

# Effect of endothelin on the regulation of the fibrinolytic system of cultured human vascular smooth muscle cells

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**Summary** The effect of endothelin-1 (ET-1) and ET-3 on the fibrinolytic system of cultured human vascular smooth muscle cells (SMCs) was investigated. When confluent cultures of human aortic SMCs (HASMCs) and human basilaris artery SMCs (HBASMCs) were conditioned with ET-1 or ET-3 (0.1 to 100 nM) plasminogen activator inhibitor-1 (PAI-1) synthesis increased up to 135% (HASMCs) or 179% (HBASMCs) of control whereas tissue-type plasminogen activator (t-PA) synthesis decreased down to 70% of control in both types of SMCs. PAI-1 mRNA levels were upregulated to 200% of control levels and t-PA mRNA decreased by 50% when the cells were treated with 100 nM ET-1 or ET-3.

We provide evidence for a new function of endothelins towards vascular smooth muscle cells. By decreasing the fibrinolytic potential of SMCs, endothelin might contribute to the development of thrombosis seen in cardiovascular diseases associated with vascular injury and/or smooth muscle cell activation such as atherosclerosis and myocardial infarction.

## INTRODUCTION

Vascular smooth muscle cells, the predominant cell type of the blood vessel wall, produce both tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) as well as PA inhibitor type-1 (PAI-1) and PAI-2.<sup>1–5</sup> Changes in the fibrinolytic capacity of smooth muscle cells might have far-reaching implications in processes like cell migration and growth, development of atherosclerotic lesions and the shift from an antithrombotic to a prothrombotic environment in case of vascular injury. The fibrinolytic system of vascular smooth muscle cells seems to be highly regulated with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\alpha$  (IL-1 $\alpha$ ), thrombin, transforming growth factor- $\beta$  (TGF- $\beta$ ) and platelet-derived growth factor (PDGF) increasing the production of PAI-1 in these cells.<sup>6–9</sup> On the other hand,

heparin has been shown to inhibit the transcription of t-PA in smooth muscle cells.<sup>10</sup> Using a rat model, increased u-PA expression has been observed in smooth muscle cells during mitogenesis, whereas t-PA expression was increased during migration.<sup>1</sup> An increase of t-PA and u-PA induced by PDGF and bFGF was reported recently in the injured rat carotid artery *in vivo*.<sup>11,12</sup>

Endothelin-1 (ET-1) seems to be an important modulator of smooth muscle cell function. It is a potent vasoconstrictor peptide released from endothelial cells which also regulates smooth muscle cell proliferation.<sup>13–15</sup> Thus, it is thought to be implicated in several pathophysiological conditions of the cardiovascular system associated with endothelial cell damage and/or smooth muscle cell proliferation such as myocardial infarction and atherosclerosis.<sup>16,17</sup> On the other hand, these pathological states have also been shown to be associated with elevated PAI-1 activity in blood and the expression of PAI-1 in atherosclerotic arteries.<sup>18,19</sup> In fact, ET-1 and ET-3 have been shown to modulate the fibrinolytic system of endothelial cells.<sup>20–22</sup> Therefore, it was the aim of this study to investigate the effect of ET-1 and ET-3 on the fibrinolytic system of vascular smooth muscle cells.

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## MATERIALS AND METHODS

### Materials

Synthetic endothelin-1 (human) and endothelin-3 (human) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). A stock solution of 10  $\mu$ M in distilled water was stored at  $-70^{\circ}\text{C}$ . Sodium dodecyl sulfate (SDS; Bio-Rad, CA, USA), morpholinopropane sulfonic acid (Serva, Germany), piperazine- $N,N'$ -bis[2-ethane sulfonic acid] (PIPES; Sigma), Seakem LE Agarose (FMC Bioproducts, ME, USA), dCTP [Aloha- $^{32}\text{P}$ ] (Amersham Inc.), were obtained as indicated. Other materials used in the methods described below have been specified in detail in the respective references.

