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Effects of endothelin on hemodynamics, prostaglandins, blood coagulation and renal function

EGBERT SCHULZ, FRANK RUSCHITZKA, STEPHAN LUEDERS, RALPH HEYDENBLUTH, JOACHIM SCHRADER, and GERHARD ANTON MÜLLER

Department of Nephrology/Rheumatology, Center of Internal Medicine University of Göttingen, Göttingen, Germany

Effects of endothelin on hemodynamics, prostaglandins, blood coagulation and renal function. The interaction of the endogenous vasoconstrictors endothelin (ET), angiotensin II (Ang II) and catecholamines with the kallikrein-kinin-, prostaglandin and renin-aldosterone systems in the pathogenesis of acute renal failure (ARF) is still to be defined. In 18 anesthesized pigs the influence of i.v. bolus applications of ET (2 μg/kg), Ang II (10 μg/kg) and norepinephrine (NE; 20 μg/kg) on hemodynamics, plasmatic coagulation and fibrinolysis system, prostaglandins and renal function was studied. ET induced a biphasic change in blood pressure, starting with an initial short-lasting reduction followed by a long-lasting elevation of systolic and diastolic blood pressure. Endothelin bolus resulted in a significant increase of 6-keto-$PGF_1\alpha$, PGE_2 and TXB_2 plasma levels ($P < 0.05$ against preinjection values), whereas prostaglandins remained unchanged in the Ang II and NE groups. There was a distinct correlation between the plasma ET and 6-keto-$PGF_1\alpha$ levels ($r = 0.82$). In contrast to Ang II or NE, ET induced a shortening of the activated partial thromboplastin time (aPTT) and increase of antithrombin III levels (ATIII), fibrin monomers (FM), prekallikrein (PKK) and factor VIII activity at the beginning. Finally a pronounced decrease of ATIII, FM and PKK occurred, indicating a consumptive coagulopathy. At the end of the experiment, elevated plasma renin activity and pCO_2, significantly decreased creatinine clearance, blood pH, pO_2, base excess, HCO_3^-, oxygen saturation ($P < 0.01$), a distinct glomerular proteinuria, and a final anuria were observated. These results reveal that ET activates the plasmatic coagulation system and induces an ARF accompanied by impairment of pulmonary function. Its coagulation activating and renal vasoconstrictive effects may be important pathophysiological factors, especially when the counteractive release of vasodilatatory and antiaggregatory prostacyclin or NO is impaired.

There is evidence that the endothelium is a highly active endocrine organ system playing an important role in the pathogenesis of renal disease because of its strategic anatomical position between the circulating blood and vascular smooth muscle, juxtaglomerular and mesangial cells.

In addition to the relaxing factors prostacyclin and NO (nitric oxide), the endothelium synthesizes and releases vasoconstrictive substances, including products derived from arachidonic acid metabolism and the recently discovered peptide endothelin. The endothelin (ET) family consists of three structurally and pharmacologically separate isopeptides [1]. Endothelin-1 is a 21-amino acid peptide arising by proteolytic processing of specific prohormones, in particular, via a preproendothelin and the 39-amino acid big endothelin. Several endothelin converting enzymes (ETCE), phosphoramidon-sensitive membrane-bound neutral metalloproteinase, pepstatin-sensitive zytosolic acid proteinases, and a newly discovered pepstatin-sensitive aspartyl protease are described as cleaving big endothelin to form the mature endothelin-1 [2–7]. According to previous studies ET is known to have a vasoconstrictive potency in the kidney vasculature bed about five times that of Ang II. ET substantially reduces renal blood flow, glomerular filtration rate and urine volume as a result of increased renal vascular resistance and mesangial cell contraction [8–12]. Significantly raised ET plasma levels in patients with acute and chronic renal failure may argue in favor of its potential involvement in renal disease [8]. As hypercoagulability and diminished fibrinolysis have been described in acute renal failure, these experiments were designed to study the interaction of ET with the coagulation and fibrinolysis system and its effects on prostaglandins in comparison with Ang II and norepinephrine.

