QUANTIFICATION OF ANDROGEN BINDING, ANDROGEN TISSUE LEVELS, AND SEX HORMONE-BINDING GLOBULIN IN PROSTATE, MUSCLE AND PLASMA OF PATIENTS WITH BENIGN PROSTATIC HYPERTROPHY

By
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ABSTRACT
The in vitro binding of 5α-dihydrotestosterone (5α-DHT) in benign prostatic hypertrophy (BPH), rectus abdominis muscle and plasma of 14 patients was characterized and quantified by agar gel electrophoresis. The respective endogenous tissue and plasma levels of 5α-DHT and testosterone (T) were determined by radioimmunoassay, and the plasmatic sex hormone-binding globulin (SHBG) concentration was estimated in the 14 patients by an (NH₄)₂SO₄ precipitation technique. Finally the in vitro conversion of 5α-DHT to the 5α-androstanediols in the BPH at 0°C after a 20–24 h incubation period was analyzed by thin-layer chromatography.

The main results were as follows: (1) In 12 out of 14 BPH cytosols three charcoal resistant binding peaks were found, of which peak 1 represents SHBG, peak 2 the specific receptor protein and peak 3 a binding protein with relatively high binding capacity and low affinity for 5α-DHT. In two cases peak 2 was absent. In 11 out of 14 muscle cytosols three binding peaks are also present, resembling those of the BPH. (2) The receptor peak is reduced on average 38% by unlabelled 5α-DHT, 23% by cyproterone acetate (CYAC) and 29% by oestradiol. The parallel data for the SHBG peak are: 62% by 5α-DHT, 22% by CYAC and 49% by oestradiol. (3) From displacement studies with unlabelled 5α-DHT the average concentration of receptor was calculated to be 12.3 fmol/mg cytosol protein (CP) in BPH, and 3.6 fmol/mg CP in muscle. Under identical conditions 39.9 fmol SHBG/mg CP and 24.1 fmol/mg CP were found in the BPH.

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1) Part of doctoral thesis.
and muscle, respectively. The mean values are significantly different ($P < 0.001$). In plasma a mean value of $4.0 \times 10^{-8}$ mol SHBG/l was found. (4) In the BPH on average 4.43 ng 5a-DHT/g tissue and 0.23 ng T/g tissue are present, in muscle 0.45 ng 5a-DHT/g tissue and 0.71 ng T/g tissue, in plasma 0.47 ng 5a-DHT/ml and 3.89 ngT/ml. (5) Statistical calculations revealed (a) a significantly ($P < 0.05$) negative correlation between the endogenous 5a-DHT and T tissue levels and the available 5a-DHT receptor sites in BPH cytosol, (b) a positive correlation between plasmatic SHBG concentration and the available SHBG concentration in BPH cytosol. (6) Compared to the rat prostate, where 36% of the incubated 5a-DHT was converted at 0°C within 20–24 h into the 5a-androstanediols, in the BPH conversion to 5a-androstanediols was negligible.

Abundant data concerning androgen metabolism and binding in the blood and prostatic tissue of patients suffering from benign prostatic hypertrophy (BPH) have been comprehensively presented at two symposia in Bethesda (USA) (1975) and Helsingborg (Sweden) (1975). Nevertheless, the aetiology of BPH remains unknown. Therefore, any contribution regarding “androgen status” at the cellular level of the BPH and muscle, and of blood, may be valuable.

In this study we have focussed our interest on the characterization and quantification of the assayable 5a-dihydrotestosterone (5a-DHT) receptor, of the sex hormone-binding globulin (SHBG) and on the quantification of the endogenous 5a-DHT and testosterone (T) levels in BPH, muscle and plasma of 14 patients. Significant correlations between the various parameters will be described. Furthermore, some metabolic studies on in vitro metabolism of 5a-DHT in the BPH are included, demonstrating striking differences from the results obtained in the rat prostate.

MATERIALS AND METHODS

Chemicals. – [1,2-3H]5α-Dihydrotestosterone (S. A. 44 Ci/mmol) was purchased from NEN Chemicals (Dreieichenhain). Before use, the radioactive steroid was dissolved in benzene:ethanol (9:1; v/v), evaporated to dryness and re-dissolved in absolute ethanol. Radiochemical purity was monitored by thin-layer chromatography and was greater than 96%. Unlabelled steroids and other reagents were obtained from Merck AG (Darmstadt). Cyproterone acetate (CYAC) was a gift from Schering AG (Berlin). The anti-testosterone antibody, a gift from Organon International (Oss), was raised in rabbits by administration of 11α-OH-testosterone-11-succinyl-BSA. Serva (Heidelberg) provided pronase, purest agarose and celite. Pure agar came from Behringwerke AG (Marburg).

