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THE INFLUENCE OF STEROIDAL AND NONSTEROIDAL ESTROGENS ON THE 5α-REDUCTION OF TESTOSTERONE BY THE VENTRAL PROSTATE OF THE RAT

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Summary—The 5α-reduction of testosterone to dihydrotestosterone (DHT) correlates with the androgen-mediated growth of the prostate under different experimental and clinical conditions. The aim was to study the regulation of the prostatic growth and enzyme activity by steroidal and nonsteroidal estrogens. Estrogens did not activate the androgen-dependent 5α-reductase activity in cultured prostate of the rat. The direct inhibition of the enzyme activity by estrogens at the concentrations achievable in the male is not probable either. However, early estrogenization of the male rats *in utero* (on Day 17 of pregnancy) with diethylstilbesterol (DES) resulted in a persistent decrease of the enzyme activity and growth of the prostate indicating a critical estrogen-sensitive period in the regulation of the ultimate enzyme activity. The similar DES-like inhibitory effect on the growth of the prostate was achieved by keeping animals from fertilization throughout the pregnancy until weaning on diet containing soy, rich in environmental estrogens. Zearalenone (Zeranol) and coumestrol, two nonsteroidal estrogens found in human and animal food mimicked estradiol action in culture, but they were not estrogenic or antiestrogenic when administered to normal adults.

INTRODUCTION

Estrogens administered prenatally or neonatally into males have long-term inhibiting effects on the growth and functional development of the male sex accessory glands [1–3]. Estrogens may also promote the growth of the prostate [4–8] and enhance the growth promoting action of androgen [9–11]. The mechanisms of these diverse estrogens actions in the prostate remain obscure. They may be based on estrogen-induced changes in androgen metabolism. Effects of estrogens on testosterone metabolism by prostatic tissue have been inhibitory, but only when high concentrations of estrogen (10^{-6} mol/l or higher) and treatments of short duration were used [12–17]. The long-term effects of estrogens on the 5α-reduction at concentrations closer to those found in the male serum have not been elucidated to date. Demonstration of such effects might provide explanatory insight into the role of estrogens in the control of normal prostatic growth as well as in the pathogenesis of prostatic tumors.

The ventral prostate of the rat was chosen as the model for the studies on the estrogenic regulation of 5α-reduction in prostate. Rat ventral prostate responds *in vitro* to androgens and a number of other hormones including estrogens [18]. Concentrations of 17β-estradiol in the range of 10^{-7}–10^{-9} mol/l were found sufficient to cause squamous metaplasia, a classical sign of estrogen action in the prostate. In addition to estradiol, nonsteroidal estrogens found in animal and human food were included in the study, because positive associations have been found between prostate neoplasia and dietary constituents (see [19] for references).

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MATERIALS AND METHODS

Experimental animals, treatments and diets

Male rats of the Sprague–Dawley strain, 10–12-weeks-old, were used throughout the study. Animals were housed in temperature- and light-controlled animal quarters on a 12/12 h light/dark cycle. In most of the experiments the rats were given commercially pelleted food (Hankkija, Turku, Finland), composed of milk products 6%, fish and meat products 14%, grain products 60%, soy 13%, vitamins and minerals (normal feed). When indicated some rats were kept on a soy-free semisynthetic diet (Astra–Ewos, Södertälje, Sweden), composed of wheat starch, casein, arachis oil, salts and vitamins. Soy flour is known to contain an abundance of phytoestrogens and is a major dietary source of equol [20]. A marked and rapid decrease in the urinary excretion of equol was found when the normal rat feed was changed to this semisynthetic diet [20].

The date of insemination was determined by vaginal smears taken the morning after 3-month-old virgin female rats were placed with a male.

When sperm cells were present in the smear, it was designated Day 0 of pregnancy.

Diethylstilbesterol (5 μg/animal) was administered in 250 μl sesame oil on Day 17. Control animals received sesame oil only. The pregnant animals were allowed to deliver normally. There were 19 DES-treated pregnant rats and the total of 165 pups were born (73 live males, 51 live females and 41 stillborn). In control group there were 6 pregnant rats, who delivered the total of 54 pups (35 males and 19 females, all alive). Larger doses of DES ($\geqslant$ 10 μg per animal given in single or in multiple injections) resulted in prolonged delivery and no live pups were born). The animals exposed to DES *in utero* were killed at the age of 10–12 weeks, and the ventral prostates were removed for further studies.

