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Temporal expression of urokinase type plasminogen activator, tissue type plasminogen activator, plasminogen activator inhibitor type 1 in rhesus monkey corpus luteum during the luteal maintenance and regression

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Abstract

Proteolytic activity generated by the plasminogen activator (PA) system has been associated with many biological processes. Using a pregnant mare serum gonadotropin (PMSG)/human chorionic gonadotropin (hCG)-induced rhesus monkey corpus luteum (CL) model, we have studied how urokinase-type plasminogen activator (uPA), tissue-type plasminogen activator (tPA), and plasminogen activator inhibitor type 1 (PAI-1), are temporally expressed in CL of rhesus monkey at the luteotropic and luteolytic periods. Slot blot analysis and in situ hybridization were performed to analyze the expression and distribution of uPA and PAI-1 messenger RNA (mRNA). Fibrin overlay was used to detect uPA and tPA activities. We found that uPA is the dominating PA in luteotropic CL in the monkey. Abundant expression of PAI-1 mRNA was detected. The highest expression of uPA and PAI-1 mRNA was observed at the luteotropic period, while their expression decreased $\approx 50\%$ at early luteal regression defined by considerably decreased serum progesterone levels, and remained at very low levels at the late stage of luteal regression. We also observed an increased tPA activity at the time of luteal regression. Moreover, the exogenous tPA could inhibit the progesterone production in cultured luteal cells from 13-day-old monkey CL. We also used LH receptor mRNA expression as a mark for the luteal phases. A highly expressed, evenly distributed LH receptor mRNA was detected in CL during the luteotropic phase, while its expression decreased at day 13 coinciding with the reduction of progesterone production. We conclude that proteolysis mediated by uPA and regulated by PAI-1 may play a role in the luteal maintenance, while tPA may participate in the luteal regression in the rhesus monkey. © 1997 Elsevier Science Ireland Ltd.

Keywords: Corpus luteum; Rhesus monkey; Urokinase-type plasminogen activator; Tissue-type plasminogen activator; Plasminogen activator inhibitor type 1

1. Introduction

Proteolytic activity mediated by the plasminogen activator (PA) system has been associated with many physiological and pathological processes, such as ovulation, embryogenesis, embryo implantation, mammary gland involution, fibrinolysis, angiogenesis, inflammation and tumor metastasis (Dano et al., 1985; Saksela and Rifkin, 1988; Vassalli et al., 1991). All these processes involve proteolytic degradation of extracellular matrix components, which must be conducted with appropriate specificity in order to prevent unrestrained

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tissue destruction. The PA system is a versatile, temporally controlled enzymatic system in which plasminogen is activated to the proteolytic enzyme plasmin by either of two physiological PAs, tissue-type PA (tPA) or urokinase-type PA (uPA). In addition, two specific PA inhibitors, PA inhibitor type 1 (PAI-1) and PA inhibitor type 2 (PAI-2) are important for regulation of the PA-system (Saksela and Rifkin, 1988; Loskutoff, 1988; Ny et al., 1993). Many in vitro studies have shown that regulation of the PA system is complex and occurs at multiple levels. The synthesis and release of tPA and uPA by specific cells in response to hormones, growth factors and cytokines initiate the activation of this system (Saksela and Rifkin, 1988; Vassalli et al., 1991). However, the net proteolytic activity is dependent on the presence of the inhibitors, which are also released in response to stimulatory factors (Saksela and Rifkin, 1988; Loskutoff, 1988; Ny et al., 1993).

Indirect evidence suggests that the PA system plays a role in the degradation of the follicular wall during ovulation in rats (Ny et al., 1993) and rhesus monkey (Liu et al., 1991a). In accordance with this, a study with PA-deficient mice indicates that ovulation efficiency is reduced significantly in double deficient mice lacking both tPA and uPA gene functions (Leonardsson et al., 1995).

