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Physiology

Ovulation efficiency is reduced in mice that lack plasminogen activator gene function: Functional redundancy among physiological plasminogen activators

(reproduction/ovary/ovarian proteases/gene targeting)

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ABSTRACT **Several lines of indirect evidence suggest that plasminogen activation plays a crucial role in degradation of the follicular wall during ovulation. However, single-deficient mice lacking tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), or PA inhibitor type 1 (PAI-1) gene function were recently found to have normal reproduction, although mice with a combined deficiency of tPA and uPA were significantly less fertile. To investigate whether the reduced fertility of mice lacking PA gene function is due to a reduced ovulation mechanism, we have determined the ovulation efficiency in 25-day-old mice during gonadotropin-induced ovulation. Our results reveal that ovulation efficiency is normal in mice with a single deficiency of tPA or uPA but reduced by 26% in mice lacking both physiological PAs. This result suggests that plasminogen activation plays a role in ovulatory response, although neither tPA nor uPA individually or in combination is obligatory for ovulation. The loss of an individual PA seems to be functionally complemented by the remaining PA but this compensation does not appear to involve any compensatory up-regulation. Our data imply that a functionally redundant mechanism for plasmin formation operates during gonadotropin-induced ovulation and that PAs together with other proteases generate the proteolytic activity required for follicular wall degradation.**

Proteolysis generated by the plasminogen activator (PA)/plasmin system has been associated with many physiological and pathological processes such as ovulation, embryo implantation and embryogenesis, mammary involution, fibrinolysis, angiogenesis, inflammation, and tumor invasion (1–3).

In mammals, ovulation is triggered by the preovulatory surge of luteinizing hormone from the pituitary, which results in liberation of the mature oocyte from the preovulatory ovarian follicle into the periovulatory space (4). A mature follicle destined to ovulate usually protrudes on the surface of the ovary. For the ovum to escape from this structure, an extensive proteolytic degradation of basement membranes and the connective tissue that constitute the follicle wall is required. Several lines of indirect evidence suggest that the PAs together with matrix metalloproteinases (MMPs) play a role in follicular rupture (for review, see refs. 5–7).

The PA system is a versatile, temporally controlled enzymatic system that comprises plasminogen, which is activated to the proteolytic enzyme plasmin by either of the two physiological PAs, tissue-type PA (tPA) and urokinase-type PA (uPA). Activation of this system is initiated by the release of tPA or uPA by specific cells in response to external signals, which leads to a locally expressed extracellular proteolytic activity (1, 3). The PA system is also regulated by specific inhibitors directed against PAs and plasmin, including PA inhibitor type 1 (PAI-1), PA inhibitor type 2 (PAI-2), protease nexin 1 (PN-1), and α_2-antiplasmin (1, 7). Many different studies have provided indirect evidence suggesting that the PA system plays a crucial role in degradation of the follicle wall during ovulation: (*i*) Plasminogen is present in the follicular fluid, and plasmin can weaken the follicle wall *in vitro* (8). (*ii*) In rats, ovulation is preceded by a transient and cell-specific expression of tPA and PAI-1, which causes a proteolytic activity localized to the surface of the ovary just prior to ovulation (9–13). (*iii*) Intrabursal injection of serine protease inhibitors, α_2-antiplasmin, or antibodies against tPA partially blocks gonadotropin-induced ovulation in rats (14, 15). (*iv*) Addition of the bacterial PA streptokinase to *in vitro* perfused rabbit ovaries induces ovulation in the absence of gonadotropins, suggesting that PAs alone can activate a broad spectrum proteolytic cascade that degrades the follicle wall (16). In spite of the large number of indirect experimental evidence that correlates ovarian PA expression to ovulation, a causative role of the PA system in ovulation remains to be established.

