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

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ABSTRACT

This work deals with the quantification of serotonin-immunoreactive prostate neuroendocrine cells (NECs) in rats exposed to prolactin in normal, cyproterone acetate-exposed, and bromocriptine-exposed animals to establish the possible influence of prolactin with or without androgenic blockade on this cell population. Thirty male peripubertal Sprague-Dawley rats were grouped as controls (CT) and those treated with cyproterone acetate (CA), cyproterone acetate plus prolactin, cyproterone acetate plus bromocriptine, prolactin (PL), and bromocriptine (BC). The volume of ductal epithelium (Vep) and total number (NSER) of the NECs serotonin-immunoreactive were measured. NECs were detected in the periurethral ducts. Compared to CT, Vep was increased in PL and BC and NSER was decreased in CA and increased in the prolactin or bromocriptine groups. The androgenic blockade decreases NSER in rat prostate; PL induces in normal and cyproterone acetate-treated rats the increase of NSER; and BC exerts a local effect over the prostate similar to that described for PL. Anat Rec, 290:855–861, 2007.

Key words: androgenic blockade; nonsteroid hormones in prostate; cell counting; neuroendocrine cells

The neuroendocrine cells (NECs) of the rat prostate are included in the so-called diffuse neuroendocrine system (Montuenga et al., 2003), which is characterized by the synthesis and secretion of polypeptides with biological activity, either locally or through the blood where they raise concentrations enough to act like circulating hormones (DeLellis and Dayal, 1997).

The amount of prostatic NECs shows abundant individual differences. Its distribution is irregular and more evident in ducts than in the acini (Rodriguez et al.,

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2003). The NECs are distributed either isolated or in little groups among the epithelial cells and can establish desmosoma-like junctions with the epithelium. Ductal NECs in the rat were shown expressing adrenomedullin, pro-adrenomedullin, serotonin, and chromogranin (Jimenez et al., 1999; Rodriguez et al., 2003), intermingled with the epithelial cells lining the ducts from all the prostate lobes; the rat NECs seem to be more abundant in the portion of ducts proximal to the urethra (periurethral ducts) and their presence in the acini was controversial (Rodriguez et al., 2003).

Structural studies in human prostate have demonstrated NECs narrowly associated with neuroendings, suggesting a direct nervous connection (Abrahamsson, 1999; Acosta et al., 2001); thus, it is supposed that the NECs could represent an intermediate link among the autonomic innervation of the prostate and the epithelial cells. Nevertheless, the role of the NECs in the prostate is not yet well known, but the hypothesis that NECs are implicated in the growth, differentiation, and regulation of prostate is plausible (Noordzij et al., 1995; Gkonos et al., 1995; di Sant′Agnese, 1998). It is possible than NECs secrete products toward the stroma and have receptors for stromal factors, which would provide the necessary interactions for the normal growth and physiology of the prostate (Montuenga et al., 2003). The NECs have been revealed as an element of relevant importance in the development of the androgen-dependent proliferative pathologies in the human prostate, either benign prostate hyperplasia or cancer (Untergasser et al., 2005a; Slovin, 2006). Some initial immunohistochemical studies found positiveness to androgen receptors in the majority of NECs in normal human prostate and prostate cancer (Nakada et al., 1993); however, subsequent studies did not confirm this fact (Bonkhoff et al., 1993; Iwamura et al., 1994; di Sant′Agnese, 1996). Now it is thought that androgens do not exert a regulating direct effect on the NECs; nevertheless, the existence of some kind of indirect regulation seems probable. This probability would explain, according to authors, the differences found by Cohen in the distribution of NECs relating to age (Cohen et al., 1993). NECs would be able to secrete their products independently of the regulation exercised by the androgens and would theoretically be able to continue secreting them during androgenic suppression (Evangelou et al., 2004; Sciarra et al., 2006). Androgens are required for the development and maintenance of rat prostate (George and Peterson, 1988); it is well known that pharmacologic castration induces relevant morphofunctional changes in both epithelial and mesenchymal compartments of rat prostate; nevertheless, the possible effect of pharmacological blocking of androgen receptors on changes in NEC population and peptidergic innervation is not well established. In the other hand, the action of nonsteroid hormones on prostate is a potential field of interest, for example, there was stated a relationship between levels of prolactin and the increase of prostate pathology in men (Bartke, 2004); moreover, prolactin stimulates the androgen-independent growth of rat prostate cells in vitro (Ahonen et al., 1999). Although the best known role of prolactin in humans is the development of the mammary gland and the lactogenesis (Buhimschi, 2004), this hormone acts like a growth factor for the prostatic tissue, having a role in the survival of the epithelial prostatic cells after castration. Prolactin also stimulates the epithelial prostatic proliferation in vitro (Nevalainen et al., 1991) and inhibits the apoptosis induced by castration (Ahonen et al., 1999). In this respect, it is interesting to note that the prolactin of pituitary origin has a powerful antagonist, which is bromocriptine. This molecule is a specific agonist of the D_2 dopaminergic receptors and a partial antagonist of the D_1 dopaminergic receptors from tubero-infundibular system, implicating that bromocriptine causes the decrease of pituitary secretion of prolactin (Factor, 1999). The rat prostate has dopaminergic receptors localized in both stromal and epithelial cells (Amenta et al., 1987), then the interaction of prolactin and bromocriptine in prostate tissues might be relevant for the maintenance and function of the gland.

