Castration Induces Apoptosis in the Ventral Prostate but not in an Androgen-sensitive Prostatic Adenocarcinoma in the Rat

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ABSTRACT

Apoptosis in the androgen-sensitive Dunning R3327 PAP prostatic adenocarcinoma was studied during the post castration period of 14 days and compared with the ventral prostate. The mRNA expression of testosterone-repressed prostatic message-2 and tissue-type plasminogen activator in the Dunning tumor and in the ventral prostate was analyzed by Northern blot experiments and immunohistochemical procedures. The degree of endonuclease-degraded genomic DNA was examined by gel electrophoresis. Apoptotic tumor epithelial cells were identified with in situ end labeling. Epithelial cells incorporating bromodeoxyuridine (BrdUrd) after castration in the ventral prostate and the Dunning tumors were localized with immunostaining. Androgen ablation resulted in an induction of testosterone-repressed prostatic message-2 and tissue-type plasminogen activator transcripts in the normal prostate with a peak at approximately 2 to 5 days post castration. These transcript levels in the Dunning prostatic tumors did not show any induction during the same period. Immunohistochemical staining for sulfated glycoprotein-2 and tissue-type plasminogen activator confirmed this difference between the tumor tissue and the ventral prostate at the transcriptional level. The determination of DNA integrity showed similar results in that the degree of DNA fragmentation in the tumor was much lower than the initial and marked degradation of DNA in the ventral prostate. The number of in situ end-labeled epithelial tumor cells were not increased by castration. BrdUrd immunodetection showed that castration induced an initial increase in the number of BrdUrd-positive epithelial cells in the ventral prostate. In the tumors, caspase resulted in a decrease in BrdUrd-positive epithelial cells. It was concluded that in the androgen-sensitive prostatic Dunning R3327 PAP adenocarcinoma, the biochemical cascade leading to apoptosis is not activated by androgen withdrawal, as in the ventral prostate.

INTRODUCTION

Endocrine treatment is first-line therapy for patients with metastatic prostatic carcinoma (1). Medical or surgical castration initially results in a clinically beneficial response in most patients. However, following this response, almost all patients sooner or later relapse, and the prostatic tumor cells become androgen independent. The progression of prostatic tumors to androgen independency has been suggested to be due to selective regrowth of an androgen-independent cell clone, while the androgen-dependent cells undergo apoptosis (1–5).

The idea that cell death is important for the effect of castration on prostatic carcinoma is supported by findings in the normal prostate. Following castration, there is a characteristic reduction in prostatic size and a dramatic decrease in androgen-dependent protein synthesis (6, 7). In the rat prostate, protein, RNA, and DNA content decrease drastically following castration, accompanied by a reduction in the androgen-dependent activities of the gland (8–10). Androgen withdrawal also initiates an active cellular program, leading to death of the glandular epithelial cells of the ventral prostate. An early event in this cell death process is the fragmentation of chromosomal DNA into low molecular weight fragments, which are integral multiples of 180–200 base pair subunits. This irreversible Ca2+/Mg2+-dependent endonuclease activity occurs within 24 h after castration (9, 10). Prostatic DNA degradation into nucleosomal size fragments reaches its maximal value by 4 days following castration and then declines over the next 10 days (10). Once initiated, apoptosis leads to a cascade of biochemical and morphological events, which finally results in an irreversible pathway towards cell elimination. The temporal sequences of events of apoptosis comprise chromatin aggregation, nuclear and cytoplasmic condensation, and eventual fragmentation of the dying cell into clusters of membrane-bound segments, called apoptotic bodies (11, 12). During prostatic regression, specific castration-induced mRNA sequences are synthesized, suggesting that prostatic involution is associated with the activation of specific genes (7, 8, 13, 14). One of the first gene products connected to apoptosis in regression of the rat ventral prostate was the TRPM-2 (15, 16). Both sequence analysis and antibody recognition studies have confirmed that the TRPM-2 gene products are homologous, if not identical, to the products of a gene expressed by mammalian Sertoli cells, the SGP-2 or clusterin gene (17–19). TRPM-2 has been used as a marker for apoptosis in many kinds of mammalian cells undergoing regression or dying including human, rat, and mouse cells, both benign or malignant. Investigations done on the content of plasminogen activators in the rat ventral prostate post castration have shown that both urokinase-type plasminogen activator and t-PA were induced (20, 21).