Correlation studies similar to those described above also suggest that MMPs play a crucial role in follicular rupture (for reviews and references, see refs. 5–7). Together these findings have led to the hypothesis that the PAs produced by ovarian cells may activate plasminogen in the follicular fluid to form plasmin, which in turn acts on pro-MMPs to generate active MMPs, resulting in a proteolytic cascade that can degrade the follicle wall (9, 17, 18).

Recently, mice with deficient tPA, uPA, and PAI-1 gene functions were created (19, 20). Surprisingly, single-deficient mice lacking tPA, uPA, or PAI-1 gene function as well as double-deficient mice lacking both the tPA and uPA genes were able to reproduce, and the progeny appeared normal at birth (19, 20). In mice with single deficiencies of tPA, uPA, or PAI-1, the litter sizes, the frequency of litters, as well as the life span were normal, but mice with combined deficiencies of tPA and uPA were significantly less fertile (20).

In addition to ovulation, successful reproduction involves several proteolytic processes where the PA system has been suggested to play a pivotal role including fertilization, embryo implantation, and embryogenesis (21–28).

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Abbreviations: PA, plasminogen activator; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; PAI-2, plasminogen activator inhibitor type 2; MMP, matrix metalloproteinase; PMSG, pregnant mare serum gonadotropin; hCG, human chorionic gonadotropin; PN-1, protease nexin 1.

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To quantitate the effects of PA deficiencies on ovulation efficiency and to study whether the inactivation of one individual PA gene leads to a compensatory up-regulation of the remaining PA gene, we have studied gonadotropin-induced ovulation in wild-type as well as tPA-, uPA-, and PAI-1-deficient mice. Our results suggest that PAs play a role in ovulation and that the physiological PAs are functionally redundant.

MATERIALS AND METHODS

Materials. Pregnant mare's serum gonadotropin (PMSG), human chorionic gonadotropin (hCG), and Triton X-100 were purchased from Sigma. The Riboprobe system was purchased from Promega. [α-^{32}P]UTP (800 Ci/mmol; 1 Ci = 37 GBq) and [α-^{32}P]dCTP (3000 Ci/mmol) were obtained from Amersham. *Taq* polymerase and restriction enzymes were purchased from Boehringer Mannheim.

Animals. Mice, as described previously (19, 20), were kept in microisolation cages on a 12-h day light cycle initiated at 0600 h and fed a regular chow and water diet. Female 25-day-old mice were injected with 1.5 units of PMSG to stimulate follicle growth and 48 h later with an ovulatory dose of hCG (5 units) to induce ovulation. For RNA and PA activity analysis, the animals were sacrificed at different time points after hCG treatment and ovaries were collected and frozen at −70°C for further analysis. For oocyte counting, animals were sacrificed 20 h after hCG injection, and the number of ova in the oviduct was recorded.

Analysis of DNA and RNA. Genomic DNA was isolated from mouse tail tips and genotyped by Southern blot analysis as described (19, 20). Total RNA from mouse ovaries was analyzed by Northern blot analysis (11) and normalized to the relative abundance of 18S rRNA. To compare the relative mRNA levels of the individual components of the PA system, antisense RNA probes of similar size and identical specific activity were used and the hybridized filters were analyzed with a PhosphorImager (Molecular Dynamics).

Synthesis of RNA and DNA Probes. As probes for Northern blot analysis an *Eco*RI fragment of mouse tPA cDNA (nucleotides 1–815) (29), a PCR fragment of mouse uPA cDNA (nucleotides 202–608), as well as a PCR fragment of mouse PAI-2 cDNA (nucleotides 460–983) (30) were subcloned into the *Eco*RI site of pGEM-3Z. An *Sph*I fragment of mouse PAI-1 (nucleotides 1088–1607) (31) was subcloned into pGEM-4Z vector. RNA probes were synthesized using an *in vitro* transcription system (Promega) with [α-^{32}P]UTP and the appropriate RNA polymerase. The following probes were used for Southern blot analysis of genomic tail DNA: a 2.0-kb genomic *Xba* I/*Hin*dIII mouse tPA fragment (described as probe C in ref. 20), a 400-bp *Pst* I/*Sma* I fragment from the 3′ noncoding region of the mouse uPA gene (a kind gift from J. Degen) (32), and a 550-bp genomic *Nhe* I mouse PAI-1 fragment (described as probe C in ref. 19). The DNA fragments were isolated and labeled with [α-^{32}P]dCTP (3000 Ci/mmol) by a random priming technique and used for hybridization (19, 20).

