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A Role of Plasminogen Activators in Animal Reproductive Cells and Organs

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ABSTRACT

Plasminogen activators (PAs) are serine proteases that convert plasminogen to plasmin. Two type of PAs are urokinase-type PA (uPA) and tissue-type PA (tPA). Plasminogen is present in most extracellular fluids. PAs play in various reproductive processes including implantation, ovulation and fertilization. In the spermatozoa, PAs and PAIs play a role in sperm motility and fertilization. PAs in the sertoli cell are stimulated spermatozoa maturation and sperm activation through the phospholipase A2. The oocyte maturation is the process for fertilization and implantation. PAs in cumulus-oocyte complexes (COCs) are related to oocyte maturation by protein kinase A and C. In the ovulatory process, PAs activity are changed and it are related to reducing the tensile strength of ovarian follicle wall. The uterine environment is important for reproduction and the uterus undergo tissue remodeling. In the uterus and oviduct of mammals, expression and activity of PAs are changed during estrous cycle. Thus, expression and activity of PAs are concerned to many reproductive functions. Therefore, PAs seem to important factor of regulator in reproductive events.

(Key words : Plasminogen activator, Reproductive organ, Reproductive cells, Plasmin, Animals)

INTRODUCTION

Generally, plasminogen is a zymogen abundant in plasma and in most of the extracellular fluids including uterine fluid (Finlay *et al.*, 1983), ovarian follicular fluid (Beers, 1975) and seminal plasma (Kobayashi *et al.*, 1992). Moreover, plasmin directly or indirectly degrades the extracellular matrix (ECM) by activating matrix matalloproteases (MMPs) (Reich, 1978) and stimulates the physiological events such as activation of growth factors in ECM (Menshikov *et al.*, 2006), angiogenesis (Olofsson *et al.*, 1998), cell migration (Ploplis *et al.*, 1998), tissue remodeling (Martin and Arias, 1982).

The conversion of plasminogen into plasmin is induced by plasminogen activators (PAs), one of serine proteases, and PAs are classified into two groups on the basis of molecule mass: urokinase-type PA (uPA), which is secreted as an inactive single chain molecule of 31-54 KDa, and tissue-type PA (tPA), which is secreted by an active form with a molecular mass of around 70 KDa. PAs play an important role not only in fibrinolysis but also in various reproductive processes including blood vessel fibrinolysis (Carmeliet *et al.*, 1994; Bugge *et al.*, 1995; Ploplis *et al.*, 1995; Carmeliet and Collen, 1996), implantation (Sappino *et al.*, 1989; Teesalu *et al.*, 1996), placentation (Liu *et al.*, 1998), ovulation (Liu *et al.*, 1991), luteolysis (Feng *et al.*, 1993; Liu *et al.*, 1996), parturition (Hu *et al.*, 1999) and fertilization (Huarte *et al.*, 1993). This review focuses on various roles of PAs in reproductive cells and organs in animals.

Tissue-Type Plasminogen Activator

tPA appears as primary mediator of vascular fibrinolysis (Collen, 1980, 1985; Bachmann, 1987). It is synthesized and secreted by endothelial cells and tumor cells such as melanoma cells (Rijken and Collen, 1981; Wallen *et al.*, 1983). This enzyme is synthesized as a single chain protein from a transcript of a gene located on chromosome 8 in the human (Rajput *et al.*, 1985; Verheijen *et al.*, 1986). tPA is synthesized as a proenzyme of molecular weight 70,000 Da. The single chain enzyme undergoes several post-translational modifications for maturation of tPA. The molecule can be proteolytically modified to yield two polypeptide chains by disulfide bonds. The smaller chain contains the catalytic site and the large chain contains several domains about other activity (Gerard *et al.*, 1986). The variants of this molecule have been reported. Ranby *et*

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al. (1982) have reported that two variants of tPA could be isolated from the Bowes melanoma. The variants differed by an Mr of 3000 and the difference is localized in the N-terminal region of the molecule. Rijken *et al.* (1985) have also reported the occurrence of two glycosylation variants from the Bowes melanoma. And Kagitani *et al.* (1985) have reported that a naturally-occurring tPA which is lack of the finger domain, can be isolated. In addition, a Mr=55,000 form of tPA can be isolated from conditioned medium from some cell lines (Dano *et al.*, 1985). This enzymatically-active degradation product is probably the result of extracellular proteolysis.

