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Role of Angiotensin I-Converting Enzyme (ACE) in the Male Reproduction: Review

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Angiotensin I-converting enzyme (ACE) is well-known component of renin-angiotensin system (RAS) and kallikrein-kinin system (KKS), both playing an important role in male reproduction. ACE exists in two isoforms – somatic (sACE) and testis-specific (tACE) being differently distributed in the male reproductive system. Knockout of tACE but not sACE caused male infertility. The tACE is expressed in germ cells during spermiogenesis in stage specific manner being a good marker for stage of spermatid differentiation. Expression of tACE in postmeiotic germ cells is an example for specific gene activation and translation during spermiogenesis. In the course of the first spermatogenic wave tACE is a marker for developmental stage of germ cell differentiation. tACE could serve as a marker for germ cell depletion during experimental and pathological conditions.

Key words: ACE, testis, germ cells, spermiogenesis

Spermatogenesis is a complex developmental process that is associated with unique patterns of gene expression. Example is the production of a testis specific isozyme of angiotensin-converting enzyme (ACE). There are two isozymes of ACE in mammals [23]. Somatic ACE is produced by endothelium and other somatic tissues and it is responsible for the conversion of angiotensin I into the potent vasoconstrictor angiotensin II. A second isozyme, testicular ACE, is a tissue-specific gene product expressed only in germ cells. In contrast to sACE, the tACE does not generate vasoconstrictor peptide AngII and it is not blocked by ACE inhibitors. Somatic ACE is composed of two homologous catalytic domains whereas testicular ACE contains a single catalytic domain identical to the carboxyl half of somatic ACE. Both ACE isozymes are encoded by the same ACE gene [11]. The studies on ACE knockout mice has demonstrated that testicular ACE is important for male fertility [10].

Angiotensin I-converting enzyme (ACE, kininase II, CD 134) is well-known component of renin-angiotensin system (RAS) and kallikrein-kinin system, both playing an important role in male reproduction [15, 17]. ACE is membrane bound Zink metalloproteinase dipeptidase that removes 2 residues from C terminus of certain peptides. The enzyme is localized on the surface of endothelial cells. ACE is responsible for the conversion of angiotensin (Ang) I to the potent vasoconstrictor Ang II and for inactiva-

tion of vasodilator peptide bradykinin. ACE acts through two G protein coupled receptors, AT I and AT II. AT I receptors are responsible for vasoconstriction and aldostere one release while AT II receptors are proposed to mediate antagonizing effects and apoptosis [5]. Therefore, ACE has been implicated in the control of blood pressure and fluid-electrolyte balance (Fig.1.)

There is another form of ACE named ACE 2 in humans and mammals. This enzyme is zink metalloproteinase with carboxypeptidase activity that shares approximately 42 % identity with the catalytic site of somatic ACE [20]. ACE 2 is involved in the generation of alternative angiotensin peptides in particular the conversation of Ang II to Ang (1-7), which is vasodilatator and Ang (1-9) [21]. This data suggested that ACE2 can be viewed as a counterbalancing tissue-specific mechanism within the activated RAS. The peptidase ACE2 was localized primary into Leydig cells of the rat testis and in both Leydig and Sertoli cells of the human testis demonstrated by immunohistochemistry. ACE2 was not present in germ cells or endothelial cells, thereby showing a different cellular distribution to the homologous peptidase testicular ACE (tACE), but overlapping with the distribution of the somatic ACE (sACE) [9].

ACE exists in two isoforms – somatic and testis-specific (germinal) and both are encoded by one and the same gene having 26 exons. Somatic ACE mw (170kDa) is responsible for the conversion of angiotensin I to the potent vasoconstrictor angotensin II. Therefore, sACE is involved in the control of blood pressure and fluid-electrolite balance [14]. Somatic ACE is produced by endothelium and several other somatic tissues [13]. The same enzyme is localized in male reproductive system mostly in endothelia cells and Leydig cells of the testis as well as in epithelial cells of the epididymis and prostate. The sACE is expressed in human germ cells during fetal life and is constant feature of germ cell cancer, analyzed by monoclonal antibodies. The ACE gene has evolved from an evolutionary duplication. The sACE consists of two homologous domains, the N- and C- domain, each of them contains an active site with a zinc-binding motif (HEMGH). The shorter molecular variant of tACE (110 kDa), in contrast of sACE, is transcribed by alternative promoter in intron 12 only during spermatogenesis being localized in developing postmeiotic germ cells, spermatids. As a result N-domain is unique in this isoform due to translated exon 13. The C-domain is identical in both, sACE and tACE (Fig.2) [6, 7].

