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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*

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ABSTRACT. The intraprostatic concentrations of testosterone (T) and dihydrotestosterone (DHT) have been measured in only a few men. We measured, in prostatic tissue obtained at surgery from seven men with benign prostatic hyperplasia, the effects of 3-month treatment with a long-acting GnRH agonist on 1) the intraprostatic concentrations of T, DHT, and 5α-androstan-3α,17β-diol (3α-diol); 2) prostatic 5α-reductase activity; and 3) the prostatic content of androgen receptors (AR). Plasma T, DHT, and 3α-diol levels also were measured. Prostatic tissue samples obtained at surgery from a group of untreated men with benign prostatic hyperplasia also were studied. The mean DHT and 3α-diol concentrations in the prostatic tissue of the treated men were about 10% of those in untreated men (n = 19; $P < 0.01$ for DHT and $P < 0.05$ for 3α-diol), and the mean intraprostatic T concentration in the treated men was about 25% of that in the control group ($0.10 > P > 0.05$). The mean *in vitro* formation of DHT by the prostatic tissue of the treated men was about 50% lower ($P < 0.05$) than that by prostatic tissue of the untreated men (n = 9). The mean cytosolic AR content in the prostatic tissue of the treated men was significantly higher ($P < 0.05$), whereas the mean nuclear content of both salt-extractable and salt-resistant AR was significantly lower ($P < 0.05$) than that in the prostatic tissue of the untreated men (n = 8). The mean plasma T levels in treated men decreased from 4.77 ± 1.79 (SD) ng/mL (16.5 ± 6.2 nmol/L) to 0.27 ± 0.42 ng/mL (0.9 ± 1.5 nmol/L) after 1 month of therapy and remained in the castrate range thereafter.

We conclude that pharmacological castration resulting from 3-month treatment with a long-acting GnRH agonist decreases the intraprostatic T concentration to about one fourth and those of DHT and 3α-diol to about one tenth of the levels in untreated men. Thus, GnRH agonist treatment may not completely abolish intraprostatic androgen concentrations in metastatic prostatic cancer patients. The decrease in prostatic 5α-reductase activity as well as the decrease in nuclear receptors are probably secondary to the decrease in plasma T concentrations. (*J Clin Endocrinol Metab* **68:** 461, 1989)

IN SOME androgen-dependent structures, including muscle and vas deferens, testosterone (T) acts as such, whereas in others, including prostate, epididymis, and hair follicles, it must be transformed into 5α-dihydrotestosterone (DHT) to exhibit its androgenic effects (1). Obviously, in these latter tissues 5α-reductase, the enzyme involved in the 5α-reduction of T, plays a key role in determining androgen action. Since DHT seems to be one of the most important factors responsible for the development of prostatic benign hyperplasia (BPH) (2), the determination of the intraprostatic concentrations of the 5α-reduced metabolites of T, and in particular of DHT, in human BPH has been the aim of several studies, which have recently been reviewed (3). Intraprostatic DHT measurements have also been used for the follow-up of patients with endocrine-dependent metastatic prostatic cancer (4).

In men with metastatic prostatic cancer, surgical or pharmacological castration reduces intraprostatic con-

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centrations to only about 50% of those in untreated men (5); for this reason, to counteract the residual androgenic activity, the additional value of antiandrogen administration has been stressed (6). However, very few data have been reported concerning the intraprostatic DHT levels in animals and in men after surgical or pharmacological castration (7, 8). To our knowledge, measurements of T and its main 5α-reduced metabolites, 5α-reductase activity, and androgen receptors (AR) in prostatic tissue after treatment with GnRH agonists have not been reported. This kind of study, due to the large amount of tissue needed, is very difficult to perform in patients with prostatic cancer, from whom only small needle biopsy fragments of tissue are usually available.

Recently, successful short term treatment with GnRH agonists has been reported in men with BPH (9–11). In the light of this observation, the aim of our study was to determine, in BPH tissue of men undergoing suprapubic prostatectomy, the effects of 3-month treatment with a long-acting GnRH agonist on the tissue levels of 1) T, DHT, and 5α-androstan-3α,17β-diol (3α-diol); 2) 5α-reductase activity; and 3) AR content. For comparison, steroid levels and 5α-reductase activity also were measured in some androgen-dependent human tissues (kidney and uterus) which do not form DHT.

