Effect of Prolactin on the Population of Epithelial Cells From Ventral Prostate of Intact and Cyproterone Acetate-Treated Peripubertal Rats: Stereological and Immunohistochemical Study

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ABSTRACT

The interactions between steroid and nonsteroid hormones in the prostate are of special interest during the growth phase of the gland. The purpose of this work is to study the influence of prolactin (PL), with or without androgenic blockade, on epithelial cells from peripubertal rat ventral prostate. Twenty male peripubertal Sprague-Dawley rats were grouped as controls, or treated with cyproterone acetate (CA), CA plus PL (CA-PL), or PL. The total number (N total) of epithelial cells, and their labeling indices to proliferative cell nuclear antigen (LI PCNA), apoptosis (LI apoptosis) and androgen receptors (LI AR) were measured. CA and PL treatment significantly decrease the N total, but the LI PCNA was unchanged. We have observed a greater LI apoptosis in pharmacologically castrated animals without PL than in the rats with androgenic blockade with PL. The LI AR does not change with CA treatment in the ventral region, but the PL significantly increases it. Androgenic blockade and PL decrease the number of epithelial cells from the ventral prostate. These changes are not attributable to the decrease of cell proliferation, rather to the increase of epithelial apoptosis. The increase of cells expressing AR after treatment with PL might be attributed to the decrease of testosterone secretion caused by the hyperprolactinemia. PL does not modulate the size of the ventral prostate in prepubertal rats. Anat Rec, 292:746–755, 2009. © 2009 Wiley-Liss, Inc.

Key words: androgenic blockade; prolactin in prostate; cell counting; cell proliferation; PCNA; apoptosis; androgen receptors

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Sex steroid hormones influence prostate development and maintenance through their roles in prostate cellular proliferation, differentiation, and apoptosis. Although suspected of being involved in prostate carcinogenesis, an association between circulating androgens and prostate cancer has not been clearly established in epidemiologic studies (Weiss et al., 2008). Although the rat prostate is an androgen-dependent organ, several studies indicate that it is androgen-sensitive, but does not require a chronic androgenic stimulus (Donjacour and Cunha, 1988). This androgen-dependence was demonstrated by the dramatic changes observed in rat prostate after surgical or pharmacological castration: androgenic deprivation causes regressive alterations due to the decrease of epithelial cells by apoptosis and to stromal reorganization (Potter et al., 2006; Augusto et al., 2008). In this sense, cyproterone acetate (CA), currently employed in antiandrogenic therapy for prostate cancer, is a potent and specific antagonist of steroid androgens in competition with dihydrotestosterone in the binding of androgen receptors (AR). Moreover, CA has antigonadotropic action due to chemical similitude to progesterone, causing a decrease in the delivery of luteinizing hormones and thus, the diminution in the production of testosterone by the testis (Barradell and Faulds, 1994; Raudrant and Rabe, 2003; Schneider, 2003).

In addition to the influence of steroid hormones, the action of nonsteroid hormones on the prostate is a potential field of interest, as a relationship between levels of prolactin (PL) and the increase of prostate pathology in men (Bartke, 2004) and rats (Wennbo et al., 1997; Kindblom et al., 2003) has been observed. Moreover, PL stimulates the androgen-independent growth of rat prostate cells *in vitro* (Ahonen et al., 1999). This hormone acts as a local growth factor for the prostatic epithelium (Crepin et al., 2007), playing a role in the survival of the epithelial prostatic cells after castration (Ahonen et al., 1999). PL also stimulates epithelial prostatic proliferation *in vitro* (Nevalainen et al., 1991). Further, the local, hormone-dependent production of PL in rat prostate was demonstrated (Nevalainen et al., 1991), together with the presence of PL receptors in the epithelium from normal (Nevalainen et al., 1997; Harris et al., 2004) and pathological prostate (Leav et al., 1999).

All these interactions between steroid and nonsteroid hormones on the prostate are of special interest during the growth phase of the gland, namely during/throughout the peripubertal stage (Vilamaior et al., 2006), because there is extensive evidence indicating that the postnatal development of rat prostate is only partially mediated by androgens: (a) surgical castration in the rat does not completely impair the growth of the prostate, (b) androgenic treatment is unable to restore the normal development of the prostate in castrated peripubertal rats, and (c) the increase of serum testosterone concentration parallels the growth phase of the prostate until 8 weeks of postnatal development (Vilamaior et al., 2006). However, when the androgen concentration levels off at the ninth week, the glandular growth still continues until week 25 (Matuo et al., 1987). It seems more interesting to study these interactions in peripubertal rats than in adults, because the prostate of the adult rat does not respond to the administration of exogenic testosterone with any increase of DNA (Berry and Isaacs, 1984), due to the homeostatic equilibrium reached in the adult stage without net glandular growth-balance between cell proliferation and apoptosis (Isaacs, 1984a). Further, a detailed (quantitative) study of the influence of nonsteroid factors on peripubertal growth is still lacking for the rat ventral prostate. In consequence, we have found it interesting to undertake an analysis of the effect of PL, with or without androgenic blockade, on the acinar epithelium from the ventral prostate in peripubertal male rats. To perform this task, the present work deals with (1) the quantification of cell proliferation by evaluation of proliferative cell nuclear antigen (PCNA) immunoreactive nuclei; (2) apoptosis (estimating the rate of nuclei showing DNA fragmentation), and (3) androgen receptor (AR) status (evaluating the amount of AR immunostained nuclei) in the ventral prostate epithelium exposed to PL in normal or pharmacologically castrated peripubertal animals.

MATERIALS AND METHODS

Experimental Protocol

Twenty male peripubertal Sprague-Dawley rats (40 to 70 days old) were used for immunohistochemical and stereological studies. The animals were fed Panlab Lab Chow (Panlab, Barcelona, Spain) and water *ad libitum*. They were kept in a controlled environment (20°C–22°C temperature and 45%–55% relative humidity) and exposed to 12 hr cycles of light and darkness. Animal protocols agree with the guidelines for the care and use of research animals adopted by the Society for the Study of Reproduction. During this work, all animal studies were conducted in accordance with the European Community's Council ruling of 24 November 1986 (86/609/EEC) (Van et al., 2001) and Spanish and local directives ruled by "Real Decreto 1201/2005."