Tissue. – Tissue and blood from 14 patients, between 63 and 78 years (mean 70 years) old and suffering from BPH were investigated. Blood samples were drawn at 8.00 a.m. one day before operation. BPH tissue and a small piece (less than 500 mg) of the rectus abdominis muscle were obtained during the prostatectomy according to Millin

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The tissue was transported in an ice cooled 0.9% NaCl solution to the pathologist, where an aliquot was taken for histological examination. Thereafter the tissue was brought to the laboratory and processed immediately.

**Processing of the tissue.** – BPH and muscle tissue were cut into small pieces and blotted on filter paper. Tissue was then frozen in liquid nitrogen and pulverized in a mortar chilled in liquid nitrogen. The fine tissue powder was then divided in two parts, one for receptor studies, the other for measuring endogenous 5α-DHT and T levels. The heparinized blood was centrifuged for 10 min at 3000 r.p.m. to obtain the plasma, which was divided in three parts in order to analyze the endogenous 5α-DHT and T levels, the SHBG concentration, and the [3H]5α-DHT-binding pattern.

**Binding studies.** – Tissue powder was transferred to centrifuge tubes and buffer (0.01 M Tris-HCl, 0.002 M EDTA, 0.005 M NaN₃, 0.01 M MgCl₂·6H₂O, 0.002 M 2-mercapto-ethanol, pH 7.4 at 2°C) was added, in the ratio of 1 g tissue to 1 ml buffer. Tissue and buffer (= homogenate) were well mixed by stirring and incubated for 20–24 h at 0°C either with 2.2 × 10⁻⁸ M [3H]5α-DHT (final concentration) or in parallel test tubes with 2.2 × 10⁻⁸ M [3H]5α-DHT plus 2.2 × 10⁻⁵ M unlabelled 5α-DHT, oestradiol or CYAC, whereby the unlabelled compounds were added to the homogenate 20 min prior to [3H]5α-DHT. The homogenate (approximately 2 ml) was then centrifuged for 1 h at 0°C and 35 000 r.p.m. in a fixed-angle rotor. The clear supernatant was defined as 100 000 g cytosol. Excess of unbound steroid in the cytosol was removed by adding 10% (v/v) of a Dextran coated charcoal suspension (2% (w/v) Norit A (Serva); 0.2% (w/v) Dextran T 70 (Pharmacia) in homogenization buffer) for 15 min. Charcoal was removed by centrifugation of the cytosol twice for 3 min at 14 000 g with a cooling period of 3 min. In order to find out differences in the binding pattern of the cytosol and plasma, the latter was incubated with 1 × 10⁻⁷ M [3H]5α-DHT and treated under identical conditions as described for the tissue.

**Anti-steroid antibody treatment of the cytosol.** – The principle of this method was first described by Castañeda & Liao (1975). The method served for the qualitative characterization of the three binding peaks found in the BPH cytosol: 10 µl of a 1:10 dilution in 0.9% NaCl of an anti-testosterone antibody was added to 100 µl of the charcoal treated cytosol obtained from the homogenate incubated with [3H]5α-DHT. The antibody has a 30% cross-reaction with 5α-DHT, and the amount used binds more than 90% of the tritiated steroid not bound specifically to the receptor protein. After incubation at 0°C for 2 h the receptor bound steroid is separated from antibody bound steroid by agar gel electrophoresis, in which the antibody migrates far to the cathode (slice nos. 20–23) and the receptor to the anode.

**Agargel electrophoresis.** – Agargel electrophoresis at low temperature was performed according to Wagner (1972). Inter- and intra-assay reproducibility of the receptor quantification was checked earlier (Krieg 1976), the coefficient of variation being 5.2% and 10%, respectively.

**Quantification of the cytosolic assayable receptor and SHBG concentration.** – The decrease of the radioactivity in peaks 1 and 2, obtained by incubation of the homogenate (a) with tritiated 5α-DHT alone and (b) with tritiated 5α-DHT plus a 1000-fold excess of unlabelled 5α-DHT, is taken as a measure of specific, i.e. SHBG (peak 1) and receptor (peak 2) bound radioactivity. The amount of SHBG and receptor bound hormone, expressed as mol/mg cytosol protein, was calculated from the peak differences, measured in cpm taking into account the efficiency of the scintillation counter, the spe-
cific activity of the labelled compound, the amount of cytosol applied and the protein concentration in the cytosol. On the assumption that one mol receptor protein or SHBG binds one mol steroid, the concentration of bound hormone corresponds to the receptor and SHBG concentration.

