulation in hyperplastic tissues is involved in hyperplasia and is due in part to an imbalance of cofactors available for 5α-reduction and 17β-oxidation of testosterone.

This conclusion from the comparison of testosterone metabolism in normal and hyperplastic human prostates immediately raises the point of DHT accumulation in hyperplastic tissues as demonstrated by Siiteri and Wilson [7] and Geller *et al.* [12]. Since testosterone is metabolized through the same pathways in both tissues (Fig. 5), DHT accumulation in the diseased gland may be explained by each or several of the following: (i) a decreased 17β-oxidation of testosterone which would determine more testosterone to be available for 5α-reduction; (ii) an increased formation of DHT from testosterone; (iii) a decreased 17β-oxidation of DHT; (iv) a decreased 3-oxo reduction of DHT; (v) and increased transformation of 3α-diol into DHT.

Our finding that testosterone 17β-oxidation was decreased and 5α-reduction increased in digests of unsupplemented hyperplastic prostate minces supports the first two points. Such data contrast with those reported by Siiteri and Wilson [7] who did not find significant differences in testosterone 5α-reduction between normal and hyperplastic glands when incubating tissue slices in glucose supplemented media. Differences in methodology are the use of slices and glucose by these authors and may contribute to explanation of our conflicting results. Nevertheless, our data from NADPH-supplemented digests support their conclusions but suggest that quantities of oxidized and reduced cofactors respectively available for 17β-oxidation and 5α-reduction of testosterone may be of importance. The effect of cofactors on the 17β-hydroxysteroid oxidoreductase activity is underlined by our findings concerning the formation of Adione from DHT substrate. In this case, unsupplemented minces digests proved to form significantly more Adione with normal than with hyperplastic tissues. Thus, a decreased formation of Adione in hyperplastic glands may have to be considered to explain DHT accumulation in such tissues and involve endogeneous cofactors in the oxidized form.

Consideration of a decreased 3-oxo reduction of DHT for explaining accumulation of this metabolite in hyperplastic prostate would agree with the findings of Geller *et al.* [12] who showed endogeneous epimeric androstanediols to be present in larger quantities in normal than in hyperplastic glands. Our data showing that no significant difference could be found in the rates of epimeric androstanediols formation between both tissues minces do not support this hypothesis and contrast with those obtained by Jacobi and Wilson [9] with microsomes and cytosol fraction from normal and hyperplastic prostates. These

authors incubated the prostatic fractions in 1 mM EDTA and 0.5 mM NADPH-supplemented buffer and found the epimeric androstanediols to be formed in significantly larger quantities by hypertrophic than by normal glands. No satisfactory explanation for this finding in regard to others could be proposed. Since steroid-metabolizing enzymes may find in isolated subcellular fractions conditions leading to activities different from that of tissue minces, we have examined the DHT metabolism in minces, homogenates, microsomes and cytosol fractions derived from the same normal and hyperplastic glands. Incubations at pH 7.4 were supplemented with NADPH in all cases and contained either 1 mM EDTA or $ZnCl_2$. Our data show that neither EDTA nor $ZnCl_2$ are effective for a consistent change of DHT metabolism in both glands but that 3-oxo reduction of DHT is significantly increased in microsomes of normal prostate when compared with that of microsomes derived from hyperplastic glands. This finding is again opposed to those of Jacobi and Wilson [9] but our procedure differed by the pH (7.4 instead of 5.5). Anyway, our data tend to show that different preparations from the same prostatic tissue lead to different rates of DHT 3-oxo reduction and possibly extend the presence of nuclear 3α-hydroxysteroid dehydrogenase in rat prostate [15] to the human gland.

Finally, the consideration of an increased transformation of 3α-diol into DHT in hyperplastic tissues may bring some agreements for interpretation of the above findings. Thus, Malathi and Gurpide [16] have proved that formation of DHT from 3α-diol was favored in superfused slices of human hyperplastic prostates. If related to the fact that such glands are depleted in endogeneous androstanediols when compared to normal [12] it appears that back transformation of 3α-diol into DHT may lead into the characteristic accumalation of DHT in hyperplastic tissues. The involved 3α-hydroxysteroid oxidoreductase would then act both as a reductase and as a dehydrogenase with appropriate endogeneous cofactors. Whole activity of this enzyme may be expressed only as DHT 3-oxo reductase when isolated in subcellular fractions and tested in the presence of NADPH [9]. The finding that 3α-diol formation is increased in the hyperplastic prostate cytosol fraction may reflect this activity [9]. In contrast, use of intact cells reflects the different conditions and cofactors locally available for enzymic action, and permits to both reductase and dehydrogenase activities to be expressed. Thus, and according to our findings, both normal and hyperplastic tissues show the same DHT-reductase activity, while 3α-diol dehydrogenase activity could be increased in hyperplastic tissues and lead to 3α-diol depletion [12] and DHT accumulation [7] in hyperplastic prostates.

In contrast to the prostatic 3α-hydroxysteroid oxidoreductions, it has been demonstrated that the 3β-hydroxysteroid oxidoreductase only forms minor quantities of DHT from 3β-diol in hyperplastic prostates [15] but mainly transform DHT into 3β-diol which is the main substrate for 7α-hydroxylation [10]. Our findings show that NADPH-supplemented or unsupplemented minces from both normal and hyperplastic glands reduce DHT to the same extent and that different NADPH-supplemented preparations from these tissues lead to different results which may explain those of Jacobi and Wilson [9].

Consideration of these data suggest that endogeneous reduced cofactors may be utilized at first in intact cells to insure the formation of constant levels of 3β-diol and of its metabolites, thus becoming limitant for 3-oxo reduction in hyperplastic tissues.

This suggestion is supported by the proposed independence of 3α and 3β-reduction of DHT from hormonal control [17, 18] and the maintenance of prostatic cells secretion by 3β-diol [14]. The use of data from canine prostate in discussion involving androstanediols formation appears questionable to us since we have shown from *in vivo* experiments [2, 3] that the 3α/3β epimers ratios are reversed in the human and canine tissues and may be implied in different modes of action [2].

The above suggestions and our data on human prostate tend to dissociate the cellular implications of DHT from those of 3β-diol. The DHT would accumulate in prostatic hyperplasia mainly from back transformations of 3α-diol and Adione involving the available endogeneous cofactors while 3β-diol formation from DHT would remain constant in normal and diseased tissues and play a role on prostatic secretion.

Accordingly, careful studies of DHT 3- and 17-oxidoreductions with tissue preparations and conditions mimicking the *in vivo* situation have to be carried out. Possibility of numerous 3α-hydroxysteroid dehydrogenases in different subcellular fractions already described in rat [15] has to be established in human prostate and quantities of available endogeneous cofactors must be taken into account [19]. These works should then help to resolve the involvement and the mechanisms of enzymic action leading to DHT accumulation and hyperplasia.

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