The animals were divided into six groups (five rats per group), according to treatment, following this schedule:

Control group (CTRL): Five rats, 47 days old, without any treatment, were killed 18 days after the beginning of the experiment.

Cyproterone acetate-treated group (CA): Five rats, 47 days old, were treated with daily subcutaneous administration of CA (50 mg/kg of body weight) during 18 days. The CA was extracted from tablets of Androcur® (Schering, Madrid, Spain), using the protocol described by Bosland and Prinsen (1990).

Cyproterone acetate-treated group plus treatment with prolactin (CA-PL): Five rats, 40 days old, were treated with CA (the same dose as Group CA) during 18 days, plus 50 IU/kg of body weight of PL (Sigma, Barcelona, Spain), administered daily by subcutaneous injection during 7 days, after treatment with CA.

Prolactin group (PL): Five animals, 58 days old, were treated exclusively with 50 IU/kg of body weight of PL, administered daily by subcutaneous injection during 7 days (Edwards and Thomas, 1980).

All rats were killed at the end of the treatments at the same age: 66 days old, that is, included in the range of the peripubertal stage, by exsanguination after CO_2 narcosis. The ventral prostate was dissected from the abdominal cavity of each animal and the bladder, deferent ducts, seminal vesicles, glands of coagulation, and the other prostate regions (dorsal, ampular) were carefully removed. The ventral prostate was weighed immediately and the total fresh volume (Vtot) was determined

by gravimetric methods (water displacement). Afterward, the prostate was cut into 3-mm-thick slices. The section plane was perpendicular to the sagittal axis of the gland. All specimens were fixed by immersion in 4% paraformaldehyde in phosphate-buffered saline (PBS) pH 7.4, during 24 hr and embedded in paraffin.

The paraffin blocks were then serially sectioned. Five-micrometer-thick sections (for routine hematoxylin-eosin staining), alternating with 10 μm-thick sections (for immunohistochemistry and stereological cell counting), were obtained from each block.

For the histological qualitative description, hematoxylin-eosin stained sections were employed.

Immunohistochemical and Apoptosis Detection Methods

In all the groups, at least 10 selected slides per animal (per prostate) and per antigen were immunostained. Deparaffinized and rehydrated tissue sections were treated for 30 min with hydrogen peroxide 0.3% in PBS pH 7.4, to block endogenous peroxidase. Mouse monoclonal and rabbit polyclonal antibodies were used as primary antibodies. To detect PCNA and AR immunoreactivities, (Pereira et al., 2006; Cordeiro et al., 2008), sections were incubated with a monoclonal anti-PCNA antibody (Biomeda, Foster City, CA) diluted at 1:200, and with a polyclonal anti-AR antibody (Calbiochem, Cambridge, MA), diluted at 1:60. Pretreatment of sections by heat in citrate buffer pH 6.0, using a pressure cooker (Martin et al., 2001), was performed to enhance AR immunostaining. All primary antisera were diluted in PBS, pH 7.4, containing 1% bovine serum albumin (BSA) plus 0.1% sodium azide. All incubations with primary antisera were carried out overnight at 4°C. When primary monoclonal antibodies were used, the second antibody employed was a biotin-caproyl anti-mouse immunoglobulin (Biomeda). Another antibody, a biotin-caproyl-anti-rabbit immunoglobulin (Biomeda), was added to primary polyclonal antibodies. The second antibodies were diluted at 1/400 in PBS containing 1% BSA without sodium azide and incubated for 30 min at room temperature. Thereafter, sections were incubated with a streptavidin–biotin–peroxidase complex (Biomeda). The immunostaining reaction product was developed using 0.1 g diaminobenzidine (DAB) (3,30,4,40–Tetraminobiphenyl, Sigma, St. Louis) in 200 mL of PBS, plus 40 mL hydrogen peroxide.

Apoptosis was studied using a technique for detecting DNA fragmentation: terminal deoxynucleotide transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL technique) (Roche, Mannheim, Germany) (Gavrieli et al., 1992). This method involves the insertion of labeled nucleotides into broken ends of DNA strands. A brief description of the method follows: at least 10 slides per prostate region were tested in the different groups. Sections were deparaffinized and rehydrated, then incubated with proteinase K (10 mL/mL in TRIS/EDTA pH 8) for 30 min at 37°C for digestion of nuclear proteins. Endogenous peroxidase was inactivated with 0.3% hydrogen peroxide in distillate water during 30 min. The sections were incubated in the TdT mixture with the addition of labeled nucleotides for 1 hr at 37°C (2:1). The sections were then incubated with Converter POD during 30 min at 37°C. The reaction was detected using 0.1 g DAB in PBS (200 mL), plus 40 mL hydrogen peroxide.

After immunoreactions and the TUNEL method, sections were counterstained with Harris hematoxylin. All slides were dehydrated in ethanol, and mounted in a synthetic resin (Depex, Serva, Heidelberg, Germany). The specificity of the immunohistochemical procedures was checked by incubation of sections with nonimmune serum instead of the primary antibody, and the specificity of the TUNEL method was also checked by incubation in the TdT mixture without labeled nucleotides.

Quantitative Methods

Estimates of the number of total epithelial cells and epithelial cells immunoreactive to PCNA and AR and positive to TUNEL were calculated, using the technique of the optical disector, an unbiased stereological method (Gundersen et al., 1988; Howard and Reed, 2005). Measurements were carried out using an Olympus microscope fitted with a motorized stage and equipped with an 100× oil immersion lens (numerical aperture of 1.4) at a final magnification of 1,200×, employing the stereologic software CAST-GRID. This program controls the XY displacement of the microscope stage and allows the selection of fields to be studied by random systematic sampling after the input of an appropriate sampling fraction. An average of 100 fields per section and per antigen was scanned in each group. The software superimposes a disector frame onto the images captured by a video camera. The Z displacement of the samples was measured by a microcator (Haidenhain, Transreut, Germany) adapted to the vertical axis of the stage.

