Journal of Cellular Biochemistry 90:138–147 (2003)

Vitamin D and Androgen Regulation of Prostatic Growth

Eddy S. Leman,[1,2] Julie A. Arlotti,[1] Rajiv Dhir,[1,2,3] and Robert H. Getzenberg[1,2,3]*

[1]Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15232
[2]Cellular and Molecular Pathology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15232
[3]University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15232

Abstract Vitamin D has been reported to inhibit the growth of prostate cancer cells and model systems. In this study, we examined the interaction between 1,25-dihydroxyvitamin D_3 (1,25 D) in the presence or absence of endogenous testosterone on the growth and development of the adult rat prostate. Male Sprague–Dawley rats (165 days old) were either kept intact or castrated. Seven days after castration, the rats were treated with vehicle (control) or 1,25 D for 3 weeks and then sacrificed. Both ventral and dorsal lateral prostates were harvested; whole tissue lysates were collected and AR and VDR protein levels were analyzed by immunoblot analyses. Administration of 1,25 D in the intact animals decreased the prostatic size by 40%, compared to control animals, whereas 1,25 D did not influence the size of the prostate in castrated rats. 1,25 D administration in intact groups also increased both the AR and VDR protein levels by ~twofold, whereas in castrated groups, 1,25 D only increased the AR protein level by 1.5–2.5-fold. 1,25 D in the presence of endogenous testosterone inhibits prostatic growth, whereas 1,25 D in the absence of endogenous testosterone does not affect prostatic growth. The growth inhibitory activity of 1,25 D in the presence of testosterone may be mediated through the ligand activated AR and VDR pathways. These studies may reveal important information about the potential efficacy of 1,25 D as well as hormonal status in understanding the development of prostate diseases. J. Cell. Biochem. 90: 138–147, 2003.

Key words: vitamin D; testosterone; AR; VDR; normal prostate

1,25-dihydroxyvitamin D_3 (1,25 D) is the most active hormonal form of vitamin D that regulates calcium and phosphate homeostasis in the body. Many studies have reported the growth inhibitory activity of 1,25 D on prostate cancer cells and that decreased circulation levels of 1,25 D have been associated with a higher risk of prostate cancer [Schwartz and Hulka, 1990; Hanchette and Schwartz, 1992; Corder et al., 1993; Braun et al., 1995; Gann et al., 1996; Ahonen et al., 2000; Tuohimaa et al., 2001]. Similar anti-proliferative effects of 1,25 D are also evident in primary cultures of normal prostate cells in vitro [Peehl et al., 1994]. When adult male rats are castrated and administered with a super-physiological dose of 1,25 D, the resulting prostates of these animals were more than twice the size of the control animals treated with vehicle alone [Konety et al., 1996]. When the rats are castrated and then treated with exogenous testosterone in addition to 1,25 D, they exhibit greater prostatic differentiation without the increase in size seen in castrated rats.

Administration of 1,25 D in rats in utero also influences the size and differentiation of the prostate throughout the life span of the animal [Konety et al., 1999]. Thus, 1,25 D has an "imprinting" effect on the prostate similar to what has been reported with androgens and estrogens [Wernert et al., 1988; Makela et al., 1990; Schulze and Claus, 1990]. When 1,25 D was administered at a super-physiological dose during the pre-pubertal period, there was no difference in prostatic weights between the control and the 1,25 D treated rats [Konety et al., 2000]. Exogenous administration of

Grant sponsor: NIH; Grant number: DK52697.

*Correspondence to: Dr. Robert H. Getzenberg, PhD, Department of Urology, UMPC Shadyside, Urology Research Laboratories, 5200 Centre Avenue Suite G40, Pittsburgh, PA 15232.
E-mail: getzenbergrh@msx.upmc.edu

Received 23 May 2003; Accepted 28 May 2003
DOI 10.1002/jcb.10605

dihydrotestosterone (DHT) after puberty further decreased the prostatic weights of control rats. However, in rats treated with 1,25 D, exogenous DHT did not have any significant effect on prostatic weight. Despite this evidence, to date, little is known about the role of 1,25 D as well as its interaction with androgens in regulating the growth and differentiation of the normal prostate.

In this study, we examined the interaction between 1,25 D in the presence or absence of endogenous testosterone on the growth and development of the adult rat prostate. 1,25 D administration in intact animals decreases their prostatic size by 40%, compared to control vehicle animals, whereas 1,25 D does not influence the size of the prostate in castrated rats. 1,25 D administration on intact animals also increases both the AR and VDR protein levels by ~twofold, whereas in castrated groups, 1,25 D only increases the AR protein level by 1.5–2.5-fold. The results suggest that 1,25 D as a steroid hormone along with androgens may have a synergistic effect in influencing prostotic development in adult rats. The synergism between 1,25 D and testosterone could possibly be mediated through the actions of both AR and VDR in the prostate.

MATERIALS AND METHODS

Hormone and Antibodies

1,25 D (Sigma, St. Louis, MO) stock concentration was prepared to 1 mg/ml in ethanol. The anti-AR, anti-VDR, and anti-actin antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA.

