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ANDROGENS AND ANDROGEN-RECEPTORS IN PROSTATE TISSUE FROM PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA: EFFECTS OF CYPROTERONE ACETATE

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Summary—Testosterone, 5α-dihydrotestosterone and cyproterone acetate (CPA) were estimated in samples of prostate tissue, obtained from benign prostatic hyperplasia (BPH) patients who were or were not pretreated with CPA. Furthermore, these steroids were estimated in various fractions of the BPH tissue, and the number of nuclear androgen-receptor sites was determined. CPA-treatment caused a 4-fold, significant suppression of 5α-dihydrotestosterone levels in total prostate tissue and its subfractions, without affecting testosterone levels or the androgen-receptor contents of the nuclear extracts. Nuclear concentrations of CPA were twice as high as those of 5α-dihydrotestosterone. It is concluded that effects of CPA may have been caused through a combination of the following mechanisms: (1) suppression of peripheral androgen levels; (2) competition with androgens for (nuclear) androgen-receptors; and (3) suppression of prostatic 5α-reductase.

INTRODUCTION

Cyproterone acetate (CPA) is a synthetic progestagen, which can suppress pituitary gonadotrophin secretion [1]. In addition, this steroid has anti-androgenic properties, as evidenced by competitive inhibition of the formation of androgen-receptor complexes in androgen target tissues [2, 3], resulting in decreased nuclear retention of 5α-dihydrotestosterone (DHT)-receptor complexes [4–6] and finally in reduced target organ weights [1].

Most of the above-mentioned studies were performed in *in vitro* systems. Studies on the effect of CPA on androgen levels in prostatic tissue are scarce, and it is difficult to extrapolate the results of these investigations in animal models to the human situation, e.g. because in animal studies care was usually taken to exclude the effect of the CPA-treatment on peripheral androgen levels by simultaneous administration of testosterone [7]. The aim of the present study was to obtain more information on the mechanism of action of CPA in men by measuring levels of androgens and androgen-receptors in prostate tissue, obtained by trans-urethral resection of this tissue from patients with benign prostatic hyperplasia (BPH), who had or had not been treated with CPA before the operation.

MATERIALS AND METHODS

Patients

All patients included in the study had BPH, as evidenced by the following parameters: (1) presence of symptoms typical for the disease; (2) enlarged prostate determined by rectal examination; (3) a free flow rate of urine of < 10 ml/s at a voided volume of 100 ml or more; and (4) presence of residual urine in the bladder after voiding. All patients gave informed consent to participate in the study.

The control group consisted of 11 untreated patients; 6 of these patients underwent trans-urethral resection (TUR) of the prostatic tissue by the cold punch technique. In addition, BPH tissue from 4 patients, who had been operated on earlier without pretreatment, was investigated. Twelve patients were treated with CPA (two doses of 100 mg daily) for a period of at least 6 weeks, 7 of these patients were operated.

Treated patients were investigated on a two-weekly basis for evaluation of prostate size, volume of residual urine, free urinary flow rate

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and plasma hormone levels, until operation by TUR took place. Because of decreased prostate size, not all treated patients were operated. This experimental protocol was part of a clinical study which was recently reported [8]. In the control patients, investigation of urodynamic functions and plasma hormone levels was only performed once.

Estimation of plasma and tissue hormones

Plasma testosterone and DHT were extracted, separated using silica columns [9] and estimated separately by radioimmunoassay, using the antiserum described earlier [10]. CPA was also estimated by radioimmunoassay, using an antiserum provided by Schering (Berlin). Cyproterone cross-reacts for 25% in this assay. Plasma LH was estimated by radioimmunoassay [11] and SHBG by binding of [^{3}H]-DHT [12]. Part of the prostatic tissue was dissolved in 1 N NaOH (1 h, 60°C) for direct estimation of protein [13], DNA [14], testosterone, DHT and when applicable, CPA. Testosterone and DHT were estimated after addition of ^{3}H-labelled recovery markers and extraction of the dissolved tissue, and chromatography on the above mentioned silica columns. CPA was estimated in separate volumes of the homogenates.

