

# Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo<sup>1</sup>

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## **Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo.**

**Background.** The fibrinolytic system plays an important role in degrading fibrin-rich thrombi and in vascular and tissue remodeling. Elevated levels of plasminogen activator inhibitor-1 (PAI-1) can reduce the efficiency of the endogenous fibrinolytic system. Angiotensin (Ang) has been shown to regulate PAI-1 expression via the Ang type 1 (AT1) receptor in some tissues and via the AT4 receptor in cultured endothelium. The purpose of this study was to examine the tissue-specific pattern of PAI-1 expression in response to infusion of Ang II in vivo.

**Methods.** Adult male Sprague-Dawley rats ( $N = 5$  in each group) were treated with four hours of intravenous infusions of Ang II or vehicle control while mean arterial pressure (MAP) was monitored: group 1, 600 ng/kg/min Ang II; group 2, Ang II + 10 mg/kg of the AT1 receptor antagonist (AT1RA) L158-809 q2 hour; group 3, Ang II + 0.01 to 0.1 mg/kg hydralazine as required to maintain normal blood pressure; and group 4, saline-infused controls. After infusion, tissue was harvested for Northern blotting, immunohistochemical analysis, and in situ hybridization.

**Results.** In group 1, Ang II infusion increased MAP from  $105 \pm 8$  to  $160 \pm 9$  mm Hg (mean  $\pm$  SE,  $P < 0.01$ ). Ang II induced increased expression of PAI-1 mRNA in all tissues examined from 5.1-fold in the heart, 9.7-fold in the kidney, 10.0-fold in the aorta, and up to 30.0-fold in the liver (all  $P < 0.01$  vs. control). While both AT1RA (group 3) and hydralazine (group 4) prevented Ang II-induced elevation in blood pressure, the Ang II-dependent expression of PAI-1 mRNA was reduced by only AT1 receptor blockade.

**Conclusions.** We conclude that in the rat, PAI-1 is induced in a variety of tissues by Ang II directly through the AT1 receptor, independent of its effects on blood pressure.

Activation of the renin-angiotensin system (RAS) is associated with vascular and glomerular sclerosis and

tubulointerstitial fibrosis [1–3]. The accumulation of extracellular matrix (ECM) in vascular and renal tissue is a product of both increased matrix synthesis and reduced ECM degradation. The cytokines and growth factor-like effects of angiotensin (Ang) II, which are largely mediated via the Ang II type 1 AT1 receptor [4], lead to increased ECM deposition. Recent studies suggest that Ang II may also play a role in regulating the activity of proteolytic pathways involved in matrix degradation. Ang II regulates the expression of protease inhibitors that reduce endogenous proteolytic housekeeping activity, including plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) [5–9]. PAI-1 is a member of the superfamily of serine protease inhibitors and is the primary physiologic inhibitor of tissue-type PA (t-PA) and urokinase-type PA (u-PA) [10]. Plasmin is generated from the inactive precursor plasminogen by the action of plasminogen activators. Plasmin's broad proteolytic activity makes it important in wound repair, ECM degradation, and fibrinolysis. Plasmin plays an additional role in regulating ECM deposition by activation of latent matrix metalloproteinases (MMPs) [11], which degrade ECM proteins in the kidney. Thus, it is not surprising that PAI-1 influences the development of fibrosis in tissues and that PAI-1 deficiency protects mice from bleomycin-induced pulmonary fibrosis [12]. We have hypothesized that the well-recognized effects of Ang on fibrosis may be augmented by its actions to induce PAI-1 [13, 14]. In vitro data have shown that the AT1 receptor mediates this Ang-induced PAI-1 expression in vascular smooth muscle cells, while the Ang II-derived hexapeptide metabolite, Ang IV, acting through the AT4 receptor, regulates PAI-1 expression in bovine aortic endothelial cells [5–7]. In contrast, in vivo data have shown that both Ang I-converting enzyme inhibitor (ACEI) and an Ang type 1 receptor antagonist (AT1RA) were associated with decreased scarring and decreased PAI-1 expression in a renal model of sclerosis [14]. However, the specific mechanisms for Ang II induction in vivo of PAI-1 have not been previously examined.

<sup>1</sup>See Editorial by Basile, p. 460

**Key words:** renin-angiotensin system, plasmin/plasminogen activator system, extracellular matrix, blood pressure.

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The present study was performed in order to identify and characterize the induction of PAI-1 by Ang II in vivo and to determine whether hemodynamic effects were involved with this effect.

## METHODS

### Experimental design and animals

Adult male Sprague-Dawley rats ( $N = 25$ , 350 to 450 g; Charles River, Nashville, TN, USA) were studied. Animals were housed under normal conditions with a 12-hour light/dark cycle at 70°F with 40% humidity and 12 air exchanges/hour. Rats received normal rat chow and water ad libitum ("5001" Purina Laboratory Rodent diet, 23.4% protein, 4.5% fat, 6.0% fiber, and 0.40% sodium) before the study. All studies were approved by the Institutional Animal Care and Use Committee.