Animal Experiments

Male Sprague–Dawley rats aged 165 days old were divided into four groups. Groups 1 and 2 of ($n = 8$ each) served as intact, non-castrated groups; animals in groups 3 ($n = 6$) and 4 ($n = 8$) were castrated. Rats in groups 3 and 4 were castrated using a sterile surgical technique on day 0. Animals in groups 2 and 4 received intraperitoneal injections of 2 μg (40 μl) 1,25 D diluted in 5 ml PBS starting from day 7, whereas animals in groups 1 and 3 received intraperitoneal injections of vehicle (40 μl of 100% ethanol in 5 ml PBS). All animals received injections on alternate days for 3 weeks. The animals were kept in the dark and fed with vitamin D deficient diet containing 0.3% calcium and 0.4% phosphate. Total body weights were measured twice a week during the treatment. At the end of the 3-week treatment, the animals were weighed, sacrificed, and serum was collected by cardiac puncture and stored for calcium and 1,25 D levels. The prostates were harvested, separated into ventral (VP) and dorsal-lateral (DLP) lobes, and sent for histopathological examination and analysis of AR and VDR protein levels as well as their DNA binding activities. The animals and experiments used in these studies were approved by the institutional animal care committee according to Public Health Service guidelines.

Total Protein Preparation

Prostate tissues from three different animals were used for immunoblot analyses. Whole tissue extracts were prepared by homogenizing the tissues with a polytron tissue homogenizer (Brinkmann, Westburg, NY) in extraction buffer [20 mM HEPES (pH 7.9), 20 mM NaF, 1 mM $Na_3P_2O_7$, 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 420 mM NaCl, 20% glycerol, 1 μg/ml leupeptin, and 1 μg/ml aprotinin], followed by snap-freezing in ethanol/dry ice for 5 min and thawing on ice for 10 min. The freeze and thaw procedures were repeated again for a total of three times. The supernatant was then centrifuged and harvested. Protein concentrations were determined by using the Coomassie plus protein assay kit (Pierce, Rockford, IL) according to the manufacturer's protocol.

Immunoblot Analyses

One hundred micrograms of whole tissue lysates were loaded and separated by 7.5% SDS/PAGE along with a separate lane containing 10 μl of Rainbow Markers (Amersham Life Sciences, Arlington Heights, IL). Proteins were then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA) utilizing a semi-dry transfer apparatus (Bio-Rad, Richmond, CA). The membranes were incubated overnight in 5% non-fat dry milk in PBS with 0.2% Tween-20. The membranes were washed several times followed by 1-h incubation with either 1:250 dilution of anti-AR primary antibody or 1:500 dilution of anti-VDR antibody in PBS with 2% non-fat dry milk and 0.2% Tween-20 at room temperature. The membranes were then washed again for three times (10 min for each wash) with PBS and 0.2%

Tween-20 and incubated for 1 h with appropriate secondary antibody conjugated with horseradish peroxidase at 1:5,000 dilution (Amersham Life Sciences) at room temperature. The membranes were then washed again with PBS and 0.2% Tween-20 (three 10-min washes). To control protein loading, all the membranes were also probed with anti-actin antibody. Proteins were detected by a chemiluminescence reaction using the ECL Immunoblot kit (Amersham Life Sciences). To quantitate the bands, both AR and VDR protein bands were then analyzed densitometrically by FX-Phosphoimager Analysis (quantity one), and normalized with the actin bands. Statistical analyses were performed with two-way ANOVA and the significant values were indicated with P value < 0.05.

RESULTS

Figure 1 shows that 1,25 D treatment results in significant weight loss in both intact and castrated groups. Both intact and castrated animals that received 1,25 D lost 36 and 37% ($P = 3.7\text{E}{-}08$ and $P = 6.39\text{E}{-}08$), respectively, of their body weight when compared to the animals that received ethanol (vehicle). Castrated animals also lost 8% ($P = 0.04$) of their body weight when compared to the ones that were intact. Figure 2 shows that in intact animals, 1,25 D reduced the mean prostatic weight by 48% in the VP ($P = 0.005$) and 30% in the DLP ($P = 0.03$). Following castration, the mean prostatic weight was reduced by 83% ($P = 8.43\text{E}{-}05$) in the VP and by 67% ($P = 1.96\text{E}{-}05$) in the DLP when compared to the intact animals. Furthermore, 1,25 D did not have any significant effect in restoring the prostatic size in the castrated groups ($P = 0.224$). 1,25 D administration in the castrated animals further decreased the mean prostatic weight in the VP by 61% ($P = 0.006$) and by 50% ($P = 0.009$) in the DLP when compared to the intact animals that also received 1,25 D.

