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# EFFECT OF PROGESTINS ON 17β HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN PROSTATE

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## SUMMARY

Addition of progesterone to the incubation medium increased the *in vitro* conversion of testosterone to androstenedione in rat prostatic tissue (as previously shown by Patwardhan and Lanthier, *J. Steroid Biochem.* **6** 137 (1975)) and in slices of human hyperplastic prostates. Direct measurements of 17β hydroxysteroid dehydrogenase activities showed that the increase is not due to a stimulation of the enzymatic activity. Therefore, the augmented conversion of testosterone to androstenedione most probably is a consequence of the inhibitory effect of progesterone on the conversion of testosterone to 5α dihydrotestosterone.

Prolonged administration of medroxyprogesterone acetate (Depo Provera®, Upjohn, 500 μg/100 g body weight, injected subcutaneously on alternate days, for a month) doubled the levels of prostatic 17β hydroxysteroid dehydrogenase activity in adult rats. However, consideration of the relatively small fractions of testosterone converted to androstenedione, or of 5α dihydrotestosterone converted to androstanedione, in rat prostates and human hyperplastic prostates, suggests that progestin-induced changes in 17β hydroxysteroid dehydrogenase activity may not be as important in the regulation of androgen action in prostatic tissue as they appear to be in the regulation of estrogenic action in human endometrium.


## INTRODUCTION

Regulation of the metabolism of steroid hormones at their target tissue may represent a physiologically and pharmacologically important mechanism of control of hormonal action. For example, induction of estradiol 17β dehydrogenase activity by progesterone influences the intracellular levels of estradiol in human endometrium [1].

Changes in the extent of prostatic conversion of testosterone (T) to 5α dihydrotestosterone (DHT), the effective androgen in this tissue [2–5], can be expected to affect the biologic action of circulating T. To a lesser extent, changes in the levels of 17β hydroxysteroid dehydrogenase (17β DH) activity in prostate may also influence the intracellular levels of DHT, both by acting on T and DHT. Patwardhan and Lanthier[6] reported that addition of some progestins to the medium in which minced rat prostates were incubated for 3 h with [$^3$H]-T increased the isotope incorporation into androstenedione (A) and reduced the formation of [$^3$H]-DHT. A reduction of the *in vitro* conversion of T to DHT in the presence of progesterone in human prostate was described by Jenkins and McCaffery[7].

The purpose of the work reported here was to determine whether the increased conversion of T to A promoted by progestins was due to a stimulation of the 17β DH activity, as suggested by Patwardhan and Lanthier, or was due to an inhibition of competing reactions. Direct measurements of the enzymatic activity were carried out in rat and human prostatic tissue incubated for 3 h in medium containing progesterone. In another series of experiments, 17β DH levels were measured in prostates from adult rats treated for a month with medroxyprogesterone acetate (MPA), using appropriate controls.

## MATERIALS AND METHODS

*Steroids.* [1,2-$^3$H]-Testosterone (50 Ci/mmol), [4-$^{14}$C]-androstenedione (50 mCi/mmol), and [4-$^{14}$C]-5α-dihydrotestosterone (50.6 mCi/mmol) were obtained from New England Nuclear Corp. The labeled steroids were purified by t.l.c. prior to use. Crystalline steroids were purchased from Steraloids.

*Progestin treatment.* Adult male rats (Sprague–Dawley) were either 60 days or 90 days old at the start of the injection schedule. Depo Provera (Upjohn, medroxyprogesterone acetate) was diluted with saline and injected subcutaneously on alternate days (500 μg/100 g body weight) for a month. Control rats were injected with saline only.

*Tissue.* Small portions of human hyperplastic prostates were obtained from patients undergoing suprapubic prostectomy. Ventral prostates were removed from adult rats sacrificed by decapitation. The tissue was minced with scissors and used immediately.

*Incubations.* Approximately 70–80 mg portions of minced rat prostatic tissue were incubated in 1 ml of oxygenated Eagle's balanced salt solution (GIBCO) containing about 1.4 × $10^6$ d.p.m. of [$^3$H]-testosterone, for 3 h in a water bath shaker at 37°. Progester-

one was used at a concentration of 5 μg/ml. In experiments involving human hypertrophic prostates, 200 mg of tissue and 2 ml of medium were used.

The incubations were terminated by adding 5 ml of methanol and 200 μg each of unlabeled T, DHT and A. Appropriate amounts (6000–10,000 c.p.m.) of either [$^{14}$C]-DHT or [$^{14}$C]-A, or both, were added in order to estimate losses occurring during the isolation of the steroids. After homogenization and centrifugation of the precipitated proteins and nucleic acids, the methanolic supernatants were concentrated to half their vol. and extracted with ethyl acetate (2 × 3 ml). The extracts were taken to dryness and the residues were subjected to thin layer chromatography.

In order to evaluate the influence of progesterone on the 17β DH activity, similar incubations were conducted without addition of radioactive testosterone. At the end of the incubation period, the tissues were quickly washed with ice-cold buffer and assayed for enzymatic activities, as described below.

