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MODE OF ACTION OF PROGESTERONE, GESTONORONE CAPRONATE (DEPOSTAT)* AND CYPROTERONE ACETATE (ANDROCUR)* ON THE METABOLISM OF TESTOSTERONE IN HUMAN PROSTATIC ADENOMA: *IN VITRO* AND *IN VIVO* INVESTIGATIONS

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SUMMARY

Selective uptake of H^3-testosterone in human benign prostatic hypertrophy (BPH) has been demonstrated by *in vitro* and *in vivo* studies. 5α-dihydrotestosterone and 5α,3α-androstanediol are by far the major metabolites of testosterone. Progesterone and gestonorone capronate are competitive inhibitors of testosterone metabolism in BPH. These progestagens are taken up in preference to testosterone into prostatic cells. The subsequent formation of dihydrotestosterone is inhibited, whereas cyproterone acetate does not influence the reduction of testosterone. Animal studies support the concept that this antiandrogen acts by interfering with the binding of dihydrotestosterone to an intranuclear receptor.

Growth and development of the prostate are dependent on androgens. This is shown by the following observations:

1. Prepubertal castration or pituitary hypogonadism hamper the growth and development of the prostate gland. Substitution of androgens in these individuals normalizes the development of this organ.

2. Postpubertal castration or hypophysectomy at the onset of the second growth period of the prostate lead to its atrophy. Exogenous androgen administration will prevent these involutional changes [1].

Androgens affect as well the onset and development of prostatic adenoma [2–6]. Our investigations were designed to scrutinize the metabolic steps of testosterone in human prostatic adenoma, comparing them with the results derived from the literature. In addition we have studied whether progesterone, gestonorone capronate or cyproterone acetate influence testosterone turnover in human benign prostatic hypertrophy and eventually a tentative explanation of their mechanism of action was sought. This was accomplished through *in vitro*- and *in vivo*-techniques of investigation.

MATERIALS AND METHODS

Chemicals

7, H^3-Testosterone (Radiochemical Center, Amersham, Buckinghamshire, England) had a S.A. of 5 Ci/mM and was more than 95% pure when examined by thin layer chromatography on silica gel sheets (E. Merck, Darmstadt, West Germany) in the system hexane methanol H_2O (5:4:1; v/v). The H^3-testosterone was dissolved in ethanol. The subsequent reference steroids were used: testosterone (Serva, Heidelberg, West Germany); 5-α-dihydrotestosterone (5-α-androstan-17-β-ol-3-one, Merck, Darmstadt, West Germany); 5-β-dihydrotestosterone (5-β-androstan-17-β-ol-3-one, Merck, Darmstadt, West Germany); androstenedione (androst-4-ene-3,17-dione; Merck, Darmstadt, West Germany); 5-α-androstanediol (5-α-androstane-3α,17β-diol); 5-β-androstanediol (5-β-androstane-3α-17β-diol); 5-α-androstanedione (5-α-androstane-3,17-dione); 5-β-androstane-3,17-dione (Ikapharm, Ramt-Gan, Israel).

All organic solvents were of analytical grade.

Study of inhibition of testosterone metabolism

The tissue of 12 histologically confirmed prostatic adenomas was used. Immediately after suprapubic prostatectomy the tissue was rinsed and stored in ice-cold isotonic saline solution. Within 2 h after removal it was processed. The standard incubation consisted of 300 mg of adenoma tissue, 10^{-7} mol/l H^3-testosterone (= 34 pmol) and 2 ml Krebs Ringer-phosphate buffer, pH 7·4. The incubation was carried out over 2 h in an O_2:CO_2 atmosphere (95:5; v/v) at 37°C under gentle shaking.

Progesterone or gestonerone capronate were added to the incubate so that a final concentration of 0·2, 2,20 or 200 μM/l was reached. In five additional tissue specimens, H^3-testosterone was employed in increasing amounts from 1·7 to 100 × 10^{-8} M with and without 1 and 4 μg gestonerone capronate or 1 and 2 μg cyproterone acetate per 2 ml medium.

Preincubation experiments

Tissue specimens from 6 prostatic adenomas were

* Schering AG, Berlin.

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incubated in the presence of progesterone or gestonerone capronate (0·2, 2,20, 200 μM/l) for 1 h without the tracer. Subsequently the medium was decanted, the tissue was rinsed in fresh medium and reincubated with medium containing 1·7 to 100 $\times$ 10^{-8} M/l H^3-testosterone, 2×10^{-2} M/l glucose and 1 mg/ml $NADPH_2$.