The hormone-sensitive Dunning prostatic R3327 PAP adenocarcinoma responds to castration with a reduced growth rate, but there is no absolute reduction in tumor volume (22, 23). It has been shown that the number of epithelial tumor cells in this tumor model is not reduced 6 weeks after castration when compared to intact control animals (22). Although it is possible that this finding can be explained by the regrowth of androgen-insensitive cells, recent results by Westin et al. (24) support the idea that the androgen ablation effect on the Dunning R3327 PAP prostatic adenocarcinoma is not due to an increased tumor cell death. Their results showed that there was no reduction in the number of epithelial cells and only a slight decrease in average epithelial cell size at 14 days after castration. Apoptotic bodies could occasionally be observed in the Dunning R3327 PAP tumors, reflecting basal levels of cell death, but the apoptotic index did not change during the period studied (24). Since one principal clinical problem with prostatic cancer is the cells that do not die as a result of androgen ablation therapy, the purpose of the present study was to investigate the effects of castration on the androgen-sensitive Dunning R3327 PAP prostatic adenocarcinoma on different markers of apoptosis over a period of 14 days after castration.

3 The abbreviations used are: TRPM-2, testosterone-repressed prostatic message-2; SGP-2, sulfated glycoprotein-2; t-PA, tissue-type plasminogen activator; SSC, standard saline citrate; SDS, sodium dodecyl sulfate; ISEL, in situ end-labeling; BSA, bovine serum albumin; PBS, phosphate-buffered saline; IgG, immunoglobulin G; TBS, Tris-buffered saline; ABC, avidin-biotin-peroxidase complex; BrdUrd, bromodeoxyuridine.
MATERIALS AND METHODS

Animals and Tissue. Male Copenhagen × Fischer F1 rats (ALAB, Uppsala, Sweden) were s.c. transplanted with pieces of Dunning R3327 PAP adenocarcinoma as described previously by Landström et al. (25) and submitted to experiment. The tumor subline, which originated from the laboratory of Dr. N. Altman (The Papanicolaou Cancer Research Institute, Miami, FL), is known to be well differentiated and androgen sensitive (3). The rats were housed at a local animal house under controlled temperature (25°C) and humidity (40–60%) on a 12-h light/12-h dark schedule. The animals had free access to water and pelleted food.

Approximately 6 months after inoculation of the tumors, animals were randomly divided into different experimental groups (intact control and 1, 2, 3, 5, 7, 10, and 14 days after castration). The animals which served as intact controls were not treated in any way before they were sacrificed. Castration was performed via scrotal incision under ether anesthesia. Rats included in this group were sacrificed at indicated times after castration. Sacrificed rats were rapidly opened, and ventral prostate and tumor tissue were dissected out and cleaned from surrounding tissues. Dissected tissue specimens were immediately frozen in liquid nitrogen and stored at −70°C until further examination.