Some adult animals were castrated under ether anesthesia and treated with testosterone (2 mg of testosterone in 200 μl of 0.9% NaCl solution s.c. per rat). 17β-estradiol (25 or 100 μg per 100 g body wt) was injected in 200 μl of sesame oil.

Prostate culture

Explants of rat ventral prostate were cultured according to Trowell with minor modifications [21]. Explants were randomly put on lens paper strips lying on stainless steel grids in petri dishes. The hormones added in the basal medium, Medium 199 with Earle's salts with or without Phenol Red (Sigma Chemical Co., St Louis, Mo.), were insulin, testosterone and corticosterone. The medium was added up to the level of explants. The petri dishes were kept at 37°C in closed chambers gassed daily with a mixture of 40% oxygen, 5% carbon dioxide and 55% nitrogen. The chambers contained water on the bottom to guarantee sufficient humidity.

The hormones used in organ cultures were insulin (Insulin Lente®, Novo Industries, Copenhagen, Denmark) at 0.08 IU/ml, testosterone (17β-hydroxy-4-androsten-3-one) at 10^{-7} mol/l, estradiol (1,3,5,(10)-estratriene-3,17β-diol) at 10^{-5}–10^{-12} mol/l and corticosterone (4-pregnene-11β,21-diol-3,20-dione) at 10^{-7} mol/l. Hormones were dissolved in propylene glycol (Fluka AG, Buchs, Switzerland) and added in 10 μl–10 ml of medium. Diethylstilbesterol (DES, 4,4′-(1,2-dietyl-1,2-ethylene-diyl)bisphenol), zearalenone (6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid lactone), zeranol (zearalanol, a synthetic derivative of zearalenone, 6-(6,10-dihydroxy-undecyl)-β-resorcylic acid lactone) and coumestrol (2-(2,4-dihydroxyphenyl)-6-hydroxy-3-benzofuran carboxylic acid 6-lactone) were purchased from Sigma Chemical Co. (St Louis, Mo., DES and zearalenone), International Minerals & Chemical Corporation (Terre Haute, Ind.), Eastman Kodak Co. (Rochester, N.Y.), respectively.

The 5α-reductase assay

Homogenization of tissue. Minced tissue or cultured explants of rat ventral prostate were homogenized in 0.5–2 ml of 0.1 M Krebs–Ringer phosphate buffer, pH 7.4, containing 1 mM dithiothreitol (Sigma Chemical Co., St Louis, Mo.) with a glass–glass homogenizer cooled with ice. The suspension of tissue was used for the assay of the enzyme activity without any filtration or centrifugation. Fresh homogenates were usually utilized for assays; if not, the homogenate was stored at -20°C where the enzyme inactivated with a half-life of about 10 days.

Incubation conditions

The incubation solutions in duplicate tubes contained, in a final vol of 400 μl, the following components; 0.5 mM NADP, 5 mM glucose-6-phosphate, 0.5 unit glucose-6-phosphate dehydrogenase, 0.1 M Krebs–Ringer phosphate buffer, pH 7.4 100–200 μl homogenate and 7.5 μl 4-[^{14}C]testosterone (52 mCi/mmol) (0.05 mCi/1.25 ml propylene glycol) (Amersham International plc, Amersham, U.K.). The reaction was started by the addition of the prostatic homogenate. The reaction mixtures were incubated at 37°C for 30 min. At these incubation conditions the amount of 5α-reduced metabolites produced was linear at least up to 60 min. The requirement of NADPH was absolute because no enzyme activity was observed when any component of the NADPH-generating system was omitted from the incubation medium.

Extraction of steroids

The reaction was stopped with 3 ml ethyl ether. The tubes were stoppered and agitated. The upper phase was removed. The extraction was repeated. The lower phase was further extracted with 3 ml chloroform. The ether and chloroform solutions were combined and evaporated under nitrogen. The steroids were taken up in 300 μl absolute ethanol containing testosterone, DHT, 5α-androstane-3α,17β-diol, and 5α-androstane-3β,17β-diol.

