Antagonistic Action of Anti-androgens on the Formation of a Specific Dihydrotestosterone–Receptor Protein Complex in Rat Ventral Prostate

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SUMMARY

Cyproterone (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dione) 17α-acetate, a potent anti-androgen, suppressed the uptake of radioactive androgens *in vivo* by the ventral prostate of rats. This was accompanied by a decrease in the retention of 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) by prostate cell nuclei. Cyproterone and its 17α-acetate (less than 0.5 μg/ml) also inhibited the formation of a specific dihydrotestosterone–protein complex in prostate cell nuclei when minced prostate was incubated with radioactive testosterone or dihydrotestosterone. Estradiol-17β, diethylstilbestrol, and progesterone, but not hydrocortisone succinate, also suppressed the retention of dihydrotestosterone by prostatic cell nuclei *in vitro*, but to a much lesser extent than cyproterone.

Recent studies have shown that cell nuclei of rat ventral prostate can selectively retain 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-17-one) *in vivo* for a prolonged period of time (1, 2). The selective retention of 5α-dihydrotestosterone by prostate cell nuclei also occurred during the incubation of minced ventral prostate with radioactive testosterone *in vitro* (2). The retention of 5α-dihydrotestosterone appeared to be due to protein(s) which can be solubilized from nuclei with a salt solution (3–5). On sucrose gradient centrifugation, the 5α-dihydrotestosterone–protein complex extracted from prostate cell nuclei migrated as a 3 S component (5, 6).[1] Formation of the nuclear 3 S 5α-dihydrotestosterone–protein complex could be achieved if the isolated prostate cell nuclei were incubated with a cytosol fraction which also contained a specific dihydrotestosterone-binding protein (5, 6). Since 5α-dihydrotestosterone, one of the most active androgens for the normal function and growth of ventral prostate, is not retained by nuclei of other tissues that are relatively insensitive to androgens, it was suggested that such retention is an important prefatory step for the action of the androgen in rat ventral prostate. This communication describes the antagonistic action of two anti-androgens, cyproterone[2] (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dione) and its 17α-acetate, on the uptake and retention of 5α-dihydrotestosterone *in vivo* and *in vitro* by cell nuclei and a specific receptor protein of rat ventral prostate.

Long-Evans or Sprague-Dawley rats 4–

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[1] In a previous communication we assigned sedimentation coefficients of 3.5 S and 2.5 S, respectively, to proteins which bind selectively 5α-dihydrotestosterone in the cytosol and nuclei. More recent studies have shown that the nuclear 5α-dihydrotestosterone-binding protein has sedimentation coefficients in the vicinity of 3 S.

[2] Cyproterone and its 17α-acetate were kindly provided by Dr. M. Friedrichs of Schering-Berlin Laboratories. Labeled androgens were products of New England Nuclear Corporation.

6 months of age were castrated via the scrotal route. Animals were killed by cervical dislocation. Ventral prostates were dissected free of their capsules and minced with scissors. For tissue immersion experiments *in vitro*, the minced prostate was incubated in 3 ml of medium 199 containing 0.5 μCi (0.0034 μg) of 7α-^{3}H-5α-dihydrotestosterone at 37° for 30 min under an atmosphere of 95% O_2 and 5% CO_2. After incubation, minced prostate was washed in 0.32 M sucrose containing 1 mM $MgCl_2$ and 0.02 M Tris-HCl buffer (pH 7.5), homogenized, and centrifuged at 500 × *g* (average) for 10 min to obtain the nuclear fraction. The nuclear fraction was washed by resuspending the sediment in 5 ml of the sucrose medium and recentrifugation. Addition of a 100-fold concentration of nonradioactive testosterone or 5α-dihydrotestosterone during the washing processes did not significantly alter the radioactivity of washed nuclear sediment, suggesting a firm association of radioactive 5α-dihydrotestosterone with the nuclei. The 5α-dihydrotestosterone–protein complex was extracted by exposing the labeled nuclei to 0.4 M KCl–1.5 mM EDTA–0.02 M Tris-HCl at pH 7.5 for 30 min on ice.[3] The mixture was centrifuged at 14,000 × *g* (average) for 40 min to obtain a clear extract. The extract was then layered on a sucrose gradient (4.6 ml, 5–20%, linear) containing 0.4 M KCl–1.5 mM EDTA–0.02 M Tris-HCl, pH 7.5. It was centrifuged at 55,000 rpm for 22 hr using an SW-65 rotor in a Spinco L-2-65B ultracentrifuge. Fractions (0.2 ml each) were collected from the bottoms of the tubes, and radioactivity was measured by the procedure described before (2). For the experiments shown in Tables 1 and 2, the washed nuclear sediment was resuspended in 3 ml of the 0.32 M sucrose solution described above, mixed with 20 ml of 2.2 M sucrose containing 1 mM $MgCl_2$, and layered on top of 5 ml of the 2.2 M sucrose solution in a cellulose nitrate tube of the SW-25.1 rotor. After centrifugation at 22,000 rpm for 1 hr, the nuclear pellet was removed, and radioactivity and DNA were measured as described before (2). The above procedure effectively minimized contamination by cytoplasmic particles and whole cells (2). The yield of nuclei was about 65%.