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Methods

Eighteen female domestic Goettingen bred pigs (body wt 32.4 ± 2.3 kg) were fasted 12 hours before the experiment, and water was available *ad libitum*. The pigs were premedicated with ketamine (20 mg/kg, i.m.; Bayer AG, Leverkusen, Germany) and atropine (0,02 mg/kg, i.m.; Hoechst, Frankfurt, Germany), and anaesthesized with sodium pentobarbital (20 mg/kg, i.v. followed by 2.5 mg/kg/hr, i.v.). After induction of sceletal muscle paralysis with pancuronium (0.5 mg/kg/hr, i.v.; Organon, Eppelheim, Germany), the animals were ventilated by a positive pressure respirator (adjusted to body wt). The right a. carotis interna was connected to a transducer system for the analysis of blood pressure and heart rate. The experiment was initiated after a 60 minute period of stable hemodynamics. Three consecutive 20-minute blood samples for measurements of baseline levels were collected from a polyethylene catheter, inserted subcutaneously in the right vena iugularis interna. Sixty, 120, 180 and 240 minutes after the start of the experiments the animals received a bolus of 2 μg ET-1/kg (group 1; $N = 6$), 10 μg Ang II/kg (both Bachem, Heidelberg, Germany) (group 2; $N = 6$) or 20 μg NE/kg (group 3; $N = 6$), all dissolved in 20 ml 0.9% NaCl via a catheter placed in the right v. subclavia. Urine volume, electrolytes and blood gas analysis (ABL 300, Radiometer, Krefeld, Germany) were performed hourly. Zero, 10, 30 and 60 minutes after each bolus

Table 1. Maximal prostaglandin values

	ET	Ang II	NE
$PGF_1\alpha$	1116.8[a] ± 336.8	151.7 ± 34.1	121.2 ± 36.0
PGE_2	262.0[a] ± 95.1	73.5 ± 5.0	83.5 ± 5.2
TXB_2	1191.8[a] ± 126.6	360.5 ± 42.4	304.7 ± 18.5

[a] $P < 0.05$

Table 2. Renin, ng/ml/hr

	Baseline	End of experiment
ET	2.20 ± 0.063	7.98[a] ± 1.10
Ang II	2.93 ± 0.970	1.35 ± 0.44
NE	2.37 ± 0.770	1.97 ± 0.29

[a] $P < 0.05$

Table 3. Creatinine clearance, ml/min

	Baseline	End of experiment
ET	114.3 ± 11.2	0.0[a] ± 0.0
Ang II	110.2 ± 8.7	97.7 ± 6.8
NE	108.8 ± 10.1	98.3 ± 11.7

[a] $P < 0.05$

application 10 ml venous blood samples were collected for determinations of serum electrolytes, plasma osmolality, plasma-renin activity (PRA), plasma concentrations of prostaglandin E_2 (PGE_2), the prostacyclin derivate 6-keto-$PGF_1\alpha$, the thromboxane derivate B_2 (TXB_2), endothelin-1, serum creatinine and coagulation parameters. Isotonic saline infusions of 2 ml/min via each of the three catheters maintained constant standards throughout the experiment and the catheters were kept open since no anticoagulant was administered. Serum and urine electrolytes were measured using a flame photometer, plasma osmolality by means of a micro-osmometer, and PRA by a radioimmunoassay [13]. Plasma concentrations of ET were measured using a ^{125}I-endothelin assay system; prostaglandin plasma levels (all assays supplied by Amersham, Braunschweig, Germany) [8, 14–16] and coagulation parameters were measured as described elsewhere [17–24]. Proteinuria was investigated by a nephelometer analyzer (Behringwerke, Marburg, Germany).

Statistical analyses were performed using the statistical analytic system (SAS Institute Inc., Cary, NC, USA). Normal distribution of the variables were tested by the Shapiro-Wilk test. If normal distribution appeared then the paired comparisons *t*-test was used; if not, then the Wilcoxon matched pairs rank test was applied. Significance was $P < 0.01$ at the arterial blood gas analysis (BGA) and $P < 0.05$ at the other parameters.