This work deals with the quantification of serotonin-immunoreactive prostate NECs in rats exposed to prolactin in normal, pharmacologically castrated, and bromocriptine-exposed animals, to establish the possible influence of prolactin with or without androgenic blockade on this cell population.

MATERIALS AND METHODS

Experimental Protocol

As the NEC in the rat prostate show a significant increase around puberty (Rodriguez et al., 2003), 30 male peripubertal Sprague-Dawley rats from 40 to 70 days old were used for immunohistochemical and stereological studies. The animals were fed with Panlab Lab Chow (Panlab, Barcelona, Spain) and water ad libitum. They were always in a controlled environment (20–22°C of temperature and 45–55% of relative humidity) and exposed to cycles of 12 hr 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. The animals were arranged in six groups according to treatment (five rats per group), following the next schedule:

Control group (CT). 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 cyproterone acetate (50 mg/kg of body weight), during 18 days. The cyproterone acetate was extracted from tablets of Androcur® (Schering, Madrid, Spain) using the protocol described by Bosland and Prinsen (1990).

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

Cyproterone acetate-treated group and posterior treatment with bromocriptine (CA-BC). Five rats, 40 days old, were treated during 18 days with cyproterone acetate (same dose as Group CA) plus

0.417 mg/kg of body weight of bromocriptine (Sigma, Barcelona, Spain) subcutaneously administered each 12 hours during 7 days after treatment with cyproterone acetate.

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

Bromocriptine group (BC). Five animals, 58 days old, were exclusively treated with 0.417 mg/kg of body weight of bromocriptine subcutaneously administered each 12 hr during 7 days (Stoker et al., 1999).

All rats were killed at the end of the treatments (at the same age for all the groups: 66 days old, i.e., included in the range of peripubertal age) by exsanguination after CO_2 narcosis. The prostate complex was dissected from the abdominal cavity of each animal and the bladder, deferent ducts, seminal vesicles, and glands of coagulation were carefully removed. Immediately, the prostate was weighted and the total fresh volume was determined by gravimetric methods (water displacement). Afterward, the prostate was cut exhaustively 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. Performed were 5-micrometer-thick sections (for routine hematoxylin–eosin staining) alternating with 10-μm-thick sections (for immunohistochemistry and stereological cell counting) on each block.

For the histological qualitative description and identification of the different regions of the prostate, the hematoxylin-eosin–stained sections were used. As the NECs from rat prostate were exclusively localized in the epithelium of periurethral ducts from dorsolateral prostate (Rodriguez et al., 2003), only this region was used in the present study.

Immunohistochemical Methods

In all the groups, at least 10 selected slides per animal (per prostate) were immunostained. Serotonin was used as a marker for NEC, because it was the best marker to visualize these cells from rat prostate, whereas the immunostaining to chromogranin A, provides a weak and diffuse signal in the rat, although it is quite good to demonstrate NECs in human prostate (Rodriguez et al., 2003). 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 serotonin antibody (Biomeda, Foster City, CA) was used as primary antibody, diluted at 1/50 in PBS pH 7.4, containing 1% bovine serum albumin (BSA) plus 0.1% sodium azide. The incubation with primary antisera was overnight at 4°C.

The second antibody used was a biotin–caproyl anti-mouse immunoglobulin (Biomeda), 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 of diaminobenzidine (3,3′,4,4′-tetraminobiphenyl, Sigma, St. Louis, MO) in 200 ml of PBS, plus 40 μl of hydrogen peroxide.

After immunoreactions, 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 procedure was checked by incubation of sections with nonimmune serum instead of the primary antibody.