The corpus luteum (CL) is a transient endocrine organ formed from an ovulatory follicle. The well developed functional CL actively secretes progesterone, and meanwhile, transports the steroid hormone by its capillary network to prepare the uterine environment for implantation provided fertilization has occurred (Niswender and Nett, 1995). If fertilization has not occurred, or if implantation is unsuccessful, the CL first loses its progesterone-producing ability (the functional luteolysis), and then is structurally degenerated (the structural luteolysis) (Niswender and Nett, 1995; Michael et al., 1994). Associated with luteal function maintenance and regression is extensive connective tissue remodeling, and tissue degeneration (Murdoch et al., 1988; Endo et al., 1993). In the rhesus monkey, the luteal life span in normal menstrual cycles is about 15 days (Knobil, 1972). This is also the case in the pregnant mare serum gonadotropin (PMSG)/human chorionic gonadotropin (hCG)-induced luteinized ovaries (Feng et al., 1993). As little is known about the roles of the PA system in CL during luteal maintenance and regression in primates, with such a model, we have studied the expression and localization of uPA and PAI-1 mRNA in monkey CL as well as the tPA activity content in CL. Since luteinizing hormone (LH) is essential to maintain luteal function, we also studied the expression and localization of LH receptor mRNA in monkey CL.

2. Materials and methods

2.1. Materials

McCoy's 5a medium (modified, without serum), PMSG and hCG were purchased from Sigma (St. Louis, MO). Human fibrinogen was purchased from American Diagnostica (New York, NY); purified human tPA, uPA and plasminogen were obtained from Biopool (Umeå, Sweden); thrombin and Triton X-100 were purchased from Sigma; bicinchoninic acid protein-assay reagents were obtained from PIERCE (Rockford, IL); the riboprobe system for slot blot analysis was purchased from Promega (Madison, WI) and α-^{32}P-labeled UTP (800 Ci/mmol) was obtained from Amersham (Aylesbury, UK); restriction enzymes, *Taq* polymerase, 4-nitro blue tetrazolium chloride, 5 - bromo - 4 - chloro - 3 - indolyl - phosphate, anti-digoxigenin-AP Fab fragments and the Dig RNA Labeling Kit for riboprobe of in situ hybridization were purchased from Boehringer Mannheim (Mannheim, Germany); goat serum was obtained from Life Technologies (Paisley, Scotland).

2.2. Animals

Female rhesus monkeys ($n = 12$, 4 monkeys/time point) aged ≈ 3–4 years old were used. All animals were obtained from the monkey colony of the Primate Research Center, Kunming Institute of Zoology, Chinese Academy of Sciences. Animals were housed under controlled environmental conditions with free access to water and food. Experimental protocols were approved by the ethical committee of the Chinese Academy of Sciences.

2.3. Monkey model

The monkey CL model and surgery procedures used were previously described (Feng et al., 1993). To induce luteinization of the ovary, an initial dose of 950 IU PMSG was given i.m. on day 1 of superovulatory induction. From day 2 to day 7, 300 IU PMSG was administered every other day followed by a 300 IU PMSG injection daily for an additional 5 days. On day 13, a single injection of 4000 IU hCG was administered and this day was designated as day 1 of CL. At different luteal stages, anaesthetization of the monkeys was maintained by ketamine and the ovaries were removed by abdominal surgery in an aseptic operation room. One of the two ovaries was kept in 2-methylbutane at -70°C for in situ hybridization. CL from the other ovary was dissected and frozen down in liquid nitrogen for total RNA extraction and fibrin overlay.

2.4. Synthesis of RNA probes

The subcloning of monkey PAI-1 complementary DNA (cDNA) fragment to pGEM-3 vectors have been previously described (Liu et al., 1995b). The monkey uPA and LH receptor cDNA fragments were obtained by RT-PCR using primers designed directly from human DNA sequences (uPA: nucleotides 1211–1651 (Verde et al., 1984); LH receptor: nucleotides 438–847 (Jia et al., 1991)) and total RNA from monkey ovaries as templates, and ligated into pGEM-3 vectors. Sequencing results showed that the rhesus cDNA fragments were equivalent to human, and similarities between them were 95.90 (uPA) and 97.55% (LH receptor), respectively. The cloning of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragment was previously described (Liu et al., 1996a). Before transcription, plasmids were linearized so that antisense or sense RNA probes could be produced. Transcription was performed using an in vitro transcription system (Promega) with ^{32}P-labeled UTP and appropriate RNA polymerase. The specific activity of the probes varied from $2–5 \times 10^8$ cpm/μg RNA. For in situ hybridization, plasmids were linearized in the same way and riboprobes were made using a Dig RNA Labeling Kit.

