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Influence of Diethylstilbestrol, Leuprolelin (a Luteinizing Hormone-Releasing Hormone Analog), Finasteride (a 5α-Reductase Inhibitor), and Castration on the Lobar Subdivisions of the Rat Prostate

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ABSTRACT: The effects of various means of interfering with androgen action on rat coagulating gland, ventral prostate, lateral type 1 prostate, lateral type 2 prostate, and dorsal prostate were examined morphologically and quantitatively by assessing DNA content, wet weight, protein content, and zinc concentrations. Adult male Sprague-Dawley rats were subjected to 2 weeks of interfering with androgen action by treatment with Leuprolelin (a luteinizing hormone-releasing hormone analog), Finasteride (a 5α-reductase inhibitor), or diethylstilbestrol (DES), or by physical castration. For all prostatic lobes, inhibition of 5α-reductase elicited the smallest reduction in prostatic wet weight, DNA and protein contents, and zinc concentration. The most profound reductions in all parameters were elicited by castration. Treatments with DES and Leuprolelin gave intermediate effects with DES being the more effective in reducing all parameters in all prostatic lobes. Morphological changes elicited by all forms of androgen blockade were reduction of epithelial height, relative increase of connective tissue, reduction in ductal diameter, length, and number. The order of effectiveness of the various treatments on morphological features was as described above.

While all forms of androgen blockade elicited similar effects throughout the prostate, differences in response to all forms of interference with androgen action were observed in different lobes of the prostate with regard to wet weight, DNA and protein contents, and zinc concentration as well as morphological effects. Regressive changes at the morphological level were particularly striking in the coagulating gland and ventral prostate, and indistinct in the lateral type 2 prostate. Prostatic zinc concentration in both normal and androgen-deprived rats was the highest in the lateral type 2 prostate and was reduced by interfering with androgen action to the greatest extent in the dorsolateral prostate (lateral type 1 and type 2, and dorsal prostate). The distribution of zinc correlated with the expression of metallothionein, which was detected by immunocytochemistry only in the lateral type 2 prostate of both normal and androgen-deprived rats. Intraprostatic heterogeneity of zinc and metallothionein expression emphasizes interlobar differences in biological function within the rat prostate. The mechanism of development of regional heterogeneity within the prostate may shed light on the pathogenesis of prostatic proliferative diseases (prostatic hyperplasia and prostatic cancer) that initially owe their development to focal changes within large cell populations.

KEY WORDS: **androgen action, prostate, castration, interlobar heterogeneity**

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INTRODUCTION

It is well known that the prostate is an androgen-dependent organ and that castration causes a net loss in prostatic weight and DNA content as a result of massive regression and loss of cells through apoptosis [1–4]. For decades the androgen dependence of the prostate has been used as a clinical strategy in the treatment of prostatic cancer through various means of androgen deprivation [5]. Interfering with androgen action has been achieved in prostate cancer patients by administration of antiandrogens, estrogen, or synthetic analogs of luteinizing hormone-releasing hormone (LH-RH). Estrogen and LH-RH analogs cause down-regulation of LH secretion by the anterior pituitary and long-term suppression of serum testosterone (T) to castrate levels [6,7], while antiandrogens are competitors at the site of the androgen receptor. Another method of impairing androgen action is through use of 5α-reductase inhibitors, which block metabolic conversion of T to dihydrotestosterone (DHT), a treatment now used for benign prostatic hyperplasia [8–11].

Like the human prostate, the rat prostate is a complex gland composed of several lobes [4]. Microdissection techniques, pioneered by our group, have revealed that the rat prostate is composed of five lobes: the coagulating gland or anterior prostate, ventral prostate, lateral type 1 prostate, lateral type 2 prostate, and dorsal prostate [12]. Individual prostatic lobes have characteristic ductal branching patterns and express different secretory proteins and other secretory products [4,12]. Prostatic lobes can be divided into three groups on the basis of secretory function by immunocytochemistry, SDS-PAGE, and Western blot studies: a) The ventral prostate expresses as its major secretory protein a tetrameric protein known as prostatein, prostatic-binding protein, or α-protein, which is composed of three subunits (C1, C2, C3) [13–18]. Probasin mRNA is also expressed at low levels in the rat ventral prostate [19]. The ventral prostate also secretes citrate, polyamines [4,20], and a spermine-binding protein [21]. b) The coagulating gland, lateral type 1 prostate, and dorsal prostate express DP-1 and DP-2 as major secretory proteins [22,23] with probasin being a minor protein [24]. c) The lateral type 2 prostate expresses probasin as its major protein and much lower levels of DP-1 [12,24]. Through the use of microdissection techniques, levels of DP-1 and probasin vary considerably on a duct-by-duct basis within the dorsal-lateral prostate [24]. Functional heterogeneity within the prostate gland is but one aspect of intraprostatic regional heterogeneity as other features (ductal morphology, DNA synthesis, regressive changes following androgen deprivation, and biochemical activity) vary regionally from lobe to lobe or proximodistally along prostatic ducts [3,12,25–29]. Since most activities within the prostate are androgen dependent, interference with androgen action through of diethylstilbestrol (DES), Leuprolelin, and 5α-reductase inhibitor as well as physical castration on DNA and protein contents, prostatic weight, and zinc concentrations were determined for the five lobar subdivisions of the rat prostate.

MATERIALS AND METHODS

Animals and Experimental Treatments

Fourteen-week-old male Sprague-Dawley rats (Japan SLC Inc., Shizuoka, Japan) were used in this study. Rats were divided into five groups: i) sham operated (control group), ii) castrated via a scrotal incision (castration group), iii) implanted subcutaneously with a 20–30 mg pellet of DES (Sigma, St Louis, MO) (DES group), iv) injected once (3 mg/kg body weight) with Leuprolelin, an LH-RH analog (5-oxo-prolyl-histidyl-tryptophyl-seryl-tyrosyl-D-leucyl-leucyl-arginyl-*N*-ethyl-prolinamide-monoacetate), a gift from Takeda Chemical Industry (Osaka, Japan) (LH-RH group), and v) injected with Finasteride (50 mg/kg/day), a 5α-reductase inhibitor (N-(1, 1-dimethylethyl)-3-oxo-4-aza-5a-androst-1-ene-3-one-17b-carboxamide), a gift from Merck Sharp and Dohme (Rahway, NJ), dissolved in ethanol/triolein (1 : 4, vol/vol) (Finasteride group). After 14 days of treatment, the animals of all groups were sacrificed. Doses of Leuprolelin [30] and Finasteride [31] were chosen based upon previously published effective doses of these drugs.

