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Role of plasminogen activator and plasminogen activator inhibitor type-1 in luteolysis——a minireview

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Abstract   This minireview summarized our recent studies on the role of plasminogen activator (PA) and inhibitor type-1 (PAI-1) in luteolysis. We have demonstrated that (1) both tissue type and urokinase type plasminogen activators (tPA and uPA) and a plasminogen activator inhibitor type-1 (PAI-1) were present in the corpus luteum of rat and rhesus monkey; (2) decrease in progesterone production in corpus luteum was well correlated with a sharp increase in tPA (but not uPA) and PAI-1 secretion; (3) exogenous tPA decreased luteal progesterone synthesis while monoclonal antibodies increased progesterone production; (4) interferon γ inhibited luteal progesterone synthesis and stimulated tPA production while LH plus prolactin increased progesterone production and decreased tPA (but not uPA) activity in cultured luteal cells; (5) increase in proteolysis in the corpus luteum was also correlated with decrease in progesterone production in mouse. These data suggest that local degradation of extracellular matrix controlled by plasminogen activator and inhibitor is involved in the processes of luteolysis.

Keywords: tissue type plasminogen activator (tPA), plasminogen activator inhibitor type-1 (PAI-1), luteolysis.

PROTEOLYSIS activity generated by plasminogen activator (PA) system is associated with many physiological and pathological processes, such as ovulation, embryogenesis, embryo implantation, mammary gland involution, fibrinolysis, angiogenesis, inflammation and tumor metastasis[1]. 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) and 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[1, 2]. It has been well documented that time-coordinated expression of tPA (produced mainly by granulosa cells) and PAI-1 (produced mainly by theca-interstitial cells) in the ovaries leads to ovulation[3, 4]. After ovulation, granulosa cells and theca-interstitial cells change into corpus luteum, a temporary endocrine organ, which mainly secretes proges-

terone for maintenance of pregnancy by priming the uterus to support the implantation and early development of the conceptus[5]. Corpus luteum formation involves dramatic morphological and biochemical changes, such as invasion of the capillary network from the theca tissue into the granulosa layers. During the luteolytic period, corpus luteum first loses its ability to produce progesterone (the functional luteolysis), followed by structural regression (the structural luteolysis)[6]. Both corpus luteum formation and luteolysis involve proteolytic processes such as antiogenesis, tissue remodeling and tissue regression. Little is known, however, about the roles of PA system during different developmental stages of corpus luteum under physiological conditions. In the study reported here, we summarized our recent findings on the role of tPA and its inhibitor type-1 in luteolysis.

1 Identification of tPA, uPA and PAI-1 activity in corpus luteum

Two types of PA——tPA and uPA, and PAI-1 have been identified in ovarian granulosa cells and theca-interstitial cells of both rat[4] and monkey[7]. Since after ovulation both ovarian cell types transform into luteal cells, it is, therefore, interesting to examine whether luteal cells are also capable of expressing the PAs and PAI-1.

The monkey corpus luteum was extracted, and the extracts were incubated with protein A-sepharose 4B beads precoated with normal rabbit serum (NRS) or antibodies against tPA or uPA. After immunoprecipitation, supernatants were analyzed for PA activities[8]. Monkey corpus luteum contained two types of PA activities in the NRS group. After precipitation with tPA antibodies only uPA activity could be observed in the supernatant, whereas after incubation with uPA antibodies, the supernatant showed only tPA activity[8], indicating that monkey corpus luteum produces tissue type and urokinase type of PA. The molecular weight of the two PAs was corresponding to human tPA and uPA respectively. We also identified the PAI-1 activity in the same samples[9]. tPA, uPA and PAI-1 were also identified in the corpus luteum of pregnant[10] and pseudopregnant[11] rats.

