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Localization of plasminogen activator and inhibitor, LH and androgen receptors and inhibin subunits in monkey epididymis

Tong Zhang, Cai-Xia Guo, Zhao-Yuan Hu and Yi-Xun Liu[1]

State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China

[1]To whom correspondence should be addressed

Epididymis is a site of sperm maturation and storage. Limited and directed-proteolysis regulated by plasminogen activator (PA), plasminogen activator inhibitor type-1 (PAI-1) and other related factors may play an essential role in these processes. Our previous studies have demonstrated that rat epididymis expressed luteinizing hormone receptor (LHR), tissue type (t) and urokinase type (u)PA, mRNAs, and tPA activity was stimulated *in vitro* by human chorionic gonoadotrophin (HCG). In the present study we further examined localization of mRNAs for tPA, uPA, LHR, androgen receptor (AR), as well as inhibin subunits α, βA and βB in rhesus monkey epididymis. Using in-situ hybridization with digoxygenin-labelled cRNA probes, we have demonstrated that tPA and PAI-1 mRNAs were localized in epithelial cells of adult monkey epididymis. uPA mRNA was localized in the same areas, but to a much smaller extent. tPA, uPA and PAI-1 mRNAs were greatly expressed in the caput and corpus of adult epididymis than in other regions. In-vitro experiments showed that both tPA and uPA activities in epididymal cells were dramatically stimulated by HCG, but not by follicle stimulating hormone (FSH). LHR (but not FSH receptor) and AR mRNAs were localized in the epithelial cells of the epididymis. However, LHR mRNA was detected in both adult and immature infant monkeys, whereas AR was found only in the adult. Inhibin α, βA and βB mRNAs were also detected in this organ, βA mRNA being more strongly expressed in the caput than in other regions of the epididymis. We suggest that LH and androgen may be the key hormones in coordination with the PA–PAI-1 system in regulating epididymal differentiation and sperm maturation.

Key words: epididymis/LH receptor/inhibin subunits/plasminogen activator and inhibitor/in-situ hybridization

Introduction

During their transit through the epididymis, spermatozoa released from the testis undergo a number of morphological and biochemical changes and acquire the capacity for motility and fertilization (Austin, 1985). The luminal fluid microenvironment, which includes specialized enzymes, nutrients and hormones, is believed to be essential for normal maturation and survival of spermatozoa in the epididymis (Bedford, 1975; Hamilton, 1975; Hinton and Palladino, 1995). Loss or modification of the surface molecules of spermatozoa is an important aspect of sperm maturation. Proteases present in epididymal luminal fluid may play an important role in this process (Eddy *et al.*, 1985; Tulsiani *et al.*, 1995). Extracellular proteolysis precisely regulated by the plasminogen activator (PA) system is associated with many physiological processes (Dano *et al.*, 1985; Saksela and Rifkin, 1988; Vassalli *et al.*, 1991). PA is a highly selective serine protease activator that catalyses the conversion of plasminogen into plasmin, a trypsin-like protease which is the key factor in initiating the extracellular proteolysis cascade (Mignatti and Rifkin, 1993).

Both tissue type (t)PA and urokinase type (u)PA, as well as plasminogen activator inhibitor type-1 (PAI-1), were identified in monkey Sertoli cells (Liu *et al.*, 1995a) and might play an essential role in the processes of spermatogenesis and spermiation in testis (Liu *et al.*, 1995b).

PA in spermatozoa has been reported to play a role in the process of fertilization (Huarte *et al.*, 1987; Rekkas *et al.*, 1991; Smokovitis *et al.*, 1992; Lison *et al.*, 1993). tPA and uPA activities were also found in the seminal plasma of human and monkey (Liu *et al.*, 1996). Treatment of the fertile men with testosterone enanthate to induce azoospermia was accompanied by an increase in seminal PA activity (Liu *et al.*, 1996). Immunocytochemical studies showed that both uPA and PAI-1 antigens were localized on the surface of human spermatozoa (Liu *et al.*, 1996). Epithelial cells of rat epididymides expressed mRNAs for tPA, uPA, PAI-1 and LHR, and both tPA and uPA activities in the tissue were stimulated by human chorionic gonadotrophin (HCG) (Zhou *et al.*, 1996, 1997), indicating that PA and PAI-1 may be involved in the process of epididymal sperm maturation. Luteinizing hormone (LH), a hormone capable of regulating both testicular and epididymal functions and so may be an important factor in coordinating the processes of spermatogenesis and sperm maturation through a local autocrine and paracrine regulatory mechanism.

Inhibins and activins are biochemically related proteins and thought to be important paracrine regulators in testes (Mather and Krummen, 1992). Inhibins are heterodimers composed of an α subunit and one of two β subunits (βA or βB), whereas activins are homodimers or heterodimers of the β subunits. Most of the work on inhibin/activin in male reproduction

reported in the literature was restricted to testes, little being known about their expression and function in sex accessory tissues.

Despite the detailed knowledge of mammalian epididymal physiology and anatomy (Robaire and Hermo, 1988; Hamilton, 1990; Setchell *et al.*, 1994), the biological significance of the function of the epididymis and the relationship between sperm maturation and the factors released from the epididymis are poorly understood (Bedford, 1994). A few systematic analyses have been made on human material obtained at surgery or after accidental death in an effort to establish a sound understanding of the epididymis in man, but the results have been rather meagre (Bedford, 1994).

