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EFFECTS OF ANDROGEN DEPRIVATION ON PROSTATE ALPHA$_1$-ADRENERGIC RECEPTORS*

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ABSTRACT

Objectives. Benign prostatic hyperplasia (BPH), the most common benign tumor in men, consists of two components—static (enlargement regulated by androgens) and dynamic (smooth muscle contraction through alpha$_1$-adrenergic receptors [alpha$_1$-ARs]). Because medical therapy of BPH involves tissue androgen deprivation, we studied the influence of androgen deprivation and replacement on regulation of rat ventral prostate alpha$_1$-ARs.

Methods. Prostate weight, alpha$_1$-AR density, autoradiographic images, histologic features, and cell-specific protein were examined before and after castration and androgen replacement.

Results. Castration decreases ventral prostate wet weight, a process reversed by testosterone administration. In contrast, there is an apparent increase in alpha$_1$-AR density (29 ± 4 versus 65 ± 6 fmol/mg total protein, mean ± SEM) after castration, returning to baseline with testosterone replacement; alpha$_1$-AR density remains constant in control liver membranes. Alpha$_1$-ARs predominate in stroma throughout androgen deprivation therapy. Epithelially derived cells decrease (83% to 67%) after castration, resulting in a relative doubling in stroma (17% to 33%); the protein content of epithelial and stromal cells remains identical. Therefore, prostate-specific increases in alpha$_1$-ARs appear to result from relative increases in the ratio of smooth muscle to epithelium after castration rather than from direct upregulation of alpha$_1$-AR protein.

Conclusions. Because alpha$_1$-AR density does not decrease with androgen deprivation, these studies suggest that alpha$_1$-AR antagonists remain an important component in BPH therapy, even when 5-alpha-reductase inhibitors are utilized. UROLOGY **48:** 335–341, 1996.

Benign prostatic hyperplasia (BPH) is characterized by enlargement of the prostate gland, with accompanying symptoms of progressive bladder outlet obstruction. The prostate is anatomically complex, consisting of fibromuscular (stroma) and glandular (epithelial) elements, both of which are involved to varying degrees in the pathogenesis of BPH. Functionally, BPH consists of two components—static (generalized epithelial gland enlargement regulated by androgens) and dynamic (stromal smooth muscle contraction mediated predominantly by alpha$_1$-adrenergic receptors [alpha$_1$-ARs]). Both clinical and experimental studies demonstrate that prostatic growth is under androgen control. Androgen deprivation (whether by castration, leutinizing hormone-releasing hormone [LHRH] agonists, or 5-alpha-reductase inhibitors such as finasteride) decreases prostate size,[1,2] providing therapy affecting predominantly the static component of BPH. Side effects, such as decreased potency and libido, are prominent with castration and LHRH agonists as a result of decreased serum testosterone. In contrast, finasteride exerts its action on prostatic tissue, decreasing dihydrotestosterone (the active metabolite of testosterone) without affecting serum testosterone, thus having fewer side effects. Finasteride has shown clinical efficacy for BPH; however, initial trials have not been as effective as originally hoped compared with standard surgical treatment.

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Increasingly, it has become apparent that the dynamic component of BPH is responsible for many of the symptoms of BPH. Contraction of prostatic smooth muscle involves alpha$_1$-ARs.[3-5] Alpha$_1$-AR antagonists rapidly increase urinary flow rates, thus treating the symptoms of BPH.[6] Alpha$_1$-ARs are G protein-coupled receptors that mediate the actions of the endogenous catecholamines norepinephrine and epinephrine. Three subtypes of alpha$_1$-ARs (alpha$_{1a}$ [previously designated alpha$_{1c}$], alpha$_{1b}$, and alpha$_{1d}$ [previously named alpha$_{1a/d}$, alpha$_{1d}$, or alpha$_{1a}$]) have been cloned and characterized[7-12]; and a fourth subtype (alpha$_{1L}$) has been described pharmacologically.[13] Recent data suggest that the alpha$_{1L}$-AR subtype may be important in prostate smooth muscle contraction.[14] Because medical therapy of BPH involves tissue androgen deprivation, we studied the influence of castration and androgen replacement on the regulation of rat ventral prostate alpha$_1$-ARs.