Materials and Methods

BPH patients

Twenty-six men with BPH undergoing suprapubic adenomectomy were divided into two groups. Nineteen men (age range, 64–86 yr) were controls. All had stopped any kind of medical therapy at least 1 month before surgery. Seven men (age range, 64–80 yr) were treated for 3 months with monthly sc injections of 3.6 mg long-acting GnRH agonist D-Ser(Bu^t)^6-Azgly^{10}-LHRH (Zoladex, ICI Pharma, Milan, Italy), dispensed in a biodegradable and biocompatible *d,l*-lactide-glycolide copolymer and supplied as a cylindrical rod (1.2 mm in diameter and 1 cm in length) in a purpose-designed applicator (12). In these men surgery was performed at the end of the third month of treatment (within 21–30 days after the last injection of the GnRH agonist). Plasma samples for steroid and LH measurements were obtained from those men before treatment, on days 30 and 60 of treatment, and on the day of surgery. Informed written consent was obtained from each man. The study was approved by the local Committee for Investigation in Humans.

Prostatic, kidney, and uterine tissues

Prostatic BPH tissue was obtained at surgery from both groups. Histological confirmation of BPH was obtained in all men. Kidney tissue samples were obtained from four men and four women undergoing surgery for renal carcinoma; none had any signs or symptoms of endocrine disease. The age ranges were 62–69 and 16–42 yr in the men and women, respectively.

Uterine tissue was obtained from 9 women undergoing hysterectomy for leiomyoma (age range, 39–74 yr).

Reagents

Mass spectrometric determination of steroids. Unlabeled T, DHT, and 3α-diol were obtained from Sigma (St. Louis, MO). Trideuterated (16,16,17-$^2H_3$) T, DHT, and 3α-diol were synthesized by a previously reported procedure (13). Tritiated T was purchased from New England Nuclear (Boston, MA). Ethanol, acetone, diethylether, cyclohexane, chloroform, acetonitrile, and heptane (Merck, Milan, Italy) were analytical grade and not redistilled before use. Sep-Pak C18 cartridges were purchased from Waters Associates (Milford, MA). Sephadex LH-20 was obtained from Pharmacia (Uppsala, Sweden) and used in standard chromatography columns obtained from Bio-Rad (Richmond, CA). Heptafluoro-butyric anhydride (HFBA) was purchased from Pierce Chemical Co. (Rockford, IL).

Measurement of 5α-reductase activity. Unlabeled steroids (DHT and 3α-diol) were purchased from Sigma. The radioactive steroids [4-^{14}C]T (SA, 58 Ci/mmol), and 5-α-dihydro-[1,2,4,5,6,7-^{3}H]T (SA, 155 Ci/mmol), and [1α,2α-N-^{3}H]3-diol (SA, 40/Ci mmol) were purchased from the Radioactive Centre (Amersham, United Kingdom).

Measurement of AR. Unlabeled and tritiated 17α-methyl-trienolone (R1881; SA, 86 Ci/mmol) was obtained from New England Nuclear. Triamcinolone acetonide was obtained from Sigma. Hydroxylapatite (Bio-Gel HTP) was purchased from Bio-Rad, and the glass fiber filters (GF/c; 2.4 cm) were obtained from Whatman (Waters, Inc., London, United Kingdom).

Methods

Steroid measurement in BPH tissue and plasma. Measurements of T, DHT, and 3α-diol in BPH tissue samples as well as in kidney and uterine tissue were performed according to a method described in detail previously (13). Tissue samples were collected at surgery and immediately stored in liquid nitrogen. Two or 3 g were homogenized, and known amounts of trideuterated T, DHT, and 3α-diol were added. After thawing and deproteinization, the homogenate was extracted twice with diethylether, and the diethylether extract was chromatographed first on a Sep-Pak C18 cartridge and then on a Sephadex LH-20 column. The Sephadex eluate was derivatized with HBFA and suspended in 25 μL heptane. For plasma samples only one diethylether extraction and one Sephadex LH-20 chromatography were performed. One- or 2-μL aliquots of the HBFA derivatives were injected in triplicate into a PYE gas chromatograph (PYE Unicam Ltd., Cambridge, United Kingdom) directly coupled to a VG 7070 EQ mass spectrometer (VG Analytical, Manchester, United Kingdom). The concentrations of T, DHT, and 3α-diol in the samples were determined using the method of isotopic dilution. A calibration curve was constructed for each steroid using the peak area ratio between the native and the deuterated steroid. The data were analyzed using a PDP8/A computer (Digital Equipment Corp., Maynard, MA). The intra- and interassay coefficients of variation (CVs) of the mass spectrometric method for steroid determinations in the prostatic tissue of treated men were 7.6%, 14.7%, and 18.7%, and 13.8%, 11.3%, and 22.2% for T, DHT, and 3α-diol, respectively. The intra- and interassay CVs of the measure-

ments performed in the plasma were 7.4%, 3.3%, and 5.0%, and 8.2%, 11.1%, and 5.4% for T, DHT, and 3α-diol, respectively. The detection limits of the method were 35 pmol/L (or g tissue) for T and 3α-diol and 70 pmol/L (or g tissue) for DHT. The mean recovery of known amounts of steroids added to tissue homogenates was 97.6% for T, 98.5% for DHT, and 99% for 3α-diol (n = 5). Plasma LH was measured by a RIA method using commercial reagents (Biodata, Milan, Italy). The intra- and interassay CVs were 3.5% and 8.4%, respectively (n = 10). The sensitivity of the method was 0.3 mU/mL (U/L).