**SHBG determination in plasma.** – SHBG binding capacity in the plasma was measured by the method of Dennis et al. (1977). After removing the endogenous steroid by charcoal absorption, SHBG was precipitated with (NH₄)₂SO₄ (28 % (w/v) final concentration) and re-dissolved in buffer containing a saturating amount of 5α-DHT plus a tracer amount of [³H]5α-DHT. After incubation for 15 min at 37°C and cooling to 0°C a differential dissociation technique (Horst et al. 1974) was applied to eliminate unspecific binding. Values were recorded in moles/l binding capacity for 5α-DHT, which corresponds to the molar SHBG concentration under the assumption of a mol to mol binding relation of SHBG and 5α-DHT. The inter-assay reproducibility of the method has been checked, the coefficient of variation being 3.8 %.

**Determination of the endogenous tissue and plasma levels of 5α-DHT and T.** – Radioimmunoassay for plasmatic T and 5α-DHT was performed as described earlier (Baritsch et al. 1977). For tissue samples all procedures prior to extraction were done at 0°C as follows: To two samples of the pulverized tissue, weighing about 300–600 mg, 1000 cpm of tritiated T and 5α-DHT were added, respectively, for estimating the steroid recovery. After adding one volume of water, the homogenate was sonicated for three times 20 seconds periods, left for 1 h in an ice bath for equilibration and then extracted three times with ether. The combined organic phases were dried under nitrogen, applied with 2 x 0.5 ml i-octane on top of i-octane-pre-washed columns (0.5 cm diameter; stationary phase: celite:ethyleneglycol:propyleneglycol (4:1:1; w/v/v)) and eluted subsequently with 3.5 ml i-octane (discarded), 3.5 ml i-octane:toluene (95:5; v/v) (discarded), 3.0 ml i-octane:toluene (3:2; v/v) (5α-DHT) and 3.5 ml i-octane:toluene (1:1; v/v) (T). The eluates containing T or 5α-DHT were collected and taken for radioimmunoassay, which was performed in triplicate by the Dextran coated charcoal method. Antibody against T was from Organon International (Oss), antibody against 5α-DHT was purchased from Miles (Slough), raised against 5α-DHT-1-BSA. The recovery in all samples, derived from less than 800 mg tissue, was 50–95 %. Dilution experiments and addition of authentic steroids revealed a good linearity. When the steroids were extracted from 500 mg BPH or muscle tissue, the sensitivity of the method was 150 pg of T and 5α-DHT per g tissue. Intra-assay coefficient of variation, estimated in BPH samples weighing 300–700 mg, was for 5α-DHT 19.6 % (n = 18), for T 14.5 % (n = 7).

**Thin-layer chromatography.** – Under identical conditions, as mentioned above for the binding studies, after 24 h of incubation at 0°C the homogenate of the BPH and rat prostate were processed into the 100 000 g cytosol. The steroids were then extracted with ether and chloroform and separated by thin-layer chromatography on silica gel in chloroform:acetone (9:1; v/v). To separate 5α-DHT from androsterone and epianandrosterone the respective fraction from the first chromatography was acetylated and then chromatographed on Al₂O₃ G (type E) in cyclohexane:ethylacetate (9:1; v/v). Details have been reported previously (Buric et al. 1972).

**Miscellaneous.** – (1) Enzyme digestion: before separation by agar gel electrophoresis, the cytosol was incubated for 90 min at 0°C with pronase in a final concentration of 1 mg/ml. (2) Heat-lability was checked by heating the cytosol at 45°C for 1 h before analysis. (3) Cytosolic protein concentration was measured by means of the biuret reaction. (4) The statistical significance of the differences of the means were checked with

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the Wilcoxon-Test, the correlations of the values with the Spearman rank correlation coefficient. (5) Measurement of radioactivity: (a) the gel slices were deep frozen in the counting vials in order to facilitate elution of the radioactivity by the scintillation fluid (240 g naphthalene, 15 g PPO, 0.15 g POPOP, made up to 3 litres with dioxan: xylene (2:1; v/v) mixture. (b) The aliquots for the plasmatic SHBG determination and for the measurement of the endogenous tissue and plasma 5α-DHT and T levels were filled up with an Instagel (Packard):toluene (1:1; v/v) mixture. (c) The thin-layer fractions were collected in counting vials, after which radioactivity was eluted with ethanol and scintillator (0.3 g POPOP, 3 g PPO, toluene to 1 litre). Because of the constant ratio to the internal standard, the radioactivity of (a) and (b) were measured as cpm and activity in the thin-layer fraction as dpm, quench corrected by automatic external standardization in a liquid scintillation counter (Packard Tri-Carb 3380 and 3390, efficiencies 40 % and 35 %, respectively).

