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Original Contribution

COMPARATIVE GENDER-RELATED ANALYSIS OF THE LOCALIZATION OF TISSUE ALKALINE AND ACID PHOSPHATASE EXPRESSION IN THE URETHRA IN DOMESTIC CATS (*FELIS SILVESTRIS CATUS*)

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ABSTRACT

AIM: Detection of the presence of acid and alkaline phosphatase, whose tissue expression indicates the course of normal and pathological events in the urethra, as well as gender-related peculiarities in the activities of studied enzymes in this anatomical area.

MATERIAL AND METHODS: The pelvic urethra from six male cats and the urethra of six female cats, all clinically healthy and sexually mature. The expression of acid and alkaline phosphatase was detected by Gomori staining.

RESULTS: The enzyme histological investigation of pelvic urethra in male cats showed that the most significant localization of acid phosphates activity was observed in the propria and adventitia of pelvic urethra, followed by apical parts of epithelial cells in disseminated part of the prostate and in the lumen of glandular tubules. In the lumen of disseminated prostatic alveoles, no alkaline phosphatase activity was detected.

In female cats, the strongenst acid phosphatase activity was established in the connective tissue located among skeletal muscle fibres, followed by a slighly detectable enzyme expression in the propria and perivascular areas of the urethra, whereas alkaline phosphatase activity was present in the connective tissue among skeletal muscle fibres. In the other connective tissue elements of the urethra and its epithelium, female cats exhibited no alkaline phosphatase activity.

CONCLUSION: The lack of tissue alkaline and acid phosphatase activity in urethral propria in female cats was considered as a proof for the absence of structures, resembling a female prostate.

Key words: alkaline phosphatase, acid phosphatase, urethra, cat.

INTRODUCTION

Acid and alkaline phosphatases are hydrolases, whose tissue expression is an indication for normal and pathological processes in dog's urethra (1) as well as in the female urethra in humans (2).

It is known that the structure of the pelvic urethra in male cats (3) and the expression of these enzymes are characterized by a number of peculiarities, while there is no data on the studied phosphatases' expression in the urethra of female cats.

The female urethra has been studied predominantly in humans. Most authors investigated the histochemical and immunohistochemical peculiarities, related to the presence and features of prostate lobules in its propria (4;5; 6).

The female prostate has been studied in relation to its predisposition towards the development of adenocarcinomas and cysts in that specific part of the human urethra (7; 8; 9; 10).

A number of authors have studied histologically and immunohistologically the female urethra and the prostate in the Mongolian gerbil and the rat (11; 12; 13; 14).

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In the propria and the submucosa of the pelvic urethra, which are made up from thickened connective tissue, some elastic fibres, smooth muscle cells and a strong infiltration of diffuse lymphoid tissue, are located the lobules of the disseminated prostate (3, 15; 16; 17).

As shown by enzymohistochemical studies of disseminated rat prostate, the activity of the lysosomal acid phosphatase increases after castration, as opposed to the secretory function, which decreases after androgenic deprivation (18).

It was established, during studies of male dog reproductive organs in relation to the tissue expression of those hydrolases, that the activity of alkaline phosphatase was the highest in the epididymis, and was significantly lower in the disseminated prostate (19).

The insufficient reference data regarding the localization of enzyme expression of the tissue alkaline and acid phosphatases in the pelvic urethra of male cats were a convincing reason to perform the current enzyme histochemical study.

Our goal was to determine the different parts in the normal pelvic urethra of male and female cats, in which the activity of these hydrolases can be observed.

MATERIALS AND METHODS

Detection of the presence of acid phosphatase per Gomori:

Pelvic urethras from 6 healthy mature male cats were studied, 2.8 kg to 4 kg in weight, as well as urethras from 6 female cats, 2.2 kg to 3.2 kg in weight, of the European shorthair breed, aged between 1 and 2 years. The animals were euthanized with 200 mg Thiopental (Biochemie, Austria) i.v.applied in the cephalic vein.