Prostatic Microdissection

The coagulating gland, ventral, lateral type 1, lateral type 2, and dorsal prostatic lobes were separated by microdissection as described earlier [12]. Briefly, after sacrifice the prostate was excised from the donor, transferred to calcium- and magnesium-free phosphate-buffered saline (CMF-PBS; Handaibiken, Osaka, Japan) containing 0.5% collagenase (Sigma Co., St. Louis, MO), and incubated for 30 min at room temperature. Microdissection was performed under a dissecting microscope (Olympus VMZ, Olympus Instrument, Tokyo, Japan) using fine forceps and a von Graefe iris knife.

Morphology

Each lobe was microdissected into ductal arrays that were fixed with 4% paraformaldehyde. Ductal wholemounts were either photographed or embed-

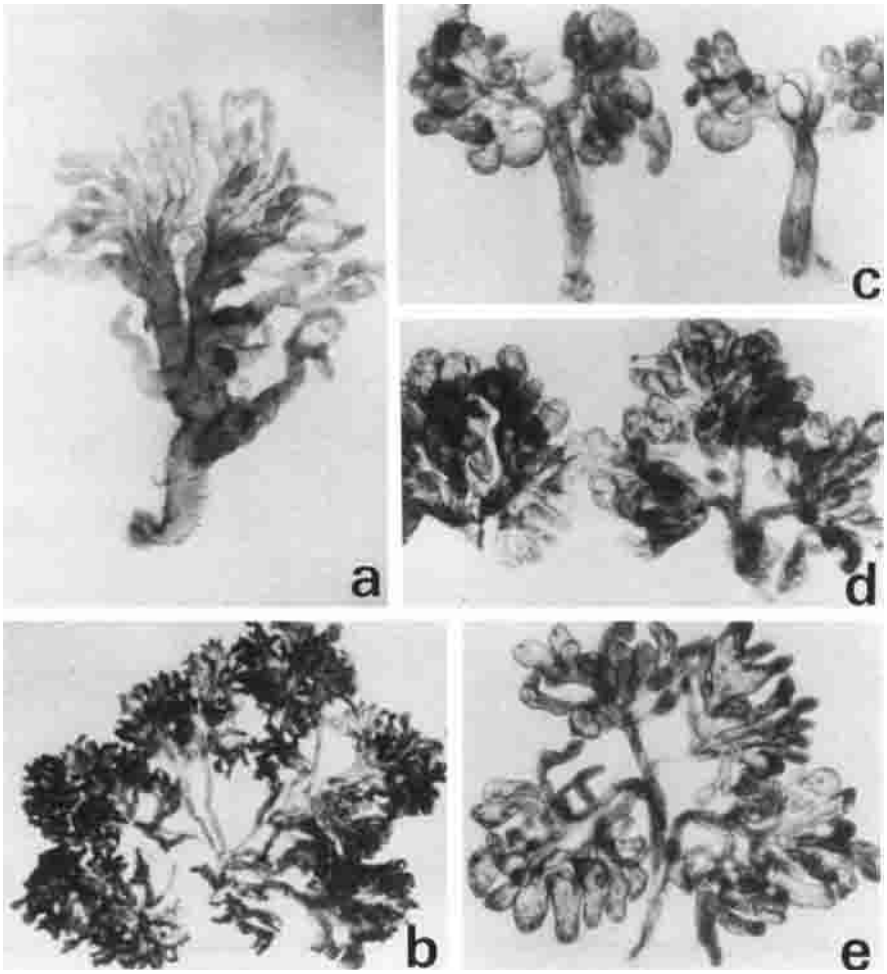

Fig. 1. Morphological appearance of prostatic ductal units from normal intact adult 14-week-old Sprague-Dawley rats. Gross appearance of a main duct with secondary branches of the coagulating gland (**a**), ductal networks of the ventral prostate (**b**), main ducts with terminal branches of the lateral type 1 (**c**), main ducts with terminal branches of the lateral type 2 (**d**), main ducts with terminal branches of the dorsal prostate (**e**). a, b = ×5.4; c–e = ×10.8).

ded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

Serum Androgens

At sacrifice after the 2-week treatment period, blood was collected, allowed to clot for several hours at 4°C, and the serum was isolated. Radioimmunoassays were performed using an antibody to T supplied by Japan DPC Corp. (Tokyo, Japan) and an antibody to DHT supplied by Teikoku Hormone Mfg. Co. (Tokyo, Japan). Two milliliters of rat serum were extracted twice with 7 ml of diethyl-ether, and the ether fractions were evaporated to dryness. T and DHT were separated by Celite chromatography. T was quantified by a direct solid-phase radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA). DHT was quantified by radioimmunoassay by the method of Hosaka et al. [32]. Interassay variances for T and DHT were 12.9% and 26.5%, and intra-assay variances for T and DHT were 8.9% and 11.7%, respectively, which in part was due to a 3.3% cross-reactivity of the anti-T for DHT and a 58.2% cross-reactivity of the anti-DHT for T.

Wet Weight and DNA Assay

Coagulating gland, ventral prostate, lateral type 1 prostate, lateral type 2 prostate, and dorsal prostate of Sprague-Dawley rats were blotted free of excess fluid and weighted on a microbalance (Shimadzu AEL-40 SM, Shimadzu Instrument, Kyoto, Japan). The DNA contents of the prostatic lobes were measured using a Hoechst dye assay [33]. Prostatic lobes