Results

The ET effect was characterized by a biphasic change in blood pressure (BP). Within the first 25 seconds after i.v. bolus injection ET produced an initial short-lasting reduction of systolic and diastolic BP from 102 ± 4.3/72.8 ± 4.0 mm Hg down to a minimum of 89 ± 5.1/65.4 ± 4.3 mm Hg, and a heart rate elevation from an average baseline of 68.3 ± 3.1 to 92.2 ± 5.8 beats/min. The hypotensive phase was rapidly followed by a gradual and long-lasting increase in systolic and diastolic BP. The rise of systolic BP was comparable in the three groups (Maximum: ET, 229 ± 13.2; Ang II, 226.2 ± 10.7; NE, 225.8 ± 19.9 mm Hg) without significant differences, only diastolic BP was significantly higher in group 1 and did not reach baseline values again (Maximum: ET, 160.2 ± 7.8; Ang II, 133.5 ± 5.6; NE, 122.7 ± 6.1 mm Hg). Systolic and diastolic BP remained significantly elevated up to 40 minutes after the ET bolus injection ($P < 0.05$), whereas they returned to the baseline after an average of five minutes in the Ang II group and three minutes in the NE group. The Ang II and NE groups showed a short-lasting heart rate elevation (75.5 ± 3.6/66.8 ± 7.5 to 114.8 ± 3.9/101.7 ± 5.4 beats/min), and the ET group showed a transient decrease in HR after bolus application (68.3 ± 3.1 to 58.0 ± 3.6 beats/min). ET plasma values rose from 33.1 ± 1.3 up to 75.7 ± 12.2 pg/ml 10 minutes after the first and up to 192 ± 63.9 pg/ml after the fourth ET bolus. In contrast, Ang II and NE did not cause any alterations of plasma ET values (Ang II, 34.4 ± 2.4; NE, 36.9 ± 0.6 pg/ml).

ET application induced elevations of all the plasma prostaglandin levels measured, resulting in a significant elevation of 6-keto-$PGF_1\alpha$, PGE_2 and TXB_2 ($P < 0.05$). In the other groups the prostaglandins remained unaltered (Table 1). There was a distinct correlation between the plasma ET and 6-keto-$PGF_1\alpha$ levels ($r = 0.828$).

PRA was found to be significantly elevated after the ET ($P < 0.05$) and decreased as expected after Ang II administration (Table 2). Creatinine clearance was significantly diminished after ET application (Table 3). Serum electrolytes and plasma osmolality did not change significantly in any group during the experiments.

In contrast to pigs given a bolus of Ang II or NE, ET induced an activation of the plasmatic coagulation system. A shortening of the aPTT was found within the first 10 minutes after the bolus injection (17.1 ± 0.1 down to 16.0 ± 0.7 seconds, correlation with ET plasma levels $r = 0.89$). Baseline values were reached after 30 minutes (Fig. 1). Factor VIII activity increased significantly after the first three bolus injections from an average of 87.7 ± 10.4 to 100.0 ± 8.9% ($P < 0.05$; Fig. 2). Thirty minutes afterwards the activity dropped again to the baseline values. However, after the third bolus factor VIII activity remained elevated. Fibrin monomers were augmented 10 and 30 minutes after the first two boluses (254.7 ± 6.1 to a maximum of 275 ± 8.7 ΔmE; $P < 0.05$; Fig. 3) and fell significantly after the third injection (218.8 ± 14.0). NE and Ang II did not alter factor VIII activity or fibrin monomers. Prekallikrein and more pronounced ATIII values initially increased (from 192.3 ± 18.2/108.0 ± 3.6% to 217.0 ± 12.3/123.0 ± 12.2%; $P < 0.05$), but decreased significantly after the third and the second ET boluses, respectively (to 177.7 ± 21.5/95.0 ± 2.8%; $P < 0.05$; Figs. 4 and 5).

The urine volume was significantly reduced after each ET bolus, resulting in a final anuria ($P < 0.05$; Table 4).

At the end of the experiments blood pH (Table 5), pO_2 (128.1 ± 9.4 to 82.3 ± 15.4 mm Hg), oxygen saturation (98.5 ± 1.1 to 87.0 ± 5.1%), standard bicarbonate (Table 6) and base excess significantly decreased (3.5 ± 1.1 to −12.9 ± 4.69 mvol/liter); a marked elevation of pCO_2 was registered ($P < 0.01$; Table 7). NE caused a slight fall of base excess (3.0 ± 0.7 to 0.8 ± 0.6 mvol/liter) and standard bicarbonate (Table 6), whereas all other parameters remained unchanged. Additionally, 32.0 ± 7.4 ml ascites and a marked glomerular proteinuria (750.7 ± 89.4 mg/liter) were found in the ET group.

