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

Acta morphologica et anthropologica, 22
Sofia ● 2015

Protective Role of Germinal Angiotensin I Converting Enzyme (gACE) for Sperm and Fertilization: Review

M. Pencheva[1], Y. Koeva[1], N. Atanassova[2]

[1] Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Bulgaria
[2] Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

The present review is focused on the processes in male reproductive system associated with the motility and maturation/capacitation of sperm and it is related to the fact that about 40% of male infertility cases have unclear nature, i.e. idiopathic infertility, which often results from compromised maturation of sperm in the epididymis and other portions of reproductive ducts. One of the enzymes involved in realization process is germinal isoform of angiotensin I converting enzyme (gACE), also known as testicular ACE (tACE). Our study revealed the key role of gACE in spermatogenesis and later in the process of fusion of sperm and eggs, suggesting a possible remodeling and protective function of the enzyme toward male gametes. This study is an attempt to demonstrate the importance of gACE in fertilization process and male reproductive health.

Key words: gACE, testis, reproduction, sperm, male infertility.

Introduction

Semen is a complex organic fluid containing sperm and a complex of proteins that are produced and secreted by male reproductive tract and its accessory glands. Much of proteins produced act in specific areas, while others accompany sperm in their long way of spermatogenesis, ducts of male and female reproductive system to final destination oocytes/ova. During that process, sperm undergo series of physiological and biochemical events associated with sperm motility and capacitation. Investigations on such key proteins are essential for better understanding of the evens responsible for infertility and more specifically called idiopathic infertility.

The present study examines the role of germinal isoform of angiotensin I-converting enzyme (gACE) in male reproduction, in particular its function in male reproductive tract. A number of different cell surface proteins, such as membrane-anchored enzymes, receptors, and adhesion molecules, are targets of specific proteolytic processing that releases their extracellular domain and may function as a posttranslational switch for their biological activity [2, 19, 51].

Structure and Function of ACE

In male mammals ACE is expressed in two isoforms encoded by a single gene and transcribed from two alternative promoters [5, 9]: a somatic ACE (sACE), which is expressed in numerous tissues (vascular endothelium, renal tubule, and intestinal epithelium), and gACE (also known as testicular ACE), which is located on the plasma membrane of sperm released from the testis [48, 55].

The structure of the ACE gene is a result of gene duplication in the distant past. ACE molecule consists of an intracellular domain, a transmembrane domain, N- and C- terminal domains. ACE is anchored to the plasma membrane near the C-terminus of the enzyme. ACE also exists in a soluble form (in blood plasma, amniotic and cerebrospinal fluid and seminal plasma). The soluble form of the enzyme is released with the mediation of unknown protease known as secretory ACE as a result of process called "shedding" [4, 20].

ACE is zinc metalloproteinase that hydrolyses multiple substrates exerting multiple specific functions, some of them are still unknown. Acting as a non-specific dipeptidase, by liberating di- and tripeptides from various proteins, the enzyme may participate, in general processes as peptide metabolism, detoxification and absorption of amino acids at cell surfaces [11].

Angiotensin converting enzyme is an integral part of renin-angiotensin-aldosterone system (RAAS) [36]. The system consists of a cascade of precursors which are transformed into final bioactive products. The main peptides involved are as follows: angiotensinogen, which is converted to angiotensin I (Ang I) by the enzyme renin secreted by the kidney. Ang I is converted by somatic isoform of ACE to potent vasoconstrictor angiotensin II [Ang II or Ang (1-9)]. Therefore, sACE has been implicated in the control of blood pressure and fluid-electrolyte balance [4, 11]. Somatic ACE also played role in male reproduction catalyzing degradation of bradykinin, which stimulated germ cell proliferation [1]. The enzyme is a marker for germ cell neoplasia [37]. Ang II is involved in Leydig cell steroidogenesis via cleavage of LHRH and substance P. Epididymal sACE is responsible for remodeling of seminal fluid [12].

