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# TESTOSTERONE AND PROGESTERONE METABOLISM AND THEIR INTERACTION IN THE HUMAN HYPERPLASTIC PROSTATE

R. F. MORFIN, J.-P. BERCOVICI, J.-F. CHARLES and H. H. FLOCH

Laboratoire de Biochimie, Faculté de Médecine, B.P. 815, 29279 Brest, Cedex, France

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SUMMARY

Studies *in vitro* of progesterone and testosterone metabolism in seven cases of human benign prostatic hyperplasia (BPH) were carried out. Major progesterone metabolites identified before and after $CrO_3$-oxidation were 5α-pregnanedione, 3β-hydroxy-5α-pregnan-20-one and 5α-pregnane-3,6,20-trione respectively.

Incubations of [$^{14}$C]-testosterone in the presence of 0·7, 3·6 and 7·1 μM non-radioactive progesterone resulted in a decrease of the total testosterone 5α-reduced metabolites and a decrease of the 5α-dihydrotestosterone/5α-androstanediols ratios (3α + 3β).

Incubations of [$^{14}$C]-progesterone in the presence of 0·7, 3·6 and 7·1 μM non-radioactive testosterone led to similar results where the total progesterone 5α-reduced metabolites and the 5α-pregnanedione/3β-hydroxy-5α-pregnan-20-one ratios decreased significantly.

These findings suggested that interactions of progesterone with testosterone metabolism pathways are limited to a direct competition for the 4-en-3-oxo-steroid 5α-reductase.

## INTRODUCTION

Both studies *in vivo* and *in vitro* have shown that testosterone metabolism in human prostates with benign prostatic hyperplasia (BPH) is mainly reductive and leads to 17β-hydroxymetabolites [1–3].

Several investigators have proved these steroids to have an important bearing on the growth maintenance and function of male target tissues to androgens. Many lines of evidence indicate that among the 5α-reduced-17β-hydroxymetabolites of testosterone, 5α-dihydrotestosterone is necessary for testosterone action at the target site [4, 5] and that BPH is associated with a higher tissue content of this steroid [6].

This evidence implies that extensive reduction of testosterone or ineffective further metabolism of 5α-dihydrotestosterone are processes leading to the accumulation of 5α-dihydrotestosterone in BPH.

On the other hand progesterone has been shown to exert through its conversion to 5α-pregnanedione an inhibition of the 5α-reduction of testosterone to 5α-dihydrotestosterone in human skin, a male target tissue for androgens [7, 8]. Progesterone has been shown to compete with testosterone [9] as substrate for the 5α-reductase of rat ventral prostate. More recently [10] caproate of 17α-hydroxy-progesterone or of progesterone has been shown both *in vivo* and *in vitro* to exert an inhibitory effect on the formation and accumulation of 5α-reduced metabolites of testosterone in human BPH. Progesterone may therefore prove to be a useful agent for the treatment of human BPH.

Characterization and in most cases identification of the principal metabolites of testosterone in human BPH tissues have been accomplished both *in vitro* [1, 11–14] by incubation experiments and *in vivo* [2, 3]. Thus the presence of prostatic 5α-reductase, 17β-hydroxysteroid oxidoreductase, 3α-hydroxysteroid oxidoreductase and 3β-hydroxysteroid oxidoreductase was demonstrated. More recently we have published evidence for 6ξ- and 7ξ-hydroxylation after incubation of labelled 5α-androstane-3β,17β-diol for one hour with minced BPH tissue [15]. Acevedo and Goldzieher[16] have studied the metabolism of progesterone in BPH tissues, providing evidence for 5α-reduction, 20α-oxidoreduction and 6β-hydroxylation of the substrate.

In this paper we shall present results of our investigation of testosterone and progesterone metabolism in surgical specimen of human BPH. Preliminary data on this work have been published [17].