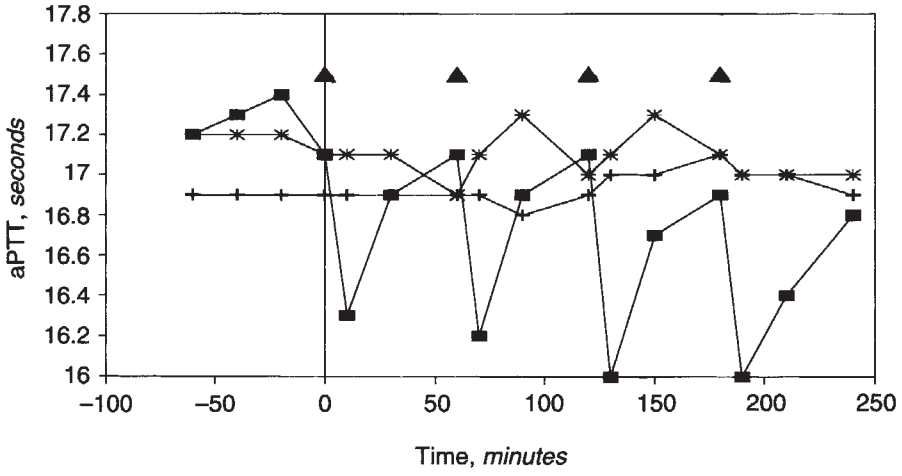

Fig. 1. *aPTT.* Symbols are: (▲) bolus; (■) endothelin; (+) angiotensin II; (✳) norepinephrine.

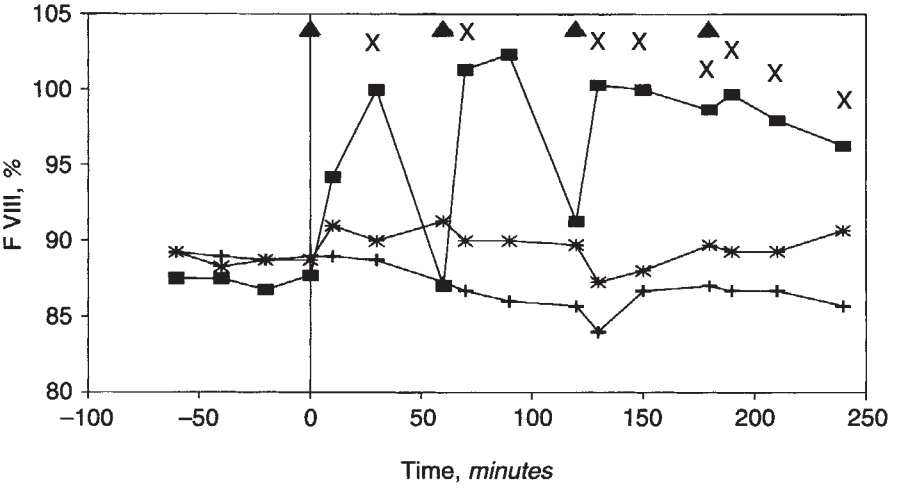

Fig. 2. *Factor VIII.* Symbols are: (▲) bolus; (■) endothelin; (+) angiotensin II; (✳) norepinephrine. (×) $P < 0.05$.

Discussion

The study compared the effects of the endogenous vasoconstrictors endothelin-1, angiotensin II and norepinephrine on hemodynamics, prostaglandins, coagulation and renal function in swine.

Although BP was elevated after the administration of the three different peptides, the hemodynamic biphasic profile caused by ET was different [25, 26]. The initial hypotension and tachycardia could be caused by stimulation of atrial extension receptors accompanied with an increased ANP release. Another explanation could be a compensatory release of prostacyclin [27] or of NO. When ET was infused intravenously in rats, its pressor activity was strongly limited by the release of prostacyclin and EDRF [28]. Indeed, in isolated preparations, ET induced a release of prostacyclin and NO, which can reverse contraction of the mesenteric artery induced by ET [29]. In pithed or chemically denervated rats, a single intravenous injection of ET elevated the blood pressure for more than one hour. In contrast to this prolonged hypertensive activity, rapid elimination of endothelins from the bloodstream has been observed in rats; over 60% disappeared after one minute, with high uptake in the lung, kidney, and liver [30–34]. The present experiments clearly demonstrate that, in contrast to Ang II and NE, ET induces the release of vasodilatatory prostaglandins, such as the prostacyclin derivative 6-keto-$PGF_1\alpha$ as well as PGE_2. Since pretreatment with indomethacin, an inhibitor of prostaglandin synthesis, caused a prolongation and enhancement of endothelin-induced hypertension without an initial blood pressure decrease [35], the concomitant release of endogenous vasodilatators might have prevented a further elevation of blood pressure. This may be of pathophysiological importance when release of 6-keto-$PGF_1\alpha$ is impaired and ET is increased, as it is in hypertension or advanced atherosclerosis [8, 36, 37].