2 Decrease in corpus luteum progesterone production is correlated with increase in corpus luteum tPA and PAI-1 secretion

To examine the corpus luteum secretary capability of tPA, uPA and PAI-1 activities, as well as progesterone levels in the corpus luteum at various stages of development, corpus luteum tissue or dispersed luteal cells obtained from the ovaries of pregnant rat or pseudopregnant rat and rhesus monkey on various days were cultured at 37℃ for 24 h in 0.5 mL McCoy's 5a medium. The medium tPA, uPA and PAI-1 activities as well as progesterone concentrations were determined. During the entire course of pregnancy from Day 1 to Day 22, the uPA activity measured in rat corpus luteum was low[10]. Similarly little tPA activity could be detected in corpus luteum before Day 12. A huge amount of tPA, however, was secreted abruptly by the corpus luteum from Day 14 to Day 22 when luteolysis took place. A marked increase in PAI-1 activity was also noted at the same time[11]. The tPA activity in corpus luteum on Days 14, 17 and 22 increased 35-, 37- and 22-fold and the PAI-1 activity on the same days increased 15-, 19- and 15-fold above that on Day 1, respectively[10]. The progesterone concentration in the conditioned medium of the corresponding cultures were high on the early days, reaching the maximum level on Day 12, and decreased sharply on Day 14 when the tPA activity reached the maximum level[10, 11]. The same reverse changes of corpus luteum tPA and progesterone levels were also obtained from studies on pseudopregnant monkey and

rat[8, 9, 12]. Corpus luteum of rhesus monkey at Day 5 and Day 10 secreted high levels of progesterone, but very low tPA activity[9]. On Day 13, when luteolysis might take place, a dramatic increase in luteal tPA activity was observed, and was correlated with a remarkable decrease in progesterone secretion. The reverse relationship between progesterone secretion and tPA activity in the corpus luteum at various days is shown in table 1.

Table 1　Inverse relationship between progesterone production and tPA activity in monkey corpus luteum at various stages

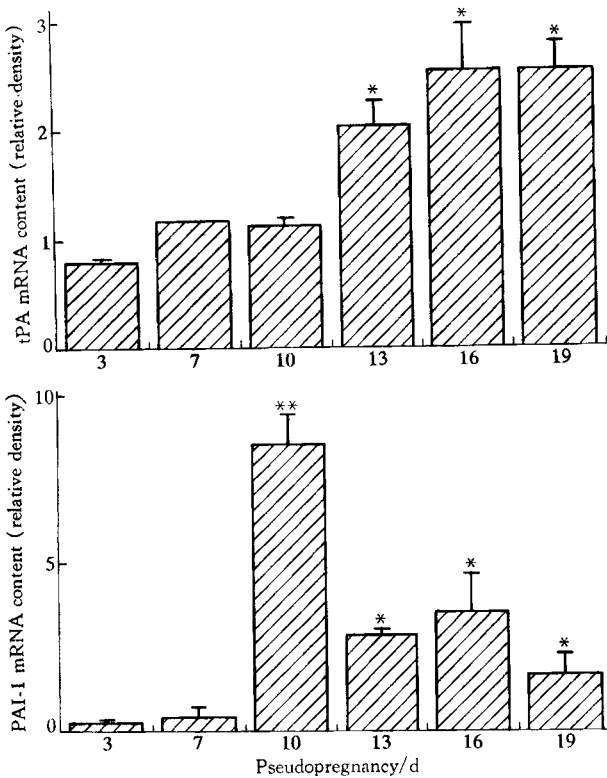| Days of pregnancy	Progesterone production /ng·mL^{-1}, mean ± SE	Relative tPA activity (%)	Days of pregnancy	Progesterone production /ng·mL^{-1}, mean ± SE	Relative tPA activity (%)
5	79.0 ± 5.9	1.0 ± 0.2	15	19.7 ± 7.5[a]	10.3 ± 1.2[b]
10	22.4 ± 6.9	1.1 ± 0.1	23	7.2 ± 0.9[b]	5.0 ± 0.9[a]
13	8.4 ± 1.8[b]	42.2 ± 2.4[b]			

$n = 3$; a) $P < 0.05$; b) $P < 0.01$.

Fig. 1.　Northern blot analysis of tPA mRNA (upper part) and PAI-1 mRNA (lower part) expression in rat corpus luteum at different stages of adult pseudopregnancy. *, $P < 0.05$; **, $P < 0.01$.