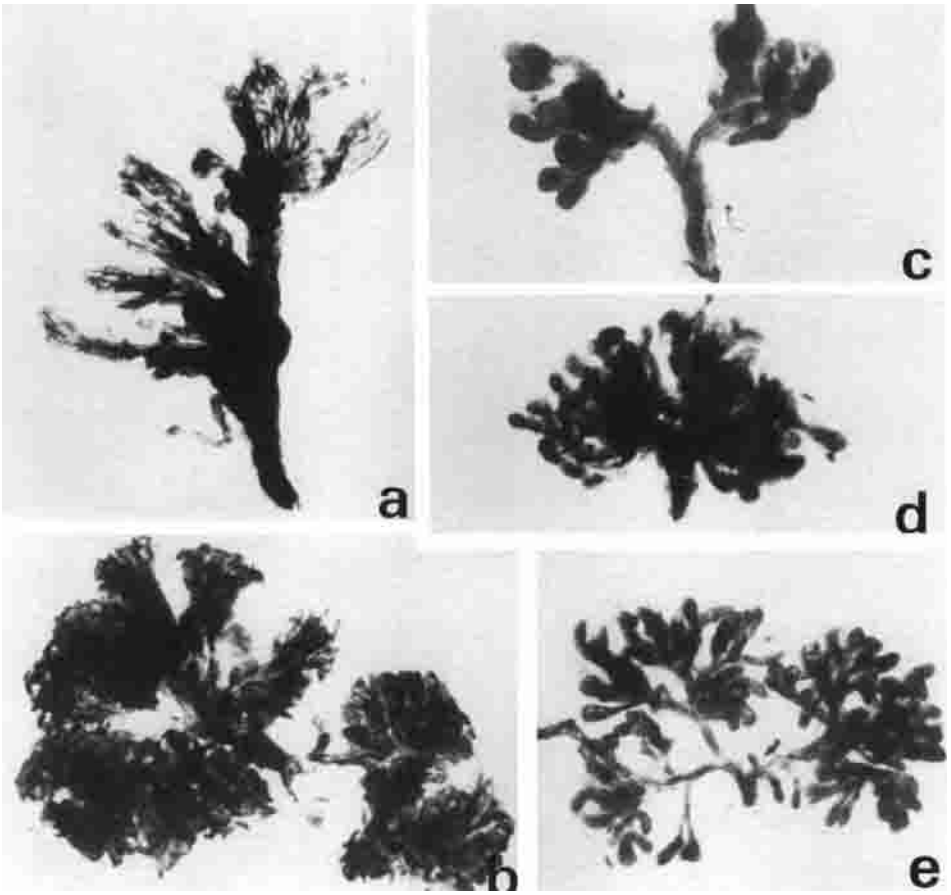

Fig. 2. Morphological appearance of prostatic ductal units from 14-week-old adult Sprague-Dawley rats that were castrated 2 weeks previously. Gross appearance of a main duct with secondary branches of the coagulating gland (**a**), ductal networks of the ventral prostate (**b**), a main duct with terminal branches of the lateral type 1 (**c**), a main duct with terminal branches of the lateral type 2 (**d**), main ducts with terminal branches of the dorsal prostate (**e**). a, b = ×7; c–e = ×15.

were homogenized in 1 ml CMF-PBS on ice in a glass homogenizer and centrifuged for 5 min at 8,000 rpm at 4°C. Supernatants were collected for protein and zinc assays. The pellets were incubated in 700 μl of 0.5 N NaOH for 2 hr at 60°C and then neutralized with an equal volume of 0.5 N HCl. Fifty microliters of the neutralized samples (from 1,400 μl of final total volume) were mixed with 1 ml of Hoechst dye 33258 (0.2 μg/ml in PBS). Fluorescence measurements were made on a fluorometer (excitation filter 7–60 and emission filter 2A, Shimadzu RF-5000, Shimadzu Instruments, Kyoto, Japan). Salmon sperm DNA was used as a standard.

Protein Assay

The protein content of individual rat prostatic lobes was measured by a Bradford assay in 0.1 ml of supernatant samples which were mixed with 5.0 ml of diluted Bio-Rad protein assay dye reagent (Bio-Rad, Richmond, CA) and incubated for 5 min at room temperature. Absorbance at 595 nm was read in a Shimadzu spectrophotometer (Shimadzu UV260, Shimadzu Instruments). Bovine serum albumin was used as a standard.

Zinc Assay

The zinc concentration of rat prostate (μg/mg tissue weight was measured by a 5-Br-PAPS [2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) phenol disodium salt dihydrate] assay. To 0.2 ml of prostatic supernatant deproteinized with trichroloacetate, 1.6 ml of 5-Br-PAPS (0.08 nM in 0.2 M carbonate buffer, pH 9.8) and 0.4 ml of salicylaldoxime (29 mM) were added and incubated for 10 min at room temperature. Absorbance at 560 nm was read in a Shimadzu spectrophotometer.

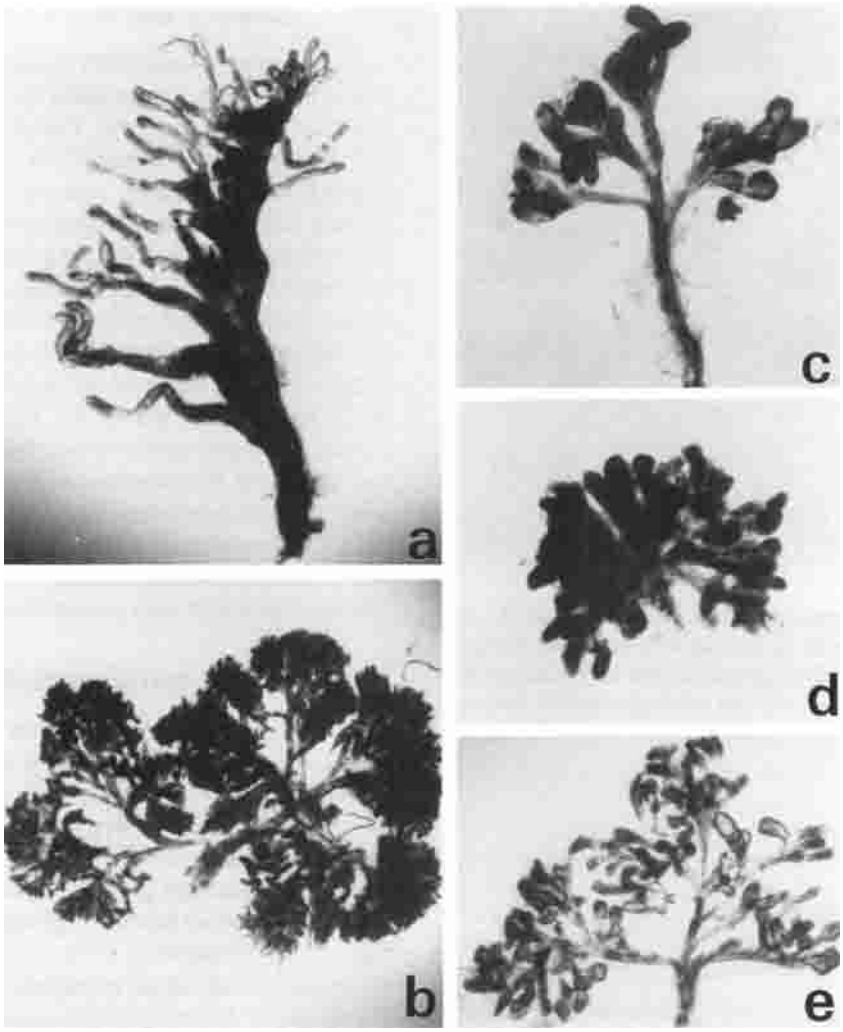

Fig. 3. Morphological appearance of prostatic ducts microdissected from a 14-week-old Sprague-Dawley rat that was treated with Finasteride for 2 weeks. Gross appearance of the coagulating gland with its main duct and secondary branches (**a**), ductal networks of the ventral prostate (**b**), a main duct with terminal branches of the lateral type 1 prostate (**c**), a main duct with terminal branches of the lateral type 2 prostate (**d**), a main duct with terminal branches of the dorsal prostate (**e**). (a, b = ×7; c–e = ×15.

