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A COMPARATIVE STUDY OF BINDING, METABOLISM AND ENDOGENOUS LEVELS OF ANDROGENS IN NORMAL, HYPERPLASTIC AND CARCINOMATOUS HUMAN PROSTATE

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SUMMARY

14 Prostatic carcinomas (PCA), 14 benign prostatic hyperplasia (BPH) and 7 normal prostates (NPR) were investigated with respect to their 5α-dihydrotestosterone (DHT)-binding, *in vitro* metabolism of testosterone (T), DHT and 5α-androstane-3α,17β-diol (3α-diol), and tissue concentration of T, DHT and 3α-diol. Binding studies were performed by agar gel electrophoresis, metabolism was investigated by thin-layer chromatography, and the endogenous androgen concentrations were measured by radioimmunoassay. The main results were as follows: 1. Qualitatively no DHT-binding differences were found between PCA and BPH. 2. In PCA, a 2.5 times higher receptor concentration was found relative to BPH. 3. For both PCA and BPH, a positive correlation was found between plasma and cytosol sex hormone-binding globulin concentration. 4. Compared with BPH and NPR, the conversion T to DHT and of DHT to 5α-androstanediols was significantly decreased in PCA. 5. Compared with NPR, in BPH the ratio 5α-reduction to 3α(β)-reduction was significantly higher, mainly due to a reduced 3α(β)-reduction. 6. In NPR, compared with BPH and PCA, the ratio 5α-reduction to 3α(β)-reduction was significantly lower, mainly due to a relatively high 3α(β)-reduction. 7. The T concentration was significantly higher in PCA than in BPH or NPR. 8. The DHT concentration was significantly lower in NPR than in PCA or BPH; in this respect there were no significant differences between PCA and BPH. 9. The 3α-diol concentration was significantly higher in NPR than in BPH. High 3α-diol levels were also found in PCA. In conclusion, the NPR seems to be protected against excessive accumulation of T and DHT due to the shift of metabolism to the 5α-androstanediols. Compared with NPR, in PCA and BPH the androgen metabolism is shifted significantly to T and DHT, both of which have, in contrast to the 5α-androstanediols, a high affinity for the cytosol androgen receptor.

INTRODUCTION

Possible differences between human normal prostate (NPR), prostatic carcinoma (PCA) and benign prostatic hyperplasia (BPH), concerning their "androgen status" at the cellular level, could lead to further ideas about the as yet completely unknown role which androgens may play with respect to the pathogenesis and hormone responsiveness of prostatic tumors. We have therefore compared androgen binding, *in vitro* metabolism and endogenous tissue concentration of androgens in PCA, BPH and NPR. The data found indicate that the NPR seems to be protected against excessive accumulation of testosterone (T) and 5α-dihydrotestosterone (DHT) due to the shift of androgen metabolism to the 5α-androstanediols,† while in BPH and especially in PCA the androgen metabolism is shifted to T and/or DHT, both of which display a high affinity for the cytosol androgen receptor.

† The following abbreviations and terms are used: 5α-androstanediols (DIOL) = 5α-androstane-3α,17β-diol + 5α-androstane-3β,17β-diol; $NADPH_2$ = reduced form of nicotinamide-adenine dinucleotide phosphate; 5α-reductase = 3-oxo-5α-steroid Δ^4-dehydrogenase; 5α-reduction = amount of DHT plus 5α-androstanediols found after DHT incubation; 3α(β)-reduction = amount of 5α-androstanediols found after DHT incubation.

MATERIALS AND METHODS

Chemicals

All chemicals used were purchased from companies mentioned in earlier publications [1, 2].

Tissue

The origin and handling of the PCA as well as BPH tissue have been described in detail earlier [1, 2]. The number of samples investigated is stated in the Results section. The 7 NPR were obtained from the Institute of Forensic Medicine. The men were in the age range 19–43 years (mean: 33), and the time span between death of the men and tissue processing lasted up to 6 h. The absence of any pathological alteration was verified by histological examination.

Binding studies

Qualitative and quantitative studies were performed as described recently [1, 2] using agar gel electrophoresis according to Wagner[3]. Homogenates of BPH and NPR were incubated in the way described earlier for BPH [1]. Incubation of the PCA tissue was slightly modified owing to the lack of sufficient material and to standardization of the procedure according to the European Group of Prostatic Cancer Research. Details have been described elsewhere [2].