RESULTS

Characterization of the [3H]5α-DHT-binding in BPH, muscle and plasma

In 12 out of 14 BPH cytosols, analysis by agargel electrophoresis revealed a [3H]5α-DHT-binding pattern as shown in Fig. 1. Besides small amounts of unbound radioactivity in slices no. 21–28, three charcoal resistant binding peaks

![5α-DHT BINDING IN HUMAN BENIGN PROSTATIC HYPERTROPHY AND PLASMA](image)

*Fig. 1.*

*In vitro* binding of [3H]5α-DHT in the 100 000 g cytosol of the BPH (left panel) and plasma (right panel), analyzed by agargel electrophoresis. BPH homogenate was incubated for 20–24 h at 0°C with 2.2 × 10⁻⁸ M (final concentration) [3H]5α-DHT. After processing the cytosol, which was charcoal-treated to remove the excess of unbound steroid, 40 µl was applied between slices no. 14 and 15. Anodic pool left, cathodic right from the start. Electrophoresis: 90 min at 10 V/cm. Radioactivity was measured in cpm/slice, each slice being 3 mm wide. Plasma (1:3 dilution with 0.9 % NaCl) was incubated with 1 × 10⁻⁷ M [3H]5α-DHT, and analyzed as for BPH cytosol.
In vitro binding of [3H]5α-DHT in the 100,000 g cytosol of BPH and muscle. BPH homogenate was incubated for 20–24 h at 0°C with 2.2 × 10−8 M [3H]5α-DHT alone (▲), or (left panel) in the presence of a 1000-fold excess of unlabelled 5α-DHT (●), cyproterone acetate (CYAC) (□), or (middle panel) oestradiol (E2) (○). Under identical conditions muscle homogenate was incubated with [3H]5α-DHT (■) (right panel). After processing the cytosol, it was treated with charcoal to remove the excess of unbound steroid. An aliquot of the BPH cytosol was incubated for 2 h at 0°C with an antibody against testosterone and 5α-DHT (+) (middle panel). Forty µl cytosol was applied between slice nos. 14 and 15. Anodic pool left, cathodic pool right from the start. Electrophoresis: 90 min at 10 V/cm. Radioactivity was measured in cpm/slice, each slice being 3 mm wide.

were found: peak 1 migrates towards the cathode (slices no. 15–18), while peak 2 and 3 were found towards the anode in slices no. 11–14 and 5–10, respectively. In two out of 14 samples analyzed in the present series peak 2 was absent. Fig. 1 (right panel) shows the regular 5α-DHT-binding pattern in human plasma, where peak 2 is absent. Fig. 2 (right panel) demonstrates that in muscle cytosol (musculus rectus abdominis) a qualitative binding pattern is found which is quite similar to the pattern obtained in the BPH. In three out of 14 cases analyzed, peak 2 was absent. Due to the lack of material, the binding pattern in the muscle has only been characterized in this series by the displacement of peak 1 and 2 with a 1000-fold excess of unlabelled 5α-DHT, in order to calculate the receptor and SHBG concentration.
The further qualitative characterization of the $[^3\text{H}]5\alpha$-DHT-binding pattern in the BPH cytosol is shown in the left and middle panel of Fig. 2. A 1000-fold excess of $5\alpha$-DHT, CYAC as well as oestradiol are effective competitors for the $[^3\text{H}]5\alpha$-DHT-binding peaks 1 and 2. Conversely peak 3 increases. Fig. 3 shows the percentage decrease of the $[^3\text{H}]5\alpha$-DHT-binding peaks in the various cases investigated. Peak 2 is on average reduced 38 %, 23 % and 29 % by $5\alpha$-DHT, CYAC and oestradiol, respectively, peak 1 on average 62 % (5α-DHT), 22 % (CYAC) and 49 % (oestradiol). Regarding peak 1 the means are significantly different ($P < 0.05$) from each other, while concerning peak 2 this holds true only when comparing the means of 5α-DHT and CYAC. Treatment of BPH cytosol with anti-testosterone antibody (Fig. 2, middle panel) removes $[^3\text{H}]5\alpha$-DHT from peak 1 and 3; peak 2 remains well defined. The antibody itself migrates towards the cathode (slices no. 20–23). Fig. 4 shows the various percentage decrease of the three $[^3\text{H}]5\alpha$-DHT-binding peaks of the BPH cytosols treated with this antibody. The means of 59 % (peak 3), 22 % (peak 2) and 38 % (peak 1) are significantly different ($P < 0.05$) from each other.

