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Stage Specific Expression of Angiotensin I-Converting Enzyme in Adult and Developing Rat Testis

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Recent studies demonstrated that testicular angiotensin-converting enzyme (tACE) is essential for fertilizing ability of spermatozoa. The aim of the present paper is to characterize localization and distribution of tACE in adult and developing rat testis. First immunoreactivity appeared in the cytoplasm of round spermatids step 8 at stage VIII of the cycle of seminiferous epithelium. Later that stage the immunostaining progressively increased and reached maximum at stages IV-VIII. Stage specific localization characterizes tACE as a marker for stages of spermatid differentiation. In the course of the first spermatogenic wave tACE is a marker for developmental stage of germ cell differentiation. Testicular ACE also could serve as a marker for germ cell depletion during experimental and pathological conditions.

Key words: angiotensin-converting enzyme, testis, spermatogenesis, rat.

Introduction

ACE is well-known component of renin-angiotensin system and exists as two isoforms – somatic and testis-specific (germinal) encoded by one and the same gene. Somatic ACE is responsible for the conversion of angiotensin I to the potent vasoconstrictor angiotensin II (Ang II) and inactivation of vasodilator peptides, bradykinin. Thus, sACE is involved in the control of blood pressure and fluid-electrolyte balance [8]. In the male reproductive system sACE is localized in testicular endothelial, Leydig cells; epithelial cells of the epididymis and prostate. The enzyme is expressed transiently in human germ cell during fetal life and is constant feature of germ cell cancer. Ang II locally produced by sACE regulated steroidogenesis in Leydig cells, secretory function of epididymis and tubular contractility in the prostate. Ang II has been shown to maintain sperm motility and to stimulate capacitation [3].

Testicular ACE is transcribed by an alternative promoter in 12 intron of ACE gene. Due to transcription of 13 exon, the N-domain of tACE is unique and C domain is identical in both isoforms [3]. The substrate preference of tACE is still unknown and this

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isoenzyme does not generate Ang II. Testicular ACE has been shown to play an essential role in the control of male reproduction [4] . Dipeptidase activity of tACE was identified as factor of sperm-zona pellucida binding [1]. On the other hand, tACE acts as releasing factor for some GPI proteins anchored on spermatozoa membrane and necessary for fertilization [6]. Based on these findings, it can be suggested that tACE may serve as marker of fertilizing ability of spermatozoa.

Testicular ACE is expressed in spermatids and spermatozoa in human and mice; in rat it is hormone dependent [11]. Consisting data on the expression of tACE in rat testis are lacking. On the other hand, rat provides various experimental models for investigation of hormonal regulation of different factors involved in spermatogenesis. In this respect the current study aimed to characterize specific pattern of localization of tACE in adult and developing rat testis.

Materials and Methods

Immunohistochemistry for tACE. Dewaxed and rehydrated 5 μm 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

Immunohistochemical analysis revealed stage-specific pattern of tACE expression in postmeiotic germ cells. First faint immunoreactivity appeared in the cytoplasm of round spermatids step 8 (stage VIII of the cycle) in a round shape manner (Fig.1A, table 1). Weak intensity was found in elongating spermatids step 9 at stage IX of the cycle of seminiferous epithelium. Later that stage the immunostaining progressively increased and was located in caudally organized cytoplasm of elongating spermatids. Medium intensity of reaction was observed in spermatids step10-11 at stages X-XI of the cycle of seminiferous epithelium (Fig.1B). Immunoexpression became strong later than steps 12 of spermiogenesis (stage XII of the cycle; Fig.1C) and reached maximum in steps 17-19 (stages IV-VIII of the cycle; Fig.1D). Strong immunoreactivity was confined to residual bodies that were numerous in the lumen of seminiferous tubules at stages VIII-IX of the cycle (Fig.1B). Residual bodies were also found in the basal part of the tubules where they were phagocytosed by Sertoli cells. Flagela of late elongated spermatids step 18-19 were also strongly reactive. No immunoexpression was observed in other germ cell types (spermatogonia, spermatocytes) as well as in somatic cells (peritubular cells, Leydig and Sertoli cells).

