Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 17
Sofia • 2011

Expression of Testicular Angiotensin I – Converting Enzyme in Ageing Spontaneously Hypertensive Rats

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Recent studies demonstrated that testicular angiotensin-converting enzyme (tACE) is essential for fertilizing ability of spermatozoa and the enzyme is regulated by androgens. Relationship between hypertension, disturbance of spermatogenesis and androgen production is suggested. Dramatic changes occurred in the testis during ageing resulting in declined testosterone and sperm production. The aim of the present paper is to investigate the effect of both ageing and spontaneous hypertension on the expression of tACE. The normal stage-specific pattern of ACE immunoreactivity was seen in aging spontaneously hypertensive rats (SHR) but lack of expression was found due to depletion of corresponding stages of spermatid maturation. Values of gonado-somatic index, blood pressure and serum testosterone levels are significantly elevated. Testicular ACE also could serve as a marker for germ cell depletion during aging and pathological conditions.

Key words: angiotensin-converting enzyme, ageing testis, SHR, androgens.

Introduction

ACE is an important component of renin-angiotensis system. Two isoforms – somatic (sACE) and testicular (tACE) are known. Substrate of tACE is not known. In contrast to sACE, tACE does not generate vasoconstrictor peptide Angiotensin II and it is not blocked by ACE inhibitors [4]. tACE is localized in developing male germ cells during elongation of spermatids. tACE plays an important role in the control of the male reproduction [5], being essential for fertilizing ability of spermatozoa [7]. Mice lacking ACE gene exhibited reduced fertility [3].

Androgens (A) are especially important for the maintenance of spermatogenesis and fertility in adulthood proved by experimental models of hormone manipulation and transgenic mice [12]. During ageing dramatic changes in the testis occurred at structural and functional levels manifested by germ cell loss, suppression of steroidogenesis, respectively testosterone (T) production in Leydig cells (LCs) and reduced responsiveness of Sertoli cells (SCs) to androgens (expression of androgen receptor) [2]. Andro-

gen regulation of tACE in germ cells is suggested using experimental ablation of LCs and hence withdrawal of testosterone production by ethane dimethanesulfoate (EDS) (our unpublished data).

Hypertension in spontaneously hypertensive rats (SHR) is androgen-dependent [11] and germ cell depletion accompanied by altered immunoexpression of tACE and elevated T levels was reported in our recent study [1]. The complex relationship between ageing and hypertension in action on spermatogenesis, both influencing androgen production and action, is not studied. In this respect the aim of present study was to investigate effect of both ageing and spontaneous hypertension on the expression of tACE.

Materials and Methods

Male Wistar rats (n=9) and SHR (n=14) at 8 months of age were used in this study. Blood pressure was measured by the tail-cuff method in conscious animals. Total serum testosterone concentrations were measured by RIA as reported previously [1]. Gonado-somatic index was calculated as ration of testis weight/body weight [8].

Immunohistochemistry for tACE: Dewaxed and rehydrated 5 μm testicular sections were subjected to antigen retrieval in 0.01 M Citrate buffer, pH 6 at 95 °C for 5 min water bath. For endogenous peroxidase block, slides were incubated in 3% H_2O_2 in methanol for 5 min at RT. Then, they were blocked for 1 hour in 1.5% donkey serum in PBS. Primary antibody against ACE (1:500) was applied for 30 min at 37 °C. After that goat biotinylated secondary antibody-ABC staining system was applied and liquid DAB was used as chromogen.

Results

The systolic blood pressure of the male SHR at age of 8 months was higher than in age- and sex-matched normotensive Wistar rats (Table 1). Testosterone level was significantly increased in serum of SHR compared to Wistar rats ($p < 0,001$). SHR displayed significantly lower body weight ($p < 0,001$) than control Wistar rats whereas absolute and relative testes weights (expressed as gonado-somatic index) in SHR were significantly higher ($p < 0,05$, resp. $p < 0,001$)

Table 1. Systolic blood pressure, mean serum testosterone level, mean body weight, mean absolute weight of the testes (right and left) and gonado-somatic index in Wistar rats and SHR