## EXPERIMENTAL

### *Steroids and solvents*

Carbon-14-labelled testosterone (57·5 mCi/mmol) and progesterone (29·3 mCi/mmol) were purchased from New England Nuclear Corporation (Boston, U.S.A.) and C.E.A. (France) respectively. Purification of the labelled substrates was carried out by thin-layer chromatography (t.l.c.) and purity checked by crystallization to constant specific activity after addition of

The following trivial names were used in this paper: 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; 5α-pregnanedione, 5α-pregnane-3,20-dione; 5α-reductase, 4-en-3-oxosteroid: NADPH 5α-oxidoreductase.

authentic carrier. Carrier steroids: testosterone, progesterone, 5α-dihydrotestosterone, 5α-pregnanedione, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 3β-hydroxy-5α-pregnan-20-one, 3α-hydroxy-5α-pregnan-20-one and 5α-pregnane-3,6,20-trione were commercial samples. Purity of carrier steroids was checked by t.l.c. and gas–liquid chromatography. All solvents were distilled before use and controlled by gas–liquid chromatography.

*Collection of tissues*

Surgical samples of human BPH obtained by the transvesical approach were immediately put in a beaker surrounded with crushed ice. Pathological examination on pieces of the tissue confirmed the diagnosis of benign prostatic hyperplasia. As soon as obtained the tissues were brought in a cold room (4°C) where all subsequent operations prior to incubation were carried out.

*Incubation procedure*

Testosterone and progesterone substrates were dried under nitrogen at the bottom of incubation tubes. Tissues were cut with scissors and minced with a Harvard tissue press through a sieve with 1 mm dia holes. Minces were weighed in 200 mg lots on a Mettler microbalance and positioned on the inner side wall of incubation tubes. Immediately before incubation 5 ml of 0·067 M phosphate buffer (pH 7·4) with or without 1 mg of NADPH were poured in the incubation tube and used to rinse down the tissue minces which were further dispersed with a glass rod.

Incubations were carried out for 10 min at 37°C. The time was increased to 60 min for preparative incubations. All incubations were stopped by addition of chilled acetone (10 ml) and cooling of the tubes at −20°C.

Further extraction with ethyl acetate (10 ml, 4 times) gave a crude extract which was concentrated under nitrogen. Recoveries of the $^{14}$C-labelled steroids (92·5–98%) were computed after determination of the radioactivity in portions of the crude extracts.

*Thin-layer chromatography*

Separation of radiometabolites contained in the crude extract was achieved by t.l.c. on silica gel G. Testosterone and progesterone metabolites were separated after two developments in benzene/95% ethanol -9:1, v/v) or one development in chloroform–methanol (99:1, v/v). Separation of 5α-androstane-3α,17β-diol from the 3β epimer was achieved on alumina with one development in benzene/95% ethanol (97:3, v/v). Metabolites were located on the chromatograms by autoradiography, eluted from the gel and identified by crystallization to constant S.A. after addition of authentic carrier as previously described [18].

## RESULTS

*Metabolism of progesterone*

Preparative incubations contained 2 g of minces in 50 ml of 0·5 μM progesterone in buffer with 10 mg NADPH and were incubated for 60 min. After t.l.c. of the extract radioactive zones isopolar with progesterone, 5α-pregnanedione, 3β-hydroxy-5α-pregnan-20-one and 3α-hydroxy-5α-pregnan-20-one were recovered and authentic carriers added before crystallization to constant specific activity. In addition the more polar metabolites were eluted and oxidized with chromium trioxide. The resulting mixture contained a major quantity of labelled derivative isopolar with 5α-pregnane-3,6,20-trione.

