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Thrombin regulates components of the fibrinolytic system in human mesangial cells

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Thrombin regulates components of the fibrinolytic system in human mesangial cells. Besides its procoagulant activity, thrombin has been shown to stimulate cell proliferation and to regulate the fibrinolytic pathway. We report here the effect of purified human alpha thrombin on the synthesis of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) by cultured human mesangial cells. Thrombin (0 to 2.5 U/ml) increased in a time- and dose-dependent manner the production of t-PA and PAI-1 (2- to 3-fold increase of secreted t-PA and PAI-1 release during a 24 hour incubation). This effect was associated with a twofold increase in DNA synthesis measured by ^{3}H-thymidine incorporation. Zymographic analysis and reverse fibrin autography showed that thrombin also increased the level of the 110 Kd t-PA-PAI-1 complex, whereas PAI-1 was present as a free 50 Kd form in the culture medium conditioned by unstimulated and thrombin-stimulated cells. Free t-PA was never observed. Both membrane binding and catalytic activity of thrombin were required since the effects of 1 U/ml thrombin were inhibited by addition 2 U/ml hirudin, which inhibits the membrane binding and catalytic activity of thrombin, and since DFP-inactivated thrombin, which has the ability to bind but which has no enzymatic activity, did not induce t-PA or PAI-1. Gamma thrombin, which does not bind to thrombin receptor, did not increase t-PA and PAI-1 releases. The effects of thrombin were probably mediated by protein kinase C activation since H7, an inhibitor of protein kinases, inhibited significantly thrombin effects on t-PA and PAI-1 production, and since addition of an activator of protein kinase A, 8-bromocyclic AMP (100 μM), induced a significant inhibition of the thrombin effect. The effects of thrombin were also suppressed by 1.25 μg/ml alpha amanitin, suggesting a requirement of de novo RNA synthesis. Northern blot analysis indicated that thrombin induced an increase in the mRNA levels of t-PA and of PAI-1. We conclude that thrombin increases DNA synthesis in human mesangial cells and enhances the synthesis of both t-PA and PAI-1. The latter is released in a large excess as compared to t-PA. Hence, thrombin may have a role in provoking a localized hypofibrinolytic state and may contribute to the persistence of glomerular fibrin deposits during proliferative glomerulonephritis.

Thrombin is a crucial factor in coagulation, but it also regulates fibrinolysis [1–3] and has a spectrum of effects on different cell types, including platelets, endothelial cells and fibroblasts [4–7]. Thrombin has been shown to stimulate DNA synthesis in cultured fibroblasts [6, 7] and to enhance the synthesis of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) by endothelial cells [1–3].

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These cellular effects require the binding of active thrombin to specific membrane receptors. Receptor occupancy, enzymatic cleavage and the signals generated by each appear to be required to stimulate cell proliferation [8]. Like many mitogens and other polypeptides, thrombin activates phospholipase C and thus causes breakdown of phosphatidylinositol 4,5 biphosphate into IP_3 and diacylglycerol [9]. IP_3 mobilizes Ca^{++} from the endoplasmic reticulum, whereas diacylglycerol stimulates protein kinase C, a Ca^{++} phospholipid dependent enzyme [10].

Coagulation processes are involved in the pathogenesis of vascular or glomerular nephropathies [11]. Intrarenal generation of thrombin can result from glomerular expression of tissue factor or thromboplastin, which has recently been described in animal [12, 13] and human glomeruli [14]. Thus thrombin could induce intravascular fibrin formation by its well-recognized coagulant effect but can also act on glomerular cells, such as mesangial cells as reported by Schultz et al [15].