RNA Preparation, Northern Blot, and Hybridization Analysis. Total RNA was prepared from tumor and prostate tissue using acid guanidium thiocyanate-phenol-chloroform extraction method (26). RNA (4 or 10 µg total RNA/sample) was denatured with glyoxal and dimethyl sulfoxide and electrophoresed on a 1% agarose gel in 10 mM sodium phosphate buffer (pH 7), before capillary transfer onto nylon filter in 20X SSC (3 M NaCl and 0.3 M sodium citrate). A 0.24–9-kilobase RNA standard (GIBCO BRL, Laboratory Design AB, Sweden) was used as a size marker in the RNA gel electrophoresis experiments. Nylon filters were prehybridized for 4 h and hybridized for 16 h at 42°C using [α-32P]dCTP random-primer DNA probes (Amersham Sweden AB, Stockholm) for TRPM-2 and β-actin. The hybridization solution contained 50% formamide, 5X SSC, 7.5X Denhardt’s solution, 0.05 M sodium phosphate (pH 6.5), and 100 µg/ml heat-denatured salmon sperm DNA. Filters were washed twice at 42°C for 15 min in 2X SSC plus 0.1% SDS, twice at 50°C for 15 min in 0.5X SSC plus 0.1% SDS, and at 50°C in 0.1X SSC plus 0.1% SDS until low background and then autoradiographed. Hybridization with [α-32P]dCTP Riboprobe Gemini II Core system labeled (Scandinavian Diagnostic Services, Falkenberg, Sweden) t-PA RNA probe was done for 16 h at 64°C in hybridization solution containing 50% formamide, 5X SSC, 0.1% SDS, 25 µg/ml heat-denatured salmon sperm DNA, 8X Denhardt’s solution, 10 mM EDTA (pH 8), 25 mM Tris-HCl (pH 8), and 250 µg/ml E. coli tRNA. Prehybridization (2 h at 62°C solution) was the same except for the labeled riboprobe. Before autoradiography, the filters were washed twice at 66°C for 15 min in 2X SSC plus 0.1% SDS, 1X SSC plus 0.1% SDS, and in 0.1X SSC plus 0.1% SDS until low background.

Analysis of RNA Integrity. Frozen tumor and prostate tissue (4–6 mg/800 µl) were cautiously minced in a homogenization buffer (0.1 M NaCl, 0.01 M EDTA (pH 8), 0.3 M Tris-HCl (pH 8), and 0.2 M sucrose) using a knife-homogenizer. The homogenates were then incubated at 65°C for 30 min in a final concentration of 0.6% SDS. Then 8 µM potassium acetate were added to a final concentration of 1.1 M. After gentle mixing, the samples were kept on ice for 1 h and then centrifuged at 5000 rpm (Eppendorf rotor, 18 × 3.5 g, 2040 g) for 10 min at room temperature. Recovered supernatants were then sequentially extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and ethanol precipitated; the recovered DNA was then resuspended in 1X Tris-EDTA (0.01 M Tris-Cl (pH 8) and 0.001 M EDTA (pH 8)). After 20 µg/ml RNase A treatment (Sigma Chemical Co., St. Louis, MO) and two extractions as before, the DNA was precipitated with a final concentration of 2 mM ammonium acetate and 2.5 volumes of ice-cold absolute ethanol. The DNA pellets were then washed once with 80% ethanol before being left to air dry and subsequent resuspension in dH2O before concentration measurement. DNA samples were held at −20°C until 3' end-labeling reaction and gel electrophoresis. The 3' end-labeling with [α-32P]dATP (Amersham Sweden AB, Stockholm) of the DNA (400 ng/reaction) was done using a DNA 3' end-labeling kit (Boehringer Mannheim, Scandinavia AB, Bromma, Sweden). Labeled DNA was then separated on a 2% agarose 1X Tris-acetate (0.04 M Tris-acetate and 0.001 M EDTA) gel. A 123-base pair DNA standard (GIBCO BRL, Laboratory Design AB, Sweden) was used as a size marker. After completion of the gel electrophoresis, the gel was dried in a slab-gel dryer at room temperature in a vacuum and then exposed to an X-ray film.