Preparation of Ovarian Extract and Casein Zymography. Ovarian extracts were prepared as described (11). For analysis of PA activity, samples of ovarian extracts containing 100 μg of protein were adjusted to 50 mM Tris·HCl, pH 6.8/2.5% SDS and were fractionated by SDS/PAGE. After electrophoresis, gels were incubated in 2.5% (vol/vol) Triton X-100 for 2 × 45 min to remove SDS from the gel. The gel was then layered on the substrate gel containing a mixture of 0.8% agarose, 1.3% nonfat dry milk, and 40 μg of plasminogen per ml in PBSA prepared as described (33). Zymographs were then allowed to develop at 37°C.

Statistical Analysis. The statistical differences between the genotype groups were determined by Student's *t* test for two independent samples with unequal variance and a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Regulation of tPA, uPA, PAI-1, and PAI-2 mRNA Expression in the Mouse Ovary During Gonadotropin-Induced Ovulation. The ovaries of rat and mouse are essentially identical in terms of their structure and physiology; yet, ovulation induction in these closely related species is preceded by secretion of two different forms of PA from the granulosa cells (11, 34). In the rat, tPA and its inhibitor PAI-1 are coordinately induced before ovulation (11, 12), whereas uPA is induced in the mouse (34).

To study whether coordinated regulation of PAs and PA inhibitors also takes place in the mouse ovary during gonadotropin-induced ovulation, we have characterized the ovarian levels of tPA, uPA, PAI-1, and PAI-2 mRNA in wild-type mice. Immature 25-day-old female mice were injected with PMSG and hCG, and at different time points after the hormone treatment ovaries were removed and total RNA was prepared and analyzed by Northern blot hybridization.

As shown in Fig. 1 PMSG-treated mice contained low amounts of uPA mRNA, but after hCG treatment the levels of uPA mRNA increased 5- to 10-fold, reaching maximum levels ≈4 h after hCG treatment. Consistent with a previous study (34), this indicates that uPA mRNA is the most abundant and most dramatically regulated mRNA in the mouse ovary. As shown in Fig. 1, low levels of tPA mRNA were also found throughout the periovulatory period, with a small 2- to 3-fold induction 4 h after hCG injection. *In situ* hybridization experiments revealed that expression of uPA mRNA was mainly localized to granulosa cells of preovulatory follicles, whereas tPA mRNA was mainly localized to oocytes and theca-interstitial tissue including apical regions of the preovulatory follicles that are located on the surface region of the ovary (ref. 28; A.-C. Hägglund, K.L., A. Ny, and T.N., unpublished data). Low levels of PAI-2 and PAI-1 are also expressed in the ovary with a slight induction 4 and 12 h after hCG treatment, respectively. In contrast to the rat, there was no dramatic