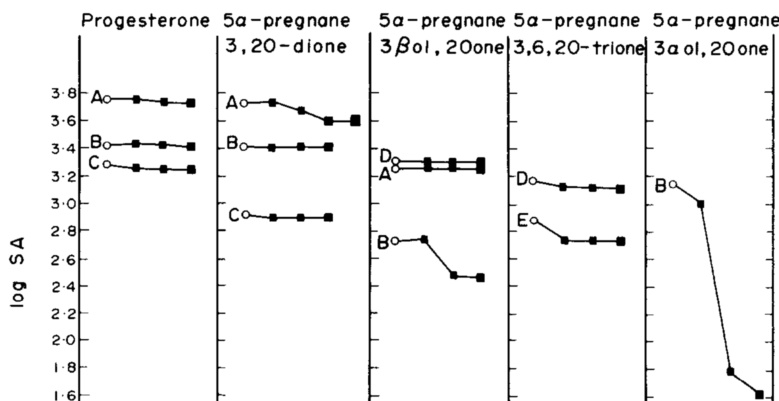


Fig. 1. Crystallization to constant specific activity (S.A.) of progesterone radiometabolites obtained from incubations with minces of human BPH. In each case the log S.A. of the crystals is expressed from left to right for consecutive crystallizations of the initial solution containing authentic carrier. In all cases the first, second, third and eventually fourth crystallization were carried out in ethyl acetate/n-hexane, acetone/n-hexane, acetone/water and methanol/water respectively. Letters refer to the prostates from which metabolites were derived. 3β-hydroxy-5α-pregnan-20-one = 5α-pregnane-3βol,20-one, 3α-hydroxy-5α-pregnan-20-one = 5α-pregnane-3αol,20-one.

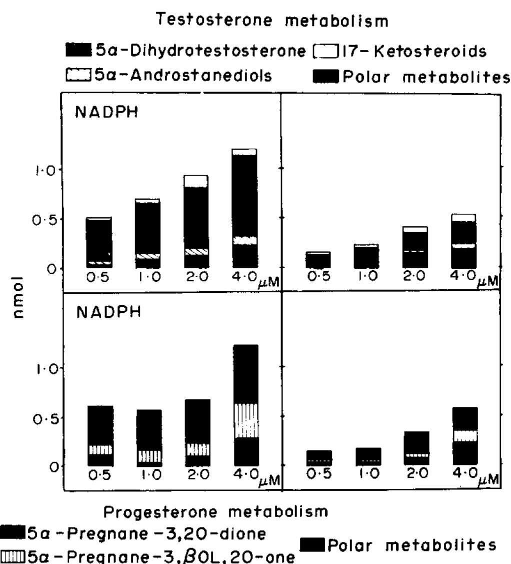


Fig. 2. Yields of metabolism after incubation for 10 min in 5 ml of [4-$^{14}$C]-labelled testosterone and progesterone at different concentrations with 0·2 g minces of human prostatic hyperplasia (case B) in the presence or without exogenous NADPH.

Crystallization to constant specific activity in different cases (Fig. 1) proved the radiochemical purity of recovered progesterone and the identity of 5α-pregnanedione, 3β-hydroxy-5α-pregnan-20-one and 5α-pregnane-3,6,20-trione.

In one case the radioactivity associated with minor metabolites isopolar with 3α-hydroxy-5α-pregnan-20-one was not retained in the crystals after three consecutive crystallizations of the carrier.

*Testosterone and progesterone metabolism in the same tissue*

Incubations of minces (0·2 g) from seven cases of BPH were carried out with 0·5, 1·0, 2·0 and 4·0 μM progesterone or testosterone. Solubility of these steroids at such concentrations in the medium were tested in the absence of tissue minces; recovery of dissolved testosterone and progesterone ranged from 99·5 to 98·5% and from 97 to 95% respectively.

Metabolites of testosterone and progesterone were mainly 5α-reduced and their formation was favoured by the presence of NADPH. In each case quantities of testosterone transformed by the prostatic enzymes closely matched those obtained from progesterone in identical conditions. Since yields of metabolism differed from case to case, only one representative example was selected for illustration (Fig. 2).

*[$^{14}$C]-testosterone metabolism in the presence of non-radioactive progesterone*

Incubations were carried out with minces from seven cases of human BPH. The [$^{14}$C]-testosterone substrate was 0·5, 1·0, 2·0 and 4·0 μM in 5 ml of buffer. Such incubations in presence of NADPH were carried out with or without 0·7, 3·6 or 7·1 μM non-radioactive progesterone.

The testosterone metabolites were separated and measured and data from the seven cases were expressed in pmol and averaged. Thus testosterone transformations in the presence of NADPH were consistently decreased when progesterone was present in the medium (Table 1).

Striking evidence of progesterone competition with testosterone as a substrate for the prostatic 5α-reductase could be noticed when quantities of 5α-dihydrotestosterone and 5α-androstanediols were computed. Formation of unidentified polar metabolites of testosterone was less affected by increasing levels of progesterone. The 5α-dihydrotestosterone/5α-androstanediols ratios were computed to relate the progesterone-induced decreases in 5α-dihydrotestosterone formation with its further metabolism (Table 1). This ratio was lowered in all progesterone-supplemented incubations thus involving both inhibition of the 5α-reductase and maintenance of the 3ξ-hydroxysteriod dehydrogenases activities in the progesterone-induced decrease of 5α-dihydrotestosterone levels.