There is another form of ACE named ACE 2. ACE 2 is involved in generation of alternative angiotensin peptides in particular, conversation of Ang (1-9) to Ang (1-7), which is vasodilatator [43].

RAS involved prorenin, renin, angiotensinogen, Ang I and II and ACE and they have been reported in reproductive organs, particularly in the ovary, epididymis and testis [50, 53]. Ang II participated in several important functions for male gametes as sperm motility, acrosome reaction and binding of sperm to the zona pellucida [54, 34].

ACE acts through two G protein coupled receptors, AT I and AT II. Activation of AT I receptors is responsible for vasoconstriction and aldosterone release while AT II receptors are proposed to mediate antagonizing effects and apoptosis [6, 8, 13, 22, 40]. AT1 receptor is present in seminiferous tubules, in particular Sertoli cells and germ cells and as well as in tails of spermatids. In mature sperm AT1 receptor is localized at the base of the tail and in the acrosome area.

Role of gACE in the Testis

Germinal isoform of angiotensin I converting enzyme (gACE) is germ cell specific isoform of male reproductive system that is a key factor for male fertility [53]. In contrast to sACE, gACE does not generate vasoconstrictor peptide AngII and substrate for gACE has not been identified [12]. Germinal ACE is expressed at high level by

developing germ cells and it is also present in mature sperm. Germinal ACE activity is possibly linked with androgens and is involved with spermatogenesis and sperm maturation.

During spermatogenesis the enzyme is detected in germ cells in rodents (mouse, rat) and men. Although, gACE mRNA present in spermatocytes, the gACE protein is found in post-meiotic germ cells (spermatids) during elongation steps in gradually increasing pattern [3, 32, 47]. In adult rat stage specific pattern of protein expression revealed [3]. Faint immunoreactivity appeared in the cytoplasm of round spermatids step 8 (stage VIII of the cycle) in a round shape manner. Later that stage, the immunostaining progressively increased and was located in caudally organized cytoplasm of elongating spermatids. Immunoexpression of gACE 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 of gACE was observed in other germ cell types (spermatogonia, spermatocytes) as well as in somatic cells (peritubular cells, Leydig and Sertoli cells). Similar pattern of cellular localization of and distribution gACE was described in mice [32, 47].

Stage-specific expression of gACE was demonstrated in human testis where gACE protein was found only in adluminal membranes of post meiotic germ cells later than step 3 round spermatids corresponding to step 7 round spermatids in rat [38]. Maximal expression of human gACE occurred in step 7-8 elongated spermatids. Germinal ACE mRNA expression was detected in pachytene spermatocytes and round spermatids. The gACE protein was always strictly confined to the adluminal membrane of differentiating spermatids, leaving the head and acrosomal regions free of detectable immunoreactivity [45]. After sperm release, gACE protein localized to residual bodies and the neck and midpiece region of normal spermatozoa within the seminiferous tubules and ejaculates.

High gACE activity has been determined in human seminal plasma [46]. Immunoelectron microscopy demonstrated that ACE is mainly located at the plasma membrane of the acrosomal region, equatorial segment, postacrosomal region and midpiece [28]. A role of the gACE in capacitation, acrosome reaction and binding to zona pellucida has been suggested by several authors [10, 49].

In spermatozoa the protein is localized in the neck and middle part of ejaculated mature spermatozoa [45]. Dipeptidase activity of gACE 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, gACE shed various GPI-anchored proteins, mostly PH-20 and Tesp5 from the cell surface of germ cells [27, 29]. Therefore, gACE may serve as marker for fertilizing ability of spermatozoa.

Stage specificity of gACE localization during spermatogenic cycle characterizes gACE as a good marker for stage of spermatid differentiation [3]. In rat testis expression of gACE starts and reaches maximum in androgen dependent stage VIII of spermatogenic cycle that implies androgen regulation of enzyme production in germ cells. Localization pattern of gACE 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 gACE in post meiotic germ cells is an example for specific gene activation and translation during spermiogenesis [12].

