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ANTAGONIST ACTION OF DIHYDROPROGESTERONE ON THE FORMATION OF THE SPECIFIC DIHYDROTESTOSTERONE–CYTOPLASMIC RECEPTOR COMPLEX IN RAT VENTRAL PROSTATE*

F. WRIGHT†, M.-O. KIRCHHOFFER† and P. MAUVAIS-JARVIS‡
† Biochemical Department, Faculty of Medicine Pitié-Salpêtrière 75634, Paris Cedex 13, and
‡ Department of Endocrinology and Gynecology, Hopital Necker 75730, Paris Cedex 15, France

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SUMMARY

The binding of progesterone and 5α-pregnane-3,20-dione (DHP) to the 8–9S specific cytoplasmic receptor protein for dihydrotestosterone (DHT) was studied in the rat ventral prostate. Binding of [1,2-^{3}H]-DHT (2×10^{-9} M) to this receptor protein was suppressed both by progesterone and DHP at a concentration which equalled 10 times that of unlabeled DHT for the same effect. Specificity of this binding was checked with estradiol, testosterone, 5α-androstane-3α,17β-diol, and aldosterone. It thus appears likely that in rat ventral prostate progesterone is an effective antiandrogen acting at two levels; firstly by inhibiting the 5α-reduction of testosterone to DHT as previously shown; secondly via its 5α-reduction into DHP which in turn competes for the binding sites of DHT on the specific cytoplasmic receptor protein; a necessary step to transfer androgens into the nucleus.

INTRODUCTION

Evidence has been obtained from different laboratories that progesterone interferes in androgen target cells with the 5α-reduction of testosterone to 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone-DHT). Such results were obtained by Massa and Martini and Jenkins and McCaffery[1, 2] during incubation of human and rat prostate, and by Voigt *et al.*[3] and Wilson[4] on human skin. The latter authors interpreted this enzymic inhibition as the result of a competition between testosterone and progesterone for the active site of the 5α-reductase present in DHT dependent androgen target cells. Indeed, 5α-pregnane-3,20-dione (dihydroprogesterone-DHP) is formed during incubations of human skin microsomes with radiolabeled progesterone [3].

The aim of the present investigation was to examine whether the antiandrogenic effect of progesterone was only due to a competition on the 5α-reduction system or if progesterone and its 5α-reduced metabolite: DHP interfered at yet another step in the sequence of androgen action: namely at the receptor level.

MATERIALS AND METHODS

Steroids. [1,2-^{3}H]-DHT (specific activity 40 Ci/mmol) was purchased from New England Nuclear and its purity checked by chromatography. Unlabeled testosterone, DHT, progesterone, DHP, estradiol, cortisol and aldosterone were purchased from Roussel-Uclaf and purified by crystallization in methanol–water. Cyproterone acetate (CPA) was a generous gift from the Schering Institute.

Animals. Wistar rats weighing 250–300 g were used in all experiments. They were castrated under ether anaesthesia 24 h before prostates were removed. On average 8 animals were used for each experiment.

Cytosol preparation. Prostates were removed and immediately put in ice cold DTT Buffer (Tris–HCl 0.05 M, pH 7.4 EDTA 0.1 mM, glycerol 10% and dithiothreitol 0.25 mM). Ventral prostates were rinsed in DTT Buffer and minced finely with scissors before homogenization with a Polytron PTS OD 10 in 4 vol. of DTT buffer. Cytosol was obtained by centrifugation at 50,000 rev./min for 120 min at 1°C in a 50 Ti Beckman Rotor and L_3 centrifuge.

Incubations. 1 ml fractions of cytosol (8–12 mg total protein as measured by the Lowry method [5]) were incubated for 2 h at 0°C (ice-cold water in cold room) in the presence of steroids.

The incubations were stopped by addition of 1 ml of a dextran-coated charcoal solution prepared in DTT buffer (DCC-charcoal 0.5%, dextran T 70 0.05%). After standing for 15 min at 0°C, tubes were centrifuged at 1500 *g* for 15 min and decanted. The supernatant was adjusted to 4–6 mg total protein per ml and 400 μl layered on a 5–25% sucrose gradient containing 10% glycerol and centrifuged overnight at