ISEL. The detection of apoptotic cells by ISEL was basically done according to the protocol by Wijmans et al. (27). Ventral prostate and tumor tissue specimens were fixed in phosphate-buffered formalin, dehydrated, embedded in paraffin, and cut in 4-µm-thick sections. After deparaffinization and rehydration following standard procedures, tissue sections were heated in 2X SSC (0.3 M NaCl and 30 mM sodium citrate, pH 7) at 80°C for 20 min and subsequently washed thoroughly in distilled water. To enable enzymatic incorporation of nucleotides, the sections were digested in 0.5% pepsin in HCl (pH 2) for 15 min under gentle shaking in a 37°C water bath. The digestion was stopped by washing several times in tap water and then in buffer A (50 mM Tris-Cl, 5 mM MgCl2, 10 mM b-mercaptoethanol, and 0.005% BSA, pH 7.5) for 5 min. After drying, the sections were incubated for 1 h at 85°C in buffer A containing 0.01 mM dATP, dCTP, dGTP, biotin-16-UTP (Boehringer Mannheim Scandinavia AB, and 4 units/ml of DNA polymerase I (Sigma). After blocking endogenous peroxidase for 5 min in 0.1% H2O2 in 0.01 M PBS, followed by 5-min washes two times in PBS, the sections were incubated with avidin dissolved 1:100 in PBS with 1% BSA and 0.5% Tween 20 for 30 min in room temperature before developing with diaminobenzidine. For negative controls, DNA polymerase I was excluded from the nucleotide mix. Normal rat prostate at 3 days after castration was used as a positive control (27).

Immunohistochemistry. A new immunohistochemical method recently described by Shi et al. (28) was used for the SGP-2 immunostaining. Tissue from Dunning R3327 PAP tumors, from ventral prostates of intact rats, and from rats castrated at different times earlier was fixed in neutral buffered formalin for 24 h, processed, and the embedded in paraffin blocks. Six-µm-thick sections were mounted on poly-L-lysine (Sigma) coated slides. The sections were then deparaffinized, rehydrated in water, and heated in a microwave oven set at 92% of maximal effect (Bio-Rad) for 2 times at 5 min in distilled water. The sections were allowed to cool and were then rinsed in PBS (pH 7.3) with 0.1% BSA (Sigma). Normal 5% goat serum was used to block unspecific binding. The sections were then incubated overnight at 4°C with a rabbit anti-rat SGP-2 antibody. This antibody recognizes the two subunits of SGP-2 and the identical protein TRPM-2 (29, 30). The antibody was used in a concentration of 20 µg IgG/ml. The immunoreaction was then visualized using the Super Sensitive Multi-Link kit (BioGenex, San Ramon, CA) using alkaline phosphatase/Fast Red as a detection system. Negative control sections were processed in an identical manner by substitution of the primary antibody with normal rabbit IgG.

The immunohistochemical assay with primary anti-tPA antibodies known to react specifically with rat t-PA was done on 6-µm-thick cryostat sections mounted on poly-L-lysine coated slides (31–33). After rinsing three times in TBS containing 0.05 M Tris-Cl (pH 7.4) with 0.15 M NaCl, the sections were immersed in 30% peroxide for 30 min in 0.3% H2O2 dissolved in 0.05 M Tris-Cl (pH 7.4). After rinsing, the sections were routinely dehydrated, lightly counterstained with Mayer’s hematoxylin, and covered. As recommended by Stemmerger (35), we included the following controls: (a) replacement of the primary antibody with buffer; and (b) substitution of the primary antibody with the same titer of preimmune IgG.

Detection of BrdUrd incorporation post castration (2, 3, 7, and 14 days) in ventral prostate and Dunning tumors was carried out on tissue recovered from rats 5 h after being given an i.p. dose of 50 mg/kg BrdUrd (Sigma) at indicated times after castration as well as in intact controls. The tissue specimens were fixed in phosphate-buffered formalin, dehydrated, and embedded in paraffin. Five-µm-thick sections were immunostained with a monoclonal antibody against BrdUrd (DAKO, Álvsjö, Sweden) using biotinylated goat anti-mouse IgG and a peroxidase-labeled ABC reagent (Vector Laboratories, Burlingame, CA).
RESULTS