2.5. RNA preparation and analysis

CL at different stages was homogenized in a guanidine isothiocyanate solution and total RNA was isolated by centrifugation through a cushion of 5.7 M CsCl (Liu et al., 1996a). Slot-blot analysis was performed as previously described (Liu et al., 1991b). The relative abundance of specific mRNA was analyzed with a PhosphorImager (Molecular Dynamics) and normalized to the relative abundance of GAPDH mRNA in corresponding samples.

2.6. In situ hybridization

In situ hybridization was performed as previously described (Liu et al., 1996a) with digoxigenin-labeled monkey uPA, PAI-1 and LH receptor riboprobes. Briefly, 10 μm cryostat sections were collected on SuperFrost*/Plus slides and fixed in 4% paraformaldehyde (in PBS) for 10 min. Prehybridization was performed in a solution of 50% formamide, 5 × SSC (standard saline citrate), 5 × Denhardt's solution, 250 μg/ml tRNA and 500 μg/ml herring sperm DNA at room temperature overnight. Hybridization was performed in the same solution, containing ≈ 1 μg/ml digoxigenin labeled riboprobe, at 72°C overnight. After hybridization the slides were washed in 0.2 × SSC at 72°C for 1 h. The slides were then incubated with blocking buffer (10% heat inactivated normal goat serum, 0.1 M Tris (pH 7.5), 0.15 M NaCl) for 1 h at room temperature before addition of the alkaline phosphatase conjugated antidigoxigenin antibody (1:5000 dilution in 0.1 M Tris (pH 7.5), 0.15 M NaCl, 1% heat inactivated goat serum). After incubation with the antibody at 4°C overnight, the slides were washed three times in Tris buffer (0.1 M Tris (pH 7.5) and 0.15 M NaCl) before being equilibrated in alkaline phosphatase buffer (0.1 M Tris (pH 9.5), 0.1 M NaCl, 50 mM $MgCl_2$). Substrate for alkaline phosphatase was then added (450 μg/ml 4-nitro blue tetrazolium chloride and 175 μg/ml 5-bromo-4-chloro-3-indolyl-phosphate in AP-buffer) and after 1–3 days of incubation, the color reaction was terminated with 10 mM Tris and 1 mM EDTA (pH 8). Slides used for comparison were finished at the same time. To monitor background levels and the specificity of hybridization, the sense strand of monkey uPA, PAI-1 and LH-receptor RNA probes were included in each experiment. Photographs were taken with an Olympus OM-2 camera on an Olympus T041 photomicroscope at a magnification of × 10–25.

2.7. Cell preparation and culture

The cell preparation and culture were performed as previously described (Feng et al., 1993). Briefly, on day 13 of CL development, the ovaries were removed, and the CL were dissected out of the ovaries. They were rinsed three times in fresh medium and cut into small pieces and incubated with 0.04% collagenase at 37°C for 30–60 min. Individual cells were obtained by repetitive pipetting of the tissue. At the end of digestion, luteal cells were washed three times by centrifugation. Cells (1×10^6) in 1 ml serum-free McCoy's 5a medium were put into poly-D-lysine coated 35 mm Falcon culture dishes and incubated in a CO_2 incubator at 37°C for 24 h. The media were collected at the end of the culture period and stored at −70°C for progesterone assay.

2.8. SDS-PAGE and fibrin overlay

To measure PA activity, monkey CL was homogenized in a buffer containing 140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4 and 8 mM KH_2PO_4 (pH 7.4) and 0.5% Triton X-100 using a homogenizer. Fifty micrograms of total protein for each sample was used for electrophoresis.

PA activity in samples was fractionated by SDS-PAGE according to Laemmli (1970). The fibrin-agar indicator gel was prepared as previously described (Ny et al., 1985).

2.9. Progesterone assay

The progesterone level in cell culture medium was assayed by RIA as previously described (Feng et al., 1993).

2.10. Data analysis

All experiments for slot-blot analysis, in situ hybridization and fibrin overlay were repeated three times. Relative mean values (mean ± SEM) from three experiments for uPA, PAI-1 and LH receptor mRNA levels were calculated. For fibrin overlay and in situ hybridization, representative results were used. The statistical significance between two groups of progesterone production was determined with Student's t-test. Comparisons with $P < 0.05$ were considered significant.