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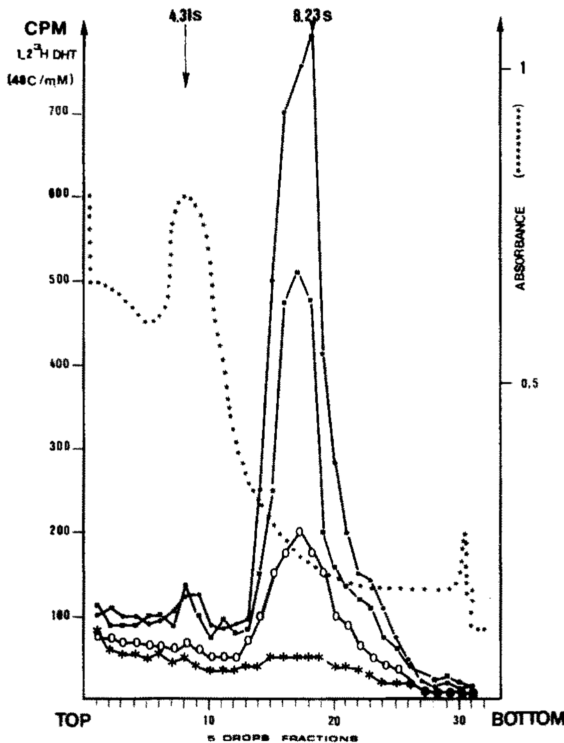

Fig. 1. [^{3}H]-DHT binding on rat ventral prostate cytoplasmic receptor and its displacement by unlabeled DHP at various concentrations: ●——●, 1,2-[^{3}H]-DHT 2×10^{-9} M; ■——■, 1,2-[^{3}H]-DHT 2×10^{-9} M + DHP 2×10^{-8} M; ○—○—○, 1,2-[^{3}H]-DHT + DHP 2×10^{-7} M; *——*, 1,2-[^{3}H]-DHT + DHP 2×10^{-6} M.

50,000 rev./min in a SW 50.1 rotor. Aliquots of the supernatant were kept for radioactivity measurements before and after charcoal addition.

Gradients analysis. Analysis of the sucrose gradients after ultracentrifugation were performed on an ISCO automatic analyser UA 5 with simultaneous protein analysis and recording.

Reference proteins. In order to measure the sedimentation coefficient of the radioactive peak of receptor, DTT buffer solutions of SAB and cytochrome C were processed as above. This allowed us to assess that the protein peak recorded on the spectrometer UA 5 (ISCO), after centrifugation of rat prostate cytosol, had a constant sedimentation coefficient of 4.31S. This protein peak was therefore used as an internal standard to measure the sedimentation coefficient of the radiolabeled peak of receptor.

RESULTS

A typical sucrose gradient profile can be seen in Fig. 1: rat prostate cytosol was incubated with tritiated DHT and then displaced by unlabeled DHP at different concentrations. The dotted line represents a recording of the non-labeled cytosolic proteins throughout the gradient. This peak of proteins was rigorously in the same position on every gradient and was used as an internal standard at 4.31 for an estimate of the triated receptor protein peak (see material and methods above). The receptor protein was found to have a mean 8.23S sedimentation coefficient. Progressive suppression of the 8.23S receptor peak was observed after incubation of [^{3}H]-DHT together with increasing amounts of DHP. Expressed in percentage of inhibition, the relative effect of DHT, progesterone, DHP and cyproterone acetate are summarized in Fig. 2. Each point represented is the mean value of six to eight determinations.

Although DHT is clearly the most potent inhibitor of [^{3}H]-DHT binding to the rat ventral prostate cytosolic protein, both progesterone and DHP none the less can inhibit this binding.

As can be seen, dilution of tritiated DHT 2×10^{-9} M with a 100-fold excess of unlabeled DHT (2×10^{-7} M) prevented the formation of the tritiated peak; progesterone dihydroprogesterone or cyproterone acetate had also a 100% inhibitory effect but at a 10-fold higher concentration (2×10^{-6} M).

Specificity was checked in this system and results are also summarized in Fig. 2. Cortisol and aldosterone have no affinity for the receptor protein as they fail to interact with the radioactive peak of [^{3}H]-DHT (2×10^{-9} M) even at a 10,000-fold excess (2×10^{-5} M). Estradiol and 5α-androstane-3α,17β-diol do not compete significantly with [^{3}H]-DHT for the binding sites of the receptor as they induce less than 50% inhibition even at 2×10^{-5} M. As for testosterone it was less competitive than DHT, progesterone, DHP and CPA as it only presented a 75% inhibition of the [^{3}H]-DHT peak even at very high concentrations (2×10^{-5} M).

