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Morphology of the epithelial cells and expression of androgen receptor in rat prostate dorsal lobe in experimental hyperprolactinemia

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Abstract: The effect of hyperprolactinemia on the prostate has not been well investigated. Since androgens play an important role in prostate development, growth and function, the goal of the present study was to estimate the influence of hyperprolactinemia on expression of the androgen receptor (AR) in rat epithelial cells of prostate dorsal lobe and on morphology of these cells. Studies were performed on sexually mature male Wistar rats. The experimental group rats received metoclopramide (MCP) intraperitoneally to provoke hyperprolactinemia. The control group animals were given saline in the same way. For light and electron microscopy the prostate dorsal lobes were obtained routinely. To evaluate the intensity of immunohistochemical reaction for AR in epithelial cells, the optical density was measured and computer-assisted image analysis system was used. Morphological observations of the dorsal lobe epithelial cells were carried out in transmission electron microscope. MCP caused over twofold increase in prolactin (PRL) serum levels. In rats with hyperprolactinemia, the testosterone levels (T) were twofold decreased. The intensity of immunohistochemical reaction for AR in epithelial cells of dorsal lobe in the experimental group was significantly lower than in the control group. In the dorsal lobe epithelial cells of experimental group animals, the transmission electron microscopy (TEM) revealed highly dilated RER cisternae and reduced number of microvilli on the cellular surface when compared to the control group. The results show that hyperprolactinemia in male rats causes morphological abnormalities in the dorsal lobe of prostate. The abnormalities are caused by elevated prolactin either directly or indirectly through decreased level of testosterone. Decreased expression of AR in epithelial cells of prostate dorsal lobe is likely to be caused by decreased testosterone level. (www.cm-uj.krakow.pl/FHC)

Key words: Androgen receptor - Testosterone - Hyperprolactinemia - Prostate dorsal lobe - Electron microscopy

Introduction

Androgens play a crucial role in the regulation of prostate growth, by stimulating proliferation and survival of the glandular epithelial cells [5]. There are two comparable sources of these hormones: testis and adrenal gland [17]. Androgen receptor (AR) has been detected in a number of cell types of male reproductive system [14, 16, 31] including epithelial and stromal cells in the rat and human prostate [5, 11, 24, 29]. In human prostate, AR has been found in columnar as well as in basal epithelial cells [11]. However, in all rat prostate lobes (lateral, dorsal and ventral) AR has been visualized only in columnar epithelial cells [29]. Expression of AR in rat prostate depends on age, androgen serum levels and varies with the type of prostate lobes. In old rats, AR expression in columnar epithelial cells of dorsal lobe is higher than in young ones [5]. Prins *et al.* [27] indicated that nuclear AR expression in dorsal lobe is regulated primarily by androgens. On the other hand, the dorsal lobe has been shown to be dependent on prolactin (PRL) that promotes the growth of prostate and its secretion in synergism with androgens or alone [1, 2, 10, 18, 23, 26, 30].

Prolactin is known to exert its biological action via membrane bound receptors [13, 23, 36]. In rat prostate dorsal lobe, two forms of PRL receptors were detected: short and long [23, 36]. To reveal the influence of elevated prolactin concentration on prostate, we used pharmacologically induced model of hyperprolactinemia. The most common causes of hyperprolactinemia

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are pituitary adenomas, hypothalamic disease, primary hypothyroidism, chronic renal disease and also certain drugs [20, 21, 37]. There are multiple mechanisms that lead to sexual and reproductive dysfunction in men suffering from hyperprolactinemia [6, 7, 18, 19, 25].

We reported previously that hyperprolactinemia following administration of MCP in male rats significantly increased the quantity of AR and caused morphological changes in the epithelial cells of lateral prostate lobe in spite of decreasing testosterone serum level [31, 32]. The dorsal lobe as well as lateral lobe of rat prostate is the most homologous to human prostate [10]. Thus, we have found it interesting to estimate expression of androgen receptors in columnar epithelial cells of prostate dorsal lobe and morphology of these cells under conditions of hyperprolactinemia induced with metoclopramide (MCP).