### Isolation of vascular smooth muscle cells

Human aortic smooth muscle cells (HASMCs) and human basilar artery SMCs (HBASMCs) were isolated from pieces of normal human aorta or human basilar artery, respectively, obtained from organ donors using the explant technique.<sup>23</sup> Briefly, the respective tissue specimen was cut into 10 to 20 pieces of 1–2 mm diameter, these pieces were placed in a Petri dish (100 mm; Costar, MA, USA) coated with 1% calf skin gelatine in PBS and covered with a drop of Medium 199 containing 20% supplemented calf serum (SCS; Hyclone, UT, USA), 100  $\mu\text{g}/\text{mL}$  streptomycin, 100 IU/mL penicillin, 250 ng/mL amphotericin B and 1 mmol/L glutamine. After 3 to 5 days the explants became adherent and the Petri dish was filled with Medium 199 containing supplements as described above. Cells were confirmed to be vascular smooth muscle cells by their typical 'hill and valley' morphology and by positive immunofluorescence staining with a monoclonal antibody against alpha-SMC-actin (Boehringer-Mannheim, Germany). Of the cells tested, 95% to 98% showed positive staining for alpha-SMC-actin.<sup>7</sup> Primary cultures were harvested at confluence with 0.05% trypsin/0.02% EDTA and plated at a split ratio of 1:3 in 75  $\text{cm}^2$  flasks. Subconfluent cells were allowed to grow to confluence under the same conditions and harvested during exponential cell growth with trypsin/EDTA and frozen in 1 mL aliquots of culture medium containing 10% dimethylsulfoxide (DMSO) in liquid nitrogen. For experiments, vials were thawed at  $37^{\circ}\text{C}$  and cells were grown in six-well plates (9.4  $\text{cm}^2$ ; Costar) in Medium 199 containing SCS, and antibiotics at concentrations as described above, until confluence was reached. Average cell densities at confluence was  $2 \times 10^5$  cells/well. All cells used in this study were in passage 4. The cells were always fed with fresh medium the day before the experiment.

### Preparation of conditioned media (CM)

Confluent cultures were rinsed twice with Hank's balanced salt solution (HBSS; Sigma) and incubated at  $37^{\circ}\text{C}$  in 1 mL/well medium M199 containing 1.25% SCS and the indicated concentrations of ET-1 or ET-3. After incubation, the culture supernatant was collected, and cell debris was removed by centrifugation and stored at  $-70^{\circ}\text{C}$  until used. Total cell number of the respective cultures was counted after trypsinization with a hemocytometer.

### Assays for t-PA and PAI-1 antigen in CM

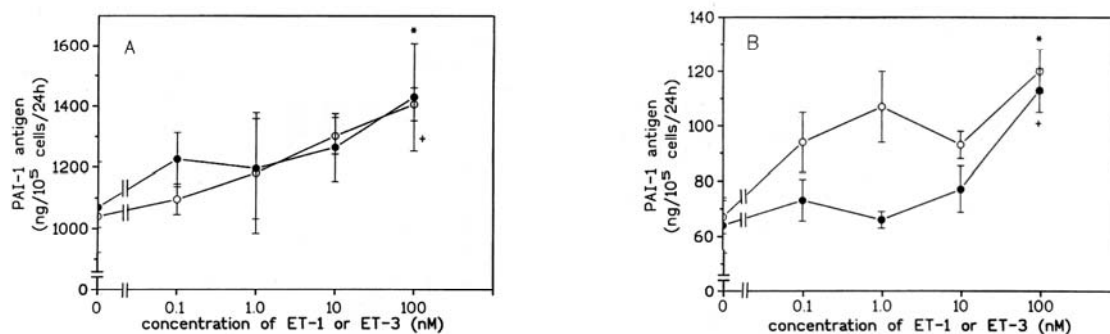
The concentrations of t-PA and PAI-1 antigens were determined by specific commercial enzyme-linked immunosorbent assays (ELISAs) (Technoclone, Austria) according to the manufacturer's instruction. The test ranges for these assays are 0.3–2.5 ng/mL for t-PA and 1.0–30 ng/mL for PAI-1. The t-PA-ELISA detects free t-PA and t-PA in complex with PAI-1. The PAI-1 ELISA measures free, complexed and latent PAI-1.