Measurement of 5α-reductase activity: incubation, extraction, and identification of the metabolites. Specimens of approximately 30 mg prostatic tissue obtained from nine untreated (age range, 64–86 yr) and the seven treated men with BPH as well as 30 mg kidney and uterine tissue were incubated in quadruplicate. A detailed description of the extraction and identification of metabolites was previously given (14). In summary, each incubation mixture of 2 mL buffer Krebs-Ringer solution equilibrated with 95% O_2 and 5% CO_2 at 37 C contained 2 mmol/L NaDPH (Sigma) and either [4-^{14}C]T or [^{14}C] DHT (6×10^{-7} mol/L) as substrates. After 2-h incubation in a metabolic shaker, the reactions were stopped by freezing, and the samples were kept at −20 C until extraction. The metabolites formed were extracted with diethylether. The dried extracts were dissolved in ethanol and chromatographed on silica gel thin layer plates using dichloromethane-diethylether. The metabolites were localized by iodine vapor staining, and the corresponding spots were scraped and counted (14). No separation of the two diols (3α-diol and 3β-diol) was performed, because 3β-diol represents only a minor metabolite of T and DHT in the human prostate (Zoppi, S., Motta M., and Martini L., unpublished observations). The amounts of various metabolites recovered were expressed as picograms per mg tissue.

AR assay. AR content was measured in periurethral tissue specimens obtained at surgery from eight (age range 66–81 yr) untreated men and the seven men with BPH treated with the GnRH agonist. Prostatic tissue specimens were stored in liquid nitrogen, and AR content was determined within 7 days after sampling. Cytosol and nuclear extracts (salt-extractable and salt-resistant fractions) were prepared, and their AR content was assayed as previously described (15, 16). Duplicate aliquots of cytosol and nuclear salt-extractable and salt-resistant fractions were incubated with 0.025–5 nmol/L methyltrienolone (R1881) as the labeled ligand with or without a 100-fold molar excess of unlabeled methyltrienolone to determine nonspecific binding. All incubations were performed in the presence of a 500-fold molar excess of triamcinolone acetonide (16). The incubations were carried out at 4 C for 72 h for cytosol fractions and for 168 h for nuclear fractions, which, in previous experiments, proved to be the optimal conditions (Fiorelli, G., De Bellis, A., Longo, A., unpublished observations). Free and receptor-bound radioactive steroids were separated using hydroxylapatite glass-fiber filters (16). The radioactivity of the washed hydroxylapatite filters was measured in a Packard scintillation spectrometer (Packard, Downers Grove, IL). The results were analyzed by the computer program LIGAND (17), and specific binding was expressed as femtomoles of steroid bound per mg protein for the cytosol fractions and femtomoles of steroid bound per mg DNA for the nuclear fractions. The specific binding of R1881 to cytosol and nuclear AR was saturable and of high affinity. The binding of the labeled ligand was specific for the cytosol and nuclear AR, as indicated by the competition studies which confirmed previous results (15, 18). The relative competition ability of the various steroids tested was as follows: R1881 (100%) = DHT > T (50%) > 3α-diol, progesterone, estradiol (3%) > cyproterone acetate (1%) > cortisol (0%).

Statistical analysis. The statistical significance of results within the same group and between the two groups of men (untreated and treated) was assessed using the Student's *t* test for paired and unpaired data where appropriate. $P < 0.05$ was considered significant.

Results

Steroid measurements

The mean plasma T, DHT, and 3α-diol concentrations in the men with BPH before and during GnRH agonist administration are shown in Fig. 1. The plasma steroid concentrations declined rapidly and remained low (in the castrate range) during the 3-month treatment period. Plasma LH concentrations also declined, although not quite so rapidly.

The intraprostatic T, DHT, and 3α-diol concentrations in the untreated and GnRH agonist-treated men with BPH are shown in Fig. 2. The mean DHT [0.476 ± 0.280 (±SE) ng/g tissue] and 3α-diol (0.018 ± 0.004) levels in the prostatic tissue of the treated men were about 10% of those in the untreated men [4.490 ± 0.380 ($P < 0.01$) for DHT; 0.188 ± 0.041 ($P < 0.05$) for 3α-diol]. The mean intraprostatic T level in the treated men (0.106 ± 0.027) was about 25% of that in the untreated men (0.404 ± 0.102; $0.10 > P > 0.05$).