The objectives of the present study were to examine the localization of the mRNAs for tPA, uPA, PAI-1, LH receptor (LHR), androgen receptor (AR) as well as inhibin α, βA and βB in rhesus monkey epididymis and attempt to examine their coordinated expression in relation to sperm maturation in the tissue.

Materials and methods

Animals and tissue collection

Infant (aged 18–24 months) and adult male rhesus monkeys were provided by Beijing Biomedical Institute and Primate Research Center, Kun-ming Institute of Zoology, China. Epididymides were collected from the animals (which had been freshly killed for other purposes) and embedded in Tissue-Tek OCT compound (Miles Inc, Elkhart, IN, USA), rapidly frozen in *n*-hexane at –80°C and stored at –80°C until use.

Probe preparation

Single-stranded RNA probes for in-situ hybridization were prepared from cDNA fragments. The preparation of plasmid vector containing cDNA fragments of monkey tPA, PAI-1 were reported previously (Liu *et al.*, 1995b). Monkey uPA, LH and FSH receptor cDNA were provided by Dr Ny (Umeå University, Sweden). AR was donated by Professor Zhang Yong-lian (Shanghai Institute of Biochemistry, Chinese Academy of Sciences). Inhibin α, βA and βB subunit cDNA plasmids were kindly provided by Dr Hsueh (Stanford University, USA). Digoxigenin-labelled RNA transcripts of sense and antisense strands were synthesized from linearized plasmids by in-vitro transcription with digoxygenin-UTP (dig-RNA labelling kit; Boehringer Mannheim GmbH, Mannheim Germany). The labelled UTP is incorporated into the transcripts at approximately every 20–25th nucleotide, which is most suitable for detection of anti-digoxygenin antibodies (Boehringer Mannheim). Restriction endonucleases were purchased from Promega (Madison, WI, USA).

In-situ hybridization

Cryosections of 15 μm were cut using a cryostat (–20°C) and thaw-mounted onto poly-L-lysine-coated glass slides. The slides were air-dried and fixed in 4% paraformaldehyde (Merck, Darmstadt, Germany) in phosphate-buffered saline (PBS). Following fixation, sections were digested with 0.2 N HCl for 25 min at 37°C, incubated in PBS containing 0.3% Triton X-100 for 15 min, rinsed in PBS and post-fixed for 5 min. After washing three times in PBS, the sections were incubated in 0.1 M triethanolamine containing 0.25% acetic anhydride for 15 min to reduce background and then prehybridized in a solution containing 2× sodium chloride/sodium citrate (SSC; 0.3 M NaCl/0.03 M Na_3 Citrate) and 50% deionized formamide for 2 h at 40°C. The sections were hybridized with digoxygenin-labelled antisense or sense cRNA probes in hybridization solution (2× SSC/50% formamide/0.5% sodium dodecyl sulphate/250 μg/ml yeast tRNA/1× Denhardt's/10 mM Tris, pH7.5). All reagents were obtained from Boehringer Mannheim. Hybridization was performed in a humidified box for 20 h at 45°C. The sections were washed at 37°C in 2× SSC for 2 h; 1× SSC for 2×30min and at 40°C in 0.1× SSC for 2×15 min. The hybridized probes were detected using alkaline phosphatase-coupled anti-digoxygenin *Fab* fragment. Colour reaction was developed by incubation with Nitro Blue Tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in colour development buffer (100 mM Tris/150 mM NaCl/50 mM $MgCl_2$, pH9.5). The reactions were terminated by immersing the slides in 0.1 M EDTA.

Cell preparation and culture

All procedures were performed under sterile conditions. Freshly removed epididymides of adult monkeys were separated from fat and other tissues and the caput region was removed and minced. The segments were washed in PBS several times to remove spermatozoa and incubated at 35°C in McCoy's 5a medium (modified without serum; Sigma, St Louis, MO, USA) containing 0.5 mg/ml collagenase and 5 μg/ml DNase I (Gibco BRL, Gaithershurg, MD, USA) with shaking to obtain the epididymal cells. The cells (1×10^6 cells/dish) were incubated for 48 h at 35°C under a water-saturated atmosphere of 5% CO_2 and 95% air in 1 ml serum-free McCoy's 5a medium in the presence or absence of FSH (F, 100 ng/ml), LH (L, 100 ng/ml) (National Hormone and Pituitary Program, Bethesda, MD, USA) and forskolin (FK, 10^{-4} M) (Sigma) (Liu *et al.*, 1995b; Zhou *et al.*, 1996, 1997). The media were then assayed for PA activity.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The activity of tPA and uPA in the cell-conditioned media were assayed following fractionation by SDS–PAGE according to Laemmli (1970) with modifications (Liu *et al.*, 1986). 150 μl cell-conditioned medium containing a final concentration of 2.5% SDS was placed in each well of the gel chamber. Electrophoresis was performed at 100 V for 30 min and then at 50 V for 16 h, until the dye front reached the bottom of the gel. After electrophoresis the gels were washed twice in a solution of 2.5% (V/V) Triton X-100 for 45 min to remove SDS, prior to analysis on fibrin gels.

Fibrin overlay method

The fibrin–agar indicator gel was prepared based on a method developed by Granelli-Piperno and Reich (1978) with modifications (Ny *et al.*, 1985). The fibrin-agar gel contained 25 μg/ml plasminogen as the zymogen for PA. Fibrinogen (2.4 mg/ml) and thrombin (0.5 IU/ml) were also added to allow the formation of fibrin as the substrate for plasmin. Washed SDS–PAGE gels containing samples which had undergone electrophoresis were laid carefully onto the fibrin-agar indicator gels and incubated at 37°C in a humidity chamber for varying periods until the lysis zones became visible, indicating the presence of PA.

