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The activity and immunoexpression of cathepsin D in rat male reproductive organs

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Cathepsin D is a cysteine endopeptidase that belongs to the lysosomal enzyme family. The aim of the study was to evaluate the enzyme immunoexpression and activity in selected male genital organs in mature Wistar rats. The activity of cathepsin D was measured spectrophotometrically in homogenates of the testis, epididymis, seminal vesicle and prostate. Immunohistochemical staining was also performed in the ductus deferens. Enzyme activity was found in the following sequence: testis>epididymis>dorsal prostatic lobe>seminal vesicle>lateral prostatic lobe>ventral prostatic lobe. Although there were differences in enzyme activity between various organs of the male reproductive system, cathepsin D immunoreactivity was seen exclusively in the Sertoli and Leydig cells in the testis.

Key words: cathepsin D, lysosomal enzyme, testis, epididymis, ductus deferens, seminal vesicle, prostate

INTRODUCTION

Both spermatogenesis and spermiogenesis are regulated by various factors, including local ones produced in the testis and epididymis. However, final sperm maturation takes place during ejaculation, when spermatozoa are mixed with fluids secreted mostly by the prostate and seminal vesicles. In previous studies it was found that cathepsins, especially cathepsin D (E.C. 3.4.23.5), play an important role in this process [13]. According to Fouchecourt et al. [8], the enzyme is one of 117 proteins secreted by the epididymal epithelium. The cathepsin D content was lower than that of albumin, lactoferrin and clusterin but similar to cholesterol transfer protein, beta-N-acetyl-hexosaminidase and prostaglandin D2 synthetase.

Cathepsin D is a cysteine endopeptidase that belongs to the lysosomal enzyme family. It regulates intracellular degradation of exogenous and endogenous proteins, activation of enzyme precursors, biosynthesis of peptide hormones, as well as cell growth and maturation. It is also involved in tumour invasion, metastasis formation, inflammatory processes, autoimmune diseases and neuronal degeneration [6, 14, 15].

The cathepsin is stored mainly in lysosomes as a bound fraction, while an extralysosomal fraction is known as a free one. The bound fraction comprises all the active forms of enzymes and proenzymes located inside the endoplasmic reticulum, Golgi apparatus and lysosomes. The free fraction contains only the active forms of enzyme present in the cytoplasm and is not associated with any endoplasmic membranes [17, 21]. The distribution and activity of cathepsin D differ between various organs. Differences were observed even between various types of cells in a single organ [1, 9–11].

The aim of the present study was to evaluate cathepsin D activity and immunoexpression in selected male reproductive organs.

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MATERIAL AND METHODS

The experiment was designed in accordance with international guidelines and guidelines No. 0038/ /2000 of the Local Bioethical Committee.

Sexually mature albino rats of the Wistar CRL:(WI)WUBR strain obtained from a commercial breeder (Rembertow, Poland) were used. The rats were acclimated for at least two weeks, housed and maintained in an animal care facility as described earlier [4–6]. All the animals examined came from the control group used in other studies and had not been exposed to any xenobiotics. The animals were sacrificed on day 84 after the quarantine, which corresponds to the 22nd week of postnatal life.

All abdominal organs were dissected and macroscopically examined. The internal genital organs were removed *en bloc*, weighed, and sectioned. The head, body and tail of the epididymis and the lobes of the prostate were separated. Samples of each genital organ from the right side were fixed in 10% buffered formalin and used for immunohistochemical studies. They were routinely processed, embedded into paraffin blocks and sectioned into 4 μm slices. The Cathepsin D Kit (NCL-Cath-Paraffin, Novocastra Laboratories Ltd.; Newcastle, UK) with monoclonal mouse anti-human cathepsin D antibody (clone C5) was used according to the directions of the manufacturer. Epitope retrieval was applied with two cycles of heating in a microwave oven at 750 W for 5 minutes. The positive control was a sample of invasive ductal breast cancer known to be strongly cathepsin D positive. The negative control was a section treated in the same way as in the study group but with the omission of the primary antibody. The evaluation of immunostaining was made by a light microscope (Olympus BX45).