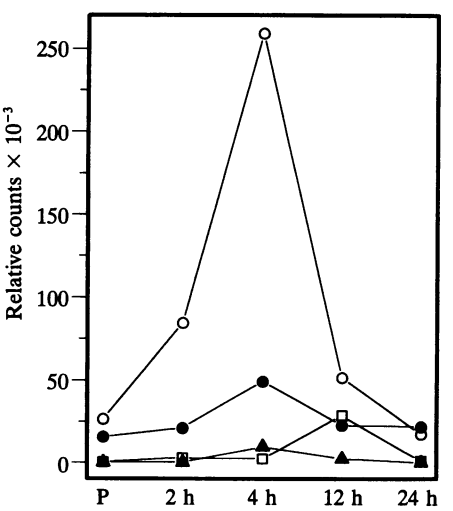

FIG. 1. Relative expression of uPA, tPA, PAI-1, and PAI-2 mRNA in the mouse ovary during gonadotropin-induced ovulation. Immature 25-day-old female wild-type mice were injected with 1.5 units of PMSG and 48 h later with 5 units of hCG. At the indicated time points after gonadotropin treatment, the ovaries were collected. Total RNA was prepared and uPA (○), tPA (●), PAI-1 (□), and PAI-2 (▲) mRNA were analyzed by Northern blot analysis as described. Results are expressed as relative counts calculated from the IMAGE-QUANT program in the PhosphorImager (Molecular Dynamics). P, ovaries treated with PMSG for 48 h; 2 h, 4 h, 12 h, and 24 h, time after hCG injection.

(10-fold) preovulatory increase of PAI-1 mRNA in the mouse (11). The species-specific difference in regulation of the PA system between mouse and rat is therefore not only reflected in the different type of PA used in ovulation induction but also in the regulation of PAI-1. The relative levels of PAI-1 and PAI-2 mRNA expression were also significantly lower than that of tPA and uPA mRNA throughout the periovulatory period, suggesting that these PA inhibitors may play a less important regulatory role during ovulation in mice. It is therefore possible that other protease inhibitors can reduce PA or plasmin activity in the ovary and thereby modulate proteolysis. Since PN-1 is expressed in the ovary, we analyzed PN-1 mRNA in the ovaries during gonadotropin-induced ovulation (35). Although PN-1 mRNA was expressed at relatively high levels in the ovary, its expression was not regulated (data not shown).

Ovulation Efficiency Is Reduced in Mice Lacking PA Function. In addition to ovulation, successful reproduction involves many biological processes in which the PA system has been claimed to play a role including fertilization, embryo implantation, as well as embryogenesis (21–28). The reduced fertility observed in mice with a combined deficiency of tPA and uPA could therefore be due to a defect in any of these biological processes; alternatively, it could be caused by the general health problems associated with the double-deficient genotype, which is manifested in growth retardation beyond the age of 3 weeks and the onset of several pathological conditions from the age of 8–12 weeks (20). To investigate whether the ovulation mechanism is impaired in PA-deficient mice, we determined ovulation efficiency at an early age before the onset of pathological conditions. The 25-day-old animals used in this study were healthy and revealed no macroscopic abnormalities or pathological defects. There was no difference in body weight or ovarian weight between the different genotypes (data not shown). Because fibrin deposition in various organs has been documented in $tPA^{-/-}/uPA^{-/-}$ mice beyond 2–3 months of age, we performed immunostaining experiments of frozen ovarian sections from the mice used in the oocyte counting experiments with a goat anti-mouse fibrinogen/fibrin-specific antibody (20). No histological abnormalities or fibrin depositions could be documented in any of the genotypes of mice at this young age (data not shown).

To determine the ovulation efficiency in mice with a single deficiency of tPA, uPA, or PAI-1 the female offspring from heterozygous $tPA^{+/-}$, $uPA^{+/-}$, and $PAI\text{-}1^{+/-}$ breeding pairs were used in ovulation-induction experiments. As shown in Table 1, no differences in ovulation efficiency were found between the respective wild-type control mice and mice that are heterozygous or homozygous for mutations in the tPA, uPA, or PAI-1 gene.