Further characterization of the binding peaks are the loss of binding ability of peak 2 when heating the cytosol for 1 h at 45°C, or incubating the cytosol with pronase at 0°C for 90 min. Peak 3 remains unaffected under these treatments, while peak 1 decreases slightly after the pronase treatment.

From these studies we are defining peak 1 as SHBG and peak 2 as the organ specific receptor.

**COMPETITOR**

<table>
<thead>
<tr>
<th>COMPETITOR</th>
<th>DHT</th>
<th>CYAC</th>
<th>E₂</th>
<th>DHT</th>
<th>CYAC</th>
<th>E₂</th>
</tr>
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<td>PEAK 2 ('RECEPTOR') OF THE BPH CYTOSOL</td>
<td>38</td>
<td>23</td>
<td>29</td>
<td>38</td>
<td>23</td>
<td>29</td>
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<tr>
<td>PEAK 1 ('SHBG') OF THE BPH CYTOSOL</td>
<td>62</td>
<td>22</td>
<td>49</td>
<td>62</td>
<td>22</td>
<td>49</td>
</tr>
</tbody>
</table>

**Fig. 3.**

Percentage decrease of the $[^3\text{H}]5\alpha$-DHT peaks 1 and 2 of the BPH cytosol by a 1000-fold excess of unlabelled 5α-DHT, cyproterone acetate (CYAC) and oestradiol (E₂), analyzed by agar-gel electrophoresis. The peak numbering is given in Figs. 1 and 2.

The mean percentual decreases are indicated by $\bar{x}$, $n = 14$. 

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Fig. 4.
Percentage decrease of the $[^3H]5a$-DHT peaks 1, 2 and 3 of the BPH cytosol by incubating the pre-labelled cytosol for 2 h at $0°C$ with an antibody against testosterone and $5a$-DHT and subsequent analysis by agar gel electrophoresis. The peak numbering is given in Figs. 1 and 2. The mean percentual decreases are indicated by $\bar{x}$, $n=14$.

Fig. 5.
Receptor and SHBG concentration in BPH, muscle and plasma of 14 patients. The receptor and SHBG bound $[^3H]5a$-DHT has been taken as the measure of receptor and SHBG concentration, assuming a mol to mol binding relation between the specific proteins and $5a$-DHT. For further details see Materials and Methods. The means are indicated by $\bar{x}$. 207
Quantification of the receptor and SHBG concentration in BPH, muscle and plasma

As shown in Fig. 5 (left and middle panels), in the BPH cytosol on average 12.3 fmol/mg protein of receptor is assayable, while in the muscle under identical conditions the value is 3.6 fmol/mg protein. The mean values are significantly different ($P < 0.01$), the ratio being 3.4. The assayable SHBG concentrations are 39.9 fmol/mg protein in the BPH cytosol and 24.1 fmol/mg protein in the muscle cytosol. The mean values are significantly different ($P < 0.01$), the ratio being 1.7.

In the right panel of Fig. 5 the SHBG concentration in 13 plasmas of this series is shown. When calculating various Spearman rank correlation coefficients, it is noticeable that there exists a significant positive correlation between the SHBG concentration in plasma and BPH cytosol (Fig. 6, left panel). When correlating the plasmatic SHBG concentration with the SHBG concentration in the muscle cytosol, the coefficient is 0.519, which is slightly outside the 95% confidence level, being $\geq 0.566$ ($n = 13$). Alternatively the SHBG concentration in the BPH and muscle cytosol correlate significantly, while this is not true for the correlation between the receptor concentration in the BPH and muscle cytosol (Fig. 6, middle and right panel). Furthermore, in the BPH as well as muscle cytosol no correlation exists between receptor and SHBG concentration.

![Fig. 6.](image-url)

Spearman rank correlation of SHBG and receptor concentrations in BPH, muscle and plasma. The points are identical with the values given in Fig. 5. Left panel: SHBG concentration in plasma versus SHBG concentration in BPH cytosol. Middle panel: SHBG concentration in muscle cytosol versus SHBG concentration in BPH cytosol. Right panel: receptor concentration in muscle versus receptor concentration in BPH cytosol.
**Table 1.**
Endogenous levels of 5α-DHT and testosterone (T) in BPH, muscle and plasma, obtained by radioimmunoassay.