Product isolation and quantitation

The ethanol solutions were applied to ready-made silica gel sheets on aluminum (E. Merck, Darmstadt, F.R.G.). The chromatogram was developed with the solvent system: hexane-1-hexanol (3:1) for 2 h using a sandwich chamber [22]. Nonradioactive steroid standards were located by spraying with Liebermann–Burchard reagent and heating. The radioactivities of the cut spots were counted in a scintillation counter.

Estimation of 5α-reductase was based on the percentage formation of DHT, 5α-androstane-3α/3β,17β-diol and androstanedione from testosterone and calculated in terms of pmol min^{-1} mg protein^{-1}. The protein content of the homogenates was assayed using the folin phenol reagent.

Morphometry

Tissue samples or cultured explants were fixed in Bouin's fixative, and prepared for histology. Sections

were cut from the blocks and stained with H-E. One section of each block was taken for morphometric analysis. Volume densities of epithelium (V_{VEP}), glandular lumina (V_{VLU}) and interacinar stromal tissue (V_{VIT}) and height of epithelium (h) were calculated with point-counting volumetry as described earlier [37]. The acini with squamous metaplasia were counted.

Statistics

The results were tested with analysis of variance following *t*-test with Bonferroni correction by using BMDP 7D program.

RESULTS

The effects of short-term castration and treatments of castrated and intact rats with estradiol on mean wet wt, protein concentration, and 5α-reductase activity of ventral prostate are summarized in Fig. 1a–c. The castration for 3 days resulted in a decrease of about 40% of the enzyme activity expressed in terms of pmols per min per mg protein. This decrease was completely reversed when testosterone was administered (2 mg s.c. daily) to the castrated rats (not shown in the figure). In the castrated animals, the 5α-reductase activity doubled in 6 days.

The administration of estradiol (25 μg s.c. daily for 3 days) to normal and castrated rats caused nonsignificant changes in mean prostate wet wt and protein concentration. However, the 5α-reductase activity was lowered significantly in noncastrated animals. In fact, the decrease was equivalent to that seen after the 3-day castration (Fig. 1). The effects of castration and the simultaneous administration of estradiol were not significantly different from that observed following castration alone suggesting that reduction of androgen concentration is involved in the estogen-induced inhibition of the 5α-reductase. No metaplastic changes were seen in the prostatic epithelium of these animals.

Zeranol (a synthetic derivative of zearalenone) or coumestrol given s.c. daily for 6 days (5 injections) to normal adults or estradiol-treated noncastrated adults had neither estrogenic nor antiestrogenic activity when measured in terms of the changed organ wet wt, protein concentration and 5α-reductase activity (Tables 1 and 2). The morphological changes (an increased volume density of interstitium and a decreased volume density of lumen) seen in estrogen-treated noncastrated adults were not reversed by zeranol or coumestrol, either, indicating again the lack of antiestrogenicity of the compounds in the test system. No metaplastic changes were seen in the prostatic epithelium of either treated or control animals.

The androgen-dependence of the 5α-reductase was confirmed in prostate culture. The 5α-reductase