In vivo *experiments*

Nine patients undergoing suprapubic, transvesical prostatectomy received 35–60 min prior to the operation 400 μCi H^3-testosterone dissolved in a 20% ethanol solution i.v. 5, 15 and 30 min after the injection and at the time of enucleation of the prostatic adenoma 10 ml blood were drawn into tubes containing heparin. In addition to the adenoma a sample of rectus abdominis muscle and of the urinary bladder was collected.

Five of these patients received five days prior to the transvesical prostatectomy 200 mg gestonerone capronate q.d.i.m. up to a total of 1000 mg. The operation was performed 24 h after the last injection.

Steroid extraction and measurement

After the *in vitro* incubation the reaction was terminated by adding chloroform–methanol (2:1; v/v). Following evaporation 2 ml normal saline (4°C) plus 10,000 d.p.m. C^{14}-testosterone (S.A. 51 mCi/mM) was added as internal standard. The tissue was fragmented by means of the Ultra-Turrax (IKA-Werke, Stauffen, West Germany). The steroids were extracted and backwashed according to the method of Folch *et al.*[7]. The extraction of steroids from plasma, from prostatic adenoma, muscular and bladder tissue in the *in vivo* experiments was accomplished similarly.

The total extract was dissolved in chloroform and spotted together with 10 μg C_{19} reference steroids on silica gel F 254 sheets (E. Merck, Darmstadt, West Germany). These sheets were developed once in a tank saturated with chloroform–methanol (97·5:2·5, v/v) and once in dichloromethane–ethyl acetate (80:20; v/v) at room temperature. The 4-steroids were localized by the aid of U.V.-light with a wave length of 254 nm. Subsequently the sheets were radiochromatographed with a thin layer chromatogram scanner II (Firma Berthold, Bad Wildungen, West Germany). Finally the sheets were sprayed with acetic acid–sulfuric acid–anisaldehyde (100:2:1; v/v) and stained by heating up to 120°C over 20 min. The steroids were identified by recrystallization to a constant specific activity [8]. The stained steroid areas consistent with the radiochromatographic peak were cut into counting vials. After adding 20 ml toluene with 0·4% diphenyloxazole and 10% methanol the radioactivity was assessed in a liquid scintillation spectrophotometer (Model 3380, Packard, U.S.A.).

The background c.p.m. was subtracted and d.p.m. was calculated after correction for quenching (accounting approximately for 35%), for C^{14}-interference in the H^3-channel (approx. 23% spillover of the C^{14}-testosterone standard), and for the recovery of the C^{14} internal standard, being between 55 and 65%.

RESULTS

The *in vivo*-experiments after i.v. injection of 400 μCi H^3-testosterone revealed a selective uptake of radioactivity into the prostatic adenoma tissue removed surgically as compared to the musculature of the urinary bladder and through the rectus abdominis muscle. When the total radioactivity found in adenomatous tissue is taken as 100% the relative radioactivity of the urinary bladder musculature measures 43% and in rectus abdominis muscle 20% (Fig. 1).

In adenomatous tissue the total radioactivity is distributed predominantly among the major metabolites 5α-dihydrotestosterone and 5α-androstanediol. Whereas 72% of testosterone remained unmetabolized in urinary bladder musculature and approx. 55% in rectus abdominis muscle, the prostatic adenoma tissue contains only 12% unmetabolized H^3-testosterone (Fig. 1). In this latter tissue 80% of the total radioactivity is H^3-5α-dihydrotestosterone and approx. 5% H^3-5α-androstanediol. The percentage of 5α-dihydrotestosterone in urinary bladder muscle is 25%, and in rectus abdominis muscle, 22% of the total radioactivity.

After the addition of progesterone at the lowest concentration used, a decrease in the formation of 5α-reduction products to approx. 50% of the control value was found. This inhibition grows with increasing concentration of progesterone. Simultaneously the