In the course of the first spermatogenesis tACE appeared in stage-specific manner. Lack of tACE expression in the testis is due to absence of corresponding type of spermatids. Mid-pubertal testis (28-day-old) is negative for tACE as germ cell development proceeds to stage round spermatids 1-3 step (Fig.1E). In late pubertal testis (42-day-old) spermatogenesis are not completed and proceeds to elongating spermatid 16 step in stage III. Imunoreactivity is observed in all the stages with an exception of stages IV-VI (Fig.1F). Lack of reaction in these stages is due to that elongating spermatids step 17-19 did not appear yet.

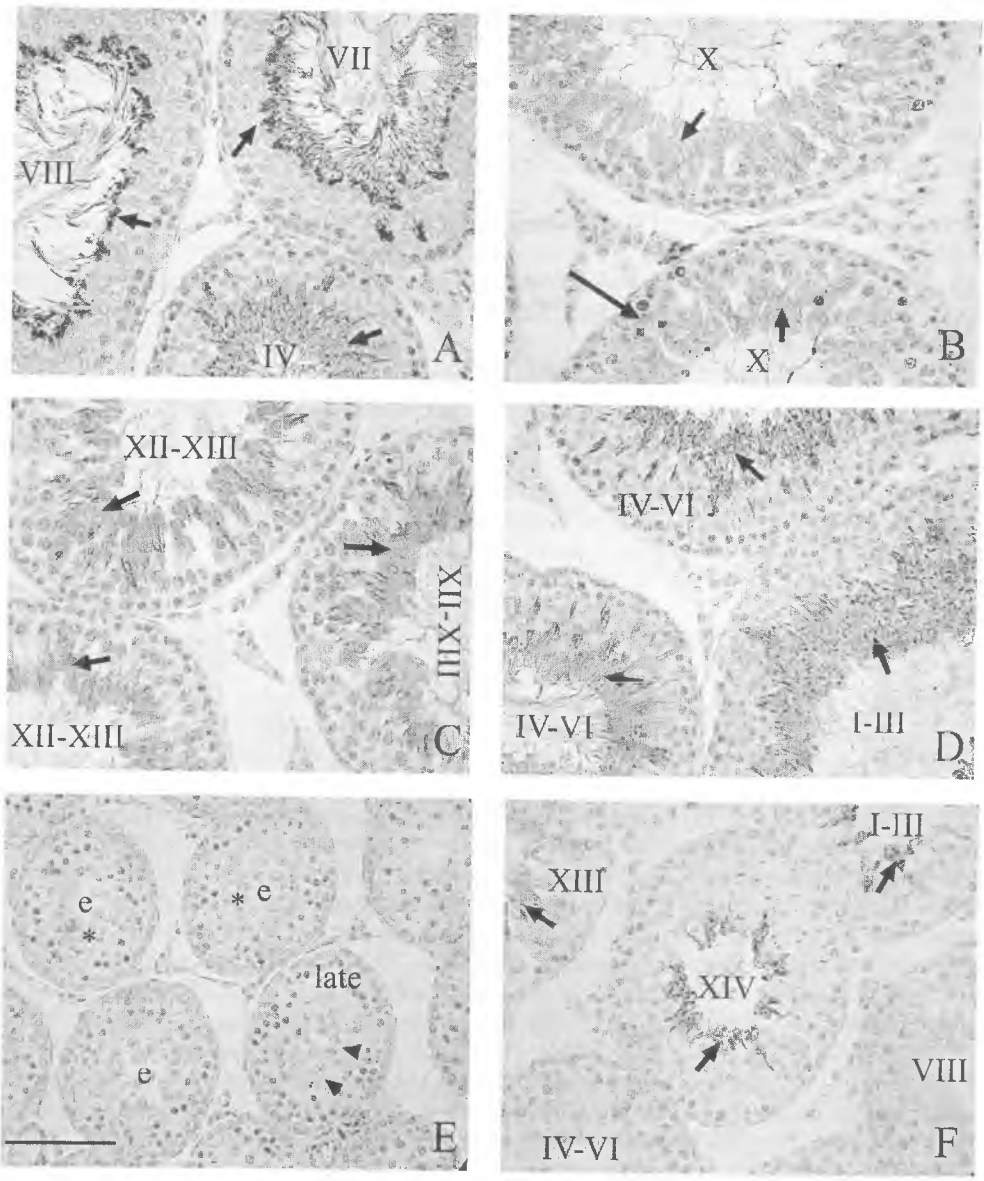

Fig. 1. Stage-specific immunoexpression of tACE in the cytoplasm of differentiating spermatids in adult (**A-D**) and developing (**E-F**) rats. Note the minimal intensity at step 8 and maximal intensity at step 19 of spermiogenesis, both at stage VIII of the spermatogenic cycle. Residual bodies are strongly positive (long arrow). Reaction product in the cytoplasm of elongating spermatids is denoted by short arrows. Negative reaction in 28-day-old rat as germ cell development proceeds to stage round spermatids 1-3 steps (asterisks) in early stages (e); late stage is identified by first and second meiotic divisions (arrowheads). In 42-day-old rat spermatogenesis are not completed and proceeds to elongating spermatids 16 step in stage III. Immunoreactivity is observed in all the stages with an exception of stages IV-VI as elongating spermatids steps 17-19 did not appear yet. Scale bar – 50 μ

Table 1. Semiquantitative evaluation of tACE immunoexpression at the stages of the seminiferous epithelium and steps of spermiogenesis in adult rats

Stages of the seminiferous epithelium								
Steps of spermiogenesis								
VII		VIII		IX	X-XI	XII-XIV	I-III	IV-VI
7	19	8	19	9	10-11	12-14	15-16	17-18
–	++++	–/+	++++	+	++	++/+++	+++	++++

Discussion

The role of tACE in male reproduction and fertilization is proved by knockout models in mice lacking ACE gene [2]. ACE null mice lacking both somatic and testicular ACE are infertile independently of normal testis weight, normal sperm count and morphology. Infertility is due to poor sperm migration in the oviduct and failure to bind zona pellucida. Mutants exhibit also low blood pressure and renal dysfunction. The expression of transgenic sACE exclusively in vascular endothelial cells of ACE-null mice restores blood pressure, but male mice remain sterile, indicated that sACE cannot substitute for tACE in supporting male fertility [5]. Interestingly, expression of testicular ACE in ACE null mice restored fertility.