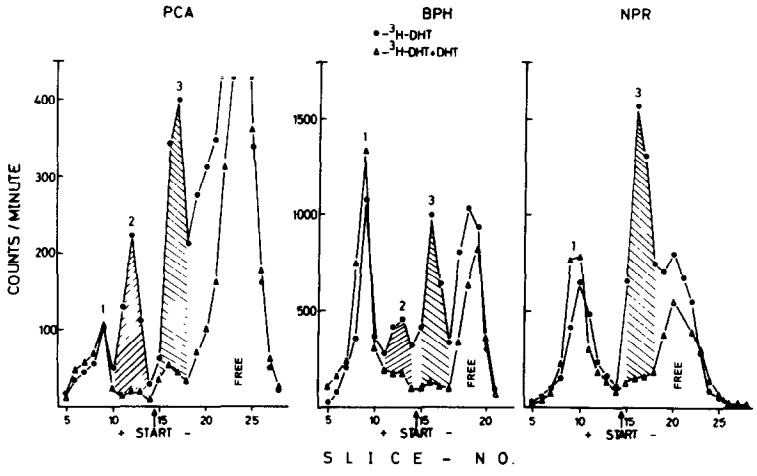

Fig. 1. *In vitro* binding of tritiated DHT in the 100,000 *g* cytosol of human prostatic carcinoma (PCA), benign prostatic hyperplasia (BPH) and normal prostate (NPR). Organ homogenates or cytosols were incubated with tritiated DHT alone (●) or in the presence of an excess of unlabelled DHT (▲). Before binding was analyzed by agar gel electrophoresis, the cytosol was treated with charcoal to remove excess of unbound steroids. Cytosol (40 μl) was applied between slices 14 and 15. The anode was to the left and the cathode to the right of the start. Electrophoresis: 90 min at 10 V/cm. Temperature within the gel <5°C. Radioactivity was measured in CPM/slice, each slice being 3 mm wide. The shaded areas of peaks 2 and 3 indicated the amounts of DHT specifically bound to the receptor protein and to the sex hormone-binding globulin, respectively.

Sex hormone-binding globulin (SHBG) binding capacity in plasma was measured by the method of Dennis *et al.*[4].

Metabolic studies

Metabolic studies were performed on PCA, BPH and NPR in the way described for PCA and BPH in an earlier publication [2]. For clarity some experimental details are outlined in the Results section.

Determination of endogenous androgen tissue levels

The tissue concentrations were measured by radioimmunoassay after thorough steroid extraction. Details for measuring T, DHT and 5α-androstane-3α,17β-diol (3α-diol) were reported elsewhere [1, 5]. The handling was exactly the same for PCA, BPH and NPR.

Miscellaneous

Further details for measuring enzyme and heat sensitivity of the binding proteins, cytosol protein concentration, and radioactivity were published earlier [1]. The statistical significance of differences between means was determined with the Wilcoxon–Mann–Whitney test ("*U*-test") and the correlation between plasma and cytosol SHBG concentrations with the Spearman rank correlation coefficient.

RESULTS

Binding studies

Figure 1 shows typical DHT-binding patterns obtained for human organ cytosols by agar gel electrophoresis. For cytosols obtained from 14 samples of PCA (left panel), three charcoal resistant binding peaks were regularly found and small amounts of unbound radioactivity were also detected in slices 18–28. While peaks 2 and 3 decreased in the presence of an excess of unlabelled DHT (shaded areas), peak 1 remained unaffected. From a series of binding studies [1, 2] we know that peak 2 represents the cytosol androgen receptor and peak 3 the plasma SHBG. In 12 out of 14 BPH cytosols (middle panel) a qualitatively identical binding profile to that found in PCA cytosols could be demonstrated; in two cytosols peak 2 was absent. In the seven NPR cytosols (right panel), only peaks 1 and 3 were regularly assayable, while receptor-bound DHT (peak 2) could not be detected. When calculating the assayable cytosol receptor (peak 2) and SHGB (peak 3) concentrations in PCA and BPH from the amount of tritiated DHT displaced by an excess of unlabelled DHT, as indicated by the shaded areas of Fig. 1, it was found (Table 1) that PCA cytosol had significantly ($P < 0.04$) higher assayable receptor and SHBG concentrations than BPH. Furthermore (Fig. 2), if the PCA are divided into a group consisting exclusively of adenocar-

Table 1. Receptor and sex hormone-binding globulin (SHBG) concentrations in the 100,000 *g* cytosol of human prostatic carcinoma (PCA), benign prostatic hyperplasia (BPH) and normal prostate (NPR)

		Receptor conc (fmol/mg protein)		SHBG conc (fmol/mg protein)	
	n	Mean	Range	Mean	Range
PCA	14	30.9*	6.0–93.5	93.2†	35.7–225
BPH	14	12.3	0–37.8	39.9	18.1–84.7
NPR	7	Not assayable		Not determined	

* Significantly different from BPH, $P < 0.04$.
† Significantly different from BPH, $P < 0.01$.