Table 1. Ovulation efficiency in wild-type, heterozygous, and single-deficient mice

Genotype	No. of mice used	No. of oocytes per mouse
$tPA^{+/+}$	11	9.2 ± 1.5
$tPA^{+/-}$	17	8.5 ± 0.9
$tPA^{-/-}$	8	9.1 ± 1.9
$uPA^{+/+}$	19	8.1 ± 1.1
$uPA^{+/-}$	33	8.3 ± 1.0
$uPA^{-/-}$	15	8.1 ± 1.1
$PAI\text{-}1^{+/+}$	13	8.6 ± 1.1
$PAI\text{-}1^{+/-}$	25	9.1 ± 0.7
$PAI\text{-}1^{-/-}$	9	9.3 ± 1.1

tPA, uPA, and PAI-1 heterozygous mice were interbred to obtain offspring of all genotypes (indicated on the left). Immature 25-day-old female mice were injected i.p. with 1.5 units of PMSG followed 48 h later by injection of 5 units of hCG. Animals were sacrificed 20 h after hCG treatment and the number of ova in the oviducts was recorded. Number of oocytes represents mean ± SE.

To determine whether ovulation is impaired in mice with a combined deficiency of tPA and uPA, we determined the ovulation efficiency in the offspring of tPA and uPA double-heterozygous ($tPA^{+/-}/uPA^{+/-}$) breeding pairs. As shown in Table 2, the number of oocytes per mouse in the wild-type ($tPA^{+/+}/uPA^{+/+}$) mice was 10.4 oocytes compared to 7.7 oocytes in mice with a combined deficiency of tPA and uPA ($tPA^{-/-}/uPA^{-/-}$). This represents a 26% reduction in ovulation efficiency of the $tPA^{-/-}/uPA^{-/-}$ mice, and analysis by student's *t* test revealed that the difference of these two values is statistically significant ($P = 0.0467$). Interestingly, a similar reduction of ovulation efficiency was obtained in rats where pharmacological inhibitors of serine proteases or antibodies against tPA or α_2-antiplasmin were injected into the ovarian bursa (14, 15).

The reduced ovulation efficiency in mice with a combined deficiency of tPA and uPA provides direct evidence that the PA system plays a role during gonadotropin-induced ovulation. In addition, our data suggest that the compromised ovulation is a likely reason for part of the reduced fertility in mice with a combined deficiency of tPA and uPA (20). The observation that tPA or uPA single-deficient mice have the same ovulation efficiency as wild-type mice suggests that there is no unique requirement for either of the two physiological PAs. Rather, our data suggest that tPA and uPA are functionally redundant in ovulation and that one PA can functionally compensate for the loss of the other. This conclusion is supported by the findings that tPA is the major PA induced in the rat, while uPA is the major PA induced in mice prior to ovulation (11, 34). The ability of one PA to complement for the other as observed here has also been noticed for the role of the PAs in fibrinolysis, where mice with a combined deficiency of tPA and uPA have significantly reduced fibrinolysis compared to the single-deficient mice (20).

Up-Regulation of the Remaining PA Gene Is Not the Basis for the Complementation Observed in Single-Deficient Mice. The data in Table 2 suggest that one PA can functionally complement for the other in ovulation induction. One mechanism to obtain functional complementation is to compensate the loss of a component by increasing the synthesis of other functionally related proteins, which was exemplified by members of the CREB/ATF transcription factor family (36).

To investigate whether deletion of a PA or PA inhibitor gene affects the regulation pattern of the remaining components of the PA system, we therefore characterized the expression of tPA, uPA, PAI-1, and PAI-2 in tPA-, uPA-, and PAI-1-deficient mice during PMSG/hCG-induced ovulation. As shown in Fig. 2, the expression pattern of tPA mRNA in

Table 2. Ovulation efficiency in wild-type, heterozygous, and tPA and uPA double-deficient mice