We have recently shown that human mesangial cells in culture synthesize tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) [16]. The latter is released in a large excess and found in the conditioned medium in a free 50 Kd form and a tPA-PAI-1-complexed 110 Kd form [16]. This large excess of PAI-1 may inhibit the fibrinolytic activity of human glomeruli which consists mainly of t-PA [17, 18], and thus may prevent or inhibit fibrin removal. While thrombin has been reported to affect the fibrinolytic activity of non-renal endothelial cells [1–3], its effects on the synthesis of fibrinolytic components by human glomerular cells have not been evaluated. We report the results of such studies in cultured mesangial cells.

Material

The following materials were used: collagenase type IV, Triton X 100, hirudin, 8-bromocyclic AMP, alpha-amanitin from Sigma Chemical Co (St. Louis, Missouri, USA); purified human alpha-thrombin (3,000 U/mg) from Doctor Freyssinet, Strasbourg, France; diisopropylfluorophosphate from Fluka (AG Chem, FRG); protein kinase C inhibitor 1 (5 isoquinolylsulfonyl)-2-methylpiperazine (H7, Seikagaku, Tokyo, Japan); bovine thrombin from Hoffman-Laroche (Basel, Switzerland), low melting point agarose from BRL (USA), polyvinyl chloride U microtiter plates from Dynatech Laboratories (Alexandria, Virginia, USA); purified fibrinogen and plasminogen from AB Kabi (Stockholm, Sweden); human urokinase reference stan-

dard grade from Choay (Paris, France), human t-PA (600,000 U/mg), goat polyclonal antihuman t-PA, goat polyclonal antihuman PAI-1 IgG and mouse monoclonal antihuman PAI-1 antibody (MAI 12) from Biopool (Sweden); alpha ^{32}P d CTP > 3000 Ci/mmol from the Radiochemical Center (Amersham, UK).

Purified gamma thrombin prepared by trypsin digestion of alpha thrombin as described [19] was from Dr. A. Bezeaud, Paris, France. Purified alpha-thrombin (200 U/ml) was incubated for 30 minutes at 37°C with 5 mM diisopropylfluorophosphate (DFP) and then dialyzed overnight against phosphate buffer saline pH 7.4 to remove free DFP. Residual thrombin activity measured by the coagulation time of a 2 mg/ml fibrinogen solution was less than 1% of native thrombin. The determination of total protein concentration in the conditioned medium was made by the method of Folin using serum albumin as standard.

Methods

Mesangial cell preparation

Glomeruli were isolated from normal human kidneys judged to be unsuitable for transplantation, as previously described [16]. In brief, thin (2 to 4 mm) strips of decapsulated kidney cortex were minced in culture medium. Small fragments of tissue were pushed through 90-mesh stainless steel screens with a glass pestle. The resulting mixture containing glomeruli was then passed over a graded series of screens, and single, unencapsulated glomeruli were finally retained on a fine mesh screen, washed and sedimented in basal medium. These steps resulted in a preparation of glomeruli virtually free of nonglomerular contaminants.

For isolation of contractile mesangial cells, isolated glomeruli were digested by collagenase type IV (750 U/ml) for 30 minutes as described [20]. The glomerular suspension was sieved over a stainless mesh screen and isolated epithelial cells were recovered in the filtrate after repeated washings. The remaining glomerular fragments were recovered and explanted in 10 mm, Petri's dishes in complete Waymouth's medium supplemented with 20% fetal calf serum (FCS). The glomeruli were incubated at 37°C in a humidified 5% CO_2 incubator. By this method smooth muscle-like mesangial cells appeared from the glomeruli approximately 8 to 14 days following attachment. They have been previously characterized by morphological and biochemical properties [16]. They appeared large and stellate. Immunofluorescence studies with specific polyclonal antibody raised against Von Willebrand factor were negative. In order to further eliminate any contamination by either epithelial or endothelial cells, all experiments were performed between the third and fifth passages.