Parameters	Wistar rats (n = 9)	SHR (n = 14)	p – value
RR (mmHg)	115,6 ± 4,6	171,8 ± 3,8	$p < 0,001$
Serum testosterone(ng/ml)	0,55 ± 0,28	1,76 ± 0,79	$p < 0,001$
Body weight (g)	411,67 ± 26,92	330,71 ± 36,26	$p < 0,001$
Absolute weight of the testes (g)	3,06 ± 0,28	3,34 ± 0,39	$p < 0,05$
Gonado-somatic index	0,37 ± 0,03	0.52 ± 0,07	$p < 0,001$

Values are expressed as mean _ SD. n= number of rats.

Immunohistochemical analysis of control 8 month rats revealed stage-specific pattern of tACE expression in postmeiotic germ cells in particular elongating spermatids steps 8-19 (Fig. 1A). Spermatogenesis and ACE expression looks as normal as in con-

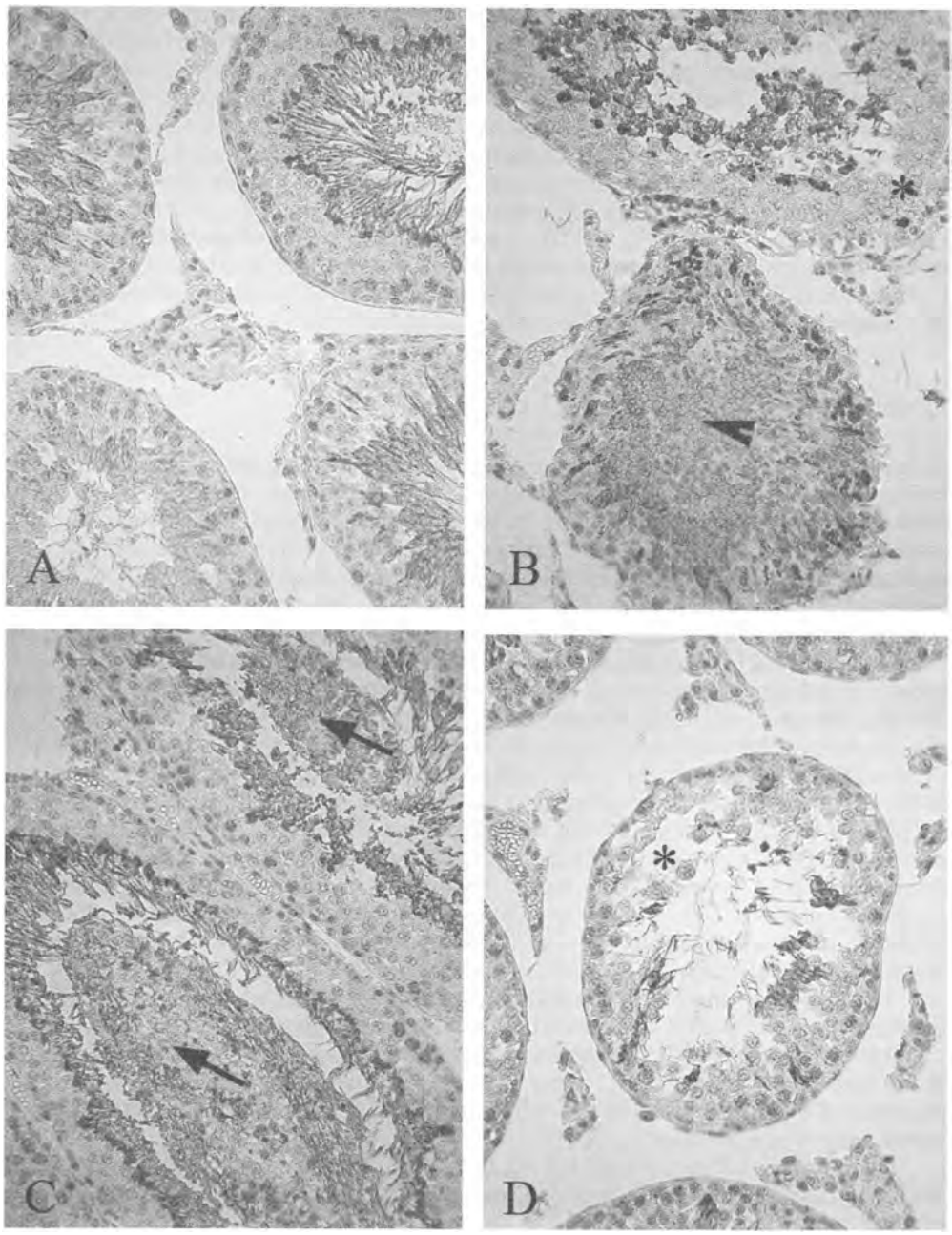

Fig. 1. Stage-specific immunoexpression of tACE in the cytoplasm of differentiating spermatids in ageing 8-month-old rat control (**A**) and SHR testis (**B-D**). **A:** Note the weaker intensity in the cytoplasm of elongating spermatids at step 10-11 in stage X-XI of the spermatogenic cycle; stronger intensity in early stages I-VI, step 15-17 and maximal intensity in middle stage VII-VIII step 19. **B-D:** Note lack of tubular lumen due to germ cells occupied its space (arrowhead); germ cells sloughed off into the lumen (arrows); germ cell depletion from the seminiferous epithelium (asterisks). × 400

trols aged of 2 months. In 8 months SHR rats the stage specific pattern of ACE was not affected (1B-C). Structural changes in the seminiferous epithelium were manifested by abnormal arrangement of germ cell type, respectively abnormal position/topography of the germ cells. Some seminiferous tubules lack lumen and its space was occupied by germ cells Most of them were elongating spermatids recognized by strong ACE immunoreactivity (Fig. 1B). In other cases tubular lumen is formed but filled with germ cells that were sloughed off seminiferous epithelium (Fig. 1C). More severe changes involved different degree of germ cell depletion and loss of mainly spermatids and some spermatocytes was found (Fig. 1B, D). Lack of defined stages of spermatid elongation as well as their slough off into the lumen is well distinguishable due to prominent expression of ACE. Therefore ACE could be used as a marker for germ cell type depleted due to aging or other pathological condition.

Discussion