The studies were performed under strict adherence to the European convention for the protection of vertebrates used for experimental and scientific purposes (Strasburg, 16.05. 1986), the European convention for protection of companion animals (Strasburg, 13.11. 1987) and the Law of animal protection in Bulgaria (Section IV- Experiments with animals, as per art. 26, art., 27, and art. 28; Section VII – Animal Euthanasia, as per art. 45, art. 46, and art. 47, as well as Section VIII – Protection and humane treatment of animals, as per art. 52, art. 53 passed on 24.01.2008 and published in State Newspaper issue 13 in 2008).

Cryostate sections of 10 μm thickness were used, fixed in 10% neutral formalin for 24 hours, at temperatures ranging between 0° - 4° C, which were afterwards mounted on glass slides. The samples were transferred into an incubator medium and placed in a thermostat at 37 °C for 3 hours. They were washed with distilled water, treated with ammonium sulfide for 1 minute, which coloured them in dark brown, and afterwards were embedded in glycerol gelatin (20).

Detection of the presence of alkaline phosphatase per Gomori:

The same cryostat sections were transferred into an incubator medium and placed into a thermostat at 37° C for 2 hours, and afterwards were treated with 2% cobalt chloride for 3 minutes, then trasnferred in ammonium sulfide, in which they stayed until turned black (Buchalova, and Kopeva, 1982).

Localization of enzyme expression of tissue alkaline and acid phosphatases were determined through a light microscope Primo Star (Zeiss, Germany), and the results were documented using a digital camera Prog Res CT3 (Germany).

RESULTS

During the enzyme histochemical study of the pelvic urethra of male cats, the most significant localization of tissue acid phosphatase activity was established in the propria and the adventitia of the pelvic urethra, followed by the apical parts of the epithelium cells in the disseminated prostate and in the lumen of its glandular tubules. Enzyme expression was also found in the basal membranes **(Fig. 1)**. In the perivascular parts of the blood vessels in the propria and the adventitia the enzyme expression was evident. The weakest acid phosphatase reaction was established in the basal and middle areas of the epithelium cells of the disseminated prostate. Weak acid phosphatase expression was also observed in the loose fibrous connective tissue between the fibres of the skeletal muscles **(Fig. 1)**.

The study on the activity of this hydrolase in the urethral walls in female animals exhibited that the strongest enzyme activity can be observed in the connective tissue between the fibres of the skeletal muscles, followed by the lack of enzyme expression in the propria and the perivascular areas of the urethra **(Fig. 2)**.

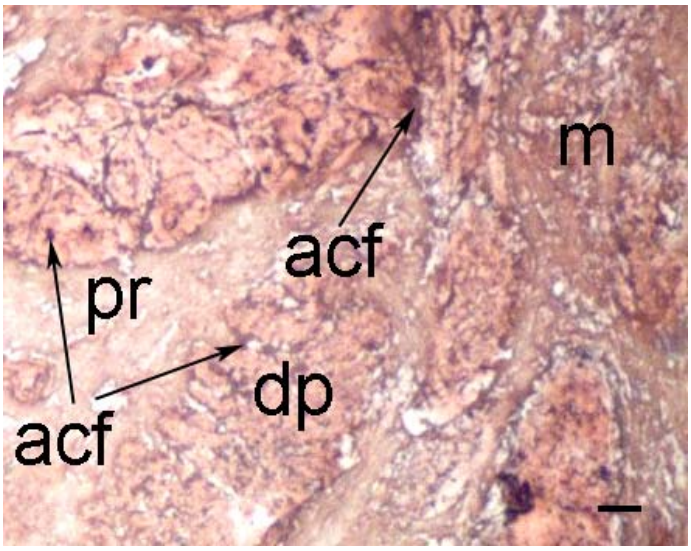

Fig. 1 Acid phosphatase expression (acf) in the wall of the pelvic urethra of a male cat – propria (pr), disseminated part of the prostate (dp), muscular layer (m). Gomori staining. Bar = 60 μm.