Nuclear extracts were prepared from separate aliquots of BPH tissue as described by Blankenstein *et al.* [15]. Briefly, tissue was thawed on ice and minced with scissors. Five hundred milligram portions of minced tissue were homogenized between two layers of stainless steel grid in 50 mmol/l Tris–HCl buffer, pH 7.5, containing 2.5 mmol/l KCl, 5 mmol/l $MgCl_2$ (TKM buffer), and 0.55 mol/l sucrose. The tissue residue was termed "stromal fraction" and stored at −80°C until steroid estimation after dissolving the tissue in 1 N NaOH. The crude nuclei were concentrated and partially purified by layering the suspension over TKM buffer containing 0.88 mol/l sucrose and centrifugation for 10 min at 1000 *g*. The sucrose containing supernatant was termed "buffer A" and stored for steroid estimation. The purified nuclear pellet was resuspended in 10 mmol/l Tris–HCl buffer, pH 7.5 containing 1.5 mmol/l EDTA, 1.5 mmol/l dithiothreitol (TED-buffer), and 50 mmol/l NaCl. After counting the nuclei, the remainder of this suspension was centrifuged for 10 min at 800 *g*. The supernatant was termed "buffer B" and stored for steroid estimation. For extraction of androgen-receptor complexes, the pellet of purified nuclei was suspended in 2 mmol/l phosphate buffer, pH 8.5, containing 1 gm/l heparin at a concentration of $1–2 \times 10^7$ nuclei/ml. After 1 h, the suspension was centrifuged for 30 min at 100,000 *g*. The supernatant was termed the nuclear extract. The androgen-receptors in this nuclear extract were estimated as described earlier [15, 16]. Steroids were extracted from a separate portion of the purified nuclei after resuspension of the nuclei in 1 ml water, containing 0.1% Tween-80, [^{3}H]testosterone and [^{3}H]DHT. This extract was stored and extracted with acetone for steroid estimation as described above.

Interassay variations for the estimations of DHT and testosterone amounted to 16.5 and 17.7% (variation coefficients), respectively. Non-steroid containing samples yielded results of 0.18 ± 0.12 (SD, $n = 8$) and 0.24 ± 0.14 pmol/sample. All reported values have been corrected for the blank value observed in the same assay.

Statistical procedures

The significance of differences between the results of various treatment groups was assessed using paired or non-paired Student's *t*-tests or, in case of absence of normal distribution of the data, by Wilcoxon's test. Differences were considered significant when $P < 0.05$ (two-tailed).

RESULTS

Plasma concentrations of testosterone, DHT, SHBG and LH before and after various periods of treatment with CPA are shown in Fig. 1, together with plasma CPA concentrations measured in samples collected during the CPA-treatment. Peripheral levels of testosterone, DHT and LH were already significantly suppressed after 2 weeks of treatment with CPA ($P < 0.01$, $P < 0.05$ and $P < 0.02$, respectively). Differences for SHBG were only significant after 4 weeks ($P < 0.02$). Finally, constant plasma levels of CPA were reached after 4 weeks of treatment.

Table 1 summarizes plasma concentrations of testosterone, DHT and CPA at the time of

Table 1. Concentrations of testosterone, DHT and CPA in plasma at the time of operation in control and CPA-treated BPH patients (means ± SEM)

	Controls (5)	CPA-treated (8)
Testosterone (nmol/l)	15.5 ± 2.7	2.7 ± 0.4
DHT (nmol/l)	2.46 ± 0.23	0.38 ± 0.05
CPA (μmol/l)	—	1.26 ± 0.10