Rats were anesthetized with 50 mg/kg inactin intraperitoneally (Byk, Gulden Konstanz, Germany) and placed on a temperature-controlled table set at 37°C. Polyethylene catheters were placed in the right femoral vein and artery for continuous infusion and monitoring of arterial blood pressure by a transducer recorder (Blood Pressure Analyzer 100; Micro-Med Inc., Louisville, KY, USA). All rats were treated with four hours of continuous intravenous infusions at 1.0 mL/h rates as specified later in this article. The dose of Ang II was chosen based on pilot studies for maximum induction of PAI-1 while being able to control blood pressure with hydralazine (doses from 200 ng/kg/min; data not shown). All compounds were dissolved in 0.9% sterile sodium chloride solution. Mean arterial pressure (MAP) was monitored continuously throughout the study period. There were five animals in each treatment group. Group 1 was infused with Ang II, 600 ng/kg/min (Ang II acetate salt; Sigma, St. Louis, MO, USA). Group 2 was infused with Ang II, 600 ng/kg/min + L158-809, 10 mg/kg every 2 hours intravenously (highly specific AT1RA, gift of Merck Research Laboratories Inc., Rahway, NJ, USA). Group 3 was infused with Ang II, 600 ng/kg/min + hydralazine, 0.01 to 0.1 mg/kg intravenously (Sigma), as required to maintain normal blood pressure. Group 4 was infused with saline, 1.0 mL/hour (control).

Rats were sacrificed immediately after the end of the four hours of infusion, and kidney, liver, heart, and aorta were harvested. Tissues for morphological studies were immersion fixed in 4% paraformaldehyde-phosphate-buffered saline (PBS) solution. Portions of tissues were homogenized in isopropanol for precipitation of RNA.

### cDNA probes

The cDNA fragment for rat PAI-1 mRNA was prepared by reverse transcription-polymerase chain reaction (RT-PCR) and cloned using a TA cloning kit (Invitrogen, Carlsbad, CA, USA), and harvested and purified from

*E. coli* as previously described [14]. Fidelity of the cDNA product was confirmed by DNA sequence analysis. The commercial human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe was used as a housekeeping gene control (Promega, Madison, WI, USA). cDNA probes were labeled with [<sup>32</sup>P]dCTP (New England Nuclear, Boston, MA, USA) by the random primer method.

### RNA isolation and Northern blot hybridization

Total RNA from left kidney, liver, heart, and aortic tissues were extracted by RNeasy<sup>TM</sup> B method (Qiagen, Houston, TX, USA). RNA pellets were resuspended in DEPC-treated water, and RNA concentration was determined by absorbance at 260 nm. RNA (15 µg) was size fractionated on 1.0% formaldehyde agarose gels. Equal loading of RNA was confirmed by visual examination of ribosomal RNA by ethidium bromide staining. RNA was transferred to nylon membranes (Hybond N; Amersham, Piscataway, NJ, USA) and cross-linked by ultraviolet illumination. The membranes were incubated in prehybridization buffer for two hours and then hybridized with [<sup>32</sup>P] dCTP-labeled cDNA probes for 18 to 24 hours at 65°C in hybridization buffer [4 × SSC, 1 × Denhardt's, 1 × sodium dodecyl sulfate (SDS), 100 mg/mL denatured salmon sperm DNA, and 10% dextran sulfate]. Membranes were washed twice in 2 × standard saline citrate (SSC), 0.1% SSC for 20 minutes at 65°C and once in 0.1 × SSC, 0.1% SSC for 20 minutes at 65°C. Membranes were air dried and exposed to XAR film (Kodak Co., Rochester, NY, USA) in intensifying screens at -70°C for three to five days. Autoradiographs were scanned by image scanner JX-330 (Sharp, Japan), and the intensity of signals was measured by NIH Image (National Institute of Health, Bethesda, MD, USA). The ratio of specific message to the housekeeping gene GAPDH was used to quantitate expression for each tissue sample.

### In situ hybridization

<sup>35</sup>S-labeled sense and antisense riboprobes for PAI-1 were prepared by transcription of the pCR<sup>TM</sup> II plasmid with insertion of cDNA fragment by SP6 or T7 RNA polymerase as previously described (Promega) [14]. Sections were dewaxed in xylene and hydrated in graded ethanols and then 4% paraformaldehyde. After treatment by proteinase K and triethanolamine/acetic anhydride, sections were dehydrated in ethanol and air dried. Hybridization was done in buffer [50% formamide, 10% dextran sulfate, 8 mmol/L dithiothreitol (DTT), 0.2 mg/mL tRNA, 300 mmol/L NaCl, 10 mmol/L Tris-HCl, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% BSA] overnight at 50°C. Sections were washed in 5 × SSC, 20 mmol/L β-mercaptoethanol at 50°C for 15 minutes, in 2 × SSC, 200 mmol/L β-mercaptoethanol, 50%

formamide at 68°C for 20 minutes, in TEN twice at 37°C for 10 minutes, treated with RNase at 37°C for 30 minutes, washed in TEN at 37°C for 10 minutes, in  $2 \times$  SSC, and  $0.1 \times$  SSC each twice at 68°C for 15 minutes. Sections were then dehydrated in ethanol and air dried, dipped in photographic emulsion, and exposed at 4°C for 10 days. The sections were developed with D-19 developer (Kodak) and counterstained with toluidine blue. Control in situ hybridization was done with sense probes.