Immunocytochemistry

Paraffin tissue sections were placed on poly-L-lysine-coated slides. Staining was performed by means of a streptavidin-biotin peroxidase complex method. After deparaffinization the slides were immersed in 3% periodic acid solution for 10 min and incubated with normal goat serum for 20 min at room temperature. The slides were incubated with the primary antibody (a mouse monoclonal Anti-Metallothionein, Dako Japan; diluted 1: 50) for 1 hr at room temperature and washed with CMF-PBS. The secondary antibody (biotinylated goat anti-mouse, diluted 1:50) was applied for 10 min, and the slides washed with CMF-PBS. The streptavidin-labeled-peroxidase ABC reagent was applied for 5 min at room temperature (Histofine SAB-Kit, Nichirei, Co., Tokyo, Japan), and the slides were washed with CMF-PBS. The slides were treated with 0.05% diaminobenzidine tetrahydrochloride in 0.05 M *Tris buffer* containing H_2O_2 (0.6%).

Statistical Analysis

The data were expressed as the mean ± SE of the mean. The statistical significance between means was determined by one-way analysis of variance. Treatment groups were compared using Student's *t* test.

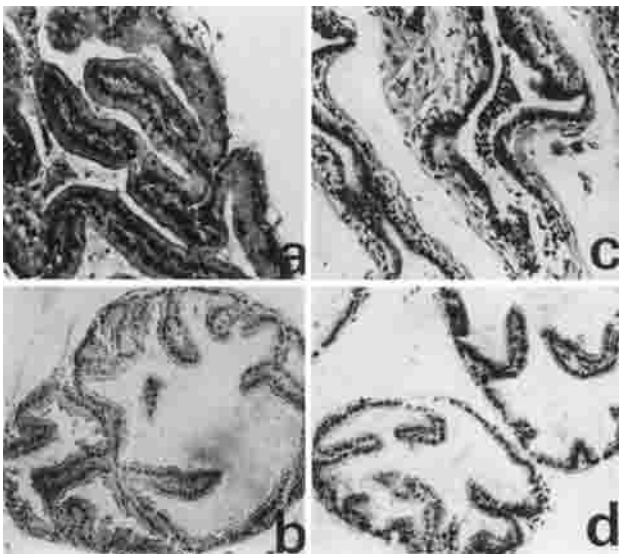

Fig. 4. Transverse sections of ducts of the ventral prostate (**a, c**) and lateral type 2 prostate (**b, d**) of intact male rats (a, b) and rats castrated (c, d) 2 weeks previously and stained with hematoxylin and eosin. Note reduction of epithelial cell height and the relative increase in the connective tissue (especially in c) elicited by castration (c, d). ×200.

RESULTS

Morphology

Gross appearance of microdissected prostatic ducts revealed reductions in the ductal architecture of all lobes following androgen deprivation by all treatment methods, although the most profound effects were observed following castration (Figs. 1, and 2). All forms of interference with androgen action elicited reduction of epithelial cell height, relative increase of connective tissue, and reductions in ductal diameter, length, and number. However, morphological regression of prostatic ducts by interfering with androgen action varied in degree between different treatments, and varied between different prostatic lobes. For example, reduction in gross ductal size was minimal following treatment with Finasteride in comparison with other treatments (Fig. 3), and more severe as a result of castration or DES treatment (Fig. 2). Reduction of epithelial height and relative increase of connective tissue were more profound following castration or DES treatment in comparison to treatment with either Leuprolelin or Finasteride. Histological changes indicative of prostatic regression were particularly striking in the coagulating gland and ventral prostate, and rather indistinct in the lateral type 2 prostate (Fig. 4).

Serum T and DHT Concentrations

Total serum T and DHT concentrations were measured at the time of sacrifice (2 weeks following initiation of the treatments). T and DHT levels were 2.83 ± 0.56 ng/ml (n = 5) and 0.20 ± 0.03 ng/ml (n = 5), respectively, in intact, untreated control rats. Rats treated with Finasteride had serum T and DHT concentrations of 3.05 ± 0.85 ng/ml (n = 5) and 0.04 ± 0.01 ng/ml (n = 5), respectively. Serum T concentrations of rats treated with Leuprolelin or DES, or simply castrated were below 0.03 ng/ml, the lower limit of the assay. While both antibodies had some degree of cross-reactivity for either T or DHT, the trend in serum androgen concentrations was as expected for the treatment in question, with 94-fold reductions in serum T concentration in castrated rats or rats treated with Leuprolelin or DES, and a 5-fold decrease in serum DHT concentration and no change in serum T concentrations in rats treated with Finasteride.

Statistical Analysis

Data are presented in Figures 5–8 on the effects of various methods of interfering with androgen action on DNA content, wet weight, protein content, and zinc concentration in the rat ventral prostate and analyzed by Student's *t* test for significance between different treatment groups. Symbols denoting *P* values of differences between groups on Figures 5–8 are indicative of statistical differences between the intact control and individual treatment groups. Table I gives *P* values for differences between each of the different forms of interfering with androgen action. On the whole in almost all prostatic lobes significant reductions were observed in DNA content, wet weight, protein content, and zinc concentration in rat ventral prostate between Finasteride and DES treatment and Finasteride and castration. By contrast, differences between Finasteride and Leuprolelin (LH-RH analog), and Leuprolelin and DES were on the whole not significant.

DNA Contents

For all prostatic lobes physical castration gave the most profound reduction in DNA content (Fig. 5). Relative and absolute reductions in DNA content varied from lobe to lobe. In castrated rats DNA content of the coagulating gland was reduced by 65% of intact controls. Inhibition of 5α-reductase by Finasteride treatment consistently gave the smallest reduction in DNA content in all prostatic lobes. Of all chemical treatments, DES consistently gave the most profound reduction in DNA content with LH-RH treatment generally being slightly less effective in reducing pro-

TABLE I. Statistical Significance Between Two Groups†

	N vs. M	N vs. L	N vs. E	N vs. C	M vs. L	M vs. E	M vs. C	L vs. E	L vs. C	E vs. C
DNA										
CG	**	**	***	***		*	*		*	*
VP	**	***	***	***	**	**	**	*		
L1			***	***			**		**	**
L2		*	*	**	*	*	*		**	**
DP	**	**	**	***		***	***	**	**	
Weight										
CG		**	***	***	**	***	***			
VP	**	**	***	***		***	***	***	***	
L1						**	**		*	
L2				*			**		*	*
DP	*	*	**	***		**	***		***	**
Protein										
CG	***	***	***	***		**	***		**	***
VP	***	***	***	***	***	***	***		**	***
L1	**	**	**	***					***	**
L2	**	**	***	***		***	***	***	***	**
DP	**	***	***	***			**	**	***	***
Zinc										
CG	**	***	***	***	***	***	***			
VP				**			*			
L1	**	**	***	***		**	**			
L2			**	**			*			
DP	*	*	**	*			*			

†N, normal group; M, 5α-reductase inhibitor group; L, LH-RH analog group; E, DES group; C, castrated group; CG, coagulating gland; VP, ventral prostate; L1, lateral prostate type 1; L2, lateral prostate type 2; DP, dorsal prostate.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

static DNA content in all lobes. In the ventral prostate, reduction in DNA content by DES equaled that of castration. For the lateral prostate (type 1 and type 2) none of the methods of chemical interference with androgen action (5α-reductase inhibitor, LH-RH, DES treatments) were as effective as physical castration in reducing DNA content (see Table I for statistical analysis).