The remaining organs, except the ductus deferens, were frozen in liquid nitrogen and stored at –20°C until biochemical study. Before being frozen, the seminal vesicles were cut along the longitudinal line and part of the fluid was evacuated by delicate palpation and washed in ice cold physiological saline. After being defrosted at melting ice temperature, the samples were separately homogenised. The total activity of cathepsin D was measured spectrophotometrically. The biochemical procedure is described in detail elsewhere [4, 5].

Comparisons of continuous quantitative data were made between all the organs examined using the Kolmogorov-Smirnov test and the results expressed as minimal and maximal values, mean, standard deviation (SD) and median. Because of continuous variables the differences in enzyme activity were evaluated by Student's t test and ANOVA followed post hoc by Duncan's test. Enzyme activity values in various parts of the male reproductive system were also presented as lg% compared to the corresponding activity of the testis. An $\alpha = 0.05$ ($p < 0.05$) was considered significant.

RESULTS

Uniformly distributed positive immunostaining of cathepsin D was observed in the Sertoli cells of the seminiferous tubule of the testis (Fig. 1A, B). The staining pattern was granular and cytoplasmic. Strong positive immunostaining was also found in the Leydig cells. Germ cells were cathepsin D negative, however, an acrosomic system was also occasionally visualised. No immunoreactivity for cathepsin D was found in the epithelial or stromal cells of the epididymis (Fig. 1C), the ductus deferens (Fig. 1D), the seminal vesicle (Fig. 1E) or the prostate (Fig. 1F). Despite negative immunoreaction in the epithelial cells of the seminal vesicle and prostate, the fluid in the glandular spaces exhibited positive staining for the enzyme. The positive reaction with cathepsin D antibody was always seen in the endothelial cells and thrombocyte plug in blood vessels (Fig. 1A–F). This reaction was an internal positive control for the slides evaluated. The intensity of immunostaining was comparable in cells of the same type.

The greatest activity of cathepsin D was found in the testis and then in the epididymis (head > body > > tail), the dorsal lobe of the prostate, the seminal vesicle, and the remaining lobes of the prostate (lateral > ventral) (Table 1). When compared to that in the testis, the enzyme activity in the seminal vesicle and in the lateral and ventral prostatic lobes was significantly lower (Fig. 2). All parts of the epididymis exhibited a similar activity of cathepsin D ($p = 0.566$; ANOVA = 0.5838). However, highly significant differences were observed between various lobes of the prostate ($p < 0.001$; ANOVA = 31.5836).

DISCUSSION

We have found strong, albeit variable, cathepsin D activity in the homogenates of some male genital organs in rats, despite highly selective immunoexpression confined mostly to the Sertoli and Leydig cells of the testis.

Different results were reported by Igdoura et al. [10], who found strong cathepsin D immunoexpression in both the testis and the epididymis of the five adult