Statistical analysis

All experiments for in-situ hybridization and PA activity were repeated at least three times. Figures show one representative experiment from three similar results.

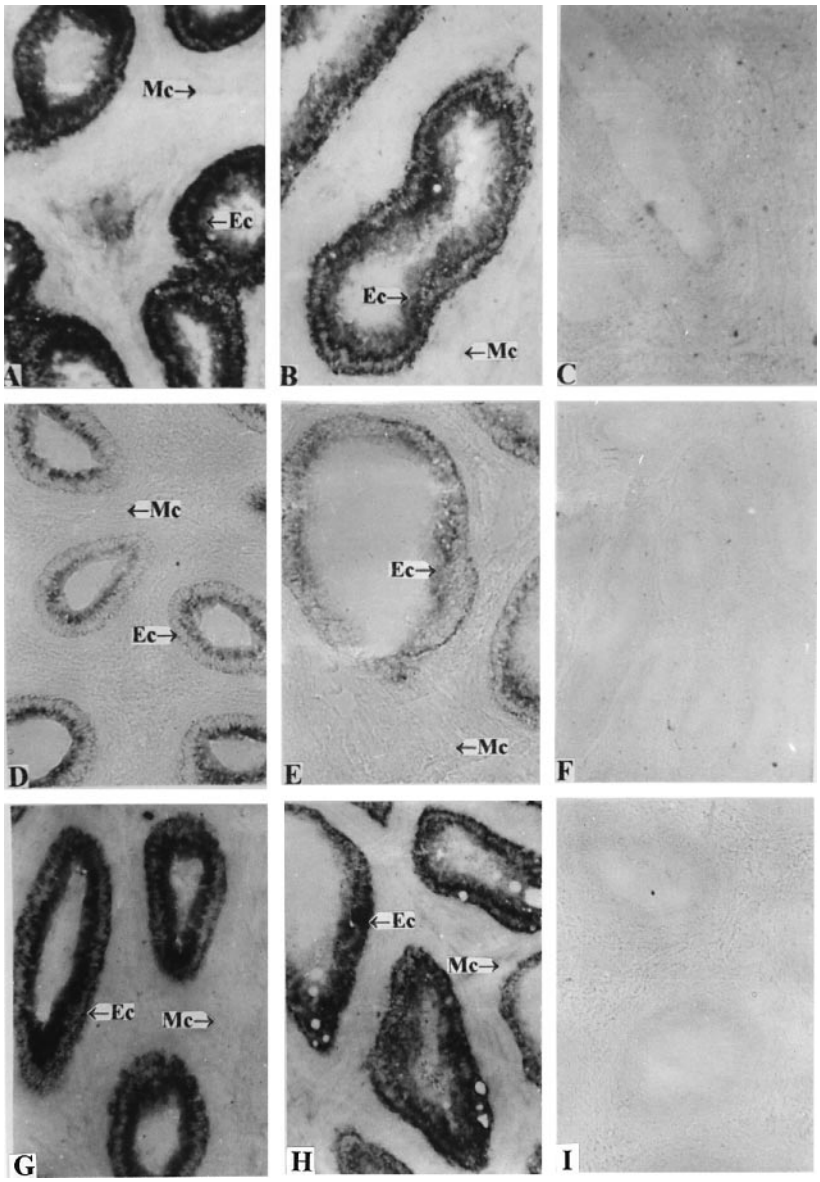

Figure 1. Localization of mRNAs for tissue type (t) plasminogen activator (PA) (**A**, **B** and **C**), urokinase type (u)PA (**D**, **E** and **F**) and plasminogen activator inhibitor type-1 (PAI-1) (**G**, **H**, **I**) in caput (**A**, **D** and **F**) and corpus (**B**, **E** and **H**) of adult rhesus monkey epididymis. **C**, **F** and **I** represent digoxygenin-labelled sense cRNA for tPA, uPA and PAI-1 respectively, serving as negative controls. Ec = epithelial cells; Mc = mesenchymal cells. (Original magnification ×320).

Results

In-situ localization of tPA, uPA and PAI-1 mRNAs in rhesus monkey epididymides

In-situ hybridization using digoxygenin-labelled antisense cRNA probes demonstrated that high levels of tPA (Figure 1A) and PAI-1 (Figure 1G) mRNAs were expressed in the caput of the epididymal epithelial cells (Ec) of adult rhesus monkey. uPA mRNA had the same localization but the amount expressed was much lower (Figure 1D). The initial and cauda regions of the epididymis expressed lower levels of mRNAs for tPA, uPA and PAI-1 than the caput and corpus regions (not shown). No detectable amount of these mRNAs could be found in the epididymis of infant rhesus monkey (not shown). Figure 1C, F and I show digoxygenin-labelled sense cRNAs for tPA, uPA and PAI-1 respectively.

In-situ localization of mRNAs for LH, FSH and AR in rhesus monkey epididymides

As shown in Figure 2, LHR mRNA was expressed in the epithelial cells (Ec) of both infant (a) and adult (b) rhesus monkey epididymides. The expression level was high in infant monkey (Figure 2a), with no obvious difference among various regions of the epididymis (A and B), whereas the caput epididymis of adult monkey (Figure 2b, B) expressed much higher amount of LHR mRNA than initial (Figure 2b, A), corpus (Figure 2b, C) and cauda (Figure 2b, D) regions. No detectable level of FSH mRNA was found in adult (Figure 3) and infant (not shown) monkey epididymis.