In each selected field, the area of acinar epithelium was scanned and the numerical densities of both total epithelial cells (N_V total), epithelial cells immunoreactive for the two antigens studied (N_V PCNA, N_V AR) and epithelial cells with TUNEL positive nuclei (N_V apoptotic cells) (per μm^3 of epithelial volume) were evaluated, counting their nuclei, according to the Sterio rule (Sterio, 1984).

The N_V is determined by the formula: $\sum Q_D^- / \sum (Vdis^+) \cdot Fr$, where: Q_D^- = number of eligible nuclei, $Vdis^+$ = volume of disectors in which the upper-right corner hits epithelial tissue, and Fr = 1.3 [shrinkage factor, resulting from the processing of the tissue (Martin et al., 2001)].

To estimate the reference space (Vep), the frames with their upper-right corner hitting epithelial tissue were registered (dis^+) and the volume fraction of epithelium represented by the ratio between the amount of dis^+ and the total of disectors used (distot) was calculated. Following this step, the prostatic-epithelial volume (Vep) was obtained by multiplying this ratio per the ventral prostate fresh volume (Vtot). The absolute number of epithelial cells (N total, N PCNA, N AR, N apoptotic cells) was then calculated by multiplying N_V by Vep. The labeling indices for PCNA, AR, and apoptotic cells were then calculated: LI PCNA = N PCNA/Ntotal; LI AR = N AR/Ntotal; LI apoptosis = N apoptotic cells/N total, and expressed as percentages. Only the Vtot, N total for the epithelial cells, and the labeling indices (PCNA, AR, and apoptosis) were employed for the statistical analysis.

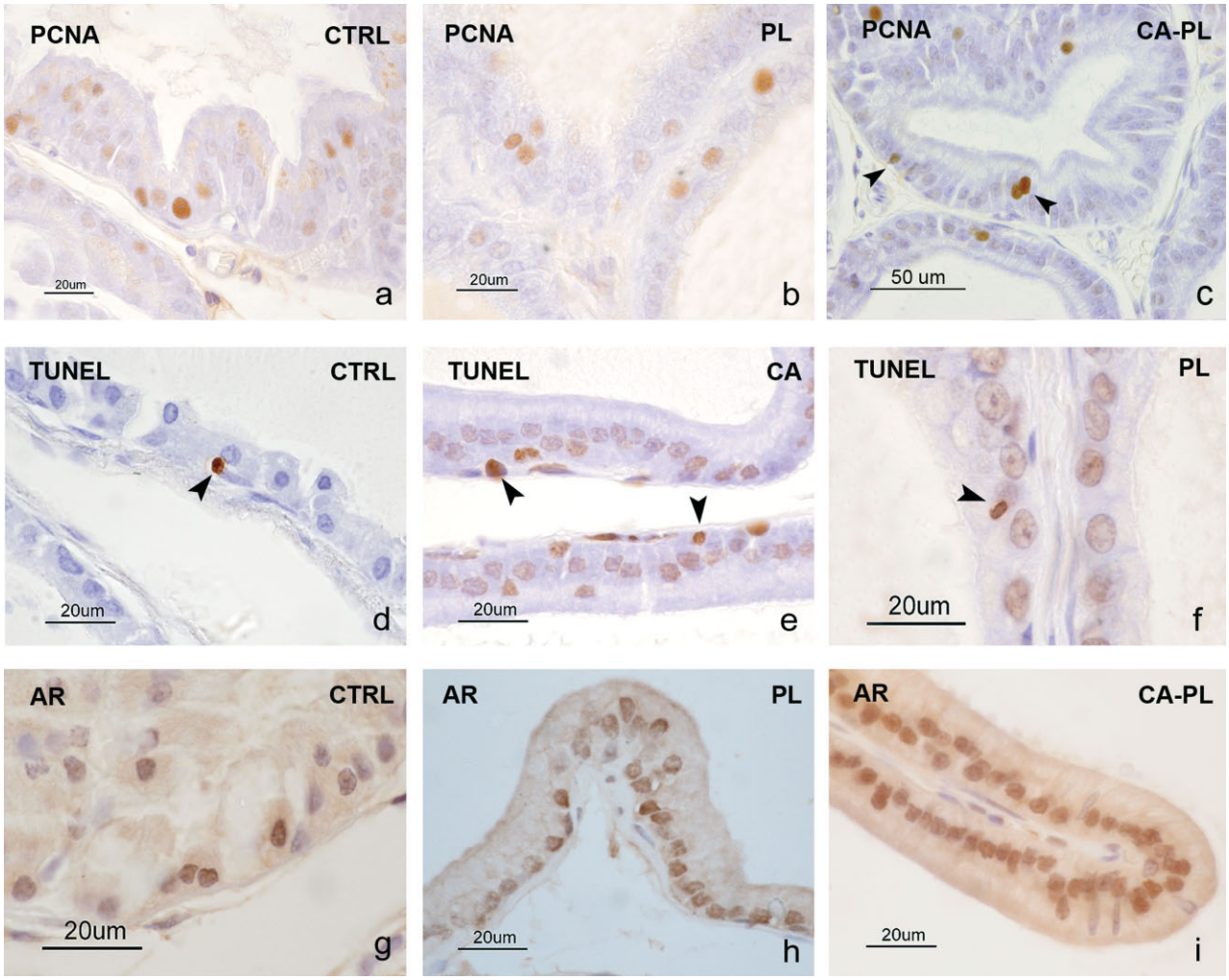

Fig. 1. (**a**) Prostate acini from the ventral lobe of a CTRL rat. Abundant epithelial nuclei immunoreactive to PCNA are seen. (**b**) A remarkable amount of nuclei immunostained for PCNA were also seen in the ventral prostate from a PL-treated rat. (**c**) In an animal treated with PL plus androgenic blockade (CA-PL group), a slight decrease of the nuclei immunoreactive to PCNA (arrowheads) were detected. (**d**) Prostate acini from the ventral lobe of a CTRL rat. Only one epithelial nucleus showing fragmented DNA visualized by TUNEL was observed (arrowhead). (**e**) Several nuclei stained by TUNEL (arrowheads) were seen in the ventral prostate of a pharmacologically castrated (CA group) rat. (**f**) In a rat treated with PL, only one nucleus stained by TUNEL (arrowhead) was detected. (**g**) A remarkable amount of epithelial nuclei immunostained for AR is seen in an acinus of the ventral prostate in a CTRL rat. Almost all the epithelial nuclei from ventral prostate in PL (**h**) and in CA-PL groups (**i**) were immunostained for AR. Calibration bars: 20 μm.

