

LOCALIZATION OF SEROTONIN- AND  
CHROMOGRANIN A-POSITIVE NEUROENDOCRINE  
CELLS IN FELINE PROSTATE GLAND

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**Summary**

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Immunohistochemical investigation of serotonin- and chromogranin A-positive neuroendocrine cells was carried out in prostate glands of 8 sexually mature, clinically healthy male European shorthair cats aged 1–2 years and weighing 2.8–4 kg. For the first time, serotonin- and chromogranin A-positive neuroendocrine cells were described by us in the feline prostate. Light microscopy showed that prostatic neuroendocrine cells were morphologically heterogeneous with irregular small processes and low density. They were located among the adjacent epithelial cells of the glandular alveoli and duct rarely extended to the alveolar lumen. Both serotonin-positive and chromogranin A-positive endocrinocytes were observed, mainly of the closed cell type, i.e. their processes did not extend to the alveolar lumen. Chromogranin A-sensitive cells demonstrated higher density and size compared to serotonin-sensitive ones. The presence of neuroendocrine cells among the cylindrical epithelial prostatic cells was probably related to their role in the regulation of prostatic fluid excretion through the stimulating effect of serotonin upon smooth muscle motility.

**Key words:** cat, chromogranin A, neuroendocrine cells, prostate, serotonin

INTRODUCTION

Neuroendocrine cells are the smallest part of prostatic epithelial cell population and are distributed in all glandular parts. They are morphologically heterogeneous cells with irregular neurite-like processes, situated among the other epithelial cell types. Endocrinocytes are identified by the presence of neurosecretory granules and their potential to express a large group of markers (serotonin, chromogranin A or B, somatostatin etc.). Endocrinocytes are involved in the homeostatic regulation of exocrine prostatic secretion, in the growth and differentiation of the

gland. In canine prostate, serotonin-positive cells were observed, resembling the findings in men. Neuroendocrine cells are characterized with a low density. They are located among the adjacent epithelial cells, extending to the alveolar lumen (open type) or remaining entirely surrounded by the glandular epithelium, and not reaching the lumen (closed type). Neuroendocrine differentiation in canine prostate resembles that of men in similar distribution and cell morphology traits. The main difference in the density of serotonin-positive cells between men and

dogs is that based on the ratio of endocrinocytes number to number of alveoli per observation field, the number of neuroendocrine cells in one glandular alveolus is lower in dogs (Ismail *et al.*, 2002; Sion-Vardy *et al.*, 2004; Sauer *et al.*, 2005; Aprikian *et al.*, 2006).

Some authors (Di Sant' Agnese *et al.*, 1987; Arrighi *et al.*, 1998; Sinowatz *et al.*, 2005) have investigated accessory sex glands in boars, bulls, stallions, donkeys and guinea pigs with regard to the localization of endocrinocytes. In their view, these cells were most commonly located in the prostate complex.

Other research groups (Angelsen *et al.*, 1998; Xue *et al.*, 1999; Amuller *et al.*, 2001; Laczko *et al.*, 2004) established that the density of endocrinocytes in human prostate was higher in the central zone of the gland as compared to the periphery. The density of cells was high around the large collicular ducts and neuroendocrine cells were almost lacking subcapsular peripheral ductules and alveoles.

Prostatic endocrinocytes are dendritic intraepithelial regulatory cells with a dual character – neuronal and epithelial. On the basis of morphological traits of open-type endocrinocytes, it is established that long apical processes extend to the alveolar lumen whereas the short dendritic ones are interwoven among the different epithelial cells. Apical processes receive signals from the luminal content, whereas dendritic ones regulate the secretion of adjacent epithelial cells through a paracrine mechanism. Both afferent and efferent nerve fibres, connected to neuroendocrine cells, are observed. Endocrinocytes do not possess androgenic receptors and that is why they are androgen-independent (Abrahamsson & Anthony di Sant'Agnes, 1993; Xue *et al.*, 1998; Santamaria *et al.*, 2002; Rodri-

ges *et al.*, 2003).

Neuroendocrine cells are established in all three zones of human prostate gland. The presence of neuroendocrine cells among the cylindrical epithelial cells of periurethral ducts is related to their role in the excretion of prostatic fluid into the urethra. Serotonin stimulates the contractility of muscle layers in periurethral ducts in a way similar to its positive role for small intestine peristalsis (Abrahamsson & Anthony di Sant'Agnes, 1993; Xue *et al.*, 1998; Santamaria *et al.*, 2002; Rodrigues *et al.*, 2003).

Neuroendocrine cells are encountered in a number of tissues and organs. Recently, they are called APUD (amine precursor uptake and decarboxylation) cells. At present, they are designated as diffuse endocrine system. Neuroendocrine tumours originate from the diffuse neuroendocrine system. Neuroendocrine carcinomas are described in dogs and cats (Patnaik *et al.*, 2005).