### Quantification of t-PA and PAI-1 mRNA levels by Northern blot analysis

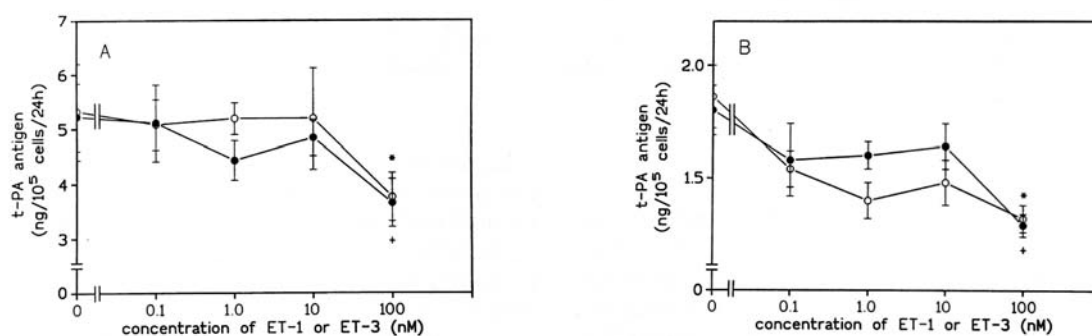
Total cellular RNA was isolated from cells by acid guanidinium thiocyanate/phenol/chloroform extraction as described by Chomczynski and Sacchi.<sup>24</sup> Northern blotting was performed as recently described.<sup>25</sup> In order to quantify differences in the specific mRNA expression, the developed films were scanned using a densitometer (Hirschmann Elscript 400; Hirschmann, Germany). The scanning data for each specific mRNA message were compared to the intensity of the GAPDH message. The following cDNA fragments were used as probes in the hybridization experiments: a 1.5 kb *Sma*I/*Hind*III fragment of human t-PA cDNA, a 1.4 kb *Eco*RI/*Bgl*II fragment of a human PAI-1 cDNA of the 3.2 kb transcript (PCR amplified coding sequence from MJZJ cDNA) and a 1.2 kb *Pst*I fragment of a rat GAPDH cDNA which was used as an internal standard probe. The cDNA fragments were radiolabelled by random-priming using a Random Prime DNA Labelling Kit (Boehringer Mannheim, Germany).

### Statistical analysis

The results are reported as means  $\pm$  standard deviation. One-way ANOVA was used to determine significance levels. *P* values of less than 0.05 were considered to indicate statistically significant differences.



**Fig. 1** Effect of ET-1 and ET-3 on the synthesis of PAI-1 antigen in cultured HASMCs (panel A) and HBASMCs (panel B). HASMCs and HBASMCs were incubated with or without different concentrations (0.1–100 nM) of ET-1 (open circles) or ET-3 (full circles) for 24 h. Conditioned media were collected and analysed for PAI-1 antigen as described in the Materials and Methods section. The results are the mean values of three experiments, each performed in triplicate. Values are given as means  $\pm$  S.D.; + or \*  $P < 0.001$  as compared to control.



**Fig. 2** Effect of ET-1 and ET-3 on the synthesis of t-PA antigen in cultured HASMCs (panel A) and HBASMCs (panel B). HASMCs and HBASMCs were incubated with or without different concentrations (0.1–100 nM) of ET-1 (open circles) or ET-3 (full circles) for 24 h. Conditioned media were collected and analysed for t-PA antigen as described in the Materials and Methods section. The results are the mean values of three experiments, each performed in triplicate. Values are given as means  $\pm$  S.D.; + or \*  $P < 0.001$  as compared to control.