To study tPA and PAI-1 mRNA expression during different stages of pseudopregnant rat corpus luteum, total RNA was extracted, and Northern blot analysis was performed. Corpus luteum stages were monitored by measuring serum progesterone levels. In accordance with our previous studies on pregnant rat, the pseudopregnant rat model also showed the same reverse pictures of tPA/PAI changes and progesterone levels in the corpus luteum[12]. As shown in fig. 1, on the early days of corpus luteum development (Day 3 and Day 7), the expression of tPA and PAI-1 mRNA was rather low. As corpus luteum entered the luteolytic period, when progesterone dropped to the low levels, both tPA (see the upper part of fig. 1) and PAI-1 (see the lower part of fig. 1) mRNAs increased. An increase in tPA mRNA expression was observed from Days 13—19 (a 2- to 3-fold increase compared to Day 7). The increase in PAI-1 mRNA expression on Day 10 occurred just before luteolysis and appeared earlier than the induction of tPA mRNA. This is consistent with the changes of tPA and PAI-1 activities measured in the corpus luteum[12].

3 Exogenous tPA decreases, while monoclonal tPA antibodies increase corpus luteum progesterone production

To examine whether tPA or tPA antibodies *in vitro* affect directly the luteal cells and change the ability of progesterone secretion, corpus luteum tissue[10, 11] or dispersed luteal

cells[9] were cultured with or without either purified tPA (100 μL/mL) or tPA monoclonal antibodies (10 μg/mL) in the presence of plasminogen (25 μg/mL), a substrate for PA activation. Addition of tPA to the culture of rat corpus luteum of pregnancy[11] significantly decreased progesterone production in the medium by 33% at 12 h and 54% at 24 h. In contrast, addition of tPA monoclonal antibodies to neutralize the endogenously produced tPA activity significantly increased the steroid production by 100% at 12 h and 40% at 24 h. The same effect of tPA and tPA antibodies on the primate luteal cells in culture was also observed[8, 9]. Exogenous tPA inhibited the basal and LH-induced progesterone production. In contrast, inclusion of tPA monoclonal antibodies to neutralize the endogenously produced tPA activity also significantly increased the medium progesterone concentration. Furthermore, an additive effect of LH and tPA antibodies on the luteal cell progesterone production was observed[9]. Addition of uPA, or uPA monoclonal antibodies to the culture had no such effect on the steroid production[8, 9].

Interferon γ(IFN-γ) has been reported to inhibit LH-stimulated progesterone production in cultured bovine luteal cells[13]. It is of interest to examine whether IFN-γ decreases corpus luteum progesterone production while increases tPA secretion. Pregnant rats on Day 4 received injections of various dosages of IFN-γ, and 24 h later the animals were killed and blood samples were collected. Serum progesterone concentration was considerably inhibited by the injection of IFN-γ. Inhibition of 69% and 89% of the steroid production was found with injection of 20 IU and 40 IU of IFN-γ, respectively. In cultured rat luteal cells in the presence or absence of IFN-γ (10 IU/mL) and hCG (100 ng/mL) alone or in combination, IFN-γ alone significantly decreased the basal level of progesterone production, while it markedly stimulated the basal tPA activity. Addition of hCG to the culture enhanced both progesterone and tPA production. The increased progesterone secretion by hCG was considerably inhibited when IFN-γ was also added. In contrast, a stimulatory effect of hCG plus IFN-γ on tPA activity was found in the culture[11].

Prolactin and LH have been reported to exert a synergistic luteotrophic effect in rat[14]. Using a monkey luteal cell culture model, we have demonstrated that LH alone seemed to inhibit tPA activity and stimulate progesterone production dose-dependently, while prolactin had no such effect. The two pituitary hormones in combination, however, completely suppressed the tPA (but not uPA) activity and synergistically dramatically increased luteal progesterone secretion[9].