MATERIAL AND METHODS

Animals and Tissues

After institutional animal care committee approval, adult rats (10 weeks old, 250 to 300 g; Harlan Sprague Dawley, Indianapolis, Ind) were housed three per cage in a temperature-controlled room (68 to 72°F) on a 12-hour light/dark schedule and given Purina Special Lab Chow and water ad libitum. Animal groups included the following (9 to 15 animals per group): group 1 = untreated control rats, killed on day 0; group 1S = sham-operated rats, injected subcutaneously with vehicle sesame oil (once a day) on days 5 to 8, killed on day 10; group 2 = castrated rats, killed on day 1; group 3 = castrated rats, killed on day 3; group 4 = castrated rats, killed on day 5; group 5 = castrated rats, killed on day 10; group 6 = castrated control rats (castration, vehicle on days 5 to 8), killed on day 9; group 7 = castrated rats, testosterone replacement on days 5 to 8 (2 mg testosterone/day, micronized powder, USP, Amend, Irvington, NJ, lot W-4563H-21 in vehicle sesame oil), killed on day 9. Animals were euthanized with carbon dioxide asphyxiation. Ventral prostatic tissue was immediately removed, freed of fat and mesentery, weighed (wet weight), and frozen in liquid nitrogen for future assays. Control liver tissue was harvested and frozen in liquid nitrogen at the same time. For in situ autoradiographic experiments, animals were euthanized by cervical dislocation, and prostate glands were removed and flash-frozen in liquid nitrogen-chilled isopentane. Tissue for all experiments was stored at −70°C until analyzed.

Alpha$_1$-AR Radioligand Binding

Frozen tissues were weighed (ventral prostate, liver, or kidney, 50 to 200 mg), then homogenized with a Polytron PT 3000 using a 7-mm foam reducing generator (Brinkmann Instruments, Westbury, NY) in 1 mL of lysis buffer (5 mM Tris/5 mM ethylenediaminetetra-acidic acid [EDTA]) with protease inhibitors (benzamidine [10 μg/mL], leupeptin [10 μg/mL], and soybean trypsin inhibitor [10 μg/mL]). The homogenate was then centrifuged in a total volume of 15 mL at 19,000 rpm for 10 minutes, supernatant decanted, and pellet resuspended in 10 vol of binding buffer (150 mM NaCl, 50 mM Tris-HCl, 5 mM EDTA, pH 7.4, with protease inhibitors; 10 mL/g tissue wet weight), then quickly frozen liquid nitrogen and stored at −70°C. Total protein was determined by the bicinchoninic acid method (Pierce, Rockford, Ill).[15] Total alpha$_1$-AR density was assessed using a saturating concentration (300 pM) of the alpha$_1$-AR antagonist [^{125}I]HEAT (2-{beta-(4-hydroxy-3[^{125}I]dophenyl)ethylaminomethyl}tetralone; Dupont-New England Nuclear, Boston, Mass) in a total volume of 0.25 mL of binding buffer with protease inhibitors (10 to 100 μg of membrane protein, depending on tissue type). Prazosin (1 μM) (Sigma Chemical Co., St. Louis, Mo) was used to determine nonspecific binding. The reaction was incubated for 45 minutes at 25°C, then terminated with ice-cold 50 mM Tris-HCl, pH 7.4, followed by rapid filtration over GF/C filters using a Brandel Harvester (Brandel Biomedical Research & Development Laboratories, Inc., Gaithersburg, Md).