DISCUSSION

The antiandrogenic activity of progesterone is well documented. Dorfman[6] has shown that progesterone applied on the combs of testosterone stimulated chicks has an antiandrogenic activity and that progesterone diminishes the growth of male accessory organs of mice treated subcutaneously with testosterone. In addition, progesterone dissolved in ethanol and topically applied to the flank organ of female hamsters, inhibits locally the enlargement of the sebaceous gland provoked by testosterone [7] whereas in human beings the topical application of progesterone on pubic skin inhibits *in situ* the 5α-reduction of testosterone to DHT [8]. The decrease in DHT binding to specific cytoplasmic receptor under the effect of progesterone was at first reported by Baulieu and Jung[9]. In addition Mangan and Mainwaring[10] and Fang *et al.*[11] observed that progesterone partially inhibited the nuclear binding of DHT in rat ventral prostate gland *in vitro* and *in vivo*. Moreover in human prostatic hypertrophy Snochowski *et al.*[12] recently used [6,7-^{3}H]-methyltrienolone (R 1881) as ligand for the cytosolic receptor. They reported that, whereas non-radioactive R 1881 or DHT displaced completely the receptor binding at

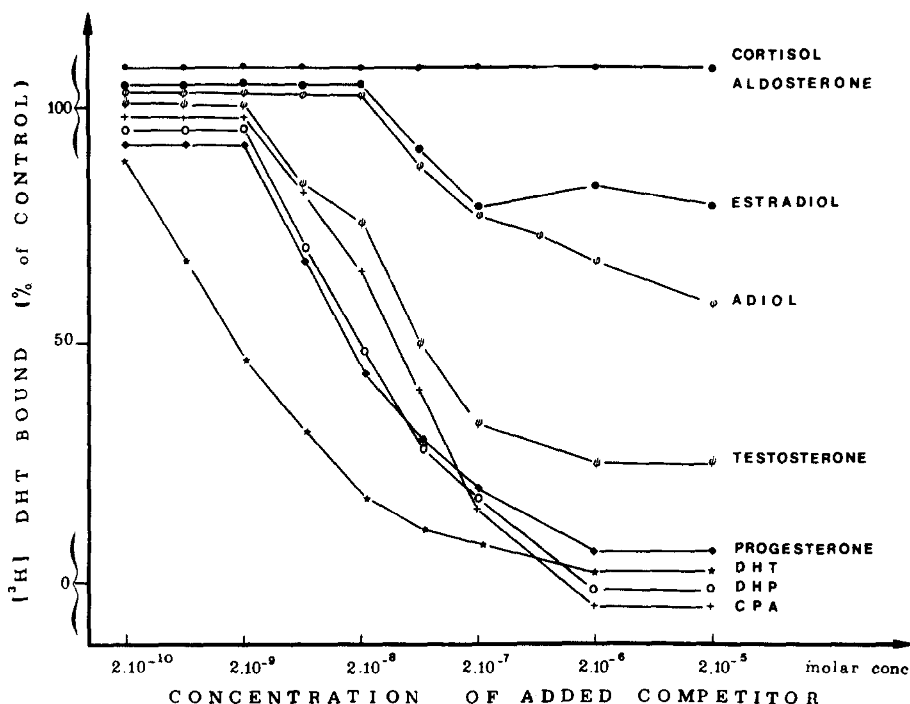

Fig. 2. Competition of DHT, progesterone, DHP, cyproterone acetate (CPA), cortisol, aldosterone, estradiol and 5α-androstanediol (adiol) for specific binding of [^{3}H]-DHT to rat prostate cytosol. Samples were incubated with 2×10^{-9} M [^{3}H]-DHT and varying concentration of unlabeled steroids.

a 100-fold excess testosterone and progesterone could also displace [^{3}H]-R 1881 from its binding sites to the receptor but not completely.

However, in these studies the metabolism of progesterone was not examined and Mangan and Mainwaring reported that "it was not known whether progesterone or its 5α-reduced metabolite: DHP was the competitor for the DHT binding sites" [10].

The present data emphasize the role of DHP as an antiandrogen, active at the level of specific receptor, in androgen target tissues. The activity of DHP seems to be very important since this steroid suppresses completely the 8.23S peak observed after incubation of [^{3}H]-DHT at the same concentration as CPA (2×10^{-7} M).

The fact that testosterone does not displace [^{3}H]-DHT binding to the receptor is sometimes subject to controversy but is in good agreement with the literature [13, 14]. Some authors even use this fact as an argument for specific characterization of the receptor as opposed to TeBG [13, 14]. Only kidney and skeletal muscle receptors seem to act differently to our knowledge [15, 16]. This is probably due to the different enzyme environment of this target organ where 5α-reductase seems to be very low if not absent.

The antiandrogenic activity of progesterone may be therefore outlined as follows: when progesterone is present in sufficient amount in androgen target cells, it may be a preferential substrate for the 5α-reductase system present in this structure [3, 4] and as a consequence DHP is formed with a higher yield than DHT. In turn, DHP produced can compete with free DHT (still present in target cells) for the active binding sites of the specific cytoplasmic receptor protein thus preventing DHT to be transfered into the nucleus.

In addition it is interesting to note that the reciprocal affinities of progesterone and testosterone for the 5α-reductase system and of DHT and DHP for the cytoplasmic receptor protein can be partly explained on the basis of structural analogies in the molecules such as the 3-oxo-4ene-structure for the first pair [17, 18] and the 5α-hydrogenated carbon for the latter [19].

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