Cyproterone acetate exhibits a very powerful anti-androgenic effect *in vivo* on the growth and function of male accessory reproductive glands (9, 10). When it was injected concomitantly with ^{3}H-testosterone into castrated rats, the extent of retention of radioactive 5α-dihydrotestosterone by prostate cell nuclei was greatly reduced. Two such experiments are shown in Table 1. Cyproterone acetate also sup-

[3] The procedure is essentially the same as that used for the extraction of estradiol receptor protein from uterine nuclei (see refs. 7 and 8). Normally more than 70% of the radioactivity associated with prostate cell nuclei can be released by one extraction. A higher yield can be obtained if the extraction is repeated.

TABLE 1

Influence of cyproterone acetate on the uptake and retention of androgens by ventral prostate of rats in vivo

Rats (body weight, 300 g) were castrated. Seventy hours later, 50 μCi (0.72 μg) of 7α-^{3}H-testosterone were injected intraperitoneally into each rat, either 35 min (experiment 1) or 60 min (experiment 2) before death. If used, cyproterone acetate (5 mg/rat in experiment 1; 1 mg/rat in experiment 2) was added to the testosterone solution for injection. The injection medium was 1,2-propanediol (1 ml). Controls received the same amount of carrier. For each group, the prostates from five rats were pooled. Nuclei were purified from a 2.2 M sucrose solution as described in the text. By thin layer and gas chromatography, 85–95% of the radioisotope associated with the nuclei was 5α-dihydrotestosterone (see ref. 2).

	Radioactivity associated with prostate			
	Expt. 1		Expt. 2	
Treatment	Nuclei	Whole tissue	Nuclei	Whole tissue
	dpm/100 μg DNA	*dpm/mg dry wt*	*dpm/100 μg DNA*	*dpm/mg dry wt*
Control	418	290	1513	380
+Cyproterone acetate	143	60	240	75

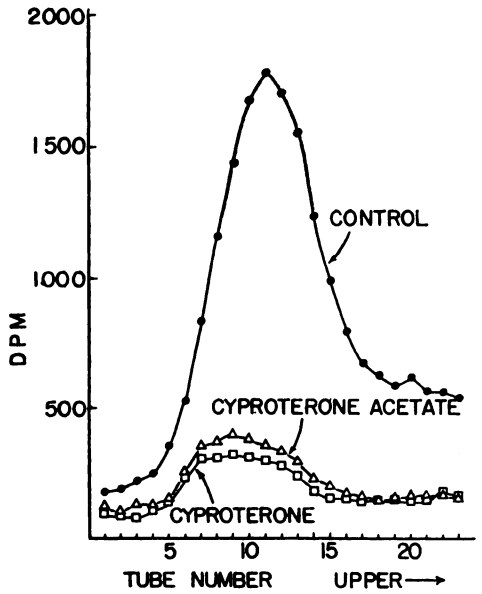

FIG. 1. *Sedimentation of 5α-dihydrotestosterone–protein complex by sucrose gradient centrifugation and the effect of cyproterone and its acetate on formation of the complex*