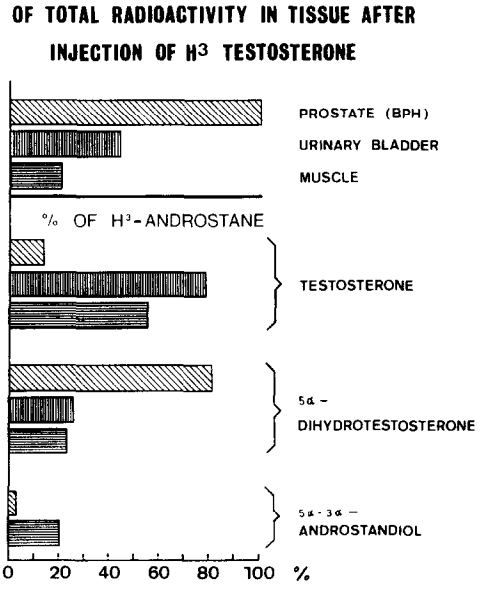

Fig. 1. *In vivo* experiments: Percentage of the total radioactivity recovered which is distributed among the individual androstanes in the tissue of the prostatic adenoma, the musculature of the urinary bladder and of the rectus abdominis muscle after i.v.-injection of 400 μCi H^3-testosterone. The total radioactivity in the prostatic adenoma tissue was set as 100%.

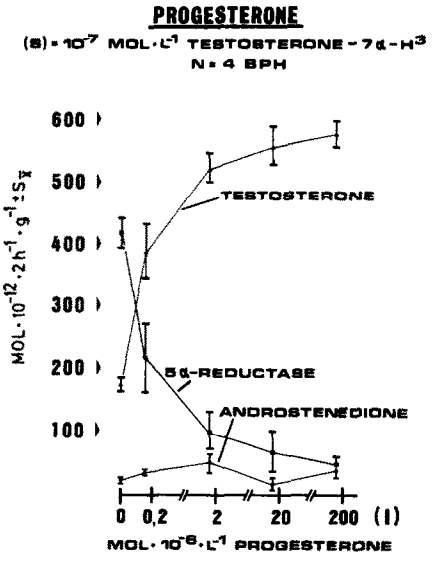

Fig. 2. Graph indicating the results of incubation after the administration of progesterone in an increasing concentration. With the progressive decrease of the 5α-reduction products, the level of unmetabolised testosterone rises.

fraction of unmetabolized testosterone rose. The oxydation of testosterone to androstenedione is not affected significantly (Fig. 2).

The administration of gestonorone capronate impedes testosterone reduction too. The oxydation pathway remains uninfluenced. This inhibition of the formation of 5α-reduction products is present already at the lowest dose of gestonorone capronate and is augmented progressively with increasing gestonorone capronate concentration (Fig. 3).

Adding cyproterone acetate in a concentration of 1 and 2 μg to the medium containing testosterone from $1{\cdot}7 \times 10^{-8}$ to 100×10^{-8} mol/l does not suppress the formation of 5α-dihydrotestosterone

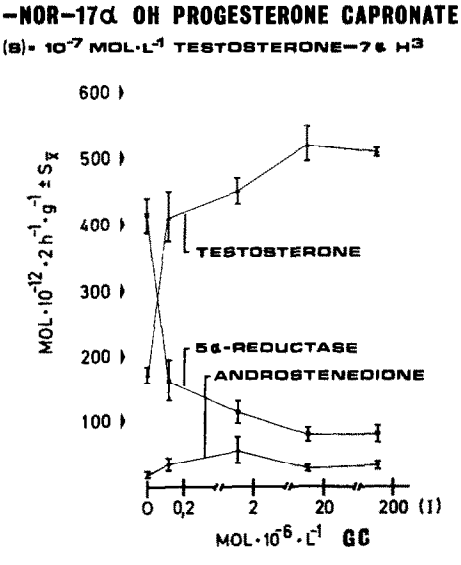

Fig. 3. Graph indicating the incubation experiments after the addition of gestonerone capronate in an increasing concentration.

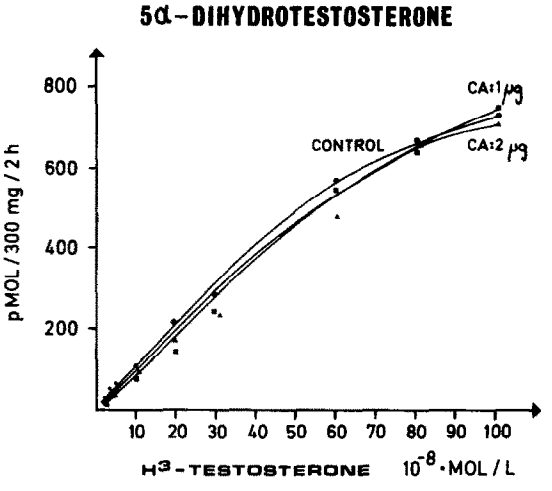

Fig. 4. Result of the incubation experiments with H^3-testosterone in an increasing concentration without and with the administration of cyproterone acetate (1 and 2 μg). The recovered amount of 5α-dihydrotestosterone is plotted as a function of the substrate concentration.