Androgen is essential for maintenance of epididymal function. As shown in Figure 4, a high level of expression of AR mRNA was present in the epithelium (Ec) of the adult monkey epididymis. The expression level was higher

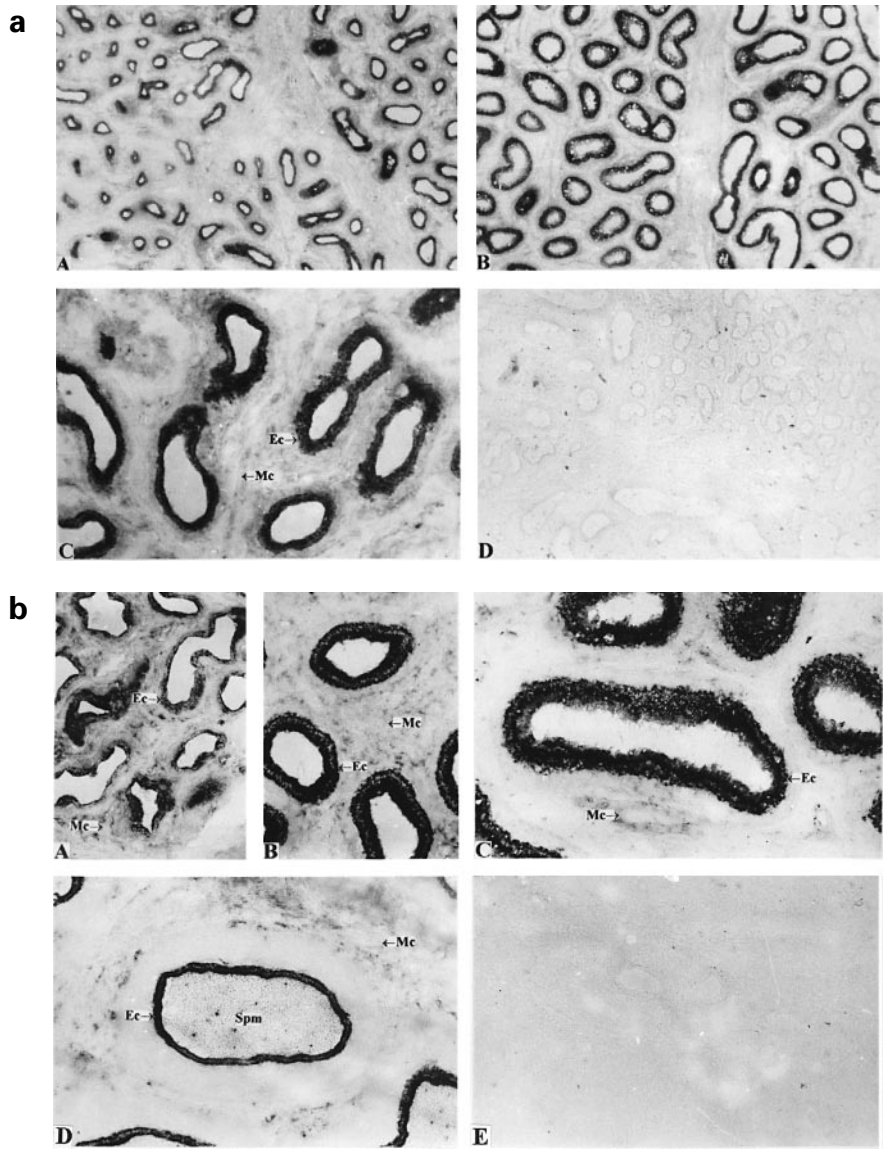

Figure 2. Localization of luteinizing hormone receptor (LHR) mRNA in infant (**a**) and adult (**b**) rhesus monkey epididymis. **A**, **B** (original magnification ×126) and **C** (original magnification ×320) represent different regions of initial, caput, corpus and cauda respectively. **D** in (**a**) and **E** in (**b**) represent digoxygenin-labelled sence LHR cRNA negative control. Ec = epithelial cells; Mc = mesenchymal cells; spm = spermatozoa.

in the caupt region (Figure 4B) in comparison with the other regions (Figure 4A, C and D).

In-situ localization of inhibin α, βA and βB mRNAs in rhesus monkey epididymides

Using in-situ hybridization with digoxygenin-labelled cRNA probes, we found that all of the three inhibin subunit genes were expressed in the epithelial cells (Ec) of the adult monkey epididymis as shown in Figure 5. The expression levels of subunit α (A, B, C) and βB (G, H, I) genes in various regions were similar, but inhibin βA was expressed to a much greater extent in caput epididymis (Figure 5D) than in other regions.

LH stimulation of tPA and uPA activities in cultured monkey epididymal cells

As shown in Figure 6, the epididymal cells in the basal culture conditions (c) secreted only uPA. Addition of FSH (F) to the culture did not change the profile of uPA secretion. Inclusion of LH (L) in the medium, however, dramatically enhanced the uPA secretion and also induced tPA secretion. Forskolin (FK), a activator of adenylate cyclase, was able to mimic the action of LH, greatly stimulating both tPA and uPA activities.

