Endogenous androgen levels in epithelium and stroma of human benign prostatic hyperplasia and normal prostate

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Abstract. 5α-Dihydrotestosterone (DHT) and 5α-androstane-3α,17β-diol (3α-diol) were extracted from epithelium and stroma of human benign prostatic hyperplasia (BPH) and of normal prostate and quantified by RIA. The main results were: (1) concerning the BPH, DHT is mainly located in the nuclear fraction of epithelium and stroma, whereas 3α-diol was completely of extranuclear origin, (2) in the nuclei derived from BPH stroma the DHT content (7.1 ± 0.88 pmol/mg DNA, mean ± SEM [n = 14]) was significantly higher (P < 0.01) than in the nuclei derived from BPH epithelium (3.8 ± 0.38 pmol/mg DNA [n = 14]). The DHT content in the nuclear fraction derived from unseparated, i.e. whole tissue, was 5.6 ± 0.60 pmol/mg DNA [n = 14]. (3) the biological significance of the overwhelming DHT accumulation in the stromal nuclei for the BPH tissue is reflected by a significant correlation between the DHT values in the nuclei from stroma and whole tissue (r = 0.710, P < 0.01) and (4) in four normal prostates the DHT content in the nuclear fraction of epithelium (1.3 ± 0.37 pmol/mg DNA) and stroma (2.2 ± 0.93 pmol/mg DNA) was significantly lower (P < 0.01, each) compared with the respective BPH fraction. These data support earlier findings which indicate that the stroma of BPH is a preferential tissue for androgen metabolism.

Despite the still unknown aetiology of the human benign prostatic hyperplasia (BPH) biochemical studies on androgen action in the diseased and normal human prostate have demonstrated profound differences at the cellular level. Tissue 5α-dihydrotestosterone (DHT) content was markedly elevated (Siiteri & Wilson 1970; Geller et al. 1976; Hammond 1978; Meikle et al. 1978; Krieg et al. 1979; Belis 1980). 5α-androstane-3α,17β-diol (3α-diol) distinctly decreased (Geller et al. 1976; Hammond 1978; Meikle et al. 1978; Krieg et al. 1979) in BPH. These findings agree well with the quantitative alterations of androgen metabolizing enzymes (Bruchovsky & Lieskovsky 1979; Krieg et al. 1979) favouring in BPH tissue the formation of DHT which is known to possess a great potency to induce cell hyperplasia in the rat prostate epithelium (Robel et al. 1971; Lesser & Bruchovsky 1973).

The human prostate is composed of two tissue components, i.e. epithelium and stroma. Growth and functional integrity of the epithelium of the rodent prostate are dependent on the presence of stroma (for review: Cunha et al. 1981) and it has been reported that such dualism might also be involved in the development of BPH (Franks et al. 1970). Therefore, recent biochemical and endocrinological studies have been focused on epithelium and stroma of BPH and normal prostate. Androgen metabolism (Harper et al. 1974; Cowan et al. 1977, 1979; Bruchovsky et al. 1980; Romijn et al. 1980; Krieg et al. 1980, 1981), androgen and oestrogen binding (Bashirelahi et al. 1980; Sirett et al. 1980; Krieg et al. 1981) and endogenous tissue DHT levels (Bruchovsky et al. 1980; Sirett et al. 1980; Bolton et al. 1981) have been investigated in both compartments of BPH. With respect to androgen metabolism, most investigators came to the conclusion that 5α-reductase, which converts testosterone to DHT, is distinctly higher in the
stroma than in the epithelium of BPH, while in the normal prostate (Bruchovsky et al. 1980; Krieg et al. 1981) this difference is less pronounced. Along this line, it has been speculated that the pathological alterations in BPH might be induced by increased DHT formation in the stroma, which in turn could stimulate the growth of the adjacent epithelium (Sirett et al. 1980).

In this paper our earlier investigations on androgen metabolism (Krieg et al. 1981) have been extended to the measurement of DHT and 3α-diol concentrations in epithelium and stroma of BPH and normal prostate in order to give further evidence that the stroma of BPH is a preferential tissue for androgen metabolism, i.e. favouring the formation of DHT.

Materials and Methods

**Human tissue specimens**

BPH tissue was obtained by suprapubic prostatectomy from patients in the age range 52–80 years. One normal prostate was received from an autopsy within 4 h after death following an accident. In addition, three normal prostates were obtained during operation from kidney donors who were brain dead between 5 and 24 h. After exstirpation, an aliquot was taken for histological examination and the remainder was immediately chilled to 0°C in 0.9% NaCl and transported to the laboratory.