(expressed as pmol/300 mg tissue/2 h) (Fig. 4), neither does cyproterone acetate affect the rate of appearance of 5α-androstanediol (Fig. 5).

The H^3-testosterone turnover can be assessed in calculating the degree of efficiency in the presence and absence of steroids to be tested. This was done according to the following formula:

$$\text{Degree of efficiency} = \frac{\text{testosterone added} - \text{testosterone recovered}}{\text{testosterone added}}$$

In the control incubations the degree of efficiency declines paralleling the increasing substrate concentration. After the addition of μg gestonorone capronate the degree of efficiency drops from 1 in the control to 0·74 when the concentration of H^3-testosterone added was 34 pmol (9·8 ng/2 ml). After the administration of 4 μg gestonorone capronate the

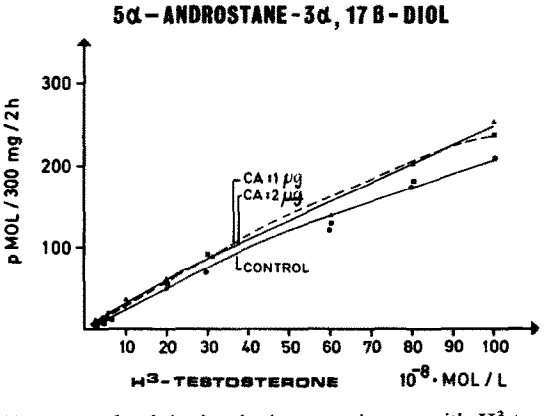

Fig. 5. Result of the incubation experiments with H^3-testosterone in an increasing concentration without and with cyproterone acetate in a concentration of 1 and 2 μg. The amount of 5α-androstanediol recovered is plotted as a function of the substrate concentration.

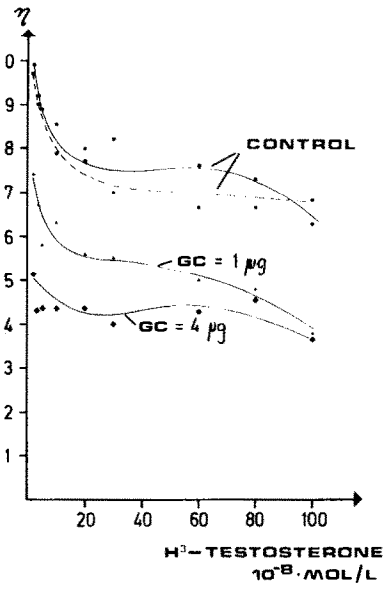

Fig. 6. Degree of efficiency in relation to increasing testosterone concentration without and with 1 and 4 μg gestonorone capronate.

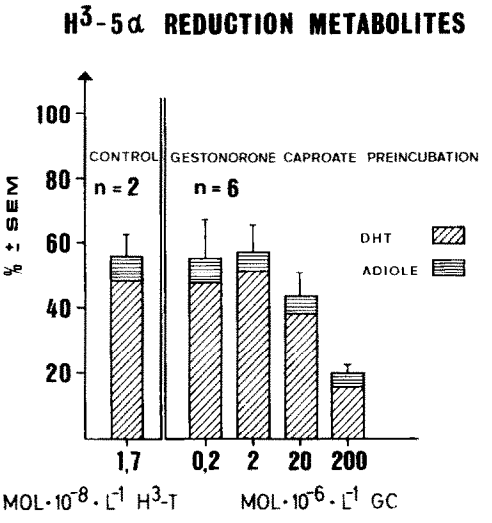

Fig. 8. Graph indicating the results after pre-incubation with gestonorone capronate. There is no significant difference as to the rate of appearance of 5α-reduction products in comparison to the control-incubations when a concentration of gestonorone capronate of 0·2, 2 and 20 × 10^{-6} mol/l is used.

initial degree of efficiency did not exceed even 0·5. The curve practically levels off at this amount of gestonorone capronate (Fig. 6).

After pre-incubation with progesterone in a concentration of 0·2 and 2 × 10^{-6} mol/l the formation of 5α-reduction products and oxydation products of testosterone is not significantly altered in comparison to the control incubations, although there is a certain inhibitory effect upon the appearance of metabolites. In the two higher progesterone concentrations the formation of H^3-5α-metabolites drops to minimal values (Fig. 7).