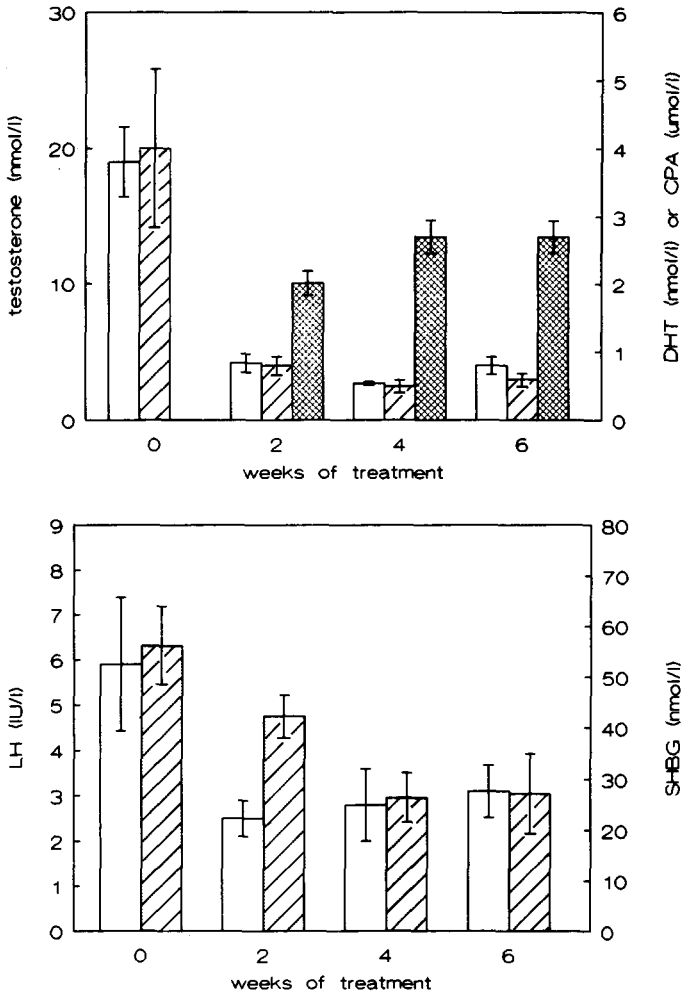

Fig. 1. Plasma concentrations of testosterone (open bars), DHT (hatched bars) and CPA (cross-hatched bars) (upper panel) and of LH (open bars) and SHBG (hatched bars) (lower panel) after various periods of treatment with CPA (means ± SEM, $n = 5$).

operation in control patients and in patients of the treated group. Here, too, treatment caused a significant ($P < 0.001$) suppression of peripheral androgen concentrations, when levels in the treated group are compared with those in the control group.

Tissue concentrations of the androgens and CPA, expressed per gram wet wt of the homogenized tissue portions, have been summarized in Fig. 2. DHT concentrations in all tissue fractions were significantly suppressed in the CPA-treated group (P always <0.05), whereas testosterone levels were not significantly influenced by the treatment. The sum of the concentrations of testosterone and DHT was 25.3 ± 3.8 (SEM) pmol/g tissue in the control group, and 8.6 ± 0.9 pmol/g tissue in the treated group ($P < 0.005$). Total tissue levels, based on a calculation of the sum of levels in the various subfractions, were 92 ± 9 (SEM)% of the amounts estimated in the non-fractionated tissue for DHT and 134 ± 22% for testosterone; there was no difference between the control and CPA-treated group in this respect. For CPA, much higher levels were detected in the combination of the separated tissue fractions, when compared with the level in the extract of the homogenized tissue.

Ratios between the levels of DHT and testosterone (DHT/T) in plasma, total tissue and tissue fractions have been summarized in Fig. 3. The non-affected DHT/T ratios in the plasma suggest that CPA has not influenced peripheral 5α-reduction of testosterone. However, in the prostate tissue and its fractions, the DHT levels were strongly suppressed, when compared with the concentrations of testosterone, resulting in significantly decreased DHT/T ratios in the stroma and cytosol fractions ($P < 0.05$). The distribution of DHT over the various tissue