Stereological Methods

Evaluation of reference volume. As the NECs from rat prostate were exclusively localized in the epithelium of periurethral ducts from dorsolateral prostate (Rodriguez et al., 2003), the volume of the epithelial compartment (Vep) only was estimated. First, the volume fraction occupied by periurethral ducts ($V_V$duct) over the total prostate volume (Vprost) was estimated on an average of 30 systematically randomly sampled microscopic fields in five systematically randomly selected sections of each animal from each group (Howard and Reed, 2005). The measurements were performed by counting the points hitting either the periurethral ducts or the reference area (i.e., prostate tissue) using the CAST-GRID software package (Interactivision, Silkeborg, Denmark), which provides a counting point frame with a point associate area A(p) = 45 μm^2 (Santamaria et al., 2002). The final magnification for these measurements was ×500. The ductal volumes (Vduct) were then calculated multiplying the $V_V$duct per the prostate volume measured by water displacement (Vprost). The Vduct was then used to estimate the epithelial volume of the ducts (Vep) that is the reference space where the NEC are.

Counting NECs. Estimation of the number of NECs immunoreactive to serotonin (NSER) was performed using the technique of the optical dissector, 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 a ×100 oil immersion lens (numerical aperture of 1.4) at a final magnification of ×1,200, and using the stereologic software CAST-GRID. This program controls the XY displacement of the 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 were scanned in each group. The software superimposes a dissector 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 periurethral ducts was scanned and the numerical density of NECs immunoreactive to serotonin (per μm^3 of epithelial volume) (N_VSER) was evaluated, counting their nuclei, according to the Sterio rule (Sterio, 1984).

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

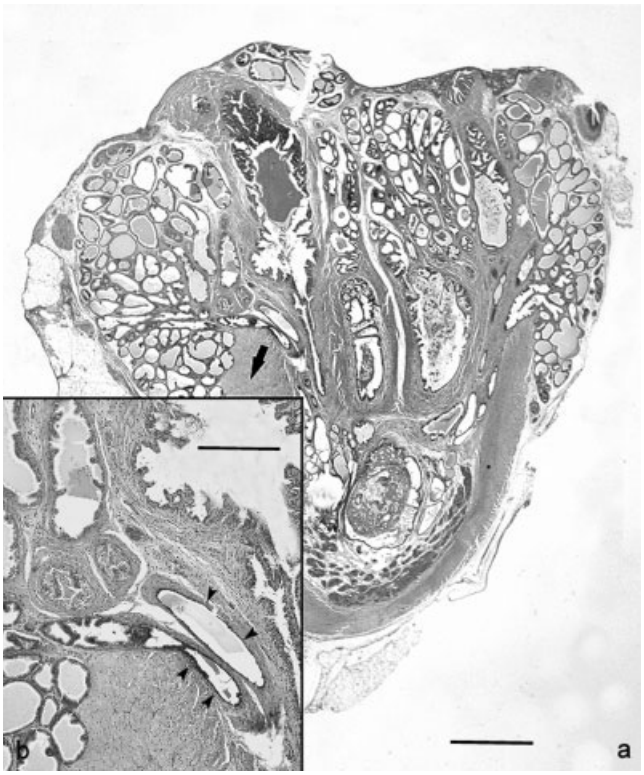

Fig. 1. **a:** Transverse section of rat dorsolateral prostate stained with hematoxylin–eosin, from the control (CT) group at low magnification, some periurethral excretory ducts are seen. **b:** These ducts are shown in more details (arrowheads). Scale bars = 1,000 μm in a, 800 μm in b.