Northern Blot Hybridization Analysis. Northern blot experiment of TRPM-2 gene expression in the regressing rat ventral prostate showed an induction of the transcription level between days 2 and 5 post castration. The degree of TRPM-2 mRNA at 14 days after androgen ablation was almost as low as in the intact control (Fig. 1). The Dunning R3327 PAP prostatic adenocarcinoma tumors from the corresponding rats did not show any significant amplification of the TRPM-2 transcript compared to the induction observed in the ventral prostate (Figs. 1 and 2). There was no obvious difference in tissue detection of the TRPM-2 message in intact tumors and tumors from different post castration times (Fig. 2). t-PA mRNA was induced in the ventral prostates from day 2 post castration and decreased again after day 5 (Fig. 1). This androgen ablation effect of the t-PA transcription levels in the ventral prostate could not be seen in tumor tissue after castration (Fig. 2). At 2 days after castration, there was a slight induction of TRPM-2 and t-PA transcripts in the tumor, which probably is due to specimen variation since it has not been seen in repeated experiments. There were very low amounts of t-PA mRNA both in intact control tumors and in tumors from castrated animals. β-Actin was used as an internal standard, and the message levels between different samples were rather constant.

Fig. 1. TRPM-2, t-PA, and β-actin transcript levels determined by Northern blot hybridization experiments on total RNA (4 µg/sample) from the ventral prostate post castration. The samples are, from the left, intact control (day 0) and 1, 2, 3, 5, 7, 10, and 14 days post castration. Lane numbers are days post castration.

Fig. 2. TRPM-2, t-PA, and β-actin transcript levels determined by Northern blot experiments on total RNA (10 µg/sample) from Dunning R3327 PAP prostatic adenocarcinoma tissue post castration. The samples are, from the left, intact control (day 0) and 1, 2, 3, 5, 7, 10, and 14 days post castration. Lane numbers are days post castration.

Electrophoretic Analysis of DNA Integrity. The apoptotic non-random degradation of DNA into oligonucleosomal fragments can be detected by agarose gel electrophoresis. The results from the agarose-gel separation of ventral prostatic DNA showed that at 1 day after castration, the ventral prostate DNA was degraded into a nonrandom pattern, as compared to the high molecular DNA in intact controls. Tumor DNA was less degraded, both in the intact control and 1 day after castration (Fig. 3). There was no increase in the level of degraded tumor DNA for the rest of the treatment period (Fig. 4). These experiments revealed that the apoptotic activity in the Dunning R3327 PAP tumor was considerably lower than in the ventral prostate after castration (Figs. 3 and 4).

In Situ Detection of Apoptotic Cells. The tumor epithelial cell ISEL index did not increase and remained below 0.5% throughout the study period, reflecting the unchanged basal level of apoptosis (Figs. 5 and 6). In contrast, the ventral prostate epithelial cell ISEL index increased from 0.3% before to 3.8% (n = 2) 3 days after castration (data not shown in the figure), in line with the results reported by Wijsman et al. (27).

Immunohistochemistry. In the intact ventral prostate, hardly any of the epithelial cells, except in occasional glands probably representing proximal parts of the glandular system (29), contained immunoreactive SGP-2 (TRPM-2; not shown in the figure). Distal parts of the glandular system with high cylindrical cells did not contain immunoreactive SGP-2 (Fig. 7a). At 1 and 2 days after castration, the number of SGP-2 immunoreactive epithelial cells increased, and at 3 days, almost all epithelial cells, except in occasional glands, showed an immunoreaction (Fig. 7b). At 5, 7, and 14 days after castration, the number of immunostained cells were lower than at 3 days but higher than in intact rats (not shown in the figures). Control sections incubated with normal rabbit IgG were always unstained. In the Dunning R3327 PAP tumors, there was no specific SGP-2 immunoreactivity before or after castration (Fig. 7, c and d).