Experiments in cell cultures

Mesangial cells were plated in multidish wells and grown in RPMI medium supplemented with 10% FCS, 100 UI/ml penicillin and 50 μg/ml streptomycin. Twenty-four hours before the experiments, medium was removed and replaced with a serum-free minimum defined medium (48.7% HAM F12, 48.7% DMEM, 1% HEPES, 1% glutamin, 0.05% penicillin) to prevent contamination of conditioned medium by t-PA or PAI-1 from FCS. The day of the experiment, the cells were incubated 24 or 48 hours with the substances to be tested. At the end of this period, supernatants were collected and adjusted to 0.1% Triton X 100 and stored at −20°C. Care was taken to ensure that the viability of the cells did not differ between experimental and control conditions. In all cases more than 90% of the cells excluded Trypan blue dye.

Thymidine incorporation assay

Subconfluent cultured cells were incubated 24 hours in minimum defined medium (MDM) supplemented with the substances to be tested and 1 μCi/ml of ^{3}H-thymidine (Amersham, UK). As a positive control of cell growth stimulation, experiments were also performed in MDM containing 1% FCS. After incubation, 1 mg/ml cold thymidine was added to the cells for 30 minutes. Cells were counted in a Malassez plate and the radioactivity was counted in a B-counter (LKB Rackbeta, Switzerland). It has been controlled that thrombin (1 U/ml) in the presence of 1% FCS had the same coagulant activity as thrombin diluted in serum-free buffer as measured by the coagulation time of a 2 mg/ml fibrinogen solution.

Enzyme-linked immunosorbent assay (ELISA) of t-PA and PAI-1

ELISA of t-PA was made as previously described [16, 18]. ELISA of PAI-1 was slightly modified: microtiter polyvinylchloride plates were coated with 5 μg/ml specific goat polyclonal antihuman PAI-1 IgG (Biopool, Sweden). After washing, samples to be tested were added for two hours at 37°C. After washing, free and complexed PAI-1 were detected with a mouse monoclonal anti-PAI-1 antibody (250 ng/ml) which recognize both free PAI-1 and complexed PAI-1 (MAI 12, Biopool). Plates were then washed with PBS Tween 0.1% and incubated with diluted horseradish peroxidase coupled with antimouse IgG (1/1000) for one hour at 22°C. Thereafter, plates were washed and the reaction revealed by addition of ophenylene diamine and hydrogen peroxide as previously described [16]. For the ELISA of t-PA, plates were coated with 5 μg/ml goat polyclonal antihuman t-PA IgG and incubated with the samples to be tested. Free and complexed t-PA bound to the plates were then revealed by biotinylated anti-t-PA antibodies as previously reported [16]. The lowest concentrations that could be detected were 0.4 ng/ml for PAI-1 and 0.14 ng/ml for t-PA. The interassay variations were respectively 16% and 12%, and the intraassay variations were less than 10%.

Zymography and reverse fibrin autography

After separation by 10% SDS polyacrylamide gel electrophoresis, plasminogen activator activity was detected on a fibrin agar underlay (fibrinogen 10 mg/ml, thrombin 300 mU/ml, agarose 1%, plasminogen 20 μg/ml) containing plasminogen as previously described [16, 18]. Incubation was performed at 37°C under humidified atmosphere. Commercial human urokinase and t-PA were also run in parallel in the gel as standards for zymography.

To detect plasminogen activator inhibitors by reverse fibrin autography [21], u-PA (0.1 U/ml final concentration) was added to the fibrin agar underlay. Progressive lysis of the fibrin film was observed after incubation at 37°C, except at the location of plasminogen activator inhibitor.

Table 1. Effect of thrombin on DNA synthesis and proliferation of human mesangial cells in 24 hours

	Incorporated ^{3}H-thymidine cpm/10^4 cells	Cell count/well
Control	4260 ± 497	30583 ± 3306
Thrombin		
0.1 U/ml	4650 ± 310	ND
0.5 U/ml	6250 ± 500	ND
1 U/ml	8360 ± 271[a]	31471 ± 3074
5 U/ml	19980 ± 1250[a]	ND
10 U/ml	21120 ± 1180[a]	ND
FCS		
(1%)	9060 ± 381[a]	34167 ± 3506
Thrombin *1 U/ml*		
+ FCS (1%)	15050 ± 844[b]	32250 ± 869

Mesangial cells (3 to 4 × 10^4 cells/well) were incubated for 24 hours in the different conditions. ND: not done. Means ± SEM of 4 experiments made in duplicate are reported.