Figure 3 shows the mean intraprostatic T, DHT, and 3α-diol concentrations in the GnRH agonist-treated men compared with those in kidney and uterine tissue, two structures that do not form DHT. In the treated men, the prostatic tissue T concentration (0.106 ± 0.027 ng/g tissue) was significantly lower than those in male and female kidney tissue (1.91 ± 0.22 and 0.93 ± 0.35; $P < 0.01$ and $P < 0.05$, respectively) as well as uterine tissue (0.257 ± 0.039; $P < 0.01$). On the other hand, the mean intraprostatic DHT level (0.476 ± 0.110) was significantly higher than that in female kidney (0.097 ± 0.031; $P < 0.05$) and uterine tissue (0.063 ± 0.020; $P < 0.01$) and slightly but not significantly higher than that in male kidney tissue (0.160 ± 0.019). The mean intraprostatic 3α-diol level (0.018 ± 0.004) was not significantly different from those in female kidney (0.033 ± 0.001) and uterine tissue (0.018 ± 0.006) and was significantly lower than that in male kidney tissue (0.055 ± 0.020; $P < 0.05$).

Measurement of 5α-reductase activity

As shown in Fig. 4a, when radioactive T was used as substrate, formation of DHT by BPH tissue of GnRH

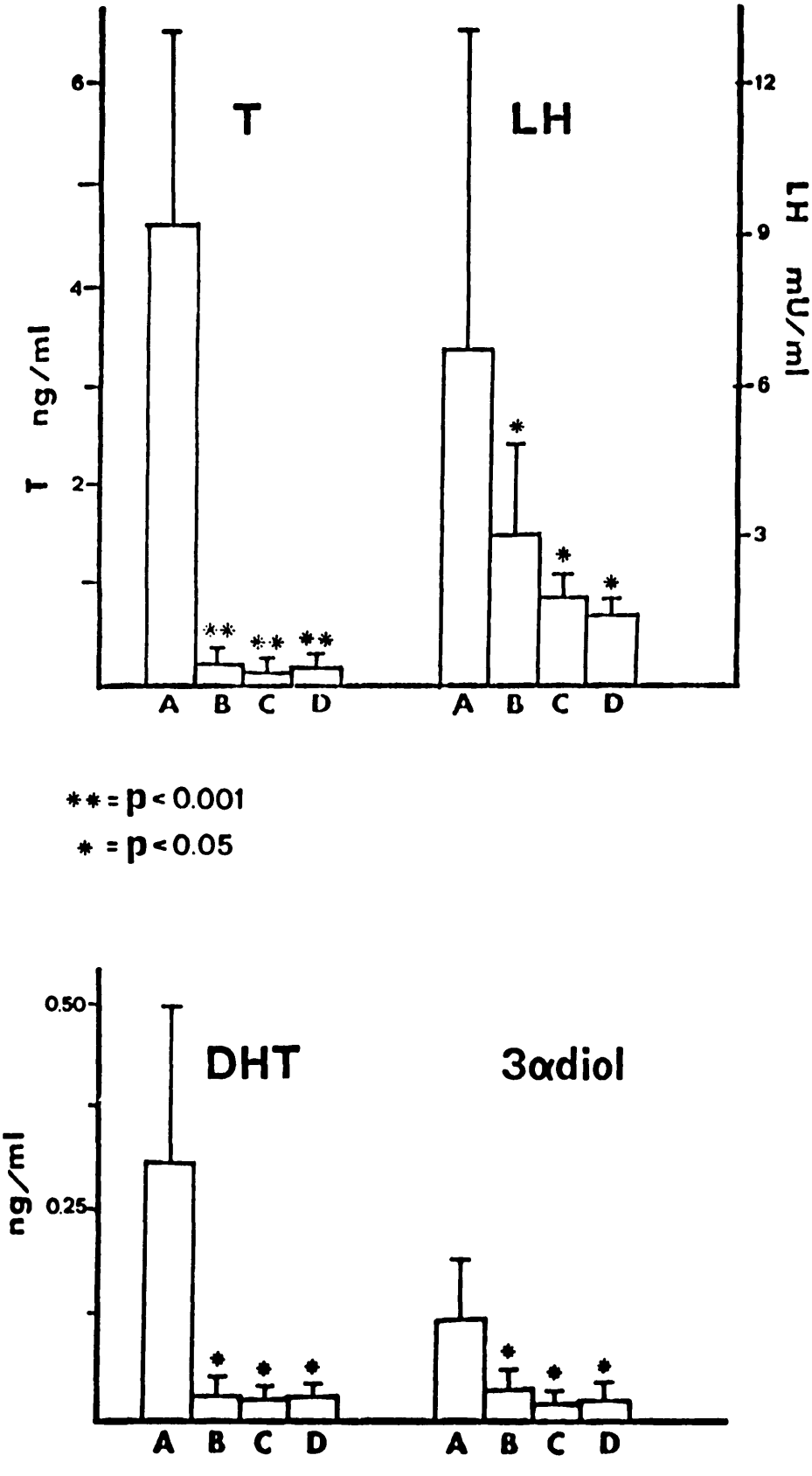

FIG. 1. Mean (±SD) plasma T, LH, DHT, and 3α-diol concentrations in seven men with BPH before (A) and during treatment with GnRH agonist (B, C, and D, 1, 2, and 3 months of treatment, respectively). *, $P < 0.05$; **, $P < 0.001$ (*vs.* A). Conversion factors: T, ng/mL × 3.467 = nmol/L; DHT, ng/mL × 3.443 = nmol/L; 3α-diol, ng/mL × 3.419 = nmol/L; LH, mU/mL × 1 = U/L.