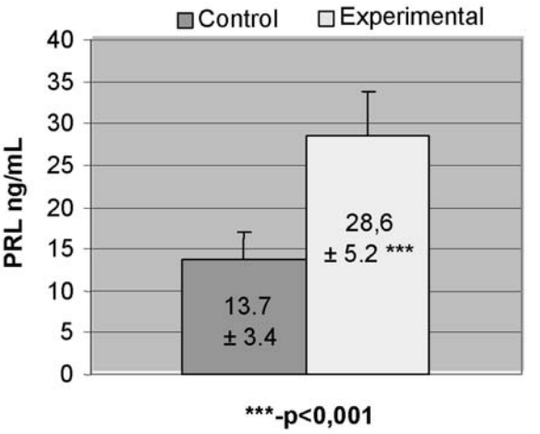

Fig. 1. Prolactin (PRL) serum concentrations in control rats and in rats receiving MCP (mean ± SD)

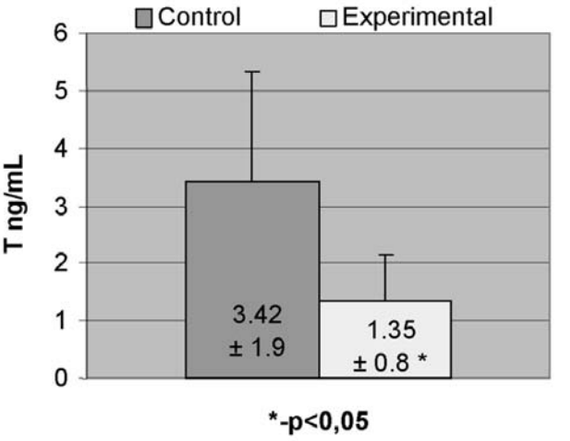

Fig. 2. Testosterone (T) serum concentrations in control rats and in rats receiving MCP (mean ± SD)

Materials and methods

Animals. Twenty sexually mature male rats of Wistar strain were divided into control and experimental group. The experimental group received metoclopramide (MCP, Polfa Starogard Poland) intraperitonealy in a dose of 2.2 mg/kg b.w. for 14 days (duration of rat seminiferous epithelium cycle) to provoke hyperprolactinemia. The rats of control group were given saline in the same way. For light and electron microscopy the prostate dorsal lobes were carefully prepared and separated from other lobes. The blood was collected from heart for evaluation of serum hormone concentrations.

Hormone analysis. Prolactin serum levels were measured with rat prolactin ELISA enzyme immunoassay kit (Spi-Bio, France). Testosterone serum levels were measured with radioimmunoassay kit (Farmos Diagnostika, Finland).

Immunohistochemistry. Immunohistochemistry was performed on deparaffinized and rehydrated sections (4 μm). The radiation of microwave oven (250 W) was applied for 30 min in citrate buffer. The endogenous peroxidase was inhibited with 3% H_2O_2 in methanol for 10 min. The nonspecific binding sites were blocked with 3% normal goat serum (Sigma, USA) for 30 min. The sections were incubated overnight at 4˚C with primary antibody: the polyclonal antibody against androgen receptor (NCL-ARp, 1:10, Novocastra Lab, Ltd Newcastle, UK) and subsequently, with secondary antibody, biotinylated goat anti-rabbit IgG (1:400, Vector Lab., Burlingame, CA, USA) for 60 min. In the next step, avidin-biotin--horseradish peroxidase complex (ABC/HRP; 1:100, Dako/AS, Denmark) was applied for 30 min. Diaminobenzidine (DAB) was used to visualize the immunohistochemical reaction. The sections were counterstained with Mayer's haematoxylin. In the control reaction, the primary antibody was replaced by normal goat serum. After each step, sections were rinsed with Tris-buffered saline (TBS). Finally, sections were examined with light microscope.