Rats were castrated 42 hr before death. For each group, the ventral prostates (0.9–1.0 g) from four rats were pooled, minced, and incubated with 0.5 μCi (0.0034 μg) of 7α-^{3}H-5α-dihydrotestosterone at 37° for 30 min in 3 ml of medium 199 (pH 7) without (●) and with 1.3 μg/ml of cyproterone (□) or cyproterone acetate (△). After incubation, the prostates were washed, homogenized, and centrifuged at 500 × *g* (average) for 10 min to obtain the nuclear fraction. The 5α-dihydrotestosterone–protein complex was extracted with 0.5 ml of salt medium (see the text), and 0.2 ml of this extract was layered on a sucrose gradient (5–20%, linear) and centrifuged (see the text). Fractions were collected from the bottoms of the tubes, and radioactivity was measured.

pressed the uptake of androgens by whole ventral prostate glands. This could be one of the reasons for the lower nuclear retention of androgen. The effect of cyproterone acetate was probably not due to a decrease of androgen in the blood supply or to a lower rate of reduction of testosterone to 5α-dihydrotestosterone, since cyproterone and its acetate also decreased the retention of 5α-dihydrotestosterone by nuclei when minced prostate was incubated *in vitro* with ^{3}H-5α-dihydrotestosterone.

TABLE 2

Effect of cyproterone acetate on the formation of nuclear 5α-dihydrotestosterone–protein complex during incubation of minced ventral prostate in vitro with ^{3}H-5α-dihydrotestosterone

Rats were castrated 19 hr before the experiment. Ventral prostates (12.7 g) were pooled from 16 rats and divided into four groups. The experiment was carried out in the same way as described in Fig. 1, except that nuclei were further purified by centrifugation in 2.2 M sucrose. The nuclear 5α-dihydrotestosterone–protein complex was then extracted and centrifuged through a sucrose gradient as described in the text. The radioactivity associated with nuclei (as measured by DNA content) and with the 3 S protein component on the gradient was determined and expressed as a percentage of the controls, which had received no cyproterone acetate.

Concentration of cyproterone acetate	Radioactivity associated with		
	Purified nuclei		3 S protein
μg/ml	*dpm/mg DNA*	%	%
0.0	12125	100	100
0.1	8928	74	69
0.5	2928	24	7
1.0	3166	26	13

As shown in Fig. 1, when minced ventral prostate was incubated *in vitro* with ^{3}H-5α-dihydrotestosterone and the nuclear 5α-dihydrotestosterone–protein complex was extracted and analyzed by sucrose gradient centrifugation, the 3 S peak of the complex was significantly lower if cyproterone or its acetate was added to the incubation mixture.

Under the conditions employed, cyproterone acetate at concentrations as low as 0.1–0.5 μg/ml (0.24–1.2 μM) significantly inhibited the nuclear retention of 5α-dihydrotestosterone and the formation of the nuclear 3 S protein–5α-dihydrotestosterone complex (Table 2). Such inhibition appeared to be specific, since hydrocortisone succinate at concentrations up to 13.3 μg/ml did not show any effect on 5α-dihydrotestosterone binding to prostate cell nuclei or the 3 S protein. Estradiol-17β, diethylstilbestrol, and progesterone at concentrations up to 13.3 μg/ml inhibited these processes by 10–30% when minced rat ventral prostate was incubated with ^{3}H-

testosterone or ^{3}H-5α-dihydrotestosterone. Whether such inhibitory action is related to their antagonistic action on the prostate (11) needs further study.

The precise mechanism by which cyproterone and its acetate inhibit the 5α-dihydrotestosterone retention processes is not clear. Although these anti-androgens could act at cell membranes by preventing the androgen from entering the target cells or intracellular organelles, they could interfere directly with the formation of 5α-dihydrotestosterone–receptor protein complex.

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