Discussion

Increasing evidence has demonstrated that a number of proteins produced by the epididymal epithelium are involved in various processes related to loss or modification of sperm surface molecules (Eddy, 1988) and motility (Turner and Giles, 1982), and sperm maturation and fertilizing ability (Orgebin-Crist and Jahad, 1978). Proteolysis locally generated in the epididymis by PA–PAI-1 system may be involved in these processes. It is, therefore, not surprising that high level of PA and PAI-1 were detected in the monkey epididymis, although a lower level of uPA mRNA than tPA was detected by in-situ hybridization.

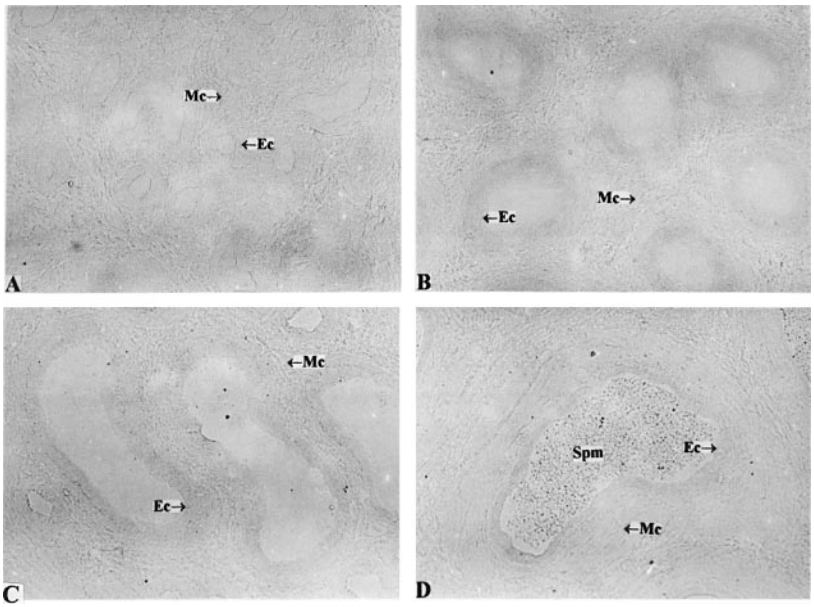

Figure 3. Localization of follicle stimulating hormone receptor (FSHR) mRNA in epididymis of adult rhesus monkey (original magnification ×320). **A**, **B** and **C** represent various regions of the epididymis. **D** serves as sense probe negative control. Ec = epithelial cells, Mc = mesenchymal cells; spm = spermatozoa.

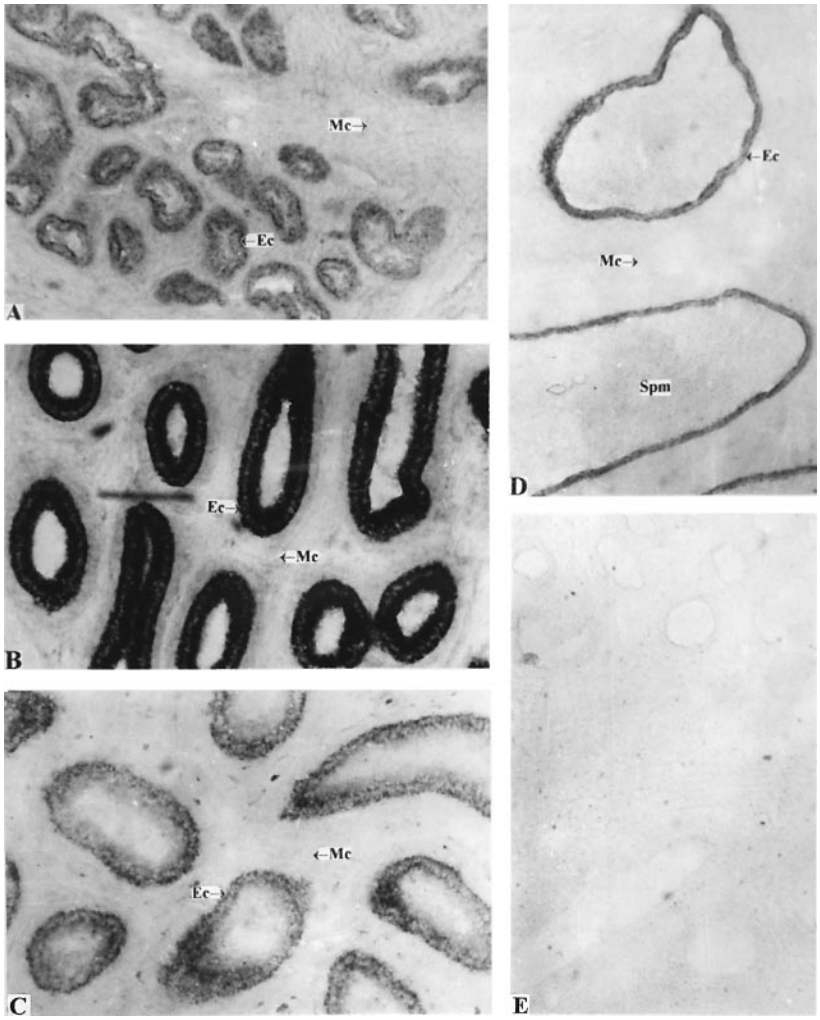

Figure 4. Localization of androgen receptor (AR) mRNA in epididymis of adult rhesus monkey (original magnification ×320). **A**, **B**, **C** and **D** represent the regions of initial, caput, corpus and cauda of the epididymis respectively. **E** serves as sense probe negative control. spm = spermatozoa.