3. Results

3.1. PA activities in monkey CL at the luteotropic and luteolytic periods

The PMSG/hCG injection induced a stable luteal phase in the rhesus monkey (Knobil, 1972; Feng et al., 1993). As shown in Fig. 1, 10 days after PMSG/hCG injection, the serum progesterone concentration is quite high, indicating that the CL is functional. At day 13, the progesterone level decreased significantly, and remained at a low level to the late luteal stage (day 15) (Knobil, 1972; Feng et al., 1993). To estimate the PA activities in monkey CL at different developmental stages, CL extracts from samples at days 10, 13 and 15 were prepared for analysis by SDS-PAGE and fibrin overlay. As shown in Fig. 2, uPA is the dominant PA in monkey CL. In 10-day-old CL, relatively high levels of uPA activity were observed. A decrease of ≈50% in uPA activity was detected at the beginning of luteal regression (day 13). As the luteal regression progressed (day 15), uPA activity decreased to a very low level. The lysis bands showed that monkey tPA and uPA have migration properties similar to human tPA and

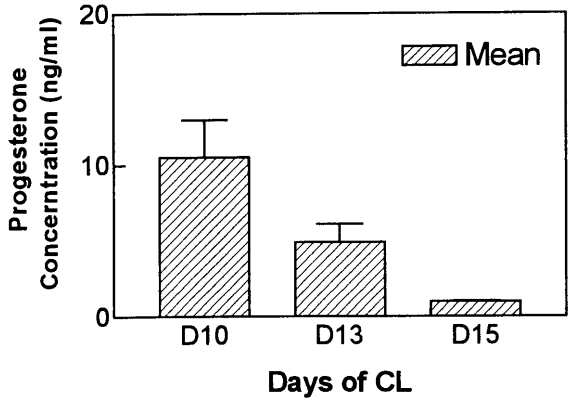

Fig. 1. Serum progesterone levels during the luteotropic and luteolytic periods. Monkey serum was collected at certain luteal stages. Progesterone concentrations were measured by RIA as described in Section 2. Values represent the mean ± SEM of three different experiments. D, day.

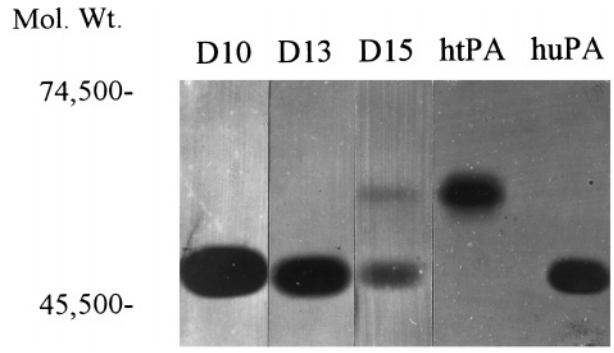

Fig. 2. PA activities in luteinized monkey luteal extracts during the luteotropic and luteolytic periods. Monkey CL during the luteotropic (day 10) and luteolytic (day 13 and day 15) phases were collected as described in Section 2. CL extracts (50 μg total protein) were analyzed by SDS-PAGE and fibrin overlay. Ages of CL are indicated at the top of each lane. The migration positions of human tPA and uPA as well as stained molecular-weight standards are indicated on the left.

uPA, indicating that they have molecular weights similar to their corresponding human molecules. In addition, the activity for tPA was low during the luteotropic period (day 10), while there was an increase in the tPA activity at day 15 which was at the late stage of luteal regression.

3.2. Expression of uPA, PAI-1 and LH receptor mRNA in monkey CL at the luteotropic and luteolytic periods

To study the mRNA expression of uPA, PAI-1 and LH receptor in monkey CL at different phases, total RNA was extracted from monkey CL and slot-blot analysis was performed. The relative content of mRNA levels are shown in Fig. 3. The uPA mRNA level at day 10 was relatively high, but decreased ≈50% when luteal regression started (day 13), which is a similar pattern to the protein level data. At day 15, as luteal regression continued, uPA mRNA levels decreased to very low levels which is also consistent with its activity (Fig. 2, day 15). The highest PAI-1 expression was in 10-day-old CL and it decreased ≈50% on day 13. At day 15, only very little PAI-1 was detected. In addition, LH receptor showed high expression in luteinized monkey ovaries at the luteotropic period (day 10). During the luteal regression, its expression decreased dramatically to very low levels (day 13 and 15). The decrease in LH receptor mRNA levels coincided with the reduction of progesterone production during luteal regression in the rhesus monkey.