### Immunohistochemistry

Tissues were routinely processed, and 4  $\mu$  sections were prepared. Sections were treated by 3% hydrogen peroxidase for 10 minutes, power block (BioGenex Laboratories, San Ramon, CA, USA) for 45 minutes, and then incubated with 20  $\mu$ g/mL rabbit anti-rat PAI-1 antibody overnight (American Diagnostica Inc., Greenwich, CT, USA). This antibody has been previously characterized and shown to be specific for PAI-1 [15]. After rinsing twice with PBS, biotinylated goat anti-rabbit Ig (BioGenex) was added, incubated for 45 minutes, followed by rinsing two times in PBS, and incubation with peroxidase conjugated streptavidin (BioGenex) for 45 minutes. After rinsing three times with PBS, diaminobenzidine was added as a chromagen. Slides were counterstained with hematoxylin. Control slides treated with nonspecific antisera instead of the primary antibody showed no staining. All sections were examined without knowledge of the treatment protocol.

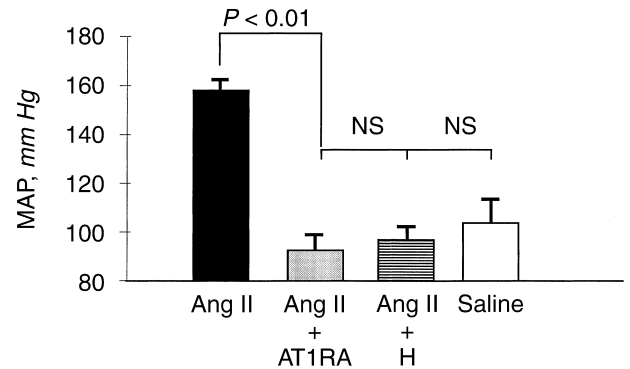
### Statistical analysis

The average of ratios of PAI-1 mRNA and GAPDH for each group was compared using Fisher's PLSD followed by analysis of variance (ANOVA). Results are presented as means  $\pm$  SE. A  $P$  value  $<0.05$  was considered significant.

## RESULTS

### Effect of Ang II on MAP

Angiotensin II infusion alone (group 1) resulted in increased MAP, from baseline  $105 \pm 8$  to  $160 \pm 9$  mm Hg ( $P < 0.01$ ; Fig. 1). In contrast, saline-infused controls (group 4) exhibited no significant change in MAP during the study period (baseline  $106 \pm 9$  vs.  $107 \pm 14$  mm Hg at end of infusion,  $P = \text{NS}$ ). In order to examine whether any induction of PAI-1 mRNA was blood pressure dependent or mediated by nonhemodynamic actions of the AT1 receptor, Ang II-infused rats were treated with an AT1RA, L158,809 (group 2), or hydralazine (group 3), to normalize MAP during Ang II infusion. These agents effectively prevented the pressor responses to Ang II (baseline  $95 \pm 9$  vs.  $93 \pm 9$  mm Hg after infusion in group 2; baseline  $103 \pm 5$  vs.  $97 \pm 8$  mm Hg after infusion



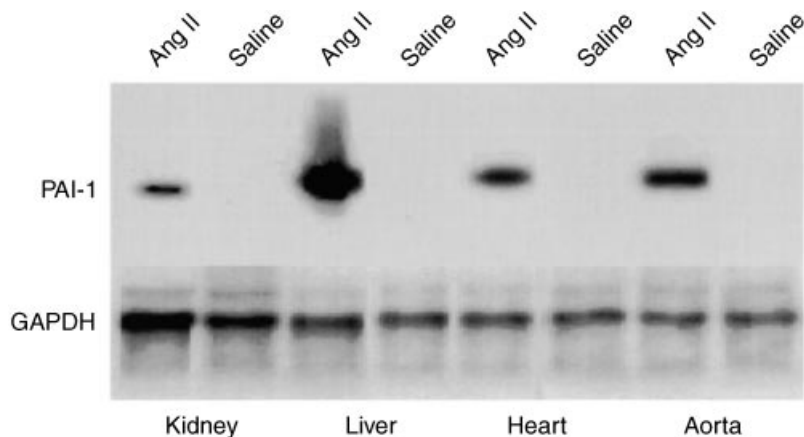
**Fig. 1.** Mean arterial pressure (MAP) data during infusions are shown. Angiotensin II (Ang II) resulted in a significant increase in MAP. Both Ang II type 1 receptor antagonist (AT1RA) and hydralazine (H) normalized MAP, although only AT1RA decreased PAI-1 expression.

in group 3;  $P = \text{NS}$  vs. control,  $P = \text{NS}$  group 2 vs. group 3; Fig. 1).