[a] $P < 0.05$ was compared to control conditions

[b] $P < 0.05$ as compared to thrombin addition alone, and to FCS alone

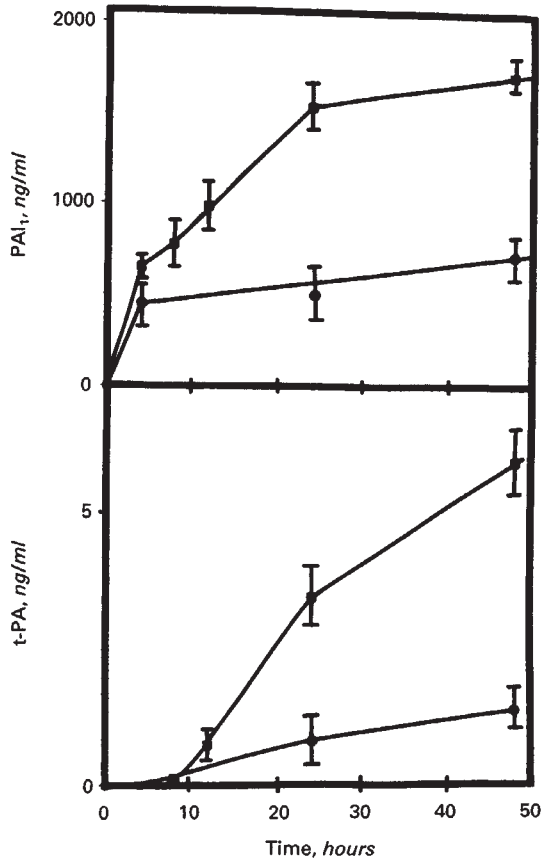

Fig. 1. *Time-course of t-PA (B) and PAI-1 (B) antigen release by human mesangial cells in the absence (●) or presence (■) of 1U/ml thrombin.* Mean ± SEM are represented ($N = 3$).

RNA extraction and Northern blot analysis

After incubation of glomerular mesangial cells total RNA was extracted by the phenol-chloroform method as described [22], and precipitated by Li Cl (3 M). Total RNA (10 μg/track) was separated by electrophoresis in 0.9% agarose gel containing

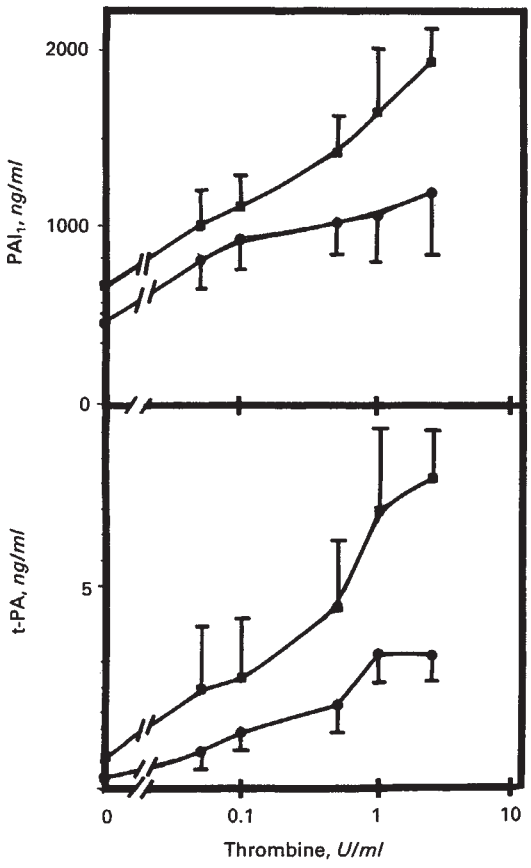

Fig. 2. *Dose-response effect of thrombin on t-PA (B) and PAI-1 (A) antigen release by human mesangial cells at 24 (●) and 48 (■) hours of incubation.* Mean ± SEM are represented ($N = 3$).