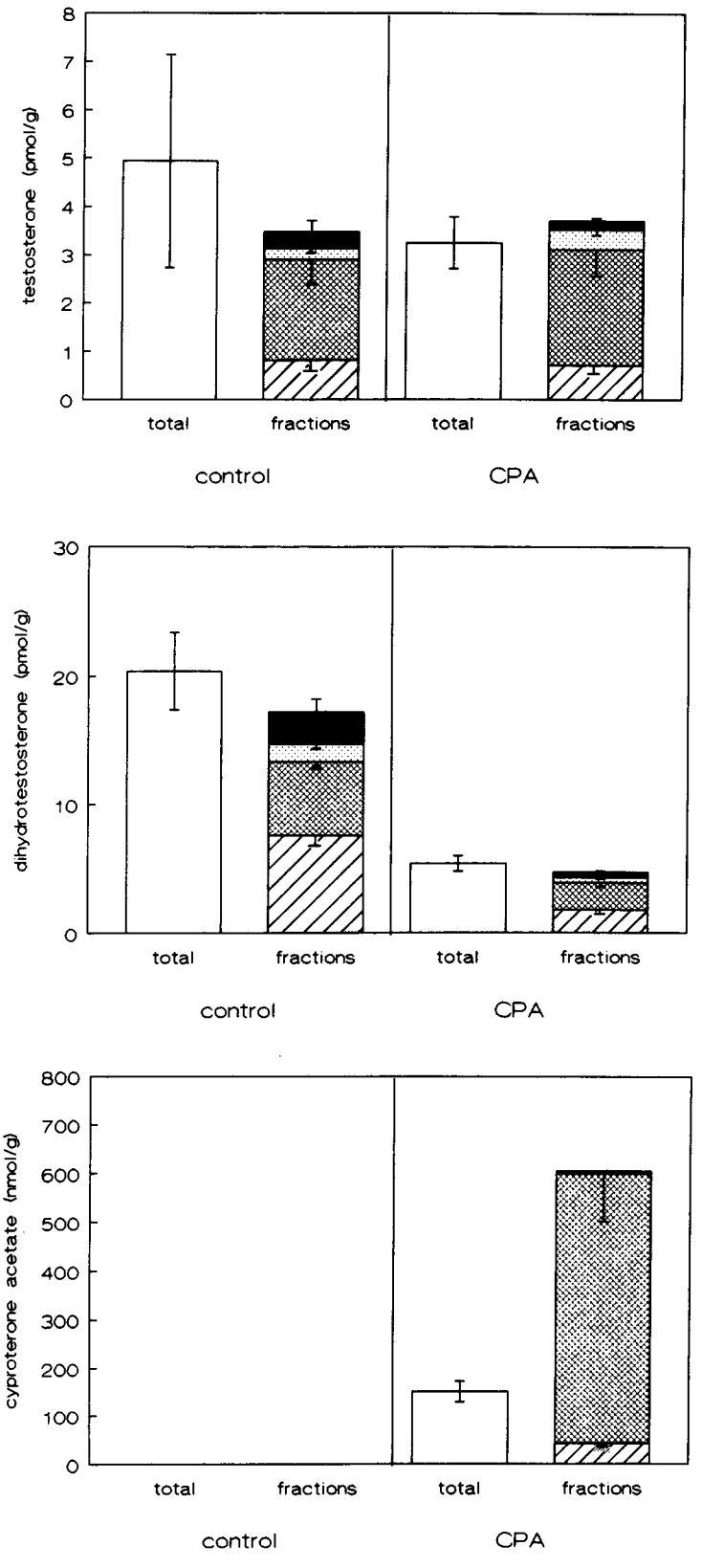

Fig. 2. Concentrations of testosterone (upper panel), DHT (middle panel) and CPA (lower panel) in (fractions of) prostatic tissue from control and CPA-treated patients with BPH. Open bars show values for total tissue, hatched bars values for stroma, cross-hatched and stippled bars concentrations in buffers A and B, and black bars values in the nuclei (means ± SEM, $n = 6$–10).