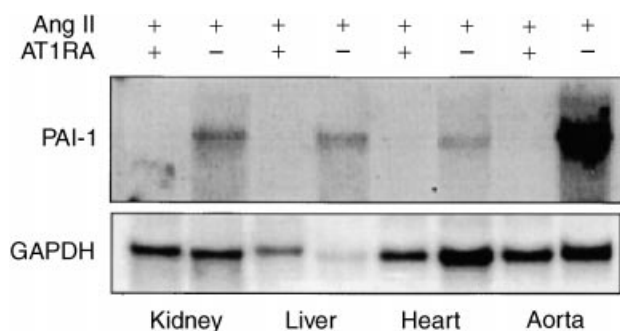
### Effects of Ang II on PAI-1 expression

**PAI-1 expression by Northern blot.** The expression of PAI-1 mRNA was increased in all tissues examined in response to Ang II infusion. The expression of PAI-1 mRNA relative to GAPDH was increased by Ang II versus saline control 9.7-fold in kidney (arbitrary density units  $1.93 \pm 0.74$  vs.  $0.20 \pm 0.08$ ), 5.1-fold in heart ( $1.63 \pm 0.29$  vs.  $0.32 \pm 0.08$ ), 10.0-fold in aorta ( $2.30 \pm 0.52$  vs.  $0.25 \pm 0.11$ ), and 30.0-fold in liver ( $3.90 \pm 0.86$  vs.  $0.13 \pm 0.02$ , all  $P < 0.01$  vs. control; Fig. 2). The PAI-1/GAPDH mRNA ratio was thus significantly increased in group 1 Ang II-infused rats versus control saline. The increased expression of PAI-1 mRNA in response to Ang II in these tissues was completely abolished by the AT1RA (group 2: PAI-1/GAPDH mRNA ratios for kidney, heart, aorta, and liver 0.8-, 0.8-, 1.2-, and 1.5-fold vs. control, arbitrary density units  $0.16 \pm 0.10$ ,  $0.25 \pm 0.13$ ,  $0.31 \pm 0.09$ , and  $0.20 \pm 0.14$  in each organ, respectively, all  $P = \text{NS}$  vs. control; Fig. 3). In contrast, the expression of PAI-1 mRNA in response to Ang II was not attenuated when MAP was normalized by hydralazine (Fig. 4). The PAI-1/GAPDH mRNA ratios were significantly higher than control saline in group 3 animal tissues (PAI-1/GAPDH mRNA ratios for kidney, heart, aorta, and liver 9.3-, 3.9-, 8.1- and 21.5-fold, respectively, vs. control; arbitrary density units  $1.85 \pm 0.43$ ,  $1.23 \pm 0.29$ ,  $2.03 \pm 0.96$ , and  $2.79 \pm 0.85$  in each organ, all  $P < 0.05$  vs. control) and not different from Ang II alone ( $P = \text{NS}$ ; Fig. 5).

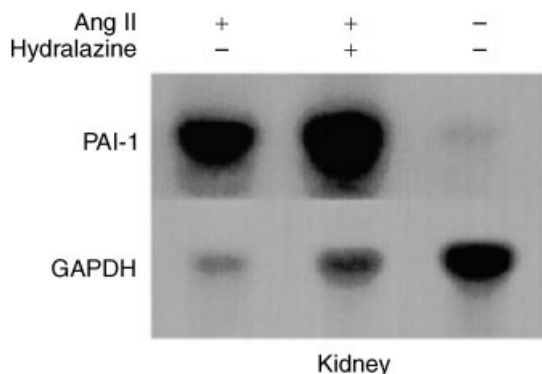
**PAI-1 expression by in situ hybridization and immunohistochemistry.** There was minimal expression of PAI-1 mRNA by in situ hybridization in group 4 saline control infusion rats, with low-level signal diffusely over all tubules in the kidney, without a specific signal in glomeruli, endothelium, collecting duct, or smooth muscle of pelvis



**Fig. 2. After infusion of Ang II, the expression of PAI-1 mRNA was increased in all tissues.** Control saline did not induce plasminogen activator inhibitor-1 (PAI-1) mRNA. PAI-1/GAPDH ratio was significantly increased in Ang II versus control saline. (Each lane represents mRNA from one rat. Northern blots from three rats representative of the entire group are shown.)



**Fig. 3. Expression of PAI-1 mRNA was attenuated in Ang II + AT1RA in all tissues analyzed.** The PAI-1/GAPDH ratio was significantly decreased compared with Ang II ( $P < 0.01$ ), but was not different from control saline ( $P = NS$  vs. control, representative Northern blots).