**Tissue processing**

All procedures up to the extraction of steroids were performed as near 0°C as possible. Epithelium and stroma were separated as reported earlier (Krieg et al. 1981). Fractions of whole tissue, epithelium and stroma were extracted with ether, the extracts were purified by celite column chromatography and the DHT and 3α-diol content of the respective fractions was measured by RIA (Krieg et al. 1977, 1978). For the quantification of androgens in the nuclear fractions, aliquots of whole tissue, epithelium and stroma homogenates were centrifuged at 800 g, the pellet was taken up in buffer (50 mmol/l Tris, 1.5 mmol/l CaCl₂, 5 mmol/l Na₃PO₄, pH 7.25) containing 0.88 mol/l sucrose and centrifuged for 15 min at 800 g. The pellet was thoroughly washed twice with buffer containing 0.25 mol/l sucrose and 0.01% Triton X-100 and once with buffer containing 0.25 mol/l sucrose and 1% BSA. The last pellet was processed as described for the homogenates.

The contamination of the nuclear fractions by extranuclear androgens was evaluated by the following experiment: BPH tissue (0.5 g) was homogenized in 2 ml of homogenization buffer (Krieg et al. 1981) containing 300 000 dpm of [³H]DHT or [³H]3α-diol, left for 1 h at 0–4°C and centrifuged. The pellet was re-suspended in 2 ml buffer, left for 2 h at 0–4°C and centrifuged. This procedure mimics the handling and maximal times for the separation of tissues into epithelium and stroma. The pellet was processed further as described for the nuclear fractions. The radioactivity of the ether extracts was counted and indicated that less than 3.0% (DHT) and 2.4% (3α-diol) of the added androgen appeared in the nuclear fractions (maximal values from two experiments, each).

**Estimation of DNA**

Samples were diluted at 0°C 1:10–1:20 (w/v) with HClO₄ of 0.2 mol/l final concentration, left for 15 min on ice and centrifuged. The pellets were washed with the same vol of cold 0.2 mol/l HClO₄ and taken up in the same vol of 0.3 mol/l KOH. After incubation for 16 h at 37°C the solution was cooled to 0°C, brought to approximately 0.2 mol/l HClO₄ by adding a respective vol of 70% (w/w) acid, kept on ice for 15 min and centrifuged. The pellet was again washed with the same vol of 0.2 mol/l HClO₄ and re-suspended in the same vol of 1.2 mol/l HClO₄, hydrolyzed for 20 min at 70°C, cooled and centrifuged. An appropriate aliquot of the supernatant was taken for the colour reaction, which was performed by the modified Burton method (Burton 1956; Giles & Myers 1965) using salmon sperm DNA as standard. Each determination was performed in duplicate using two different

| Table 1. Androgens in whole tissue and in separated epithelium and stroma of BPH (mean ± SEM, n = 12). |
|------------------------------------------------------|------------------------------------------------------|
| | DHT | 3α-diol |
| a) | pmol/g* | pmol/g* |
| Whole tissue | 17 ± 2.6 | 4.6 ± 0.52* |
| Epithelium | 16 ± 2.4 | 3.3 ± 0.44 |
| Stroma | 16 ± 3.8 | 2.1 ± 0.15 |
| b) | fmol/mg protein | fmol/mg protein |
| Whole tissue | 109 ± 14 | 30 ± 2.8* |
| Epithelium | 285 ± 65 | 64 ± 15.6 |
| Stroma | 271 ± 56 | 45 ± 5.6 |
| c) | pmol/mg DNA | pmol/mg DNA |
| Whole tissue | 7.1 ± 1.07 | 1.9 ± 0.21* |
| Epithelium | 4.5 ± 0.68 | 0.9 ± 0.12 |
| Stroma | 10.6 ± 2.52 | 1.4 ± 0.10* |

* Wet weight of the respective fraction.

Significantly different: 1 from epithelium and stroma at least $P < 0.05$; from epithelium $2 P < 0.05$, $3 P < 0.01$. 

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Table 2.
DNA content of whole tissue and of separated epithelium and stroma from BPH and normal prostate (mean ± SEM [n]).