3.3. Localization of uPA, PAI-1 and LH receptor mRNA expression in monkey CL at the luteotropic and luteolytic periods

In order to obtain dynamic and detailed pictures of

the PA system and LH receptor in monkey CL, in situ hybridization was performed on sections of luteinized monkey ovaries using digoxigenin-labeled riboprobes. The results are summarized in Fig. 4.

Monkey CL expressed high levels of LH receptor mRNA 10 days after ovulation (Fig. 4A), indicating it is functionally active. The LH receptor mRNA signals were evenly distributed throughout the luteal tissues. A higher magnification image is shown in Fig. 4J. At this time, uPA mRNA was also evenly distributed (Fig. 4B). PAI-1 mRNA showed abundant expression (Fig. 4C) and had an expression pattern similar to LH receptor (Fig. 4A,K). When the monkey CL enters its luteolytic period (day 13), CL lost most of its LH receptor mRNA expression (Fig. 4D). The uPA mRNA expression appeared as randomly distributed spots (Fig. 4E). The PAI-1 mRNA expression showed the same distribution pattern as that of day 10 (Fig. 4F). As luteal regression progressed (day 15), CL exhibited low LH receptor mRNA expression (Fig. 4G). The uPA mRNA expression decreased to a very low level (Fig. 4H), which was in agreement with the slot-blot analysis (Fig. 3) and the fibrin overlay data (Fig. 2). Also in agreement with the slot-blot analysis (Fig. 3), in situ hybridization showed low levels of PAI-1 expression in 15-day-old CL, which appeared as randomly distributed signals in the CL (Fig. 4I, arrowheads).

3.4. Effect of exogenous tPA on progesterone production in cultured monkey luteal cells

Luteal cells (1×10^6) obtained from the CL on day 13 were incubated at 37°C in 1 ml serum free McCoy's 5a medium in the presence of plasminogen (10 μg/ml), and with or without LH (100 ng/ml), tPA (100 ng/ml) and uPA (0.5 μg/ml) for 24 h. The medium progesterone content is shown in Fig. 5. LH significantly increased the progesterone secretion ($P < 0.01$). The addition of exogenous tPA to the culture medium inhibited the basal level of progesterone ($P < 0.01$). No such effect was seen by adding uPA to the culture medium ($P > 0.05$), indicating that tPA, but not uPA, is capable of directly decreasing the luteal progesterone production.

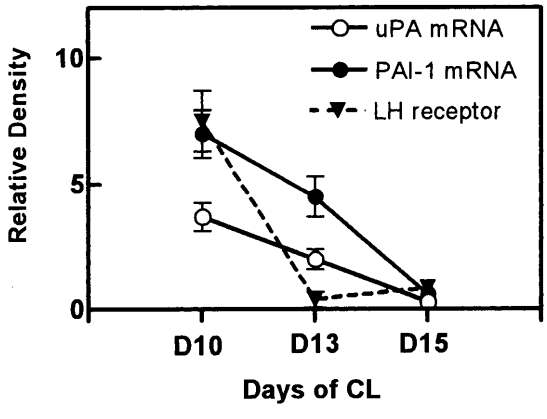

Fig. 3. uPA, PAI-1 and LH receptor mRNA levels in monkey CL during the luteotropic and the luteolytic periods. Monkey CL during the luteotropic (day 10) and luteolytic (day 13 and day 15) phases were collected as described in Section 2. Total RNA was extracted and applied to nylon filters. The filters were hybridized with a ^{32}P-labeled uPA, PAI-1, LH receptor or GAPDH antisense RNA probe before being analyzed in a PhosphorImager. Values (mean ± SEM) from three experiments normalized against the relative levels of GAPDH mRNA in the corresponding samples are shown. The ages of CL are indicated at the bottom.