Genotype	No. of mice used	No. of oocytes per mouse
$tPA^{+/+}/uPA^{+/+}$	12	10.4 ± 1.0
$tPA^{+/+}/uPA^{+/-}$	28	10.4 ± 0.9
$tPA^{+/-}/uPA^{+/+}$	25	11.3 ± 1.0
$tPA^{+/+}/uPA^{-/-}$	10	11.2 ± 1.9
$tPA^{+/-}/uPA^{+/-}$	59	11.2 ± 0.6
$tPA^{-/-}/uPA^{+/+}$	14	11.1 ± 1.5
$tPA^{+/-}/uPA^{-/-}$	35	10.3 ± 0.5
$tPA^{-/-}/uPA^{+/-}$	22	9.4 ± 0.6
$tPA^{-/-}/uPA^{-/-}$	18	7.7 ± 0.9

tPA and uPA double heterozygous mice ($tPA^{+/-}/uPA^{+/-}$) were interbred to obtain offspring of all genotypes (indicated on the left). Animals were treated as described in Table 1. Number of oocytes represents mean ± SE. $P = 0.0467$ (Student's *t* test) for number of oocytes in $tPA^{+/+}/uPA^{+/+}$ vs. $tPA^{-/-}/uPA^{-/-}$ mice.

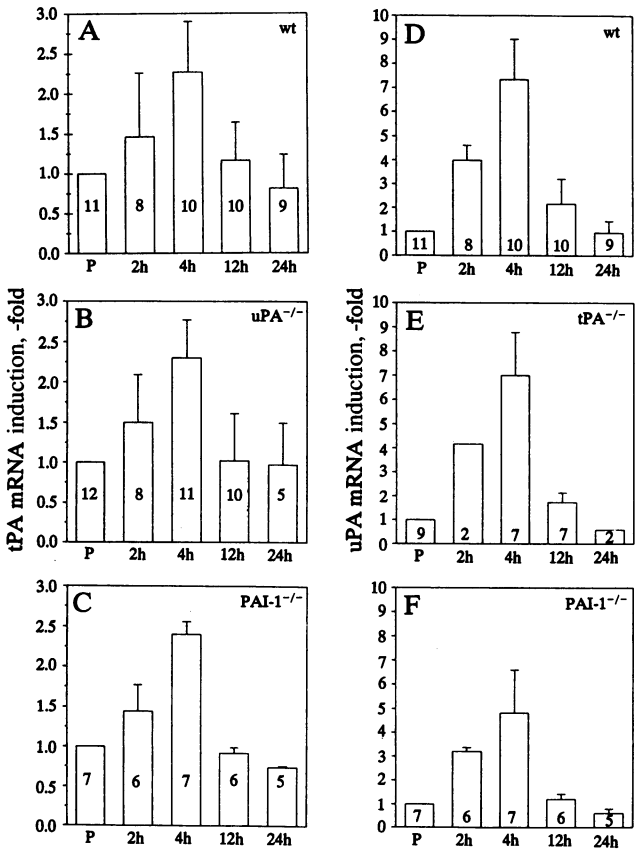

FIG. 2. Comparison of tPA and uPA mRNA levels in ovaries of wild-type, tPA$^{-/-}$, uPA$^{-/-}$, and PAI-1$^{-/-}$ mice during gonadotropin-induced ovulation. Animals were treated, and total RNA from ovaries of wild-type (*A* and *D*), uPA$^{-/-}$ (*B*), tPA$^{-/-}$ (*E*), and PAI-1$^{-/-}$ (*C* and *F*) mice was prepared and analyzed for tPA mRNA (*A–C*) and uPA mRNA (*D–F*) as described in the legend to Fig. 1. Relative amounts of mRNA are expressed as means ± SD of three individual experiments with the PMSG-treated group set as 1.0. Numbers inside columns indicate number of mice analyzed.

uPA$^{-/-}$ and PAI-1$^{-/-}$ mice and the expression pattern of uPA mRNA in tPA$^{-/-}$ and PAI-1$^{-/-}$ mice are essentially the same as in wild-type controls. These results reveal that there is no significant difference in the levels of expression or in the regulation pattern of tPA and uPA mRNA between wild-type mice and mice with single deficiencies of tPA, uPA, or PAI-1.

