ESTRADIOL-17β INHIBITION OF ANDROGEN UPTAKE, METABOLISM AND BINDING IN EPIDIDYMIS OF ADULT MALE RATS IN VIVO: A COMPARISON WITH CYPROTERONE ACETATE

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Received 5-19-80

ABSTRACT

The effects of estradiol-17β on androgen uptake, metabolism and binding were studied in rat epididymis in vivo in comparison with cyproterone acetate. Steroids (250 ug/100 g body weight) were injected 5 min prior to ^{3}H-testosterone in castrate rats. Estradiol-17β inhibited ^{3}H-testosterone uptake into epididymal cytosol by 58% as compared to 38% by cyproterone acetate. ^{3}H-Testosterone uptake into epididymal nuclei was inhibited 95% by estradiol-17β and 83% by cyproterone acetate. Total bound radioactivity in cytosol fractions was reduced to a greater extent by estradiol-17β than cyproterone acetate when either ^{3}H-testosterone or ^{3}H-dihydrotestosterone was injected. Binding of ^{3}H-dihydrotestosterone to nuclear receptors was completely abolished by estradiol-17β ; whereas approximately 20% binding remained in the nuclear extract after cyproterone acetate treatment. Metabolism of ^{3}H-testosterone in vivo was also altered by estradiol-17β , resulting in diminished conversion to ^{3}H-dihydrotestosterone. Cyproterone acetate, on the other hand, did not affect ^{3}H-testosterone metabolism. Estradiol-17β and cyproterone acetate inhibited in vitro binding of ^{3}H-dihydrotestosterone to the intracellular cytoplasmic receptor, but not the intraluminal androgen binding protein (ABP). These data suggest that estradiol-17β may have a more potent antiandrogenic effect on the epididymis than cyproterone acetate due to inhibition of 5α reduction of testosterone as well as binding to the androgen receptor.

INTRODUCTION

Estrogens have long been used as antiandrogens in the treatment of prostatic carcinoma. Cyproterone acetate, a synthetic progestational agent, has been shown to have potent antiandrogenic activity. Both estradiol-17β and cyproterone acetate affect the accessory sex organs by reducing levels of circulating testosterone in the male. The mechanism

of this effect appears to be directed through feedback to the hypothalamus (1). In addition, it has been shown that these antiandrogens have direct effects on ventral prostate (2,3), seminal vesicle (4), epididymis (2,5,6) and testis (2) by competitive inhibition of androgen receptors in these target organs.

In addition to the intracellular androgen receptor, epididymal cytosol also contains an intraluminal androgen binding protein (ABP), which is secreted by the testis. Previous studies in vivo have shown that cyproterone acetate inhibits binding of testosterone or dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one) to the epididymal androgen receptor, but has no effect on binding to ABP (5,6).

It was of interest to compare the in vivo effects of estradiol-17β and cyproterone acetate on the uptake, metabolism and binding of labeled androgen to receptor and ABP in epididymis.

MATERIALS AND METHODS

Materials

[1,2,6,7-^{3}H]testosterone, S.A. 91 Ci/mmole, and [1,2-^{3}H]dihydrotestosterone, S.A. 44 Ci/mmole, were obtained from New England Nuclear. The radiochemical purity was checked by thin-layer chromatography on silica gel GF in benzene:methanol (9:1) and on aluminum oxide in 100% diethyl ether. Cyproterone acetate (6-chloro-17-hydroxy-1,2 α -methylene-4,6-pregnadiene-3, 20-dione 17-acetate) was obtained from Schering, A.G., Berlin. Acrylamide, N,N,N^{1},N^{1}-tetramethylethylenediamine (TEMED) and N, N^{1}-methylene-bis-acrylamide (Bis) were obtained from Eastman Kodak Co. Testosterone, dihydrotestosterone, estradiol-17β and cortisol were obtained from Mann Research Laboratories.

Preparation of animals

Sprague-Dawley rats weighing 350-450 g were castrated 18 hours or 8 days prior to the experiments. Rats were obtained from Hormone Assay Laboratories, Chicago, Illinois. For in vivo studies rats were functionally hepatectomized and eviscerated (5) prior to intravenous injection of ^{3}H-testosterone. Each rat received a subcutaneous injection of 5% dextrose/ saline (5 ml) after the evisceration procedure. Labeling of binding components in vivo: Non-radioactive competing steroids (1 mg in

0.1 ml ethanol) were injected into the left femoral vein, and five minutes later radioactive testosterone or dihydrotestosterone (60 uCi) was injected into the right femoral vein. Rats were killed 3 h later.