Statistical Analysis

The means ± SD of all the estimated values were calculated from each experimental group and the differences among groups were evaluated by analysis of variance for each parameter. Comparisons between the means for all the groups studied were performed by the Newman–Keuls test. To study the influence of pharmacological castration (CA treatment) on the rats exposed to PL, a two-way ANOVA was performed, considering three grouping factors: (1) pharmacological castration (animals with and without CA treatment); (2) hormonal treatment, that is, no treatment at all (without PL); and (3) treatment with PL (with PL). For the statistical analysis, the groups of rats described earlier were regrouped as follows: Animals without CA: CTRL + PL groups; animals with CA: CA + CA-PL groups; animals without PL: CTRL + CA groups; animals with PL: PL + CA-PL groups. The sources of variation were: pharmacological castration (CA treatment), hormonal treatment (PL), and the interaction of both factors. Comparison between the means for treatment with or without CA was performed by the Bonferroni test. The level of significance selected was $P < 0.05$. The statistical program used was SPSS 9.0 (SPSS, Chicago, IL, 1995).

RESULTS

Qualitative Results

No remarkable changes were observed in the prostate epithelium from any group of treatment, in comparison with controls, with respect to the size of the epithelial lining or glandular lumen (Fig. 1a–i). Variable amounts

of epithelial nuclei show immunoreactivity to PCNA in all the groups of treatment. Some diminution of PCNA immunostained nuclei was apparently detected in pharmacologically castrated rats, in comparison with either controls or animals treated by PL without CA exposition. This slight decrease affects both luminal and basal nuclei equally (Fig. 1a–c). The amount of apoptotic nuclei visualized by the TUNEL method was apparently increased in pharmacologically castrated rats without hormonal treatment, in comparison with the other groups (Fig. 1d–f). The nuclei immunostained for AR were more abundant in PL-treated animals when compared with controls (Fig. 1g–i).

Quantitative Results

Ventral prostate volume. The volume decreases significantly ($P < 0.05$) in the ventral prostate of the rats with pharmacological castration, but PL treatment did not change the volume of the region significantly (Fig. 2a,b). For each group of hormonal treatment (with and without PL), the intact rats and the pharmacologically castrated rats were considered. No significant differences were detected between the intact animals (with and without PL) and the pharmacologically castrated rats (with and without PL) (Fig. 2c).

Total number of epithelial cells. Pharmacological castration and the PL treatment significantly decrease ($P < 0.05$) the N total in ventral prostate (Fig. 3a,b). For each group of hormonal treatment (with and without PL) intact rats and pharmacologically castrated rats were considered. Significant differences were detected among the intact animals (with and without PL), and no significant differences were observed among the pharmacologically castrated rats (with and without PL) (Fig. 3c). Moreover, a significant interaction between pharmacological castration and hormonal treatment was observed: the decrease in the number of epithelial cells was significantly greater in the group without PL than in the PL group when the animals were pharmacologically castrated previously (Table 1, Fig. 3c).

LI PCNA. Pharmacological castration and hormone treatment did not affect the LI PCNA of epithelial cells in the ventral region, either when pharmacological castration and PL treatment were considered alone or combined (Fig. 4a–c).

LI apoptosis. No changes in LI apoptosis attributable to pharmacological castration or hormone treatment were detected in the ventral region (Fig. 5a,b). However, a notable interaction between the two factors was visualized (Table 1): a significant increase of the LI apoptosis in pharmacologically castrated animals without hormone treatment, compared to that in the rats with androgenic blockade and treated by PL (Fig. 5c).

LI AR. The rate of expression of AR (LI AR) does not change in the ventral region with pharmacological castration (Fig. 6a), but the treatment with PL increases the LI AR significantly ($P < 0.05$), in comparison with the group without PL, independent of pharmacological castration (Fig. 6b,c).