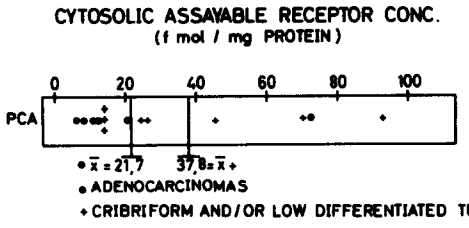

Fig. 2. Assayable cytosol receptor concentrations found in the 100,000 *g* preparations from 14 prostatic carcinomas (PCA). The PCA were divided in two groups: ●-group consisting exclusively of adenocarcinoma; +-group showing predominantly a cribriform or cribriform and low differentiated tumour pattern. Differences between the means were statistically significant with $P < 0.05$.

cinomas and a group consisting predominantly of a cribriform and/or low differentiated tumour type, the latter have a significantly ($P < 0.05$) higher mean receptor concentration. For SHBG, a significant difference between the PCA groups could not be demonstrated. In Fig. 3 the statistically significant Spearman rank correlations between corresponding cytosol and plasma SHBG concentrations are shown; the higher the assayable cytosol concentration of SHBG, the higher also the corresponding plasma SHBG concentration.

Metabolic studies

Table 2 summarizes the main metabolites found after incubation of PCA, BPH and NPR homogenates with either tritiated T, DHT or 3α-diol. Four points seem remarkable: 1. In PCA, significantly more of the added T remained unmetabolized than in BPH and NPR, and formation of DHT plus 5α-androstanediols was approximately 15% lower. However, the amount of DHT formed was as high as in BPH or NPR. 2. In PCA, significantly more of the added DHT remained unmetabolized than in BPH or NPR, and formation of 5α-androstanediols was significantly lower. 3. For all three organs, there was very little conversion of added 3α-diol to DHT. 4. Comparing BPH and NPR, the latter converted much more of the added DHT to 5α-androstanediols, although the difference is slightly above the 5% confidence limits.

Figure 4 shows a further difference between BPH and NPR. In BPH, in the absence of $NADPH_2$ in the incubation medium, a measurable amount of T is converted to DHT plus 5α-androstanediols, but in NPR this conversion stops dramatically; this is shown by the shaded bars and the difference is significant. In contrast, after DHT incubation in the absence of $NADPH_2$, the conversion of DHT to the 5α-androstanediols stops nearly completely and identically in both homogenates. In Table 3, the data are compiled according to relative metabolic activities. Besides the significantly higher ratios in PCA compared with BPH and NPR, which confirm the significant differences in metabolism mentioned above, it seems remarkable that the ratio 5α-reduction to 3α(β)-reduction is significantly lower in NPR than in BPH.

Endogenous androgen tissue levels

Table 4 summarizes the endogenous androgen concentrations found in the PCA, BPH and NPR homogenates. The following differences are statistically significant: 1. The high T concentration in PCA compared with BPH and NPR. 2. The low DHT concentration in NPR compared with PCA and BPH. 3. The low 3α-diol concentration in BPH compared with PCA and NPR.

DISCUSSION

This comparative study clearly demonstrates statistically significant quantitative differences between human PCA, BPH and NPR with respect to their androgen binding, *in vitro* metabolism and endogenous androgen concentrations.

Concerning androgen binding in BPH four points should be discussed:

1. We have characterized and quantified the binding of tritiated DHT to the cytosol androgen receptor in BPH very carefully [1] and could find no qualitat-

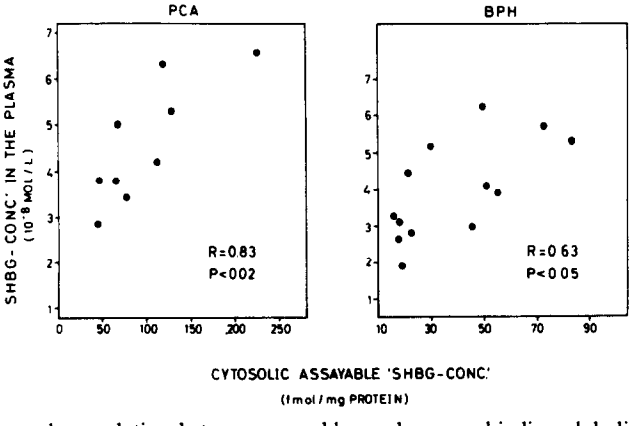

Fig. 3. Spearman rank correlation between assayable sex hormone-binding globulin (SHBG) concentration in plasma and in the corresponding prostatic carcinoma (PCA) or benign prostatic hyperplasia (BPH).