Preparation of cytosols and nuclear extracts

Cytosols (105,000 g supernatants) and extracts from purified nuclei following in vivo injection of labeled steroid were prepared as described previously (5). Cytosols were prepared in 3 volumes of 50 mM Tris-HCl (pH 7.4 at 4°C) containing 3 mM $MgCl_2$ (buffer A) and 0.32 M sucrose using an all glass Duall homogenizer. Purified nuclei were prepared by centrifugation through 2.2 M sucrose and washing with Triton X-100 (7). Extraction of nuclear radioactivity was achieved with 0.4 M KCl in buffer A containing 1.5 mM EDTA. Labeling of cytosol in vitro: Epididymal cytosol from 24 h or 8 d castrate rats was incubated with 0.69 nM ^{3}H-dihydrotestosterone (44 Ci/mmole) alone or in the presence of competitors for 15 hrs at 0°C.

Polyacrylamide gel electrophoresis

The preparation of 5% polyacrylamide gels (either 11 x 60 mm or 6 x 50 mm) containing 10% glycerol has been described previously (6). Upper and lower buffers contained 0.6% Tris and 2.9% glycine in water (pH 8.6 at 4°C). Bromphenol blue (BPB) was added to the upper buffer as an electrophoretic marker. Aliquots of supernate of 500 ul or 100 ul were layered directly over the running gels. Gels were electrophoresed at 6-8 mAmp/cm^2 (i.e. 5 mAmp/tube for large gels or 3 mAmp for small gels) in a 5°C cold room with the lower buffer immersed in ice. Gels were sliced into 2 mm sections, the sections placed in counting vials, and 10 ml scintillation fluid added. Greater than 98% of the radioactivity in the gels was extracted into the scintillation fluid after standing 17 hrs at room temperature.

DEAE filter assay of cytosol receptor

The method of Santi et al. (8) was used in assaying bound radioactivity on DE-81 filters. The assay was performed at 4°C in buffer A.

Other analytical methods

Radioactivity was measured in a Packard Tri-Carb scintillation spectrometer in scintillation fluid containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene in toluene. Protein concentrations in the supernatant fractions were measured by the method of Lowry et al. (8) using bovine serum albumin as a standard.

Identification of labeled metabolites

A modification of the procedure of Stern and Eisenfeld (9) was used for the extraction of steroids from tissue. Epididymides were homogenized in 6 volumes of acetone, containing 3,000 dpm [4-^{14}C]testosterone for determination of recovery. The following unlabeled steroids (100 ug) were added as carriers: androstanediol (5α-androstane-3α,17β-diol), test-

osterone, dihydrotestosterone, androsterone (3α -hydroxy-5α -androstan-17-one), androstenedione (4-androstene-3,17-dione) and androstanedione (5α -androstane-3,17-dione). After 18 h of extraction at 4°C, the homogenates were filtered through 2 layers of filter paper (Whatman #1) and the acetone evaporated under nitrogen to 1 ml. Steroids were extracted in 9 ml diethyl ether:water (2:1) twice and chromatographed on silica gel GF in benzene:methanol (9:1). After detection of carrier steroids by short wave UV light and iodine vapor, the entire plate was scraped in zones, and the radioactivity corresponding to each zone was determined by liquid scintillation counting. Dihydrotestosterone was separated from androsterone by TLC on aluminum oxide in 100% diethyl ether and identified by crystallization to constant specific activity.

RESULTS

A series of experiments was planned to examine the acute effects of a single injection of estradiol-17β or cyproterone acetate on ^{3}H-testosterone accumulation, metabolism and nuclear binding in the epididymis *in vivo* . Figure 1 compares the effects of testosterone, dihydrotestosterone, cyproterone acetate, estradiol-17β and cortisol on accumulation of radioactivity in cytosol (Fig. 1A) and nuclear (Fig. 1B) fractions following intravenous injection of competitor and ^{3}H-testosterone. Cyproterone acetate and dihydrotestosterone reduced the accumulation of radioactivity in cytosol by about 40%, estradiol-17β by 58% and testosterone by 79% (Fig. 1A). Nuclear accumulation was inhibited by 83% with cyproterone acetate, 95% with estradiol-17β or dihydrotestosterone and 98% with testosterone (Fig. 1B). Cortisol did not inhibit androgen accumulation in either cytosol or nuclear fractions.