There was a 50% difference ($P = 0.0018$) in the mean prostate to total body weight ratios in groups 3 and 4, and a 53% difference ($P = 0.0067$) in the mean prostate to total body weight ratios between animals in groups 2 and 4. There was also a 78% difference ($P = 2.22\text{E}{-}05$) between the mean prostate to total body weight ratios between intact animals and castrated animals. However, there was no significant difference ($P = 0.74$) in the mean prostate to total body weight ratios between control-intact animals and 1,25 D-intact animals. Table I summarizes the differences in mean total body weight, prostatic size, mean prostate to total body weight ratios, seminal vesicles, as well as

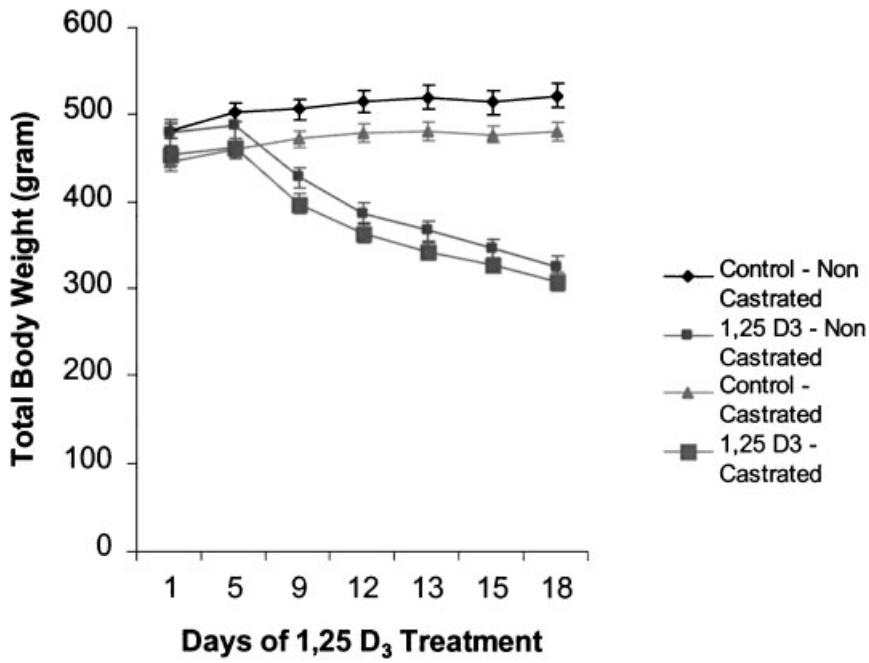

Fig. 1. Comparison of total body weight during 1,25 D treatment.

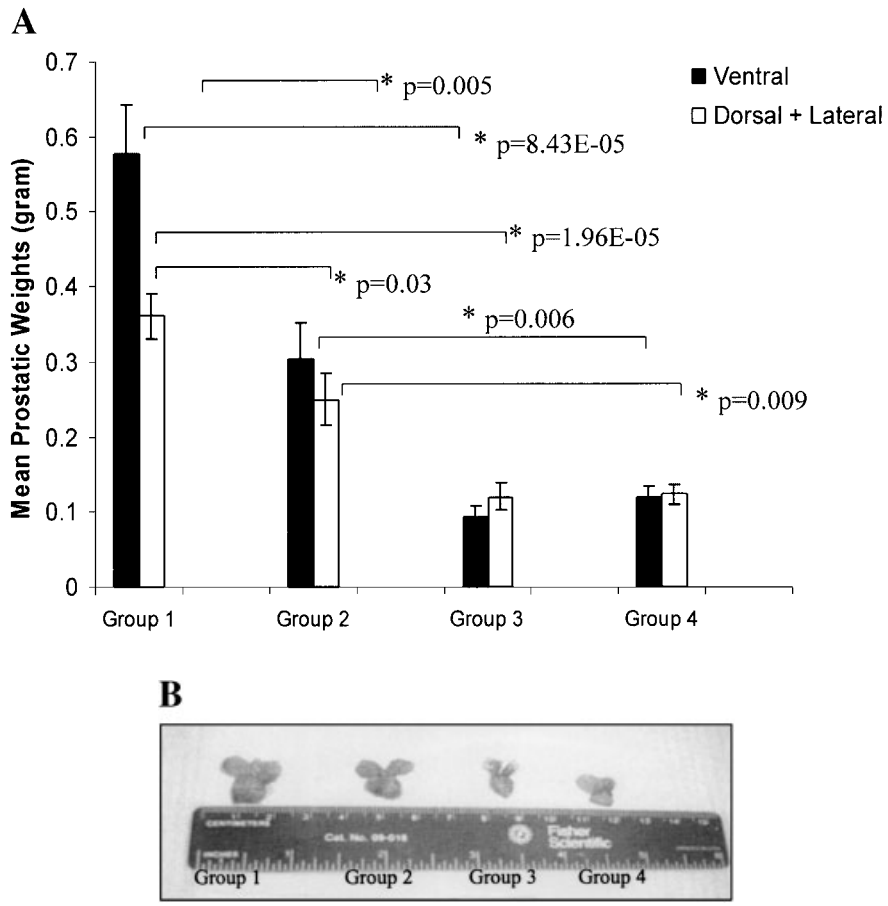

Fig. 2. **A**: Weights of both ventral and dorsal-lateral (DLP) lobes of the prostates (group 1, control-intact; group 2, 1,25 D-intact; group 3, control-castrated; and group 4, 1,25 D-castrated). **B**: Comparative examples of ventral and DLP prostates.

differences in serum parameters. There was a significant increase in total calcium levels in animals that received 1,25 D treatment ($P = 0.003$), which has been reported as a side effect of 1,25 D. Intact animals that received 1,25 D had eightfold excess of serum 1,25 D levels ($P = 0.03$) compared to control-intact animals. In castrated animals that received 1,25 D, the serum 1,25 D levels were 14-fold ($P = 0.002$) more than that of the control-castrated animals. Interestingly, when the rats were castrated, there was a 2.75-fold decrease ($P = 0.0005$) in serum 1,25 D levels when compared to the intact animals.