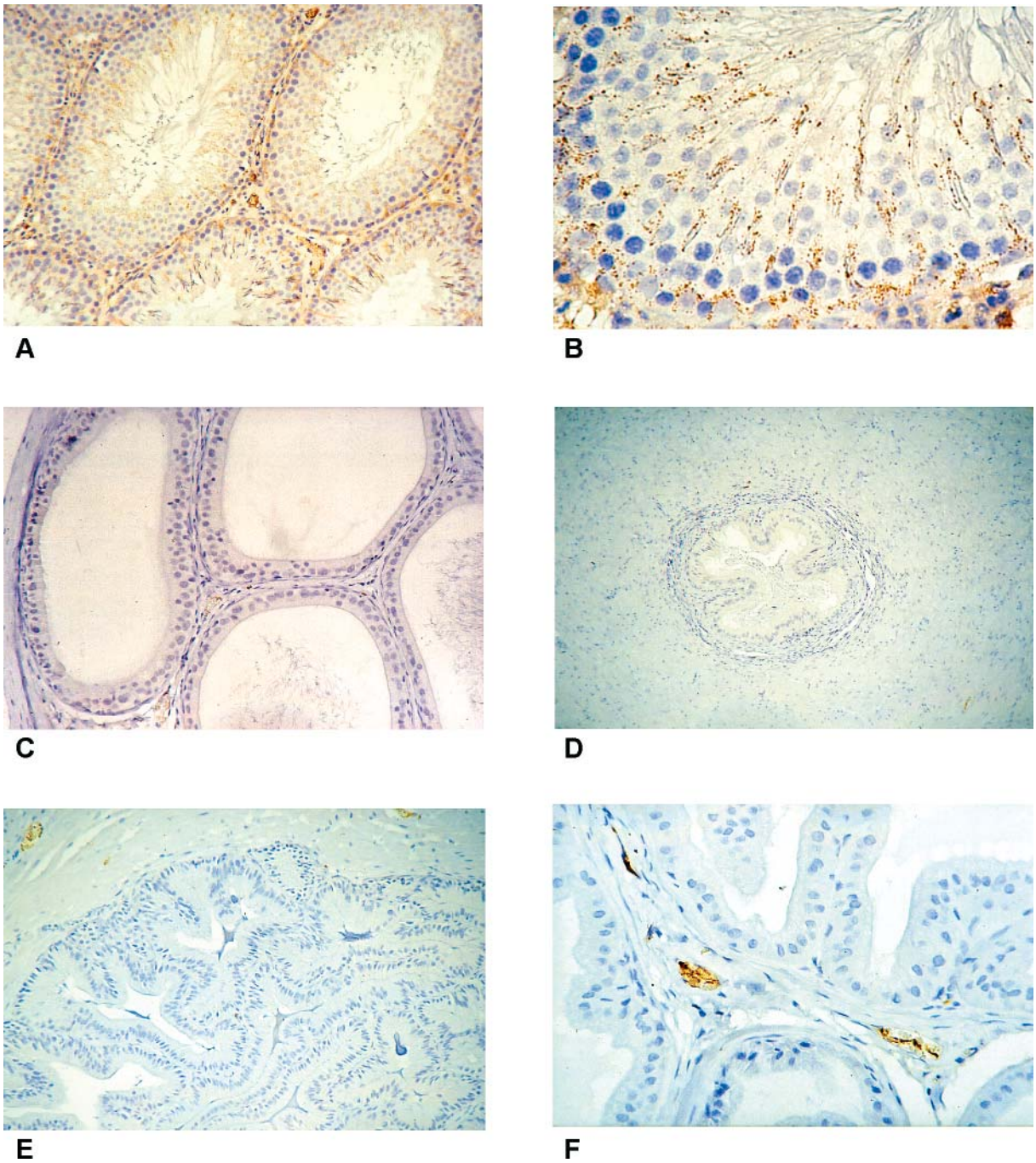

Figure 1 A, B. Positive immunostaining of cathepsin D in the Sertoli and Leydig cells but negative in the germ cells of the rat testis. **C.** Negative immunostaining in the tail of the epididymis. Positive reaction in the endothelial cells. **D.** Negative immunostaining in the ductus deferens. **E.** Negative immunostaining in the seminal vesicle. Positive reaction in the endothelial cells and thrombocyte plugs. **F.** Negative immunostaining in the lateral lobe of the prostate. Positive reaction in the endothelial cells and thrombocyte plugs. (ABComplex/HRP, magn. A, C, E — × 200; B — × 630; D — × 100; F — × 400).

rats examined. The most intense reaction was observed in the basal region of the Sertoli cells, the Leydig cells and the cuboidal epithelial cells in the rete testis. A granular pattern of reaction in the non-ciliated and ciliated epithelial cells was found in the epididymal efferent ducts. The only group of cells without cathepsin D immunoreactivity, with the exception of the acrosomic system of spermatids, was the germ cells. In the epididymis, cathepsin D was found mainly in the principal cells of the body and the proximal part of the tail, the clear cells of the proximal part of the head and the basal cells of the

Table 1. The total activity of cathepsin D (nmol/mg of protein/h) in selected organs of the male genital system of the rat

	Min	Max	Mean	SD	Median	p
Testis	39.670	76.200	61.535	12.618	64.580	–
Epididymis						
Head	38.410	78.330	58.360	13.918	58.470	0.9164
Body	29.000	86.946	59.016	20.578	59.448	0.9164
Tail	34.953	66.000	51.286	11.749	50.136	0.0831
Seminal vesicle	15.040	46.000	36.835	10.781	40.660	0.0063
Prostate						
Dorsal lobe	23.170	76.190	56.778	18.840	58.285	0.8336
Lateral lobe	23.000	48.000	34.428	9.802	33.381	0.0033
Ventral lobe	5.250	12.370	7.845	2.234	7.218	0.0008