Using gestonorone capronate in the preincubation experiments did not significantly affect the formation of 5α-reduction products in the two lowermost concentrations tested. However, when performing a pre-incubation with the two higher amounts of testosterone capronate the impairment of testosterone turnover is significant (Fig. 8).

In vivo *studies*

After i.v.-injection of 400 μCi H^3-testosterone the total radioactivity in plasma of the control patients rises within the first 5 min rapidly, but there is a subsequent progressive disappearance up to the time of prostatectomy. The curve of the concentration of total radioactivity in patients pretreated with gestonorone capronate has a similar course (Fig. 9). After isolation of H^3-testosterone and its radiometabolites in plasma the control group displays after 5 min a higher H^3-testosterone level as compared to the gestonorone

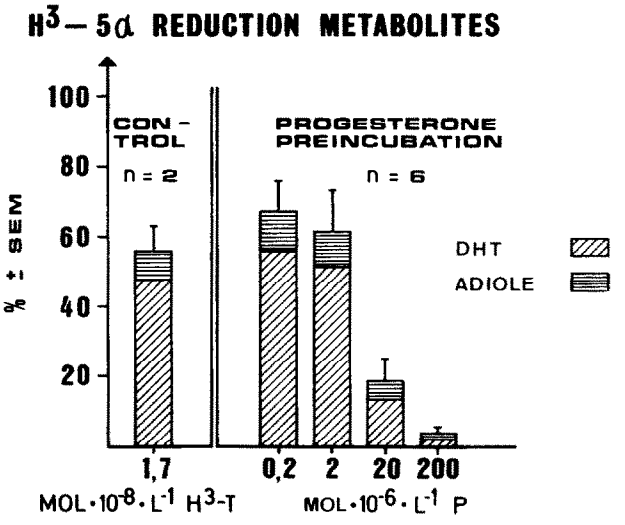

Fig. 7. Graph indicating the results after preincubation with progesterone in comparison to the control incubations. After the addition of 0·2 and 2 × 10^{-6} mol/l progesterone no significant difference as to the formation of 5α-reduction products could be found which is present, however, at the two higher progesterone concentrations.

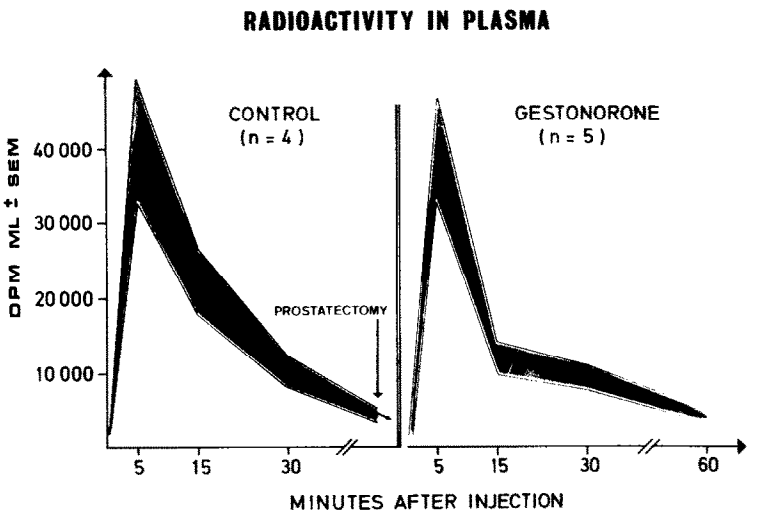

Fig. 9. Total radioactivity in the plasma of both groups of patients not displaying a significant difference.

capronate group. The 5α-dihydrotestosterone concentration in the control patients at 5 min is significantly lower than in the pretreated patients. After 30 min this relationship is reversed ($P < 0{\cdot}05$, Fig. 10). The course of the 5α-dihydrotestosterone concentration is similar to the course of the total H^3-5α-reduction products in plasma (Fig. 11). The amount of H^3-androstenedione does not display significant differences in the two groups of patients (Fig. 11). The total radioactivity recovered in prostatic adenoma tissue of the control patients is approx. 30 times higher than in the adenoma tissue of the pretreated patients or in the muscular tissue of either patient group. Gestonorone capronate pretreatment does lower significantly the amount of H^3-testosterone recovered, of its 5α-reduction metabolites, and of H^3-androstenedione in prostatic adenoma tissue as compared to the same tissue of the control patients (Fig. 12). The percentage of the H^3-androgen distribution in the prostatic adenoma tissue of either group of patients is, however, not significantly altered (Table 1).