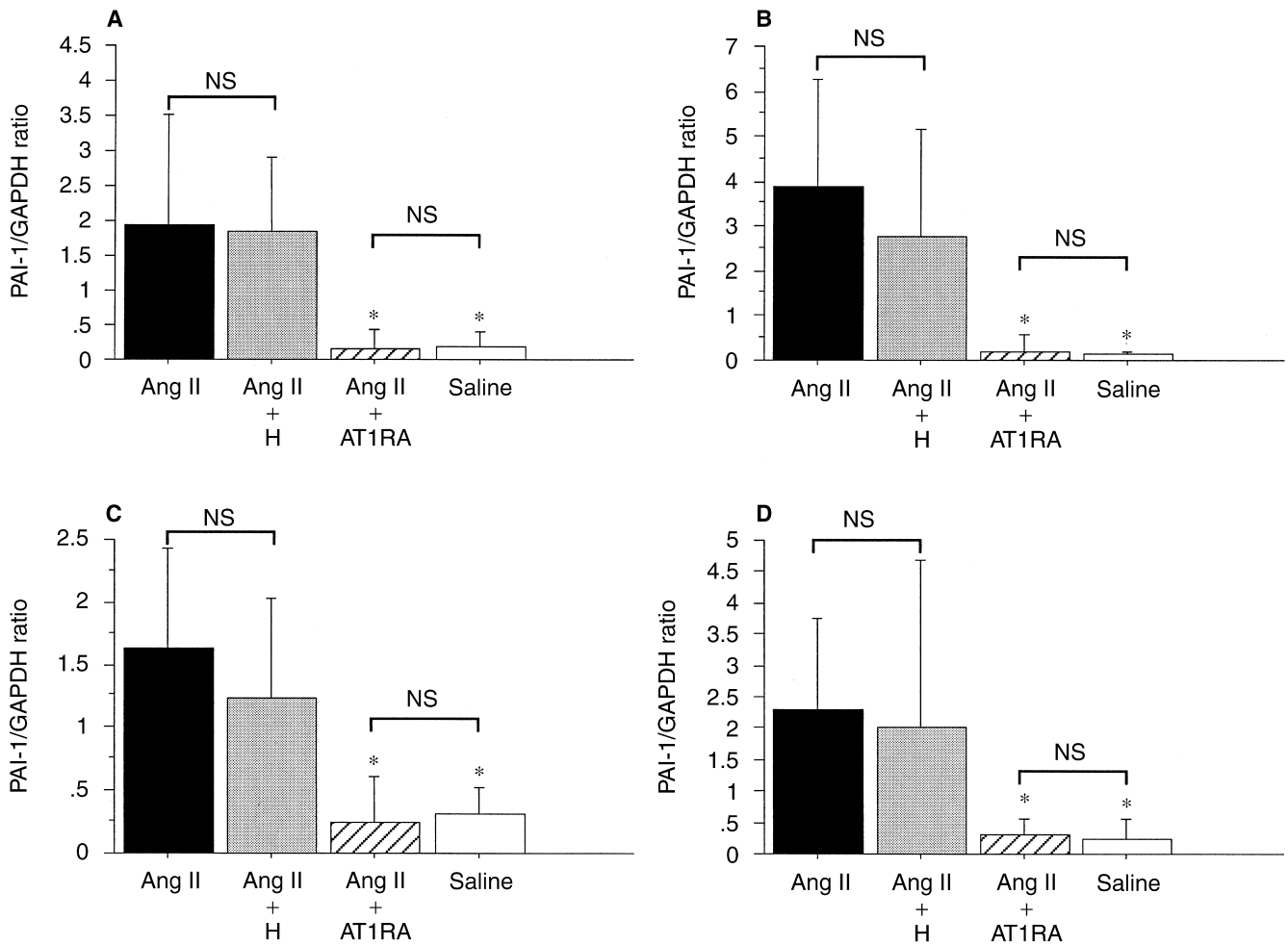
**Fig. 4. The increased expression of PAI-1 mRNA in response to Ang II was not attenuated when MAP was normalized by hydralazine (representative Northern blots).**

(Fig. 6 g, i, k). Minimal signal was present in the heart with occasional signal over endocardium and myocardium, and likewise, minimal signal was present in aorta and liver (data not shown). In Ang II-treated animals (group 1), PAI-1 mRNA signals in the kidney were present in the smooth muscle layer of pelvis, collecting duct,

at the vascular pole of the glomerulus, mesangial and parietal epithelial cells, and endothelial cells (Fig. 6 b, d, f, j, l). The strongest signals were present in collecting duct, smooth muscle layer of pelvis, and fat tissue around the pelvis. In the heart in group 1 rats, PAI-1 signal was diffusely distributed in myocytes, with even stronger signal in myocytes at the boundary between right and left ventricle, and portions of right ventricular wall (Fig. 6f). In the aorta, PAI-1 signal was present in endothelial cells and in the outer portion of the vascular smooth muscle cell layer after Ang II infusion (Fig. 6d). In the liver, PAI-1 signal was expressed diffusely in hepatocytes after Ang II infusion. These signals were decreased with AT1RA in group 2 to patterns comparable to control (Fig. 6h). Immunostaining in saline-infused control animals showed low-level staining in all tissues without increased staining at any specific sites (data not shown). Immunohistochemistry in Ang II-infused rats showed PAI-1 protein present in a distribution similar to that of its mRNA (Fig. 6 a, c, e), while staining was indistinguishable from control saline-infused rats when animals also received hydralazine (data not shown). No specific signal was detected with sense probe (data not shown). No specific immunostaining was detected when nonspecific primary antibody was used (data not shown).