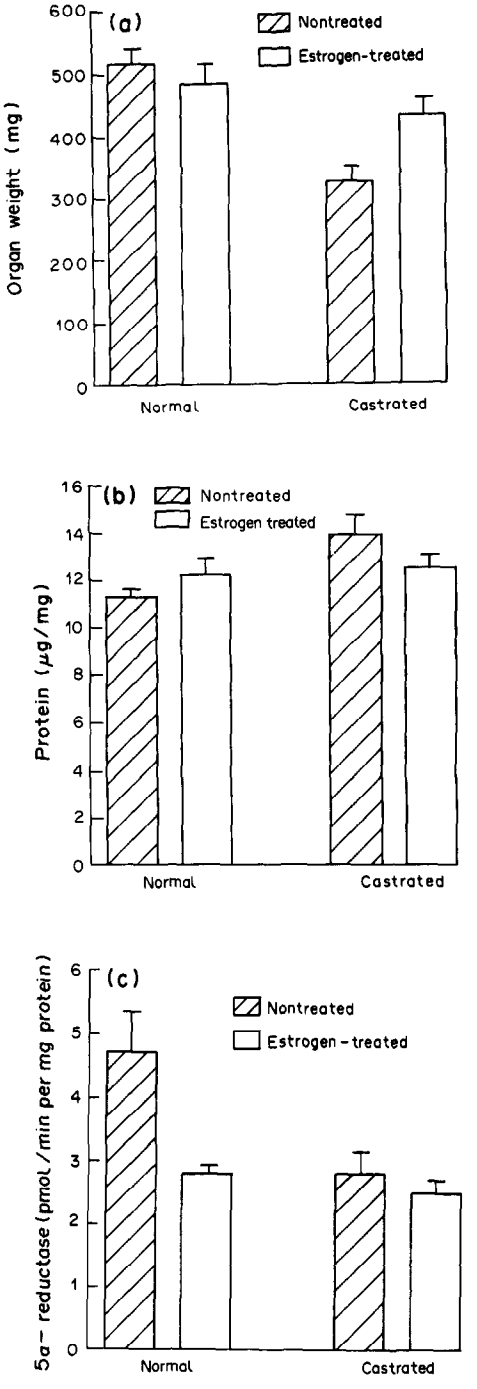

Fig. 1. Effects of 3-day castration and treatments of castrated and intact adult rats with 17β-estradiol (25 μg/100 g body weight s.c. daily for 3 days) on mean wet weight (a), protein concentration (b) 5α-reductase activity (c) of ventral prostate. Hormone treatment began on day of castration. Each value represents the mean of 8 animals.

activity was more than doubled by testosterone at the concentration of 10^{-7} mol/l found to be optimal for the maintenance of rat ventral prostate morphology for at least 14 days (11.75 pmol/min/mg protein in the presence of testosterone vs. 4.99 pmol/min/mg protein without testosterone, Fig. 2a and b). The

Table 1. Effect of 6-day treatment with estradiol and zeranol on prostatic wet weight, protein content, 5α-reductase content and morphology of adult normal rats (mean ± SEM)

	Weight (mg)	Protein (μg/mg)	5α-reductase (pmol/min/ mg protein	V_{VEP} (mm^3/mm^3)	V_{VLU} (mm^3/mm^3)	V_{VIT} (mm^3/mm^3)	D_{LU} (μm)
Control	333 ± 48	133 ± 10	5.08 ± 0.67	0.281 ± 0.015	0.530 ± 0.036	0.188 ± 0.024	208 ± 20
Estradiol (50 μg/day)	140 ± 22**	169 ± 13	5.83 ± 0.64	0.272 ± 0.029	0.420 ± 0.021	0.309 ± 0.042	154 ± 10
Zeranol (400 μg/day)	314 ± 18	116 ± 9	6.30 ± 0.52	0.251 ± 0.041	0.497 ± 0.035	0.253 ± 0.021	188 ± 16
Estradiol + Zeranol	131 ± 17***	116 ± 12	6.87 ± 0.71	0.272 ± 0.030	0.430 ± 0.048	0.296 ± 0.026	138 ± 13

Each value represents the mean of 5 animals. V_{VEP}, V_{VLU}, V_{VIT} = volume densities of epithelium, lumina and interacinar tissue, respectively. D_{LU} = diameter of lumina. ** = differs significantly from control, $P < 0.01$. *** = differs significantly from control, $P < 0.001$.

Table 2. Effects of estradiol and coumestrol on prostatic wet weight, protein content and morphology of adult normal rats (mean ± SEM)

	Weight (mg)	Protein (μg/mg)	V_{VEP} (mm^3/mm^3)	V_{VLU} (mm^3/mm^3)	V_{VIT} (mm^3/mm^3)	D_{LU} (μm)
Control	387 ± 22	115 ± 8	0.245 ± 0.029	0.639 ± 0.040	0.122 ± 0.013	227 ± 19
Estradiol (50 μg/day)	256 ± 21**	144 ± 6	0.236 ± 0.029	0.621 ± 0.030	0.144 ± 0.011	200 ± 14
Coumestrol (500 μg/day)	413 ± 31	103 ± 6	0.213 ± 0.022	0.673 ± 0.023	0.115 ± 0.015	247 ± 9
Estradiol + Coumestrol	289 ± 26	118 ± 9	0.201 ± 0.015	0.632 ± 0.028	0.167 ± 0.025	204 ± 7

Each value represents the mean of 5 animals. V_{VEP}, V_{VLU}, V_{VIT} = volume densities of epithelium, lumina and interacinar stromal tissue, respectively. D_{LU} = diameter of lumina. ** = differs significantly from control, $P < 0.01$.