<table>
<thead>
<tr>
<th></th>
<th>BPH DNA (mg/g*)</th>
<th>Normal prostate DNA (mg/g*)</th>
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</thead>
<tbody>
<tr>
<td>Whole tissue</td>
<td>2.46 ± 0.11 [14]</td>
<td>1.72 ± 0.25 [3]</td>
</tr>
<tr>
<td>Epithelium</td>
<td>3.57 ± 0.26 [14]</td>
<td>3.27 ± 0.49 [4]</td>
</tr>
<tr>
<td>Stroma</td>
<td>1.49 ± 0.08 [14]</td>
<td>0.83 ± 0.17 [4]</td>
</tr>
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* Wet weight of the respective fraction.
1 Significantly different from normal prostate $P < 0.01$.

aliquots of tissue in order to control parallelity of samples and standards at different dilutions. Comparison of DNA values or androgen data related to the DNA content was performed under the assumption that the recovery of DNA from epithelial and stromal origin was identical.

Other methods
Protein was measured by the biuret reaction as reported earlier (Krieg et al. 1981). Tests for statistical significance were carried out using two tailed, non-parametric methods (Wilcoxon U-test, Spearman rank correlation coefficient).

Results
Table 1 summarizes the total amounts of DHT and 3α-diol found in the unseparated whole BPH tissue as well as in the isolated epithelium and stroma. Whether the steroid content is expressed per g tissue, mg tissue protein or mg DNA, the DHT content is distinctly higher than the 3α-diol content in each case. Furthermore, Table 1 demonstrates that the DHT and 3α-diol content is either equal in the epithelium and the stroma (Table 1a, b) or significantly higher in the stroma than in the epithelium (Table 1c) depending on the chosen basis. Controversial data were also found concerning the steroid concentration in the unseparated whole BPH tissue. The values were equal, intermediate, lower or higher when compared with the epithelium and stroma.

In order to circumvent these discrepancies which allow no conclusion concerning differences either between the tissue fractions or between BPH and normal prostate, we measured the DHT and 3α-diol concentration per mg DNA in the nuclear fraction of the unseparated whole tissue as well as of epithelium and stroma of BPH and normal prostate. In Table 2 the DNA contents per g wet weight of tissue are summarized. In the unseparated whole BPH tissue as well as in the BPH stroma a significantly higher DNA concentration was

Table 3.
Nuclear DHT content in whole tissue and in separated epithelium and stroma of BPH and normal prostate (mean ± SEM [n]).

<table>
<thead>
<tr>
<th></th>
<th>BPH DHT (pmol/mg DNA)</th>
<th>Normal prostate DHT (pmol/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole tissue</td>
<td>5.6 ± 0.60 [14]</td>
<td>2.7 ± 1.66 [3]</td>
</tr>
<tr>
<td>Epithelium</td>
<td>3.8 ± 0.38 [14]</td>
<td>1.3 ± 0.37 [4]</td>
</tr>
<tr>
<td>Stroma</td>
<td>7.1 ± 0.88 [14]</td>
<td>2.2 ± 0.93 [4]</td>
</tr>
</tbody>
</table>

Significantly different$^1$ from whole tissue $P < 0.05$, from stroma $P < 0.01$,

$^1$ from normal prostate $P < 0.01$. 

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found when compared with the normal prostate, while in the epithelium of BPH and normal prostate the DNA content was rather similar. Based on these DNA data, the DHT concentrations in the nuclear fractions of various tissue components of BPH and normal prostate are summarized in Table 3. Concerning the BPH, the DHT data were only slightly lower than those measured in the total homogenates of the various tissue components (Table 1c), indicating that the major part of DHT is located in the nuclei. Otherwise, in BPH and normal prostate 3α-diol could not be detected (< 0.7, < 0.3, < 0.6 pmol/mg DNA for whole tissue, epithelium and stroma, respectively) in any of the fractions, indicating the extranuclear localization of 3α-diol in Table 1. With respect to BPH, a significantly higher DHT content was found in the stroma and whole tissue than in the epithelium. Furthermore, as shown in Fig. 1, a significant positive correlation was found between DHT content in the stroma and whole tissue nuclei, while respective correlation studies between epithelium and whole tissue \( r_s = 0.374, \) NS as well as epithelium and stroma \( r_s = 0.081, \) NS remained insignificant.

Turning to the DHT content in the various nuclear fractions of normal prostate, significant differences between the whole tissue, epithelium and stroma could not be found, although in the normal prostate the stroma nuclei too showed a tendency to higher DHT concentration. Finally, when comparing the BPH with the normal prostate, the nuclear fractions of the BPH tissue contained higher DHT concentration, the differences being statistically significant for epithelium and stroma.