Neuroendocrine cells are also present in prostatic neoplasms and are considered prognostic signs for the development of androgen-independent prostatic lesions. One such tumour is the carcinoid (Di Sant' Agnese, 1995; Deftos *et al.*, 1996; Amorino & Parsons, 2004; Huang *et al.*, 2006).

As seen from the cited reports, prostate gland is investigated in detail by means of immunohistochemistry in men, dogs, and some laboratory rodents. In cats, the data about prostate gland are less complete and are mainly interpreted on the basis of the knowledge about canine prostate. This lack of information was a serious reason for this study.

The purpose of the present study was to perform an immunohistochemical investigation of feline prostate to determine the localization of serotonin- and chromogranin A-positive neuroendocrine cells.

## MATERIALS AND METHODS

The investigation was performed with 8 sexually mature, clinically healthy male European shorthair cats (*Felis silvestris catus*), at the age of 1–2 years, weighing 2.8 to 4 kg. The animals were obtained from a licensed animal shelter.

The experiment was approved by the University Committee on Animal Experimentation and performed in strict compliance with the ethical guidelines for humane treatment of animals as per European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the European Convention for the Protection of Pet Animals, and Law on Animal Protection in the Republic of Bulgaria – part VII (Euthanasia of Animals, art. 45, 46, 47, 48; Section IV- Experiments with animals, as per art. 26, 27, and 28).

Cats were euthanized with intravenous injection of 200 mg Thiopental (Biochemie, Austria) into the cephalic vein. The material was obtained immediately by incision of abdominal and pelvic cavity and removal of prostate gland.

Organ specimens were fixed into 10% neutral formalin, dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin. Immunohistochemical staining was done for detection of serotonin- and chromogranin A-positive neuroendocrine cells using avidin-biotin peroxidase method applied to prostatic tissues. Cross sections of 5 µm were cleared in xylene at 56°C in a thermostat for one hour and rehydrated in a descending ethanol series. Then, they were put into 10% sucrose and distilled water for 24 hours. Further, they were washed in 0.1M PBS (phosphate buffer with pH 7.4), incubated in 1.2 % hydrogen peroxidase and methanol for 30 min and washed in 0.1M phosphate buffer at pH

7.4 for 15 min. Afterwards cross sections were placed in normal mouse serum (DAKO LSAB®) for 30 min, incubated with primary mouse/rabbit anti-human antibodies for 24 hours and washed in 0.1M phosphate buffer (pH 7.4). Then they were reincubated with anti-mouse antibodies (DAKO LSAB® 2 system, HRP K0675) for 4 hours and streptavidin-HRP complex (DAKO LSAB 2 system, HRP K0675) for 4 hours and washed in 0.1M phosphate buffer (pH 7.4) and 0.05M Tris-HCL buffer at pH 7.5 for 10 min.

The reaction was developed by a mixture of 3 mg 3,3'-diaminobenzidine (DAB) (Sigma, St. Louis MO, USA) and 15 ml 0.05 Tris-HCL buffer, pH 7.5 and 36 µL 1 % hydrogen peroxide for 10–20 min and washing in 0.1M phosphate buffer (pH 7.4). Cross sections were dried overnight at room temperature, dehydrated in 95% ethanol, cleared in xylene and embedded in Entellan (Merck, Darmstadt, Germany).

The negative control was run with incubation of control cross sections with non-immune serum instead of the primary antibody (Gulubova & Vlaykova, 2008; Gulubova *et al.*, 2008).

The following antibodies were used:

- polyclonal rabbit anti-cow S-100 proteins (N1573, DAKO A/S Denmark);
- monoclonal mouse anti-HLA DR antibodies (M0746, DAKO) diluted 1:40;
- monoclonal mouse anti-human CD83 antibodies (N1573, Serotec, Oxford, UK) diluted 1:20;
- monoclonal mouse anti-human CD1 antibodies (sc-18885, Santa Cruz Biotechnology, USA) diluted 1:100.

Immunostaining kit DAKO LSAB®2 System, HRP (K0675, DAKO) and DA-

KO® DAB Chromogen tablets (S3000, DAKO) was used as detection system.

Light microscopy was performed with a light microscope Leica DM 2500 (Germany), and data were photographed with a digital camera Leica DSC 290 (Germany). The density (in mm<sup>2</sup>) and dimensions (in µm) of the neuroendocrine cells were measured with an eyepiece micrometer.