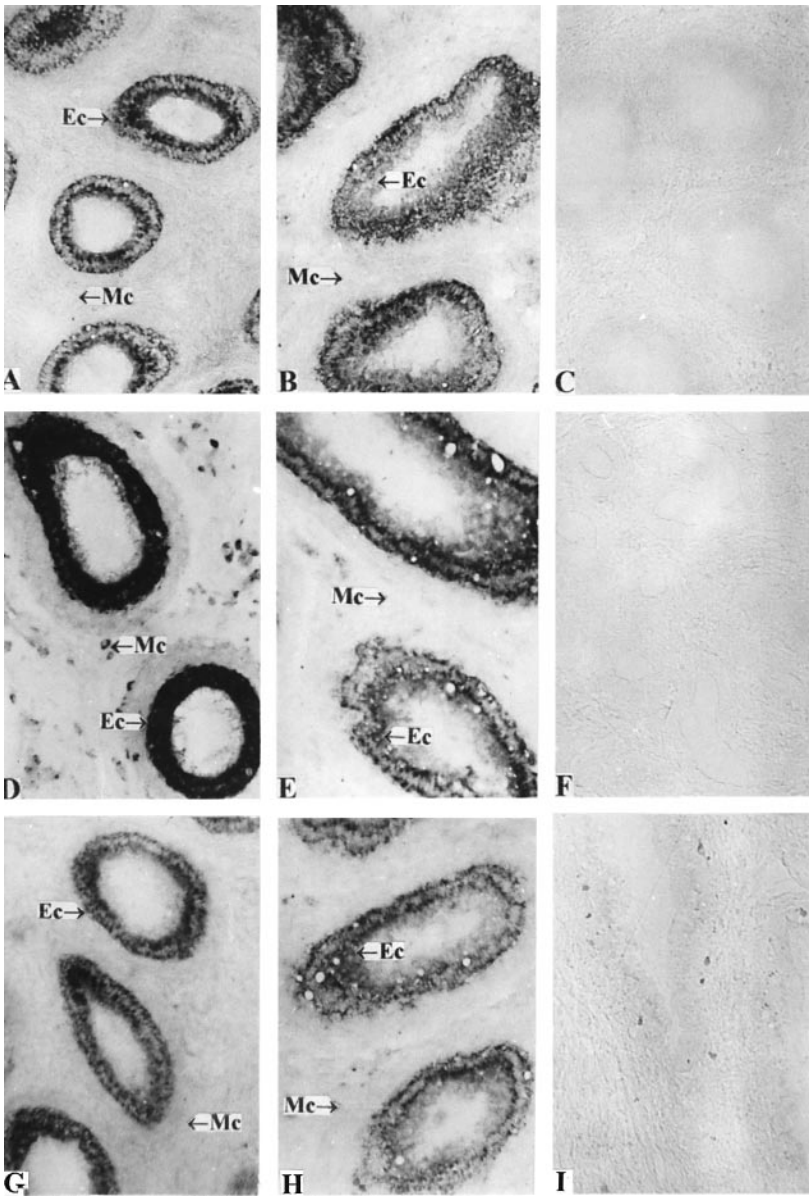

Figure 5. Localization of mRNAs for inhibin α (**A**, **B** and **C**), βA (**D**, **E** and **F**) and βB (**G**, **H** and **I**) in caput (**A**, **D** and **G**) and corpus (**B**, **E** and **H**) of adult monkey epididymis. **C**, **F** and **I** represent the sense probes for inhibin α, βA and βB respectively serving as negative controls. Ec = epithelial cells, Mc = mesenchymal cells.

Both tPA and uPA in spermatozoa have been reported to play a role in the process of fertilization (Huarte *et al.*, 1987; Rekkas *et al.*, 1991; Smokovitis *et al.*, 1992; Lison *et al.*, 1993). Mouse (Huarte *et al.*, 1987) and human (Liu *et al.*, 1996) spermatozoa have been found to be capable of binding uPA with a species-specificity, similar to other cell types bearing uPA receptors (Vassali, 1994). uPA receptor mRNAs were detected in the germ cells of monkey testis (Zhang *et al.*, 1997). These data suggest that uPA present in epididymis and seminal plasma (Liu *et al.*, 1996) may also partially bind to the surface of the spermatozoa and participate in the process of sperm maturation, storage and fertilization.

Caput and corpus epididymides expressed higher levels of tPA and PAI-1 than other regions. It is known that the physiological function of the epididymal epithelium changes continuously along the duct. The difference in the messenger expression levels suggests that local proteolysis generated by the interaction of PA and PAI-1 in the caput and corpus may play a more important role in some aspects of sperm maturation than the other regions. The precise mechanism of the action remains to be clarified.