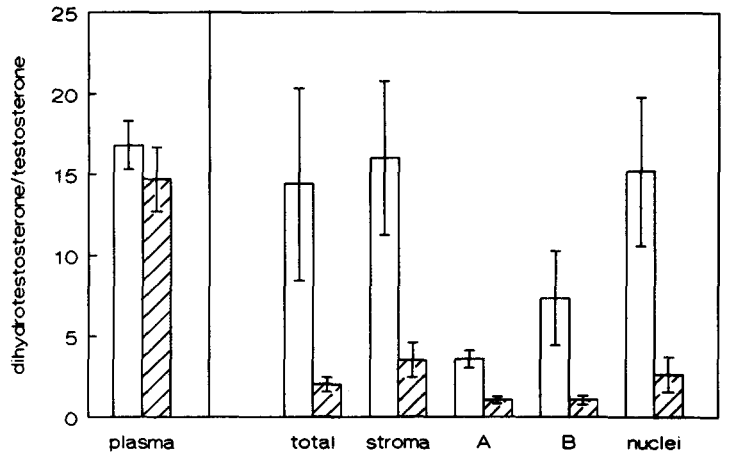

Fig. 3. DHT/T ratios in plasma, total prostate tissue and fractions of prostate tissue from control (open bars) and CPA-treated patients (hatched bars) with BPH. For the sake of clarity, ratios for plasma have been multiplied by 100 (means ± SEM, $n = 6$–10).

fractions was not influenced by CPA-treatment: in both the control and CPA-treated groups approx. 7% of the total amount of DHT was found in the nuclear fraction. For sake of clarity, nuclear steroid concentrations, expressed as fmol/10^6 nuclei on the basis of the assumption that 10^6 cells constitute 1 mg tissue, have been summarized in Table 2.

Finally, the amounts of nuclear androgen-receptors have also been summarized in Table 2. No significant differences between amounts of receptor per nucleus from prostate tissue from control or CPA-treated patients could be detected. When expressed per mg DNA, no significant differences were detected either; mean levels were in the order of 0.60 pmol/mg DNA.

DISCUSSION

Peripheral plasma levels of testosterone and DHT were effectively suppressed after administration of CPA (Fig. 1). Since suppression of the androgen levels was stronger than that of SHBG, the concentration of free androgens in the circulation also decreased as a result of the CPA-treatment.

Androgen levels in BPH tissue from untreated patients agree well with concentrations reported by other investigators [17–20]. Summation of the concentrations in the sub-fractions yielded the results obtained for total tissue, indicating the validity of the assay system for DHT and testosterone. However, CPA in total tissue was appreciably lower than that in the sum of the separate fractions. This may have been caused by hydrolysis of the CPA under the strongly alkaline conditions used for dissolving the tissue: the amount of immunoreactive CPA after this treatment agrees with the cross-reactivity of cyproterone in the CPA immunoassay. It is unlikely that hydrolysis of CPA plays an important role in the normal metabolism of this compound [21, 22].

Treatment with CPA did not significantly affect tissue levels of testosterone (Fig. 2). The distribution over the various tissue fractions was not affected either. In contrast, tissue levels of DHT were significantly suppressed, resulting in a 3-fold decrease of concentrations of testosterone-plus DHT. No change in the distribution of DHT over the various fractions was detected either. Comparable differences were obtained when the results were expressed on the basis of DNA rather than on wet weight (data not shown).

The distribution of CPA over the various tissue fractions differs appreciably from that of testosterone or DHT (Fig. 2). A very high concentration of CPA was found in the wash buffer after the first centrifugation of the nuclei (buffer A). This observation suggests a rapid exchange between CPA and testosterone or DHT during the isolation of the nuclei, followed

Table 2. Nuclear steroid levels and androgen-receptors (fmol/10^6 nuclei) in prostate tissue from control and CPA-treated BPH patients (means ± SEM)

Parameter	Control	CPA-treated
Testosterone	0.34 ± 0.24 (10)	0.18 ± 0.06 (7)
DHT	2.52 ± 0.98 (10)	0.40 ± 0.08 (7)*
CPA	—	0.72 ± 0.27 (6)
Androgen-receptors	10.6 ± 2.9 (9)	9.3 ± 2.6 (5)

*Significantly different from data for control group ($P < 0.05$).

by nuclear retention of the newly formed androgen-receptor complexes. This indicates the presence of a major technical difficulty in obtaining a definite answer to the question of the distribution of the anti-androgen between the subcellular fractions of prostatic tissue *in vivo*. The redistribution, however, is likely to have caused an underestimation, rather than an overestimation of nuclear CPA concentrations. Even after this redistribution the nuclear levels of CPA were twice as high as those of the most active androgen, DHT. A binding affinity for CPA to the androgen-receptor of 50% of that of DHT [23] would be enough to explain an appreciable suppression of the activity of the endogenous androgens which are still present in the prostatic tissue after CPA treatment.