Computer-assisted image analysis. To enable the quantitative evaluation of the intensity of immunohistochemical reaction of AR, the optical density was measured and analyzed with the computer image analyzer (Quantimet 600 S, Leica, UK). The optical density of the immunohistochemical reaction product was matched to the density of AR. The system was calibrated, light transmittance replacing grey levels (range 0-255) with the value of optical density in the range of 0-2. The microscopic image was analyzed at × 400 magnification and next transmitted as a monochromatic (grey) image to the computer system. For statistical analysis, integrated optical density (IOD) and mean optical density (MOD) values were counted to estimate the intensity of immunohistochemical reaction (for AR) in nuclei of 600 columnar epithelial cells in each group.

Transmission electron microscopy (TEM). Prostate dorsal lobe was cut into 1 mm^3 pieces, fixed in 0.25 mol/l glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) for 2 h at 4˚C, postfixed in 1% OsO_4, dehydrated in ethyl alcohol (30-96%) and 100% acetone, and subsequently embedded in Spurr's resin (Polysciences, Inc.). The blocks were cut with Reichter OmU2 ultramicrotome. The ultra-thin sections were contrasted with uranyl acetate as well as lead citrate, and examined under JEM-1200 EX transmission electron microscope.

Statistical analysis. Serum prolactin and testosterone concentrations were analyzed by the Cochran-Cox test. Parameters of integrated and mean optical density (IOD and MOD) were analyzed by the Mann-Whitney U-test. Statistical significance was accepted at $p<0.05$.

Results

In rats of the experimental group as compared to the control group, the mean PRL serum concentration was

Table 1. Parameters of AR immunostaining of nuclei in the epithelial columnar cells of prostate dorsal lobe in control rats and in those receiving MCP for 14 days (M14)

Columnar cells in prostate dorsal lobe	n	Integrated optical density (IOD)			Mean optical density (MOD)		
		LQ-UQ	M	Mean ± SD	LQ-UQ	M	Mean ± SD
Control	597	554-1205	810	904 ± 429	0.76-0.90	0.84	0.82 ± 0.11
M14	598	557-1027	763	835 ± 373*	0.74-0.89	0.81	0.81 ± 0.13*

*p<0.05; n - number of examined cells with immunostained nuclei; p - level of statistical significance; LQ-UQ - (lower-upper) quartiles; M - median

more than 2 times higher (28.6±5.2 vs. 13.7±3.4 ng/ml) whereas the mean T concentration was about 2 times lower (1.35±0.8 vs. 3.42±1.9 ng/ml, Figs. 1 and 2).

Immunohistochemical study showed expression of AR in the nuclei of columnar epithelial and stromal cells of prostate dorsal lobe in the experimental as well as in the control group. In rats with hyperprolactinemia, the nuclei of epithelial cells revealed lower optical density of immunocytochemical reaction (Fig. 3B,D) as compared to control rats (Fig. 3A,C). A significant decrease ($p<0.05$) in both IOD and MOD was noted in the experimental animals (Table 1).

Transmission electron microscopy revealed two different types of epithelial secretory cells in the rat prostate dorsal lobe. The first one presented small, separated profiles of rough endoplasmic reticulum (RER), whereas in the second one the cisternae of RER were slightly widened (Fig. 4A). In both cell types, the apical parts contained well developed Golgi apparatus, vacuoles, lysosomes and microvilli on the surface, typically for simple columnar epithelium. In the glandular lumina of the prostate dorsal lobe, we observed blebs containing the structures presented in the apical part of these cells. The occurrence of the blebs suggests apocrine mechanism of secretion in dorsal lobe of prostate. Besides apocrine secretion, merocrine secretion was also observed (Fig. 4C).

In rats with hyperprolactinemia, transmission electron microscopy revealed in the majority of columnar epithelial cells highly dilated RER cisternae (Fig. 4B). On the surface of some columnar cells, we observed small number of microvilli in the experimental group (Fig. 4B). In the majority of cases, the apical blebs were observed in glandular lumen both in control as well as in experimental group (Fig. 4D).