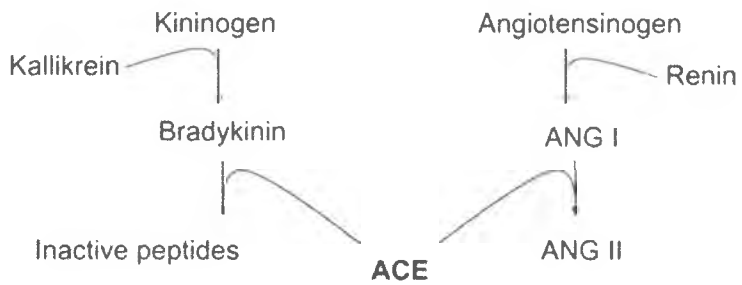

Fig.1. Angiotensin-converting enzyme as key component of rennin-angiotensin-converting system (RAS) and kallikrein-kinin system (KKS). In RAS, the enzyme is the main producer of vasoconstrictor angiotensin II. In KKS, ACE degrades/inactivates vasodilatator kinin such as bradykinin. Thus, the dual effect of ACE in angiotensins and kinins resuls in vasoconstriction.

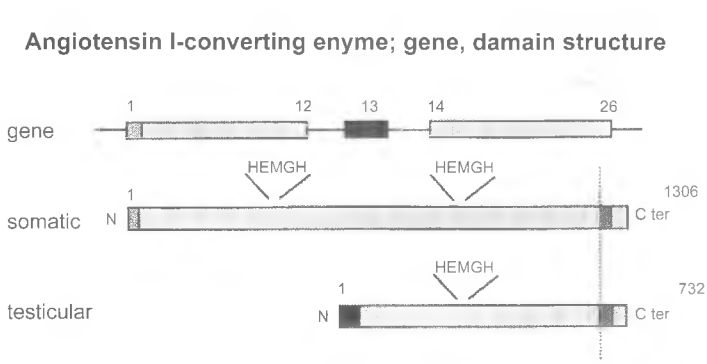

Fig.2. Gene structure of ACE. Two isoforms of ACE are presented, somatic and testicular. They are encoded by a single gene with 26 exons. The sACE consist of two homologous domains (N- and C-domain) each of them with an active site (HEMGH). Difference between both isoforms was observed in tACE which is transcribed by alterantive promoter during spermatogenesis. Thus, only testicular ACE contains of unique N-terminal sequence.

Both of ACE isoenzymes play an essential role in male reproduction system. Main function of sACE is local production of AngII that modulates Leydig cell steroidogenesis and regulates tubular contractility in the prostate. Somatic ACE and generated AngII may contribute to sperm motility, capacitation and acrosome reaction via the angiotensin II type (AT II) receptor [8]. Furthermore, ACE, and mostly its product angiotensin II, is supposed to be involved in the regulation of fluid- and electrolyte transport in the epididymis. Ang II secreted from the basal cells in the epididymis may exert its effect on electrolyte transport by acting on Ang II receptors on the basolateral membrane of the principal cells [22]. Somatic ACE is localized on apical portion of epididymal epithelial cells suggesting participation in remodeling of seminal fluid as well as in detoxification. In addition, sACE cleaves and inactivates LHRH (Luteinizing-hormone-releasing hormone) and substance P, both neuropeptide hormones involved in testosterone production by Leydig cells. Somatic ACE is expressed mainly in human germ cell during fetal development and it is constant feature of intratubular germ cell neoplasia (CIS), being oncofoetal marker [6]. Served as peptidase, the sACE cleaves and inactivates the tetrapeptide goralatide (N-acetyl-seryl-aspartyl-aspartyl-lysil-proline - ACSDKP), which is a natural and circulating inhibitor of proliferation of hematopoietic stem cell and other progenitor cells. ACSDKP blocks the S phase entry of normal but not of neoplastic cells and thus promotes survival and resistance of stem cells to chemotherapy and radiation. Therefore, inhibition of sACE may open new strategies in the prevention of side-effect during cancer therapy [7]. In KKS, sACE is responsible for degradation of bradykinin which stimulates germ cell proliferation [1] suggesting negative role of sACE for germ cell mitotic division.