Histologic and In Situ Autoradiographic Analysis of Rat Ventral Prostate

To examine changes in prostatic histologic features and distribution of alpha$_1$-ARs in stroma versus epithelial cells with castration and hormone replacement, six groups were chosen for further analysis: group 1 (intact control rats), group 2 (1-day castration), group 3 (3-day castration), group 4 (5-day castration), group 5 (10-day castration), and group 7 (castration, testosterone replacement). Serial 10-μm sections were cut on a cryostat, thaw-mounted on gelatin-coated microscope slides, and then stored at −70°C until processing. Histologic examination was performed after slides were lightly counterstained with hematoxylin-eosin. Radioligand autoradiography was performed as previously described.[16] Briefly, 10-μm sections were incubated with 2 nM [furoyl-5-^{3}H]prazosin (Dupont-New England Nuclear) in ligand buffer (170 mM Tris-HCl [pH 7.6] containing 10 μM phenylmethylsulfonylfluoride [PMSF]) for 30 minutes at room temperature; 100 μM prazosin (Sigma Chemical Co.) was used to determine nonspecific binding. To stop the reaction, sections were washed three times in ligand buffer (1, 5, and 5 minutes), rinsed briefly in double-distilled water, then air-dried for 2 hours at room temperature. Slides were dipped in NBT-2 nuclear-track photographic emulsion (Interscience, Fairview, Ore) and exposed for 28 days at 4°C. After exposure was complete, slides were developed by incubation for 5 minutes in Kodak D19 developer (Kodak, Rochester, NY), 30 seconds in 2% acetic acid, 5 minutes in Kodafix (Kodak), and 30 minutes in running water. Sections were then lightly counterstained with hematoxylin-eosin, and photographed under bright- and dark-field microscopy.

Determination of Rat Ventral Prostate Cell Type and Cell-Specific Protein Content

Because the calculation of alpha$_1$-AR density is influenced by cell membrane protein content, and regression of prostatic tissue occurs with castration, we determined cell type and cell-specific protein content in rat ventral prostates after castration in a second group of rats. Prostates were excised from Sprague-Dawley rats (250 to 275 g) at day 0 (intact control group, n = 6) and day 5 (n = 12) after castration. Prostates were weighed, pooled for each group, minced, disaggregated by enzymatic digestion, and cell types separated on discontinuous Percoll gradients as previously described.[17] The purity of each fraction was monitored visually (for example, to determine cell size) and by immunofluorescence using antibodies specific for simple epithelial cells (PKK-1, which recognizes cytokeratin 8 and 18), basal epithelial cells (EAB 903, which recognizes cytokeratins 1, 5, 10, and 11), and "stromal" cells (anti-smooth muscle actin). After spinning, Percoll gradient fractions were diluted in isotonic phosphate-

buffered saline (PBS), centrifuged briefly, and resuspended in fresh ice-cold PBS; this washing was repeated three times. Cells were counted on a hemocytometer, and an aliquot of cells was lysed by ultrasound followed by determination of protein content using the Bradford method.[18]

Statistical Analysis

Analysis of variance was used to determine differences in group mean values. Dunnett's multiple comparison test was then performed to determine differences between control group 1 and all other groups (1S – 7). A P value of 0.05 was considered significant.

RESULTS

Rat Ventral Prostate Wet Weight

As expected, total rat ventral prostate weights decreased significantly from control group 1 (308 ± 10 mg) to day 3 after castration (209 ± 15 mg, $P < 0.01$), reaching a nadir by day 10 after castration (51 ± 2 mg, $P < 0.01$) (Table I). Testosterone replacement resulted in significant restoration of prostate weight (225 ± 7 mg, $P < 0.01$ versus group 5 [10-day castration]). Ventral prostate weights from sham-operated control rats injected with vehicle and killed on day 10 were not statistically different from group 1 control rats (killed on day 0).

Histologic Analysis of Ventral Prostatic Tissue from Castrated Rats

Detailed histologic analysis of prostatic tissue was performed in groups 1, 3, 4, 5, and 7. Changes in prostatic histologic tissue are typical of that seen with castration and hormone replacement (data not shown). Specifically, by 3 days after castration (group 3), increasing loss of prostate epithelial cells occurs without changes in stroma. Prostatic tissue 5 days after castration (group 4) reveals death of tall columnar luminal epithelial cells with remodeling of surviving basal epithelial and stromal components. Prostatic tissue 10 days after castration (group 5) consists of cuboidal luminal epithelium with an increased number of basal cells on a stromal support layer. Testosterone replacement returns prostatic histologic tissue to normal, with slightly enlarged epithelial cells.