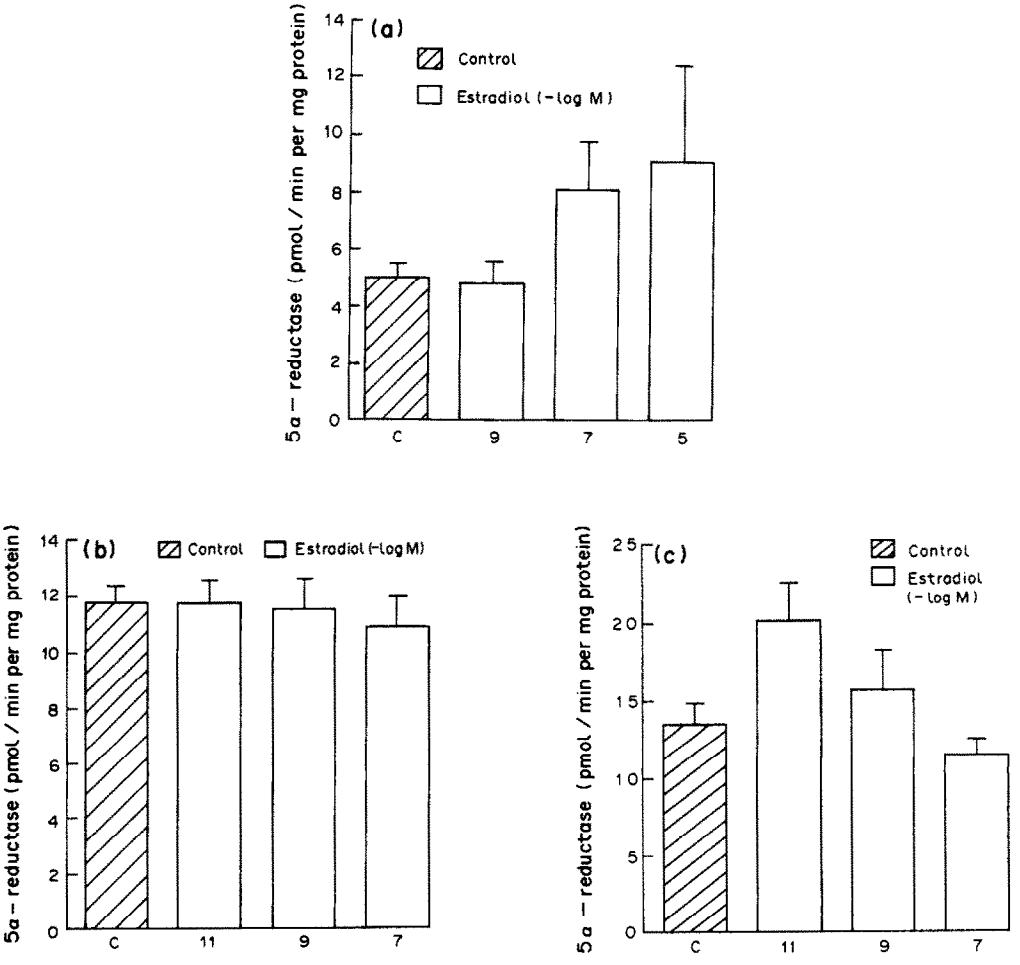

Fig. 2. Effects of 17β-estradiol added in different concentrations to the basal culture medium in explants from ventral prostate of intact adult rats cultured for 7 days. (a) With Phenol Red and without testosterone. (b) With Phenol Red and with testosterone (10^{-7} mol/l). (c) Without Phenol Red and with testosterone (10^{-7} mol/l). Each value represents the mean of 3–20 culture dishes.

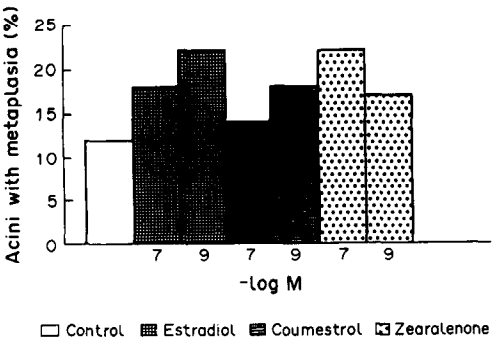

Fig. 3. Percentage of acini with metaplastic changes in explants cultured with 17β-estradiol, coumestrol and zearalenone for 7 days in basal culture medium (with Phenol Red). Values represent means of 6 cultures (for control and 17β-estradiol-treated groups) or 3 cultures (for coumestrol- and zearalenone-treated groups).

enzyme activity began to increase by 12 h and reached its maximum by 24 h after addition of testosterone to culture medium, well before any morphometric changes were recorded.

