Endothelial Dysfunction in Acute Renal Failure: Role of Circulating and Tissue Endothelin-1

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Abstract. The kidney is an important target and source of the potent vasoconstrictor and mitogen endothelin-1 (ET-1). However, its exact role in acute renal failure (ARF) remains to be determined. ARF was induced in male Wistar-Kyoto rats (n = 7) in a 2-kidney, 2-clip model of 30-min clamping. Twenty-four hours after clamp release, contractions to angiotensin I (AngI) and II, ET-1, and big ET-1 were studied in isolated aortic and renal artery rings. Endothelium-dependent and -independent relaxations were assessed by acetylcholine and sodium nitroprusside. ET-1 clearance, tissue uptake, plasma levels, and vascular and kidney content were investigated. In addition, ET_{A} and ET_{B} receptor mRNA expression was determined. Sham-operated animals served as controls (n = 7). In ARF, ET-1 plasma levels and tissue content of the renal artery, the aorta, and the kidney markedly increased (P < 0.01). Plasma half-life of radiolabeled \(^{125}\)I-ET-1 was markedly prolonged, whereas \(^{125}\)I-ET-1 tissue uptake decreased in the kidney in ARF. Contractions to AngI and AngII were blunted (P < 0.05) and those to KCl were unchanged, whereas vascular responses to big ET-1 and ET-1 were enhanced in the renal artery and also in the aorta in ARF (P < 0.05 to 0.001). Correspondingly, ET_{A} and ET_{B} receptor gene expression occurred, which induces endothelial dysfunction and enhanced vasoconstriction in different vascular beds in ARF.

An intact endothelium produces a variety of vasoactive substances that control enhanced vasoconstriction, modulate platelet–vessel wall interaction, adherence of monocytes, and migration and proliferation of vascular smooth muscle cells. Strategically located between the circulating blood and the vascular smooth muscle, the renal endothelium also closely interacts with mesangial, tubular, and juxtaglomerular cells and may thus contribute to the regulation of renal vascular tone and kidney function (1). Hence, the endothelium is not just a permeability barrier and target, but is increasingly recognized as a mediator in the pathogenesis of cardiovascular and renal disease (1). Impairment of endothelial function develops long before structural changes occur and is associated with cardiovascular risk factors such as hypercholesterolemia (2), hypertension (3), diabetes (4), and aging (5). In addition, an increasing body of evidence indicates that endothelial dysfunction can be added to the list of risk factors that contribute to vascular injury in patients with chronic renal failure (6). However, the pathogenesis of endothelial dysfunction in acute renal failure (ARF) remains elusive (7).

Endothelial cells release numerous vasoactive substances, such as the potent vasodilator nitric oxide (NO), which also inhibits cellular growth and migration (1). The effects of NO are counterbalanced by endothelium-derived vasoconstrictors, such as angiotensin II (AngII), superoxide, prostaglandin H\(_{2}\), thromboxane, and the 21-amino acid peptide endothelin-1 (ET-1) (8).

The endothelins have been implicated in the pathogenesis of several diseases of the kidney, including renal injury following ischemia (9–11), administration of nephrotoxic agents (12,13), glomerulonephritis (14), and hepatorenal syndrome (15). Three different isopeptides (ET-1, ET-2, and ET-3) exist that are encoded by different gene loci and are all cleaved from proendothelins (big ET) by endothelin-converting enzymes (16). Endothelins exert their biologic effects via activation of specific membrane-bound receptors that consist of seven transmembrane domains and are coupled to G proteins (17). Two different receptor subtypes have been cloned in mammalian tissues, termed ET_{A} and ET_{B} receptors (18,19). The two receptors have different structures, functions, and distributions (17,20). However, little is known regarding the regulation and expression of both receptors in vascular and renal tissue during ARF.