The effect of estradiol-17β on metabolism of ^{3}H-testosterone in epididymis *in vivo* was next examined in comparison with cyproterone acetate. Figure 2 shows radioactive metabolites in whole epididymis after injection of either estradiol-17β or cyproterone acetate prior to ^{3}H-testosterone. In the control epididymis the most prominent metabolite was dihydrotestosterone (50%) with unmetabolized testosterone representing

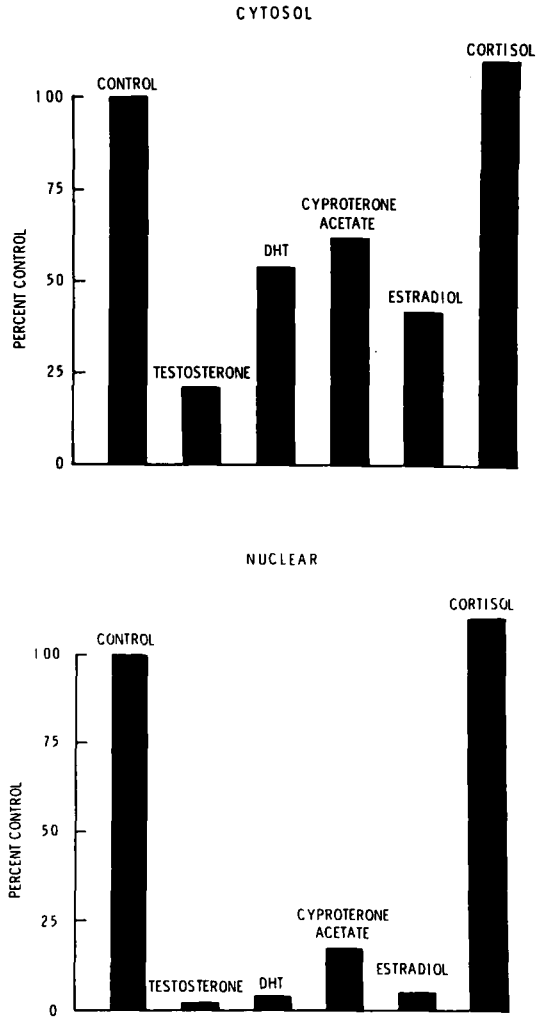

Figure 1: Effects of various steroids on in vivo accumulation of radioactivity in cytosol and nuclei of epididymis following injection of ^{3}H-testosterone. Eighteen hour castrate rats were eviscerated and functionally hepatectomized. Each of three rats was injected intravenously with either testosterone, dihydrotestosterone, cyproterone acetate, estradiol-17 β , or cortisol (250 ug/100 g body weight) in 0.1 ml ethanol or ethanol alone five minutes prior to injecting 50 uCi ^{3}H-testosterone intravenously. Three hours later the rats were killed and the epididymides were homogenized in 3 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 4°C) containing 3 mM $MgCl_2$ and 0.32 M sucrose in an all-glass Duall homogenizer. The homogenates

were centrifuged at 600 g for 10 min. Cytosols and purified nuclei were prepared as described in Materials and Methods. Radioactivity was extracted directly into scintillation fluid.

22% of recovered radioactivity. In the cyproterone acetate-treated epididymis there was very little effect on metabolism. However, estradiol-17β markedly inhibited the in vivo conversion of testosterone to dihydrotestosterone. Consequently, 47% of recovered radioactivity was ^{3}H-testosterone and only 17% ^{3}H-dihydrotestosterone. Unidentified polar water-soluble metabolites comprised about 12% of the total recovered activity and did not change significantly with either estradiol-17β or cyproterone acetate treatment.