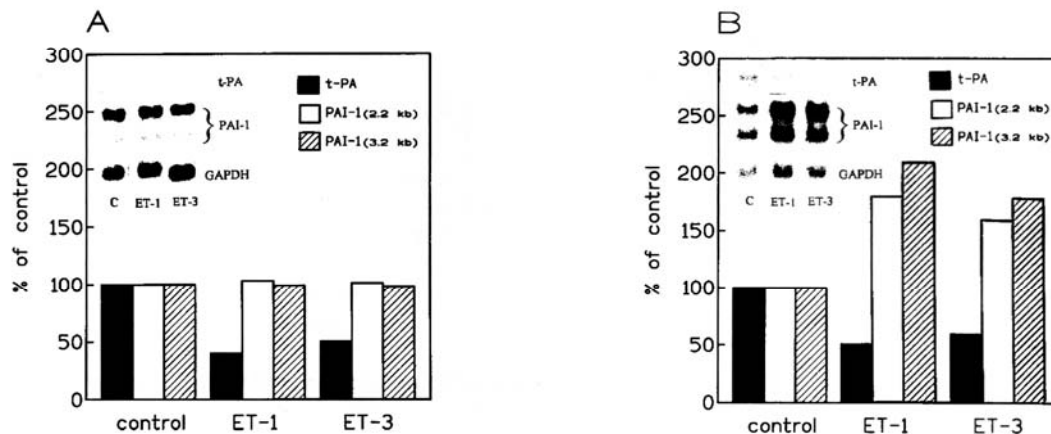
## RESULTS

As shown in Figure 1, PAI-1 antigen increased in a dose-dependent manner with increasing doses of ET-1 and ET-3 over the concentration range employed in the conditioned media of such treated HASMCs (Fig. 1A) or HBASMCs (Fig. 1B). Cultures treated with 100 nM ET-1 or ET-3 released up to 35% (in HASMCs) or up to 79% (in HBASMCs) more PAI-1 than control cultures. In contrast, t-PA antigen in the CM of HASMCs or HBASMCs decreased after exposure of cells to 100 nM of ET-1 and ET-3, respectively (Fig. 2A and 2B). In HASMCs, t-PA antigen decreased to 70% of control after treatment with ET-1 or ET-3 whereas in HBASMCs t-PA antigen was reduced to 63% under these conditions. Human umbilical vein endothelial cells (HUVECs) responded in a similar way when treated with ET-1 or ET-3, respectively (PAI-1: 100 nM ET-1:  $956 \pm 21$  ng/10<sup>5</sup> cells/24 h, 100 nM ET-3:  $975 \pm 90$  ng/10<sup>5</sup> cells/24 h, control:  $782 \pm 27$  ng/10<sup>5</sup> cells/24 h;  $n = 9$ ,  $P < 0.05$ ; t-PA: 100 nM ET-1:  $5.8 \pm 0.3$  ng/10<sup>5</sup>

cells/24 h, 100 nM ET-3:  $5.5 \pm 0.4$  ng/10<sup>5</sup> cells/24 h, control:  $7.7 \pm 0.1$  ng/10<sup>5</sup> cells/24 h;  $n = 9$ ,  $P < 0.001$ ).

As shown in Figure 3, the effects of ET-1 or ET-3 on upregulation of PAI-1 and downregulation of t-PA production in SMCs were also reflected on the levels of specific mRNA. When HBASMCs were incubated for 6 h with 100 nM of ET-1 or ET-3, levels of PAI-1-specific mRNA were not affected whereas t-PA-specific mRNA decreased down to 40% and 50% of control, respectively. After 12 h of incubation with 100 nM of ET-1 or ET-3, levels of PAI-1 specific mRNA increased up to 200% (100 nM ET-1) and up to 190% (100 nM ET-3) of control levels, respectively. Under the same conditions, t-PA-specific message decreased down to 50% and 60% of control levels, respectively. No effect of ET-1 or ET-3, respectively, on t-PA or PAI-1 mRNA levels in HBASMCs was observed after 18 h of incubation (data not shown).

No effect of ET-1 or ET-3 on u-PA expression in HASMCs or HBASMCs on either the antigen level or the mRNA level was seen (data not shown).