agonist-treated men was significantly lower than that in BPH tissue of untreated men. On the contrary, formation of the diols in the prostatic tissue of treated men was not significantly different from that in untreated men. Similarly, no significant difference was found in the formation of diols in BPH tissue of treated and untreated men when radioactive DHT was used as substrate (Fig. 4b).

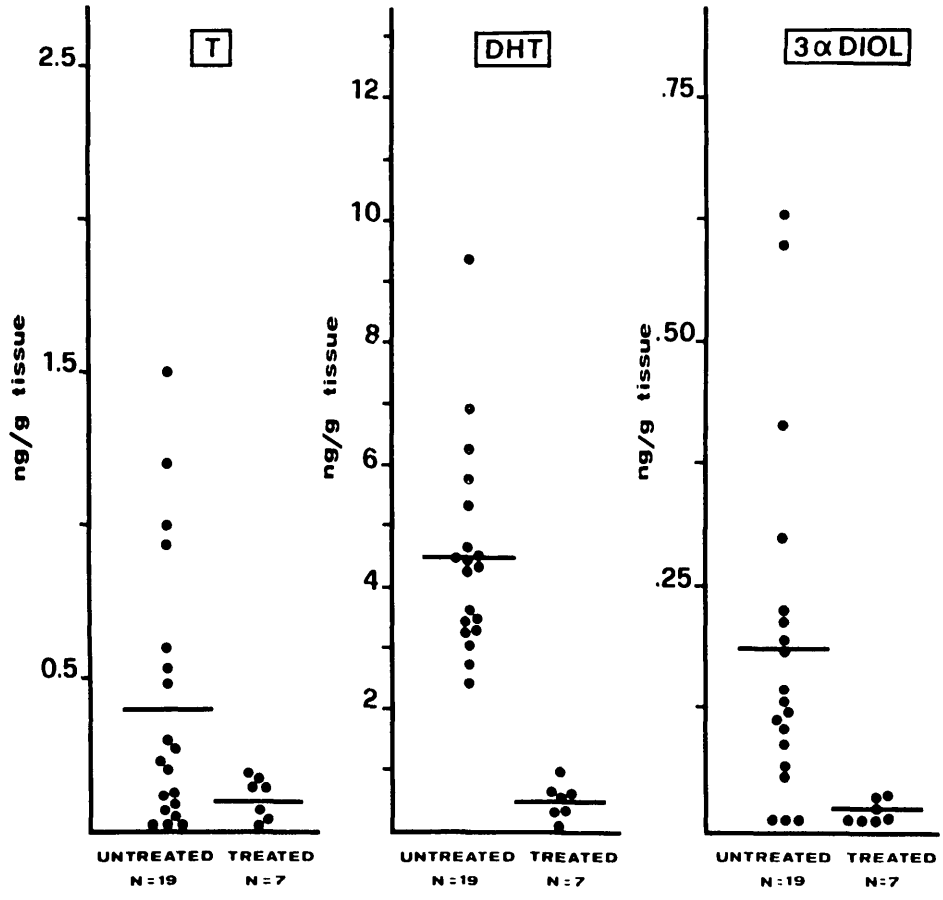

FIG. 2. Intraprostatic tissue levels of T, DHT, and 3α-diol in untreated men with BPH and in men with BPH treated for 3 months with GnRH agonist. The mean values (*horizontal bar*) of DHT and 3α-diol in the untreated men are significantly higher ($P < 0.05$) than the mean values in the treated men. Conversion factor: T, ng/g × 3.467 = pmol/g; DHT, ng/g × 3.443 = pmol/g; 3α-diol, ng/g × 3.419 = pmol/g.

The amounts of DHT obtained by incubating radioactive T with male and female kidney tissue were, respectively, about 50- and 100-fold lower than that in prostatic tissue (Fig. 5; note the difference in scales). Similar results were obtained in uterine tissue (Fig. 5). It is interesting to note that DHT formation in female kidney tissue was significantly lower than that in male kidney tissue.