Wet Weight

The decrease of prostatic wet weight for all prostatic lobes paralleled the values for DNA content in relative and absolute terms for all androgen manipulations (Fig. 6). In order of effectiveness wet weight was reduced maximally by castration, followed by treatment with DES, LH-RH, and Finasteride for all lobes. As above the greatest effects were seen in the ventral prostate and the smallest reductions in wet weight were observed in the lateral type 1 prostate (see Table I for statistical analysis).

Protein Content in Prostatic Tissue

Protein content in all prostatic lobes showed the largest relative and absolute reduction following physical castration and the smallest reduction following treatment with Finasteride (Fig. 7). Of the three types of interference with androgen action, DES was the most effective, Finasteride the least effective, and LH-RH treatment was intermediate in effectiveness. The ventral prostate gave the largest relative and absolute reduction in protein content for both chemical interference with androgen action and physical castration (see Table I for statistical analysis).

Zinc Concentration

The lateral type 2 prostate had the highest zinc concentration (μg/mg tissue weight) in the intact control rat in comparison to other lobes (Fig. 8). Lateral type 1 and dorsal prostates had intermediate zinc levels, and the coagulating gland and ventral prostate had the lowest zinc levels. While androgen manipu-

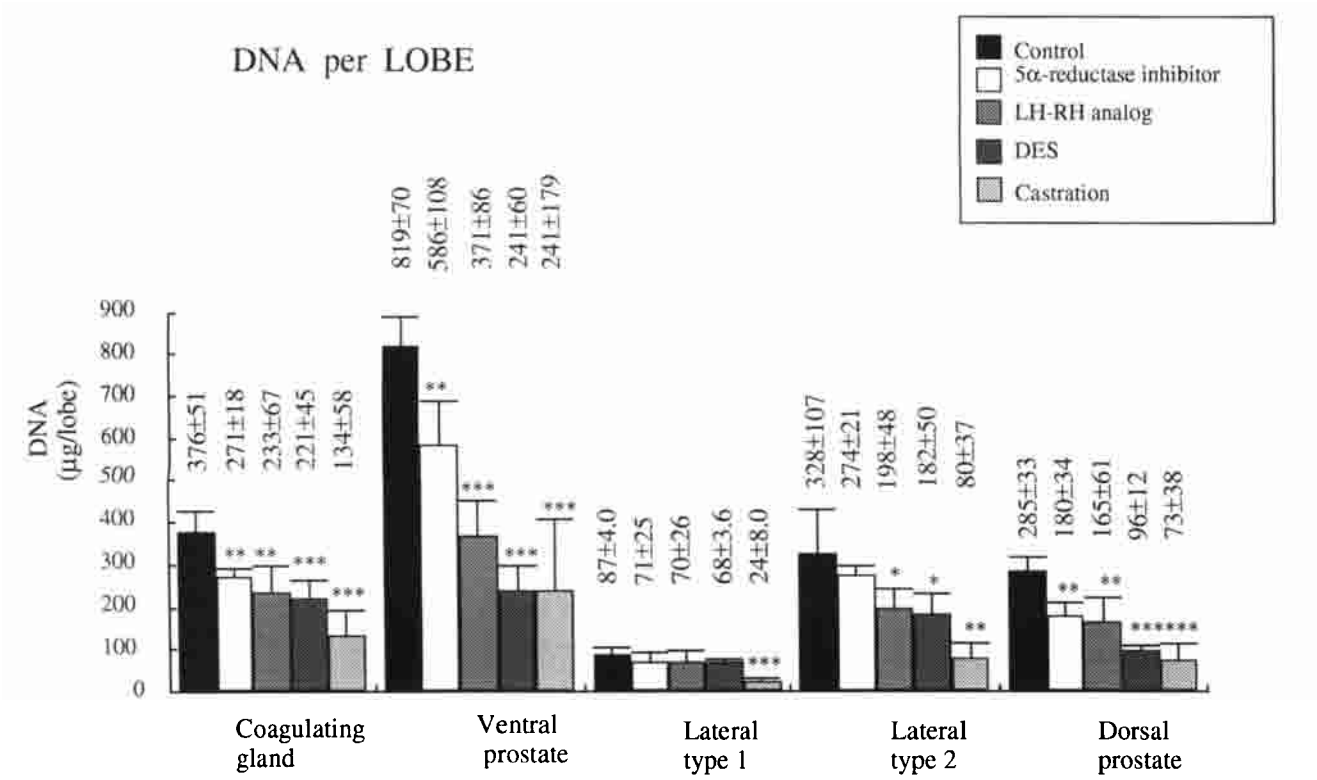

Fig. 5. DNA contents of prostatic lobes (n = 5). Prostatic DNA contents of androgen-deprived rats were statistically different from those of intact control rats (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) by the Student's t test. Error bar, standard deviation.

lations reduced zinc levels generally in the prostate, each lobe responded differently to interference with androgen action. The most profound reductions were seen in the dorsolateral prostate, especially in the lateral type 1 and 2 prostates in which zinc content was reduced approximately 50%. As before, physical castration was most effective in reducing zinc concentration followed in order by DES, LH-RH, and Finasteride (see Table I for statistical analysis). Even following castration, the zinc concentration in the lateral type 2 prostate remained relatively high. The ventral prostate exhibited the smallest relative reduction in zinc concentration following androgen deprivation maintaining more than 85% of its normal zinc concentration.

Immunocytochemistry With Anti-Metallothionein Antibody

In both control and androgen-deprived prostates, small focal areas of immunocytochemical staining for metallothionein were only detected in the lateral type 2 prostate. Staining of the lateral type 2 prostate with anti-metallothionein antibody was patchy, and positive staining was not affected by androgen manipulation (Fig. 9).