<table>
<thead>
<tr>
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<th>Testosterone</th>
<th>5α-DHT</th>
<th>Total (T + 5α-DHT)</th>
</tr>
</thead>
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<td></td>
<td>(ng/g tissue or ml plasma)</td>
<td>(ng/g tissue or ml plasma)</td>
<td>(ng/g tissue or ml plasma)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>x ± sd</td>
<td>n</td>
</tr>
<tr>
<td>BPH</td>
<td>11</td>
<td>0.23 ± 0.12</td>
<td>13</td>
</tr>
<tr>
<td>Muscle</td>
<td>12</td>
<td>0.71 ± 0.80</td>
<td>10</td>
</tr>
<tr>
<td>Plasma</td>
<td>14</td>
<td>3.89 ± 1.42</td>
<td>14</td>
</tr>
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</table>

BPH = benign prostatic hypertrophy.
5α-DHT = 5α-dihydrotestosterone.
T = testosterone.

**Fig. 7.**
Spearman rank correlations between the receptor concentration in BPH cytosol and the BPH tissue concentration of 5α-DHT and testosterone (upper panels) or the plasma concentration of 5α-DHT and testosterone (lower panels). The units of the values can be determined from Fig. 5 and Table 1, respectively.
**Interrelationship between endogenous androgen levels and receptor concentration**

Table 1 summarizes the endogenous T and 5α-DHT concentrations in BPH, muscle and plasma. Three points seem remarkable: (1) Compared to the values in plasma, there is in the BPH a tremendous increase in the 5α-DHT concentration, paralleled by a sharp drop of T. (2) The total amount of both steroids is slightly higher in the BPH compared to plasma. (3) The total amount of both androgens is significantly lower in the muscle than in BPH and plasma; however, the DHT:T ratio is in the muscle higher than in plasma (0.63 versus 0.12). As shown in Fig. 7 (upper panel) there exists a significant negative correlation between the assayable cytosolic 5α-DHT receptor concentration and the 5α-DHT and T concentration in the BPH. Such a negative correlation does not exist between the receptor concentration and the plasma T and 5α-DHT values (Fig. 7, lower panels). Furthermore, no correlation was found between the steroid concentration in the tissues (BPH and muscle) and plasma. Correlating the receptor concentration in muscle with the respective T and 5α-DHT tissue levels a negative, but not significant, correlation was calculated.

**In vitro metabolism of [3H]5α-DHT in the BPH homogenate**

To evaluate, how much of the added [3H]5α-DHT is metabolized during the 20–24 h incubation period at 0°C, the main metabolites were analyzed, as summarized in Table 2. For comparison the rat prostate was incubated under

<table>
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<tr>
<th>Metabolites found (%)</th>
<th>[3H]5α-DHT added</th>
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<tbody>
<tr>
<td></td>
<td>Human BPH</td>
</tr>
<tr>
<td>5α-DHT</td>
<td>80 ± 1 (n = 3)</td>
</tr>
<tr>
<td>Diol</td>
<td>4 ± 1 (n = 3)</td>
</tr>
</tbody>
</table>

**Table 2.**
Percentage distribution of 5α-DHT and 5α-androstanediols (Diol) obtained by thin-layer chromatography in the 100 000 g cytosol of the BPH and rat prostate after 20–24 h incubation of the organ homogenates at 0°C with 2.2 x 10⁻⁸ M [3H]5α-DHT.

BPH = benign prostatic hypertrophy.
5α-DHT = 5α-dihydrotestosterone.
Diol = 5α-androstanediols.
identical conditions. It seems remarkable that the conversion of $5\alpha$-DHT to the $5\alpha$-androstanediols is negligible in the BPH, while in the rat prostate 36% of the extracted radioactivity was found in the $5\alpha$-androstanediols fraction.