p value when compared with the testis

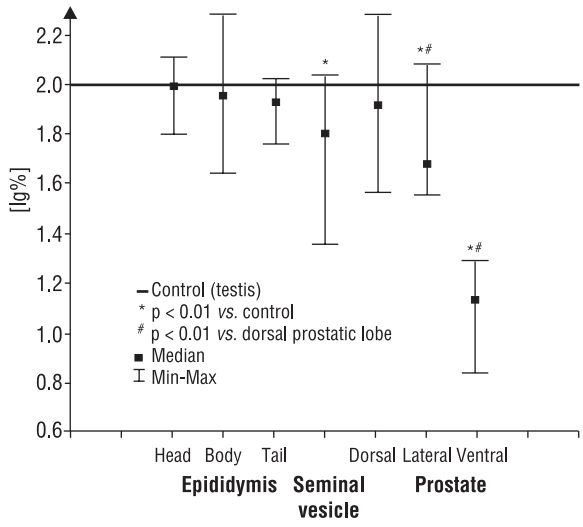

Figure 2. The relative total activity of cathepsin D (lg% when compared to the activity of the testis) in selected organs of the male genital system of the rat.

intermediate zone and proximal head. Similar results were presented in rats after castration and hypophysectomy by Hermo and Andonian [9]. However, in contrast to the previously cited data, no immunoexpression was revealed in the clear cells of the epididymal body and the tail. In another paper the same authors also detected cathepsin D in the ductus deferens [2]. In contrast to our results, weak staining of the epithelial cells of the proximal part and highly intense staining in the middle and distal parts of the ductus deferens were reported. A testicular and epididymal localisation of cathepsin D has also been found in humans [16] and mice [20].

No information on cathepsin D localisation in the seminal vesicles was found in the available literature. However, the enzyme activity was detected in the organ homogenates [20] and seminal fluid absorbed from patients with prostate carcinoma [14]. It seems that the strong immunostaining of the seminal vesicle and prostatic fluid observed in our study explains the enhanced activity of the enzyme revealed in the organ homogenates in the presence of negative immunoreactivity in the tissue.

Cathepsin D was occasionally detected by Wilson et al. [22] in perinuclear lysosomes in the secretory cells of the pancreas, in capillary endothelial cells, and in stromal cells in untreated rats. The number of positively stained cells and the intensity of the staining increased after castration. The effect was time-dependent and after 48 hours autophagolysosomes formed within secretory cells and apoptotic bodies appeared in the epithelium. Although the apoptotic bodies generally exhibited immunoreactivity of cathepsin D, a subpopulation of larger apoptotic bodies which commonly rested on the basal lamina and contained multiple inclusions, were more variable in cathepsin D expression. After 4 days the enzyme was also detected in the basally oriented dendritic cells [22]. Lee et al. [12] found the greatest expression of cathepsin D in periurenthral cells. The staining decreased in the prostatic intermediate and distal segments. However, the staining increased rapidly after castration. Such results were also confirmed by Sensibar et al. [18], who in agreement with our data, found that the activity of the enzyme is lowest in the ventral lobe compared with the lateral. The greatest activity was measured in the dorsal lobe. The same findings were observed in a human study in the normal gland and prostatic cancer specimens [11, 15].

The differences in immunoreactivity are probably secondary to the various antibodies and fixation solutions used in the present study and those cited above [1, 2, 10, 11, 17]. In spite of a high degree of homology in the enzyme amino acid sequences among the various species [3, 7, 17, 19] and in cross-reactivity between human and rat cathepsin D, the antibody used seems to be less sensitive than one prepared specifically for the rat. This explanation remains theoretical, since highly intensive staining was found in the testis, endothelial cells and thrombocytes in various organs of the male reproductive system as well as in the Browicz-Kupffer cells of the liver obtained from the same group of rats [6]. For this reason, attention should also be drawn to potential strain, housing and diet differences.

It could be stressed that, unlike the differences in enzyme activity between various organs of the male reproductive system, the greatest tissue-specific immunoexpression of cathepsin D was observed in the testis. The enzyme activity was found in the following sequence: testis > epididymis > dorsal prostatic lobe > seminal vesicle > lateral prostatic lobe > ventral prostatic lobe.

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