Since we had shown previously (7) that ^{3}H-dihydrotestosterone is essentially the only androgen retained in epididymal nuclei following injection of ^{3}H-testosterone (7), we injected ^{3}H-dihydrotestosterone instead of ^{3}H-testosterone to determine whether the decreased accumulation of labeled androgen following estradiol-17β treatment might have resulted from a decreased concentration of ^{3}H-dihydrotestosterone available for receptor binding. Injection of ^{3}H-dihydrotestosterone circumvented the effect of estradiol-17β on metabolism. Table 1 shows the effects of estradiol-17β and cyproterone acetate on nuclear accumulation and binding following the injection of ^{3}H-dihydrotestosterone. Although there was ample ^{3}H-dihydrotestosterone available for binding, essentially no bound androgen was found in the nucleus after estradiol-17β treatment. This indicated that estradiol-17β may inhibit binding of androgen in vivo by competition with the receptor. As expected, cyproterone acetate also decreased the amount of ^{3}H-dihydrotestosterone bound to nuclear receptor (Table I).

Competition for receptor binding of ^{3}H-dihydrotestosterone in epi-

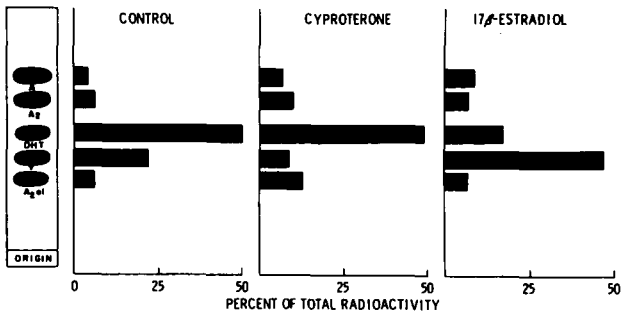

Figure 2: Effects of cyproterone acetate and estradiol-17β on ^{3}H-testosterone metabolism. Rats were injected with cyproterone acetate, estradiol-17β and ^{3}H-testosterone as described in Figure 1. Epididymides were homogenized in 6 volumes of acetone and extracted as described in Materials and Methods. The metabolites were then chromatographed on silica gel GF in benzene: methanol (9:1). The entire plate was scraped in zones and the radioactivity corresponding to each zone was determined by liquid scintillation counting. A, androstanedione; A_2, androstenedione; DHT, dihydrotestosterone; T, testosterone; A_2ol, androstanediol.

didymal cytosol was examined in vitro with increasing concentrations of dihydrotestosterone, cyproterone acetate or estradiol-17β, and binding was examined on polyacrylamide gels. Figure 3A shows the inhibition by dihydrotestosterone of androgen binding to the cytoplasmic receptor (CR) and ABP separated by polyacrylamide gel electrophoresis. A decrease in bound ^{3}H-dihydrotestosterone to CR was seen with increasing concentrations of unlabeled dihydrotestosterone. Binding to CR and ABP was inhibited by a 100-fold excess (20 ng) unlabeled dihydrotestosterone; however, only CR was inhibited by a 10-fold excess (2 ng), indicating the presence of a larger number of ABP binding sites. Estradiol-17β inhibited binding of ^{3}H-dihydrotestosterone to the cytoplasmic receptor at 10- and 100-fold excess, however, there was no displacement of the ^{3}H-dihydrotestosterone bound to ABP at these concentrations of competitor (Fig. 3B). Figure 3C

shows significant inhibition of receptor in the presence of 10- and 100-fold excess cyproterone acetate but no inhibition of ABP binding. In other experiments (data not shown), there was no competition for ABP binding by cyproterone acetate with even a 200-fold excess concentration.