Urokinase-Type Plasminogen Activator

As a one of the PAs, uPA is the product of a gene that is reported to be located on human chromosome 6 (Kucherlapati *et al.*, 1978) or chromosome 10 (Rajput *et al.*, 1985). This enzyme is synthesized as a single chain molecule and is secreted from cells as a single-chain glycoprotein. In contrast to tPA, single chain uPA has low activity (Pannell and Grewich, 1987), and must undergoes a proteolytic cleavage to yield a disulfide linked 20 chain molecule which expresses high levels enzymatic activity. The non-catalytic chain of uPA contains only kringle domain (Gerard *et al.*, 1986; Collen and Lijnen, 1986). The non-catalytic subunit of uPA contains the domain required for interaction with cellular receptors (Fibbi *et al.*, 1986; Appella *et al.*, 1987). While membrane-bound uPA maintains its fibrinolytic activity, it has a greatly reduced interaction with inhibitors and this activity is increased extracellular proteolytic activity by various cell types expressing urokinase-receptor (Bachmann, 1987). This enzyme contains the catalytic subunit and a small peptide of the non-catalytic subunit. This form of the enzyme is generated by extracellular proteolytic cleavage and low Mr uPA does not bind to cellular receptors (Vassalli *et al.*, 1985). The molecular weight of intact urokinase shows extensive species variation (Hart *et al.*, 1986a,b). The differences between high and low molecular weight uPA, are probably located on both the catalytic and non-catalytic subunits of the enzyme.

Plasminogen Activators in Male Reproduction

PAs and their inhibitors (PAIs) play a role in spermatogenesis, capacitation, sperm motility and fertilization. Sertoli cells in animal testis regulate spermatogenesis by producing various factors including PAs system (Zhang *et al.*, 1997) that plays a role in mammalian sperm motility. Sertoli cells secrete the PAs and FSH stimulates tPA, but not uPA, in the rat testis. Epithelial cells in epididymis and vas deferens secrete uPA, tPA and PAI for spermatozoa maturation (Huarte *et al.*, 1987). PAs are present on sperm surface of ejaculated spermatozoa (Huarte *et al.*, 1987; Smokovitis *et al.*,1987, 1992). uPA and tPA are located in the seminal plasma and the outer acrosomal membrane of human and boar spermatozoa (Smokovitis *et al.*, 1992) and are released during the acrosome reaction (Taitzoglou *et al.*, 1996). Also spermatozoa expresses urokinase-type PA receptor (u-PAR) and they may accept PAs from seminal plasma. That accept may be cause of capacitation, hyperactivation and fertilize capacity in sperm (Liu, 2007). Plasmins localized on spermatozoa act similarly to trypsin-like enzyme that stimulates the acrosome reaction.

Sperm motility is stimulated by uPA (Zheng *et al.*, 2001) and sperm-bound PA is involved in the fertilIzation process (Liu *et al.* 1996; Huang *et al.*, 1997; Ebisch *et al.*, 2007). This hyperactivation can be mediated by plasmin and plasmin induces the acrosome reaction and influences various motility parameters in bull spermatozoa (Taitzoglou *et al.*, 2004). The addition of plasmin to spermatozoa activates the phospholipase A2 (Guerette *et al.*, 1988). The phospholipase A2, a calcium-dependent enzyme, is present in sperm of mammals and it is activator of the acrosome reaction (Llanos *et al.*, 1982). Lysolipids are generated by phospholipase A2, such as LPC, and cis-unsaturated fatty acids are known to be fusogenic substances that accelerate the rate of the acrosome reaction in the presence of calcium (Yanagimachi and Suzuki, 1985).

Limited proteolysis is necessary for sperm surface modifications occurring during capacitation (Talbot and Franklin, 1978; Talbot and Chacon, 1981) and plasmin, as trypsin-like protease, induces the acrosome reaction in capacitated bovine spermatozoa (Taitzoglou *et al.*, 2003). PAs bind to the cell membrane of the spermatoza and increase of local plasmin. Thus, proteolysis is increased locally in spermatozoa, this action may be a cause of step for the initiation of sperm capacitation (Smokovitis *et al.*, 1987; Huarte *et al.*, 1993). And addition of plasmin to fertilization medium increases spermatozoa binding to the ZP (Sa *et al.*, 2006).