## DISCUSSION

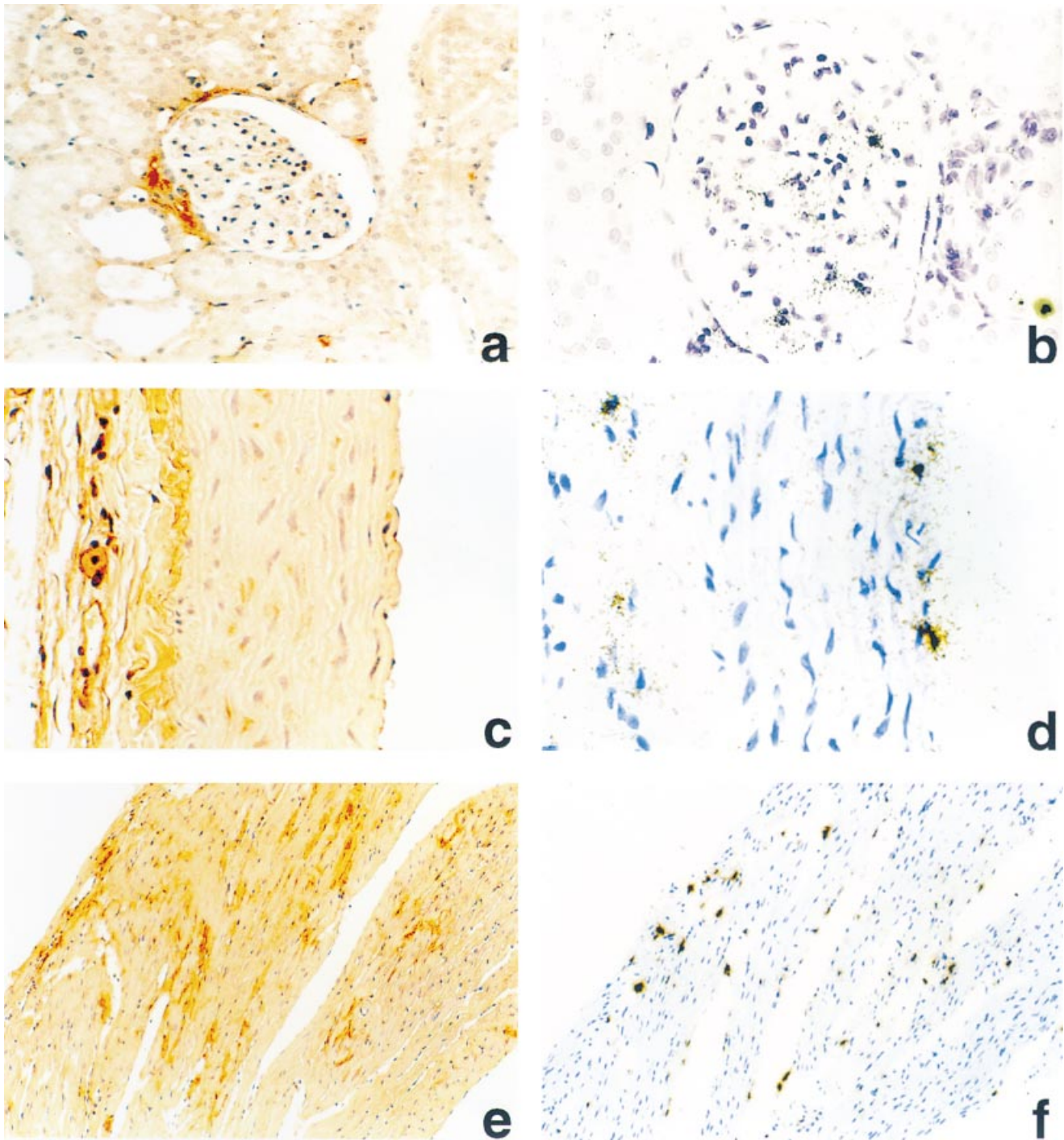
In the current study, we demonstrate that Ang II acutely induces PAI-1 expression in the rat. This effect of Ang II is largely mediated via the AT1 receptor and appears to be independent of blood pressure effects, although a minor component contributed to by local pressure effects cannot be definitively excluded based on our measurements of systemic blood pressure. Thus, normalization of Ang II-induced hypertension with hydralazine had no effect on PAI-1 induction. This induction by Ang II of PAI-1 may be a key contributor to the effects of Ang II in promoting tissue injury and/or