**DISCUSSION**

Our studies with rat skeletal muscle (Krieg 1976) showed that steroid incubation of the homogenate prior to the processing of the cytosol gave a better yield of available cytosolic binding sites than processing the cytosol first and subsequently incubating it with the steroid. The reason given for this is that the receptor protein is stabilized by its ligand during further processing of the tissue by ultracentrifugation etc. (Bell & Munck 1973; Liao et al. 1974). Therefore we applied this procedure for assaying receptor sites in the BPH cytosol. Furthermore, because of the very small amounts of unoccupied receptor sites available (Rosen et al. 1975; Bonne & Reynaud 1976) and the slow dissociation rate of the ligand from the receptor (Attramadal et al. 1975) we used a relatively long incubation period of 20–24 h at 0°C to allow the excess of added tritiated steroid a longer time period to compete for specific receptor binding sites. We were now able to characterize and quantify in 12 out of 14 BPHs a specific receptor protein, while in an earlier report from this laboratory (Steins et al. 1974) using a 2 h cytosol incubation a receptor protein was not detectable. Other workers (Mainwaring & Milroy 1973; Mobbs et al. 1975; Cowan et al. 1976; Nijs et al. 1976), using various methods have also commented on the difficulty in characterizing a receptor protein in the BPH cytosol.

Three charcoal resistant binding peaks are present in the organ cytosols whereas in human male plasma only two peaks are found, of which peak 1 has been characterized by Wagner (1972) as the binding peak due to SHBG. The identical migration behaviour of the cytosolic binding peak 1 allows us to conclude that it is SHBG, supported furthermore by (a) the significant differences shown by the displacement studies, which indicate that $5\alpha$-DHT has the highest affinity to this binding protein, followed by oestradiol and CYAC, a ranking which is also found for the plasmatic SHBG (Westphal 1971; Steins et al. 1974), (b) the heat stability of the binding, which is also the feature of plasmatic SHBG, (c) the significant positive correlation between the SHBG concentration in plasma and in the respective organ cytosols and (d) the correlation between the cytosolic SHBG concentration in BPH and muscle. The latter two findings may indicate that the cytosolic SHBG merely reflects the plasma contamination of the cytosols, which is in excellent agreement with the results of Cowan et al. (1976), but which does not support the discussion of an intracellular or extravascular accumulation of SHBG as an aetiological factor.
for this disease (Hansson et al. 1975; Rosen et al. 1975). It is furthermore remarkable that the ratio of 1.66 of the mean SHBG concentration in BPH and muscle is nearly the same as the ratio of 1.68 which we have found when comparing the mean percentual plasma contamination in both cytosols, based on the estimation of their IgG content (Steins et al. 1974).

It must be stressed that our quantification of SHBG-binding sites (= binding capacity) in the cytosols by agar gel electrophoresis are not absolute values because SHBG will still be partly occupied by endogenous steroids, and because we did not take into account the dissociation rate of the ligand during the 90 min run, which is on the average 19% per h, estimated in human plasma (Arning, unpublished). Regarding this dissociation rate, our mean value for the SHBG concentration in the BPH cytosol of about 40 fmol per mg protein is comparable with the 64 fmol per mg protein found by Cowan et al. (1976) by means of steady state polyacrylamide gel electrophoresis.

Peak 2 of the cytosolic binding pattern is not present in plasma, and therefore an organ specific binding peak. Its migration distance, heat-lability and pronase sensitivity are quite similar to the behaviour of the receptor protein characterized by us in various rat androgen target organs (Krieg & Voigt 1976). The displacement studies revealed also similarities to respective findings in our animal experiments, whereby the displacement effect of CYAC at the receptor level may partially reflect the mechanism, by which the observed regressive processes are induced in the BPH of patients treated with this anti-androgen (Scott & Wade 1969). The biological consequences of the surprisingly high displacement of 5α-DHT from the receptor by oestradiol are completely unclear and raise again the frequently discussed question of the role which this oestrogen plays in older men whose oestradiol blood levels are relatively increased compared to younger men.

Binding peak 2 was further characterized by incubation of the cytosol with anti-steroid antibody for elimination of non-specifically bound radioactivity (Castañeda & Liao 1975). While peaks 1 and 3 were almost completely reduced to background levels by antibody treatment of cytosol, peak 2 remained well defined, indicating its high affinity for 5α-DHT and furthermore its complete independence from the SHBG peak.

Our results also indicate for the first time, the presence of peak 2 in the rectus abdominis muscle in 11 out of 14 patients; its characterization is, however, incomplete due to the lack of sufficient material. The peak is heat labile and displaceable by a 1000-fold excess of unlabelled 5α-DHT. In an earlier report (Voigt et al. 1975), which as far as the muscle data was concerned was based on a single case, this peak could not be detected. From this series we now believe that human muscle contains an assayable androgen receptor, which resembles the specific receptor protein characterized by us (Krieg 1976) in the rat skeletal muscle.
So far the qualitative characteristics of the receptor protein, represented by peak 2, are similar to those of the well characterized specific protein in various androgen target organs of the rat. The same similarities between the receptor protein in rat target organs and human BPH have been found by Mainwaring & Milroy (1973), Davies & Griffiths (1975), Rosen et al. (1975) and Bonne & Raynaud (1976), using the density gradient ultracentrifugation technique, and by Attramadal et al. (1975) and Geller et al. (1975) using Sephadex column chromatography.