**Fig. 3** Effect of ET-1 and ET-3 on PAI-1 and t-PA mRNA expression in cultured HBASMCs. Confluent HBASMCs were incubated for 6 (panel A) and 12 (panel B) h in absence or presence of 100 nM ET-1 or ET-3. Equal amounts of total RNA extracted from untreated and treated cells were loaded to the gel and Northern blot analysis was performed using  $^{32}\text{P}$ -labelled cDNA probes for t-PA, PAI-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The intensity of the bands present on the autoradiogram was assessed by densitometry. Results were expressed as percentages of control values after normalization to GAPDH mRNA and represent the means from two independent experiments.

## DISCUSSION

ET-1 is a potent vasoconstrictor peptide which also induces smooth muscle cell proliferation.<sup>13–15</sup> Thus, vascular smooth muscle cells seem to be a major target for endothelin. In this study, we show that by increasing the expression of PAI-1 and simultaneously decreasing the expression of t-PA in cultured smooth muscle cells, ET-1 and ET-3 seem to be implicated in the regulation of the fibrinolytic system of these cells. Similar to our observations, ET-1 has been shown recently to increase the expression of PAI-1 in mesangial cells which are considered to be related to smooth muscle cells.<sup>26</sup> Changes in the fibrinolytic system of smooth muscle cells correlate with proliferation and migration of these cells.<sup>1,10</sup> Furthermore, a role for smooth muscle cell-derived PAI-1 in the progression of atherosclerosis has been suggested, based on the findings that PAI-1 is expressed in human atherosclerotic arteries.<sup>19</sup>

However, a recent study demonstrated that neointima formation after injury in PAI-1-deficient mice was increased whereas it was decreased in u-PA-deficient mice.<sup>27</sup> These findings suggest a role for u-PA-mediated extracellular proteolysis by smooth muscle cells in the development of atherosclerotic lesions whereas PAI-1 by inhibiting u-PA would serve as a defence mechanism limiting the progression of such lesions.

Furthermore, recent evidence suggests that PAI-1 inhibits smooth muscle cell migration by blocking integrin  $\alpha_v\beta_3$  binding to vitronectin.<sup>28</sup> The expression of ET-1 in endothelial cells is increased by thrombin, TGF- $\beta$  and TNF- $\alpha$ .<sup>13,29,30</sup> Because endothelial cells release ET-1 in a

polarized fashion predominantly to the abluminal side, one could speculate that, in the case of vascular injury, the activated endothelium would serve as a source of ET-1 which then would act in a paracrine way to activate the underlying smooth muscle cells.<sup>31</sup> Such activation of the smooth muscle cells would not only result in vasoconstriction and smooth muscle cell proliferation, but – as shown in this study – might also contribute to an antifibrinolytic and antiproteolytic environment. An autocrine role for ET-1 in this latter process has been suggested recently because ET-1 has been shown to decrease basal- and thrombin-stimulated t-PA release from cultured endothelial cells.<sup>20</sup> One could also speculate on an autocrine/paracrine effect in which ET-1, upregulated by angiotensin II in smooth muscle cells and endothelial cells, would contribute to the upregulation of PAI-1 expression in these cells induced by angiotensin II.<sup>32–35</sup> In general, such an antifibrinolytic, prothrombotic environment caused by upregulation of PAI-1 by ET-1 in smooth muscle cells might favour thrombus formation. However, *in vivo* data, indicate an antithrombotic role for ET-1, albeit by inhibiting platelet aggregation.<sup>36–38</sup> Nevertheless, the ET-induced modulation of the fibrinolytic system of smooth muscle cells, as shown in this report, might more significantly impact on the regulation of proteolytic matrix degradation during cell proliferation and migration.

In conclusion, our data provide evidence for a new function of ET-1 and ET-3 in modulating the fibrinolytic system of smooth muscle cells. If operative *in vivo*, such an effect might contribute in cardiovascular diseases associated with endothelial cell injury and/or smooth

muscle cell activation to thrombus formation seen in thrombosis or myocardial infarction on the one hand or, on the other hand, to the regulation of smooth muscle cell-dependent extracellular proteolysis in the progression of atherosclerosis.

