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Feed-forward control of prostate growth: Dihydrotestosterone induces expression of its own biosynthetic enzyme, steroid 5α-reductase

(androgen action/testosterone metabolism/4-azasteroid inhibitors)

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ABSTRACT **Dihydrotestosterone, the primary mediator of prostate growth, is synthesized in target tissues from the circulating androgen testosterone through the action of steroid 5α-reductase (EC 1.3.99.5). The expression of 5α-reductase and the level of 5α-reductase messenger RNA in rat ventral prostate are regulated by androgens. To determine whether this control is mediated by dihydrotestosterone or testosterone, we investigated the effect of finasteride, a potent inhibitor of steroid 5α-reductase, on the expression of 5α-reductase in the prostate. The administration of finasteride to intact rats for 7 days caused a 55% decrease in prostate weight and an 87% decrease in 5α-reductase enzyme activity. Furthermore, the restoration of prostate growth after castration and the enhancement in 5α-reductase enzyme activity and 5α-reductase messenger RNA level by testosterone administration were blocked by finasteride, whereas the inhibitor had no effect on dihydrotestosterone-mediated increases in 5α-reductase activity or messenger RNA level. These findings indicate that dihydrotestosterone itself controls prostate growth and 5α-reductase activity. They further suggest that prostate growth is controlled by a feed-forward mechanism by which formation of trace amounts of dihydrotestosterone induces 5α-reductase, thereby increasing dihydrotestosterone synthesis and triggering a positive developmental cascade.**

Both the differentiation of the prostate gland during male embryogenesis (1) and the subsequent growth of the tissue during postnatal life (2) are controlled by androgenic hormones synthesized in the testes. Some androgen actions, such as the promotion of spermatogenesis and the enhancement of muscle growth, are believed to be mediated by the testicular androgen testosterone (2). In other target tissues, including the prostate, testosterone is converted to dihydrotestosterone by the enzyme steroid 5α-reductase (EC 1.3.99.5) (3, 4). Dihydrotestosterone is a more potent androgen than testosterone in some bioassay systems (5), and dihydrotestosterone formation is believed to be essential for many androgen actions. For example, studies of subjects with hereditary steroid 5α-reductase deficiency (6) and investigations of the effects of inhibitors of steroid 5α-reductase (7–11) indicate that dihydrotestosterone formation is necessary for both the embryonic differentiation and the postnatal growth of the prostate. Furthermore, unregulated dihydrotestosterone action is believed to cause hyperplastic prostate growth in aging dogs and men (12).

In addition to the role in the control of differentiation and growth, androgens also control the expression of a number of genes in the prostate, including the gene for steroid 5α-reductase (13). In the ventral prostate of the rat both the steady-state level and the recovery of 5α-reductase after castration are regulated by androgens (13), and this regulation is mediated at the level of transcription of 5α-reductase mRNA (14).

To determine whether testosterone or dihydrotestosterone is responsible for the control of this crucial process for androgen-mediated prostate growth, we evaluated the effect of the 4-azasteroid finasteride, a potent competitive inhibitor of the enzyme (15–17), on the level of expression of 5α-reductase and its mRNA in the rat ventral prostate. The findings demonstrate the existence of a dihydrotestosterone-mediated feed-forward control of prostate growth. (The findings have been partially published in abstract form.§)

MATERIALS AND METHODS

Treatment of Animals. Two experimental protocols were utilized. In the first, intact and castrated rats (weighing approximately 250 g) were treated with daily injections of the 5α-reductase inhibitor finasteride (17β-*N*-*t*-butylcarbamoyl-4-aza-5α-androst-1-en-3-one, a gift from Merck Sharp & Dohme) dissolved in ethanol/triolein (1:4, vol/vol) (25 mg/kg of body weight) from the day of castration. After 7 days, the animals were killed (ether overdose), and the ventral prostates were removed, weighed, and analyzed for 5α-reductase activity. In a second set of experiments, male (250-g) rats were castrated for 7 days. During this period of castration, the average weight of the ventral prostate decreases approximately 90%. The rats were then given by subcutaneous injection each day for 4 days either oil vehicle (20% ethanol in triolein) or oil vehicle containing testosterone propionate (Tp; 1 mg/kg of body weight per day), dihydrotestosterone propionate (Dp; 1 mg/kg of body weight per day), or Tp or Dp plus finasteride (25 mg/kg of body weight per day). The animals were then killed, and ventral prostates were removed, weighed, and analyzed for 5α-reductase activity, 5α-reductase mRNA, total RNA, DNA, and protein.