In four cases 5α-androstane-3α,17β-diol was separated from the 5α-androstane-3β,17β-diol. Ratios of the epimers (3α/3β) were measured and averaged (Table 1) and found not to be significantly changed by the presence of non-radioactive progesterone in the incubation media. Nevertheless amounts of incubated testosterone were found to affect these ratios which increased with the molarity of the substrate.

*[$^{14}$C]-progesterone metabolism in the presence of non-radioactive testosterone*

Incubations with 0·5, 1·0, 2·0 and 4·0 μM [$^{14}$C]progesterone were carried out with tissue minces from seven cases of human BPH in 5 ml of buffer. NADPH-supplemented digests were incubated in the presence or without 0·7, 3·6 or 7·1 μM non-radioactive progesterone.

The labelled progesterone metabolites were separated and measured and data from the seven cases were expressed in pmoles and averaged (Table 2). In all cases the progesterone substrate was transformed mainly to 5α-reduced metabolites. This transformation was decreased to a large extent when non-radioactive testosterone was present in the medium. Thus quantities of major radiometabolites (5α-pregnanedione and 3β-hydroxy-5α-pregnan-20-one) were lowered by increasing amounts of testosterone. Levels of polar metabolites which contained 3ξ,6ξ-dihydroxy-5α-pregnan-20-one were affected to a lesser extent.

The 5α-pregnanedion/3β-hydroxy-5α-pregnan-20-one ratios were computed to relate the testosterone-induced decreases in 5α-pregnanedione formation with its further metabolism (Table 2). This ratio was lowered in most of the testosterone-supplemented incubations thus involving both inhibition of the 5α-reductase and maintenance of the 3β-hydroxy dehyd-

Table 1. [4-$^{14}$C]-testosterone metabolites (in pmol) formed at different concentrations in the presence or without exogenous NADPH and varying quantities of non-radioactive progesterone

| Radioactive substrate | 0·5 μM Testosterone | | | | | 1·0 μM Testosterone | | | | | 2·0 μM Testosterone | | | | | 4·0 μM Testosterone | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NADPH 1 mg) | − | + | + | + | + | − | + | + | + | + | − | + | + | + | + | − | + | + | + | + |
| 0·7 μM Progesterone | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − |
| 3·6 μM Progesterone | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − |
| 7·1 μM Progesterone | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + |
| Total metabolites | 134 | 531 | 325 | 191 | 184 | 312 | 738 | 602 | 494 | 345 | 436 | 1073 | 921 | 730 | 540 | 988 | 1514 | 1413 | 1272 | 1474 |
| ± S.E.M. | ±9 | ±35 | ±24 | ±19 | ±21 | ±37 | ±61 | ±38 | ±99 | ±51 | ±62 | ±116 | ±82 | ±73 | ±54 | ±349 | ±167 | ±137 | ±209 | ±271 |
| 5α-Dihydrotestosterone | 76 | 399 | 232 | 105 | 74 | 116 | 467 | 339 | 203 | 128 | 127 | 603 | 478 | 331 | 234 | 216 | 861 | 778 | 503 | 498 |
| ± S.E.M. | ±9 | ±29 | ±20 | ±13 | ±4 | ±17 | ±63 | ±39 | ±12 | ±30 | ±16 | ±74 | ±47 | ±31 | ±26 | ±18 | ±70 | ±87 | ±32 | ±72 |
| 5α-Androstanediols | 8 | 54 | 39 | 29 | 27 | 40 | 79 | 59 | 71 | 51 | 62 | 137 | 118 | 108 | 74 | 268 | 223 | 153 | 187 | 220 |
| ± S.E.M. | ±1 | ±8 | ±16 | ±6 | ±6 | ±11 | ±14 | ±10 | ±24 | ±11 | ±16 | ±32 | ±25 | ±36 | ±14 | ±194 | ±64 | ±36 | ±47 | ±52 |
| Polar metabolites | 37 | 60 | 58 | 45 | 67 | 125 | 149 | 138 | 136 | 127 | 194 | 257 | 227 | 232 | 185 | 469 | 415 | 335 | 386 | 444 |
| ± S.E.M. | ±4 | ±11 | ±12 | ±7 | ±15 | ±28 | ±28 | ±27 | ±26 | ±15 | ±45 | ±52 | ±46 | ±49 | ±33 | ±139 | ±72 | ±35 | ±81 | ±67 |
| 5α-Dihydrotestosterone / 5α-Androstanediols | 9·5 | 7·4 | 5·9 | 3·6 | 2·7 | 2·9 | 5·9 | 5·7 | 2·9 | 2·4 | 2·0 | 4·4 | 4·1 | 3·1 | 3·2 | 0·8 | 3·9 | 5·1 | 2·7 | 2·3 |
| 5α-Androstane-3α,17β-diol / 5α-Androstane-3β,17β-diol | 5·5 | 6·0 | 6·2 | 6·4 | 6·7 | 6·1 | 9·5 | 8·6 | 8·5 | 7·3 | 11·4 | 14·5 | 12·1 | 12·5 | 11·1 | 14·8 | 9·8 | 9·0 | 9·4 | 9·1 |