Table 2. Main metabolites (mean ± SD) obtained by thin layer chromatography of homogenates of human prostatic carcinoma (PCA), benign prostatic hyperplasia (BPH), and normal prostate (NPR), which had been diluted 1:2 with buffer and incubated with tritiated testosterone (TESTO), 5α-dihydrotestosterone (DHT) or 5α-androstane-3α,17β-diol (3α-DIOL) for 30 min at 37°C in the presence of $NADPH_2$ (for TESTO and DHT) or NADP (for 3α-DIOL)

Metabolites found (pmol)		Tritiated steroids added per g homogenate TESTO (15.4 ± 2.4 pmol)	DHT (16.8 ± 2.5 pmol)	3α-DIOL (23.2 ± 1.6 pmol)
PCA	TESTO	5.1 ± 2.8* (10)	<0.5 (5)	<0.5 (4)
	DHT	6.6 ± 3.2 (10)	13 ± 2.5 (5)	1.8 ± 0.2 (4)
	DIOL	3.3 ± 2.4† (10)	3.9 ± 2.1† (5)	20 ± 0.6 (4)
BPH	TESTO	0.8 ± 0.5 (16)	<0.5 (16)	<0.5 (6)
	DHT	5.5 ± 1.7 (16)	6.8 ± 1.9 (16)	1.2 ± 0.4 (6)
	DIOL	6.2 ± 2.7 (16)	7.3 ± 3.0 (16)	21 ± 1.3 (6)
NPR	TESTO	1.2 ± 0.8 (7)	<0.5 (7)	<0.5 (2)
	DHT	4.3 ± 1.7 (7)	5.5 ± 2.7 (7)	<0.5 (2)
	DIOL	7.3 ± 2.5 (7)	10.5 ± 3.6 (7)	22.6 ± 1.8 (2)

DIOL = 5α-androstane-3α,17β-diol + 5α-androstane-3β,17β-diol.
* Significantly different from BPH and NPR, $P < 0.01$.
† Significantly different from BPH and NPR, $P < 0.05$.

ive differences between these and similar studies with rat prostate [6]. Such species similarities have been observed by others [7–14], and review of the literature [7–25] shows that DHT-binding data in human BPH generally display characteristics identical to those found in accessory sexual glands of experimental animals. However, as also emphasized very recently by Wagner[26], some doubts still exist as to whether, in studies in which gel column chromatography, density gradient ultracentrifugation or charcoal adsorption techniques were applied to characterize the androgen binding in crude BPH cytosols, a precise discrimination between DHT-binding to the cytosol receptor and to the cytosol contaminating plasma SHBG was really possible. With crude cytosol from BPH, using the ultracentrifugation technique, we were unable to discriminate between the SHBG and the cytosol receptor [34]. Mobbs *et al.*[27] in an earlier paper demonstrated, using a charcoal adsorption technique, that the DHT-binding in crude human tissue cytosols is probably more related to SHBG than to an androgen receptor protein. Various authors [10, 16–18, 23, 24, 28, 29] circumvented this problem by using tritiated methyltrienolone (R1881), which does not bind to the SHBG [30], as binding marker. However, it has become recognized [16, 24, 28, 29, 31] that in BPH cytosol, R1881 binds with high affinity to a binding protein which resembles the progestin receptor of the uterus, and very recently Gustafsson *et al.*[32], using a new synthetic progestin (R5020), actually characterized such a progestin receptor in both BPH and PCA cytosols. One approach might overcome this problem of interference between androgen and progestin binding sites, namely the saturation of the progestin binding component with unlabelled R5020, in order to prevent tritiated R1881 interacting with such sites. Using this procedure Sirett *et al.*[18] found only very small amounts of unoccupied androgen receptor sites (about 4 fmol/mg cytosol protein). The measurement of occupied specific androgen receptor sites, however, seems possible using tritiated R1881 [10, 17, 24], probably due to the heat denaturation of the interfering progestin component in the course of the exchange procedure; their concentration are 5–10 fold higher than the unoccupied. We believe that the use of tritiated DHT in combination with agar gel elec-