Expression of gACE was investigated by application of various experimental models in rat as spontaneous hypertension (SHR), androgen deprivation and hyperglycaemia (diabetes mellitus) [3, 31]. Data suggested that gACE can be used as a marker for germ cell depletion in experimental and pathological conditions. In particular, gACE can be recommended for precise visualization and evaluation of spermatid loss that is not optional by routine histological technique.

Developmentally, with the advent of puberty, gACE increased significantly in the testes and ductuli efferentes and to a lesser extent in the more distal regions of the male reproductive tract. The gACE concentration in the testicles is the highest among of the all organs [15].

Studies on human males revealed time-related changes in the cell specific expression of sACE. Switch of both ACE isoforms in human germ cells occurred: sACE is found in foetal gonocytes but only gACE is exclusively expressed in spermatids and spermatozoa. Generally, Sertoli cells show only a weak and markedly diffuse immunoreactivity for sACE. This labelling disappears towards the end of gestation but may be maintained in some seminiferous tubules for months after birth. Both of Leydig cell populations – fetal and adult expressed sACE [38].

Role of gACE in the Epididymis

Once sperm have undergone process of formation and differentiation in the testis, they enter the epididymis. Epididymis is divided into three regions: caput, corpus and cauda. The proximal part of caput epididymis is designated as initial segment. Segmentation of epididymis contributed to establishment of morpho-functional compartments, each of them maintain unique microenvironment due to segment-specific expression of genes encoding signaling molecules, transcription factors, receptors, regulatory and transport molecules. Such segment-specific microenvironment is essential for adequate response of epididymal epithelium to hormones and regulatory factors [52].

Epididymis is a target androgen regulation that is important for segment specific physiology and stimulation of production and secretion of factors interacting with surface proteins located on sperm membrane [15].

Turner et al. suggested that whole amount of gACE in luminal fluid of the epididymis derived from the testis resulting from an active proteolytic process and release of extracellular domain of the enzyme from the sperm surface occurred in the initial segment. Using PCR and Northern blot the author did not found mRNA of gACE suggesting that gACE protein is not produced in the epididymis [14, 35]. Moreover, the amount and sACE mRNA is very low in the epididymis and the protein is hardly discernible. Germinal ACE is absent from the liquid fraction in the epididymis in azoospermic men thereby demonstrating that ACE in luminal fluid of the epididymis is derived from membrane-binding gACE of the sperm [14, 39].

High ACE activity has been determined in human benign prostatic hyperplasia, whereas normal prostates apparently have low enzymatic activity [46]. Vas deferens and seminal vesicles showed low ACE activity [18].

Role of ACE in Female Reproductive Physiology

ACE plays a significant role in ovarian physiology as follicular development, steroidogenesis and formation of corpus luteum, egg maturation, ovulation and follicular atresia. Appearance of ACE in the ovary is regulated by gonadotropins. Angiotensin II bioactive octa-peptide is involved in paracrine and autocrine regulation of different phases of female reproductive cycle [17]. Angiotensin II receptors are AT2 subtype and they are located primarily on granulosa and theca interna cells of atretic follicles [7, 41]. Kuji et al. [30] reported that Ang II facilitates follicular development and ovulation in the rabbit via AT2 receptor, since an AT2 receptor antagonist inhibited hCG- and Ang

II-induced ovulation, oocyte maturation in vitro, as well as, Ang II-induced estrogen and prostaglandin production.

High concentrations of renin and Ang II were found in all utero-placental tissues. The presence of renin mRNA in the endometrium, choroid and fetal part of the placenta proves local renin synthesis. Ang II is important for the contraction of uterine muscles. Ang II is also committed in the regulation of utero-placental blood flow [16].