AR assay

The mean AR dissociation constant (K_d, × 10^{-10} mol/L) in the cytosol fractions of BPH tissue of untreated men [0.7 ± 0.1 (±SE); n = 8] was similar to that in the GnRH agonist-treated men (0.6 ± 0.1; n = 7). The K_d values in the salt-extractable and salt-resistant nuclear fractions of BPH tissue of untreated men (1.3 ± 0.5 and 0.8 ± 0.3, respectively) also were similar to those in treated men (0.2 ± 0.1 and 0.3 ± 0.1, respectively).

Cytosol AR levels in prostatic tissue from treated men were significantly higher than those in prostatic tissue of untreated men [18.5 ± 4.3 (±SE) *vs.* 8.3 ± 1.5; fmol/mg protein; $P < 0.05$; Fig. 6]. The nuclear content of both salt-extractable and salt-resistant AR was significantly lower in prostatic tissue of treated men [28.8 ± 10 (range, 2–70) and 28.0 ± 8 (range, 5–59) fmol/mg DNA] than in that of untreated men [274 ± 51 (range, 152–549) and 61 ± 10 (range, 29–96); $P < 0.05$; Fig. 6].

Discussion

During treatment with the GnRH agonist the plasma T and DHT levels of men with BPH declined to castrate levels, in agreement with data previously reported (19, 20). A parallel decrease in plasma 3α-diol, to about 25% of baseline values, also occurred. The mean BPH tissue T, DHT, and 3α-diol levels in the treated men were, respectively, about 10% (DHT and 3α-diol) and 25% (T) of the corresponding mean levels in BPH tissue from untreated men. The intraprostatic T, DHT, and 3α-diol concentrations in the untreated men are similar to those reported by others (21–23). Nevertheless, the intraprostatic mean T level in the GnRH agonist-treated men was relatively higher than expected, particularly in consideration of the fact that in these men plasma T levels had decreased to less than 5% of pretreatment values, probably due to the significantly lower levels of 5α-reductase activity (as indicated by decreased DHT formation) in prostatic tissue of treated men (~50% of that in untreated men). On the other hand, in tissues not forming DHT, such as kidney and uterus, in which 5α-reductase activity was nearly undetectable, the tissue T concentra-