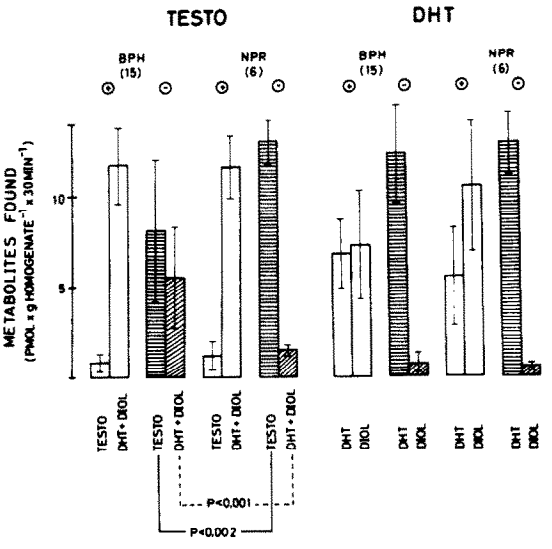

Fig. 4. $NADPH_2$-dependency of the *in vitro* metabolism of testosterone (TESTO) and 5α-dihydrotestosterone (DHT) in human benign prostatic hyperplasia (BPH) and normal prostate (NPR) as analyzed by thin layer chromatography. Homogenates (1:2 in buffer) were incubated for 30 min at 37°C with tritiated TESTO (15.4 ± 2.4 pmol/g homogenate) or DHT (16.8 ± 2.5 pmol/g homogenate) in the presence (⊕) or absence (⊖) of 30 μmol/g homogenate $NADPH_2$. The conversion of TESTO to DHT + DIOL (DIOL = 5α-androstane-3α,17β-diol plus 5α-androstane-3β,17β-diol) in the absence of $NADPH_2$ was significantly higher in BPH than in NPR.

Table 3. Relative metabolic activities found in human prostatic carcinoma (PCA), benign prostatic hyperplasia (BPH) and normal prostate (NPR) after incubation of the organ homogenates with tritiated testosterone (TESTO) or 5α-dihydrotestosterone (DHT) for 30 min at 37°C in the presence of 30 μmol/g homogenate $NADPH_2$

	TESTO / 5α-Reduction	5α-Reduction / 3α(β)-Reduction	DHT / DIOL
PCA	0.62 ± 0.42* (10)	3.1 ± 1.8 (5)	4.3 ± 2.4† (5)
BPH	0.07 ± 0.04 (16)	2.0 ± 1.4 (16)	1.4 ± 1.4 (16)
NPR	0.10 ± 0.08 (7)	1.2 ± 0.4‡ (7)	0.7 ± 0.5 (7)

DIOL = 5α-androstane-3α,17β-diol + 5α-androstane-3β,17β-diol.
5α-Reduction = DHT + DIOL found after TESTO-incubation.
3α(β)-Reduction = DIOL found after DHT-incubation.
DHT/DIOL = DHT and DIOL found after DHT-incubation.
* Significantly different from BPH and NPR, $P < 0.01$.
† Significantly different from BPH and NPR, $P < 0.02$.
‡ Significantly different from BPH and PCA, $P < 0.05$.

trophoresis, which discriminates precisely between receptor protein and SHBG, allows a reliable comparative quantitative assay of the cytosol androgen receptor protein in various human prostates. Furthermore, the progesterone receptor has only a very low affinity for DHT, as shown by competition studies in calf mammary gland [26].

2. The published cytosol androgen receptor concentrations in BPH [1, 10, 16–18, 21–24, 26, 33] show great variation within a series; there are also large differences between the mean concentrations obtained by various authors, due, at least partly, to quite different methodological approaches. The great variation of the values within one series is mainly due to the individual biological status of the patients. In this respect, the endogenous androgen concentrations and the tissue composition must be carefully considered. Concerning endogenous androgen concentration we could demonstrate [1] that there is a significant negative correlation between the androgen concentration in the tissue and the assayable receptor sites, that is to say the higher the tissular androgen concentration the lower the assayable cytosol androgen receptor concentration. As regards the variation in tissue composition, it is well known that BPH consists of a mixture of epithelial and stromal elements; Cowan *et al.* could demonstrate striking differences in R1881-binding [31] and DHT-metabolism [35] in these two elements.