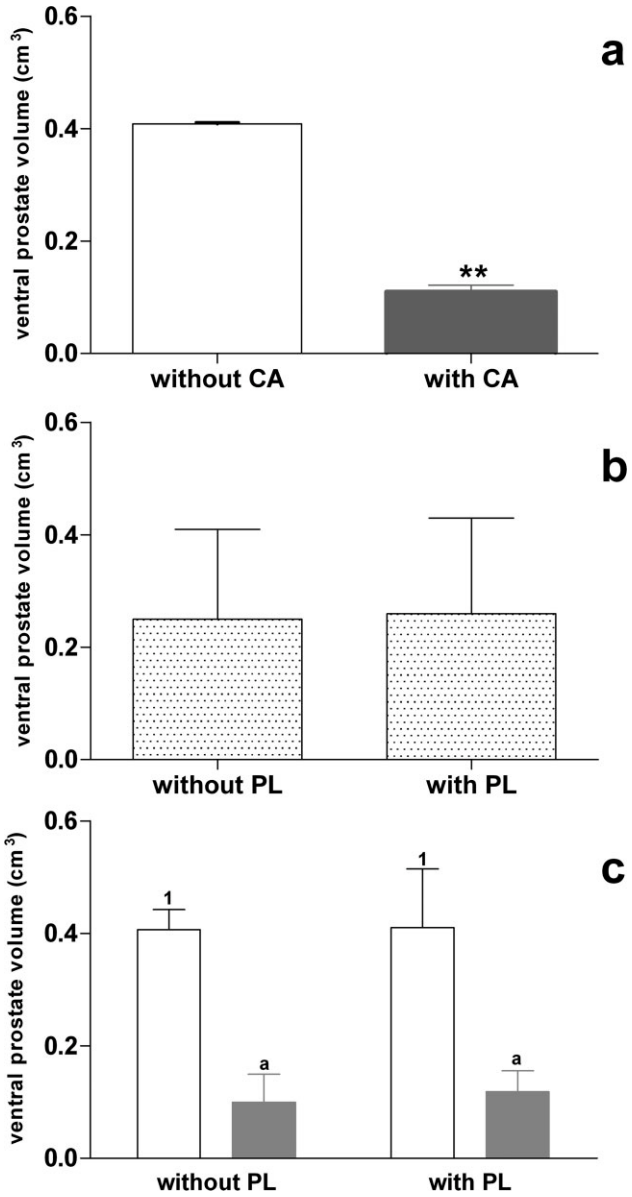

Fig. 2. (**a**) Bar diagrams indicating means ± SD for ventral prostate volume (in cm^3) in rats free of androgenic blockade (without CA), compared with pharmacologically castrated rats (with CA), irrespective of treatment with PL. The significant differences are indicated by the asterisks on the error bars. (**b**) Bar diagrams indicating means ± SD for ventral prostate volume (in cm^3) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL), irrespective of treatment with CA. There are no significant differences between them. (**c**) Bar diagrams indicating means ± SD for ventral prostate volume (in cm^3) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL). Each group of hormonal treatment (without or with PL) was divided into intact rats (empty bars) and pharmacologically castrated rats (gray bars). The same numbers on the empty bars and the same letters on the gray bars indicate the absence of significant differences between the empty or the gray bars, respectively.

DISCUSSION

Remarkable changes in ventral prostate volume of the treated peripubertal rats were detected in the present study. The changes in the size of the gland might be due

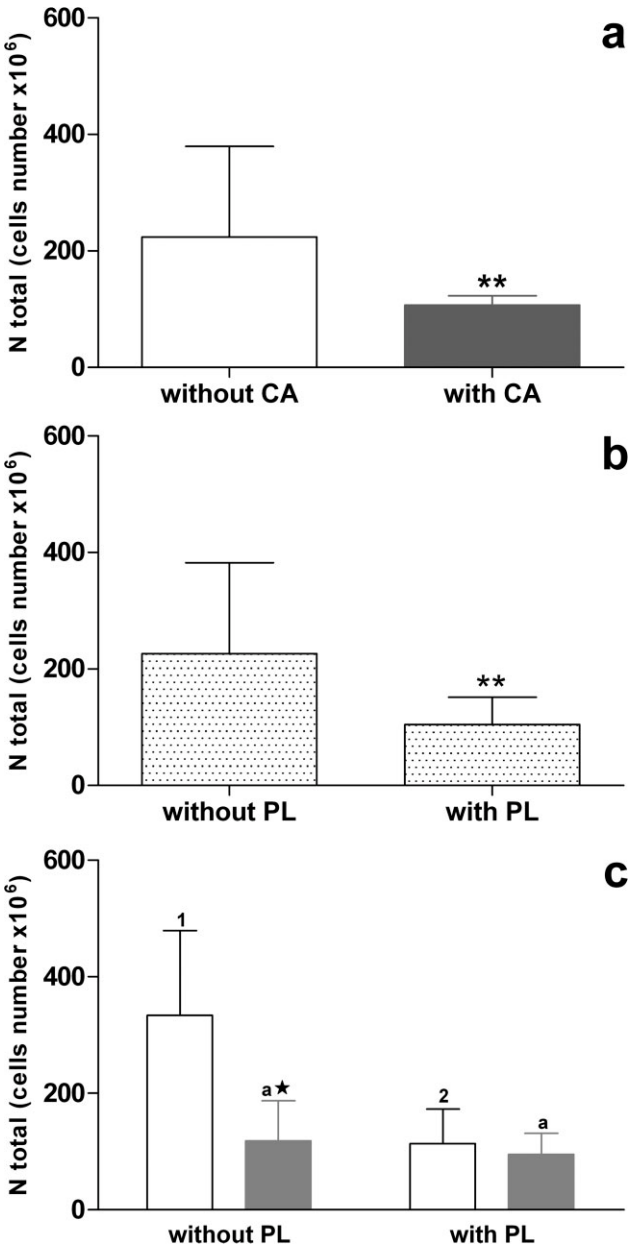

Fig. 3. (**a**) Bar diagrams indicating means ± SD for N total (epithelial cell number × 10^6) in rats free of androgenic blockade (without CA), compared with pharmacologically castrated rats (with CA), irrespective of treatment with PL. The significant differences are indicated by the asterisks on the error bars. (**b**) Bar diagrams indicating means ± SD for N total (epithelial cell number × 10^6) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL), irrespective of treatment with CA. The significant differences are indicated by the asterisks on the error bars. (**c**) Bar diagrams indicating means ± SD for N total (epithelial cell number × 10^6) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL). Each group of hormonal treatment (without or with PL) was divided into intact rats (empty bars) and pharmacologically castrated rats (gray bars). The same numbers on the empty bars and the same letters on the gray bars indicate the absence of significant differences between the empty or the gray bars, respectively. The star on the gray bar in the group without PL indicates the presence of significant differences between the intact and pharmacologically castrated rats that were not treated with PL.