We reported previously that adult rat (Zhou *et al.*, 1996, 1997) and monkey epididymis expressed LHR, tPA and uPA mRNAs. Both tPA and uPA activities were significantly stimulated *in vitro* by HCG/LH. LH is an essential pituitary hormone controlling both mammalian gonad steroid synthesis and epididymal functions, and so may be an important regulatory hormone involved in coordinating the processes of both spermatogenesis and sperm maturation. LHR was also expressed in infant monkey epididymis. This finding is consistent with the report of Schlatt and Arslan (1995) who demonstrated that HCG given to infant monkeys stimulated dramatically epididymal growth, indicating that HCG/LH participates in the regulation of epididymal development and growth via its

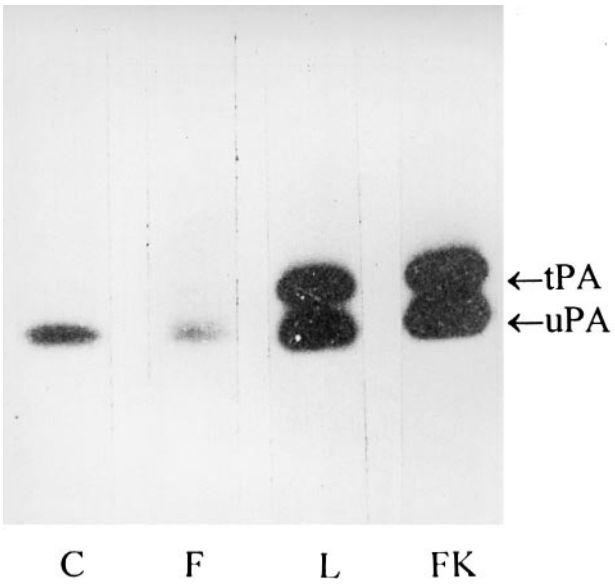

Figure 6. Stimulatory effect of luteinizing hormone (LH) and forskolin (FK) on plasminogen activator (PA) activities in cultured epididymal epithelial cells of rhesus monkey. Epididymal epithelial cells were obtained from epididymides of adult rhesus monkey by collegenase/DNAase digestion as described in the Materials and methods section. The cells (5×10^5 cells/well) were incubated at 37°C in the presence or absence (c) of follicle stimulating hormone (F, 100 ng/ml), LH (L, 100 ng/ml) or FK (10^{-4} M) for 48 h, The medium PA activities were measured by the fibrin overlay technique.

receptor. It has been well documented that epididymis cannot achieve its structural and functional maturation until the onset of LH and the first appearance of spermatozoa (Setty and Jehan, 1977), implying a role for LH in coordinating the development of epididymis and testis. High levels of AR mRNAs were also localized in the epithelium of monkey epididymis. Like LH, androgen may be also essential for regulation of epididymal function.

FSH is another important pituitary hormone responsible for regulating gonad function. We did not detect the presence of FSH receptor mRNA in the epididymis. Furthermore, FSH had no effect on epididymal growth of infant monkey (Schlatt and Arslan, 1995). In-vitro studies have also shown that FSH did not affect PA secretion in cultured rat epididymis (Zhou *et al.*, 1997) or monkey epididymal cells. These data suggest that FSH may be not involved in the regulation of epididymal function.

We report for the first time in this study that all of the three subunits of inhibin α, βA and βB are expressed in epididymal epithelium of adult monkey, implying that the epididymis is capable of producing both inhibin and activin proteins. However, no conclusion can be made concerning the mode of secretion and function of inhibin and/or activin in epididymis without further investigation. It is interesting to note, however, that the epididymis expresses much higher levels of inhibin βA in the caput than in other regions, suggesting that inhibin A or the homodimer activin βA in the caput may play an essential role in the process of sperm maturation.

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References

Austin, C.R. (1985) Sperm maturation in the male and female genital tracts. In Metz, C.B. and Monroy, A. (eds), *Biology of Fertilization.* Vol. 2 Biology of the Sperm. Academic Press, Orlando, USA, pp. 121–155.

Bedford, J.M. (1975) Maturation, transport and fate of spermatozoa in the epididymis. In Hamilton, D.W. and Greep, R.O. (eds), *Handbook of Physiology.* American Physiological Society, Washington DC, USA, pp. 303–317.

Bedford, J.M. (1994) The status and the state of the human epididymis. *Hum. Reprod.*, **9,** 2187–2199.

Dano, K., Andreasen, P.A, Grondahl-Hansen, J. *et al.* (1985) Plasminogen activators, tissue degradation, and cancer. *Adv. Cancer Res.*, **44,** 139–266.

Eddy, E.M. (1988) The spermatozoon. In Knobil, E. and Neill, J.D. (eds), *Physiology of Reproduction.* Vol. 1. Raven Press, New York, USA, pp. 27–68.

Eddy, E.M., Vernon, R.B., Muller, C.H. *et al.* (1985) Immunodissection of sperm surface modifications during epididymal maturation. *Am. J. Anat.*, **174,** 225–237.

Granelli-Piperno, A. and Reich, E. (1978) A study of proteases and protease-inhibitor complexes in biological fluids. *J. Exp. Med.*, **148,** 223–234.

Hamilton, D.W. (1975) Structure and function of the epithelium lining the ductuli efferentes, ductus epididymis, and ductus deferens in the rat. In Hamilton, D.W. and Greep, R.O. (eds), *Handbook of Physiology.* American Physiological Society, Washington DC, USA, pp. 259–301.

Hamilton, D.W. (1990) Anatomy of mammalian male accessory reproductive organs. In Lamming, G.E. (ed.), *Marshall's Physiology of Reproduction.* 4th edn, Vol. 2. Churchill Livingstone, London, UK, 691 pp.

Hinton, B.T. and Palladino, M.A. (1995) Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment. *Microsc. Res. Tech.*, **30,** 67–81.

Huarte, J., Belin, D., Bosco, D. *et al.* (1987) Plasminogen activator and mouse spermatozoa: urokinase synthesis in the male genital tract and binding of the enzyme to the sperm cell surface. *J. Cell. Biol.*, **104,** 1281–1289.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227,** 680–685.