DISCUSSION

This is the first study to examine the influence of various forms of chemical interference with androgen action (DES, an LH-RH analog, and a 5α-reductase inhibitor) and physical castration on each of the five lobes of the rat prostate. The relevance of the present studies to the human prostate rests on the strength of homologies between individual lobes of the rat prostate and the various anatomical subdivisions of the human. In this regard, the dorsal and lateral lobes of the rat prostate appear to be homologous to the peripheral zone of the human prostate. The anterior lobe of the rat prostate (coagulating gland) probably represents the central zone of the human prostate, while the rat ventral prostate does not have a human counterpart. All forms of interference with androgen action elicited prostatic regression, but to different degrees. In addition, the different lobes of the rat prostate responded to interference with androgen action differently in both a morphological and biochemical sense. Prostatic structure and secretory function are androgen dependent. Castration induces rapid regression of the prostate, and after a few weeks an atrophic state is achieved and maintained until androgen is replaced [34]. The mechanism of prostatic

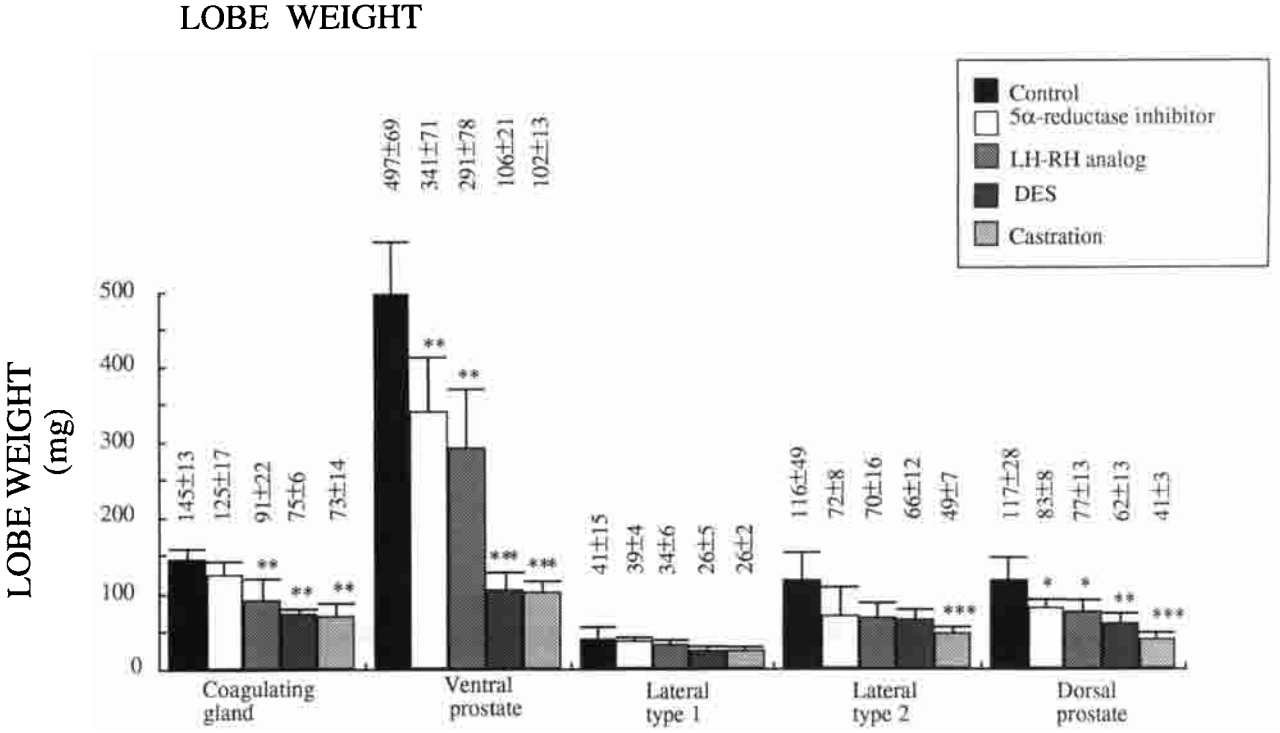

Fig. 6. Wet weight of prostatic lobes (n = 5). Prostatic wet weights of androgen-deprived rats were statistically different from those of intact control rats (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) by the Student's t test. Error bar, standard deviation.

regression in response to androgenic withdrawal is still poorly understood. The rapid decline in serum androgens following castration induces programmed cell death in the prostate, which is an active process involving macromolecular synthesis and a cascade of biochemical and morphological changes [1,35–37]. Morphologically, prostatic regression begins at the distal ductal tips near the prostatic capsule and results in a progressive loss of ductal architecture from the distal ductal tips inward towards the urethra [3]. As ductal tips and branch-points are lost, DNA content drops by 80–90% of precastrate levels by 14 days after castration [2,38–40]. This loss of DNA content is accounted for primarily by loss of epithelial cells [41] through a process of apoptosis in which nucleases degrade nuclear DNA [1,42].

From a mechanistic perspective prostatic regression has been studied almost exclusively in the rat ventral prostate. Undoubtedly this is due to the fact that the ventral lobe of the prostate is easily accessible with minimal dissection. Fourteen days after physical castration, we observed that DNA content was reduced to 24–37% of precastrate levels in the five lobes of the rat prostate, with the value for the ventral prostate being 29%. These data demonstrate a similarity in the magnitude of prostatic regression elicited by castration as measured by DNA content between the five lobes of the rat prostate. However, when other forms of interference with androgen action are considered, marked interlobe differences in response were observed. For instance, whereas DES treatment reduced DNA content by 66% and 71% of intact controls in the ventral and dorsal prostates, respectively, DES treatment only reduced DNA content by 22–45% of intact controls in the coagulating gland, lateral prostate type 1 and 2. Thus, even though physical castration and DES treatment both reduced serum T to below 0.04 ng/ml, the effects of such interference with androgen action were substantially different in different prostatic lobes. Indeed, different forms of interference with androgen action reduced prostatic DNA contents to different degrees in different lobes with inhibition of 5α-reductase with Finasteride being the least effective, followed by Leuprolelin treatment, DES treatment, and physical castration being the most effective in reducing DNA content in all lobes. Statistical analysis (Table I) confirmed this impression. In the present study the treatment period was for all treatment groups only 2 weeks. In the case of long-term chronic treatment with Finasteride profound atrophy of the prostate can be achieved [43]. Of the three forms of interference with androgen ac-