3. Our incubation procedure (0°C, 2–24 h, high excess of tritiated DHT) does not allow discrimination between occupied and unoccupied binding sites, therefore we used the term "assayable". Whether this parameter has any biological significance, in terms of hormone responsiveness of the tissue, remains as yet unclear. However, the same holds true if the cytosol binding sites are measured as occupied and unoccupied, particularly since in a series of 15 analyses the ratio occupied to unoccupied was constant in all but two cases [17]. At present, as far as the biological significance of the cytosol androgen receptor in BPH is concerned, it may only be tentatively concluded that its role is similar to the known action of the androgen receptor of the rat prostate [36], *i.e.* the BPH cytosol displays a receptor apparatus which allows the translocation of DHT into the nucleus. This conclusion is substantiated by the very recent findings of several authors [8, 15, 16, 18, 20] who have reported the presence in BPH of specific nuclear binding sites which have similar characteristics to those found in rat prostate nuclei.

4. Finally, concerning the hormones which might be involved in the development of BPH, oestrogens must also be considered in addition to androgens and

Table 4. Endogenous levels of testosterone (TESTO), 5α-dihydrotestosterone (DHT) and 5α-androstane-3α,17β-diol (3α-DIOL) in homogenates of human prostatic carcinoma (PCA), benign prostatic hyperplasia (BPH) and normal prostate (NPR) as measured by radioimmunoassay

	Endogenous androgen level (ng/g tissue)		
	PCA	BPH	NPR
TESTO	1.2 ± 0.8* (7)	0.3 ± 0.1 (11)	0.2 ± 0.1 (7)
DHT	3.9 ± 0.3 (3)	4.5 ± 1.4 (14)	1.6† ± 1.0 (6)
3α-DIOL	1.6 ± 0.8‡ (7)	0.6 ± 0.7 (14)	1.7 ± 0.3‡ (3)

* Significantly different from BPH and NPR, $P < 0.002$.
† Significantly different from PCA and BPH, $P < 0.05$.
‡ Significantly different from BPH, $P < 0.05$.

progestins. We could demonstrate significant competition between triated DHT and unlabelled oestradiol for the receptor sites [1], and furthermore, using tritiated oestradiol, various authors have described a specific binding [33, 37–39] which seems to be able to translocate the oestradiol into the BPH nuclei [39].

Compared with binding studies in BPH, fewer data have been published concerning PCA, mainly due to the difficulty in obtaining sufficient tissue in which carcinoma had been confirmed histologically, as we have stressed in a recent paper [2]. Characterization of the cytosol androgen binding therefore remains fragmentary. From the investigations so far however, the androgen receptor of PCA seems to be qualitatively identical to those of BPH and rat prostate. The receptor quantitation performed by us [2] and others [13, 23, 26, 33] revealed a wide range of values. This is also known to be the case for the oestrogen receptor in mammary breast cancer and it was suggested that cellular heterogeneity and endogenous steroid levels might be the main factors responsible for the variation [40]. As discussed for BPH, it is too early to make predictions with respect to hormone responsiveness of this malignant tumour on the basis of quantitation of cytosolic androgen receptor sites, although Mobbs *et al.*[13] and Wagner[26] have made some suggestions. However, it seems remarkable that we found that in PCA the assayable cytosol receptor concentrations were statistically higher than in BPH. This finding might be important since Lieskovsky and Bruchovsky[15] found more nuclear binding sites in PCA than in BPH or NPR. Furthermore, the higher cytosol receptor concentration in cribriform and/or poorly differentiated prostatic tumours is evident and has been confirmed by Snochowsky *et al.*[23]. In their series of 8 BPH and 3 PCA specimens, the highest receptor concentration was found in a punch biopsy of a cancer which had a low degree of differentiation. Further investigations at the cellular level will give an answer as to whether this difference in cytosol androgen receptor concentration between low and highly differentiated carcinomas is reflected by the same difference in the nuclei. Finally, besides androgens, oestrogens and progestins must also be regarded as steroid hormones influencing the growth and hormone responsiveness of the carcinomas. Using tritiated oestradiol, we [2] and others [26, 33, 41] found significant amounts bound to a cytosol protein of low binding capacity. We observed this oestradiol-binding in each of the 7 cytosols analyzed; the binding of oestradiol was however, significantly lower than DHT-binding, as shown in parallel assays. Wagner[26], on the other hand, found specimens which either bind both steroids or which bind only DHT or oestradiol. This may indicate that two different receptor proteins are involved. Concerning progestin-binding, Gustafsson *et al.*[32] have very recently reported specific binding of tritiated R5020 in three cytosols of human prostatic carcinomas. We have incubated aliquots of 4 carcinomas either with tritiated DHT or with tritiated R1881, and although the affinity of R1881 for the progesterone receptor was evident as discussed above, displacement of tritiated R1881 with either R1881 or DHT revealed identical amounts of limited binding sites; these amounts were also identical to the number of binding sites found with tritiated DHT. Thus, in our specimens, a progestin binding component seems to be absent.