TABLE 1. Two-way ANOVA for pharmacological castration and hormonal treatment

Variable	CA treatment	Hormonal treatment	Interaction
Ventral prostate volume	<0.0001**	0.68	0.55
Ntotal	0.0011**	0.002**	0.01*
LI pcna	0.29	0.52	0.92
LI apoptosis	0.31	0.14	0.02*
LI AR	0.08	<0.0001**	0.98

Summary of the *P* values for each variable studied for the two factors considered. (*) Significant differences; (**) highly significant differences.

to alterations either in the stromal or epithelial compartments or both. As we have focused our study on the epithelium, the epithelial regressive changes observed (i.e., a decrease in the amount of epithelial cells) could be either due to a low rate of cell proliferation, an increase of the apoptosis, or both. The androgenic blockade causes a decrease in the global size of the peripubertal ventral prostate, according to that described by other authors, which concern the regressive changes shown in the prostate after androgenic deprivation in both adult and prepubertal rats (Vilamaior et al., 2006; Pereira et al., 2006; Augusto et al., 2008). Controversy exists regarding the influence of PL on the prostate of pharmacologically castrated rats (either immature or mature). Thus, it is unclear if the stage of postnatal development has a significant influence on the effect of PL. For some authors, PL modulates the size of ventral and dorsal prostate in immature animals (Negro-Vilar et al., 1977). For others, (Kolbusz et al., 1982; Assimos et al., 1984; Smith et al., 1985; Prins, 1987), the influence of PL was more evident on the lateral lobes of mature rats. Nevertheless, still others (Jones et al., 1983; Chang, 1984) found no influence of PL on the prostate volume of adult rats. In the present work, either PL alone or PL plus androgenic blockade did not change the volume of ventral prostate in peripubertal rats.

We have found that androgenic blockade causes a significant decrease in epithelial cells from the peripubertal ventral prostate, therefore possibly contributing to the loss of ventral prostate volume. PL has a remarkable effect (although to a minor degree than the androgenic blockade) on the decrease in the amount of epithelial cells from the peripubertal ventral prostate. This agrees with the findings in adult rats (Sluczanowska-Glabowska et al., 2003, 2006), indicating that the hyperprolactinemia induced by metoclopramide causes a decrease of testosterone secretion that might explain the atrophy of prostate epithelium. The effect of CA may not be solely attributable to the androgen blockade, because CA also exerts antigonadotropic action (Raudrant and Rabe, 2003) and even a possible hyperprolactinemic action (Fonzo et al., 1977). These responses of the epithelial population of rat prostate to androgenic blockade and PL treatment, were, in part, different from those observed for other lineages of cells from peripubertal prostate epithelium, as, for instance, the amount of neuroendocrine cells decreases after androgenic blockade but increases with treatment with PL (Ingelmo et al., 2007).

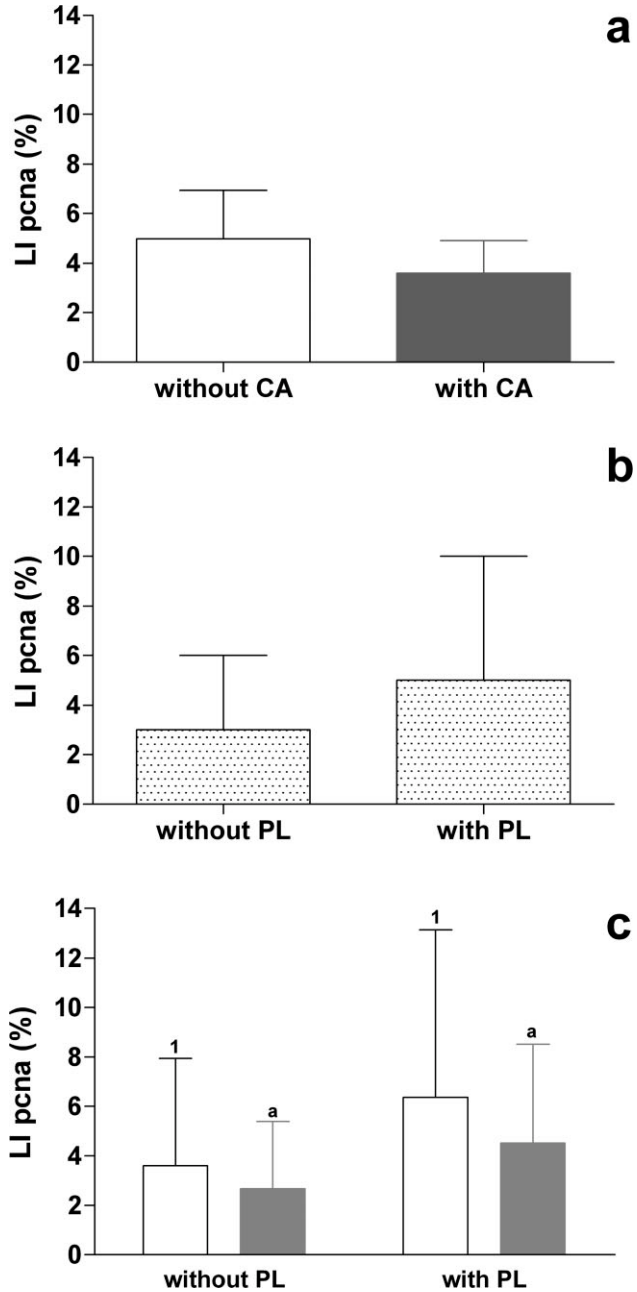

Fig. 4. (**a**) Bar diagrams indicating means ± SD for LI pcna (expressed as %) in rats free of androgenic blockade (without CA), compared with pharmacologically castrated rats (with CA), irrespective of treatment with PL. There are no significant differences between them. (**b**) Bar diagrams indicating means ± SD for LI pcna (expressed as %) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL), irrespective of treatment with CA. There are no significant differences between them. (**c**) Bar diagrams indicating means ± SD for LI pcna (expressed as %) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL). Each group of hormonal treatment (without or with PL) was divided into intact rats (empty bars) and pharmacologically castrated rats (gray bars). The same numbers on the empty bars and the same letters on the gray bars indicate the absence of significant differences between the empty or the gray bars, respectively.