20% formaldehyde and transferred to a nylon membrane (Gene Screen Plus). Hybridization was performed using human alpha ^{32}P-labeled human cDNA probes specific for t-PA or PAI-1 as described [23]. Autoradiograms were developed after 72 to 96 hours.

The probes used were the 1,000-bp Pst1 insert of human PAI-1 cDNA (provided by Dr. David Loskutoff, Scripps Clinic and Research Foundation, La Jolla, California, USA), the Bgl II fragment of the pPA 11 4B cDNA harboring 1948-bp of the human t-PA cDNA as described [24], and the 1000-bp Pst1 fragment of β actin cDNA as previously described [25] and provided by Stephania Petrucco (ISREC, Epalinges, Switzerland).

Statistical analysis

Data are reported as means ± SEM. Results were compared with Student's *t*-test or analysis of variance (ANOVA) when appropriate.

Results

Thymidine incorporation

Table 1 shows the results of thymidine incorporation in a 24 hour incubation. Addition of THR to cultured cells induced an increase in thymidine incorporation. This effect was dose-dependent and magnified following addition of 1% FCS. In

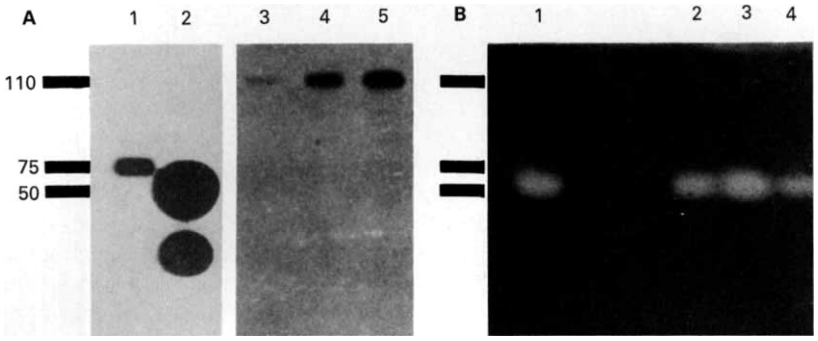

Fig. 3. *Effect of thrombin on t-PA and PAI-1 activities by zymography* (**A**) and reverse fibrin autography (**B**). A. Zymography. Lane 1: control human t-PA; lane 2: control human u-PA; lane 3: culture medium of unstimulated mesangial cells; lanes 4 and 5: culture medium of mesangial cells stimulated by 0.5 U/ml (lane 4) or 1 U/ml (lane 5) thrombin for 24 hours. B. Reverse fibrin autography. Lane 1: control human 50 Kd PAI1; lane 2: culture medium of unstimulated mesangial cells; and lanes 3 and 4: culture medium of mesangial cells stimulated by 0.5 U/ml (lane 3); or 1 U/ml (lane 4) thrombin for 24 hours.

Table 2. Inhibition of thrombin effect on mesangial cells by huridin addition and DFP inactivation

	t-PA	PAI-1
	ng/ml	
Control	3.76 ± 0.97	456 ± 111
Thrombin *1 U/ml*	7.94 ± 1.26[a]	1066 ± 258[a]
Hirudin *2 U/ml*	3.94 ± 1.19	410 ± 120
Thrombin *1 U/ml* + Hirudin *2 U/ml*	3.42 ± 0.37[b]	551 ± 190[b]
DFP-thrombin *1 U/ml*	4.62 ± 1.04[b]	463 ± 80[b]
Gamma thrombin 0.5 U/ml	3.84 ± 0.52[b]	466 ± 60[b]
1 U/ml	4.14 ± 0.32[b]	504 ± 36[b]

Mesangial cells (3 to 4 × 10^4 cells/well) were incubated for 24 hours in the different conditions. Mean ± SEM of 4 experiments made in duplicate are reported.