The androgen-activated 5α-reductase activity was further enhanced by estradiol at the low concentrations of 10^{-9}–10^{-12} mol/l in the absence of Phenol Red, known to be weakly estrogenic, but the increase was small and statistically nonsignificant at the end of 7-day culture (Fig. 2c). The slight decrease of the enzyme activity at the estradiol concentration of 10^{-7} mol/l (medium concentration) was not significant, either. This decrease was independent of Phenol Red addition. Phenol Red decreased the net formation of 5α-reduced compounds in all groups (Fig. 2b and c). The decrease became statistically significant ($P < 0.05$) when all groups were combined.

The addition of estradiol alone (without testosterone) to the basal culture medium showed a dose-dependent increase in 5α-reductase activity during 7-day culture (Fig. 2a). The changes were, however, not statistically significant. The pattern of response was different from that seen after testosterone addition. No increase in the enzyme activity was seen by 24 h when androgen-induction had reached its maximum. The volume density of epithelium was concomitantly increased by estrogen, and acini with squamous metaplasia appeared (Fig. 3) [18]. The low concentrations of estradiol (10^{-9}–10^{-12} mol/l) and Phenol Red had no effects on morphology of explants cultured for 7 days.

Estradiol action was mimicked by coumestrol and zearalenone at concentrations of 10^{-7} and 10^{-9} mol/l. Both induced metaplastic transformation in epithelium of cultured explants. The number of acini with metaplastic changes was increased, too (Fig. 3). The changes induced by estradiol (10^{-7} and 10^{-9} mol/l) and zearalenone (10^{-7} mol/l) were statistically significant ($P < 0.05$, $P < 0.001$ and $P < 0.001$, respectively). The effects of estradiol and zearalenone were not additive (not shown in the figure).

Long-term effects of exposure to estrogen were studied by treating animals prenatally with diethylstilbesterol (DES). DES was elected because it can reach the fetal tissues in an active form. Prenatal exposure to DES (5 μg per mother animal on Day 17 of pregnency) caused a small but reproducible and significant decrease in the 5α-reductase activity and prostatic weight demonstrable at the age of 10–12 weeks (Table 3). No corresponding decrease in the enzyme activity or the mean organ wet weight was seen after the postnatal estrogenization at the age of 3 weeks (100 μg estradiol s.c. per animal on Days 20 and 22), (Table 3). There was no change in the mean body weight of DES- or estradiol-treated animals and in any recorded morphometric parameters of the prostate (Table 3). No metaplastic changes were seen in the prostatic epithelium of treated animals.

In order to study possible effects of dietary estrogens the normal rat feed was changed to a semisynthetic diet devoid of estrogenic substances (see Materials and Methods) one week before fertilization. The pregnant rats were first kept on this semisynthetic diet throughout the pregnancy until weaning of the pups on Day 21 when the pups were transferred onto the normal diet. There were 3 pregnant rats in the experimental group and 2 controls. All rats were delivered normally and all pups appeared to be normal. When the pups were killed at the

Table 3. Effects of prenatal exposure to DES[1], neonatal estrogenization[2] and estrogen-free diet during prenatal and neonatal period[3] on prostatic wet weight, protein content, 5α-reductase content and morphology of adult normal rats (mean ± SEM)