Assays. 5α-Reductase activity was measured in unfractionated homogenates as described previously (13). In brief, pools of prostate tissue from each of the treatment groups were homogenized in 14 vol of 0.88 M sucrose/1.5 mM $CaCl_2$ with a Brinkman tissue homogenizer, filtered through cheesecloth, and rehomogenized by hand in a Dounce homogenizer. An aliquot of this whole cell homogenate containing approximately 0.3 mg of protein was assessed for 5α-reductase activity in 0.2 ml containing 0.05 M potassium phosphate buffer at pH 6.5, 0.5 mM NADPH, and 0.25 μM [^{3}H]testosterone. The incubation was carried out for 1 h at 25°C. The reactions were stopped by shaking the tubes with 5 vol of ice-cold chloroform, and the 5α-reduced products in the

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chloroform extract were separated by thin-layer chromatography and assessed for radioactivity as described (13). In separate experiments, prostate membranes from finasteride-treated rats were subjected to several washes to determine whether residual inhibitor interfered with the assay.

Total RNA was isolated by a guanidinium isothiocyanate procedure from ventral prostates of animals subjected to hormonal treatments, purified by centrifugation through CsCl, and measured spectrophotometrically (18). Thirty-microgram aliquots were size-fractionated by electrophoresis through 1.5% (wt/vol) agarose gels, transferred to nylon filters by capillary blotting, and subjected to prehybridization, hybridization, and washing as described by Lehrman *et al.* (19). A combination of three single-stranded ^{32}P-radiolabeled probes prepared by the method of Church and Gilbert (20) and corresponding approximately to nucleotides 22–223, 386–586, and 682–802 of the rat 5α-reductase cDNA (14) were used in the hybridization. Autoradiography was carried out for the indicated times with Kodak XAR-5 film and two Cronex Quanta III (DuPont) intensifying screens. Scanning densitometry of films derived from multiple timed exposures was performed on a Hoefer GS300 instrument. One densitometric unit is defined as 1 cm^2 of area under a scanned autoradiogram signal. As a loading control, 10-μg aliquots of RNA from each group were size-fractionated by electrophoresis on the same gel, stained with ethidium bromide, and then photographed to demonstrate that similar amounts of ribosomal RNA (rRNA) were analyzed for each of the groups.

DNA was measured by the method of Burton (21), and protein was measured by the method of Lowry *et al.* (22). The dihydrotestosterone content of prostates in the various treatment groups was measured in homogenate aliquots by radioimmunoassay after chromatographic separation of testosterone and dihydrotestosterone on small celite columns (23).

RESULTS

The administration of finasteride to intact rats for 7 days caused a 55% decrease in the mean weight of the ventral prostate (50 vs. 120 mg/100 g of body weight) (Fig. 1). Prostate 5α-reductase activity was reduced by 87% in inhibitor-treated rats and was only slightly higher than that in rats that had been castrated for 7 days (Fig. 1). The combination of finasteride and castration produced effects similar to castration alone. The activity of 5α-reductase was similar in unfractionated homogenates and washed membranes, indicating that any finasteride remaining in the diluted homogenate did not interfere with the 5α-reductase assay. We concluded that finasteride treatment causes a true decrease in prostate 5α-reductase activity.

To determine if the reduction in prostate 5α-reductase in finasteride-treated rats is a direct consequence of dihydrotestosterone deficiency, we investigated the effect of the inhibitor on androgen-mediated regrowth of the prostate and enhancement of 5α-reductase enzyme activity in the castrated rat. The protocol utilized involves the administration of androgen to rats that have been castrated for 7 days; such a regimen causes an increase in the weight, in the content of protein, RNA, and DNA, and in the activity of steroid 5α-reductase in rat ventral prostate (13).

Since finasteride is a competitive inhibitor of 5α-reductase, it was necessary to choose the minimal dose of androgen that would cause a significant regrowth and induction of 5α-reductase activity of the prostate but could still be blocked by finasteride. Therefore, a dose–response study was performed in which 0.1–10 mg of testosterone propionate per kg of body weight per day were administered for 4 days to male rats, beginning 7 days after castration. The administration of 1 mg of testosterone propionate per kg of body weight per day for 4 days caused an approximate 3-fold increase in prostate weight and a 20-fold increase in 5α-reductase activity (results not shown).