*Assay of 17β hydroxysteroid dehydrogenase activity.* Minced prostate tissue was homogenized in 3 vol. of 0.02 M sodium phosphate buffer, pH 7.5. The homogenate was passed through two layers of gauze and centrifuged at 800 × *g* for 6 min in a Sorvall RC-2B refrigerated centrifuge. The supernatant was used for the enzyme assay. The assay mixture contained, per ml, 0.3 ml enzyme preparation (approximately 3–4 mg protein), 2 mg of oxidized cofactor in 0.1 ml water, tritiated substrate ([$^{3}$H]-T, 1.4 × $10^6$ d.p.m., 50 nmol) in 0.02 ml of methanol, and 0.5 ml of 0.1 M glycine buffer. The final pH of the mixture was 9.5. The reaction was started by the addition of the enzyme and it was stopped by adding 5 ml of methanol containing carriers (T and A, or DHT and 5α androstanedione) and either [$^{14}$C]-A or [$^{14}$C]-5α-androstanedione. The mixture was incubated at 37° and samples were taken for analysis at 10, 20 and 30 min.

Maximal enzymatic activity was noted at pH 9.5. Both NAD and NADP were effective as cofactors in the oxidation of T and DHT in the rat prostate, whereas NAD was the preferred cofactor for these reactions in human hyperplastic prostates. NADP was used with rat tissue and NAD with human prostates.

*Thin layer chromatography.* Silica Gel plates (Merck GF 254) were developed with chloroform–acetone–hexane, 4:1:3 (v/v). Adequate separation of T, DHT, A and 5α-androstanedione was achieved (distance from origin: 5, 7, 9, 12 cm, respectively). Testosterone and androstenedione bands were marked under U.V. light and 5α reduced metabolites were located by exposing a reference plate to iodine vapors. Radioactivity zones were localized with a radiochromatogram scanner (Packard Instr., Model 7201). Selected zones of the chromatogram were scraped and eluted with ethyl acetate.

*Measurement of radioactivity.* Samples were dissolved in 10 ml of Econofluor (New England Nuclear) and counted in a liquid scintillation spectrometer (Isocap 300, Nuclear Chicago) at an efficiency of 35% for $^{3}$H and 64% for $^{14}$C.

*Estimation of amounts of products formed during the incubations.* The amount of a product in the reaction was calculated from the $^{3}$H/$^{14}$C ratio in the isolated compound, using as data the amount of $^{14}$C indicator added and the S.A. of the incubated substrate. The amount of product formed during the enzyme assay was related to the amount of protein in the homogenate, as determined by colorimetry [8]. Results were expressed as nmol of product formed per h and per mg of protein.

## RESULTS

*Effect of progesterone on the* in vitro *metabolism of testosterone by prostatic tissue*

a. *Rat.* The results presented in Fig. 1 confirm the data reported by Patwardhan and Lanthier[6], i.e. the amount of DHT formed from T which is found in the tissue after 3 h incubation is drastically reduced when progesterone is present in the medium. Concomitantly, a larger incorporation into A of $^{3}$H derived from [$^{3}$H]-T is found in tissue incubated in the presence of progesterone.

b. *Human.* Figure 1 also shows the results obtained during incubation of slices of human hyperplastic prostate with [$^{3}$H]-T. It can be seen that also in this

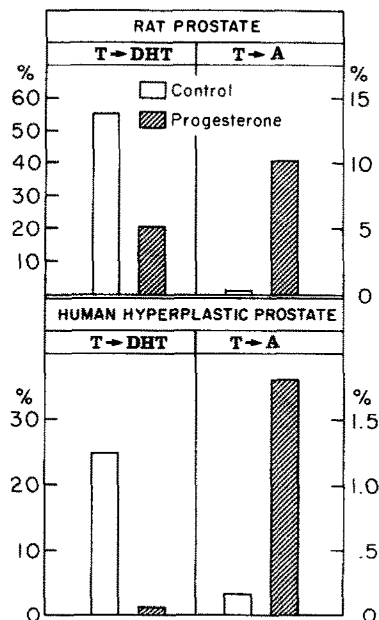


Fig. 1. *In vitro* metabolism of testosterone in rat and human hyperplastic prostate. The conversion of [$^{3}$H]-testosterone (T) to 5α-dihydrotestosterone (DHT) and androstenedione (A) was measured by incubating, for 3 h at 37°, minced prostatic tissue in medium with or without 5 μg/ml progesterone. Values are expressed as percentage of [$^{3}$H]-T converted to DHT or A during the incubation period. The values for murine tissue is the average of 2 runs, those for human are the average of duplicated experiments with tissue from 2 patients.