4 Proteolysis and luteolysis in mouse

In our previous studies we have demonstrated that rat and monkey granulosa cells secrete mainly tPA (90% of total PA activity in the overy) and a little amount of uPA[4, 15] and a PA inhibitor, PAI-1[3]. However, mouse granulosa cells secrete both uPA (about 70%) and tPA (about 30%)[16]. Furthermore, no measurable amount of PAI-1 mRNA and activity could be detected in the granulosa cells, but an α_2-antiplasmin, an inhibitor specific for plasmin, was measured in the cell-conditioned medium[17]. These data suggest that species-specific differences in the plasmin-generating system in the ovaries may be present. Therefore, in addition to the PA system, we also studied the other proteolytic system, matrix metalloproteinases (MMPs), in the corpus luteum of pseudopregnant mouse. The MMPs are a family of zinc- and calcium-dependent endopepidases. So far 11 different MMPs with different substrates
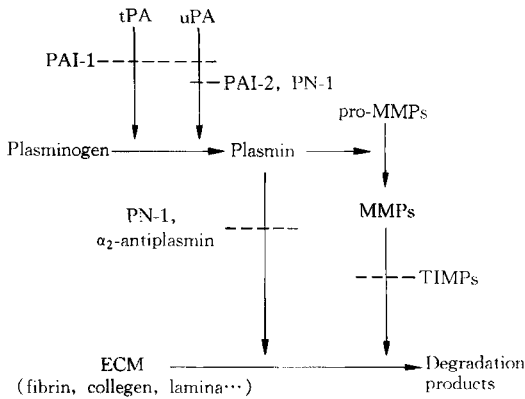
Fig. 2. PA/PAI-1 and MMP/TIMP systems.

specificity have been discovered. The PA and MMPs systems are supposed to be interactive and closely related (fig. 2). The two systems appear to form a lytic cascade which can completely denature interstitial molecules. We have demonstrated that uPA, tPA, membrane type MMP (MT-MMP), and a tissue inhibitor of metalloproteinase type-1 (TIMP-1) mRNA levels were considerably increased in pseudopregnant mouse corpus luteum when luteolysis took place (unpublished data). These data show that proteolysis in mouse corpus luteum generated by MMPs system, in addition to tPA and uPA, may play a role in luteolysis.

5 Paracrine and autocrine factors in corpus luteum

Corpus luteum is the most active steroidogenic organ, producing not only progesterone, but also androgens and estrogens. The endogenously produced testosterone and estrogen are capable of maintaining corpus luteum progesterone production[18]. In our previous studies, we have demonstrated that progesterone produced by granulosa cells is utilized by theca-interstitial cells to form androgens that are further aromatized into estrogens by granulosa cells. Thus synergistic interactions between granulosa cells and theca-interstitial cells are the prerequisites for estrogen biosynthesis[19]. Therefore, luteal estrogen production may also be derived from the two transformed luteal cells. Two types of luteal cells have been reported: large luteal cells derived from granulosa cells, and small luteal cells derived from theca-interstitial cells[20]. Granulosa cells are known to produce ovarian tPA activity[4, 6], whereas theca-interstitial cells secrete the majority of PAI-1 activity in the ovary[21]. Both granulosa cells and theca-interstitial cells are capable of producing uPA[22]. So they give rise to speculation that large luteal cells might be responsible for tPA production, while small luteal cells might be connected with the PAI-1 activity found in the corpus luteum. Coordinated expression of tPA and PAI-1 in the two cell types in the later phase of corpus luteum development may be closely related to the luteal regression. Much evidence accumulated in the past several decades clearly reveals that in addition to androgens and estrogens, corpus luteum is also capable of secreting various other peptides such as vasoactive intestinal peptide (VIP)[23], relaxin, inhibin/activin, oxytocin and growth factors in significant amounts throughout pregnancy, acting as important paracrine luteotropic factors[5]. It has also been reported recently that cytokines may act as local paracrine and autocrine luteotropic or luteolytic regulators of ovarian function[5]. IFN-γ was capable of affecting luteal function by enhancing prostaglanding $F_{2\alpha}(PGF_{2\alpha})$ production and by inhibiting progesterone synthesis[13, 24]. Furthermore, IFN-γ and $PGF_{2\alpha}$ were found to stimulate the basal and hCG-stimulated tPA secretion in cultured rat luteal cells[11, 13]. However, the mechanism by which cytokines decrease corpus luteum progesterone but increase tPA secretion is not known. On the basis of the data provided here, we speculate that the endogenously produced tPA in corpus luteum in the control of hormone production and stimulation may regulate luteal regression through local autocrine or paracrine action. However, questions

remain concerning the identity of certain intraluteal factors linking tPA/PAI-1 expression and progesterone production. In addition to PA/PAI-1 system, cytokines, $PGF_{2\alpha}$ and inhibin/activin may be the most important regulators in the mechanism of autocrine and paracrine regulation of luteal regression.

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