DISCUSSION

Our *in vitro*- as well as *in vivo*-experiments regarding the uptake and metabolism of testosterone in human prostatic adenoma confirm the findings of other investigators [2, 4, 9, 10, 11, 12, 14, 15]. We have been able to demonstrate that testosterone is taken up selectively into human prostatic adenoma tissue and metabolized almost exclusively to 5α-dihydrotestosterone and 5α,3α-androstanediol. The oxidation pathway leading to the formation of androstenedione is only of minor importance.

Progesterone and its synthetic derivative gestonorone capronate impede significantly the formation of these 5α-reduction products *in vitro*. The results of the experiments after preincubation or simultaneous incubation of human prostatic adenoma tissue with progesterone or gestonorone capronate in the pres-

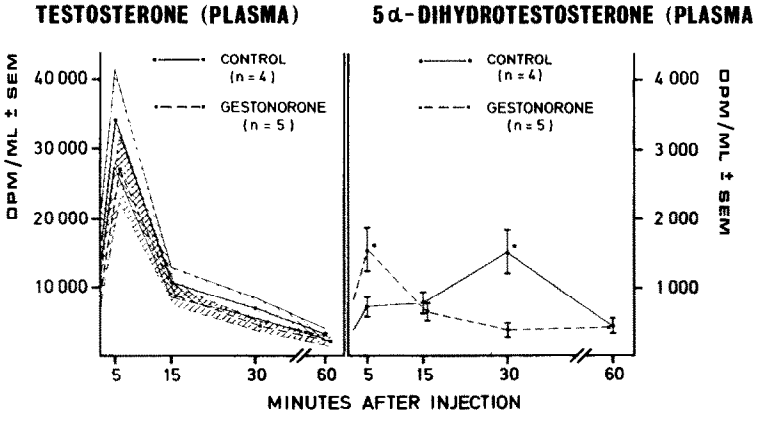

Fig. 10. The left panel represents a graph reflecting the H^3-testosterone activity in the plasma of both groups of patients. After 5 min there was a higher activity in the control group as compared to the pretreated group of patients. In the right panel the 5α-dihydrotestosterone activity in plasma is depicted. The 5 min-values of the pretreated patients are significantly higher than the untreated. This relationship is reversed after 30 min.

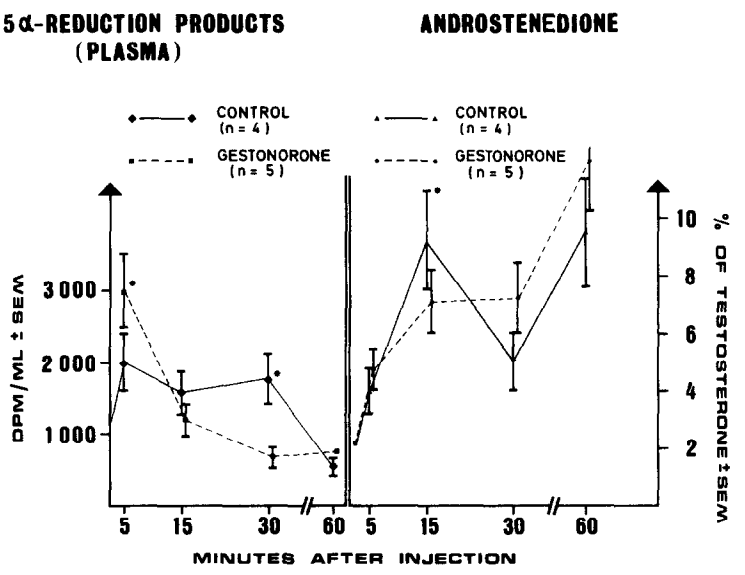

Fig. 11. In the left panel the radioactivity of the 5α-reduction products (5α-dihydrotestosterone and 5α, 3α-androstanediol) in the plasma of both group of patients is depicted. After 5 min the values in the control group are significantly lower than those pretreated. After 30 min this relationship is reversed. In the right panel the androstenedione values in the plasma are depicted. The curves are parallel in both groups of patients.