The changing ratio of DHT over testosterone concentrations in prostatic tissue after treatment with CPA (Fig. 3) might be explained by displacement of DHT rather than testosterone from the androgen-receptor, by increased prostatic metabolism of DHT or by decreased production of DHT under the influence of CPA. This first possibility is not likely, since the affinity of DHT for the receptor is higher than the affinity of testosterone. Increased prostatic metabolism of DHT cannot be excluded on the basis of the present data. However, decreased production of DHT appears to be the most likely explanation for the observation of the decreased DHT over testosterone ratio, since Andersson *et al.* [24] showed that expression of the 5α-reductase gene in prostatic tissue is induced by testosterone; blocking of androgen action by CPA would therefore suppress 5α-reductase. Liver 5α-reductase mRNA was not affected by androgen treatment [24], in accordance with our observation that there is no general inhibition of 5α-reductase: the ratio between testosterone and DHT levels in peripheral plasma was not affected by the CPA-treatment. This is also in agreement with our observation [25] that peripheral DHT levels were not affected by radical prostatectomy in prostatic carcinoma patients or TUR of prostate tissue in patients with BPH.

Finally, the number of nuclear androgen-receptors has not changed after treatment with CPA (Table 2). Receptor numbers, in the order of 10 fmol/10^6 nuclei, exceed the amounts of anti-androgen present in the purified nuclei, again suggesting that redistribution of steroids occurred during the preparation of the nuclear pellets.

Summarizing, it appears that the biological effect of CPA (i.e. decrease of prostate size, see [8]) may have been caused by a triple action on the concentration of the most active androgen, DHT, in the nucleus of the prostate cell. Firstly, CPA causes a suppression of peripheral androgen levels. Secondly, the conversion of testosterone to DHT in the prostate is suppressed. Thirdly, the levels of CPA in the purified nuclear preparations are higher than those of the endogenous androgens, indicating that the CPA can compete directly at the receptor level.

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REFERENCES

1. Neumann F. and Steinbeck H.: Antiandrogens. In *Handbook of Experimental Pharmacology* (Edited by O. Eichler, A. Farah, H. Herken and A. D. Welch). Springer Verlag, Berlin, Vol. 35(2) (1974) pp. 235–484.
2. Fang S. and Liao S.: Androgen receptors—steroid-specific and tissue-specific retention of a 17β-hydroxy-5α-androstan-3-one protein complex by cell-nuclei of ventral prostate. *J. Biol. Chem.* **246** (1971) 16–24.
3. Krieg M., Steins P., Szalay R. and Voigt K. D.: Characterization of a specific androgen receptor in rat prostate cytosol by agar gel electrophoresis—*in vivo* and *in vitro* studies. *J. Steroid Biochem.* **5** (1974) 87–92.
4. Callaway T. W., Bruchovsky M., Rennie P. S. and Comeau T.: Mechanisms of action of androgens and antiandrogens: effects of antiandrogens on translocation of cytoplasmic androgen receptor and nuclear abundance of dihydrotestosterone. *Prostate* **3** (1982) 599–610.
5. Brinkmann A. O., Lindh L. M., Breedveld D. I., Mulder E. and van der Molen H. J.: Cyproterone acetate prevents translocation of the androgen receptor in the rat prostate. *Molec. Cell. Endocr.* **32** (1983) 117–129.
6. Bartsch W., Klein H., Nehse G. and Voigt K. D.: *In vivo* model for uptake, metabolism, and binding of androgens in prostatic tissue. *Prog. Cancer Res. Ther.* **31** (1984) 441–452.
7. Huang J. K., Bartsch W. and Voigt K. D.: Interactions of an anti-androgen (cyproterone acetate) with the androgen receptor system and its biological action in the rat ventral prostate. *Acta Endocr.* **109** (1985) 569–576.
8. Bosch R. J. L. H., Griffiths D. J., Blom J. H. M. and Schroeder F. H.: Treatment of benign prostatic hyperplasia by androgen deprivation: effects on prostate size and urodynamic parameters. *J. Urol.* **141** (1989) 68–70.
9. Hämäläinen E. K., Fotsis T. and Adlercreutz H.: Rapid and reliable separation of 5α-dihydrotestosterone from testosterone on silica gel microcolumns. *Clin. Chim. Acta* **139** (1984) 173–177.
10. Verjans H. L., Cooke B. A., de Jong F. H., de Jong C. M. M. and van der Molen H. J.: Evaluation of a radioimmunoassay for testosterone estimation. *J. Steroid Biochem.* **4** (1973) 665–676.