4. Discussion

The luteal extracellular matrix contains type I and type IV collagen, laminin, and fibronectin (Wordinger et al., 1983; Luck et al., 1995; Zhao and Luck, 1995). Like many other physiological processes involving tissue remodeling and tissue involution, the luteal maintenance and regression are dependent on the action of specific and highly regulated proteolysis, which locally digests tissue proteins according to a certain pattern and without associated overlysis. In this paper, we demonstrated that the expression of the PA system undergoes considerable changes in the monkey CL, suggesting that the gonadotropin-controlled, coordinately expressed uPA, tPA and PAI-1 may be important for luteal maintenance and regression in the rhesus monkey.

We have demonstrated that functional luteal tissues express both uPA mRNA and a large amount of PAI-1 mRNA. This implies that proteolysis mediated by uPA and regulated by PAI-1 is necessary for the luteal maintenance in the rhesus monkey. It is likely that, uPA or plasmin plays a role supporting the monkey CL to function actively. The extracellular matrix is known as a dynamic and complex network that not only provides a structural support for the organism, but also actively participates in regulating the behavior of the cells that attach to it. Changes in composition of extracellular matrix can modulate many complex cellular processes, including cell motility and migration, cell differentiation and proliferation, cell metabolic functions as well as gene expression (Alexander and Werb, 1989; McDonald, 1989; Getzenberg et al., 1990; Zetter and Brightman, 1990; Liotta et al., 1991; Hynes and Lander, 1992). As a robust endocrine organ, the luteotropic CL needs to maintain itself in a dynamic metabolic condition, which is essential for active steroid hormone-producing/secreting activity. Therefore, basement membranes and connective tissues may be modified frequently, by the proteolysis mediated by uPA and regulated by PAI-1, to take part in the