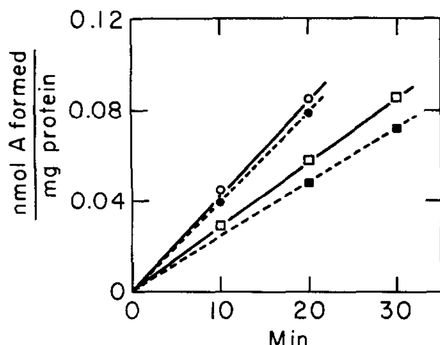


Fig. 2. 17$\beta$ hydroxysteroid dehydrogenase activity in rat and human hyperplastic prostatic tissue. Minced prostatic tissue was incubated with progesterone for 3 h at 37°, in medium with or without progesterone. At the end of the incubation, hydroxysteroid dehydrogenase activity was assayed in tissue homogenates using saturating concentrations of [$^3$H]-testosterone and NADP (rat) or NAD (man), in pH 9.5 buffer, Symbols: ○ rat, control; ● rat, progesterone; □ man, control; ■ man, progesterone.

tissue progesterone reduced the conversion of T to DHT and increased the conversion of T to A.

The conversion of [$^3$H]-DHT to [$^3$H]-androstanedione was also elevated in the presence of progesterone under the same incubation conditions. However, this increase was only one-tenth of the increase observed in the conversion of [$^3$H]-T to [$^3$H]-A.

*Effect of progesterone on prostatic 17$\beta$ DH activity during 3 h incubations*

One of the possibilities suggested by the data in Fig. 1 is that the 17$\beta$ DH activity is increased by progesterone during the 3 h incubations. However, the kinetic data shown in Fig. 2 demonstrates that the enzymatic activity is not significantly altered by progesterone *in vitro* during this period of time. Addition of progesterone to the assay system for 17$\beta$ DH did not affect the activity of the enzyme, as it was shown using rat prostate tissue.

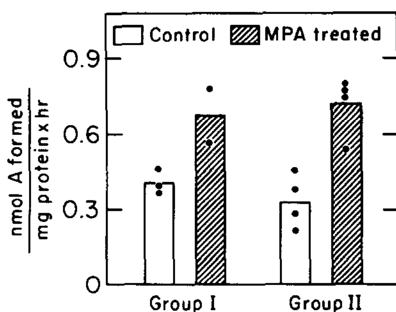


Fig. 3. Effect of MPA administration on the level of 17$\beta$ dehydrogenase in rat prostate. Rats were treated with MPA for a month; group I rats were 60 days old and group II rats were 90 days old at the start of the experiment. Each point in the graph indicates the level of prostatic 17$\beta$ hydroxysteroid dehydrogenase in individual animals.

*Effect of treatment of rats with medroxyprogesterone acetate on prostatic 17$\beta$ DH activity*

A reduction in the weight of ventral prostates of rats treated for one month by subcutaneous injection of Depo Provera on alternate days, was noted in both groups of animal studies (60 and 90 days old at the beginning of the treatment). Activities of 17$\beta$ DH were measured separately in each animal of the treated and control groups. The results are shown in Fig. 3. Enzymatic activities were approximately doubled in the treated animals.

## DISCUSSION

The antiandrogenic effects of progestins on prostatic tissue is suggested by the observed reduction in prostate weight during treatment of mature rats with MPA and by the therapeutic effectiveness of progestins in patients with benign prostatic hyperplasia [9, 10]. Progestins can exert their antiandrogenic action by inhibiting gonadotropin secretion [11] and by augmenting the metabolic clearance rate of testosterone through the induction of 5$\alpha$ reductase activity in the liver [12, 13]. *In vitro* experiments suggest that progesterone can also act at the target tissue level by decreasing the conversion of T to DHT by competitive inhibition [14, 15]. However, MPA appears to be much less effective than progesterone in inhibiting the conversion of T to DHT in both rat prostate [6] and human hyperplastic prostate [16], as well as in human skin fibroblasts [17].

The larger *in vitro* conversion of labeled T to A in the presence of progesterone appears to be a consequence of the increased availability of the labeled substrate not converted to DHT, since 17$\beta$ DH activity in tissue incubated in medium containing progesterone was not higher than control values.

The increased 17$\beta$ DH activity in the prostate of rats treated with MPA suggests another possible mechanism for the antiandrogenic action of the progestin, as increased conversion of T to A, or of DHT to 5$\alpha$ androstanedione, will lower the intracellular concentration of DHT derived from circulating T. However, a doubling of the 17$\beta$ DH activity may not be sufficient to alter the extent of conversion of T to DHT significantly, since a relatively small fraction of T is converted to A (Fig. 1). Similarly, the *in vitro* conversion of DHT to 5$\alpha$-androstanedione in human hyperplastic prostates was found to be small [18]. Therefore, an induction of prostatic testosterone 17$\beta$ DH by MPA may not be physiologically as important as the induction of estradiol 17$\beta$ DH in human endometrium, where increases of up to 6-fold are noted upon administration of MPA [1] and where the conversion of estradiol to estrone is the main route of metabolism of the hormone.

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