Discussion

Hyperprolactinemia in men is associated with dysfunction of reproductive system and causes impotence and infertility [6, 7, 18, 19, 25, 33]. Treatment with certain drugs is a well known cause of hyperprolactinemia [15, 20]. We found significant increase in prolactin serum level following administration of MCP in male rats. It is consistent with previous reports [3, 6, 8, 19, 32]. Additionally, in rats suffering from hyperprolactinemia we noticed decreased testosterone serum level. Similar results have been observed previously [6, 15, 19]. A number of hypotheses have been developed to explain the mechanisms of lowering the level of testosterone in serum in animals with hyperprolactinemia [12, 22]. Gunasekar *et al.* [12] observed reduced cholesterol fractions in Leydig cells following ovine prolactin (oPRL) administration in monkeys and suggested lower utilization of cholesterol for steroidogenesis. Munabi *et al.* [22] demonstrated decreased activity of major steroidogenic enzymes in Leydig cells of rats with hyperprolactinemia. These findings partly explain how hyperprolactinemia impairs the synthesis of steroids in testis and subsequently reduces the serum testosterone level.

In our study, the intensity of immunohistochemical reaction for AR in the rat prostate dorsal lobe was assessed by optical density measurements. We found that the expression of AR in columnar epithelial cells of prostate dorsal lobe was decreased in rats with hyperprolactinemia but this seems to be rather the effect of decreased serum testosterone level. Other studies showed the same relation between expression of AR in columnar epithelial cells of dorsal lobe and androgen serum level [5, 27, 28, 31]. On the other hand, in our previous studies we found that AR expression in the lateral lobe of prostate increased with elevation of prolactin serum level [31, 32]. Our findings are consistent with Prins [26] who has reported that AR expression in lateral lobe of prostate is related to serum PRL concentration, opposite to the dorsal lobe.

The ultrastructure of the prostate cells was the other parameter evaluated in the present study. It was previously reported that chronic hyperprolactinemia following administration of sulpiride caused enlargement and inflammation but only in the lateral lobe of rat prostate [33]. Ahonen *et al.* [2] using long-term organ cultures of rat prostate observed that prolactin induced hyperplasia of epithelium in rat dorsolateral lobe, but had no effect on epithelial morphology of the ventral lobe. However, Prins [26] demonstrated that higher PRL levels in pituitary grafts increased weight and content of protein and DNA in the lateral lobe without such effect in ventral and dorsal lobes. We have now shown the morphological changes in columnar epithelial cells of