1,25 D increased the AR protein (MW 110 kDa) expression by twofold (Fig. 3A) in both VP ($P = 9.4E{-}04$) and DLP ($P = 0.013$) of the intact groups (groups 1 and 2). In the castrated groups, 1,25 D increased the AR protein level by 1.5-fold ($P = 0.004$) in the VP and 2.2-fold ($P = 5.8E{-}04$) in the DLP. The AR protein level in the VP of the castrated animals was decreased by 1.3-fold ($P = 0.007$) when compared to the intact animals, whereas AR protein levels remained unchanged in the DLP. 1,25 D increased VDR protein (MW 52–55 kDa) expression by 1.8-fold ($P = 0.0001$) in the VP and by 1.3-fold ($P = 0.03$) in the DLP of the intact animals (Fig. 3B). However, 1,25 D did not have any significant effect in increasing the VDR protein expression in both the VP ($P = 0.43$) and DLP ($P = 0.27$) of the castrated groups. The VDR protein level in the castrated animals that received 1,25 D was decreased by 1.9-fold ($P = 0.003$) in the VP and by 1.2-fold ($P = 0.04$) in the DLP when compared to the intact animals that received 1,25 D.

Microscopic examination of hematoxylin–eosin (H&E)-stained sections of the VP from the intact control animals revealed well-differentiated glands with maximum epithelium development forming small vacuoles with

TABLE I. Differences in Weight and Serum Parameters

Parameter	Group 1 (intact-control)	Group 2 (intact-1,25 D)	Group 3 (castrated-control)	Group 4 (castrated-1,25 D)	*P*-value
Mean total body weight (±SEM) (g)	522 ± 13.87	325.88 ± 11.85	482 ± 10.71	308 ± 10.36	<0.05 (groups 1 and 2; 3 and 4; 1 and 3)[a]
Mean prostatic weight (±SEM) (g)	0.94 ± 0.06	0.55 ± 0.07	0.21 ± 0.014	0.24 ± 0.02	<0.05 (groups 1 and 2; 2 and 4; 1 and 3)[a]
% prostate to total body weight ratio (±SEM)	0.18 ± 0.015	0.17 ± 0.024	0.044 ± 0.003	0.08 ± 0.008	<0.05 (groups 1 and 3; 2 and 4; 3 and 4)[a]
Mean seminal vesicle weight (±SEM) (g)	0.70 ± 0.05	0.47 ± 0.05	0.17 ± 0.03	0.23 ± 0.03	<0.05 (for all groups)[a]
Mean serum calcium level (±SEM) (mg/dl)	9.7 ± 0.08	16.34 ± 0.47	9.78 ± 0.13	17.31 ± 0.21	<0.05 (for groups 1 and 2; 3 and 4)[a]
Mean serum 1,25 D level (±SEM) (pg/ml)	80.33 ± 3.92	652.67 ± 93.67	29.33 ± 2.91	403.33 ± 16.95	<0.05 (for groups 1 and 2; 3 and 4; 1 and 3)[a]

[a]Indicates statistically significant (the actual *P*-values are reported in the text).

apparent ratio of cytoplasmic and nuclei fractions, as well as loose connective stroma (Fig. 4A). Intact animals that received 1,25 D showed less well-differentiated glands in the VP that contained dilated basal-like epithelium with loose cytoplasmic area, as well as less amount of stroma (Fig. 4B). VPs from the castrated control animals demonstrated less well-differentiated glands with variability in the cytoplasmic and nuclear ratio in the epithelium and small amount of loose stroma (Fig. 4C), whereas in castrated animals that received 1,25 D, the VP showed a more prominent nuclear fraction with maximum development of stroma (Fig. 4D). In addition, VPs from 1,25 D-castrated animals also revealed that there was lots of concretion in the glands, which could be calcified debris from the 1,25 D administration.

Histology of the DLP sections from the control-intact animals demonstrated well-differentiated glands (Fig. 5A). In the intact animals that received 1,25 D, the DLP contained maturation of basal-like epithelium forming capillaries and vacuoles with reasonable amount of stroma (Fig. 5B). DLP of the control-castrated animals (Fig. 5C) revealed that the glands contained active basal epithelium forming columnar epithelium with capillary infolding, as well as stromal condensation with loose stromal around the glands. In castrated animals that received 1,25 D (Fig. 5D), the DLP showed basal like epithelium with stromal surrounding the glands, as well as the presence of calcified debris in the lumen as possible side effects from 1,25 D administration.