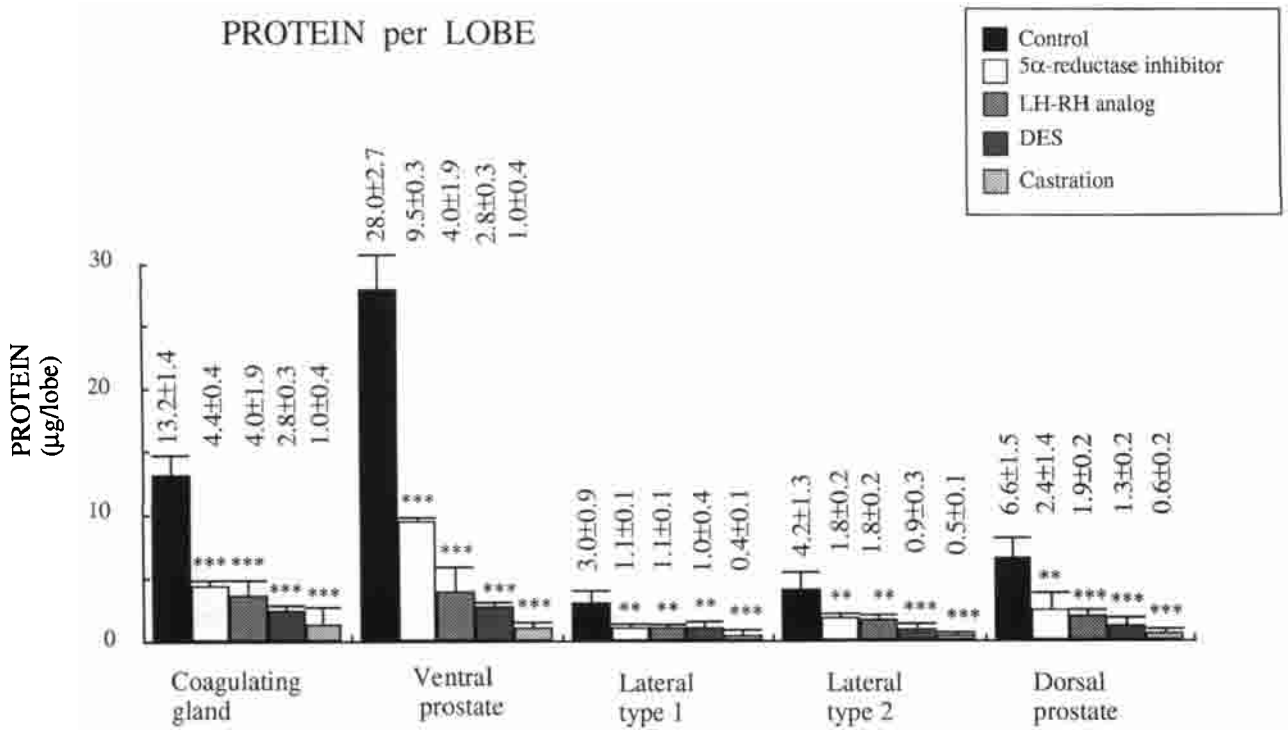

Fig. 7. Protein content of prostatic lobes (n = 5). Prostatic protein contents of androgen-deprived rats were statistically different from those of intact control rats (**$P < 0.01$, ***$P < 0.001$) by the Student's t test. Error bar, standard deviation.

tion the greater effectiveness of DES may be due to direct estrogen receptor-mediated influences of DES on the prostate as well as indirect systemic influences involving down-regulation of pituitary LH secretion and subsequent reduction in T biosynthesis by the testes [44]. Previous reports have documented estrogen receptors in prostatic stroma [45,46], and stimulation of prostatic stroma by exogenous estrogen [47–49]. On the other hand, Leuprolelin presumably affects the prostate only via reduction in systemic androgen levels, while inhibition of 5α-reductase should primarily act within the prostate to reduce intraprostatic DHT levels. Differences in these three forms of interference with androgen action on the various lobes of the prostate are, therefore, likely to be due to intrinsic interlobar biochemical differences. Indeed, interlobar and regional differences in the levels of androgen receptors [50–53] and estrogen receptors [45,46] have been reported previously within the prostate. Such differences in steroid receptors may account for interlobar response to exogenous hormones and to interference with androgen action reported previously and in this report [47–49,54,55]. Another possibility is that the differential response of prostatic lobes to sex steroids or hormone deprivation may be due to interlobar differences in steroid metabolism [56].

Reductions in prostatic protein content elicited by interference with androgen action were more profound than effects on prostatic DNA content. For instance, in the ventral prostate whereas castration elicited a 71% reduction in DNA content, protein levels were reduced by 96% of precastrate levels. Indeed, for all prostatic lobes protein levels were reduced to levels substantially below reductions in relative DNA content. This may mean that the androgen threshold for maintenance of secretory function is higher than that for prevention of apoptosis. In this regard, androgen thresholds for eliciting prostatic growth are higher than those required for prevention of programmed cell death [34]. As above, with the exception of physical castration, DES treatment was statistically the most effective form of interference with androgen action in reducing protein content in all lobes followed in order by Leuprolelin and Finasteride. Reduction in protein content by DES varied considerably on a lobe-by-lobe basis with protein levels reduced by 96% of precastrate levels in the ventral prostate, 67% in the lateral type I, and to around 80% in all of the remaining lobes. These interlobar differences in reduction in DNA and protein levels among the rat coagulating gland, ventral prostate, lateral type 1 prostate, lateral type 2 prostate, and dorsal prostate suggest interlobar heterogeneity of andro-