The present study demonstrated stage-specific expression of tACE in the cytoplasm of male germ cells of 16-week-old normal Wistar rats. Gradual increase of immunostaining was evident from step 8 to step 19 of spermiogenesis. With one exception our results are consistent with data by S i b o n y et al. [10] in 8-10-week-old Sprague-Dawley rats. Discrepancy is related to weaker immunoreactivity in elongating spermatids at steps 15-17 compared to earlier steps. As a result gradual increased immunoexpression of tACE during spermiogenesis is not observed. In another study by L a n g f o r d et al. [7] tACE immunoreactivity in mouse testis was detected later than step 10 spermatids. The differences between our data and those by both author groups could be explained by using different antibodies against the portion common to the testicular and somatic ACE isoforms. Species-specific expression of tACE was demonstrated in human testis where reaction was found only in adluminal membranes of postmeiotic germ cells later than step 3 round spermatids [9] corresponding to step 7 round spermatids in rat.

Stage specificity of tACE localization during spermatogenic cycle characterizes tACE as a good marker for stages of spermatid differentiation. Expression of tACE starts and reaches maximum in androgen dependent stage VIII of the spermatogenic cycle that implies androgen regulation of enzyme production in postmeiotic germ cells. In this respect, future investigations involving proper experimental models for androgen ablation is needed and such studies are in progress by our group.

Localization pattern of tACE revealed the importance of elongation phase of spermatids in male germ cell differentiation with respect to gene expression and not only to morphological modifications. Expression of tACE in postmeiotic germ cells is an example for specific gene activation/translation during spermiogenesis. In the course of the first spermatogenic wave tACE is a marker for developmental stage of germ cell differentiation. Testicular ACE also could serve as a marker for germ cell depletion during experimental and pathological conditions.

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