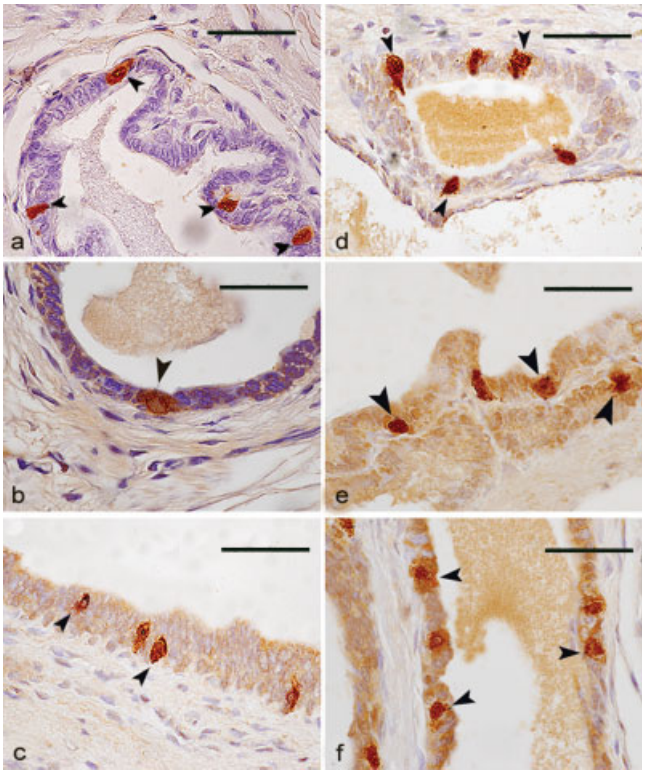

Fig. 2. **a–f:** Neuroendocrine cells immunostained to serotonin observed in the prostate excretory periurethral ducts (arrowheads), from an untreated rat (CT group, a), a pharmacologically castrated rat (CA group, b), a prolactin-treated animal after androgenic blockade (CA-PL group, c), a bromocriptine-treated animal after androgenic blockade (CA-BC group, d), a prolactin-treated rat (PL group, e), and a bromocriptine-treated animal (f). Scale bars = 20 μm in a–f.

To estimate the reference space (Vep), the frames with its upper-right corner hitting epithelial tissue were registered (dis^+) and the volume fraction of ductal epithelium represented by the ratio between the amount of dis^+ and the total of dissectors used (distot) was calculated. After that step, the ductal epithelial volume (Vep) was obtained by multiplying this ratio per the ductal volume (Vduct) previously calculated. The total number of NSER was then calculated by multiplying N_VSER by Vep.

Statistical Analysis

The mean ± SD of NSER was calculated from each experimental group, and the differences among groups were evaluated by analysis of variance. Comparisons between the means for all the groups studied were performed by the Newman–Keuls test. The level of significance selected was $P < 0.05$. The statistical program used was SPSS 9.0 (SPSS, Inc., Chicago, IL, 1995).

RESULTS

Histological Results

All the prostate acini drain secretions toward the urethra by means of terminal ducts (periurethral ducts) Figure 1a,b. NECs immunoreactive to serotonin were exclusively detected in the epithelial lining of the periurethral ducts in all the experimental groups (Fig. 2a–f). They were rounded or triangular in shape, and with occasional apical prolongations (Fig. 2a). These cells were apparently more abundant in animals treated with prolactin or with bromocriptine (Fig. 2e,f).

Quantitative Results

The Vep of the periurethral ducts, was significantly decreased in animals treated with cyproterone acetate (CA group), the addition of either prolactin or bromocriptine after androgenic blockade (CA-PL and CA-BC groups) recovers the Vep to levels similar to those observed in the CT group. A significant increase of the Vep relative to the controls was shown in the groups treated either with prolactin (PL) or bromocriptine (BC, Fig. 3).

The absolute number of cells (NSER), was significantly increased in PL group in comparison with CT animals, although the NSER from BC rats was higher than the NSER from controls; the differences were not significant (Fig. 4a). In animals treated by cyproterone acetate, the NSER was significantly decreased in comparison with the CT group. When the androgenic blockade was accompanied by prolactin or bromocriptine treatment (CA-PL and CA-BC groups), the NSER was recovered to levels significantly higher than those observed in both CT and CA groups (Fig. 4b).

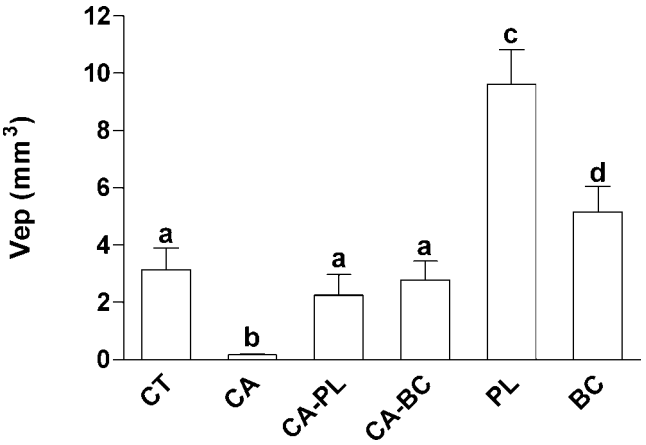

Fig. 3. Bar diagrams indicating means ± SD for volume of the epithelium of prostate excretory periurethral ducts (Vep) expressed in mm^3 in control rats (CT), cyproterone acetate exposed animals (CA), prolactin-treated animals after exposure to cyproterone acetate (CA-PL), bromocriptine-treated animals after exposure to cyproterone acetate (CA-BC), prolactin (PL), and bromocriptine (BC) -treated rats. Bars that share the same letter are not significantly different, but those with different letters are significantly different from each other ($P < 0.05$).