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#### REFERENCES

- Clowes A W, Clowes M M, Au Y P, Reidy M A, Belin D. Smooth muscle cells express urokinase during mitogenesis and tissue-type plasminogen activator during migration in injured rat carotid artery. *Circ Res* 1990; 67: 61–67.
- Levin E G, Loskutoff D J. Comparative studies of the fibrinolytic activity of cultured vascular cells. *Thromb Res* 1979; 15: 869–878.
- Booyse F M, Scheinbuks J, Radek J, Osikowicz G, Feder S, Quarfoot A J. Immunological identification and comparison of plasminogen activator forms in cultured normal human endothelial cells and smooth muscle cells. *Thromb Res* 1981; 24: 495–504.
- Laug W E, Aebersold R, Jong A, Rideout W, Bergman B L, Baker J. Isolation of multiple types of plasminogen activator inhibitors from vascular smooth muscle cells. *Thromb Haemost* 1989; 61: 517–521.
- Bell L, Madri J A. Influence of angiotensin system on endothelial and smooth muscle cell migration. *Am J Pathol* 1990; 137: 7–12.
- Gallicchio M, Wojta J, Hamilton J A, McGrath K. Regulation of plasminogen activator inhibitor type 1 in cultured smooth muscle cells by interleukin 1 $\alpha$  and tumor necrosis factor- $\alpha$ . *Fibrinolysis* 1995; 9: 145–151.
- Wojta J, Gallicchio M, Zoellner H, Hufnagl P, Last K, Filonzi E L, Binder B R, Hamilton J A, McGrath K. Thrombin stimulates expression of tissue-type plasminogen activator and plasminogen activator inhibitor type 1 in cultured human vascular smooth muscle cells. *Thromb Haemost* 1993; 70: 469–474.
- Noda-Heiny H, Fujii S, Sobel B E. Induction of vascular smooth muscle cell expression of plasminogen activator inhibitor-1 by thrombin. *Circ Res* 1993; 72: 36–43.
- Reilly C F, McFall R C. Platelet-derived growth factor and transforming growth factor-beta regulate plasminogen activator inhibitor-1 synthesis in vascular smooth muscle cells. *J Biol Chem* 1991; 266: 9419–9427.
- Au Y P, Kenagy R D, Clowes A W. Heparin selectively inhibits the transcription of tissue-type plasminogen activator in primate arterial smooth muscle cells during mitogenesis. *J Biol Chem* 1992; 267: 3438–3444.
- Jackson C L, Raines E W, Ross R, Reidy M A. Role of endogenous platelet-derived growth factor in arterial smooth muscle cell migration after balloon catheter injury. *Arterioscler Thromb* 1993; 13: 218–226.
- Jackson C L, Reidy M A. Basic fibroblast growth factor: its role in the control of smooth muscle cell migration. *Am J Pathol* 1993; 143: 1024–1031.
- Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yakaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332: 411–415.
- Inoue A, Yanagisawa M, Takawa Y, Mitsui Y, Kobayashi M, Masaki T. The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J Biol Chem* 1989; 264: 14954–14959.
- Komuro I, Kurihara H, Sugiyama T, Yoshizumi M, Takaku F, Yazaki Y. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. *FEBS Lett* 1988; 238: 249–252.
- Miyauchi T, Yanagisawa M, Tomizawa T, Sugishita Y, Suzuki N, Fujino M, Ajsaka R, Goto K, Masaki T. Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* 1989; 2: 53–54.
- Lerman A, Edwards B S, Hallett J W, Heublein D M, Sandberg S M, Burnett J C. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med* 1991; 325: 997–1001.
- Hamsten A, Wiman B, deFaire U, Blombäck M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N Engl J Med* 1985; 313: 1557–1563.
- Schneiderman J, Sawdey M S, Keeton M R, Bordin G M, Bernstein E F, Dilley R B, Loskutoff D J. Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. *Proc Nat Acad Sci USA* 1992; 89: 6998–7002.
- Yamamoto C, Kaji T, Sakamoto M, Koizumi F. Effect of endothelin on the release of tissue plasminogen activator and plasminogen activator inhibitor-1 from cultured human endothelial cells and interaction with thrombin. *Thromb Res* 1992; 67: 619–624.
- Pruis J, Emeis J J. Endothelin-1 and -3 induce the release of tissue-type plasminogen activator and von Willebrand factor from endothelial cells. *Eur J Pharmacol* 1990; 187: 105–112.
- Kaji T, Yamamoto C, Sakamoto M, Koizumi F. Endothelin modulation of tissue plasminogen activator release from human vascular endothelial cells in culture. *Blood Coagul Fibrinolysis* 1992; 3: 5–10.
- Chamley-Campbell J, Campbell G R, Ross R. The smooth muscle cell in culture. *Physiol Rev* 1979; 59: 1–61.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156–159.
- Zhang W, Wojta J, Binder B R. Effect of notoginsenoside R1 on the synthesis of tissue-type plasminogen activator and plasminogen activator inhibitor-1 in cultured human umbilical vein endothelial cells. *Arterioscler Thromb* 1994; 14: 1040–1046.
- Iwamoto T, Tamaki K, Nakayama M, Okuda S, Fujishima M. Effect of endothelin 1 on fibrinolysis and plasminogen activator inhibitor 1 synthesis in rat mesangial cells. *Nephron* 1996; 73: 273–279.
- Carmeliet P, Collen D. Gene targeting and gene transfer studies of the biological role of the plasminogen/plasmin system. *Thromb Haemost* 1995; 74: 429–436.
- Stefansson S, Lawrence D A. The serpin PAI-1 inhibits cell migration by blocking integrin  $\alpha_5\beta_3$  binding to vitronectin. *Nature* 1996; 383: 441–443.
- Kurihara H, Yoshizumi M, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Hamaoki M, Kato H, Yazaki Y. Transforming growth