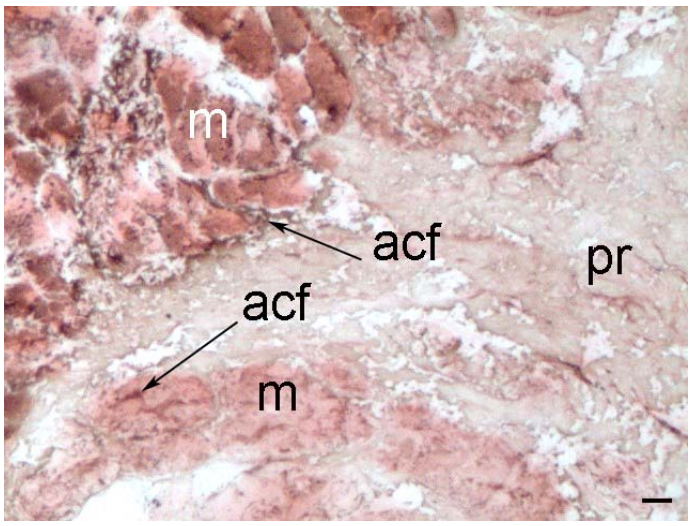

Fig. 2 Acid phosphates expression (acf) in the wall of the urethra of a female cat – propria (pr), muscular layer (m). Gomori staining. Bar = 50 μm.

Significant expression of tissue alkaline phosphatase in the pelvic urethra of male cats was observed in the basal parts of the epithelial cells and the peripheral zones of prostatic lobules from the disseminated prostate. The perivascular areas and the urethral adventitia also showed higher enzyme activity. No enzyme reaction was found in the disseminated prostate tubules' lumen. Weak alkaline phosphatase reactivity was observed in the prostatic epithelial cells **(Fig. 3).**

When the activity of this enzyme was tested in urethra of female animals, expression in the connective tissue between the skeletal muscles fibers was found. No alkaline phosphatase activity could be detected in the rest of the connective tissue elements of the urethra and its epithelium (Fig. 4).

DISCUSSION

The results of the enzyme histochemical study of the pelvic urethra of male cats have proven that the most significant expression of tissue acid phosphatase could be found in the propria and the adventitia of the pelvic urethra, compared to the interstitium of the disseminated prostate, the reason for which is believed to be the lower secretory activity of the prostatic lobules in the urethra's wall.

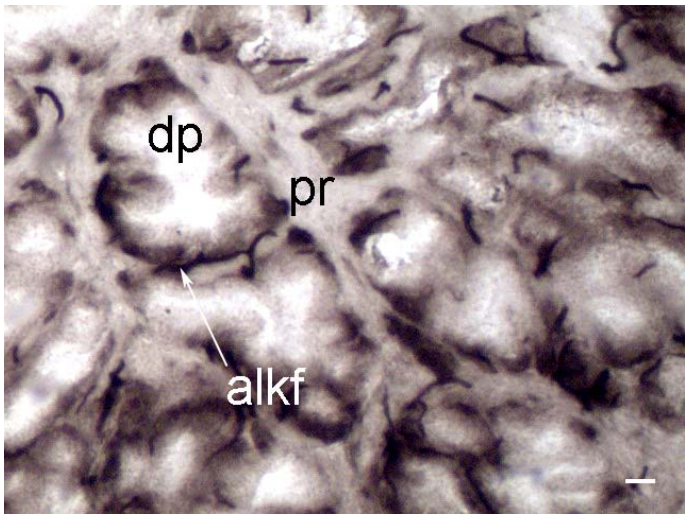

Fig. 3 Alkaline phosphatase expression (alkf) in the wall of the pelvic urethra of a male cat – disseminated part of the prostate (dp), propria (pr). Gomori staining. Bar = 25 μm.