**Discussion**

In a previous publication (Bartsch et al. 1980) we have discussed in detail that the measurement of endogenous androgen concentrations in whole target organs and their compartments should theoretically reflect the actual in vivo, i.e. steady state conditions, but that several methodological aspects might falsify the interpretation of the RIA data with respect to the actual in vivo situation. Particularly, the continuation of the steroid metabolism and steroid diffusion from one compartment into the other during the tissue processing might lead to unpredictable sources of error. Furthermore, the isolation of various tissue components, i.e. the separation of the human prostate in epithelium and stroma as well as the preparation of the nuclear fractions is not complete. In a recent paper (Krieg et al. 1981) in which we have described the isolation procedure, we found on average 17% contamination of the epithelium by stroma and 6% of stroma by epithelium. In addition, concerning the purity of the nuclear fractions we are aware that despite thorough washing steps this fraction represents only a crude preparation.

Besides these methodological considerations, which must be borne in mind at any stage of the discussion of the data, a further unsolved problem becomes obvious when measuring DHT and 3α-diol in the total tissue fractions (homogenates), namely the controversial values when relating the steroid content on the protein or DNA basis. This discrepancy might be explained by differences in the effectiveness of protein (Krieg et al. 1981) and DNA (Table 2) recovery when isolating epithelium and stroma. Therefore, the interpretation of the data of Table 1 with respect to their possible biological relevance is problematical. Similar discrepancies were reported by others (Sirett et al. 1980) and it is not improbable that, mainly due to differences in the methodological procedures,
our RIA data on homogenates of epithelium and stroma are only in part identical to those of other groups (Cowan et al. 1979; Sirett et al. 1980; Bruchovsky et al. 1980; Bolton et al. 1981).

An alternative approach to compare the accumulation of DHT in the epithelium and stroma of BPH and normal prostate would seem to be the measurement of DHT in the nuclei. This fraction is better defined than the homogenate and the convenient basis to which the DHT content has to be related is DNA. Furthermore, the above mentioned unavoidable contamination of the crude nuclear fraction was minimized (see Materials and Methods) by washing the nuclear preparation extensively with Triton X-100 and BSA containing buffer. Two further findings support the assumption of minimum cytoplasmic contamination of the nuclear preparation: (1) the absence of 3α-diol in each nuclear fraction, considering that 3α-diol is not retained in the nuclei of rat prostates (Bruchovsky et al. 1975; Bartsch et al. 1980) and (2) the mean DHT content in our nuclear fractions of BPH and normal prostate epithelium is in accordance with the values found by Bruchovsky et al. (1980) in highly purified nuclear fractions of BPH and normal prostate epithelium.

Thus, a comparison of the DHT content in the nuclei of whole tissue, stroma, and epithelium of BPH and normal prostate seems to be justified. From the data several biological implications may be tentatively discussed:

(1) The fact, which has been found also by others (Ghanadian et al. 1976; Meikle et al. 1980; Verdouw et al. 1980) that most of the DHT is located in the BPH nuclei might indicate an immediate translocation of the DHT-receptor complex into the nuclei either after DHT has entered the cell from the blood stream or after being formed intracellularly by extensive 5α-reduction of testosterone. Both the presence of androgen receptors and 5α-reductase in BPH tissue has been found by various groups (for review see: Krieg et al. 1979). Furthermore, the absence of measurable amounts of 3α-diol in the nuclear fraction, although it is detectable in the whole tissue, could indicate that the nuclear DHT accumulation is a selective process which fits well with the assumed steroid receptor mechanism (Fang & Liao 1971; Mainwaring 1977). Thus, DHT could play an overwhelming role with respect to gene expression at the nuclear level of the BPH.

(2) If distinct BPH tissue fractions are considered, the significantly higher nuclear DHT content in the stroma compared with the epithelium is remarkable. This finding reflects the androgen metabolism in so far as significantly higher 5α-reductase activity was found in the stroma compared to the epithelium (see: introduction). Thus the cellular 5α-reductase might be a pacemaker for nuclear DHT accumulation and finally for androgen action in human prostate. Furthermore, the significant correlation of DHT content between stroma and whole tissue is remarkable. This correlation underlines the central role which the stroma might play for the whole BPH at least concerning the regulation of the androgen milieu.

(3) The higher DHT content in the stroma of BPH when compared with normal prostate is a further indication that DHT as well as the stroma might be an important factor for tumour growth. The higher DHT content in the epithelium of BPH compared with normal prostate is not in accordance with the very similar 5α-reductase activity in BPH and normal prostate epithelium. However, this difference could be easily explained by excessive DHT formation in the stroma and its subsequent diffusion into the epithelium. This process was first postulated by Sirett et al. (1980).

In conclusion, these data indicate that the stroma might be the preferential tissue of the BPH for androgen metabolism. Furthermore, the stroma and DHT might be two dominating regulators for the growth of the BPH.

Acknowledgments

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References


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