Our negative results in unequivocally demonstrating cytosol androgen receptors in 7 NPR must be discussed primarily in the light of the time span between death of the young men and tissue processing, which could be up to 6 h. Simulating this time span using rats, the number of assayable binding sites decreased dramatically in castrated and uncastrated animals (our unpublished data). Thus in humans the very labile cytosol receptor protein will probably be extensively denaturated during this time span, so that the receptor becomes undetectable. Contrary to this assumption is the finding of Davies and Griffiths[8] who demonstrated cytosol androgen binding by sucrose gradient ultracentrifugation in one normal prostate specimen removed from a cadaver within 12 h of death. Mobbs *et al.*[74] also found a very small amount of cyproterone acetate-inhibitable androgen-binding in three NPR from cadavers of subjects who had died within 6–24 h of tissue analysis. Further cytosol binding studies were performed in NPR specimens obtained by needle biopsy or during the course of an open operation [21, 26]. Both groups demonstrated androgen binding sites in varying amounts, although the data of Shimazaki *et al.*[21] seem to be overestimated due to interference by binding of DHT to SHBG. Therefore, considering these data, the presence in NPR cytosol of an androgen receptor, which, as far as has been investigated does not show striking differences between the receptors of human BPH, PCA or rat prostate, can tentatively be assumed. This receptor is probably translocated into the nucleus, since Lieskovsky and Bruchovsky[15] demonstrated significant amounts of androgen receptor molecules in NPR nuclei; the amounts were, however, statistically lower than found in BPH or PCA nuclei. Further quantitative binding studies on NPR, PCA and BPH are needed to confirm these differences in receptor content.

Besides androgen receptor studies, there has been much interest in androgen metabolism in human BPH, PCA and NPR since 1963, when Farnsworth and Brown[42] first described the formation of DHT as the main metabolite after incubation of human BPH tissue slices with tritiated T. This finding was later confirmed by *in vivo* studies of Becker *et al.*[43] and by Pike *et al.*[44]. The first report concerning quantitative differences between BPH, PCA and NPR in the conversion of T to DHT was published by Shimazaki *et al.*[45], who found a ranking of meta-

bolic activity in the order NPR > BPH > PCA. Thereafter numerous publications dealt with the *in vitro* metabolism of T in these tissues [11, 35, 46–57], as well as the extent to which it can be influenced by oestrogens, progestins and antiandrogens [45, 57–66]. Without going into detail, only the decreased androgen metabolism in carcinomatous tissue was unequivocal. However, the number of specimens investigated was often very low and thus a statistical evaluation of differences between the androgen metabolism in BPH, PCA and NPR was not possible.

Therefore, our study can only be compared with a few partially similar studies recently carried out by others [46, 50, 51, 53–56]. Concerning the PCA, irrespective of the degree of differentiation of the tissue, a statistically lower 5α-reductase activity than in BPH or NPR may be assumed. This is in accordance with findings of others [11, 46, 55, 56], though Morfin *et al.*[54] and Jenkins and McCaffery[62] found decreased formation of 5α-reduced metabolites only in poorly differentiated carcinoma. Furthermore we demonstrated for the first time a statistically lower 3α(β)-hydroxysteroid dehydrogenase activity in PCA as compared with BPH and NPR. This drastically decreased conversion of DHT to 5α-androstanediols leads to a relatively higher 5α-reductase than 3α(β)-hydroxysteroid dehydrogenase activity in the PCA, thus shifting the androgen metabolites to T as well as DHT. Concerning the comparison of androgen metabolism in BPH and in NPR, Bruchovsky and Lieskovsky[46] demonstrated a statistically higher 5α-reductase and lower 3α(β)-hydroxysteroid dehydrogenase activity in BPH. This is in contrast to the findings of Jacobi and Wilson[51], who found just the opposite for 3α(β)-hydroxysteroid dehydrogenase activity, that is significantly higher activity in BPH than in NPR. When considering the absolute amounts of metabolites formed after incubation of BPH and NPR with T, DHT or 3α-diol, we could not demonstrate statistically significant differences, although the trend towards higher 5α-reductase and lower 3α(β)-hydroxysteroid dehydrogenase activity in BPH relative to NPR was evident. The same is also apparent from the data of Djöseland *et al.*[56]. However, other groups [50, 62] could not find any difference.