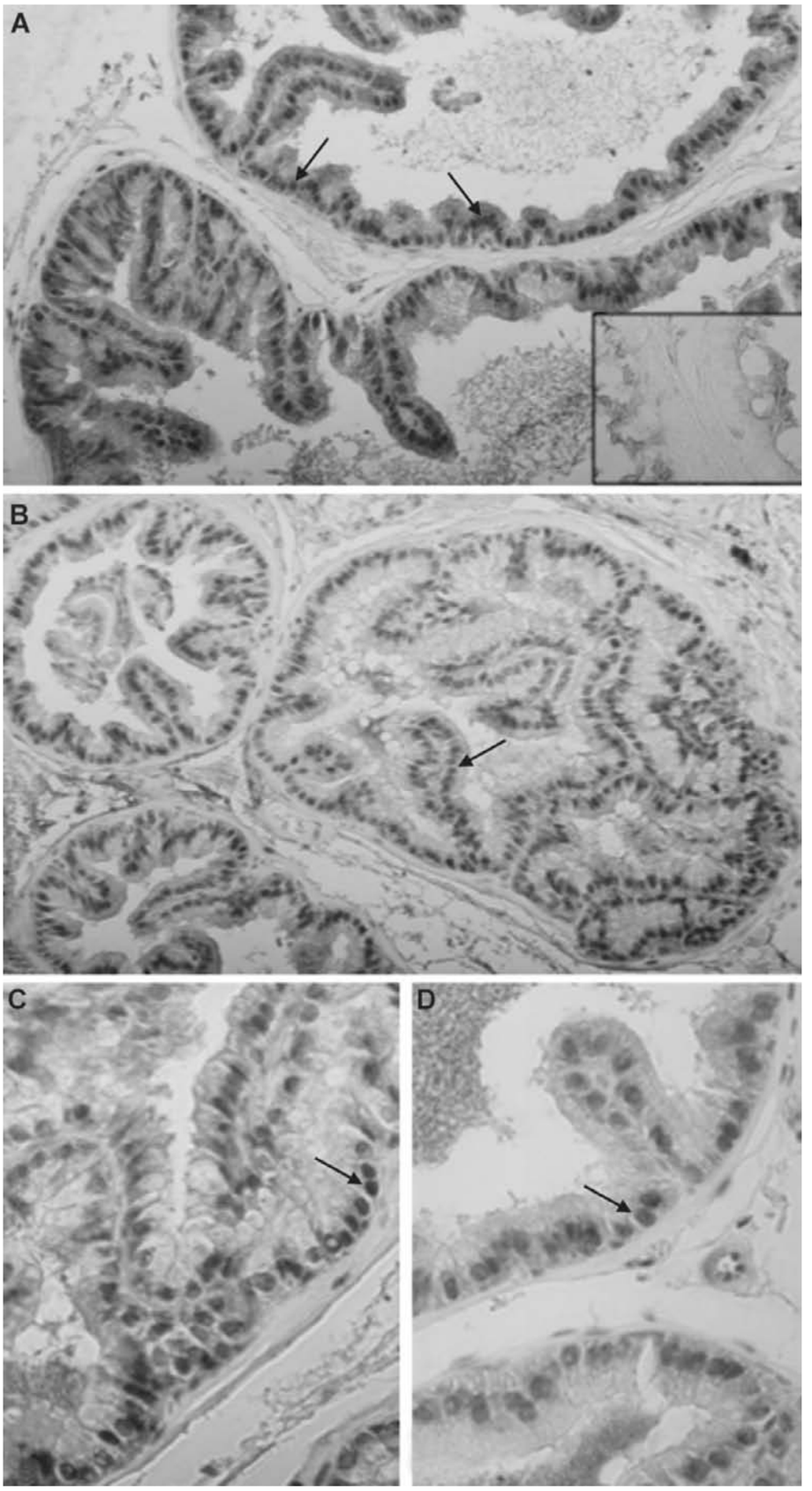

Fig. 3. Immunostaining of AR (arrows) in epithelial cells of prostate dorsal lobe of control rat **(A, C)** and rat receiving MCP **(B, D)**. × 330 (A, B); × 670 (C, D). Insert represents the control of immunohistochemical reaction. × 670.