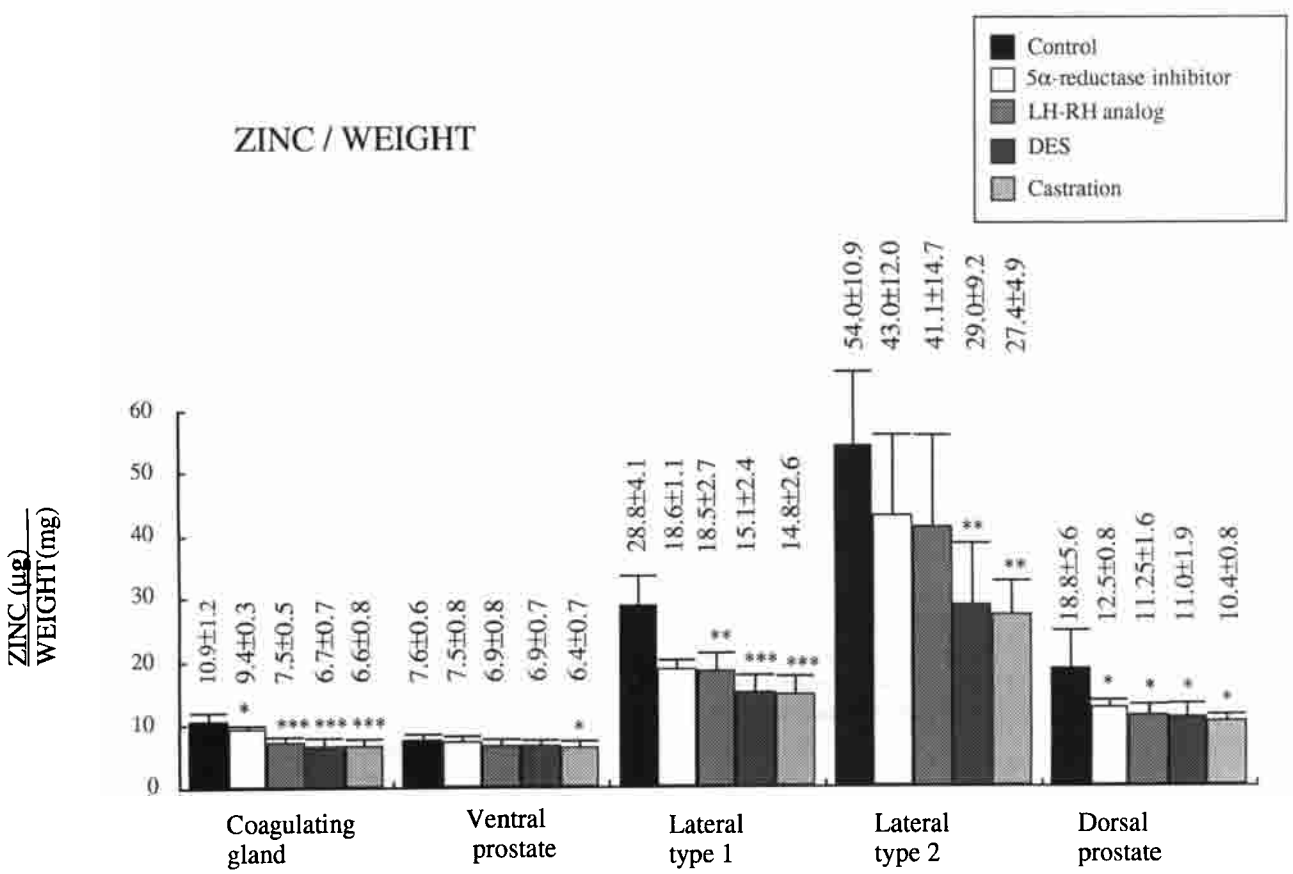

Fig. 8. Zinc concentration of prostatic lobes (n = 5). Prostatic zinc concentrations of androgen-deprived rats were statistically different from those of intact control rats (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) by the Student's t test. Error bar, standard deviation.

gen dependency perhaps based upon interlobar differences in steroid receptors or steroid metabolism among the different prostatic lobes.

Functional heterogeneity amongst the different prostatic lobes has been recognized for some time as lobe-specific differences in secretory proteins as mentioned above. In comparison to other lobes, Gunn et al. [57] demonstrated particularly high zinc concentrations in the lateral prostate as well as high levels of fructose in the dorsal prostate. The function of zinc in the prostate is unknown. However, zinc concentration is markedly decreased in human prostatic adenocarcinomas, while cadmium, a metabolic competitor of zinc, is elevated above normal in prostatic tumors [58]. Another interlobar difference in functional activity is the ability to produce citrate. Grayhack's group was the first to demonstrate that prolactin stimulates citrate production in adult rats [59], an effect that was specific to the lateral prostate. In this lobe prolactin also stimulates expression of ornithine decarboxylase and androgen receptors [60,61]. This effect of prolactin on the lateral prostate is mediated by prolactin receptors [62–64].

Metallothionein is a metal-binding protein with a molecular weight of approximately 6 kD and high cystein and sulfur content. It is unique in its ability to bind class II-B metals, such as zinc and cadmium, and may be required for the metabolism of essential metals and the detoxification of toxic metals. Correlating with the high levels of zinc in the lateral prostate, metallothionein is strongly expressed in the rat dorsolateral prostate, particularly the lateral prostate [65–67]. In this study, in which the lateral prostate was subdivided into the lateral type 1 and 2 prostates, only lateral type 2 prostate exhibited staining with the anti-metallothionein antibody both in intact and androgen-deprived rats. Thus, subdivision of the rat lateral prostate into two morphologically and biochemically distinct glands is supported by the fact that the lateral type 2 prostate differs from the lateral type 1 prostate in having a unique ductal morphology [12], a unique pattern of secretory proteins [12,24], the highest level of zinc of all prostatic lobes, and prominent expression of metallothionein.

In adult male animals, the structure and function of prostatic epithelial cells are regulated by andro-

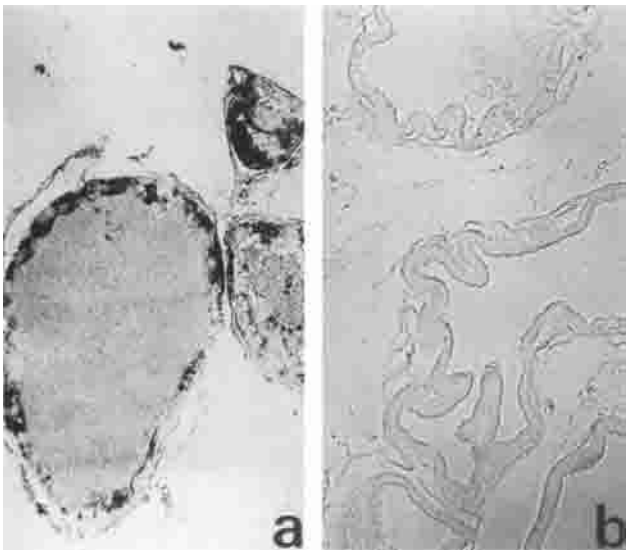

Fig. 9. Immunocytochemical staining with anti-metallothionein antibody of the (a:) lateral type 2 prostate of a control rat and (b:) ventral prostate of a control rat. The lateral type 2 prostate of both control and androgen-deprived rats exhibited a patchy positive staining. The ventral prostate does not stain with anti-metallothionein antibody. ×200.

gens. Our study demonstrates for the first time that prostatic regression exhibits substantial heterogeneity among the five subdivisions of the rat prostate following interference with androgen action in regard to reduction of DNA and protein content, wet weight, and zinc concentrations, and that different modes of interference with androgen action affect different prostatic lobes to different extents. At the functional level intraprostatic heterogeneity has been demonstrated in regard to secretory proteins, fructose, citrate, zinc, and metallothionein expression. Given the fact that the pathobiology of the human prostate also demonstrates important regional heterogeneity, with the periurethral zone being the site of development of benign prostatic hyperplasia and the peripheral zone being the predominant site of cancer development [68], it is important to explore how morphological and functional heterogeneity develops in the first place in the prostate. Tissue recombination studies have demonstrated that while fetal urogenital sinus mesenchyme is the general inducer of prostatic development, the dorsal and ventral portions of the urogenital mesenchyme have different inductive properties. Only the ventral urogenital mesenchyme is an effective inducer of ventral prostatic differentiation [69]. Presumably, the dorsal urogenital mesenchyme induces the dorsolateral prostate although this has not been tested. Of greater significance is the observation that the lobe specificity of functional activity of adult prostatic epithelial cells is not irreversibly fixed, but that secretory protein expression of adult prostatic epithelial cells can be induced to change dramatically by the mesenchyme of the urogenital sinus, seminal vesicle, and bulbourethral gland [12,24]. Taken as a whole, such observations suggest that regional patterns of prostatic ductal morphogenesis and secretory function are induced and maintained by the mesenchymal or stromal component of the gland. An understanding of the signaling pathways involved in the development and maintenance of intraprostatic heterogeneity in androgen dependency, morphology, apoptosis, secretory protein expression, and metal concentration may provide insights into the development of prostatic disease (benign prostatic hyperplasia and prostatic cancer) as both normal differentiation and proliferative disease of this gland initially owe their development to focal changes within large cell populations.

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