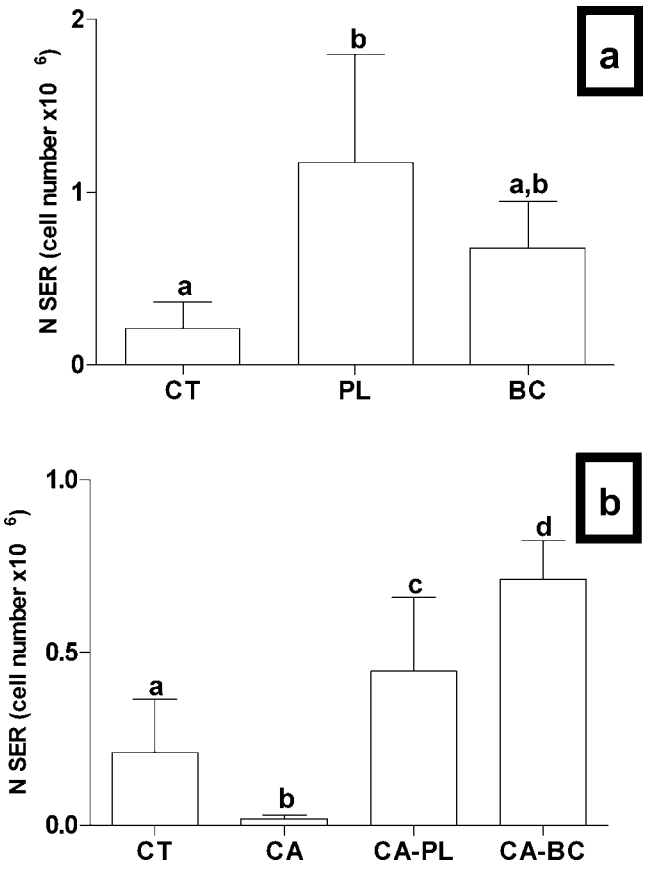

Fig. 4. **a,b:** Bar diagrams indicating means ± SD for absolute number (NSER) of neuroendocrine cells immunostained to serotonin expressed in number of cells × 10^6 per prostate in control rats (CT), prolactin (PL), and bromocriptine (BC) -treated rats (a); and cyproterone acetate exposed animals (CA), prolactin-treated animals after exposure to cyproterone acetate (CA-PL), bromocriptine-treated animals after exposure to cyproterone acetate (CA-BC; b). Bars that share the same letter are not significantly different, but those with different letters are significantly different from each other ($P < 0.05$).

DISCUSSION

Several authors (Xue et al., 2000; Aumuller et al., 2001) suggest that the amount of NECs per prostate is quite constant, but their density show a remarkable interindividual variability (Jongsma et al., 1999; Xue et al., 2000); the present study agrees with this finding, because the coefficient of variation observed for NSER was 62%. The significant decrease of NSER observed in the rats exposed to androgenic blockade (CA group) does not agree with the increase of NECs described for some authors in surgically castrated animals (Bonkhoff et al., 1993; Acosta et al., 2001; Jimenez et al., 2001; Ismail et al., 2002). These differences might be due to the different methodology used for estimation of the number of cells. Most the authors (Jongsma et al., 1999; Acosta et al., 2001) count the cells expressing the results in number per unit of area; these estimates are suspected to be biased, because of mistaken estimates of tridimensional particles (cells) per area (bidimensional reference space). In fact, when an amount of cells is estimated from a single section, this quantity is affected by cell size, because the chance to count a cell is proportional to its volume. Nevertheless, our results were obtained using unbiased stereological tools that estimate either relative (per unit volume) or absolute (per prostate) cell numbers (Howard and Reed, 2005).

The decrease of absolute number of neuroendocrine serotoninergic cells detected in the CA group was accompanied by a significant diminution of the epithelial volume of the periurethral ducts. The effect of cyproterone acetate on the prostate might be explained not only by the specific blockade of androgen receptors, because this agent has an antigonadotropic action due to chemical similitude to progesterone, causing a decrease in delivery of luteinizing hormones and, thus, the diminution in the production of testosterone by the testis (Raudrant and Rabe, 2003).

There are several possibilities to account the decrease of NSER by the action of cyproterone acetate: (1) The absence of an androgenic stimulus could cause the decrease of neuroendocrine differentiation from the population of basal cells, but this explanation does not agree with the increase of neuroendocrine differentiation found in cancer prostate cells under androgenic deprivation (Yuan et al., 2006). This disagreement might be explained because differences between in vitro and in vivo cell behavior, it is possible that the epithelial atrophy caused by cyproterone acetate disrupts the relationships maintained in vivo between epithelial secretory cells and NEC originating their loss; (2) the cyproterone acetate might promote the apoptosis of NEC, in a similar way to that observed for the epithelial secretory cell (Shao et al., 1994; Kimura et al., 2001); (3) the cyproterone acetate could not impair the integrity of the NEC population but mediates a decrease of the serotonin expression in these cells, rendering it more difficult to localize and count them.