For better understanding of the role of tACE and sACE in the male reproduction, an insertional disruption of the somatic but not the testicular ACE gene was generated. Males homozygous for this mutation have normal amounts of testicular ACE mRNA and protein but completely lack of somatic ACE and like the mice with complete knockout of sACE they have severe kidney pathology. Nevertheless, homozygous for sACE mutation males have normal fertility, proving conclusively that somatic ACE in males is not essential for their fertility [10]. ACE null mice lacking both somatic and testicular

ACE are infertile suggesting that only tACE has critical importance for male fertility by acting differently compared to sACE [15].

Testicular ACE is germ cell specific isoform that is essential for male fertility. This isoform is expressed in germ cells during spermiogenesis and tACE is localized only in elongating spermatids and spermatozoa. In contrast to sACE, tACE does not generate vasoconstrictor peptide AngII and substrate for tACE has not been identified. Acting as dipeptidase tACE is responsible for release of GPI proteins from sperm membrane that is important for sperm-zona pellucida binding, necessary for fertilization. Acting like a GPI-anchored protein releasing factor, tACE shed various GPI-anchored proteins, mostly PH-20 and Tesp5 from the cell surface of germ cells [12]. Therefore, tACE may serve as marker for fertilizing ability of spermatozoa. The role of tACE in fertilization is proved by knockout models in mice lacking ACE gene. 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 altered sperm migration in the oviduct and their ability to bind zona pellucida [19]. Mutants exhibits also low blood pressure and renal dysfunction. Experiments with transgenic expression of testicular ACE in ACE null mice restored fertility, whereas transgenesis of somatic ACE in ACE mutants does not and mice are infertile. Therefore sACE cannot substitute tACE in male reproduction.

Studies on the human germ cells showed that tACE-mRNA was present in spermatocytes and the mRNA levels increased in spermatids. The gene for tACE could be activated by C-AMP response element modulators (CREMα and CREMτ). In vitro analyses of the testis ACE promoter have identified two important transcriptional motifs within the promoter region TATA box and the other motif highly homologous to the consensus cAMP-response element (CRE). The consensus CRE or its variants have been found in the promoter regions of cAMP-responsive genes. Upon hormonal stimulation, a signal transduction cascade leads to the phosphorylation of a number of CRE-binding proteins, which then exert positive or negative effects on the transcription of cAMP-responsive genes. A unique member of this group of transcription factors is CREM. The CREM gene encodes both transcriptional repressors and activators. CREMτ, functioning as a transcription activator is abundant in male germ cells [23]. Premeiotic male germ cells express only the repressor isoforms of CREM. However, as these cells mature into pachytene spermatocytes, large amounts of CREMτ mRNA are expressed. This developmental switch of CREM expression is induced by follicle-stimulating hormone (FSH). Therefore CREMτ function as a positive regulator in the unique tissue-specific expression of testicular ACE.

ACE activity in the testicular complex is possibly linked with androgens and is involved with spermatogenesis and sperm maturation. The testicular ACE is expressed at high level by developing germ cells and is present in mature sperm. Using of indirect immunofluorescence and immunoperoxidase method, tACE was found in elongating spermatids in the testicular seminiferous tubules as well as in spermatozoa within the epididymal tubular lumen in sexually mature, but not in immature rabbits, suggesting that the presence ot tACE is dependent on sexual maturation on stage-specific manner [15]. The same results were observed in mice and human tACE in testis. Species-specific expression of tACE was demonstated in human testis where ACE was found only in adluminal membranes of postmeiotic germ cells later than step 3 round spermatids corresponding to step 7 round spermatids in rat [7, 16] (Fig.3). The same cellular distribution was described in mice.

Detailed immunohistochemical analysis in our previous studies [2, 3] revealed stage-specific pattern of tACE expression in postmeiotic germ cells in rat testis. The cycle of the seminiferous epithelium in the rat comprises of fourteen stages and spermi-