Thus, its vasoconstrictive effect is modified by the sympathic/parasympathic reaction, its own pharmacokinetics and the release of counteractive endothelial hormones.

In animal experiments intravenously infused ET preferentially affected the renal circulation. As a result of the increased vascular resistance and contraction of mesangial cells, the glomerular filtration rate and urine volume were substantially reduced [9–11], resulting in a marked glomerular proteinuria and a final anuria as signs of an ET induced ARF. These findings correspond with the metabolic acidosis presenting a decreased base excess, blood pH and standard bicarbonate as a consequence of impaired renal acid elimination and base generation.

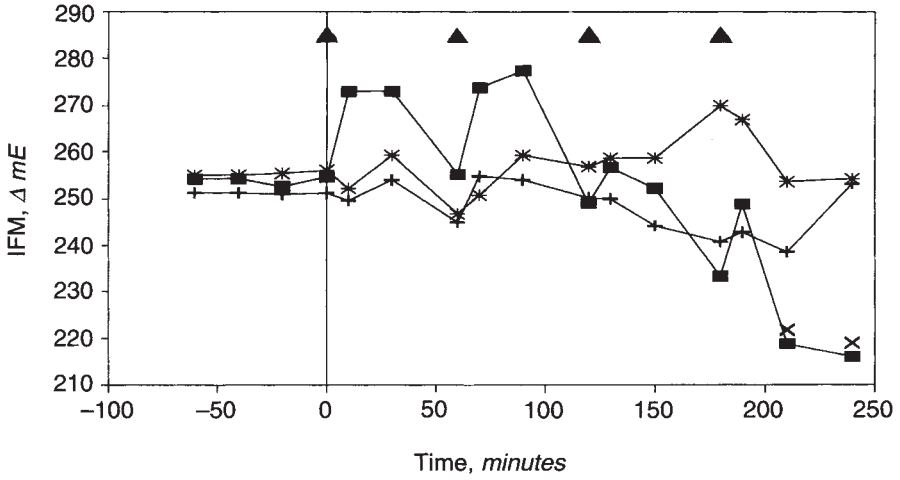

Fig. 3. *Soluble fibrin monomers.* Symbols are: (▲) bolus; (■) endothelin; (+) angiotensin II; (✳) norepinephrine. (×) $P < 0.05$.

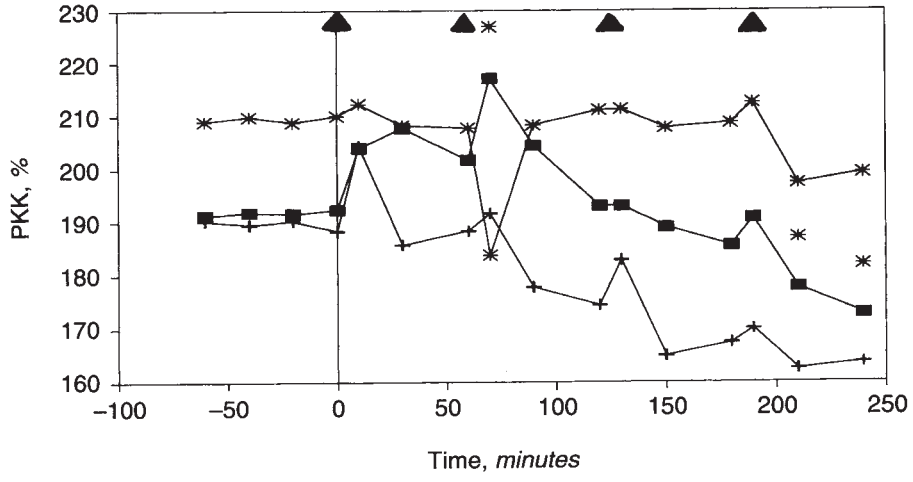

Fig. 4. *Prekallikrein.* Symbols are: (▲) bolus; (■) endothelin; (+) angiotensin II; (−✳−) norepinephrine. (✳) $P < 0.05$.