[a] $P < 0.05$ as compared to respective control values

[b] $P < 0.05$ as compared to alpha thrombin-stimulated levels

Table 3. Effect of H7 and 8-bromocyclic AMP addition to thrombin stimulated mesangial cells

	t-PA	PAI-1
	ng/ml	
Control	3.50 ± 1.05	372 ± 89
H7 *12.5 μM*	3.08 ± 1.05	296 ± 105
8-bromo cAMP *100 μM*	2.91 ± 1.46	231 ± 82
Thrombin *1 U/ml*	6.58 ± 0.48[a]	1211 ± 333[a]
Thrombin (1 U/ml) + H7 *5 μM*	6.49 ± 0.76[a]	828 ± 190
Thrombin (1 U/ml) + H7 *12.5 μM*	4.12 ± 0.53[b]	558 ± 90[b]
Thrombin (1 U/ml) + H7 *25 μM*	3.23 ± 1.53[b]	463 ± 111[b]
Thrombin (1 U/ml) + 8-bromo cAMP *100 μM*	2.4 ± 0.62[b]	610 ± 62[b]

Mesangial cells (3 to 4 × 10^4 cells/well) were incubated for 24 hours in the different conditions. Mean ± SEM of 3 to 5 experiments made in duplicate are reported.

[a] $P < 0.05$ as compared to respective control values

[b] $P < 0.05$ as compared to thrombin stimulated levels

contrast to the increase in thymidine incorporation induced by thrombin and/or FCS, the cell number was not significantly increased, suggesting that a longer time was required to observe cell division. The total protein concentration in conditioned culture medium was not modified by 1 U/ml thrombin (not shown).

Effect of thrombin on t-PA and PAI-1 synthesis

Thrombin stimulated t-PA and PAI-1 synthesis in a time- and dose-dependent manner. Over a 48 hour period, the time course of production of t-PA and PAI-1 were different (Fig. 1). A rapid increase of PAI-1 release induced by 1 U/ml thrombin was observed at four hours. In contrast, 24 hours of incubation was required to observe a significant increase of secreted t-PA mediated by thrombin, and the rate of release remained high until 48 hours. Both t-PA and PAI-1 syntheses were increased twofold in a 24 hour incubation in the presence of 1 U/ml thrombin compared to unstimulated conditions.

As shown in Figure 2, the effect of thrombin was dose-dependent. After 24 and 48 hours of incubation, the thrombin effect was significant ($P < 0.05$ for PAI-1 synthesis, $P < 0.001$ for t-PA), and there was a significant difference between 24 and 48 hours of incubation concerning the production of both PAI-1 and t-PA ($P < 0.001$ for both). There was no significant statistical interaction between the effects of thrombin and time of incubation, indicating that thrombin had a significant effect whatever the time of incubation considered.

By zymography (Fig. 3A), the previously described 110 to 120 Kd t-PA-PAI-1 complex [16] was found to increase when cells were incubated with thrombin. No free t-PA was observed, but free 50 Kd PAI-1 was detected in the conditioned medium by reverse fibrin autography (Fig. 3B), indicating that PAI-1 was released in excess as compared to t-PA. The amount of this free form was increased at lower thrombin concentrations but seemed to be decreased for thrombin concentrations of 1 U/ml or more (Fig. 3B).