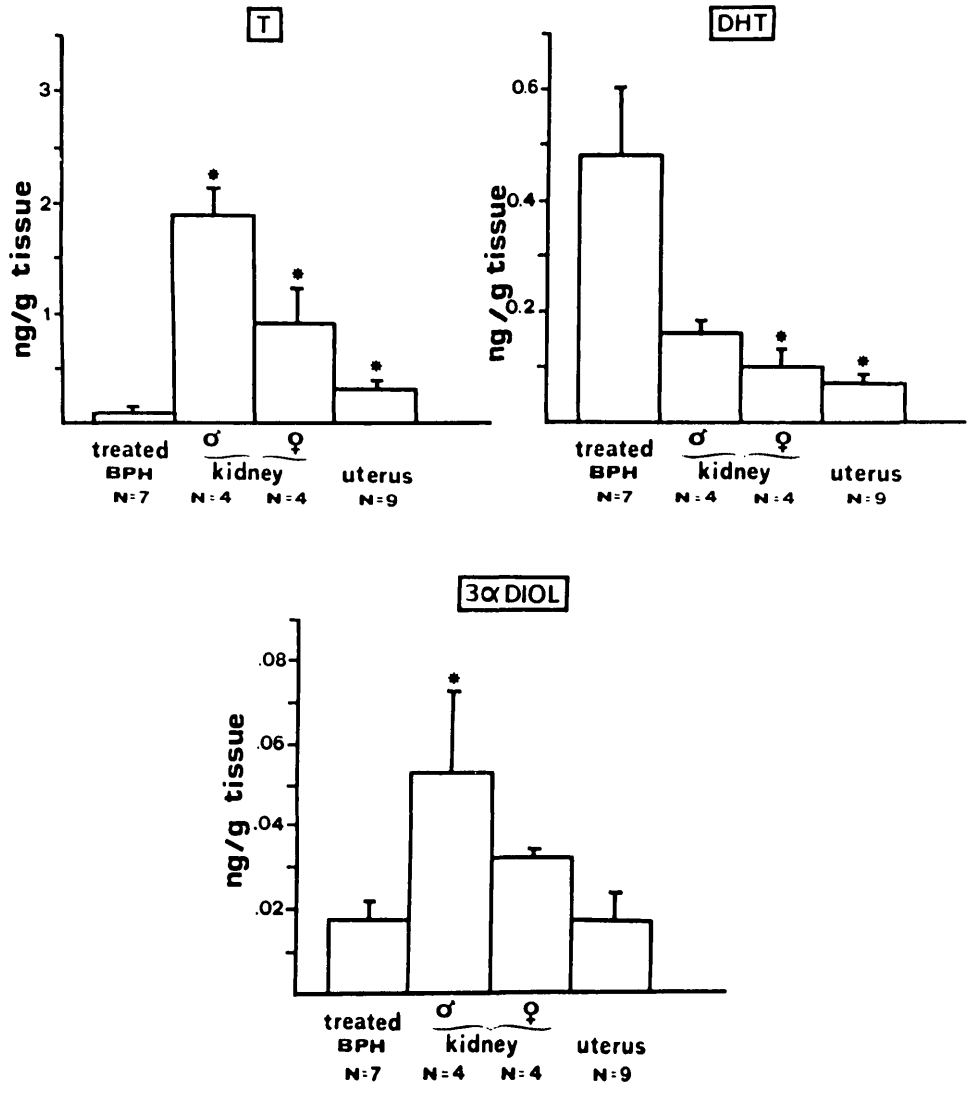

FIG. 3. Mean (±SE T, DHT, and 3α-diol levels in prostatic tissue of men with BPH treated with GnRH agonist and in specimens of human male and female kidney and uterine tissue. *, $P < 0.05$ *vs.* treated BPH. Conversion factors: T, ng/g × 3.467 = pmol/g; DHT, ng/g × 3.443 = pmol/g; 3α-diol, ng/g × 3.419 = pmol/g.

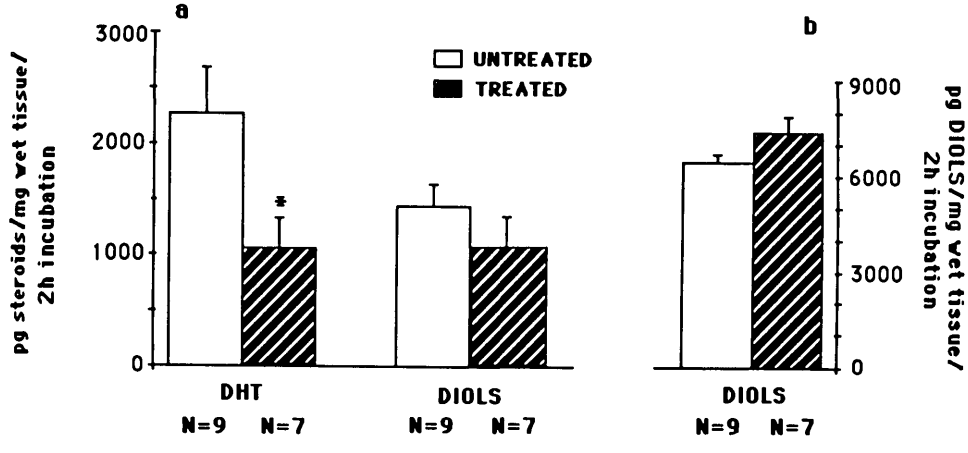

FIG. 4. Mean (±SE) *in vitro* formation of DHT and the diols (3α- and 3β-diol) by BPH tissue from untreated men and men treated with GnRH agonist incubated with radioactive T (a) and with radioactive DHT (b) as substrates. *, $P < 0.01$ *vs.* untreated BPH. Conversion factors: DHT, pg/mg·2-h incubation × 3.443 = pmol/g·2-h incubation; diols, pg/mg·2-h incubation × 3.419 = pmol/g·2-h incubation.

tion was significantly higher than that in prostatic tissue of the treated men. Moreover, the mean intraprostatic DHT concentration in the GnRH agonist-treated men (even if only about 10% of that in prostatic tissue of untreated men) was still higher than those in male and female kidney and uterine tissue.

As peripheral plasma T levels in the treated men were decreased to less than 5% of baseline, the residual DHT in the prostate might be due to the action of residual 5α-reductase activity on adrenal androgens, as previously suggested (24, 25). Since 5α-reductase has been reported to be an androgen-dependent enzyme in the ventral

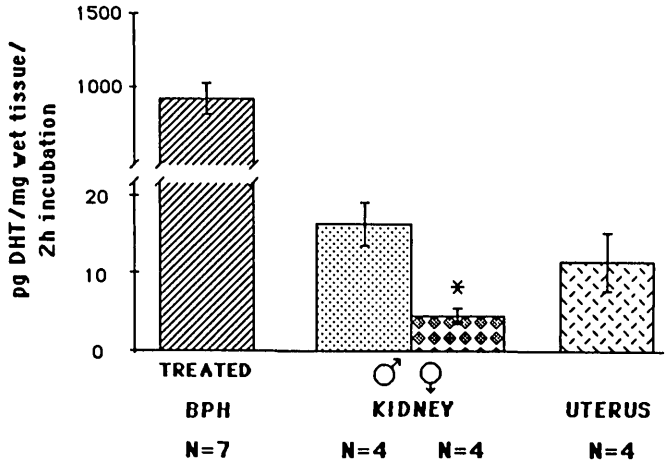

FIG. 5. Mean (±SE) *in vitro* formation of DHT by BPH tissue from men treated with GnRH agonist and by male and female kidney and uterine tissue. *, $P < 0.05$ *vs.* male kidney and uterus. Conversion factor: DHT, pg/mg·2-h incubation × 3.433 = pmol/g·2-h incubation.