In several recent papers we reported profound structural and functional changes in the main cell types of the testicular interstititum (perirubular and Leydig cells) and seminiferous epithelium (germ and Sertoli cells) [2, 6]. Aging of seminiferous epithelium is associated with thickening of basal lamina and blood vessels, indicative for disturbed communication between Seminiferous epithelium and interstititum as well as altered testicular trophic during aging. The thickening of the basement membrane in ageing rats and humans was coincidental with changes in the blood-testis barrier and germ cells depletion [10]. In the present study we found disorganization of seminiferous epithelium in particular abnormal arrangement of germ cells probably due to disruption of Sertoli cells–germ cells communications.

Androgens are especially important for maintenance of spermatogenesis in adulthood and their effects on germ cells are mediated via androgen receptor (AR) localized in Sertoli cells [12]. Reduced expression of AR in SCs reported by us in previous studies [2, 6] is probably associated with the functional alterations (decreased responsiveness to androgens) in SCs. It is likely that SCs from aging testis are unable to provide adequate support for germ cells and to respond to selective signal from them. This suggestion is in concert with our current data for elevated serum T levels providing evidence for decreased responsiveness of central nervous system to T. In spite of high T levels in SHR male they show a low sexual behavior [9].

Recently we reported [1] mildly altered expression of tACE in adult SHR (4 month-aged) associated with elevated T levels and high gonado-somatic index. In the current study we found similar trends of changes in aging SHR testis (8 month-aged). In the latter experimental model hypertensive condition is long lasting of 6 months but the value of blood pressure was not extremely high. This could explain relatively unaffected pattern of expression of tACE.

In conclusion, long lasting SHR could be considered as a potential risk factor for male infertility. ACE could be used as a marker for germ cell type depleted due to aging or other pathological condition.

Acknowledgements. We thank Mrs Tereza Dineva and Valeri Ivanov for technical assistance. This study was supported by a grant 7/2009 from Medical University of Pleven.

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