Alpha$_1$-AR Density in Rat Ventral Prostate and Control Liver Tissue

Rat ventral prostate alpha$_1$-AR density in hormonally intact animals (group 1) is 24 ± 3 fmol/mg total protein (mean ± SEM); sham-operated control rats (group 1S) are indistinguishable from hormonally intact control animals in terms of alpha$_1$-AR expression (Table I). Apparent prostate alpha$_1$-AR density increases significantly by 5 days after castration (group 4) to 41 ± 4 fmol/mg total protein ($P < 0.01$). Alpha$_1$-AR density increases further to 65 ± 6 fmol/mg total protein by 10 days after castration ($P < 0.01$); this is confirmed in group 6 (vehicle administration after castration), with alpha$_1$-AR expression 67 ± 6 fmol/mg total protein. Testosterone replacement 4 days after castration (group 7) returned prostatic alpha$_1$-AR expression to baseline levels. In contrast to ventral prostate, alpha$_1$-AR expression is higher at baseline in rat liver (94 ± 6 fmol/mg total protein). With the exception of small elevations in alpha$_1$-AR density with vehicle administration, liver alpha$_1$-AR expression did not change significantly with castration or testosterone therapy (Table I).

In Situ Autoradiography of Rat Ventral Prostate

It has been previously demonstrated that alpha$_1$-ARs predominate in human prostatic stroma;[19] we recently demonstrated that the alpha$_{1a}$-AR subtype is present in human prostate smooth muscle.[20] To examine whether castration affects alpha$_1$-AR ex-

TABLE I. ***Rat ventral prostate weight and alpha$_1$-adrenergic receptor density in control and liver tissue with castration***

Group No./Description*	Wet Weight (mg)†	Wet Weight/Total Body Weight ($\times 10^{-3}$)	Alpha$_1$-AR Density (fmol/mg total protein)†	
			Ventral Prostate	Liver
1/intact control	308 ± 10	1.20	24 ± 3	94 ± 6
1S/control (sham)	272 ± 11	1.02	29 ± 4	98 ± 13
2/1-day castration	284 ± 14	1.03	27 ± 3	78 ± 5
3/3-day castration	209 ± 15‡	0.76	29 ± 4	88 ± 5
4/5-day castration	128 ± 5‡	0.47	41 ± 4‡	93 ± 6
5/10-day castration	51 ± 2‡	0.19	65 ± 6‡	96 ± 8
6/castration + vehicle	51 ± 3‡	0.20	67 ± 6‡	129 ± 13§
7/castration + testosterone	225 ± 7	0.88	29 ± 2	111 ± 6

Key: AR = adrenergic receptor.
* *See text for detailed group descriptions.*
† *Mean ± SEM.*
‡ *P < 0.01,* § *P < 0.05 versus intact control group.*

pression in rat prostate stromal versus epithelial cells, in situ localization of [^{3}H] prazosin binding was examined in noncastrated and castrated rats. Figure 1 demonstrates that alpha$_1$-ARs predominate in the stroma in intact control rat prostate, with very little alpha$_1$-AR binding occurring in epithelial cells; this pattern remains constant with castration and/or hormone replacement. Specificity for alpha$_1$-ARs is demonstrated by competition with excess nonradiolabeled prazosin before autoradiography in normal and castrated rat ventral prostate (Fig. 2). No change in stromal alpha$_1$-AR density was noted during high-power microscopic examination of autoradiographic results (data not shown).

Determination of Protein Content and Analysis of Cell Types in Prostatic Tissue after Castration

To investigate whether increased alpha$_1$-AR expression in rat ventral prostate after castration is artifactual secondary to changes in prostatic cell types, a detailed analysis of cell types and cellular protein content of rat ventral prostate was performed in intact and 5-day postcastration animals (Table II). Secretory epithelial cells decrease significantly with castration, whereas stromal (predominantly smooth muscle) cells remain virtually unchanged. Basal epithelial cell number increases with castration. The overall percent of epithelially derived cell protein in prostate (secretory and basal epithelial) decreases from 83% to 67% with castration, resulting in a relative doubling in the percentage of stroma (17% to 33%); protein content of epithelial and stromal cells (on a per-cell basis) remains unchanged. In addition, combining individual cell protein content and the percentage present in the intact versus the castrated animal overall protein content remains remarkably stable.