	Weight (mg)	Protein (μg/mg)	5α-reductase (pmol/min/mg protein)	V_{VEP} (mm^3/mm^3)	V_{VLU} mm^3/mm^3)	V_{VIT} (mm^3/mm^3)	D_{LU} (μm)
(1) Control							
	442 ± 29	114 ± 6	4.07 ± 0.33	0.209 ± 0.022	0.683 ± 0.028	0.109 ± 0.015	238 ± 14
DES	362 ± 19	131 ± 7	3.02* ± 0.24	0.180 ± 0.011	0.713 ± 0.013	0.108 ± 0.011	255 ± 12
(2) Control	443 ± 26	139 ± 5	2.06 ± 0.17	0.214 ± 0.021	0.632 ± 0.040	0.156 ± 0.029	226 ± 15
	375 ± 23	144 ± 8	2.21 ± 0.16	0.274 ± 0.078	0.609 ± 0.069	0.118 ± 0.018	192 ± 9
(3) Control	334 ± 17	106 ± 4	7.07 ± 0.46	0.235 ± 0.018	0.644 ± 0.020	0.121 ± 0.007	231 ± 8
Semisynthetic diet	467 ± 21***	97 ± 3	8.06 ± 0.17	0.279 ± 0.032	0.584 ± 0.029	0.115 ± 0.007	247 ± 7

V_{VEP}, V_{VLU}, V_{VIT} = volume densities of epithelium, lumina and interacinar stromal tissue, respectively. * = differs significantly from control, $P < 0.05$. *** = differs significantly from control, $P < 0.001$. (1) Prenatal exposure to DES; pregnant rats were injected 5 μg DES on Day 17 of pregnancy. 23 animals per group. (2) Neonatal estrogenization; rats were injected 100 μg estradiol on Days 20 and 22. 11 animals per group. (3) Estrogen-free diet; rats were kept on semisynthetic, estrogen-free diet throughout pregnancy until weaning of the pups on Day 21 when the pups were transferred onto normal diet. 13 animals in control group and 14 animals in experimental group.

age of 10 weeks, the mean prostate weight of pups in the experimental group was increased significantly compared to those whose mothers had been on normal pelleted food containing soy products (Table 3). There was no change in 5α-reductase activity. In the second series of experiments the pups were on the soy-free diet from weaning onwards until the age of 10 weeks. The results of these experiments were negative. No significant changes were seen in the recorded parameters (not shown in the table).

DISCUSSION

The enzyme 5α-reductase (the enzyme 3-oxo-5α-steroid: NADP + 4-ene-oxido-reductase EC 1.3.1.22) catalyzes the irreversible conversion of testosterone to 5α-dihydrotestosterone (DHT), a hormonally more potent androgen in the prostate. The enzyme activity correlates with the androgen-mediated growth of the prostate under different experimental and clinical conditions. First, a hereditarily determined 5α-reductase deficiency leads to inhibition of urogenital sinus development to prostatic glands [23–24]. Second, the ability of various androgenic steroids to promote prostatic growth closely follows their capacity to enhance 5α-reductase activity [25]. Third, the DHT-induced increase in prostatic cell numbers occurs as a quantal process when a critical threshold of prostatic DHT is reached [26]. Fourth, studies with 5α-reductase inhibitors show a dose-dependent inhibition of testosterone-stimulated growth of the ventral prostate [27–30]. Furthermore, a hyperplastic growth of human as well as canine prostate occurs concomitantly with an increased net formation of DHT [31–35].

The present study confirmed the androgen-dependency of the prostatic 5α-reductase *in vivo*. It also showed that the enzyme activity is increased by androgen in tissue culture. The activation of the 5α-reductase in cultured explants was rapid. It became evident by 12 h after testosterone administration, and approached its maximum by 24 h. Estradiol further enhanced 5α-reductase of some testosterone-treated cultures when added at the low concentrations (10^{-9} μmol/l or less). However, the increase in the presence of testosterone was not significant by 7 days of culture. Moreover, the activating effect of estradiol on the 5α-reductase in the absence of testosterone, albeit dose-dependent and significant, is probably indirect, the consequence of the increased volume density of the epithelium and/or its metaplastic transformation. Androgens may thus be the only activators of 5α-reductase activity in the prostate. Neither thyroid, adrenal, nor pituitary hormones seem to play major roles in the regulation of the prostatic 5α-reductase [25]. Nevertheless, it is still possible that prolonged (weeks, months) treatment with estrogen leads to changes in androgen-dependent 5α-reductase, which would be more striking than those following short-term cultures.