Lison, D., Tas, S., Gemmart, J.P. *et al.* (1993) Plasminogen activator activity and fertilizing ability of human spermatozoa. *Int. J. Androl.*, **16,** 201–202.

Liu, Y.X., Ny, T, Sarker, D. *et al.* (1986) Identification and regulation of tissue plasminogen activator activity in rat cumulus–oocyte complexes. *Endocrinology*, **119,** 1578–1587.

Liu, Y.X., Du, Q., Liu, K. *et al.* (1995a) Hormonal regulation of plasminogen activator in rat and mouse seminiferous epithelium. *Biol. Sign.*, **4,** 232–240.

Liu, Y.X., Liu, K., Zhou, H.M. *et al.* (1995b) Hormonal regulation of tissue-type plasminogen activator and plasminogen activator inhibitor type-1 in cultured monkey Sertoli cells. *Mol. Hum. Reprod.*, **1**, see *Hum. Reprod.* **10,** 719–727.

Liu, K., Liu, Y.X., Du, Q. *et al.* (1996) Preliminary studies on the role of plasminogen activator in seminal plasma of human and rhesus monkey. *Mol. Hum. Reprod.*, **2,** 99–104.

Mather, J.P. and Krummen, L.A. (1992) Inhibin, activin, and growth factors: paracrine regulators of testicular function. In Nieschlag, E. and Habenicht, U.-F. (eds), *Spermatogenesis–Fertilization–Contraception: Molecular, Cellular and Endocrine Events in Male Reproduction.* Springer-Verlag, New York, USA, pp. 169–200.

Mignatti, P. and Rifkin, D.B. (1993) Biology and biochemistry of proteinases in tumor invasion. *Physiol. Rev.*, **73,** 161–195.

Ny, T., Bjersing, L. and Hsueh, A.J.W. (1985) Cultured granulosa cells produce two plasminogen activators and an antiactivator each regulated differently by gonadotropin. *Endocrinology*, **116,** 1666–1668.

Orgebin-Crist, M. and Jahad, N. (1978) The maturation of rabbit epididymal spermatozoa in organ culture: inhibition by antiandrogens and inhibitors of ribonucleic acid and protein synthesis. *Endocrinology*, **103,** 46–53.

Rekkas, C., Belibasaki, S., Taitzoglou, I. *et al.* (1991) Increased plasminogen activator activity and plasminogen activator inhibition in spermatozoa and seminal plasma of the ram after serum gonadotropin (PMSG) administration. Correlation with the increased level of testosterone in the blood. *Andrologia*, **23,** 273–278.

Robaire, B. and Hermo, L. (1988) Efferent ducts, epididymis, and vas deferens:

structure, functions and their regulation. In Knobil, E. and O'Neill, J.P. (eds), *The Physiology of Reproduction.* 1st edn. Vol. 1. Raven Press, New York, USA, 999 pp.

Saksela, O. and Rifkin, D.B. (1988) Cell-associated plasminogen activation regulation and physiological function. *Ann. Rev. Cell. Biol.*, **4,** 93–126.

Schlatt, S. and Arslan, M. (1995) Endocrine control of testicular somatic and premeiotic germ cell development in the immature testis of the primate *Macaca mulatta*. *Eur. J. Endocrinol.*, **133,** 235–247.

Setchell, B.P., Maddocks, S. and Brooks, D.E. (1994) Anatomy, vasculature, innervation, and fluids of the male reproductive tract. In Knobil, E. and O'Neill, J.D. (eds), *The Physiology of Reproduction.* 2nd edn. Vol. 1. Raven Press, New York, USA, pp. 1063–1176.

Setty, B.S. and Jehan, Q. (1977) Functional maturation of the epididymis in the rat. *J. Reprod. Fertil.*, **49,** 317–322.

Smokovitis, A., Kokolis, N., Taitzoglou, I. and Rekkas, C. (1992) Plasminogen activator, the identification of an additional proteinase at the outer acrosomal membrane of human and boar spermatozoa. *Int. J. Fertil.*, **37,** 308–314.

Tulsiani, D.R.P., NagDas, S.K., Skudlarek, M.D. *et al.* (1995) Rat sperm plasma membrane mannosidase: localization and evidence for proteolytic processing during epididymal maturation. *Dev. Biol.*, **167,** 584–595.

Turner, T.T. and Giles, R.D. (1982) Sperm-motility inhibiting factor in rat epididymis. *Am. J. Physiol.*, **242,** R199–R203.

Vassalli, J.D., Sappino, A.P. and Belin, D. (1991) The plasminogen activator/plasmin system. *J. Clin. Invest.*, **88,** 1067–1072.

Vassalli, J.D. (1994) The urokinase receptor. *Fibrinolysis*, **8** (Suppl. 1), 172–181.

Zhang, T., Zhou, H.M. and Liu, Y.X. *et al.* (1997) Expression of plasminogen activator and inhibitor, urokinase receptor and inhibin subunits in rhesus monkey testes. *Mol. Hum. Reprod.*, **3,** 223–231.

Zhou, H.M., Zhang, T. and Liu, Y.X. (1996) Rat epididymis expresses luteinizing hormone receptor (LHR). *Chinese Sci. Bull.*, **41,** 1608–1610.

Zhou, H.M., Zhang, T. and Liu, Y.X. (1997) Expression and regulation of plasminogen activator and plasminogen activator inhibitor type-1 in rat epididymis. *Chinese Sci. Bull.*, **42,** 779–783.

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