COMMENT

The pathogenesis of BPH is a multifactorial process, with complex interactions between static and dynamic components. Medical therapy of BPH currently targets both static component (with 5-alpha-reductase inhibitors designed to decrease prostate dihydrotestosterone levels, leading to decreases in overall prostate size[21]) and dynamic (with alpha$_1$-AR antagonists to decrease prostate smooth muscle contraction) components. Despite the clinical availability of both 5-alpha-reductase inhibitors and alpha$_1$-AR antagonists, little is known regarding the influence of androgen deprivation on the expression of prostatic alpha$_1$-ARs. Hence, we studied the influence of androgen deprivation and replacement on regulation of rat ventral prostatic alpha$_1$-ARs. Our results are both paradoxical and interesting. We demonstrate that, concurrent with known decreases in prostate size predominantly due to regression of secretory epithelial cells, androgen deprivation leads to significant and progressive *increases* ($P < 0.01$) in prostatic alpha$_1$-AR density when normalized to total protein; alpha$_1$-AR density returns to normal with testosterone replacement. Prostate specificity is demonstrated by a lack of concurrent change in rat liver alpha$_1$-AR density in response to androgen deprivation or replacement.

The first question that must be asked regarding these results is whether increased prostatic alpha$_1$-AR expression with androgen deprivation is real or artifactual. Because alpha$_1$-AR density is measured as femtomoles of receptor per milligram of total protein, it is important to confirm that increases in measured alpha$_1$-AR density are not simply due to decreased overall prostatic protein content resulting from androgen deprivation–mediated epithelial cell regression. To examine this possibility, we determined cell type and cell-specific protein content in rat ventral prostates after castration. Overall, the percent of epithelially derived cell protein in the prostate decreases from 83% to 67% after castration, resulting in a relative doubling in the percent of stroma (17% to 33%); the protein content of epithelial and stromal cells (on a per-cell basis), however, remains unchanged. Previous human studies have shown that alpha$_1$-ARs predominate in the prostatic stroma,[19,20] a finding confirmed by autoradiography in the present study in normal and castrated rats, heightening the importance of relative increases in the stromal compartment with castration. Because increased prostatic alpha$_1$-AR expression parallels the percent of stromal tissue (predominantly smooth muscle in the present study), increased relative smooth muscle expression with castration is probably the mechanism underlying increased alpha$_1$-AR ligand binding rather than direct upregulation of alpha$_1$-AR protein. This finding fits recent data demonstrating that castration does not change alpha$_1$-AR–mediated contraction in dog prostate.[22] Because strips of smooth muscle are used for these experiments, and our studies suggest that upregulation of alpha$_1$-AR protein does not occur, then changes in alpha$_1$-AR–mediated contraction would not be expected in these studies. However, extrapolating to human prostate where smooth muscle surround the urethra, relative increases in stroma with androgen deprivation might possibly augment effectiveness of alpha$_1$-AR-mediated contraction, even without a change in absolute alpha$_1$-AR density.

Serum testosterone levels decrease to nondetectable levels after castration in rats, with pros-