The effect of finasteride (25 mg/kg of body weight per day) on androgen-mediated (1 mg/kg of body weight per day of either testosterone propionate or dihydrotestosterone propionate) regrowth and enhancement of 5α-reductase was then investigated. Testosterone and dihydrotestosterone caused increases in prostate weight (from 14 ± 1 to 37 ± 2 and 33 ± 2 mg/100 g of body weight, respectively) (Fig. 2). Finasteride effectively blocked testosterone-mediated growth but had no effect on dihydrotestosterone-mediated growth. As previously reported (13), testosterone treatment of castrates also causes a profound increase in 5α-reductase activity (from 16 to 389 pmol·h^{-1}·prostate^{-1}), an increase that was prevented almost completely by finasteride (Fig. 2). Administration of dihydrotestosterone also increased 5α-reductase activity but not as much as testosterone (165 vs. 389 pmol·h^{-1}·prostate^{-1}), and the dihydrotestosterone-mediated increase was not prevented by finasteride. These results are similar if expressed as pmol·h^{-1}·(mg of protein)$^{-1}$ or pmol·h^{-1}·(g of prostate)$^{-1}$ (results not shown).

We concluded that androgen-mediated regrowth of the prostate and a large part of the enhancement of 5α-reductase are mediated by dihydrotestosterone. The fact that testosterone at this dosage caused similar increases in prostate weight but higher 5α-reductase enzyme activity than dihydrotestosterone suggests that a portion of the testosterone-mediated increase in 5α-reductase activity may be due to stabilization of the enzyme by substrate binding (24). On the basis of competition studies, dihydrotestosterone is believed

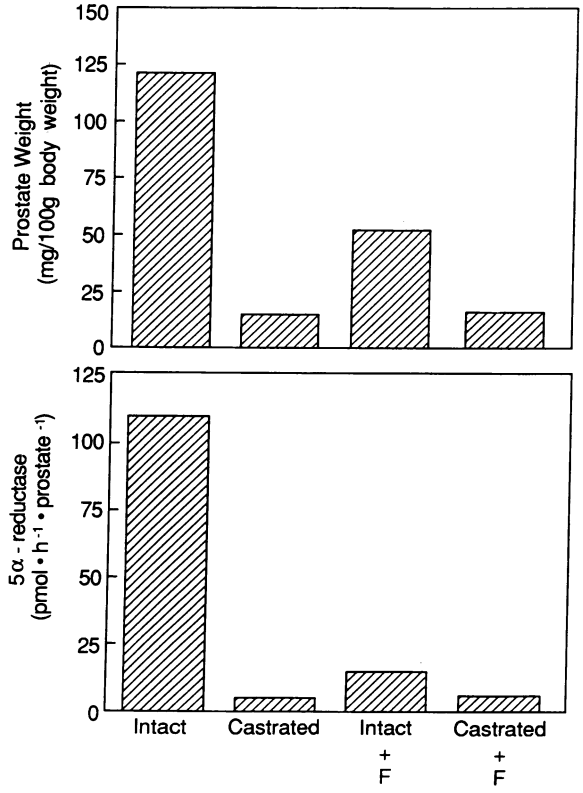

FIG. 1. Effect of finasteride (F) treatment for 7 days in intact and castrated male rats on ventral prostate weight and prostate 5α-reductase activity. The assays were performed as described in the text. Each bar in *Upper* represents a mean of two or three animals. The results in *Lower* represent single assays of a pool of tissues from the animals in each group.