11. Klijn J. G. M., Lamberts S. W. J., de Jong F. H., Docter R., van Dongen K. J. and Birkenhäger J. C.: The importance of pituitary tumour size in patients with hyperprolactinaemia in relation to hormonal variables and extracellular extension of tumour. *Clin. Endocr.* **12** (1980) 341–355.
12. Hammond G. L. and Lähteenmäki P. L. A.: A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin capacities. *Clin. Chim. Acta* **132** (1983) 101–110.
13. Bradford M. M.: Rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
14. Hinegardner R. T.: Improved fluorimetric assay for DNA. *Analyt. Biochem.* **39** (1971) 197–201.
15. Blankenstein M. A., Bolt-de Vries J. and Foekens J. A.: Nuclear androgen receptor assay in biopsy-size specimens of human prostatic tissue. *Prostate* **3** (1982) 351–359.
16. Foekens J. A., Bolt-de Vries J., Mulder E., Blankenstein M. A., Schröder F. H. and van der Molen H. J.: Nuclear androgen receptors in human prostatic tissue—extraction with heparin and estimation of the number of binding-sites with different methods. *Clin. Chim. Acta* **109** (1981) 91–102.
17. Habib F. K., Lee I. R., Stitch S. R. and Smith P. H.: Androgen levels in the plasma and prostatic tissues of patients with benign hypertrophy and carcinoma of the prostate. *J. Endocr.* **71** (1976) 99–107.
18. Geller J., Albert J., Loza D., Geller S., Stoeltzing W. and de La Vega D.: DHT concentrations in human prostate cancer tissue. *J. Clin. Endocr. Metab.* **46** (1978) 440–444.
19. Petrangeli E., Sciarra F., Di Silverio F., Toscano V., Lubrano C., Conti C. and Concolino G.: Effects of two different medical treatments on dihydrotestosterone content and androgen receptors in human benign prostatic hyperplasia. *J. Steroid Biochem.* **30** (1988) 395–399.
20. Forti G., Salerno R., Moneti G., Zoppi S., Fiorelli G., Marinoni T., Natali A., Costantini A., Serio M., Martini L. and Motta M.: Three-month treatment with a long-acting gonadotropin-releasing hormone agonist of patients with benign prostatic hyperplasia: effects on tissue androgen concentration, 5α-reductase activity and androgen receptor content. *J. Clin. Endocr. Metab.* **68** (1989) 461–468.
21. Gerhards E., Gutsche H. and Riemann J.: Biodynamik von 1,2α-Methylen-6-chlor-pregna-4,6-dien-17α-acetoxy-3,20-dion (Cyproteron-acetat) nach oraler Verabreichung beim Menschen. *Arzneim.-Forsch.* **23** (1973) 1550–1555.
22. Bhargava A. S., Seeger A. and Günzel P.: Isolation and identification of 15-β-hydroxy cyproterone acetate as a new metabolite of cyproterone acetate in dog, monkey and man. *Steroids* **30** (1977) 407–418.
23. Ojasoo R., Delettré J., Mornon J. P., Turpin-van Dijcke C. and Raynaud J. P.: Towards the mapping of the progesterone and androgen receptors. *J. Steroid Biochem.* **27** (1987) 255–269.
24. Andersson S., Bishop R. W. and Russell D. W.: Expression cloning and regulation of steroid 5α-reductase, an enzyme essential for male sexual differentiation. *J. Biol. Chem.* **264** (1989) 16249–16255.
25. De Jong F. H., Oishi K., Hayes R. B., Bogdanowicz J. F. A. T., Raatgever J. W., van der Maas P. J., Yoshida O. and Schroeder F. H.: Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch–Japanese Case-Control Study. *Cancer Res.* **51** (1991). 3445–3450.