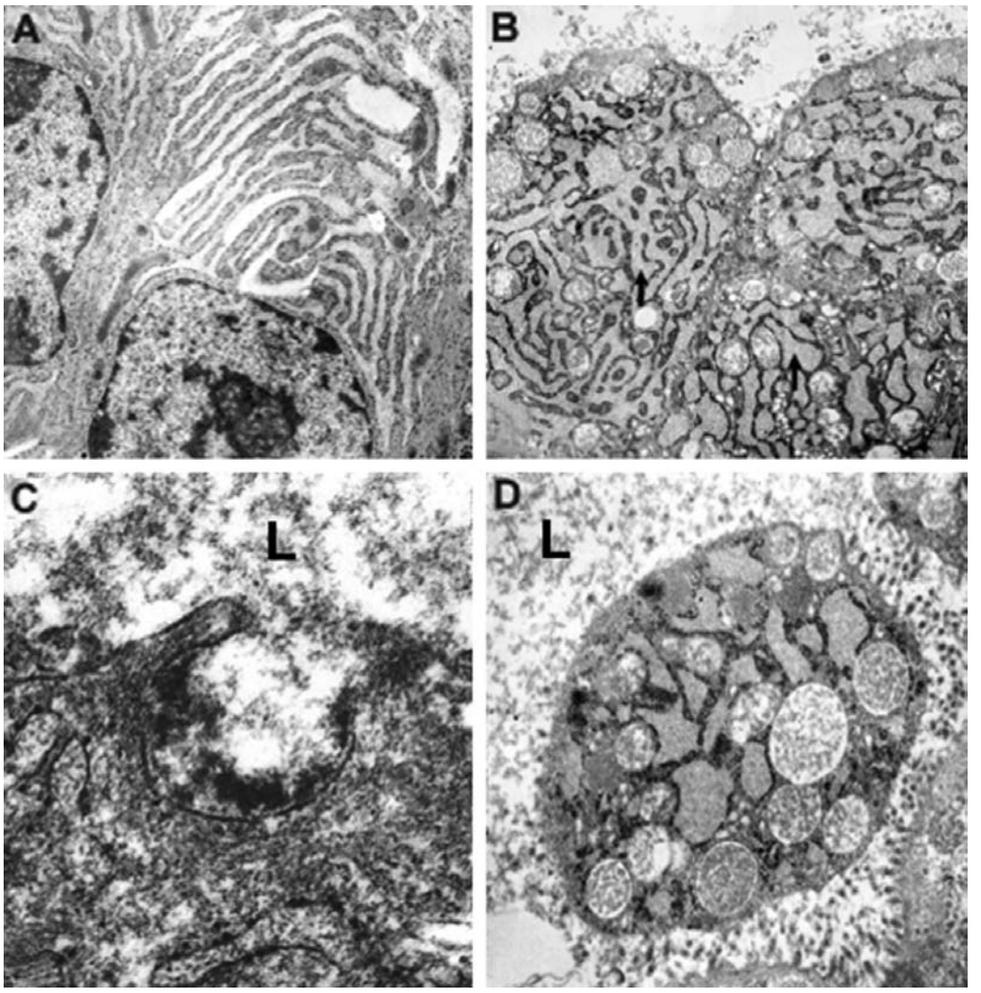

Fig. 4. Ultrastructure of the epithelial cells of the prostate dorsal lobe. Cisternae of rough endoplasmic reticulum and nuclei of epithelial cells of the prostate dorsal lobe in control rat **(A)**. Highly dilated cisternae of rough endoplasmic reticulum (arrow) of epithelial cells of the prostate dorsal lobe in experimental rat **(B)**. Merocrine secretion on the surface of epithelial cell of the prostate dorsal lobe of control rat **(C)**. A bleb containing the structures of the apical part of epithelial cells of the prostate dorsal lobe of experimental rat **(D)**. L - Lumen. TEM, × 9750 (A, B), × 75000 (C), × 18000 (D).

dorsal prostate lobe in rats with hyperprolactinemia. Examination of columnar epithelial cells of dorsal prostate lobe under TEM revealed in majority of columnar epithelial cells highly dilated cisternae of rough endoplasmic reticulum and small number of microvilli on the apical surface when compared to control rats. Since the morphological alterations were observed in cellular structures engaged in protein synthesis and release - the androgen serum level dependent processes in the dorsal lobe [35], it seems likely that they were caused by decreased level of testosterone and reduced expression of AR. Previously we reported similar ultrastructural changes in the lateral prostate lobe after administration of MCP [32]. We additionally observed apical blebs containing the structures presented in the apical part of the cells in glandular lumina but both control as well as experimental group. The blebs reflect the apocrine secretion of the cells. Although the merocrine secretion in rat dorsal lobe has been also suggested, our results are consistent with the hypothesis that rat dorsal prostatic epithelium secretes mainly in apocrine manner [4, 9, 34].

In conclusion, we postulate that hyperprolactinemia in male rats alters morphology of prostate dorsal lobe similarly to the effect previously reported in lateral lobe [32]. Ultrastructural changes are either direct consequence of elevated prolactin or the effect of decreased level of testosterone. However, decreased expression of AR in columnar epithelial cells of prostate dorsal lobe seems to be caused by decreased testosterone level. The latter is in contrast with relations influencing the AR expression existing in lateral prostate lobe [32].

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