Immunoreactive t-PA was localized in stromal blood vessels in the
ventral prostate of intact rats. The amount of vascular immunoreactive t-PA gradually increased after castration and peaked 7 days after androgen deprivation (Fig. 8). There was no specific t-PA immunoreactivity in ventral prostatic epithelial cells before or after castration. The Dunning R3327 PAP tumors in intact and castrated animals did not contain immunoreactive t-PA (not shown in the figures).

In the ventral prostates of intact animals, only a few epithelial cells were BrdUrd immunoreactive, but the number of immunoreactive epithelial cells was considerably higher in the corresponding tumors (Fig. 9). Castration induced a slight initial increase in the number of BrdUrd-immunoreactive ventral prostatic epithelial cells and some were morphologically apoptotic, as earlier described by Colombel et al. (36). In the tumors, castration resulted in a decrease in BrdUrd-immunoreactive epithelial cells. Most of the nuclei stained were apparently normal, but BrdUrd-immunoreactive apoptotic epithelial cells were also observed.

DISCUSSION

The involution of the ventral prostate after orchietomy is an actively initiated process involving a genetically programmed series of events resulting in apoptosis and a major loss of epithelial cells (9, 10, 37). In a recent study, it was suggested that an early step in castration-induced apoptosis in the ventral prostate is a reentry of quiescent (G0) epithelial cells into the cell cycle. This cycle is, however, not completed, and the cells eventually die of apoptosis (36). The present finding of an increased BrdUrd labeling index in the ventral prostate some days after castration may confirm this observation (Fig. 9). However, incorporation of BrdUrd into epithelial cells may not always indicate that these cells are in the cell cycle because cellular repair of damaged DNA, which is increased after castration in the ventral prostate, can also lead to BrdUrd uptake (38). Nevertheless, in contrast to the ventral prostate, there is no castration-induced increase in BrdUrd incorporation in the androgen-sensitive Dunning R3327 PAP tumors (Fig. 9).

Another early event in castration-induced cell death in the ventral prostate is the activation of a Ca2+/Mg2+-dependent endonuclease which causes fragmentation of DNA into nucleosomal fragments (9). Our results from the electrophoretic DNA analysis revealed a marked DNA degradation in the ventral prostate 1 day post castration, but in contrast there were no signs of a castration-induced DNA fragmentation in the Dunning tumors at this or any other time of the observation period (Figs. 3 and 4). The ISEL index in the Dunning R3327 PAP tumors post castration was decreased rather than increased during the treatment period (Figs. 5 and 6). This preliminary observation has to be confirmed in a larger number of tumors. Taken together, these data show that castration treatment alone does not induce epithelial cell apoptosis in this tumor.

TRPM-2 is, generally with few exceptions, expressed in cells undergoing apoptosis (39–42). TRPM-2 probably inhibits immune responses around apoptotic cells, but it is not involved in the effecting of cell death (40). Confiming the observations of Sensibar et al. (29), we observed an increase in SGP-2 immunostaining in the ventral prostate after castration with a marked peak on day 3 post castration (Fig. 7b). Three days after castration, about 4% of the ventral prostatic epithelial cells were in situ end-labeled, corresponding with other
results with this (27) and other (9, 24) methods. In contrast, there were no SGP-2 immunoreactive cells in the Dunning R3327 PAP tumors of intact or castrated rats (Fig. 7, c and d), and there was no castration-induced change in TRPM-2 mRNA expression (Fig. 2); nevertheless, about 0.5% of the epithelial cells in Dunning R3327 PAP prostatic tumors of intact animals were apoptotic (24), suggesting that apoptosis is initiated in tumors in intact rats in the presence of androgens.