Table 2. [4-$^{14}$C]-progesterone metabolites (in pmol) formed at different concentrations in the presence or without exogenous NADPH and varying quantities of non-radioactive testosterone

| Radioactive substrate | 0·5 μM Progesterone | | | | | 1·0 μM Progesterone | | | | | 2·0 μM Progesterone | | | | | 4·0 μM Progesterone | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NADPH (1 mg) | − | + | + | + | + | − | + | + | + | + | − | + | + | + | + | − | + | + | + | + |
| 0·7 μM Testosterone | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − |
| 3·6 μM Testosterone | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − |
| 7·1 μM Testosterone | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + | − | − | − | − | + |
| Total metabolites | 124 | 437 | 307 | 175 | 118 | 229 | 656 | 502 | 311 | 237 | 354 | 951 | 880 | 518 | 410 | 633 | 1476 | 1326 | 1003 | 723 |
| ± S.E.M. | ±25 | ±63 | ±43 | ±26 | ±20 | ±62 | ±49 | ±59 | ±28 | ±29 | ±56 | ±108 | ±103 | ±60 | ±63 | ±156 | ±278 | ±200 | ±55 | ±42 |
| 5α-Pregnanedione | 63 | 293 | 213 | 104 | 47 | 92 | 457 | 306 | 189 | 116 | 121 | 580 | 505 | 303 | 217 | 160 | 733 | 723 | 468 | 315 |
| ± S.E.M. | ±6 | ±49 | ±34 | ±18 | ±11 | ±9 | ±39 | ±34 | ±27 | ±13 | ±19 | ±81 | ±63 | ±30 | ±24 | ±28 | ±129 | ±123 | ±50 | ±35 |
| 5α-Pregnane-3βol,20-one | 14 | 71 | 37 | 29 | 19 | 27 | 98 | 65 | 56 | 42 | 54 | 123 | 133 | 129 | 109 | 102 | 266 | 240 | 233 | 170 |
| ± S.E.M. | ±3 | ±18 | ±8 | ±7 | ±5 | ±7 | ±21 | ±20 | ±14 | ±10 | ±16 | ±28 | ±22 | ±25 | ±43 | ±16 | ±30 | ±35 | ±38 | ±23 |
| Polar metabolites | 46 | 72 | 54 | 42 | 51 | 103 | 92 | 124 | 78 | 82 | 180 | 245 | 236 | 97 | 96 | 362 | 468 | 365 | 302 | 245 |
| ± S.E.M. | ±21 | ±20 | ±12 | ±12 | ±17 | ±50 | ±26 | ±56 | ±18 | ±35 | ±46 | ±62 | ±86 | ±27 | ±23 | ±115 | ±168 | ±83 | ±77 | ±54 |
| 5α-Pregnanedione / 5α-Pregnane-3βol,20-one | 4·5 | 4·1 | 5·8 | 3·6 | 2·5 | 3·4 | 4·7 | 4·7 | 3·4 | 2·8 | 2·2 | 4·7 | 3·8 | 2·3 | 2·0 | 1·6 | 2·8 | 3·0 | 2·0 | 1·9 |

rogenase activity in the testosterone-induced decrease of 5α-pregnanedione levels.