DISCUSSION

Administration of 1,25 D in adult intact rats significantly decreases the size of both VP (45% compared to control) and DLP (29% compared to control). This could be due to the loss of epithelium (reflected by the loose cytoplasmic area and dilated glands), as well as the absence of the stromal compartment. This translates to an overall decrease in both the VP and DLP of the prostate in intact animals. We previously demonstrated that 1,25 D and exogenous testosterone administration in castrated rats did not change the prostatic size [Konety et al., 1996]. It is important to note that in our previous study, 1,25 D and exogenous testosterone were administrated at the same time to the animals following castration. The effects of exogenous

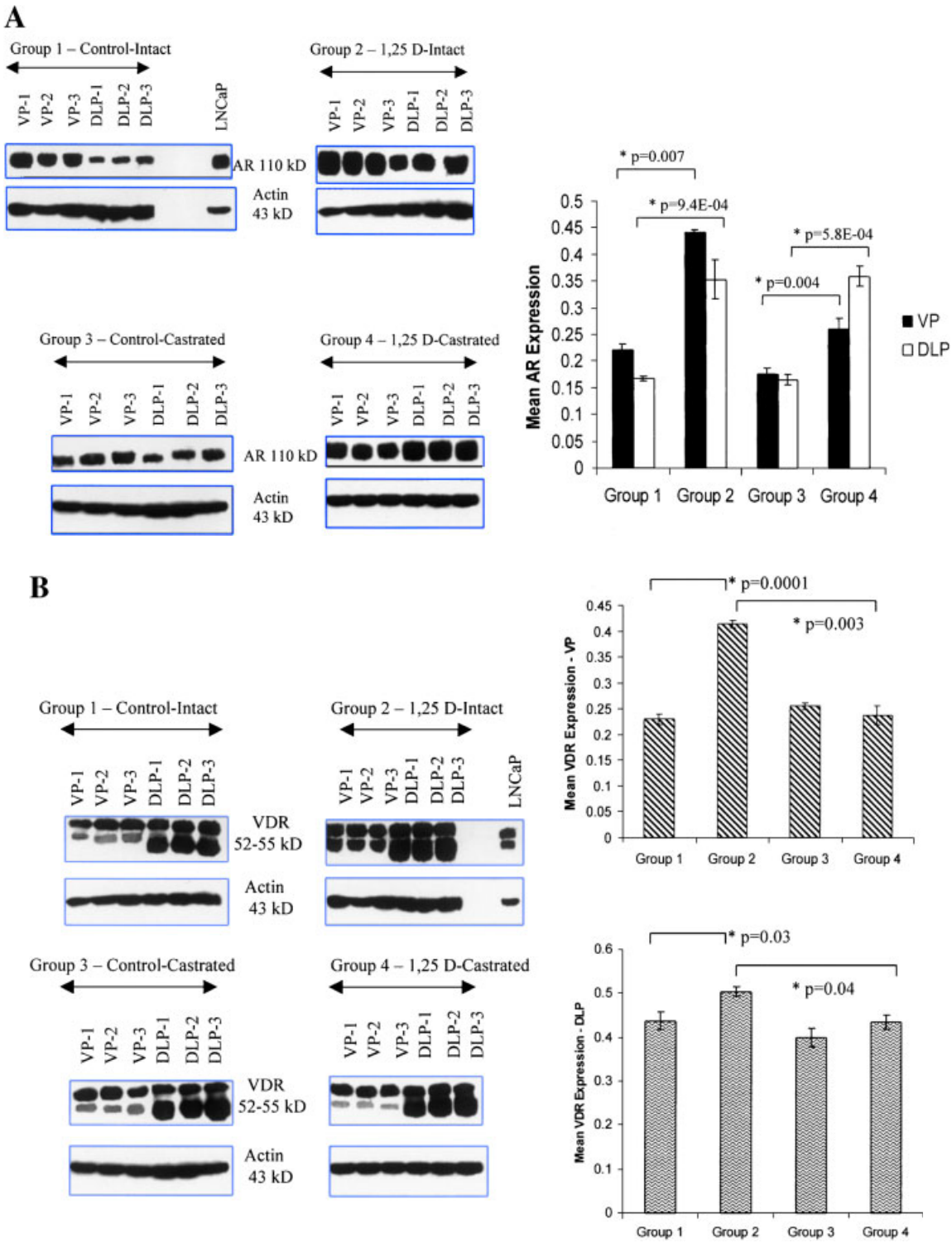

Fig. 3. Representative immunoblot analyses on: (**A**) androgen receptor (AR) and (**B**) vitamin D receptor (VDR) from whole tissue extracts from three different prostate tissues. The AR (110 kDa) and VDR (52–55 kDa) were analyzed densitometrically and normalized with actin (43 kDa). Error bars represent standard error of the mean (n = 3). Group 1, control-intact; group 2, 1,25 D-intact; group 3, control-castrated; and group 4, 1,25 D-castrated. VP, ventral prostate; DLP, dorsal–lateral prostate.