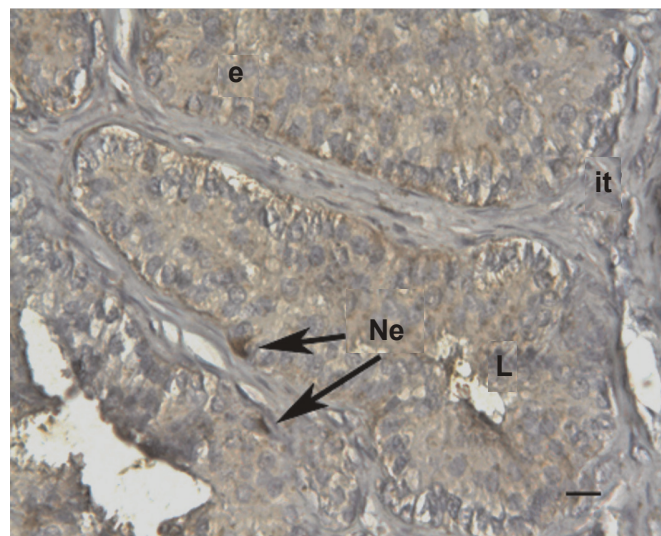
## RESULTS

The neuroendocrine cells in stroma were absent, whereas they were found in the parenchyma – 1.26±0.4 per mm<sup>2</sup> for serotonin-positive neuroendocrine cells and 1.04±0.3 for chromogranin A-positive neuroendocrine cells. The length of the serotonin-positive cells reached 7.1 µm (mean 5.26±0.23 µm) and the width (thickness) varied between 3.8–5.4 µm

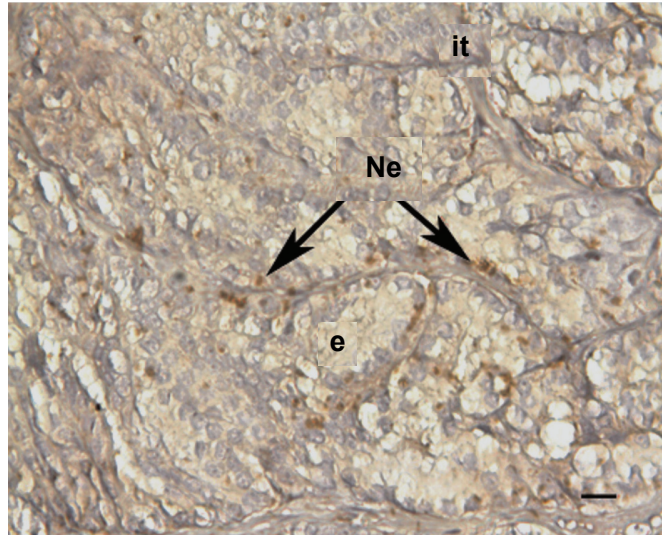
(mean 4.14 ± 0.16 µm). The length of the chromogranin A positive cells reached 8.2 µm (mean 5.93±0.35 µm) and the width (thickness) varied between 4.0–5.6 µm (mean 4.43±0.19 µm).

Prostate neuroendocrine cells were morphologically heterogeneous, with irregular, small processes and low density. Most commonly, the cell body was with an oval shape. These cells were located among the adjacent epithelial cells and more rarely, extended to the alveolar lumen (Fig. 1 and Fig. 3). They were located in the alveolar and ductal part of the gland (Fig. 2 and Fig. 4). Both serotonin- and chromogranin A-positive endocrinocytes were observed, mainly of the closed type, i.e. whose processes did not reach the alveolar lumen.

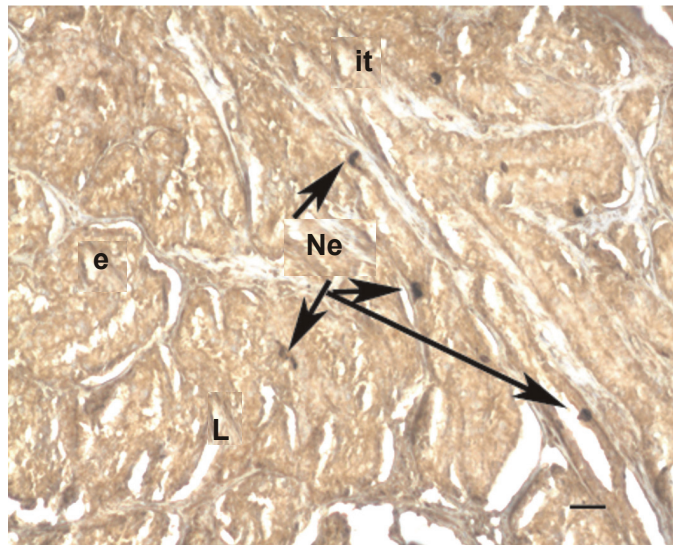
Serotonin-sensitive neuroendocrine cells were located intraepithelially and basally, and frequently reached the basal membrane (Fig. 1 and Fig. 2).



**Fig. 1.** Feline prostate gland: serotonin-positive neuroendocrine cells (Ne) among the glandular epithelium (e); alveolar lumen (L), septum (it). Bar = 7.5 µm.