When prolactin or bromocriptine were administrated to cyproterone acetate-treated animals, a significant increase of NSER was detected, which indicates a true volume-independent increase of the serotoninergic NECs. These findings suggest that prolactin acts as a trophic hormone (Costello and Franklin, 1994) to normal ductal epithelium, and/or a survival factor to epithelium exposed to androgenic blockade (Ahonen et al., 1999). It is known that prolactin interacts with specific prostate receptors (Nevalainen et al., 1997) to increase cell proliferation (Reiter et al., 1999); in addition, it was also demonstrated that prolactin induces up-regulation of prostate Bcl-2, inhibiting apoptosis (Van Coppenolle et al., 2001). The proliferative stimulus mediated by prolactin might induce an increment of basal intermediate cells from the epithelium that can become both columnar epithelial and NECs (Untergasser et al., 2005b; Signoretti and Loda, 2006). Moreover, these cells are, at least in humans, androgen-independent (Schalken and van Leenders, 2003). This finding could explain the increase of NSER in the CA-PL group.

Surprisingly, bromocriptine has similar effects in prostate as prolactin: it is well known that bromocriptine is a dopaminergic agonist that inhibits the release of pituitary prolactin. Nevertheless, the present study suggest a prostatic local effect of bromocriptine, independent and contrary to that described for its systemic (pituitary) action. The cytodifferentiating effect of bromocriptine onto the stem cells toward the neuroendocrine population might be mediated by D_1 and D_2 dopaminergic receptors detected in the epithelium and the smooth muscle cells of the prostate (Amenta et al., 1987). The catecholaminergic effects of bromocriptine might be similar to those observed in a rat hypertension model, where the increase of sympathetic activity correlates with up-regulation of androgen receptors and with spontaneous development of prostate hyperplasia (Golomb et al., 1998; Matityahou et al., 2003). It was also intriguing why cyproterone acetate shows an enhancing effect on the increase of NECs mediated by bromocriptine; the rise of cAMP levels caused by cyproterone acetate (Kvissel et al., 2007) might exert an additive effect with the catecholaminergic action of bromocriptine to potentiate neuroendocrine differentiation. Summarizing, we can conclude that (1) The androgenic blockade mediated by cyproterone acetate decreases the total number of NECs immunoreactive to serotonin in rat prostate; (2) Treatment with prolactin induces an increase of NECs immunoreactive to serotonin in rat prostate in normal and cyproterone acetate exposed rats; and (3) Bromocriptine exerts a local effect over the prostate similar to that described in (2) for prolactin but enhanced by cyproterone acetate.

LITERATURE CITED

Abrahamsson PA. 1999. Neuroendocrine differentiation in prostatic carcinoma. Prostate 39:135–148.

Acosta S, Dizeyi N, Pierzynowski S, Alm P, Abrahamsson PA. 2001. Neuroendocrine cells and nerves in the prostate of the guinea pig: effects of peripheral denervation and castration. Prostate 46:191–199.

Ahonen TJ, Harkonen PL, Laine J, Rui H, Martikainen PM, Nevalainen MT. 1999. Prolactin is a survival factor for androgen-deprived rat dorsal and lateral prostate epithelium in organ culture. Endocrinology 140:5412–5421.

Amenta D, Cavallotti C, De Rossi M, Ferrante F, Amenta F. 1987. Autoradiographic localization of the dopaminergic agonist 3H-dihydroergotoxine within the male reproductive system. Funct Neurol 2:207–216.

Aumuller G, Leonhardt M, Renneberg H, von Rahden B, Bjartell A, Abrahamsson PA. 2001. Semiquantitative morphology of human prostatic development and regional distribution of prostatic neuroendocrine cells. Prostate 46:108–115.

Bartke A. 2004. Prolactin in the male: 25 years later. J Androl 25:661–666.

Bonkhoff H, Stein U, Remberger K. 1993. Androgen receptor status in endocrine-paracrine cell types of the normal, hyperplastic, and neoplastic human prostate. Virchows Arch A Pathol Anat Histopathol 423:291–294.