A positive correlation between ET and creatinine plasma values in patients with chronic renal failure [38] and high ET plasma levels in patients with acute renal failure were observed [39]. In patients with liver cirrhosis and ascites a negative correlation between ET levels and creatinine clearance was described [40]. Proteinuria was also seen in rats [41] and may serve as an explanation for the observated ascites combined with ET-induced portal hypertension.

In the present study ET enhanced renin release, indicating that the peptide takes part in the regulation of the release of systemic vasoactive hormones. ET also increases renin release in anaesthesized dogs, but inhibits the release of renin from isolated kidney preparations [42, 43]. The enhanced renin release *in vivo* may be due to the increase in arterial resistance opposing the direct inhibitory action of ET on the renin-angiotensin system [44]. Alternatively, ET may activate renin by releasing prostacyclin—one of the most potent activators of renin release—or via decreasing delivery of sodium to the macula densa, which is consistent with its action on GFR and proximal tubule reabsorbtion as GFR and proximal tubule reabsorbtion decreases [2, 8, 36, 45]. Beyond that, the finding that NO inhibits renin production in canine renal cortical slices supports the hypothesis that ET may function physiologically to antagonize the effects of NO [2, 45, 46].

The renal effects of ET may be brought about either by a direct vasoconstrictor effect or by synergism with other vasoactive substances. It is of cardinal importance that damage or flow pertubations of cell membranes of the endothelial lining of blood vessels cause an increased production of prostaglandins and NO. However, smooth muscle cells underlying the endothelial lining also synthesize prostacyclin. This mechanism is thought to be held in reserve to reinforce local production of NO and prostacyclin and vasodilatation when cell damage to the endothelial lining occurs [47]. The observed elevations of thromboxane values can be explained by its enhanced release from platelets due to thrombin induced activation of platelet aggregation because ET itself does not affect platelet function [48–50]. Only in spontaneously hypertensive rats (SHR), but not in normotensive animals, the reduction of endothelin-induced contraction of aortic rings by dazoxiben, an inhibitor of thromboxane synthase, and SQ-29,548, an antagonist of thromboxane A2 receptors, was endothelium-dependent. These findings suggest that endothelium-derived thromboxane contributes to vasoconstriction evoked by endothelin only in SHR [51]. In addition to that, solutroban, a selective thromboxane A2 receptor antagonist, failed to reduce mesenteric vasoconstrictor responses to endothelin [52]. The observated

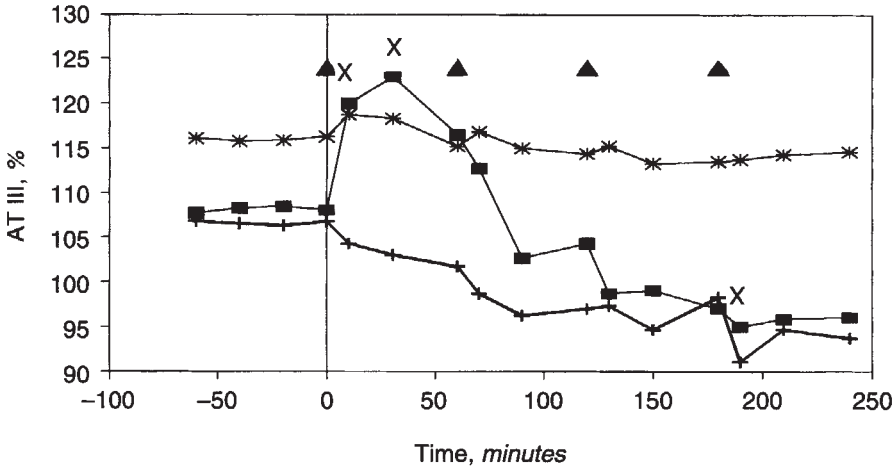

Fig. 5. *Antithrombin III.* Symbols are: (▲) bolus; (■) endothelin; (—) angiotensin II; (✳) norepinephrine. (×) $P < 0.05$.