The binding behaviour of cytosolic peak 3 indicates a high androgen binding capacity and relatively low affinity compared to peak 2, shown by the peak increase in the displacement studies and the steepest decrease of the three peaks after antibody treatment of the cytosol, respectively. The peak is in part charcoal resistant, neither heat-labile nor pronase sensitive, the latter, however, probably due to too little enzyme added. The migration distance is similar to the position of the plasmatic peak 3 and of pure human serum albumin. Therefore the peak may represent merely plasma contamination or a storage protein, first postulated by Giorgi et al. (1971) for the BPH.

Turning to the quantification of available receptor sites in the cytosol, the mean value of about 12 fmol per mg protein in the BPH is comparable to the mean value of 27 fmol per mg tissue protein found by Wagner et al. (1975) in 11 samples, taking into account that those investigators corrected their values by subtracting from the total cytosol protein the amount which is approximately due to plasma contamination. This correction leads principally to higher values. Twelve fmol per mg protein is also comparable to about 19 fmol per mg prostate cytosol protein of uncastrated rats, while in 24 h castrated rats about 170 fmol are present (Krieg et al. 1976). This low value in the human is best explained by occupation of the vast majority of the receptor sites by endogenous 5α-DHT and T, as we found for the first time a significant negative correlation between endogenous tissue 5α-DHT and T concentration and assayable receptor sites in the human BPH. That most of the cytosolic receptor might be occupied, has also been assumed by Bonne & Raynaud (1976) who found in the BPH cytosol by an exchange labelling technique with tritiated methyltrienolone (R 1881) 120 fmol per mg protein, and by Rosen et al. (1975) who have measured by radioimmunoassay in one case the endogenous 5α-DHT in the 8 S region of the glycerol gradients and calculated that more than 90% of the receptor binding sites might be occupied by this steroid. In the muscle we found on average 3.6 fmol 5α-DHT-binding sites, which is significantly less than in the BPH, but higher than the 0.8 fmol/mg protein obtained on average in the skeletal muscle cytosol of the uncastrated rat (Krieg 1976). Whether the SHBG peak influences slightly the receptor peak, especially when only small peaks are present as in the muscle, cannot be ruled out. It should, however, be stressed that there is neither a correlation between the receptor
concentration in the muscle or BPH and the respective SHBG values, nor between the receptor concentrations in the BPH and muscle.

Measurement of the endogenous androgen levels shows no correlation between the various tissue and respective plasma values indicating that the tissues themselves regulate their final androgen concentration. Furthermore it is obvious that not only in the BPH is the $5\alpha$-DHT/T quotient far greater than in plasma, which has been found by various investigators (Siiteri & Wilson 1970; Farnsworth & Brown 1976; Albert et al. 1976; Habib et al. 1976) but also in the muscle. The latter finding reflects the significant interaction of $5\alpha$-DHT with the three androphiles in the muscle.

Because extensive metabolism of $5\alpha$-DHT into $5\alpha$-androstanediols, which are not bound to the rat prostate receptor (Krieg et al. 1975), will mislead the interpretation of quantitative binding studies, we evaluated how much of the added tritiated $5\alpha$-DHT has been converted to other androgens under the incubation conditions used for the binding studies. From the rat it is known (Michel & Baulieu 1975; Verhoeven et al. 1975; Bonne & Raynaud 1976; Krieg et al. 1976) that even at $0^\circ$C within 2 h $5\alpha$-DHT is extensively converted to the $5\alpha$-androstanediols. As shown in Table 2, compared to the rat prostate, in the BPH cytosol after a 20–24 h incubation of the homogenate, only negligible amounts of $5\alpha$-androstanediols are found, whereas 80% remains as $5\alpha$-DHT. This finding might be of biological importance insofar as it indicates, although rather in a preliminary manner that the accumulation of $5\alpha$-DHT is not necessarily due only to receptor proteins but also to a possible decrease in the activity of the $3\alpha$-hydroxy-steroid-dehydrogenase. Therefore it might be, and some very recent reports underline this (Bruchovsky 1976; Geller et al. 1976), that a better understanding of the aetiology of the BPH will be found by looking for acquired errors of metabolism at the cellular level than by receptor studies or alterations in blood parameters like $5\alpha$-DHT or SHBG.

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