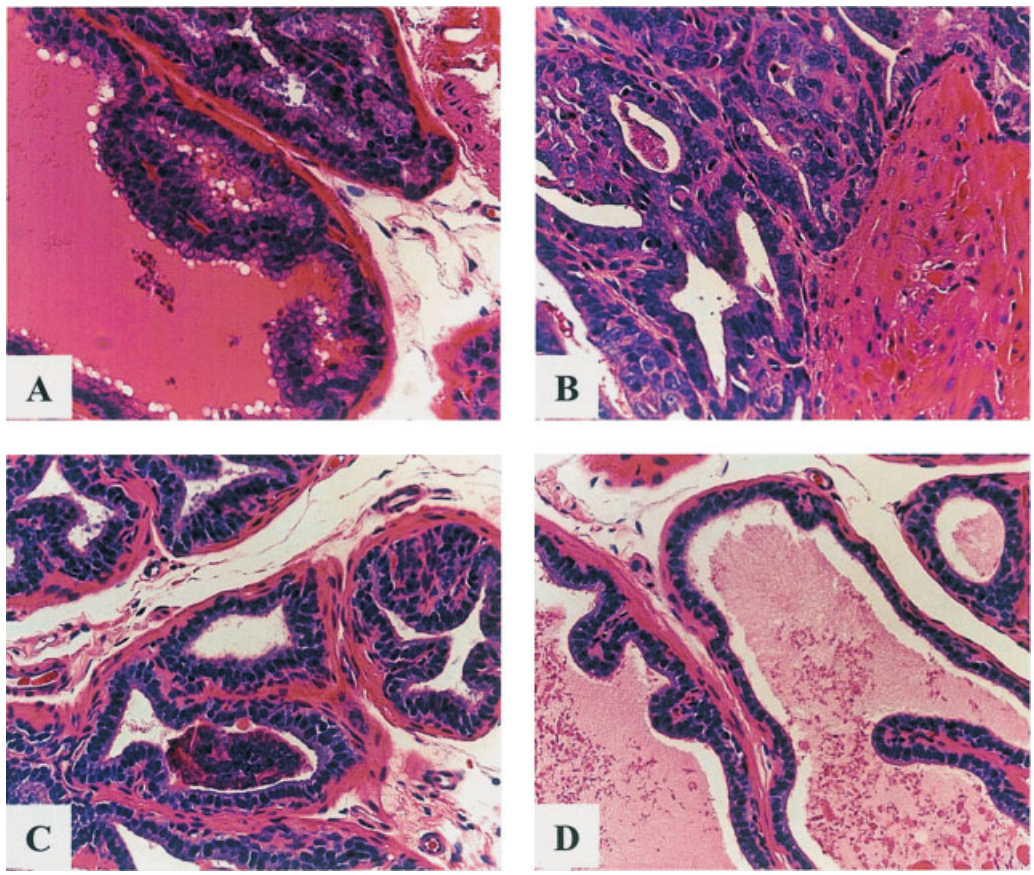

Fig. 4. Histology of ventral prostate tissues (×40) from animals in: (**A**) group 1 (control-intact), (**B**) group 2 (1,25 D-intact), (**C**) group 3 (control-castrated), and (**D**) group 4.

testosterone could be so overwhelming that the antiproliferative effect of 1,25 D may be overshadowed. In this study, 1,25 D was administrated without exogenous testosterone, and, therefore, 1,25 D was able to elicit its growth inhibitory activity. When the human prostate cancer cell line LNCaP was grown in charcoal stripped serum medium and co-treated with 1,25 D and a low concentration of androgen, substantial growth inhibition was observed [Zhao et al., 1997]. The present results support the concept that when 1,25 D is administered during adulthood when the levels of androgen are not at their maximum peak (i.e., there is no testosterone surge), 1,25 D may be able to inhibit prostatic growth.

Following castration, both VP and DLP from animals in group 3 shrink to 75 and 67%, respectively, as compared to those in intact animals. We previously reported that 1,25 D alone increased the overall prostatic size, whereas 1,25 D in the presence of exogenous testosterone did not change the prostatic size [Konety et al., 1996]. As shown by the H&E staining of the VP tissue sections from the castrated control animals, castration results in loss of epithelium and less well differentiated glands compared to control intact animals. However, when the castrated animals are treated with 1,25 D, there is a further loss of the cytoplasmic area in the epithelium that form smaller glands, whereas there is a maximum stromal development around the glands compared to the control-castrated groups. These results indicate that 1,25 D in the absence of endogenous testosterone stimulates the growth of the stroma. Similarly, the DLP also shows shrinkage of the glands with the presence of only basal-like epithelium. Following 1,25 D treatment, there is a further loss in the cytoplasmic area of the epithelium, but prominent presence of stromal development surrounding the glands. This translates to an overall similarity in the prostatic size between the 1,25