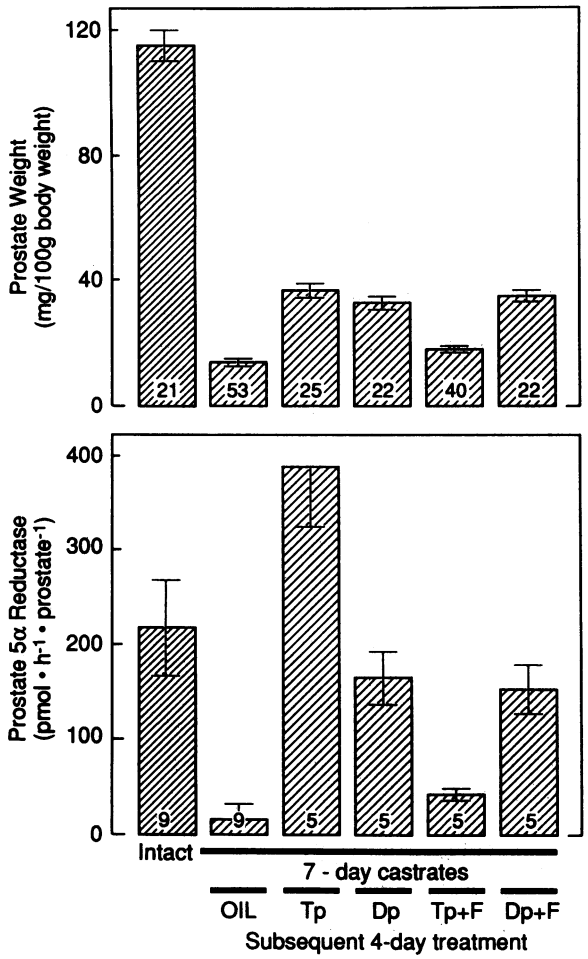

FIG. 2. Effect of finasteride on androgen-mediated regrowth of the castrated rat prostate. Intact male rats and rats that had been castrated for 7 days were then treated for 4 days with triolein (oil) or triolein containing testosterone propionate (Tp) dihydrotestosterone propionate (Dp), or finasteride (F) + Tp or Dp as described in the text. In *Upper* the mean ± SEM for the prostate weights are illustrated for all animals studied (number shown in each bar). In *Lower* the values represent the mean ± SEM for 5α-reductase assays performed on pools of prostates (number shown in each bar) in separate experiments.

to have a low affinity for the enzyme and presumably lacks this stabilizing effect (24).

To provide insight into the mechanism by which dihydrotestosterone controls 5α-reductase activity, we examined steady-state levels of 5α-reductase mRNA by blotting analysis of RNA from the ventral prostates of control rats, 7-day

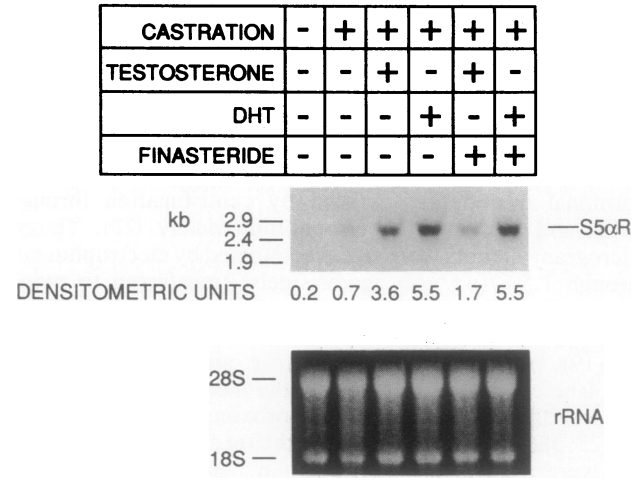

FIG. 3. Effect of finasteride on 5α-reductase mRNA levels in ventral prostate. Castrated rats (4–16 per group) were subjected (+) or not subjected (−) to the treatments indicated at the top of the figure as described in the text. DHT, dihydrotestosterone. In the upper gel, total ventral prostate RNA (30 μg) from each group was analyzed by blot hybridization to steroid 5α-reductase (S5αR) cDNA and autoradiographic analysis for 48 h. kb, Kilobases. The lower gel is a loading control in which 10-μg samples from each group were electrophoresed on the same gel as the samples of the upper gel, stained with ethidium bromide, and photographed under UV light.

castrates, and 7-day castrates treated for 4 days with testosterone propionate or dihydrotestosterone propionate at 1 mg/kg of body weight per day with or without finasteride (25 mg/kg of body weight) (Fig. 3). The administration of testosterone or dihydrotestosterone caused a large increase in 5α-reductase mRNA consistent with previous findings (14). Finasteride blocked the testosterone-mediated increase in 5α-reductase mRNA by about 65% when the results are expressed as density units per 30 μg of RNA (Fig. 3) and had an even more profound effect when the results were expressed as density units per prostate (Table 1). Finasteride also prevented an increase in prostate dihydrotestosterone levels in testosterone-treated rats (Table 1). We conclude that the androgen-mediated increase in prostate 5α-reductase enzyme activity is due in large part to a dihydrotestosterone-mediated increase in steady-state levels of 5α-reductase mRNA. Whether this action of dihydrotestosterone is due to an effect on the synthesis or the stability of 5α-reductase mRNA cannot be determined from these data.