- factor-beta stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochem Biophys Res Com* 1989; 159: 1435-1440.
30. Kanse S M, Takahashi K, Lam M C, Rees A, Warren J B, Porta M, Molinatti P, Gathe M, Bloom S R. Cytokine stimulated endothelin release from endothelial cells. *Life Sci* 1991; 48: 1379-1384.
  31. Wagner O F, Christ G, Wojta J, Vierhapper H, Parzer S, Nowotny P J, Schneider B, Waldhäusl W, Binder BR. Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem* 1992; 267: 16066-16069.
  32. Emori T, Hirata Y, Ohta K, Kanno K, Eguchi S, Imai T, Shichiri M, Marumo F. Cellular mechanism of endothelin-1 release by angiotensin and vasopressin. *Hypertension* 1991; 18: 165-170.
  33. Hahn A W, Resink T J, Scott-Burden T, Powell J, Dohi Y, Buhler F R. Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. *Cell Regul* 1990; 1: 649-659.
  34. Vaughan D E, Lazos S, Tong K. Angiotensin II regulates the expression of plasminogen activator inhibitor-1 in cultured endothelial cells. A potential link between the renin-angiotensin system and thrombosis. *J Clin Invest* 1995; 95: 995-1001.
  35. Feener E P, Northrup J M, Aiello L P, King G L. Angiotensin II induces plasminogen activator inhibitor-1 and -2 expression in vascular endothelial cells and smooth muscle cells. *J Clin Invest* 1995; 95: 1353-1362.
  36. Leadley J R, Humphrey W R, Erickson L A, Shebuski R J. Inhibition of thrombus formation by endothelin-1 in canine models of arterial thrombosis. *Thromb Haemost* 1995; 74: 1583-1590.
  37. Thiemermann C, Lidbury P, Thomas R, Vane J. Endothelin inhibits ex vivo platelet aggregation in the rabbit. *Eur J Pharmacol* 1988; 158: 181-182.
  38. Herman F, Magyar K, Chabrier P E, Braquet P, Filep J. Prostacyclin mediates antiaggregatory and hypotensive actions of endothelin in anaesthetized beagle dogs. *Brit J Pharmacol* 1989; 98: 38-40.