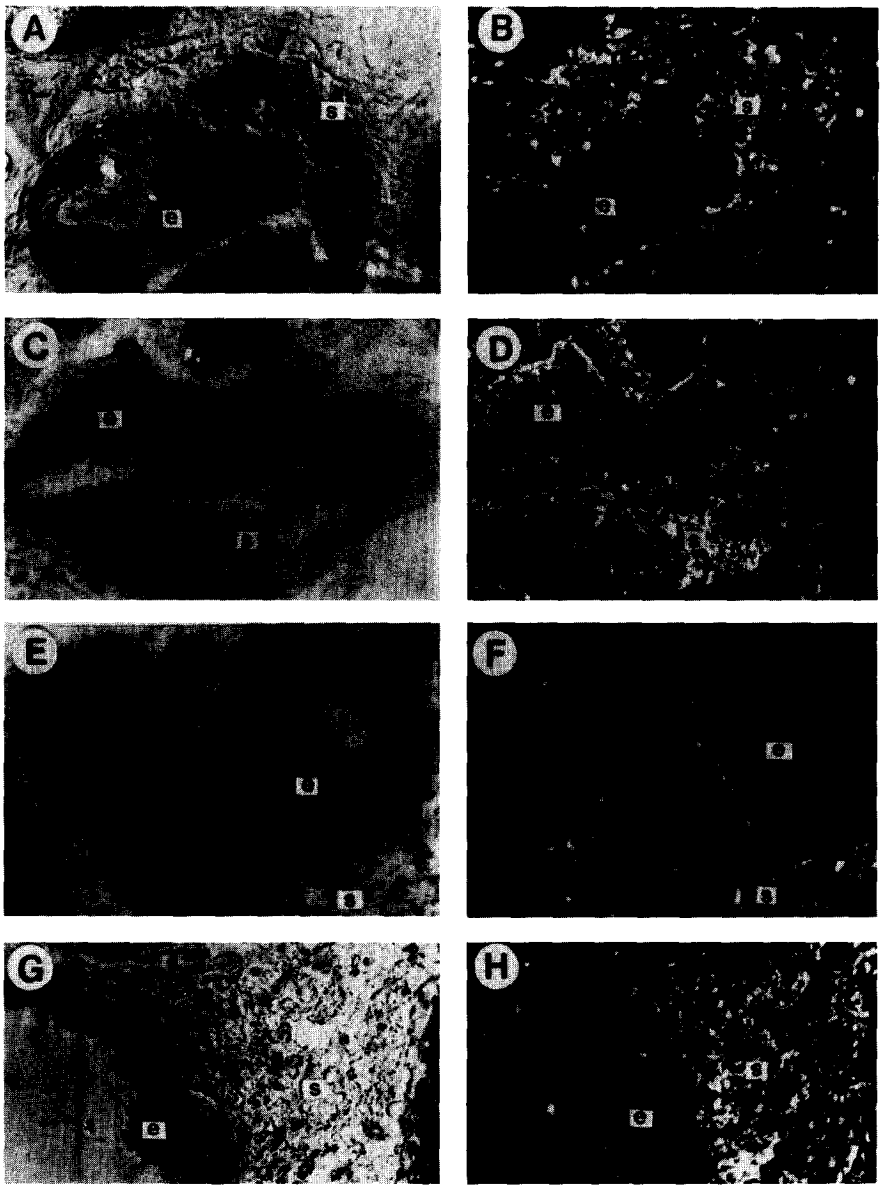

FIGURE 1. *Autoradiograms of rat ventral prostate with [^{3}H]prazosin reveal localization of alpha$_1$-adrenergic receptors to the stromal compartment at all time points (baseline, after castration and hormone replacement). (A and B) Intact control prostate (group 1). (C and D) One day after castration (group 2). (E and F) Three days after castration (group 3). (G and H) Five days after castration (group 4). (B, D, F, and H) Bright-field microscopy, hematoxylin-eosin, ×250. (A, C, E, and G) Dark-field microscopy, ×250. s = stroma, e = epithelium.*

tatic tissue dihydrotestosterone levels decreasing significantly in direct relation to testosterone.[23] However, not all tissue and receptor systems are sensitive to androgen deprivation therapy. Accordingly, regulation of various neurotransmitter receptors by androgens has been examined in many tissues.[23–27] Of particular interest to the present study, rat ventral prostatic beta$_2$-ARs and muscarinic receptors downregulate with androgen deprivation, returning to baseline with testosterone replacement.[23,26] In dog urethra, both castration and estrogen administration decreased alpha$_1$-AR and muscarinic receptors but increased alpha$_2$-AR density.[25] Another study in rabbit bladder base and proximal urethra demonstrated a 59% reduction in alpha$_1$-AR density, with no change in either beta-AR or muscarinic receptor density with castration.[27] We now demonstrate that there is no change in the absolute density of alpha$_1$-ARs after castration.