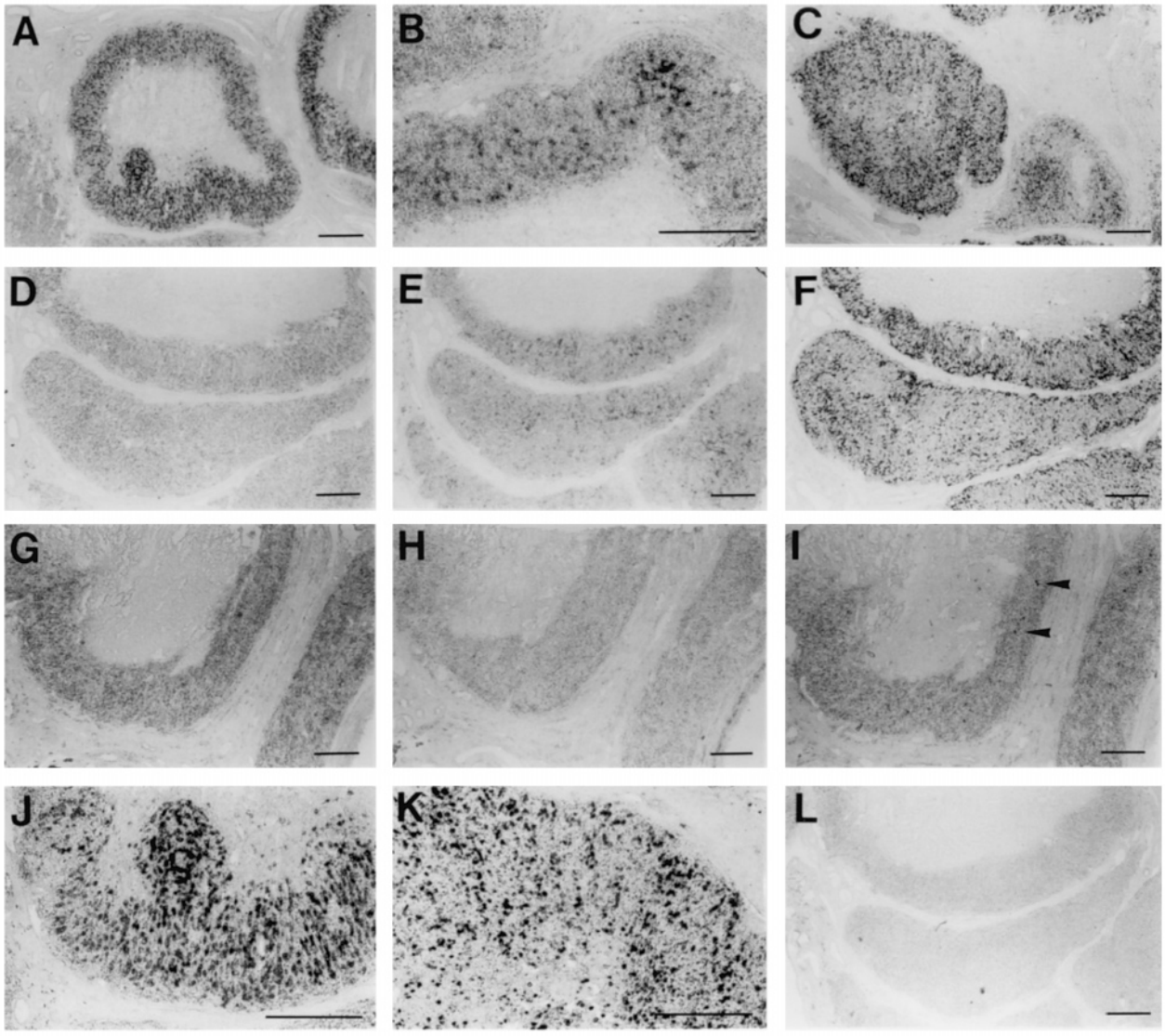

Fig. 4. In situ localization of uPA, PAI-1 and LH receptor mRNA in monkey CL during the luteotropic and the luteolytic periods. Luteinized monkey ovaries at different luteal stages were collected as described in Section 2. A series of 10 μm cryostat sections of ovaries were hybridized with digoxigenin UTP labeled uPA, PAI-1 or LH receptor antisense and sense RNA probes. After the color reaction, photographs were taken with an Olympus OM-2 camera on an Olympus T041 photomicroscope at a magnification of $\times$ 10–25. The horizontal black bars on the photographs represent 400 μm. The hybridization signal appears in brown/black. All sections in the first and second vertical row were hybridized with a LH receptor or uPA antisense RNA probe. Sections in the third vertical row were hybridized with a PAI-1 antisense RNA probe except that section L was hybridized with a PAI-1 sense probe. Sections in the first horizontal row were from 10-day-old CL. Similarly, sections in the second and third horizontal row were from 13- and 15-day-old CL, respectively. Pictures of section A and C at higher magnification are shown in J and K.

regulation of metabolism. The similar temporal expression of LH receptor, uPA and PAI-1 mRNA by functional luteal cells indicate that uPA might act to enhance luteal function under the manipulation of gonadotropins and the regulation by its inhibitor.

Our results on the expression of uPA and PAI-1 mRNA in luteotropic monkey CL are supported by our unpublished data from humans that both uPA and PAI-1 are found to be expressed in luteotropic CL. The expression levels of PAI-1 mRNA in human CL were also found to be high. Together with the monkey data in this paper, similar expression patterns of uPA and PAI-1 mRNA are seen in primates. Nevertheless, when compared with the expressions of uPA and PAI-1 in rats and mice, substantial differences exist. In the rat, no uPA (our unpublished data) or PAI-1 (Liu et al., 1996a) were observed in luteotropic CL. In the mouse, however, highly expressed and evenly distributed uPA mRNA was seen in the luteotropic CL, and PAI-1 was only expressed in newly formed CL at a low level (Liu et al., 1996b). The highly expressed uPA in luteotropic mouse CL indicates it may be used to maintain CL formation. Nevertheless, uPA might be just one of the factors which serve the luteal maintenance because uPA

gene deficient mice are able to have normal CL function and can still reproduce normally (Liu et al., 1996a). The lack of uPA does not result in total abnormal luteal function. Generally, our data obtained from monkey, human, rat and mouse indicate that the PA system exhibits various expression patterns in different species.