It has been shown previously that the tissue- and urokinase-type plasminogen activator gene expressions and enzyme activities are increased in the ventral prostate after castration (20, 21). Confirming these observations, we observed an induction of t-PA mRNA (Fig. 1) and an increased t-PA immunoreactivity (Fig. 8) in the ventral prostate after castration. Interestingly, the t-PA immunoreactivity was localized in the stromal blood vessels and not in the glandular epithelial cells, suggesting that t-PA is not directly involved in apoptosis but in tissue remodeling of a shrinking organ. There was no detectable t-PA immunoreactivity in Dunning R3327 PAP tumors from intact or castrated animals, which is in line with the results from the Northern blot analysis showing no postcastrational induction of the t-PA transcript (Fig. 2).

The present demonstration that castration does not induce DNA fragmentation or induction of apoptosis-related genes in the highly differentiated androgen-sensitive Dunning R3327 PAP tumor and previous observations of an unchanged number of apoptotic cells and an unchanged number of epithelial cells after castration (24) clearly indicate that apoptosis is probably not induced by castration in this prostatic tumor. However, it should be noted in this context that this tumor responds rapidly to castration with an arrest in tumor growth, a decrease in the number of BrdUrd-labeled (Fig. 9) and mitotic (24) cells, an accumulation of cytoplasmic lipid droplets, and a decrease in tumor epithelial cell size (23, 24).

The lack of castration-induced cell death in an androgen-sensitive tumor is surprising and suggests that this prostatic cancer model could be different from other prostatic cancers, but this is not necessarily the case. Gleave et al. (43) have shown that castration results in a decreased prostate-specific antigen secretion from the androgen-sen-
sitive human LNCaP cell and bone fibroblast chimeric tumors s.c. transplanted into athymic mice. This effect was, however, not accompanied by morphological or biochemical signs of apoptosis, and tumor volume was not reduced (43). Moreover, in a large proportion of patients with localized prostatic adenocarcinoma treated with complete androgen blockade (castration plus flutamide), there was apparently no morphological signs of cell death (44). Morphological and molecular (induction of the TRPM-2 and transforming growth factor β genes) signs of a castration-induced apoptosis are, however, observed in the androgen-dependent PC-82 human prostatic cancer grown in nude mice (45).

The differentiation, regulation of growth, and function of epithelial prostatic cells is to a large extent controlled by local regulatory factors (growth factors, growth inhibitors, and extracellular matrix components) from the surrounding stroma. Effects of hormones and hormonal withdrawal on the prostatic epithelium may be mediated via effects on stromal cells (40, 46). It has recently been shown that there are marked regional differences in androgen sensitivity along the prostatic ducts (7, 29, 40, 47). After castration, most epithelial cells in the distal part of the ducts die, but cells in the proximal parts survive. There are, however, no differences in androgen receptor content, binding activity, or 5α-reductase activity between distal and proximal ductal regions (47). Interestingly, basal epithelial cells of the prostate are primarily localized in the proximal part of the ducts (7); therefore, it has been suggested that apoptosis mainly takes place in the part of the prostatic duct system where the columnar epithelial cells are in direct contact with the neighboring stroma (40). Hypothetically, this observation may be of importance for the lack of castration-induced epithelial cell death in the Dunning R3327 PAP tumor since a continuous basal epithelial layer is present in the glands of this tumor (48).

In conclusion, the present study demonstrates major differences in the response to castration between the ventral prostate and the Dunning R3327 PAP prostatic tumor in the same animal. Both types of

Fig. 8. Cryostat sections immunostained to localize t-PA (400 ×). In the ventral prostate of intact rats, some t-PA immunoreactivity was observed in blood vessels (a, arrow). t-PA immunoreactivity in vessels was increased 7 days after castration (c, arrow). b (intact ventral prostate) and d (ventral prostate 7 days after castration), controls incubated with t-PA antibody neutralized with excess of antigen. No specific staining is observed.
tissue rapidly responded to the androgen withdrawal with regressive changes, but an induction of apoptosis and an amplification of genes related to apoptosis were only observed in the ventral prostate. The reasons for these differences are unknown, but the present study clearly suggests that the control of normal prostatic epithelial cell proliferation and death may not always be identical to that in androgen-sensitive prostate tumors.

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