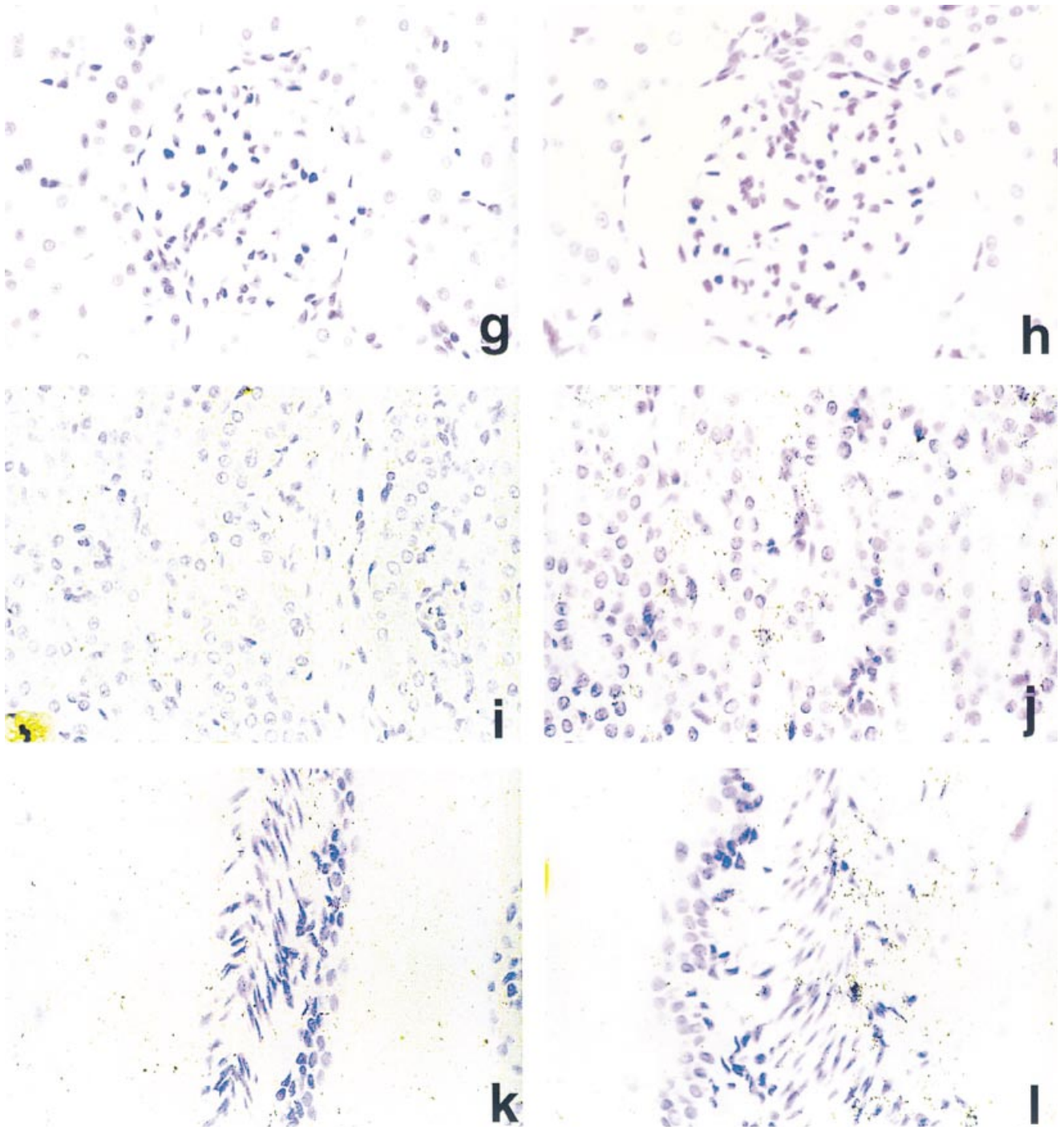
**Fig. 5.** Quantitative assessment for PAI-1/GAPDH ratios from all rats in all groups in (A) kidney, (B) liver, (C) heart, and (D) aorta is shown. \* $P < 0.01$ ; # $P < 0.05$  vs. Ang II or Ang II + hydralazine (H).

blunting reparative responses. Although activation of the RAS is generally associated with increased fibrosis, there is substantial evidence that inhibition of the RAS ameliorates progressive renal injury beyond that which can be attributed to blood pressure effects alone [1, 3, 16]. In the 5/6 nephrectomy hypertensive rat model of progressive renal disease, simple blood pressure reduction with hydralazine failed to attenuate glomerulosclerosis, while ACEI or AT1RA (with similar systemic blood pressure effect) were effective in this regard [17]. In the nonhypertensive radiation nephropathy rat model, both ACEI and AT1RA completely prevented tissue injury [14]. These results show that inhibition of the RAS can attenuate tissue injury via the AT1 receptor independently of antihypertensive actions and suggest that merely controlling blood pressure with nonspecific antihypertensives may not have the same antifibrotic potential. The implication follows that the local RAS, rather than only systemic blood pressure, may play a predominant role in the development of sclerosis.