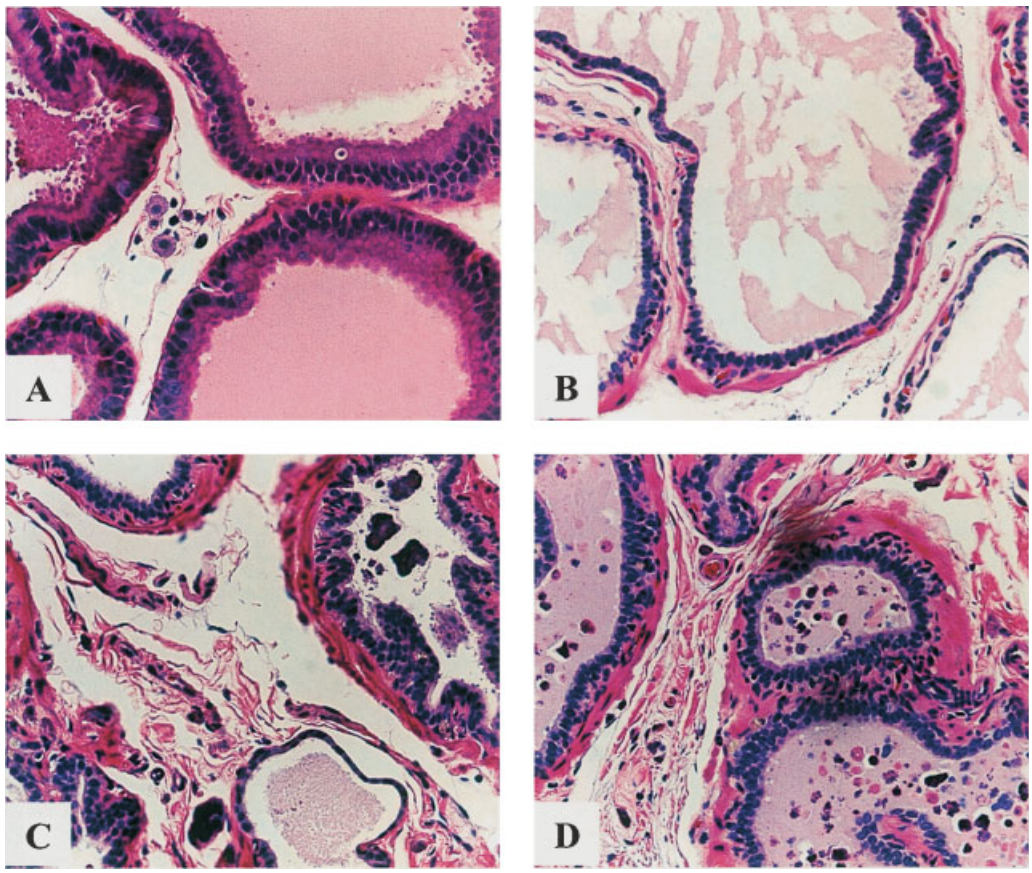

Fig. 5. Histology of DLP prostate tissues (×40) from animals in: (**A**) group 1 (control-intact), (**B**) group 2 (1,25 D-intact), (**C**) group 3 (control-castrated), and (**D**) group 4 (1,25 D-castrated).

D-castrated and control-castrated groups. It is speculated that 1,25 D treatment following castration further stimulates stromal growth and this increase is reflected in the overall prostatic size.

1,25 D administration in intact animals increased the AR protein level by twofold in both the VP and DLP. 1,25 D also increased VDR expression by 1.8-fold in the VP and by 1.25-fold in the DLP of the intact animals. It is possible that the decrease in prostatic size following 1,25 D treatment could at least be mediated through AR and VDR (as reflected by an increase in protein expression). Since both AR and VDR act as transcription factors, we speculate that binding of these receptors to their respective hormone responsive elements (ARE and VDRE) could activate and/or repress transcription of certain genes that are involved in growth/proliferation of either the epithelium or stroma. In castrated animals, 1,25 D increases AR protein in both VP and DLP. Several studies have shown that 1,25 D increases the AR expression in LNCaP cells at both the protein and mRNA levels [Hsieh et al., 1996; Hsieh and Wu, 1997; Zhao et al., 1999]. Our results suggest that 1,25 D may be able to regulate the AR protein in the absence of testosterone. However, it is important to note that the effects described in LNCaP cells were observed in vitro. Cells grown in culture often change their characteristics and properties, including their responsiveness to hormones such as testosterone and 1,25 D. The in vivo effects of 1,25 D observed in this study could be due to the presence of other factors that may be absent in culture media.

The presence or absence of endogenous testosterone also seems to influence the circulating 1,25 D levels in the serum. In intact animals, 1,25 D administration resulted in eightfold increase of serum 1,25 D levels, whereas in castrated animals, 1,25 D administration resulted in 14-fold increase of serum

1,25 D levels. When the intact and castrated animals were compared, there was a 2.75-fold decrease in serum 1,25 D levels in the castrated animals. These results indicate that when the animals are castrated, there could be a loss of epithelium in the prostate (as reflected by the decrease in prostatic size), which could result in the loss of functional VDRs. Thus, there is more free 1,25 D circulating in the serum that could not bind to the VDRs to form receptor–ligand complex in order to activate/repress transcription of certain genes.