DISCUSSION

We have investigated the role of dihydrotestosterone in the expression of the 5α-reductase enzyme in prostate. Inhibition

Table 1. Effect of finasteride on the induction by androgens of 5α-reductase and 5α-reductase mRNA in ventral prostates of castrated rats

Group	No.	Treatment on days 7–11	Mean wt of ventral prostate, mg	Nucleic acids, μg/prostate		Dihydrotestosterone, ng/prostate	5α-Reductase activity, pmol·h^{-1}/prostate	5α-Reductase mRNA, density units/prostate
				DNA	RNA			
Intact	2	Oil	350	270	450	6.2	220	15
Castrate	10	Oil	44	28	24	0.2	16	1
Castrate	4	Tp	110	120	190	2.8	330	68
Castrate	4	Dp	82	77	150	1.5	200	61
Castrate	8	Tp and F	50	44	41	0.2	41	5
Castrate	4	Dp and F	100	92	120	1.3	200	38

Beginning 7 days after castration, rats were treated with oil vehicle (oil), or oil containing testosterone propionate (Tp) or dihydrotestosterone propionate (Dp) (1 mg/kg of body weight per day in triolein) or Tp or Dp plus finasteride (F) (25 mg/kg of body weight per day). Animals were killed on day 11, and the ventral prostates in each group were divided into pools for enzyme assay (Fig. 1), mRNA measurements, dihydrotestosterone assay, and DNA measurements as described in the text. The results are means from two separate experiments.

of dihydrotestosterone formation in intact rats with the 5α-reductase inhibitor finasteride caused an 87% decrease in the activity of the enzyme, and administration of the inhibitor to castrated rats prevented testosterone-mediated increase in 5α-reductase activity and mRNA but did not prevent dihydrotestosterone-mediated increase in either parameter. These findings imply that dihydrotestosterone controls its own rate of synthesis in a positive manner. The finasteride-mediated decrease in both 5α-reductase enzyme and mRNA may explain the exquisite sensitivity of prostate dihydrotestosterone levels to finasteride and may play a crucial role in the pharmacological blockade of dihydrotestosterone production by azasteroids (25).

The present findings also demonstrate that the effect of dihydrotestosterone on prostate enzyme activity is secondary to enhancement of 5α-reductase mRNA levels. Thus, the presence of dihydrotestosterone, but not testosterone, increases the quantity of 5α-reductase mRNA (Fig. 3). In the presence of testosterone, finasteride partially blocks this increase. We do not know if the slight stimulation of 5α-reductase mRNA with testosterone treatment is due to a failure of the drug to block completely the conversion of testosterone to dihydrotestosterone or to a direct effect of testosterone itself.

Several mechanisms may underlie the mRNA response, including stabilization of the mRNA or direct enhancement of gene transcription mediated by dihydrotestosterone coupled to the androgen receptor. The androgen receptor is a member of the steroid hormone receptor superfamily (26), and much precedent exists for the ability of hormone–receptor complexes to stimulate transcription by interacting with steroid response elements (27). In the case of the androgen receptor, the picture is more complex, as there are two possible ligands, testosterone and dihydrotestosterone (6). Prior to this report, a differential response of a specific gene to these two ligands *in vivo* has not been demonstrated, to our knowledge. Nevertheless, strong physiological and genetic evidence (6) supports separate roles. For example, in mammalian sexual differentiation, testosterone coupled to the androgen receptor stimulates a program of differentiation leading to the formation of the internal male genitalia, whereas dihydrotestosterone coupled to the same receptor promotes formation of external genitalia and the prostate (6). These separate developmental programs suggest the existence of at least two gene networks (28), one responsive to dihydrotestosterone and a second responsive to either testosterone or dihydrotestosterone. Our results suggest that 5α-reductase may belong to the former network. Alternatively, the unique effect of dihydrotestosterone could be due to some undefined difference in the rate of catabolism.

A striking finding is that dihydrotestosterone, the product of 5α-reductase enzyme, positively regulates the expression of the 5α-reductase gene. Feed-forward or autocatalytic regulation has been postulated to play a key role in several developmental systems, especially in situations in which the local concentration of a molecule such as a morphogen must be dramatically increased to bring about a defined pattern of expression (29). This type of regulation has been explored at the molecular level in several systems. The protein encoded by the bacteriophage lambda *cI* gene stimulates its own transcription when bound to operator sequences in the *cI* promoter (30). In turn, the levels of *cI* in an infected cell determine the lysis versus lysogeny pattern of the virus. In *Drosophila*, the product of the fushi tarazu gene, a protein involved in determining embryonic polarity and spatial organization, stimulates transcription from its own promoter via interaction with an upstream enhancer in a process of autocatalytic activation (31, 32). Similarly, the establishment and maintenance of the female state in *Drosophila* is accomplished by a positive autoregulation of the sex-lethal gene (33). The finding of a feed-forward type of regulation imparted to the 5α-reductase gene underscores the central role played by this enzyme in prostate development and growth.

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