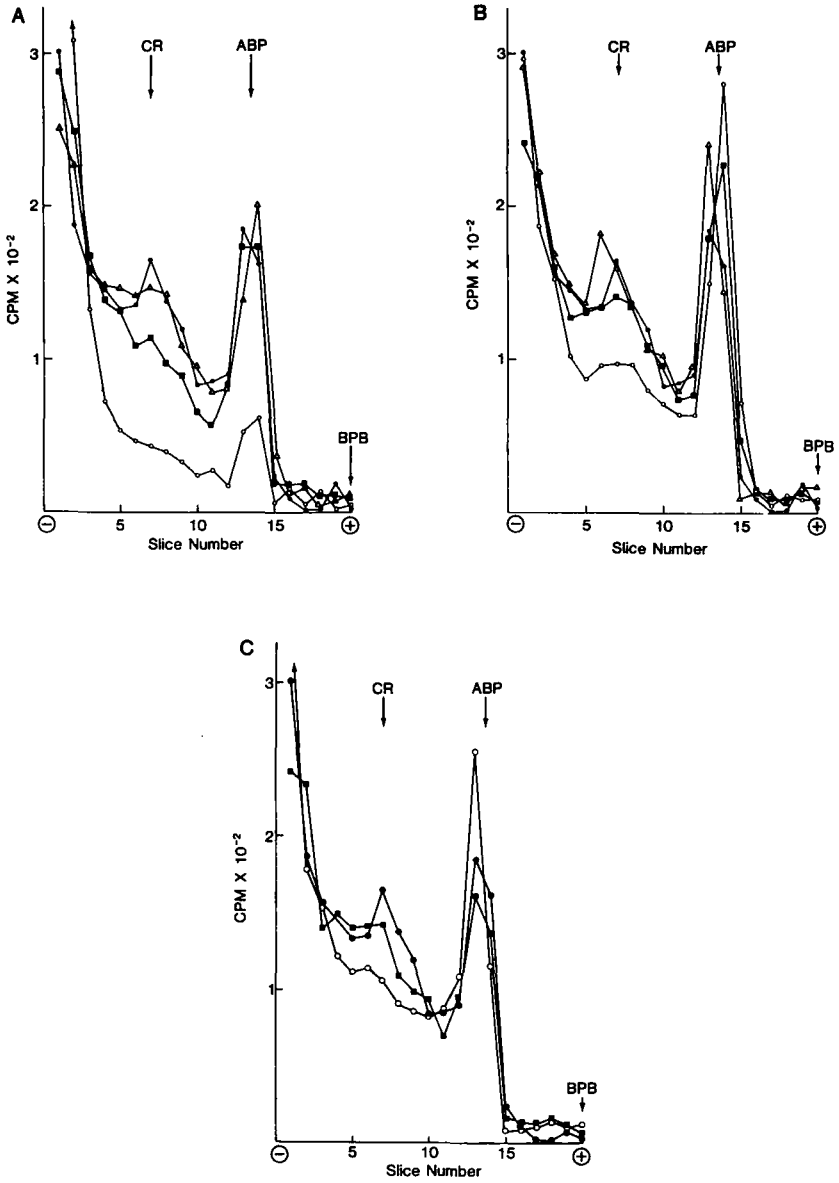

Figure 3: In vitro inhibition of ^{3}H-dihydrotestosterone binding to epididymal cytoplasmic receptor by: A. dihydrotestosterone; B. estradiol-17 β ; and C. cyproterone acetate. Epididymides from 24 h castrate adult rats were homogenized in 50 mM Tris-HCl buffer (pH 7.4 at 4°C) containing 1 mM EDTA and a 105,000 g supernate was prepared. To 1 ml aliquots of cytosol was added 0.2 ng ^{3}H-dihydrotestosterone (SA 40 Ci/mmole) with or without unlabeled steroids. The samples were equilibrated overnight at 0°C and 100 ul aliquots were applied to polyacrylamide gels. Electrophoresis was carried out at 0-4°C at 2 mAmp per gel, the gels were sliced into 2 mm segments and radioactivity was extracted with scintillation fluid. Competitor concentrations: ● —— ● None; Δ —— Δ .2 ng; ■ —— ■ 2 ng; o —— o 20 ng.

Figure 4 shows in vitro competition of ^{3}H-dihydrotestosterone for androgen receptor as measured on DEAE-cellulose filters. Epididymides were obtained from rats castrated 8 days previously to eliminate ABP. Cytosols were prepared and labeled with ^{3}H-dihydrotestosterone in the presence or absence of unlabeled competitors and receptor binding was assayed. Both estradiol-17 β and cyproterone acetate were direct competitors for ^{3}H-dihydrotestosterone binding.