Plasminogen Activators in Oocytes

The oocyte maturation and embryo development are the process for fertilization and implantation. When the ovulation, oocytes in many species including human are stopped the meiotic maturation and initiate the maturation after ovulation. The PAs activity in rat (Liedholm and Astedt, 1975), mouse (Strickland *et al.*, 1976; Sherman, 1980), porcine (Fazleabas *et al.*, 1983) and bovine (Menino and Williams, 1987) embryos, and trypsin-like activity in hamster and mouse oocytes (Gwatkin *et al.*, 1973) have been reported. The cumulus cells have a

closely connection during the oocyte maturation in mammals. Cumulus-oocyte complexes (COCs) are related to mature of oocytes and PAs are related with oocyte maturation and embryo development in mammals. PAs are expressed in cumulus cell, theca cell, oocyte, and early embryo and PAs activity is increased. The increase of PA activity in COCs during maturation *in vitro* or *in vivo* is also reported in rat (Liu *et al*., 1986) and pigs (Kim and Menino, 1995) and tPA has been detected in the ooplasm of mouse (Huarte *et al*., 1985) and pig (Kim and Menino, 1995) oocytes. The rat and mouse oocytes and cumulus-oocyte complexes produce uPA and tPA during *in vitro* culture (Liu *et al*., 1986; Liu *et al*., 1987) and *in vitro* meiotic maturation (Huarte *et al*., 1985). In rat, tPA is detected a low amounts in COCs and tPA and uPA activity increases during *in vitro* oocytes maturation (Liu *et al*., 1986). tPA activity is stimulated by FSH and GnRH in both oocytes and cumulus cells of rat (Liu *et al*., 1986; Ny *et al*., 1987) and stimulation of PA activity in rat COCs by FSH and GnRH is through protein kinases A and C (Ny *et al*., 1987; Salustri *et al*., 1985). The PA production by porcine COCs is influenced by protein kinases A and C and kinase inhibitors and both tPA and tPA-PAI activity in *in vitro* cultured COCs increases during IVM (Kim and Menino, 1995). These PAs activity are due to the stimulus with protein kinase A and C of oocyte.

The mammal's blastocysts produce PAs (Sherman *et al*., 1976; Strickland *et al*., 1976; Mullins *et al*., 1980). And plasmin could act as the zona lysin (Mintz, 1972) and effect a "sublysis" of the zona pellucida to facilitate escape by the expanding blastocyst (Baskar *et al*., 1981; Hurst and MacFarlane, 1981). The PAs are detected in embryo development in the rat, and uPA is expressed in the cell cytoplasm and plasma membrane while tPA was detected on cell membrane (Aflalo *et al*., 2005). PAs expression in porcine blastocyst are related to tissue remodeling and tissue proliferation (Fazleabas *et al*., 1983). In bovine embryo, PAs activity were not detected until the blastocyst stage and increases during blastocelic expansion and hatching (Menino and Williams, 1987). The plasminogen concentration in the medium affected the timing at which a particular cell stage (Menino and Williams, 1987).

Plasminogen Activators in Ovulation

Changes of PAs during the preovulatory period initiate the ovulatory process and subsequent corpus luteum (CL) formation. The tensile strength of ovarian follicle wall is reduced by plasmin (Beers, 1975), which requires the action of proteolytic enzymes and plasmin play a role in degradation of ECM through activation of other ECM-degrading enzymes. Also plasmin can directly degrade ECM components including collagenⅣ. In the ovulatory process, the granulosa cell layer around the follicle wall is ruptured and the entire layer is removed (Parr, 1974). Finally, the thecal cell layers are degraded from follicle wall on the point of rupture.

PAs activity in the follicular fluid increases period of ovulation and decrease after ovulation. Changes of PAs activity in ovary are correlated with the ovulatory process and PAs and plasmin activity in follicular fluid are increased by gonadotropin surge. The gonadotropin induced follicle rupture is accompanied by regulation of PAs activity by different cell types in ovary (Liu *et al*., 1987). In the mouse, sheep, and the pig, PAs are markedly increased near the time of ovulation and regulation of PAs in preovulatory follicles appears species-specific.