Table 4. Urine volume, ml/hr

	Baseline	End of experiment
ET	93.3 ± 5.5	0.0[a] ± 0.0
Ang II	91.7 ± 9.0	29.2[a] ± 11.7
NE	79.2 ± 12.4	50.0 ± 9.6

[a] $P < 0.05$

Table 5. Blood pH

	Baseline	End of experiment
ET	7.430 ± 0.028	7.093[a] ± 0.103
Ang II	7.441 ± 0.038	7.434 ± 0.044
NE	7.437 ± 0.047	7.424 ± 0.042

[a] $P < 0.01$

Table 6. Standard bicarbonate, mmol/liter

	Baseline	End of experiment
ET	27.9 ± 0.5	12.0[a] ± 2.7
Ang II	27.0 ± 0.3	27.1 ± 1.1
NE	27.3 ± 0.5	24.7[a] ± 0.9

[a] $P < 0.01$

Table 7. PCO_2, mm Hg

	Baseline	End of experiment
ET	39.4 ± 4.6	56.9[a] ± 6.6
Ang II	38.2 ± 4.6	38.9 ± 3.4
NE	37.5 ± 4.1	36.3 ± 1.8

[a] $P < 0.01$

enhanced thromboxane release is not regarded as pathophysiologically important in the ET-induced renal hypoperfusion and hypofiltration status [53], since the reduction of renal blood flow evoked by endothelin-1 is not mediated by the secondary release of thromboxane, as evidenced by the lack of effect by an appropriate thromboxane receptor antagonist [54]. The diminution of glomerular filtration during endothelin infusion was not preserved by selective thromboxane antagonism but by cyclooxygenase inhibition, indicating other cyclooxygenase products, possibly $PGF_2\alpha$, play a key role in sustaining ET-induced renal vasoconstriction [55]. Thus, locally, especially in the kidney, the endothelium-supported balance of vasoconstrictive ET and vasodilatatory prostaglandins regulates vasotonia [51, 56] as well as coagulation and fibrinolysis activity.

Another striking result of the experiments was the alteration of the coagulation system after ET administration. ET induced a state of hypercoagulability with shortening of aPTT, elevation of factor VIII activity and fibrin monomers, and finally, a consumption of ATIII levels.

The factor VIII elevation after the third ET bolus did not correlate with ET plasma levels as well as before, and reached higher levels without any transient decline to the baseline, indicating endothelial damage resulting in ET-induced ARF. The decrease of ATIII values at the end of the experiment could be due to a consumption by the beginning dissiminated intravascular coagulation since elevated plasma ET levels in patients with disseminated intravascular coagulation (DIC) are reported [57]. The decline of fibrin monomers and prekallikrein values after the third ET bolus might be interpretated as a development of a status of hyperfibrinolysis. This may be explained by a direct effect of ET on fibrinolysis or the consequence of a fibrinolysis activation by the starting DIC. ET could primarily cause an enhanced thrombin release resulting in an elevation of factor VIII activity and fibrin monomers and compensatory ATIII formation.

Hypercoagulability and platelet hyperactivity may be associated with increased ET production what may sustain and promote the activation of the coagulation cascade because thrombin has been shown to result in an augmented ET production [45, 58–61]. Additionally, plasma levels of both thrombin-antithrombin III complex and beta-thromboglobulin are correlated with the plasma ET level in acute myocardial infarction [62]. However, after the start of DIC and formation of fibrin monomer complexes local factors, most likely of endothelial origin, determine the deposition of a fibrin thrombus in the kidney, especially in the glomeruli [63–66]. Furthermore, thrombin induces vasoconstriction and reduction of renal blood flow apart from intraglomerular thrombosis [67, 68] that may be explained by ET release.

The results give evidence that ET can activate the coagulation

process. The imbalance of PGI2/NO and ET may promote vascular occlusion and cardiovascular complications in hypertensive, diabetic and atherosclerotic blood vessels [69–72]. In this situation an additional effect on the coagulation system can bring the hemostatic balance to a prethrombotic state. Especially patients with impaired fibrinolysis, as shown in some hypertensives and advanced atherosclerosis, are endangered by hypercoagulability and microthrombosis [22, 73].

In summary, when endothelial damage with a loss of compensatory secretion of prostaglandins and NO occurs ET may lead to a status of renal hypoperfusion, hypofiltration and intrarenal thrombus formation, as it is in acute renal failure.

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Reprint requests to E. Schulz, M.D., Department of Nephrology/Rheumatology, Center of Internal Medicine, University of Goettingen, Robert-Koch-Str. 40, 37075 Goettingen, Germany.

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