Consideration of the ratio of 5α-reductase to 3α(β)-hydroxysteroid dehydrogenase activity showed that in our series, as in the series of Bruchovsky and Lieskovsky[46], it was statistically higher in BPH than in NPR, indicating that the T metabolism is shifted towards DHT in BPH but in NPR towards 5α-androstanediols. Further proof of a higher 5α-reductase activity in BPH compared with NPR is given by the significantly different $NADPH_2$ dependency of the T conversion to DHT. While in BPH, in the absence of cofactor supplementation during incubation, a significant 5α-reduction of T occurs, in NPR this reduction is nearly completely stopped. If it is assumed that the metabolism in organ homogenates without cofactor supplementation is a better reflection of the actual *in vivo* condition, this finding might indicate that the 5α-reductase is better supplied with reduced cofactors in BPH than in NPR. Bearing in mind the longer time span up to the final tissue processing of NPR as compared with BPH, one might argue that this is the reason for the differences. However, Morfin *et al.*[53] could demonstrate the same difference when NPR and BPH specimens were both processed immediately. Furthermore, we could not find such differences between BPH and NPR with respect to 3α(β)-hydroxysteroid dehydrogenase activity.

If it is assumed that the *in vivo* metabolism at the cellular level of the human prostate regulates the endogenous androgen concentrations at least partly in these tissues, then from our metabolic data the following predictions can be made. In PCA relatively high levels of T and DHT and low levels of 3α-diol, in BPH relatively high levels of DHT and low levels of T and 3α-diol, and finally in NPR relatively high levels of 3α-diol and low levels of T and DHT should be found. Concerning the T concentration, in PCA the levels were actually statistically higher than in BPH or NPR. This finding has been convincingly confirmed by Habib *et al.*[67] and with less statistical significance by Farnsworth and Brown[68], while Albert *et al.*[69] found a higher mean T level in BPH than PCA. The equally low concentration in BPH and NPR has also been reported by Siiteri and Wilson[49]. With respect to the DHT tissue concentrations, it has been known since 1970 [49] that BPH has significantly higher levels than NPR, a finding confirmed later by us [70] and Geller *et al.*[71]. The significantly higher DHT level in PCA as compared with NPR was, however, reported only very recently by Geller *et al.*[72]. Conflicting results were published when considering comparative DHT values in BPH and PCA. While Geller *et al.*[72] and Habib *et al.*[67] found significantly lower DHT levels in PCA than in BPH, which was confirmed at the nuclear level by Bruchovsky *et al.*[73], Farnsworth and Brown[68] reported just the opposite. We could not find significant differences between PCA and BPH in this respect, consistent with our metabolic findings. As regards the 3α-diol concentrations, our finding of significantly higher levels in NPR than in BPH was in accordance with a report of Geller *et al.*[71]. For PCA, the unexpectedly high 3α-diol levels found by us, and the even higher concentrations reported by Albert *et al.*[69] and Farnsworth and Brown[68], cannot, at the moment, be explained by our metabolic studies.

With only one exception our predictions of endogenous tissue concentrations, from the *in vitro* metabolic data, were fulfilled, thus indicating that the *in vitro* metabolism might reflect the *in vivo* situation. Finally, taking together these comparative data on androgen binding, *in vitro* metabolism and androgen concentrations at the cellular level for BPH, PCA and

NPR, the following may be stated:

1. The NPR seems to be protected against excessive accumulation of T and DHT due to the shift of androgen metabolism to the 5α-androstanediols, which are, in general, not bound with high affinity to the cytosol androgen receptor.

2. Compared with NPR, in PCA and BPH the androgen metabolism is shifted significantly to T and DHT, both of which have a high affinity for the cytosol androgen receptor.

3. It is attractive to speculate that this acquired error in androgen metabolism could play an important role in the development and hormone responsiveness of BPH and PCA.

4. Further studies on androgen binding and metabolism must be correlated with the histological type of the tumour and also extended to the nuclear level of these tissues. Then predictions with respect to hormone responsiveness of the individual tumours might be possible in the future.

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