Fig. 3 shows the expression pattern of PAI-1 and PAI-2 mRNA in the ovaries of wild-type, PAI-1$^{-/-}$, tPA$^{-/-}$, and uPA$^{-/-}$ mice during the periovulatory period. In wild-type mice ovaries, PAI-1 mRNA remained at rather low levels before ovulation but increased around the time of ovulation 12 h after hCG treatment, whereas PAI-2 mRNA was detected only 4 h after hCG treatment (Fig. 3*A*). Compared with wild-type mice, similar expression levels and regulation patterns of PAI-1 and PAI-2 mRNA were observed during hCG-induced ovulation in the ovaries of the tPA$^{-/-}$ and uPA$^{-/-}$ mice (compare Fig. 3 *C* and *D* with Fig. 3*A*). In the ovaries of PAI-1$^{-/-}$ mice, the level and expression of PAI-2 mRNA remained similar to that in ovaries of wild-type mice (compare Fig. 3*B* with Fig. 3*A*). In addition, the expression level of PN-1, which has the capacity to inhibit PAs, was similar in wild-type and PAI-1-deficient mice (data not shown).

Comparison of PA Activity in the Ovarian Extract of Mice with a Single Deficiency of tPA, uPA, or PAI-1. To investigate whether regulation of tPA and uPA in wild-type and single-deficient mice also was reflected at the activity level, extracts

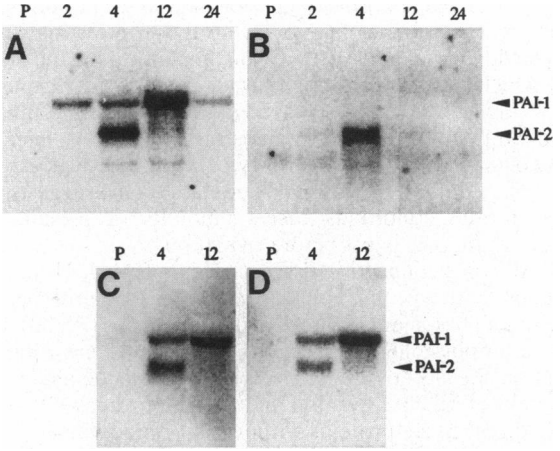

FIG. 3. Comparison of PAI-1 and PAI-2 mRNA levels in ovaries of wild-type, PAI-1$^{-/-}$, tPA$^{-/-}$, and uPA$^{-/-}$ mice during gonadotropin-induced ovulation. Total RNA from ovaries of wild-type (*A*), PAI-1$^{-/-}$ (*B*), tPA$^{-/-}$ (*C*), and uPA$^{-/-}$ (*D*) mice was prepared as described in the legend to Fig. 1 and analyzed for PAI-1 and PAI-2 mRNA by Northern blot analysis. Representative autoradiographs demonstrate PAI-1 and PAI-2 mRNA levels in the ovaries of each type of mice at different time points after treatment with gonadotropins. P, ovaries treated with PMSG for 48 h; 2, 4, 12, and 24, time (h) after hCG injection. Migration of PAI-1 and PAI-2 is indicated on the right.

of whole ovaries were separated by SDS/PAGE and analyzed for PA activity by casein zymography (33).

As shown in Fig. 4, ovarian extract from PMSG-treated wild-type mice contained relatively high levels of uPA but undetectable amounts of tPA. Confirming the regulation of their corresponding mRNAs, the activities of both tPA and uPA were increased after hCG treatment, reaching the highest levels at 4 h followed by a decline at 12 h.

The level and expression pattern of tPA activity in ovaries of wild-type, uPA$^{-/-}$, and PAI-1$^{-/-}$ mice as well as the level and expression pattern of uPA activity in wild-type, tPA$^{-/-}$, and PAI-1$^{-/-}$ mice were found to be similar, suggesting that no obvious compensatory increase of PA activity takes place in mice lacking tPA, uPA, or PAI-1 gene function. These results suggest that the apparent normal ovulation efficiency of single-deficient mice does not result from any significant compensatory up-regulation of mRNA or activity levels for remaining components of the PA system.