Germinal ACE and Male Infertility

Role in sperm–zona pellucida binding

Experiments with genetically manipulated mice provided new data about essential role of gACE for male reproduction and fertility. For better understanding of the role of gACE 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 (equal to complete knockout of sACE) is responsible for 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. ACE null mice lacking both somatic and testicular ACE are infertile suggesting that only gACE has critical importance for male fertility by acting differently compared to sACE. Infertility, independently of normal testis weight, sperm count and morphology, is due to altered sperm migration in the oviduct and their ability to bind zona pellucida. In mutant mice lack of expression of gACE on sperm membrane may result in lack of processing of some cell surface proteins of the sperm. Mutants exhibit 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 did not and mice were infertile. Therefore, sACE cannot substitute gACE in male reproduction [42].

Sperm phenotype, i.e., failure of sperm to bind to the zona pellucida, was found not only in mice deficient in ACE gene but in genetic model of disruption of a number of genes, e.g., calmegin (CLGN), calsperin (CALR3), fertilin alpha (ADAM1) and beta (ADAM2) and ADAM3. Data from these knockout models provided new understanding of molecular mechanisms of gACE function in male fertility. In particular, gACE is not a direct mediator of the connection between sperm-zona pellucida. Interactions and relationships of ADAMs proteins with calmegin and calsperin are possibly responsible for zona binding ability of sperm. Because calmegin functions as a testis-specific molecular chaperone for membrane transport of target proteins, there is a possibility of misfolding of membrane surface ADAMs proteins of sperm from CLGN-/- mice. As gACE showed normal activity in CLGN-/- mice, CLGN seems to be not involved in transporting ACE to the sperm membrane. The role of ADAM3 protein and its regulation was investigated using both of GLGN- and ACE- deficient mice. ADAM3 was absent from GLGN-/- and ACE-/- sperm suggesting that both of gACE and GLGN are involved in distributing ADAM3 to a location on the sperm surface where it can participate in sperm-zona pellucida binding [21, 24, 56].

Role in sperm–egg fusion

Similar approach of genetic mouse models was applied for identification of genes responsible for sperm-egg fusion. It is known that only acrosome reacted sperm have an ability to fuse with eggs. During acrosome reaction, sperm shed the plasma membrane

in their acrosomal cap area and expose their inner acrosomal membrane. So far, two essential proteins have emerged to be involved in sperm-egg fusion – CD9 on egg membrane and IZUMO1 on sperm [25]. IZUMO1 is a transmembrane protein with an extracellular region, a single transmembrane region and a short cytoplasmic tail [26]. Mice lacking IZUMO gene (IZUMO -/-) were healthy but males were sterile. They produced normal-looking sperm that bound to and penetrated the zona pellucida but were incapable of fusing with eggs. Human sperm also contain Izumo and addition of an antibody against human Izumo left the sperm unable to fuse with zona-free eggs [23]. In attempt to find an IZUMO1-interacting protein, Inoue et al. [26] proposed gACE3 that has been previously reported by Rella et al [44] as a novel homologue of testis specific, but only at mRNA level. In mice lacking ACE3, the only phenotype found in ACE3-deficient sperm is that the localization of IZUMO1 extended to a broader area than in wild-type sperm having no effect on sperm fertilizing ability. Therefore, elimination of gACE3 did not result in a loss of sperm fertilizing ability, differing from the case of gACE disruption.

Recent data by Li et al. [33] outlined a possible application of gACE as a marker for sperm selection for assisted reproductive technologies. Using Western blot and immunofluorescence, the author suggested that absence of gACE expression is responsible for total fertilization failure and lower fertilization rates by in vitro fertilization (IVF). Therefore, sperm lacking gACE may be recognized before commencing IVF and that the patients may be directed instead to consider intracytoplasmic sperm injection.

In conclusion, our review provides broad understanding of the role of gACE as a novel marker for male fertility. Protein interactions between gACE and other important key factors located on sperm surface underlie molecular events/mechanisms of fertilization. The identification and understanding of the role of gACE in maturation mechanisms of key sperm proteins will pave the way toward novel approaches for both contraception and treatment of unexplained male infertility. Clinical application of gACE in assisted reproductive technologies promises new perspective for development of reproductive medicine.

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