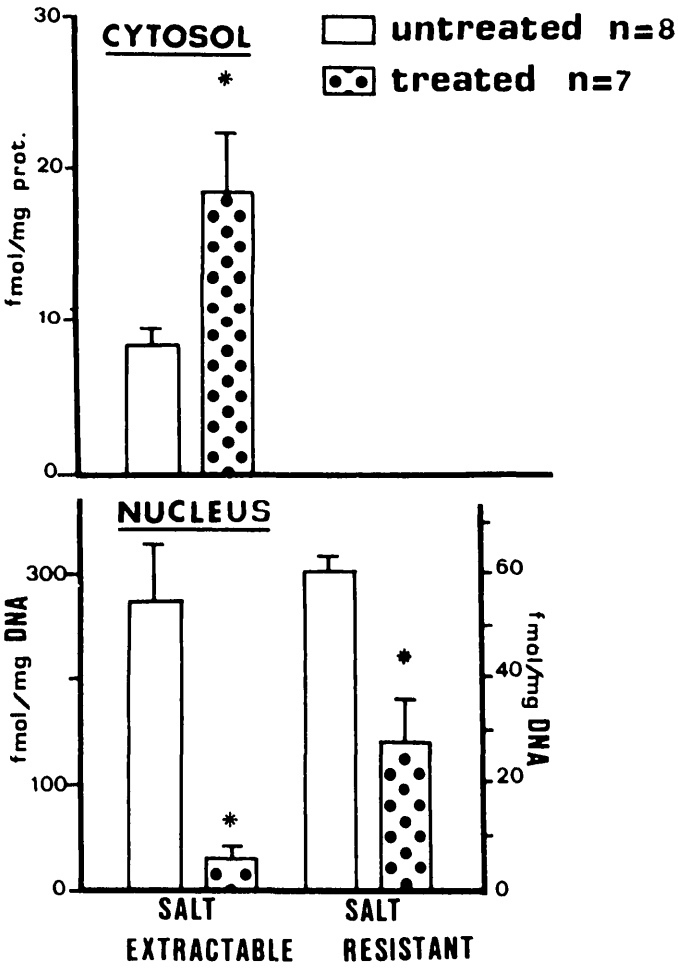

FIG. 6. Mean (±SE) cytosol and nuclear AR contents in BPH tissue from untreated men and men treated with GnRH agonist. *, $P < 0.05$ *vs.* untreated men.

prostate of the rat (26) as well in human skin (27), it is possible that the decreased 5α-reductase activity in the treated men might be due to decreased T secretion, although a direct effect of the GnRH agonist on the enzyme cannot be ruled out at present.

The results of the assays of cytosol and nuclear AR in prostatic tissue of GnRH-agonist treated men are similar to the results previously reported in castrated rats (16), and they confirm that AR content is androgen dependent in hyperplastic human prostatic tissue. In fact, the 10-fold lower intraprostatic DHT levels in the treated men parallel the 10-fold reduction of AR in the salt-extractable nuclear fraction, which seems to be strictly related to the cellular DHT content and/or its translocation into the nucleus (28). AR bound to the nuclear matrix seem to be less sensitive to the decrease in intraprostatic DHT. This finding may be explained by the fact that salt-resistant nuclear AR are not involved in the process of androgen translocation from the cytosol and that they bind steroid entering the nucleus by diffusion (29). On the contrary, the higher prostatic cytosol AR levels in the treated men may be explained by a decrease in the translocation process. This result is in agreement with data obtained in castrated rats (16, 28).

In conclusion, treatment for 3 months with a long-acting GnRH agonist had the following effects in prostatic tissue of men with BPH. 1) Prostatic tissue T levels decreased to about 25% and those of DHT and 3α-diol to about 10% of the values in untreated men. 2) Prostatic 5α-reductase activity decreased to about 50% of that in untreated men. 3) Both salt-extractable and salt-resistant nuclear prostatic AR decreased to about 10% and 50%, respectively, of the values in untreated men. It is premature to extrapolate from these results the effects of long term treatment with GnRH agonists in prostatic tissue of men with prostatic cancer. However, the results suggest that GnRH agonist treatment may not reduce intraprostatic androgen concentrations to undetectable levels.

Acknowledgment

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