Bosland MC, Prinsen MK. 1990. Induction of dorsolateral prostate adenocarcinomas and other accessory sex gland lesions in male Wistar rats by a single administration of N-methyl-N-nitrosourea, 7,12-dimethylbenz(a)anthracene, and 3,2′-dimethyl-4-aminobiphenyl after sequential treatment with cyproterone acetate and testosterone propionate. Cancer Res 50:691–699.

Buhimschi CS. 2004. Endocrinology of lactation. Obstet Gynecol Clin North Am 31:963–79, xii.

Cohen RJ, Glezerson G, Taylor LF, Grundle HA, Naude JH. 1993. The neuroendocrine cell population of the human prostate gland. J Urol 150:365–368.

Costello LC, Franklin RB. 1994. Effect of prolactin on the prostate. Prostate 24:162–166.

DeLellis RA, Dayal Y. 1997. Neuroendocrine System. In: Sternberg SS, editor. Histology for pathologists. Philadelphia: Lippincott Raven Publishers. p 1133–1151.

di Sant′Agnese PA. 1996. Neuroendocrine differentiation in the precursors of prostate cancer. Eur Urol 30:185–190.

di Sant′Agnese PA. 1998. Neuroendocrine cells of the prostate and neuroendocrine differentiation in prostatic carcinoma: a review of morphologic aspects. Urology 51:121–124.

Edwards WD, Thomas JA. 1980. Morphologic and metabolic characteristics of ventral, lateral, dorsal and anterior prostate transplants in rats. Effect of testosterone and/or prolactin. Horm Res 13:28–39.

Evangelou AI, Winter SF, Huss WJ, Bok RA, Greenberg NM. 2004. Steroid hormones, polypeptide growth factors, hormone refractory prostate cancer, and the neuroendocrine phenotype. J Cell Biochem 91:671–683.

Factor SA. 1999. Dopamine agonists. Med Clin North Am 83:415–443, vi–vii.

George FW, Peterson KG. 1988. 5 alpha-dihydrotestosterone formation is necessary for embryogenesis of the rat prostate. Endocrinology 122:1159–1164.

Gkonos PJ, Krongrad A, Roos BA. 1995. Neuroendocrine peptides in the prostate. Urol Res 23:81–87.

Golomb E, Kruglikova A, Dvir D, Parnes N, Abramovici A. 1998. Induction of atypical prostatic hyperplasia in rats by sympathomimetic stimulation. Prostate 34:214–221.

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A. 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379–394.

Howard CV, Reed MG. 2005. Unbiased stereology: three-dimensional measurement in microscopy. 2nd ed. Oxford: Bios Scientific Publishers.

Ismail AH, Landry F, Aprikian AG, Chevalier S. 2002. Androgen ablation promotes neuroendocrine cell differentiation in dog and human prostate. Prostate 51:117–125.

Iwamura M, Abrahamsson PA, Benning CM, Cockett AT, di Sant′ Agnese PA. 1994. Androgen receptor immunostaining and its tissue distribution in formalin-fixed, paraffin-embedded sections after microwave treatment. J Histochem Cytochem 42:783–788.

Jimenez N, Calvo A, Martinez A, Rosell D, Cuttitta F, Montuenga LM. 1999. Expression of adrenomedullin and proadrenomedullin N-terminal 20 peptide in human and rat prostate. J Histochem Cytochem 47:1167–1178.

Jimenez N, Jongsma J, Calvo A, van der Kwast TH, Treston AM, Cuttitta F, Schroder FH, Montuenga LM, van Steenbrugge GJ. 2001. Peptidylglycine alpha-amidating monooxygenase- and proadrenomedullin-derived peptide-associated neuroendocrine differentiation are induced by androgen deprivation in the neoplastic prostate. Int J Cancer 94:28–34.

Jongsma J, Oomen MH, Noordzij MA, Van Weerden WM, Martens GJ, van der Kwast TH, Schroder FH, van Steenbrugge GJ. 1999. Kinetics of neuroendocrine differentiation in an androgen-dependent human prostate xenograft model. Am J Pathol 154: 543–551.

Kimura K, Markowski M, Bowen C, Gelmann EP. 2001. Androgen blocks apoptosis of hormone-dependent prostate cancer cells. Cancer Res 61:5611–5618.

Kvissel AK, Ramberg H, Eide T, Svindland A, Skalhegg BS, Tasken KA. 2007. Androgen dependent regulation of protein kinase A subunits in prostate cancer cells. Cell Signal 19:401–409.

Martin JJ, Martin R, Codesal J, Fraile B, Paniagua R, Santamaria L. 2001. Cadmium chloride-induced dysplastic changes in the ventral rat prostate: an immunohistochemical and quantitative study. Prostate 46:11–20.