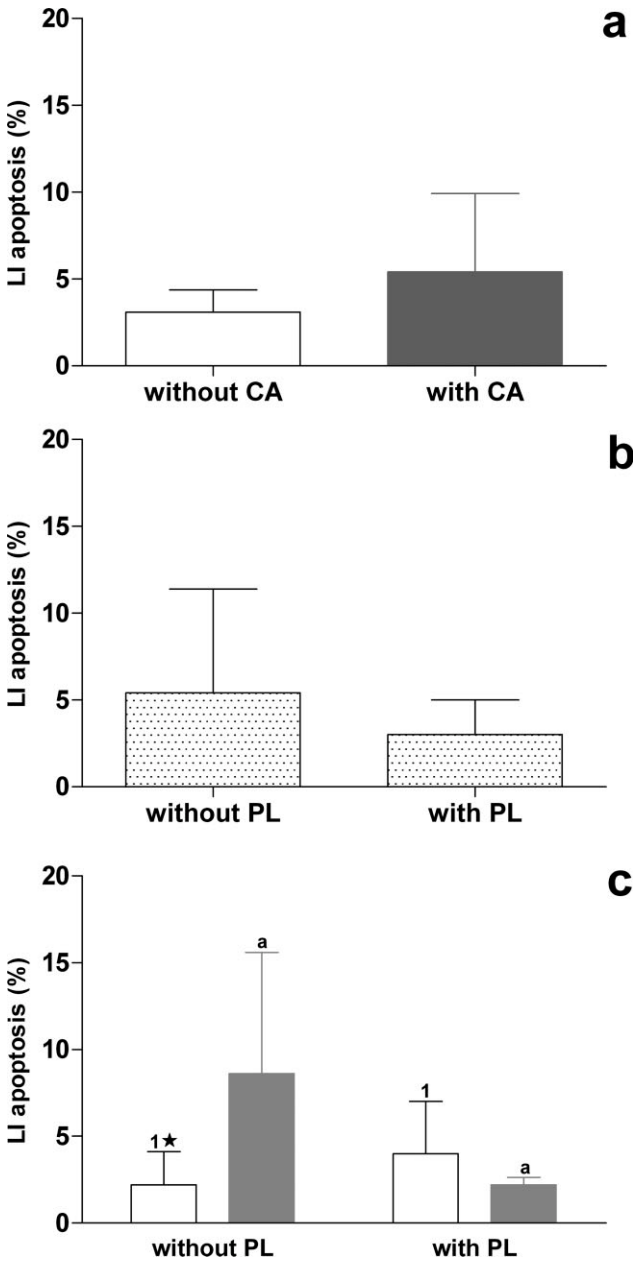

Fig. 5. (**a**) Bar diagrams indicating means ± SD for LI apoptosis (expressed as %) in rats free of androgenic blockade (without CA), compared with pharmacologically castrated rats (with CA), irrespective of treatment with PL. There are no significant differences between them. (**b**) Bar diagrams indicating means ± SD for LI apoptosis (expressed as %) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL), irrespective of treatment with CA. There are no significant differences between them. (**c**) Bar diagrams indicating means ± SD for LI apoptosis (expressed as %) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL). Each group of hormonal treatment (without or with PL) was divided into intact rats (empty bars) and pharmacologically castrated rats (gray bars). The same numbers on the empty bars and the same letters on the gray bars indicate the absence of significant differences between the empty or the gray bars, respectively. The star on the empty bar in the group without PL indicates the presence of significant differences between the intact and pharmacologically castrated rats that were not treated with PL.

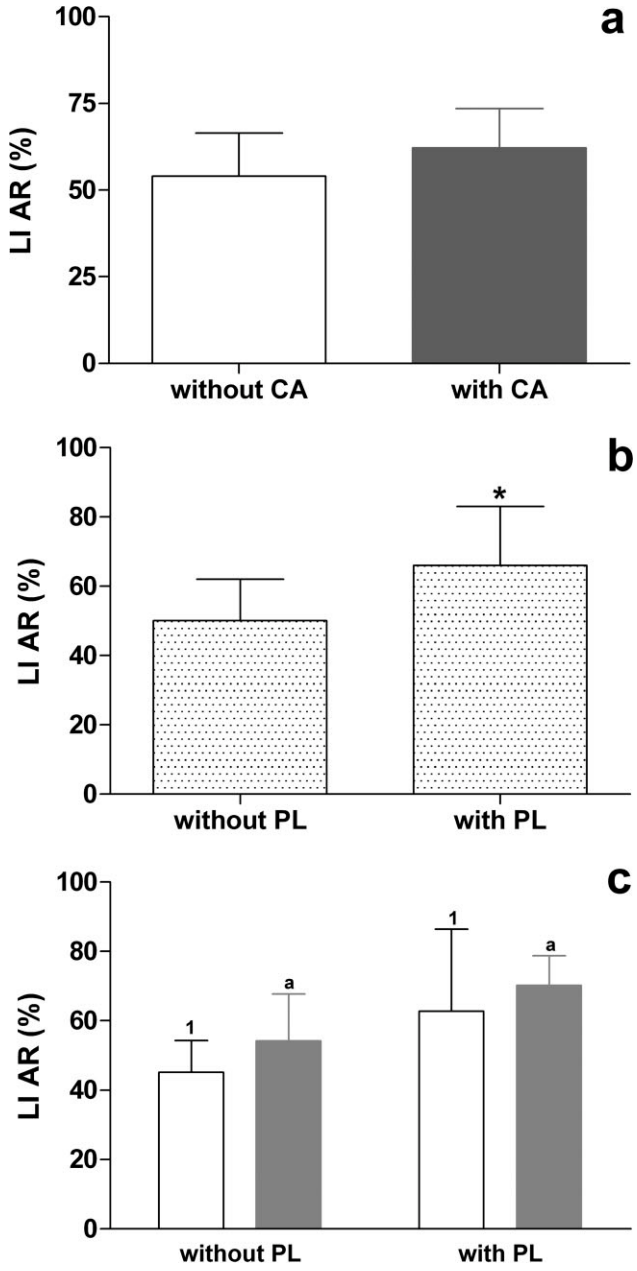

Fig. 6. (**a**) Bar diagrams indicating means ± SD for LI AR (expressed as %) in rats free of androgenic blockade (without CA), compared with pharmacologically castrated rats (with CA), irrespective of treatment with PL. There are no significant differences between them. (**b**) Bar diagrams indicating means ± SD for LI AR (expressed as %) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL), irrespective of treatment with CA. The significant differences are indicated by the asterisk on the error bars. (**c**) Bar diagrams indicating means ± SD for LI AR (expressed as %) in rats untreated by PL (without PL) compared with PL-exposed rats (with PL). Each group of hormonal treatment (without or with PL) was divided into intact rats (empty bars) and pharmacologically castrated rats (gray bars). The same numbers on the empty bars and the same letters on the gray bars indicate the absence of significant differences between the empty or the gray bars, respectively.