Since uPA is the most abundant and most dramatically induced PA just prior to ovulation it was surprising that

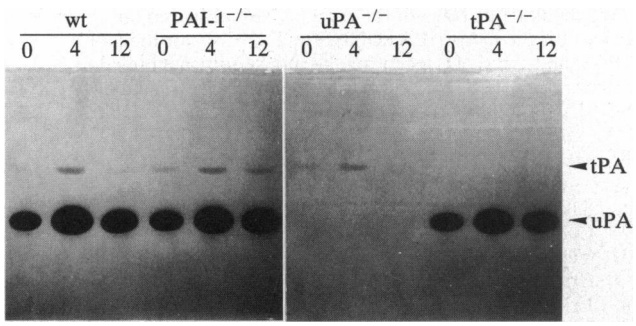

FIG. 4. Plasminogen activator activity in ovarian extracts of wild-type, PAI-1$^{-/-}$, uPA$^{-/-}$, and tPA$^{-/-}$ mice during gonadotropin-induced ovulation. Samples of ovarian extracts (100 μg of total protein) prepared from mice treated with gonadotropins for different lengths of time as described in the legend to Fig. 1 were analyzed by SDS/PAGE followed by casein zymography. Genotypes of mice used for preparation of ovarian extracts are indicated on the top. 0, 4, and 12, time (h) after hCG injection. Migration of tPA and uPA is indicated on the right.

uPA$^{-/-}$ mice had a normal ovulation efficiency. However, the low expression of tPA mRNA in the theca-interstitial tissue may provide enough PA activity to compensate for the loss of uPA, which may explain the normal ovulation efficiency in uPA$^{-/-}$ mice. Using a caseinolytic method, we have estimated the amount of plasmin in ovarian extracts of uPA$^{-/-}$ mice just before ovulation to <20% of what we find in wild-type extracts (A. Ny, L.N., and T.N., unpublished data). This suggests that under normal conditions excess amounts of plasmin are formed in the ovary just before ovulation.

The ovarian connective tissue contains type I, II, and III collagens, and type IV collagen, laminin, and proteoglycan make up the basement membrane of the follicles (37, 38). Like many other physiological processes involving cell invasion and migration through tissue barriers, ovulation is dependent on the action of highly regulated and specific proteases that can locally digest tissue proteins without associated damage (1, 3, 7). In view of the variety of substrate specificity, this targeted proteolysis cannot be performed solely by plasmin; rather, it likely requires the action of different proteolytic enzymes. One important pathway for extracellular matrix (ECM) degradation is considered to be the proteolytic cascade generated from cooperation of both the PA system and MMPs (1, 39). At least two members of MMPs (procollagenase and prostromelysin) have been shown to be activated by plasmin (17, 18, 40). Plasmin is therefore considered to be an upstream regulator of proteolytic cascades responsible for the degradation of ECM in a variety of physiological and pathological processes.

Both interstitial collagenase and gelatinase A activity and mRNA have been detected in the ovary, and a preovulatory increase of the expression of these proteases has been observed in the rat ovary during gonadotropin-induced ovulation (41–43). Although our data suggest that plasmin-independent pathways of pro-MMP activation operate in the ovary, their existence remains to be demonstrated. The recent isolation of a membrane MMP that is able to activate gelatinase A indicates that, in addition to plasmin, other unidentified activators of pro-MMPs may operate in the ovary (44).

In conclusion, our results suggest that the PA system plays a role in ovulation but that other proteases, with redundant or overlapping functions, may also participate in ovulation. In the absence of PAs, other proteases seem to provide a proteolytic activity sufficient for ovulation to take place, although with a reduced efficiency. Loss-of-function mutations in a wide variety of genes in mice have been shown to give rise to no or benign phenotypes, suggesting that functional redundancy may represent a general principle in the control of biological processes (36, 45–47).

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