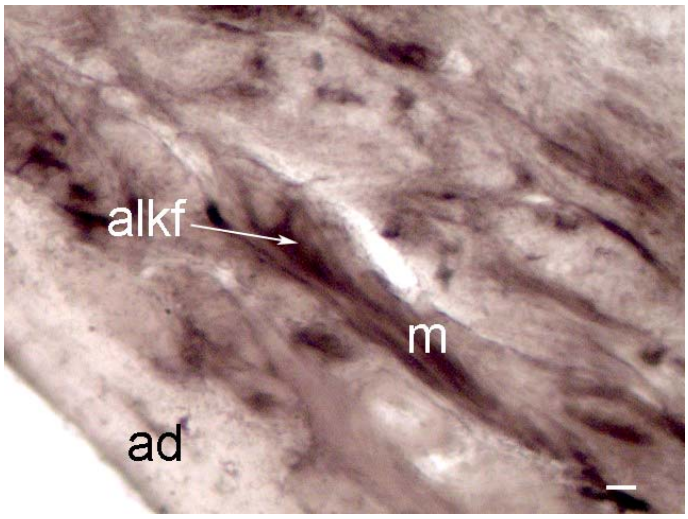

Fig. 4 Alkaline phosphatase expression (alkf) in the wall of a female cat's urethra – adventitia (ad), muscular layer (m). Gomori staining. Bar = 50 μm.

The observed enzyme activity in the apical parts of the epithelial cells of the disseminated prostate and in the lumen of the glandular alveoli corresponded to established data on the prostates of other species and was probably due to the secretory processes in the area (1, 18; 19).

The discovered enzyme expression in the perivascular parts of the vessels in the propria, in the basal membranes and in the zone under the adventitia was similar to the results with human and rat prostates (18).

The weak acid phosphatase activity observed in the loose fibrous connective tissue between the skeletal muscle fibres was probably due to the lack of secretory structures in that part of the organ.

The higher enzyme activity of acid phosphatase that was found in the connective tissue between the skeletal muscle fibres, unlike the lower activity of this enzyme in the propria and the perivascular parts of female feline urethra, was caused by the lack of prostate lobules in the female urethra of this species. The observed low enzyme expression and the accompanying secretory functions in the area are caused by the lacking secretory processes in this part of the female urethra's wall, in contrast with the case with humans (4; 8; 5; 6), rats (12), and gerbils (11; 14).

Our data pointed out that a strong expression of tissue alkaline phosphatase in the pelvic urethra of male cats was histochemically identified in the basal parts

of the epithelial cells and the peripheral zones of the prostate lobules from the disseminated prostate, which is probably due to the role of this enzyme in the transport and absorption processes in this area of the disseminated prostate epithelium. The established enzyme activity in the perivascular areas of urethral adventitia correponded to the activity in canine prostate (1, 19). Alkaline phosphatase expression, however, was not detected in the lumen of the disseminated prostatic alveoli, while the epithelial cells showed a weak alkaline phosphatase activity, the cause for which was the insignificant role of the studied enzyme on the secretory functions in the area.

The alkaline phosphatase expression found in the connective tissue between the skeletal muscle fibres, unlike the other connective or epithelial tissue elements of female cat's urethra, was probably due to the lack of prostate structures, in contrast with their presence in the female urethra in humans (4; 5, 6 , 8), rats (12), and gerbils (11;14).

The collected data on the tissue localization of acid and alkaline phosphatase in the intact pelvic urethra of male cats and the urethra of female cats could be useful in the diagnostics and interpretation of urethral lesions for both sexes of this carnivore species.

Therefore, the results of our study can be used in the differentiation of the normal from the pathologically altered urethra in cats.

CONCLUSION

The lack of tissue alkaline and acid phosphatase activity in the propria of the urethra of the examined female animals is evidence of the absence of structures resembling a female prostate.

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