Activation of the RAS has previously been shown to induce multiple growth factors that have been implicated in fibrosis, such as transforming growth factor- $\beta$  (TGF- $\beta$ ) and platelet-derived growth factor-B (PDGF-B) [3, 18–20]. Induction of these growth factors appears to be mediated by Ang II acting at the AT1 receptor [21]. TGF- $\beta$  itself is known to regulate PAI-1 expression through a Smad-dependent signaling pathway [22]. However, the early induction of PAI-1 by Ang II in vitro in mesangial cells is TGF- $\beta$  independent [23]. The link between the RAS and the PAI-1 proteolytic pathway has only been recognized more recently, but may be a key mechanism of Ang II-induced injury [24]. We have previously shown that the hexapeptide metabolite of Ang II, Ang IV, stimulates PAI-1 expression in vitro [6, 7]. This relationship between Ang and PAI-1 was also demonstrated in animal studies with aortic injury [25] or renal microvascular injury induced by radiation [14]. The colocalization of injury with increased PAI-1



**Fig. 6.** Rats infused with Ang II (group 1) showed increased PAI-1 expression by in situ hybridization and immunohistochemistry in kidney (*a*, *b*, *j*, and *l*), aorta (*c* and *d*), and heart (*e* and *f*). In the glomerulus, PAI-1 immunostaining was detected at the vascular pole, in mesangial areas, endothelial cells, and parietal epithelial cells (*a*). The distribution of glomerular PAI-1 mRNA expression (*b*) was in a pattern similar to that of the protein. Treatment with the AT1RA (*h*) decreased these signals to patterns comparable to control saline treated animals (*g*). Strong signals were also present in kidney in response to Ang II in the collecting duct (*j*), smooth muscle layer of pelvis (*l*), and fat tissue around the pelvis, in comparison with minimal background signal in control saline-infused animals (*i* and *k*). Aortic endothelial cells and outer layer vascular smooth muscle cells showed PAI-1 mRNA expression and positive immunostaining (*c* and *d*). Myocytes showed scattered intense signal for PAI-1 mRNA and positive immunostaining (*e* and *f*). (*a*, *c*, and *e*) Immunostaining with anti-PAI-1 antibody. (*b*, *d*, and *f*) In situ hybridization with antisense probe for PAI-1 mRNA (magnifications *a*,  $\times 200$ ; *b*-*d* and *g*-*l*  $\times 400$ ; *e* and *f*,  $\times 100$ ).



**Fig. 6. (Continued).**

expression supports a pathogenic role for PAI-1 in tissue injury [14]. Furthermore, the prevention of injury in the radiation model by either an AT1 antagonist or ACEI was tightly linked to decreased, albeit not normalized, PAI-1 expression [14]. Studies in PAI-1 knockout mice also support a role for PAI-1 in tissue injury. Pulmonary fibrosis in response to bleomycin was significantly re-

duced in PAI-1 knockout mice when compared with wild-type controls [12]. Studies in humans also show an effect of Ang II on PAI-1. Infusion of Ang II in patients specifically induced PAI-1 plasma levels and activity without affecting t-PA levels [26]. Activation of the endogenous RAS is also associated with increased plasma PAI-1 levels in humans [27]. Conversely, ACEI in pa-

tients after acute myocardial infarction resulted in decreased PAI-1 levels and activities [28]. Epidemiological studies point to adverse consequences of increased PAI-1 levels, with an association with increased cardiovascular disease [29–31].

The association of PAI-1 with either thrombotic or fibrotic injuries in both animal models and humans thus points to this molecule as a key target in progressive renal and/or cardiovascular injuries. However, a more precise understanding of the mechanisms of induction of PAI-1 by the RAS in vivo may yield more optimal targets for intervention. Our studies indicate that in vivo induction in the rat of PAI-1 by Ang II is mediated by the AT1 receptor, contrasting our previous observations of the role of the AT4 receptor in this induction in vitro. Obviously, species and cell and/or tissue differences may exist to account for these results. It is appropriate to caution that the specific receptor(s) that regulates the interaction of the RAS and PAI-1 in humans has not been definitively determined. The current studies have not directly tested a possible contribution of the AT4 receptor to the PAI-1 induction. A cell-specific contribution of the Ang IV metabolite via the AT4 receptor cannot be excluded by the current study. Further delineation of the potential role of the AT4 receptor thus awaits its cloning and the availability of specific tools with which to regulate this receptor in vivo.

These studies implicate PAI-1 as an important factor activated by Ang II and demonstrate yet another facet of the broad spectrum of Ang actions. These results also indicate that inhibition of Ang-induced PAI-1 can be accomplished by antagonism of the type 1 receptor in the rat. The link between the RAS and PAI-1 provides an additional mechanism whereby the RAS might promote sclerotic mechanisms, namely by increasing PAI-1 activity and the consequent activation of both thrombotic and sclerotic injuries. Thus, strategies that effectively inhibit the pathological increases in PAI-1 consequent to increased RAS activity are likely to be particularly efficacious in preventing end organ damage. These in vivo studies point to the feasibility of manipulating the PAI-1 induction with specific Ang antagonists. Future approaches may include PAI-1 specific antagonists, instead of or in addition to RAS inhibitors in patients with pathological activation of these systems. The current findings thus have important implications for strategies to optimize the prevention and treatment of Ang and PAI-1-mediated adverse events in human disease.

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