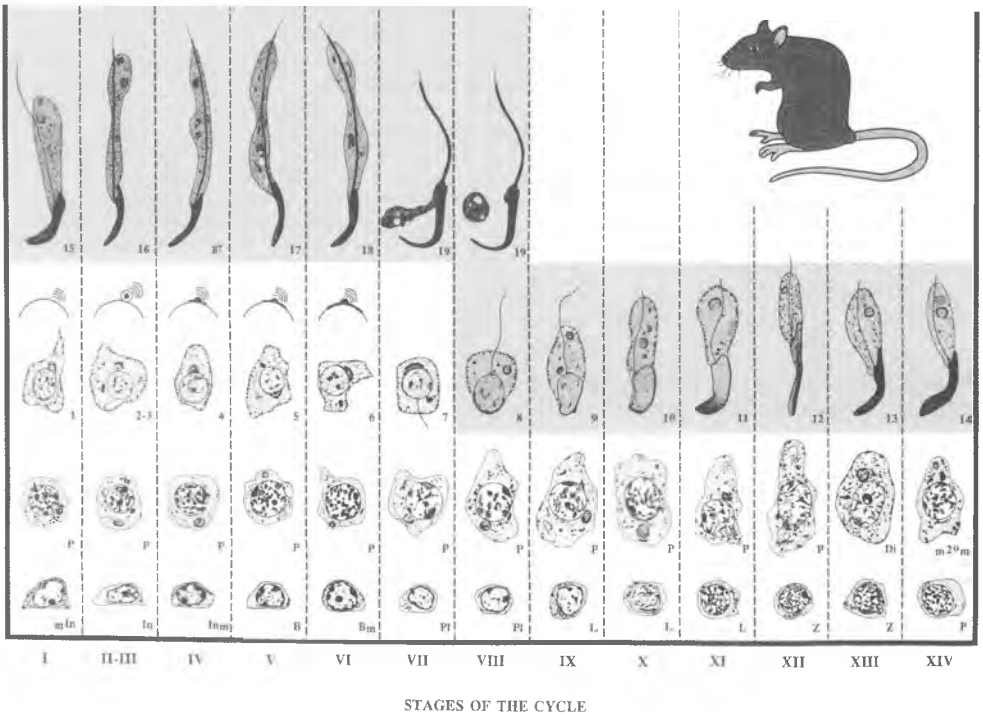

Fig.3. Schematic presentation of expression of ACE during stages of seminiferrous epithelium. On the scheme of the rat spermatogenesis all germ cell types and fourteen stages of the spermatogenesis are illustrated. Expression of tACE spanned from step 8 to step 19 of spermiogensis (marked with grey)

ogenesis involved 19 steps. Schematic and semi-quantitative expression of tACE was shown on Fig.3 and Table 1, respectively. First faint immunoreactivity appeared in the cytoplasm of round spermatids step 8 (stage VIII of the cycle) in a round shape manner. 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. Immunoexpression became strong later than steps 12 of spermiogenesis (stage XII of the cycle) and reached maximum in steps 17-19 (stages IV-VIII of the cycle). No immunoexpression was observed in other germ cell types (spermatogonia, spermatocytes) as well as in somatic cells (peritubular cells, Leydig and Sertoli cells). With one exception our results are consistent with the data by Sibony et al. [18]. Discrepancy is related to weaker expression of tACE in elongating spermatids at step 15-17 compared to earlier steps. In another study by Langford et al [13] tACE immunoreactivity in mouse testis was detected later than step 10 of spermatogenesis. The difference between these author groups could be explained by using different protocols antibodies against the portion common to the testicular and somatic ACE.

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. In late pubertal testis (42 day-old)

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
-	++++	-/+	++++	+	++	++/+++	+++	++++

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. Lack of reaction in these stages is due to that elongating spermatids step 17-19 did not appear yet.

Changes in the expression of tACE were reported in some pathological conditions such as hypertension and cancer. Our previous data [2, 4] in spontaneously hypertension rats (SHR) suggested relationship between hypertension, disturbance of spermatogenesis and elevated androgen production. In 20% of adult SHR (4 month-old) destructive changes in testicular histology were seen manifested by germ cell depletion, and reduced diameter of seminiferous tubules. In experimental group immunoexpression of tACE in spermatids steps 9-14 were more intensive than corresponding steps of the controls. As a result stage-specificity in SHR was not as prominent as in control. Loss of tACE expression in germ cell depleted tubules in SHR is due to absence of corresponding stages of spermatid differentiation. Therefore, tACE can be used as a marker for germ cell depletion due to hypertension. Expression of tACE in postmeiotic germ cell, specifically altered by SHR, suggested possible involvement of component of RAS in the process of spermiogenesis.

In conclusion, stage specificity of tACE localization during spermatogenic cycle characterizes tACE as a good marker for stage of spermatid differentiation. In the rat testis expression of tACE start and reaches maximum in androgen dependent stage VIII of spermatogenic cycle that implies androgen regulation of enzyme production in germ cells. 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 and translation during spermiogenesis. In the course of the first spermatogenic wave tACE is a marker for developmental stage of germ cell differentiation. tACE could serve as a marker for germ cell depletion during experimental and pathological conditions.

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