What are the clinical implications of stable prostatic alpha$_1$-AR expression with androgen deprivation? The most obvious clinical implication involves the interaction of 5-alpha-reductase inhibitors and alpha$_1$-AR antagonists in the treatment of BPH. Because absolute alpha$_1$-AR density does not decrease with androgen deprivation, these studies suggest that alpha$_1$-AR antagonists

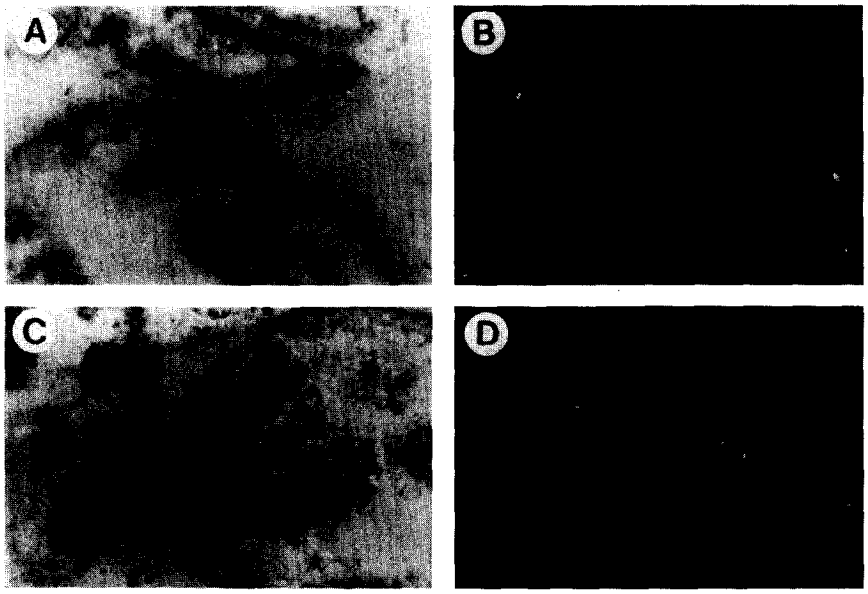

FIGURE 2. *Lack of signal in autoradiograms of rat ventral prostate with [^{3}H]prazosin after competition with excess prazosin (1 μM) confirms specific binding to alpha$_1$-adrenergic receptors. (A and B) Normal, intact prostate (group 1). (C and D) Five days after castration (group 4). (A and C) Bright-field microscopy, hematoxylin-eosin, original magnification ×250. (B and D) Dark-field microscopy, original magnification ×250.*

TABLE II. ***Protein content and analysis of cell types in rat ventral prostate after castration****

Cell Type	No. of Cells	Protein Concentration (pg/cell)	Total Protein in Fraction (mg)	% Total Protein (Individual Cell Types)	% Total Protein (Epithelial Versus Stromal)
Intact control rates†					
Secretory epithelial	6.6×10^9	2.8	18.5	63%	
Basal epithelial	3.6×10^9	1.6	5.8	20%	83%
Stromal	4.0×10^9	1.25	5.0	17%	17%
Total			29.2	100%	100%
Castration for 5 days‡					
Secretory epithelial	0.49×10^9	2.8	1.4	8%	
Basal epithelial	4.4×10^9	2.2	9.7	59%	67%
Stromal	3.6×10^9	1.5	5.4	33%	33%
Total			16.5	100%	100%

* *Percoll gradients were utilized to separate distinct populations of prostate cells (see text for details).*
† *Day 0, n = 6, total prostatic weight 3.5 g.*
‡ *n = 12, total prostate weight 3.8 g.*

remain an important component in BPH therapy even when 5-alpha-reductase inhibitors are utilized. The present study also demonstrates that changes in functionally important prostatic proteins need to be normalized to more than total protein levels in androgen-deprivation experiments.

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