Matityahou A, Rosenzweig N, Golomb E. 2003. Rapid proliferation of prostatic epithelial cells in spontaneously hypertensive rats: a model of spontaneous hypertension and prostate hyperplasia. J Androl 24:263–269.

Montuenga LM, Guembe L, Burrell MA, Bodegas ME, Calvo A, Sola JJ, Sesma P, Villaro AC. 2003. The diffuse endocrine system: from embryogenesis to carcinogenesis. Prog Histochem Cytochem 38:155–272.

Nakada SY, di Sant′Agnese PA, Moynes RA, Hiipakka RA, Liao S, Cockett AT, Abrahamsson PA. 1993. The androgen receptor status of neuroendocrine cells in human benign and malignant prostatic tissue. Cancer Res 53:1967–1970.

Nevalainen MT, Valve EM, Makela SI, Blauer M, Tuohimaa PJ, Harkonen PL. 1991. Estrogen and prolactin regulation of rat dorsal and lateral prostate in organ culture. Endocrinology 129:612–622.

Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, Harkonen PL. 1997. Prolactin and prolactin receptors are expressed and functioning in human prostate. J Clin Invest 99:618–627.

Noordzij MA, van Steenbrugge GJ, van der Kwast TH, Schroder FH. 1995. Neuroendocrine cells in the normal, hyperplastic and neoplastic prostate. Urol Res 22:333–341.

Raudrant D, Rabe T. 2003. Progestogens with antiandrogenic properties. Drugs 63:463–492.

Reiter E, Hennuy B, Bruyninx M, Cornet A, Klug M, McNamara M, Closset J, Hennen G. 1999. Effects of pituitary hormones on the prostate. Prostate 38:159–165.

Rodriguez R, Pozuelo JM, Martin R, Henriques-Gil N, Haro M, Arriazu R, Santamaria L. 2003. Presence of neuroendocrine cells during postnatal development in rat prostate: immunohistochemical, molecular, and quantitative study. Prostate 57:176–185.

Santamaria L, Martin R, Martin JJ, Alonso L. 2002. Stereologic estimation of the number of neuroendocrine cells in normal human prostate detected by immunohistochemistry. Appl Immunohistochem Mol Morphol 10:275–281.

Schalken JA, van Leenders G. 2003. Cellular and molecular biology of the prostate: stem cell biology. Urology 62:11–20.

Sciarra A, Cardi A, Dattilo C, Mariotti G, Di Monaco F, Di Silverio F. 2006. New perspective in the management of neuroendocrine differentiation in prostate adenocarcinoma. Int J Clin Pract 60:462–470.

Shao TC, Kong A, Cunningham GR. 1994. Effects of 4-MAPC, a 5 alpha-reductase inhibitor, and cyproterone acetate on regrowth of the rat ventral prostate. Prostate 24:212–220.

Signoretti S, Loda M. 2006. Defining cell lineages in the prostate epithelium. Cell Cycle 5:138–141.

Slovin SF. 2006. Neuroendocrine differentiation in prostate cancer: a sheep in wolf's clothing? Nat Clin Pract Urol 3:138–144.

Sterio DC. 1984. The unbiased estimation of number and sizes of arbitrary particles using the dissector. J Microsc 134(Pt 2):127–136.

Stoker TE, Robinette CL, Cooper RL. 1999. Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. Toxicol Sci 52:68–79.

Untergasser G, Madersbacher S, Berger P. 2005a. Benign prostatic hyperplasia: age-related tissue-remodeling. Exp Gerontol 40:121–128.

Untergasser G, Plas E, Pfister G, Heinrich E, Berger P. 2005b. Interferon-gamma induces neuroendocrine-like differentiation of human prostate basal-epithelial cells. Prostate 64:419–429.

Van Coppenolle F, Slomianny C, Carpentier F, Le Bourhis X, Ahidouch A, Croix D, Legrand G, Dewailly E, Fournier S, Cousse H, Authie D, Raynaud JP, Beauvillain JC, Dupouy JP, Prevarskaya N. 2001. Effects of hyperprolactinemia on rat prostate growth: evidence of androgeno-dependence. Am J Physiol Endocrinol Metab 280:E120–E129.

Xue Y, van der LJ, Smedts F, Schoots C, Verhofstad A, de la Rosette J, Schalken J. 2000. Neuroendocrine cells during human prostate development: does neuroendocrine cell density remain constant during fetal as well as postnatal life? Prostate 42:116–123.

Yuan TC, Veeramani S, Lin FF, Kondrikou D, Zelivianski S, Igawa T, Karan D, Batra SK, Lin MF. 2006. Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. Endocr Relat Cancer 13:151–167.