ence of H^3-testosterone reveal a clear impairment of H^3-testosterone metabolism in the prostatic cell to these gestagens. It is apparent that these gestagens are preferentially incorporated into the prostatic cell as compared to testosterone itself. The effect of progesterone is more pronounced and longer lasting than the effect of gestonorone capronate. Possibly the intracellular progesterone degradation occurs slower than gestonorone capronate or the gestagens are retained within the prostatic cell attached to a receptor. The degradation and the retention of these steroids within the cell is clearly dependent upon their concentration. These findings are in accordance with the *in vitro* studies using the rat prostate by Frederiksen and Wilson[16], Stern and Eisenfeld[17], Massa and Martini[18], Tveter and Aakvaag[19]. After the incubation of human prostatic adenoma tissue with H^3-testosterone and cyproterone acetate in a concentration of 1 and 2 μg, respectively, no inhibition of the formation of 5α-dihydrotestosterone or 5α-androstanediol was found. Obviously cyproterone acetate does not influence testosterone reduction in the prostatic cell, which has been demonstrated for the rat prostate by Neal[20]. *In vitro-* and *in vivo*-animal experiments did demonstrate its effectiveness. Grigorescu and Villee[21] found decreased RNA synthesis in the rat prostate and vesicular glands studying adult, castrated rats *in vivo* when dihydrotestosterone and cyproterone acetate was given at the same time. The inhibition of the conjunction between

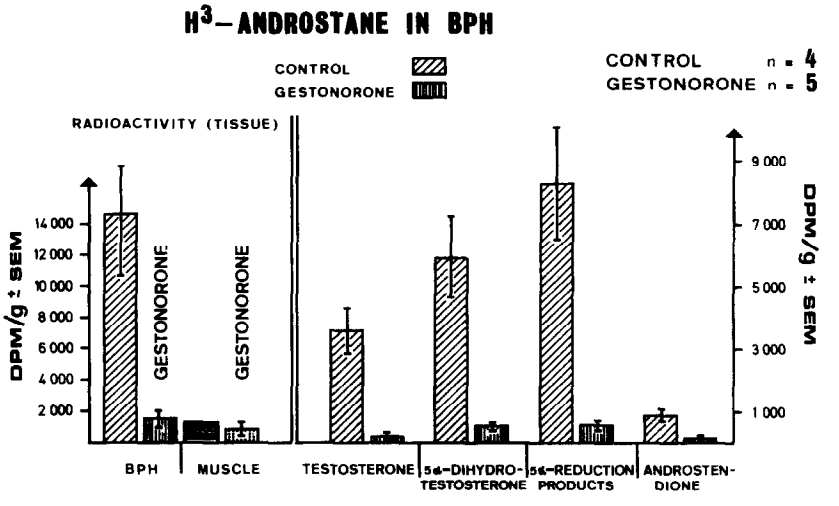

Fig. 12. Graphic representation of the total radioactivity recovered in the prostatic adenoma tissue and in muscular tissue of both groups of patients. On the right side the H^3 androstanes in prostatic adenoma tissue of both groups of patients are depicted.

Table 1. Per cent distribution of the H^3-androstanes in prostatic adenoma tissue of both groups of patients. There is no significant difference of the individual androstanes and of the 5α-reduction products without and with gestonorone pretreatment in both groups of patients

% OF H^3-ANDROGEN IN BPH

	T	5α DHT	5α 3α ADIOL	ANDROSTENE DIONE
CONTROL n = 4	26%	43%	18%	6%
GESTONORONE n = 5	18%	67%	10%	5%

dihydrotestosterone and an intra nuclear receptor of the rat prostate cell has been discovered by Walsh and Korenman[22]. Up to date there have been no similar studies using human prostatic adenomas, since the problem of isolation of nuclear receptors from prostatic adenoma cells has not been solved [23].

The findings in our *in vivo* experiments after pretreatment of patients with gestonorone capronate in a total dose of 1000 mg support the results obtained through the *in vitro* studies. It is interesting to note that the concentration of H^3-testosterone and its 5α-reduction products varies at different time intervals in the plasma of the control and pretreated patients. The initial 5α-reduction of H^3-testosterone occurs in the liver. As evidenced by the studies of Gordon *et al.*[24] gestagen derivatives stimulate the 5α-reductase activity of the liver. This would explain the higher level of 5α-reduction products in plasma of the pretreated patients as compared to the control group. At a later time interval (30 min) the formation of androgen metabolites in peripheral tissue is overt. Apparently gestonorone capronate hampers the formation of 5α-reduction products in the target organs. Thus the level of 5α-reduction products in the plasma of the control patients at 30 min exceeds the value of the pretreated group. There is no difference in the course of the curve of H^3-androstenedione in plasma in either group of patients reflecting a peripherally increasing oxidation of H^3-testosterone, however, not being quantitatively relevant. The total radioactivity recovered from prostatic adenoma tissue after gestonorone capronate pretreatment is significantly lower than the radioactivity of the control group and is in the range of the amount found with and without pretreatment in muscular tissue. It may be deduced from these findings that H^3-testosterone is taken up selectively into prostatic adenoma tissue as compared to muscular tissue and that gestonorone capronate affects specifically the uptake of H^3-testosterone into the prostatic adenoma.