The loss of epithelial population cannot be attributed to the decrease of cell proliferation because, in spite of the qualitative observation of a slight decrease of the amount of PCNA immunoreactive epithelial nuclei from pharmacologically castrated rats, no changes in the LI pcna were detected in any of the groups of treatment employed in this study, in accordance with the absence of changes in DNA in ventral and dorsal lobes of rats exposed to PL described by other authors (Prins, 1987). However, Kim et al. (2002) found diminution in cell proliferation evidenced by immunostaining for Ki-67 in castrated rats with implants of prostate cancer cell lines. The differences were remarkable when comparing our PCNA results with those from other studies, because in surgically castrated and/or estrogen-treated adult rats, the index of PCNA-positive cells decreased significantly according to other authors (Garcia-Florez et al., 2005; Pereira et al., 2006). These discrepancies could be either due to the stage of the development of the animals (prepubertal versus adult), the model of androgen deprivation (pharmacological versus surgical castration), or the methodology employed for counting cells. Although in the present study, no comparison of PCNA results with Ki 67 estimates of proliferating cells were carried out, other authors indicate that the index of Ki 67-positive cells in the ventral region presented a pattern similar to that of the PCNA-index (Pereira et al., 2006).

According to several authors (Kimura et al., 2001; Omezzine et al., 2003), the effect of androgens on cell death occurs predominantly by interference with caspase activation and the inhibition of caspase cleavage in both the intrinsic and extrinsic cell death pathways. These data suggest that androgen induced the blockade of caspase activation in both cell death pathways and was thereby able to protect prostatic cells from apoptosis induced by diverse stimuli. However, in the present study, only a slight increase of the apoptosis rate in prepubertal rats with androgenic blockade and without PL was detected. Therefore, the diminution of epithelial cells mediated by PL in this stage of development cannot be fully explained by an increase in cell death. These results agree in part with those of some authors (Isaacs, 1984b; Kyprianou and Isaacs, 1988), which indicate an increase of apoptosis in the prostate acini in castrated rats. Other studies also show that the increase of apoptosis in response to androgen deprivation occurs predominantly in the ventral lobe (Banerjee et al., 1995, 2000). Nevertheless, the increase of the apoptotic index detected in the present study was less than that observed by other authors (Garcia-Florez et al., 2005; Pereira et al., 2006). This difference might be explained by several circumstances: (a) the different stage of development of the animals (peripubertal versus adult); (b) the different model of androgen deprivation (androgenic blockade versus surgical castration). Regarding the latter, there is evidence that antiandrogen flutamide, unlike surgical castration, does not induce apoptosis in the rat ventral prostate epithelium (Limaye et al., 2007). Finally, (c) the different timing in estimating the apoptosis may explain the difference, because surgical castration causes a peak of epithelial apoptosis on the third day after castration (Garcia-Florez et al., 2005), and declines 1 week after (Pereira et al., 2006), whereas in our model, the rats were exposed to androgenic blockade (CA treatment) for 18 days from the beginning of the

experiment. It also seems that PL protects the prostate epithelium of pharmacologically castrated peripubertal rats against apoptosis, which agrees with the findings of several authors (Assimos et al., 1984; Isaacs, 1984b; Smith et al., 1985; Ahonen et al., 1999), indicating that PL was able to suppress the apoptosis *in vitro* in prostate cells from mature castrated animals.

According to some authors (Prins, 1989; Prins and Birch, 1993; Mora et al., 1996), the amount of nuclear AR in adult rat prostate decreases with surgical castration, and is restored by testosterone treatment. The results of our study disagree with these findings: no changes in the rate of epithelial cells expressing AR were found in peripubertal animals with androgenic blockade. This was therefore in agreement with the studies of other authors, indicating that the treatment with antiandrogens resulted in a loss of weight of ventral prostate from adult rats but did not alter the distribution of AR-immunoreactivity (Paris et al., 1994). Moreover, the changes in expression of AR in animals with androgen blockade might be species-specific, because in other rodents, such as gerbils (*M. unguiculatus*), pharmacological castration decreases the AR expression (Cordeiro et al., 2008). Nevertheless, PL, independent of androgenic blockade, causes an increase in the ratio of epithelial cells expressing AR. This might be considered in relation to the effect of hyperprolactinemia on the inhibition of testosterone levels, which might induce the increase of AR expression, as was also described in other studies (Sluczanowska-Glabowska et al., 2003). This compensatory increase could explain the protection from apoptosis described in the rats with androgenic blockade plus treatment with PL.

In summary, regarding the effect of PL with or without androgenic blockade on the acinar epithelium from the ventral prostate in peripubertal male rats, we conclude that: (1) The androgenic blockade mediated by CA and the treatment with PL decrease the total number of epithelial cells from ventral prostate in rats. (2) These changes in the epithelial population are not attributable to the decrease of cell proliferation, rather to the increase of epithelial apoptosis. (3) The increase in the rate of cells expressing AR after treatment with PL might be attributed to a compensatory effect of the decrease of testosterone secretion caused by hyperprolactinemia. (4) PL does not modulate the size of ventral prostate in prepubertal rats.

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