Mechanisms of action of thrombin on human mesangial cells

The specificity of the thrombin effect was tested by addition of hirudin, a natural inhibitor of thrombin, which blocks both proteolytic and membrane receptor binding functions of thrombin [26, 27]. As shown in Table 2, a significant inhibition of thrombin effect on mesangial cells was obtained by addition of 2 U/ml hirudin to mesangial cells incubated with 1 U/ml thrombin. Hirudin alone at this dose had no significant effect on t-PA and PAI-1 productions. DFP-thrombin, which can bind to

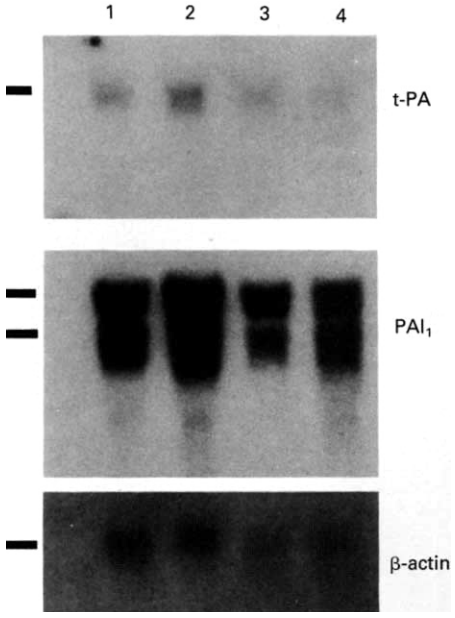

Fig. 4. *Northern Blot analysis with a t-PA cDNA probe and PAI-1 cDNA probe.* Ten μg of total RNA were used from mesangial cells incubated for 4 hours in control conditions (lane 1), in the presence of 1 U/ml thrombin (lane 2), in the presence of 12.5 μmol H7 (lane 3), and in the presence of 1 U/ml thrombin and 12.5 μmol H7 (lane 4). The control made with the βactin cDNA probe shows that the same amount of total RNA was transferred in each lane.

thrombin receptor but which has no enzymatic activity [27–29], did not stimulate PAI-1 or t-PA synthesis. Furthermore, gamma thrombin, which has enzymatic activity but does not bind to thrombin receptors, did not stimulate t-PA and PAI-1 synthesis. In separate experiments hirudin (2 U/ml) inhibited the thrombin-stimulated thymidine incorporation by 90%. DFP-thrombin did not stimulate thymidine uptake compared to control conditions (respectively 2850 ± 969 and 3435 ± 710 cpm/10^4 cells, $N = 3$, $P > 0.05$).

To investigate the intracellular pathways which were stimulated by thrombin we used H7, an inhibitor of protein kinases [30]. As shown in Table 3, H7 inhibited the thrombin-stimulated releases of PAI-1 and t-PA in a dose-dependent manner. To determine if protein kinase A activation was involved after thrombin addition as reported by others [31, 32], we added 100 μmol 8-bromocyclic AMP, which was found to inhibit the thrombin mediated increase of secreted PAI-1 and t-PA without significantly changing the basal rate of synthesis (Table 3). A 70% inhibition of the thrombin mediated increase of secreted PAI-1 and t-PA was also found in the presence of 1.25 μg/ml alpha-amanitin (from 8.58 ± 1.53 to 5.13 ± 0.57 ng/ml for t-PA and 1658 ± 358 to 666 ± 155 ng/ml for PAI-1; $N = 6$; $P < 0.05$ and $P < 0.01$, respectively). By Northern blot analysis thrombin was shown to increase the levels of t-PA and PAI-1 mRNA. This effect was also inhibited by 12.5 μmol H7 (Fig. 4).

Discussion

This study has been initiated to determine the possible effect of thrombin on the mesangial fibrinolytic system. We have previously found that human mesangial cells in culture can synthesize t-PA and PAI-1, and that PAI-1 was released in a large excess as compared to t-PA [16]. These results suggested that these cells can play a role in the pathogenesis of glomerular thromboses and the presence of glomerular fibrin deposits.