In this paper, we also demonstrated that the expression of uPA and PAI-1 mRNA were $\approx 50\%$ lower in CL at the early luteal regression (day 13) than the luteotropic period (day 10). Thus, the proteolytic activity mediated by uPA and regulated by PAI-1 might not be necessary for the initiation of luteolysis. However, the increased tPA activity on day 15 implies that it may play a role in the luteal regression. Proteolysis mediated by tPA may initiate the gradual process of luteal tissues degeneration. Furthermore, we demonstrated that exogenous tPA could significantly reduce the progesterone production by 13-day-old luteal cells in vitro, indicating that tPA, but not uPA, may be the factor that participates in luteal regression in the monkey. Our suggestion is supported by our previous study with cell culture experiments (Feng et al., 1993). We have also demonstrated that the PA system may be engaged in the luteal regression in the rat and mouse. In the rat, both tPA mRNA activity and PAI-1 mRNA levels were increased considerably during the luteolytic period than the luteotropic period (Liu et al., 1996a). Cultured rat luteal organs from the luteolytic period secrete more tPA activity than those from the luteotropic period (Liu et al., 1995a). Increased tPA and uPA mRNA expression was also seen in mouse CL during the luteal regression and the expression patterns were similar to those in the rat (Liu et al., 1996b). Hence, differences in the expression and function of the PA system in CL between the primates and the rodents are possible. Further experiments on the expression and localization of tPA mRNA in monkey luteolytic CL is underway.

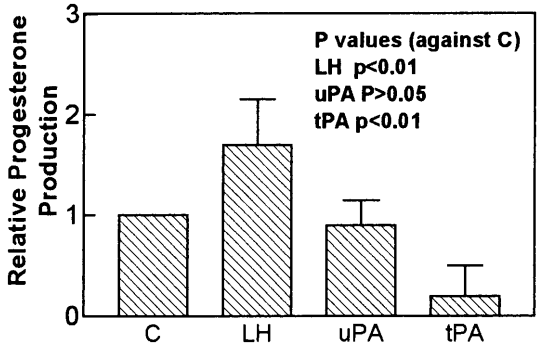

Fig. 5. Effect of exogenous tPA on progesterone production in cultured monkey luteal cells. Monkey luteal cells on day 13 were prepared as described in Section 2. Luteal cells (1×10^6) were incubated at 37°C in 1 ml serum-free McCoy's 5a medium in the presence of plasminogen (10 μg/ml) and with or without LH (100 ng/ml), tPA (100 ng/ml) and uPA (0.5 μg/ml) for 24 h. Progesterone concentrations in the cultured medium were measured by RIA. Values represent the mean $\pm$ SEM of three different experiments.

The PA system is not the only protease system that is involved in luteal function and regression. It is known that PA can activate collagenase and other metalloproteinase (MMPs), collagenase can activate stromelysin, stromelysin and plasmin can activate procollagenase (Alexander and Werb, 1989; Birkedal-Hansen, 1995). Thus, other proteases and protease inhibitors may also play a role during different luteal developmental stages. Smith et al. (1994) have reported the expression of a tissue inhibitor of metalloproteinases (TIMP-1) in ovine luteal tissues. Nothnick et al. (1995) have reported the expression and regulation of TIMP-1, 2 and 3 in pseudopregnant rat CL.

Matrix metalloproteinase (MMP) activities with molecular weights of 72 000 and 66 000 have also been detected in gonadotropin-induced rat luteal tissues (Endo et al., 1993). Moreover, a broad spectrum protease inhibitor, α2-macroglobulin whose substrates include both plasmin and collagenase, is hormonally regulated and produced mainly during the luteotropic period in pregnant rat CL (Gaddy Kurten et al., 1989). Our recent experiments with mouse CL showed that TIMP-1, MTMMP and stromelysin-3 mRNA were expressed and regulated in mouse CL at different luteal developmental stages (Liu et al., 1996b). Together with this paper, these reports suggest the important role of proteases in CL of various species.

In summary, our results suggest that proteolysis mediated by uPA, tPA and regulated by PAI-1 may play a role in luteal maintenance and regression in the rhesus monkey. However, in view of the varieties of substrate specificity and the complexity of protease families, the targeted proteolysis cannot be performed solely by protease of one kind, but rather by the combined action of different proteolytic enzymes. Together with our previous work (Liu et al., 1995a, 1996a,b), we propose that regardless of various expression patterns in different species, protease activation might be generally involved in luteal maintenance and regression.

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