Furthermore, there is a significant difference in the concentration of H^3-testosterone metabolites in prostatic adenoma tissue with and without gestonorone capronate pretreatment. The amount of H^3-testosterone recovered as well as 5α-dihydrotestosterone, androstenedione and the total 5α-reduction products in the untreated group significantly exceed the amount of the gestonorone capronate pretreated group. When comparing the per cent-distribution of H^3-androgens in prostatic adenoma tissue no difference was noted between the control group and the pretreated group of patients. Apparently gestonorone capronate did not affect the turnover of testosterone to dihydrotestosterone, but is influencing already the uptake of testosterone into the prostatic cell.

This study does not allow us to distinguish whether gestonorone capronate is preferentially incorporated into the prostatic adenoma cell instead of testosterone and whether it blocks the influx of testosterone in building up a steroid gradient, or whether this substance is bound specifically to a cytosol–receptor instead of testosterone.

REFERENCES

1. Mostofi F. K.: In *Urology* (Edited by M. F. Campbell and J. H. Harrison). Saunders Company, Philadelphia (1970) 1065.
2. Moore R. J. and Wilson J. D.: *J. biol. Chem.* **247** (1972) 958–967.
3. Fang S., Anderson K. D. and Liao S.: *J. biol. Chem.* **244** (1969) 6584–6595.
4. Siiteri P. and Wilson J. D.: *J. clin. Invest.* **49** (1970) 1737–1745.
5. Gloyna R. E., Siiteri P. K. and Wilson J. D.: *J. clin. Invest.* **49** (1970) 1746–1753.
6. Becker H., Buric L., Petersen C. and Voigt K. D.: *Acta endocr., Copenh.* **152** (1971) 28.
7. Folch J., Lees M. and Sloane-Stanley G. H.: *J. biol. Chem.* **226** (1957) 497.
8. Altwein J. E., Rubin A., Klose K., Knapstein P. and Orestano F.: *Urologe A* **13** (1974) 41–46.
9. Moore R. A.: In *Endocrinology of Neoplastic Disease* (Edited by G. H. Twombly and G. T. Pack). Oxford University Press, New York (1947) 194.
10. Farnsworth W. E. and Brown J. R.: In *National Cancer Institute Monogr.* **12** (1963) 323.
11. Becker H., Kaufmann J., Klosterhalfen H. and Voigt K. D.: *Acta endocr., Copenh.* **71** (1972) 589–599.
12. Briggs M. H. and Briggs M.: *J. clin. Endocr. Metab.* **36** (1973) 600–604.
13. Giorgi E. P., Stewart J. C., Grant J. K. and Scott R.: *Biochem. J.* **123** (1971) 41–55.
14. Giorgi E. P., Stewart J. C., Grant J. K. and Shirley J. M.: *Biochem. J.* **126** (1972a) 107–121.
15. Giorgi E. P., Grant J. K., Stewart J. C. and Reid J.: *J. Endocr.* **155** (1972b) 421–439.
16. Frederiksen D. W. and Wilson J. D.: *J. biol. Chem.* **246** (1971) 2584–2593.
17. Stern J. M. and Eisenfeld A. J.: *Endocrinology* **88** (1971) 1117–1125.
18. Massa R. and Martini L.: In *Hormones and Antagonists* (Edited by P. O. Hubinont, S. M. Hendeles and P. Preumont). Karger, Basel (1972) 253–270.
19. Tveter K. J. and Aakvaag A.: *Endocrinology* **85** (1969) 683–689.
20. Neal G. E.: In *Advances in the Study of the Prostate* (Edited by D. C. Williams, M. H. Briggs and M. Staniford). Verlag William Heinemann Medical Books Ltd., London (1970).
21. Grigorescu A. and Villee C. A.: *Biochim. biophys. Acta* **319** (1973) 165–173.
22. Walsh P. and Korenman S. G.: *J. Urol.* **105** (1971) 850–857.
23. Mainwaring W. I. P. and Milroy E. J. G.: *J. Endocr.* **57** (1973) 371–384.
24. Gordon G. G., Southern L. A., Tochimoto S., Olivo J., Altmann K., Rand J. and Lemberger L.: *J. clin. Endocr. Metab.* **30** (1970) 449–456.