Our present results report that thrombin increases the synthesis of both t-PA and PAI-1 by these cells. ELISA assays show an increase of both antigens in the presence of thrombin and PAI-1 was always released in a large excess compared to t-PA which was never found in a free form. Accordingly, increased amounts of t-PA-PAI-1 complexes were demonstrated by zymography and of free PAI-1 by reverse fibrin autography. By this latter method, however, PAI-1 seemed to be decreased in the presence of the highest doses of thrombin. This discrepancy with the results of ELISA can be explained by a direct inactivation of PAI-1 by thrombin which has been shown to induce a limited proteolysis of PAI-1 [33]. Thus, thrombin increases the release of both t-PA and PAI-1 and PAI-1 is always found in excess.

Recently an increased secretion of t-PA and PAI-1 by human umbilical vein endothelial cells in response to thrombin was reported. It was mediated through an increase in mRNA levels of t-PA and PAI-1 [3]. We also demonstrated an increase of the steady state levels of these mRNA in mesangial cells treated by thrombin. This result and the inhibitory effect of alpha amanitin suggest that thrombin stimulates tPA and PAI1 gene transcription in mesangial cells.

The effect of thrombin on human mesangial cells is probably mediated by an interaction with specific membrane receptors. Although we did not demonstrate directly their presence on mesangial cells, our results are consistent with the previously reported properties of these receptors: hirudin, which blocks both catalytic activity and binding of thrombin to a high-affinity receptor [26, 27], and DFP-inactivation of the thrombin-catalytic site both blocked the thrombin-mediated increase of t-PA and PAI-1. Gamma thrombin which did not bind to membrane receptors of thrombin had no effect on t-PA and PAI-1 synthesis. Thrombin also increases DNA synthesis in mesangial cells [15], and we found that this effect was additive with 1% FCS, which at this low concentration did not block the proteolytic activity of thrombin. As previously reported for t-PA and PAI-1 synthesis by endothelial cells [1–3] and for regulation of mitogenesis in fibroblasts [6,7], both the ability to bind and catalytic activity must be intact for thrombin to induce its effects on human mesangial cells. Interaction of active thrombin with the cell membrane results in phospholipase C activation, calcium mobilization which has been demonstrated in fibroblasts and in human mesangial cells [15] and protein kinase C activation [10]. This intracellular pathway is probably stimulated by thrombin in mesangial cells since we reported previously that phorbol myristate acetate, a known activator of protein kinase C, increases t-PA and PAI-1 released by human mesangial cells [34] and since H7, an inhibitor of protein kinases, inhibited thrombin effect at the level of t-PA and PAI-1 messenger RNAs and the level of t-PA and PAI-1 antigen productions. H7 is an isoquinoline sulfonamide derivative with a Ki value for purified protein kinase C of 6 μM [30]. At higher concentrations, it also inhibits cyclic nucleotide-dependent protein kinases A and G [28]. Thrombin has also been shown to stimulate adenylate cyclase in some cell types [31] and in rat isolated glomeruli [32]. However, 8-bromocyclic AMP had an inhibitory effect on

thrombin stimulation of t-PA and PAI-1 productions by mesangial cells. We did not study the adenylate cyclase activity of mesangial cells after thrombin addition, but our results suggest that protein kinase C activation was required for the effects on t-PA and PAI-1 whereas protein kinase A activation, if any, had rather an inhibitory effect.

In summary, thrombin can induce DNA synthesis in human mesangial cells and may be involved in the pathogenesis of proliferative glomerulonephritis. Furthermore, thrombin increases t-PA and PAI-1 synthesis at least in part by increasing gene transcription. Although at high doses thrombin partially inactivates PAI-1, an excess of PAI-1 is released as compared to t-PA. Thus thrombin may promote fibrin formation in glomeruli by its procoagulant effect and may also inhibit fibrin degradation by increasing release of free PAI-1 from mesangial cells.

Acknowledgments

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