When the animals were castrated, there was an 8% decrease in the mean body weight and a 78% decrease in the mean prostatic weight. Administration of 1,25 D caused a 37% decrease in the mean body weight and a 40% decrease in the mean prostatic weight in intact animals when compared to control animals. There was also a 36% decrease in the mean body weight but no changes in the mean prostatic weight in castrated animals that received 1,25 D. The large amount of body weight loss has been reported in our previous study [Konety et al., 1996] although there was only a 20% decrease in the mean body weight of the 1,25 D treated animals. We reason that this could be due to the difference in the 1,25 D dose administrated. In this study, the animals were treated with 2 μg of 1,25 D/animal, whereas previously, the animals were treated with 1 μg of 1,25 D/animal [Konety et al., 1996]. Taken together, the increase in the serum 1,25 D levels as well as the large amount of weight loss in the animal indicate that the higher dose of 1,25 D used in this study may play a role in affecting the total body weight.

To summarize, 1,25 D administration in adult intact rats suppresses growth of both VP and DLP, whereas in castrated rats, 1,25 D does not have any effects in the prostatic size, which could be mediated through AR and VDR. Further investigations are being directed in elucidating the effects of 1,25 D and testosterone on prostatic cells. These studies may reveal important information about the potential efficacy of 1,25 D as well as hormonal status in understanding the development of prostate diseases.

REFERENCES

Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. 2000. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control 11:847–852.

Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. 1995. Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States). Cancer Causes Control 6:235–239.

Corder EH, Guess HA, Hulka BS, Friedman GD, Sadler M, Vollmer RT, Lobaugh B, Drezner MK, Vogelman JH, Orentreich N. 1993. Vitamin D and prostate cancer: A prediagnostic study with stored sera. Cancer Epidemiol Biomarkers Prev 2:467–472.

Gann PH, Ma J, Hennekens CH, Hollis BW, Haddad JG, Stampfer MJ. 1996. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev 5:121–126.

Hanchette CL, Schwartz GG. 1992. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. Cancer 70:2861–2869.

Hsieh T, Wu JM. 1997. Induction of apoptosis and altered nuclear/cytoplasmic distribution of the androgen receptor and prostate-specific antigen by 1alpha,25-dihydroxyvitamin D3 in androgen-responsive LNCaP cells. Biochem Biophys Res Commun 235:539–544.

Hsieh TY, Ng CY, Mallouh C, Tazaki H, Wu JM. 1996. Regulation of growth, PSA/PAP, and androgen receptor expression by 1 alpha,25-dihydroxyvitamin D3 in the androgen-dependent LNCaP cells. Biochem Biophys Res Commun 223:141–146.

Konety BR, Schwartz GG, Acierno JS, Jr., Becich MJ, Getzenberg RH. 1996. The role of vitamin D in normal prostate growth and differentiation. Cell Growth Differ 7:1563–1570.

Konety BR, Nangia AK, Nguyen TS, Thomas A, Getzenberg RH. 1999. Effect of prenatal vitamin D (calcitriol) exposure on the growth and development of the prostate. Prostate 41:181–189.

Konety BR, Leman E, Vietmeier B, Arlotti J, Dhir R, Getzenberg RH. 2000. In vitro and in vivo effects of vitamin D (calcitriol) administration on the normal neonatal and prepubertal prostate. J Urol 164:1812–1818.

Makela S, Santti R, Martikainen P, Nienstedt W, Paranko J. 1990. The influence of steroidal and nonsteroidal estrogens on the 5 alpha-reduction of testosterone by the ventral prostate of the rat. J Steroid Biochem 35:249–256.

Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. 1994. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. Cancer Res 54:805–810.

Schulze H, Claus S. 1990. Histological localization of estrogen receptors in normal and diseased human prostates by immunocytochemistry. Prostate 16:331–343.

Schwartz GG, Hulka BS. 1990. Is vitamin D deficiency a risk factor for prostate cancer? (hypothesis). Anticancer Res 10:1307–1311.

Tuohimaa P, Lyakhovich A, Aksenov N, Pennanen P, Syvala H, Lou YR, Ahonen M, Hasan T, Pasanen P, Blauer M, Manninen T, Miettinen S, Vilja P, Ylikomi T. 2001. Vitamin D and prostate cancer. J Steroid Biochem Mol Biol 76:125–134.

Wernert N, Gerdes J, Loy V, Seitz G, Scherr O, Dhom G. 1988. Investigations of the estrogen (ER-ICA-test)

and the progesterone receptor in the prostate and prostatic carcinoma on immunohistochemical basis. Virchows Arch A Pathol Anat Histopathol 412:387–391.

Zhao XY, Ly LH, Peehl DM, Feldman D. 1997. 1-alpha,25-dihydroxyvitamin D3 actions in LNCaP human prostate cancer cells are androgen-dependent. Endocrinology 138:3290–3298.

Zhao XY, Ly LH, Peehl DM, Feldman D. 1999. Induction of androgen receptor by 1alpha,25-dihydroxyvitamin D3 and 9-*cis* retinoic acid in LNCaP human prostate cancer cells. Endocrinology 140:1205–1212.