In the rat, PAs and PAI are secreted by cultured granulosa cells (Ny *et al*., 1985) and injection of serine protease inhibitors suppresses ovulation (Reich *et al*., 1985). After gonadotropin treatment, granulosa cells of rat expressed uPA and tPA but secrete mainly tPA. (Liu *et al*., 1987). In contrast, thecal cells expressed only tPA during preovulatory period (Liu *et al*., 1987). Level of uPA in bovine oviduct and oviductal fluid increases before ovulation and decrease after ovulation (Gabler *et al*., 2001). PAs and uPAR are up-regulated in preovulatory follicles after gonadotropin surge (Dow *et al*., 2002). Especially, tPA mRNA is expressed in granulosal layer of follicles and low level of expression are detected in the thecal layer (Dow *et al*., 2002). In pigs, PAs and PAI are also detected in follicular wall and fluid and granulosa cell (Smokovitis *et al*., 1988). Before ovulation, tPA was induced in porcine follicles (Smokovitis *et al*., 1988).

Plasminogen Activators in Uterus and Oviducts

The uterine environment is important for reproduction. The uterus undergo tissue remodeling such as angiogenesis (Mignatti and Rifkin, 1993) and increase of the number of blood vessel and secretory glands and thickness of uterine wall during the estrous cycle, Implantation and pregnancy. Tissue remodeling requires a balance between levels of protease and their inhibitors and the uterine environment is changed when PAs system is activated. Plasmins may play a role in tissue remodeling through the degradation of ECM and activation of growth factors (Menshikov *et al*., 2006). The PAs system is important factor in various physiologic processes including tissue growth and remodeling. In human, PAs are detected in female reproductive tissue including the endometrium and uterus of pig and rat secrete the PAs during estrous cycle. These endometrial PAs expression are hormonally regulated, and the level

of uPA is increased by progesterone during luteal phase (Casslen *et al.*, 1995). In rat, PAs activity are stimulated by estrogen in uterus (Peltz *et al.*, 1983).

The mammalian oviduct provides a microenvironment for fertilization, early embryo development and gamete transport. Interaction between fertilization process and stages of embryo development differ in oviduct during estrous cycle. The plasminogen system plays an important role in environment of oviduct. In rat and pig oviduct, PAs are activated in the oviductal fluid and are changed during the estrous cycle (Jimenez Diaz *et al.*, 2000; Roldán-Olarte *et al.*, 2005). uPA is expressed higher in the isthmus than in the ampulla (Tsantarliotou *et al.*, 2005) and it is synthesized and secreted into the oviductal lumen when the embryo go through the oviduct. The role of uPA may be involved in the degradation of ECM and in the release of growth factors such as FGF and VEGF (Saksela and Rifkin, 1988, 1990; Plouët *et al.*, 1997).

CONCLUSION

The reproductive events in male and female, such as maturation of sperm and oocyte, capacitation, ovulation and uterine tissue remodeling, are important to animal reproduction. The PAs are secreted by many cell type and stimulate the various physiological and productive events. In male reproduction, PAs regulate spermatogenesis, sperm maturation, capacitation and acrosome reaction. PAs in the sertoli cell were stimulated spermatozoa maturation and sperm activation. The addition of plasmin to spermatozoa activates the phospholipase A2. Moreover, in female reproduction, PAs are related with ovulation, maturation, development of ovum and tissue remodeling of uterus. COCs and expression of PAs are related to maturation of oocytes and embryo development in mammals. PAs activity are due to the stimulus with protein kinase A and C of oocytes. The mammalian oocyte and COCs secreted mainly uPA and tPA. When ovulation, ovarian follicle wall is reptured and this action is related to PAs activity. PAs activity is increased before the ovulation, however, it is decreased after ovulation. The mammalian uterus and oviduct are important for implantation, early embryo development and pregnancy. The uterus and oviduct of mammals undergo the remodeling such as angiogenesis, PAs are changed during estrous cycle, implantation and pregnancy. Thus, expression and activity of PAs are related with the various reproductive events and it is regarded as an important regulator in animal reproduction.

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