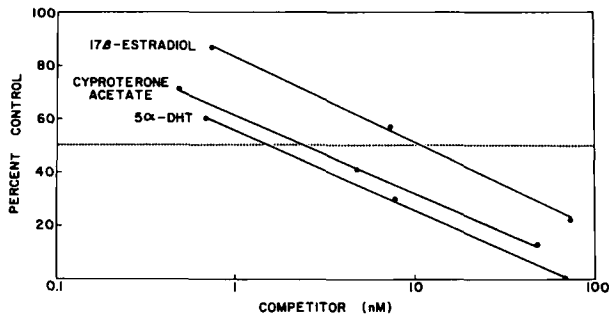

Figure 4: Competitive inhibition of ^{3}H-dihydrotestosterone binding to epididymal cytoplasmic receptors by cyproterone acetate and estradiol-17 β . Epididymides from 8 day castrate rats were homogenized in 50 mM Tris-HCl buffer (pH 7.4 at 4°C) containing 1mM EDTA and 12 mM thioglycerol (TESH buffer) and a 105,000 g cytosol was prepared. The cytosol was precipitated at 40% ammonium sulfate saturation and the pellet was redissolved and dialyzed overnight against TESH buffer containing

0.69 nM ^{3}H-dihydrotestosterone alone or in combination with various concentrations of unlabeled steroids. Aliquots of 50 ul were applied to DEAE-cellulose (DE-81) filters and eluted with 0.1 M KCl in TESH buffer as described in Materials and Methods. The ordinate represents the bound radioactivity as percent of control containing no competitor. The abscissa represents the molar concentration of competitor.

DISCUSSION

A single pharmacological dose of estradiol-17β inhibits 5α reduction of testosterone as well as accumulation of androgens within cytosol and nuclei of rat epididymis. The decreased uptake of labeled androgen in the epididymis after estradiol-17β injection appears to result from a combination of effects on metabolism and binding in the epididymis. Inhibition of metabolism by estradiol-17β was not associated with an alteration in the pattern of products formed. This is likely a direct

TABLE I

EFFECT OF CYPROTERONE ACETATE AND ESTRADIOL-17β ON THE IN VIVO UPTAKE AND BINDING OF ^{3}H-DIHYDROTESTOSTERONE IN EPIDIDYMAL NUCLEI

Treatment[a]	Total Activity[b] (cpm)		Nuclear Bound Activity[c] (cpm)
	Cytosol	Nucleus	
Control	197,000	2790	1710
Cyproterone Acetate	112,000	610	350
Estradiol-17β	97,000	100	0

[a] Either cyproterone acetate or estradiol-17β (1 mg in 0.1 ml ethanol) or 0.1 ml ethanol alone was injected intravenously into 18 h castrate rats immediately after evisceration and functional hepatectomy. Within 5 minutes, 60 uCi ^{3}H-dihydrotestosterone was injected intravenously and after 3 h the rats were killed. Three rats were used for each group treatment.

[b] Total radioactivity recovered from purified nuclei or cytosol.

[c] Bound radioactivity extracted from equal numbers of nuclei with 0.4 M KCl and measured by sucrose gradient centrifugation.

effect on the epididymis since it has been demonstrated that estradiol-17β decreases formation of dihydrotestosterone from testosterone in rat ventral prostate in vitro (10). Cyproterone acetate in a similar dosage did not inhibit metabolism of ^{3}H-testosterone. These results are in agreement with previous findings (10) that cyproterone (1 mg/100 g body weight) does not alter the ratio of dihydrotestosterone to testosterone in either ventral prostate or seminal vesicle of the rat. Since conversion of ^{3}H-testosterone to ^{3}H-dihydrotestosterone was not inhibited, treatment with cyprotereone acetate permitted a larger amount of ^{3}H-dihydrotestosterone to become available for binding.

Previous studies have shown that estradiol-17β has direct peripheral effects on androgen target tissues (10-16). Fang and Liao (11) have demonstrated that estradiol-17β inhibits in vitro translocation of a 3S receptor complex into prostate nuclei after incubation with ^{3}H-testosterone. Estradiol-17β and cyproterone acetate were bound by the androgen receptor in epididymis as indicated by competition with ^{3}H-dihydrotestosterone in vitro. The relative steroid affinities suggest that the more complete inhibition of nuclear uptake by estradiol-17β may have resulted from diminished conversion of ^{3}H-testosterone to ^{3}H-dihydrotestosterone. Testosterone and dihydrotestosterone have similar equilibrium binding constants for the epididymal androgen receptor; however, their kinetics of binding differ. The more prolonged dissociation rate of dihydrotestosterone from receptor may account for its longer retention in nuclei relative to testosterone (2).

ACKNOWLEDGEMENTS

This work was supported by USPHS Grants HD-04466 and HD-12788 and a grant from the Rockefeller Foundation. DJT is the recipient of a Research Career Development Award from the National Institutes of Health.

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