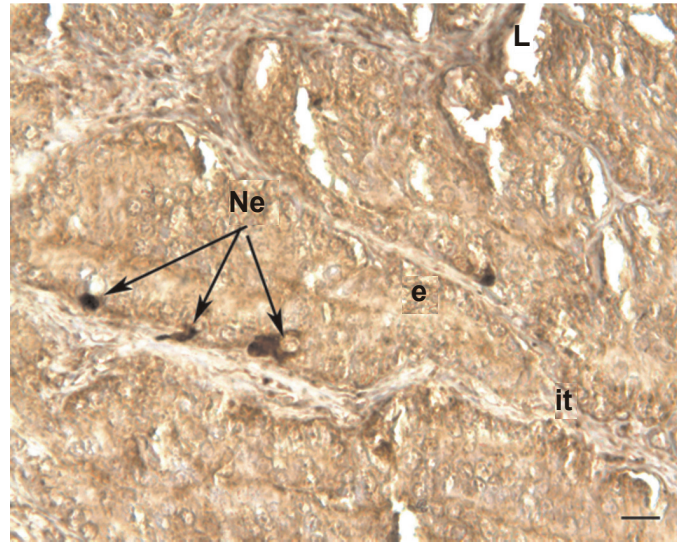
**Fig. 2.** Feline prostate gland: serotonin-positive endocrinocytes (Ne), located basally to the glandular epithelium (e); septum (it). Bar = 10  $\mu$ m.



**Fig. 3.** Feline prostate gland: chromogranin A-positive neuroendocrine cells (Ne) in the glandular epithelium (e); interstitium (it), lumen (L). Bar = 5  $\mu$ m.

Chromogranin A-sensitive endocrinocytes, situated in glandular alveoli and ducts, also showed an intraepithelial localization (Fig. 3 and Fig. 4).

Less frequently, open-type serotonin- and chromogranin A-positive endocrinocytes were observed, that were also located intraepithelially. Their dendritic-



**Fig. 4.** Feline prostate gland: chromogranin A-positive endocrinocytes (Ne) in the glandular epithelium (e); interstitium (it); lumen (L). Bar = 10  $\mu$ m.

like processes extended to the alveolar lumen, i.e. they were of the open type, whereas the cell body was in the apical or middle zones of epithelial layer, covering the glandular alveoli (Fig. 2 and Fig. 3). Chromogranin was more often expressed in neuroendocrine cells showing open-type morphology unlike those where serotonin was expressed (Fig. 3).

#### DISCUSSION

For the first time, serotonin- and chromogranin A-positive neuroendocrine cells were described by us in the feline prostate.

Chromogranin A-sensitive cells were of a higher density and greater size compared to serotonin-sensitive ones.

Our findings that endocrinocytes were the smallest prostatic epithelial cell population, with low density and distributed in the alveolar duct part of the gland, were similar to that the results of Patnaik *et al.*

(2005) about the localization of these cells in other canine parenchymal organs.

The density of neuroendocrine cells in feline prostate gland, observed by us, was relatively lower like their density in human and canine glands (Angelsen *et al.*, 1998; Xue *et al.*, 1999; Amuller *et al.*, 2001; Laczko *et al.*, 2004).

Prostatic neuroendocrine cells were morphologically similar to those of human prostate endocrinocytes described by Ismail *et al.* (2002), Sion-Vardy *et al.* (2004), Sauer *et al.* (2005) and Aprikian *et al.* (2006).

Our findings about feline prostatic serotonin- and chromogranin A-positive endocrinocytes, that were mainly of the closed type and considerably less frequently of the open type, confirmed the conclusions of Patnaik *et al.* (2005) about the morphology of neuroendocrine cells in the dog.

The intraepithelial and basal localization of serotonin- and chromogranin A-

sensitive endocrinocytes supports the views of Xue *et al.* (1998), Santamaria *et al.* (2002), Rodrigues *et al.* (2003) and Di Sant'Agnese (1995), about neuroendocrine cells in human and rat prostate.

The presence of neuroendocrine cells among the cylindrical epithelial cells of periurethral prostatic ducts was probably related to their role in the regulation of prostatic fluid excretion in urethra, via the stimulating effect of serotonin on smooth muscle tissue motility. These findings correspond to the hypotheses of Abrahamsson & Anthony di Sant'Agnese (1993), Xue *et al.* (1998), Santamaria *et al.* (2002) and Rodrigues *et al.* (2003), about the relationship of endocrinocytes to prostatic stroma.

The presence and the localization of neuroendocrine cells in feline prostate gland allowed us to presume their active role in the development of androgen-independent prostatic lesions in this carnivore species.

On the basis of our results, we suggest that feline prostate could be a suitable animal model for investigation of both normal and abnormal androgen-independent growth of human prostate gland.

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