## DISCUSSION

We have identified the major prostatic metabolites of progesterone as 5α-pregnanedione and 3β-hydroxy-5α-pregnan-20-one. Such identifications confirm previous evidence for a 4-en-3-oxosteroid-5α-reductase active on progesterone [16] and imply the presence of a very active 3β-hydroxy-$C_{21}$ steroid dehydrogenase. The 6ξ-hydroxylation suggested by the identification of 5α-pregnane-3,6,20-trione is in good agreement with the determination of progesterone 6β-hydroxylation [16]. Such enzymic activities are identical with those which were demonstrated with testosterone in human BPH tissue [1, 13, 15]. Nevertheless it was noticeable that 3α-hydroxysteroid dehydrogenase activities were extensive in testosterone metabolism and could not be demonstrated with the progesterone substrate.

In identical conditions, human BPH preparations produced quantities of testosterone metabolites which were similar to those obtained with progesterone. In both cases metabolites were mainly 5α-reduced.

We have proved that 5α-reduction of testosterone was significantly decreased when progesterone was present in the incubation medium. Such a decrease has been recently established by Orestano *et al.*[10] with concentrations of progesterone ranging from 0·2 to 200 μM.

We have tried to investigate further the mechanisms involved in the decrease of 5α-reductase activity by carrying out cross experiments where we showed that the 5α-reduction of progesterone was significantly decreased when testosterone was present in the incubation medium. This type of inhibition pointed toward a direct competition between progesterone and testosterone as substrates for the 5α-reductase. Further metabolism of 5α-reduced progesterone and testosterone led to 3β-hydroxy-5α-pregnan-20-one and 5α-androstane-3β,17β-diol respectively, and suggest the involvement of a 3β-hydroxysteroid dehydrogenase. In that case 3β-hydroxy-5α-pregnan-20-one should interfere with the transformation of 5α-dihydrotestosterone into 5α-androstane-3β,17β-diol. Findings that such interference did not take place are the following: (i) The epimeric 5α-androstanediols ratios (3α/3β) were not significantly modified when increasing quantities of progesterone were present in incubations media (Table 1); (ii) The 5α-dihydrotestosterone/5α-androstanediols ratios were decreased when progesterone quantities were increased in incubations media (Table 1); (iii) The 5α-pregnanedione/3β-hydroxy-5α-pregnan-20-one ratios were decreased when increasing quantities of testosterone were added in incubations media (Table 2).

Furthermore we have used the described conditions to carry out in one case incubations of [$^{14}$C]-5α-dihydrotestosterone in the presence of increasing quantities of progesterone. Epimeric 5α-androstanediols ratios (3α/3β) were found constant and the largest decrease of the transformation to 5α-androstanediols was only of 15% in incubations with 7·1 μM progesterone.

An explanation of these findings could be that the enzyme involved in the formation of 3β-hydroxy-5α-pregnan-20-one is not active on 5α-dihydrotestosterone. In summary, progesterone actively competes with testosterone at the level of the prostatic 5α-reductase but does not significantly interfere with the transformations of 5α-dihydrotestosterone.

The resulting effect is to lower the content in 5α-dihydrotestosterone in the prostatic cells, since both 5α-dihydrotestosterone and 5α-pregnanedione are eliminated by enzymes involved in different pathways.

These findings may be of importance in the treatment of human BPH since such tissues have been shown to contain higher levels of 5α-dihydrotestosterone than normal [6] and that 5α-dihydrotestosterone plays an important role in the determination of prostatic cells growth [19]. Nevertheless it is not possible yet to ascertain that 5α-dihydrotestosterone alone is involved in the triggering of human BPH [20] nor to prove that progesterone would systemically only compete with the 5α-reductase.

For the study of competing substrates, this work has been carried out with relatively large quantities of testosterone when compared to those which are involved at the physiological level or in the mechanism of action of androgenic steroids at the target site [6, 21].

Even if the preferential uptake of progesterone by the human diseased tissue *in vivo* [10] is taken into account, treatment of BPH in man by progesterone would certainly involve much lower quantities of these hormones. Further investigations on the use of progesterone for treatment of human BPH taking these problems into account are in progress.

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