Estradiol proved also a poor inhibitor of 5α-reductase in tissue culture, even in the absence of Phenol Red, which had a weak inhibitory effect on 5α-reductase. When added to the culture medium at the concentration of 10^{-5} mol/l medium or less, estradiol had only a weak inhibitory effects (significant only at the concentration of 10^{-7} mol/l) on 5α-reductase of explants cultured with testosterone for 7 days, and no effect in the absence of testosterone, thus confirming earlier findings of Kadohama and coworkers [15]. Although administering estradiol to intact animals considerably reduced the mean 5α-reductase activity, the effects of estradiol in castrated animals were not significantly different from that observed following castration alone, or following the administration of estradiol to intact animals. These results suggest that any short-term effects of estradiol in inhibiting the enzyme in the normal animal is probably a sequence of reducing the circulating biologically active androgen and concomitantly the 5α-reductase activity.

Estrogen has been shown to inhibit directly testosterone metabolism by prostatic tissue minces [9, 12], homogenate [11, 16–17], cellular fractions [13] or in short-term tissue culture [10, 14–15]. Farnsworth[9] found a general depression of testosterone metabolism and, more specifically, Groom *et al.*[10] reported inhibition of 5α-reductase. 5α-dihydrotestosterone formation within rat and canine prostatic explants was directly suppressed by estrogens such as estradiol and diethylstilbesterol (at the final concentration of 1 μmol/l or more) [10, 15]. The addition of estradiol-17β or diethylstilbesterol *in vitro* also inhibited the formation of 5α-dihydrotestosterone and androstanediols by normal human, benign hyperplastic and carcinomatous tissue, but only when high concentrations of estrogen (3×10^{-6} mol/l or higher) were used [12, 14, 16]. In general the K_i-values of estrogens are orders of magnitude higher than endogenous steroid concentrations as showed by Krieg *et al.*[17].

It thus seems justifiable to conclude that, in the dosages used clinically and at the hormone concentrations found in the male, estrogens have no direct inhibitory effects on the 5α-reductase in the prostate. The same conclusion was also reached by Usui and coworkers from their *in vivo* findings [16].

Both zearalenone and coumestrol induced metaplastic transformation in cultured explants of rat ventral prostate and acted thus like estrogen in the prostate. This confirms the earlier *in vivo* findings showing induction of squamous metaplasia and hyperplasia of the prostate in wethers by zeranol implants [36]. The effects of estradiol and zearalenone were not additive. This suggests that in the absence of estradiol these nonsteroidal estrogens could bind to estrogen receptors and possibly act in the manner of estradiol. In contrast to *in vitro* findings, neither of the compounds could mimick estradiol action or were antiestrogenic when administered to adult animals.

This may be due to metabolic inactivation of these compounds in the organism or impaired transport to hypothalamus. They may also be inactive in central nervous system [37].

Prenatal exposure to diethylstilbesterol has been shown to result in structural and functional alterations in the male genital tract [38–39]. DES has also produced changes in the prostate [40]. A single dose of DES (5 μg per rat) administered to a pregnant rat on Day 17 was shown to have long-term inhibitory effects on 5α-reductase, and on the postnatal growth of the prostate. There were no structural abnormalities in the prostate. Because estrogenization at the age of 3 weeks did not produce similar long-term effects, there exists a critical period in the control of the enzyme activity and growth of the prostate. The experiments with the animals kept on different diets produced similar results. Animals exposed *in utero* and neonatally showed a response to dietary factors. It is possible that estrogenic substances present in high amounts in normal food pellets exerted the DES-like inhibitory effect on the prostate.

It is not known, how DES acts during the prenatal period. The most plausible explanation for DES action would be the direct or indirect inhibition of the testis, known to be active in the perinatal period. Inhibition of testicular androgen secretion at a critical stage of development would slow down the synthesis of androgen dependent proteins such as 5α-reductase. However, the direct inhibitory effect of DES on the prostate cannot be excluded.

In conclusion, it is unlikely that estrogens directly influence the androgen-dependent 5α-reductase in the rat ventral prostate. Morphological changes induced by estrogen in prostate culture may very well account for the slight increase seen in 5α-reductase activity. The direct inhibition of the enzyme by estrogens at the concentrations achievable *in vivo* is not probable, either. However, early estrogenization of the animals resulted in a persistent decrease of the enzyme activity and the growth of the prostate, indicating a critical estrogen-sensitive period in the regulation of the ultimate enzyme activity. Nonsteroidal estrogens found in human and animal food mimicked estradiol action except that they did not produce castration-like effect when administered to normal adults.

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