In addition to endothelial cells, a variety of cell types in the kidney are able to produce ET-1, such as the mesangial, glo-
merular epithelial, and medullary collecting duct cells (21–23). Although ET-3 production was demonstrated in rat mesangial and glomerular epithelial cells (24), no ET-3 production in the human kidney could be shown so far (22). As ET-2 seems to be exclusively produced in the intestine, ET-1 is considered the clinically most important endothelin in human kidney disease. Previous studies have suggested that ET-1 acts predominantly as a paracrine-autocrine substance and can be tightly regulated at the tissue level by several factors such as thrombin, AngII, arginine vasopressin, cytokines, oxidized LDL, and transforming growth factor-B1 (16,25–27). In contrast, mesangial ET-1 synthesis is inhibited by NO and atrial natriuretic peptide (25,26). ET-1 also exerts potent growth-promoting properties (27) and increases the expression of extracellular matrix proteins in mesangial cells (23). ET-1 is rapidly internalized by vascular smooth muscle cells through clathrin-mediated endocytosis (28,29) and cleared from the circulation by receptor-operated mechanisms in the lung and kidney (30,31). The kidney exhibits a particular organotropy for the effects of the endothelins. Indeed, ET-1 induces constriction in the renal vascular bed with a potency five times greater than that of AngII and ten times that of norepinephrine (32). In humans, infusion of ET-1 exerts potent biologic actions on the kidney through activation of $\text{ET}_\text{A}$ receptors, as it decreases renal blood flow, GFR, urine volume, and natriuresis (33,34). Experimental studies suggest that ET$\text{A}$ as well as combined ET$\text{A/B}$ receptor antagonism is protective in experimental models of chronic (35) and ARF (9,36–39).

Hence, in this study we have evaluated the pathophysiologic role of the circulating and tissue ET-1 system, as well as that of its receptors, the mechanisms involved in its activation, and its impact on endothelial function in different vascular beds in ARF.

**Materials and Methods**

**Animals**

Male Wistar-Kyoto rats (13 wk of age) were obtained from IFFA Credo (L’Arbresle, France). ARF was induced in a 2-kidney, 2-clip model of 30-min clamping ($n = 7$). Age-matched animals underwent sham operation (manipulation of the renal pedicles) and served as controls ($n = 7$). In additional experiments, the same protocols were performed in rats in which the clamps remained in situ for 24 h. The rats were fed a standard chow (Nafag, Gossau, Switzerland). BP was measured in unanesthetized rats by the tail-cuff method (BP Recorder 8005 W + W Münchenstein, Switzerland) before and 24 h after clamping. Resting tension was gradually increased and rings were repeatedly exposed to KCl (100 mmol/L) until the optimal resting tension was reached (aorta: 2.5 ± 0.1 g [CTL], 2.4 ± 0.1 g [ARF]; renal artery: 1.0 ± 0.1 g [CTL], 0.9 ± 0.1 g [ARF]). Cumulative concentration–response curves to ET-1 ($10^{-10} – 3 \times 10^{-7}$mol/L) and bigET-1 ($10^{-9} – 3 \times 10^{-7}$mol/L) were obtained in quiescent preparations. Because of the rapid development of tachyphylaxis, only single concentrations of AngI and AngII were given (1.0–7 mol/L). In additional experiments, rings were preconstricted with norepinephrine (approximately 70% of KCl 100 mmol/L; precontraction did not differ between the groups), and relaxations to acetylcholine ($10^{-10} – 3 \times 10^{-5}$ mol/L) and to sodium nitroprusside ($10^{-10} – 3 \times 10^{-5}$ mol/L) were obtained. Additional concentrations of drugs were added when contractions to the previous concentration were stable.

**Drugs**

Acetylcholine, N$\text{\textsuperscript{G}}$-nitro-$\text{L}$-arginine methyl ester, potassium chloride, norepinephrine, and sodium nitroprusside were from Sigma (Buchs, Switzerland). ET-1 and bigET-1 were from Novabiochem (La¨ufelfingen, Switzerland), and AngI and AngII were from Novartis (Basel, Switzerland). Pentobarbital was from Abbott Laboratories (Chicago, IL). All concentrations of the drugs used are expressed as final molar concentration in the organ chamber bath solution. All drugs were prepared daily and dissolved in distilled water.

**Measurement of ET-1 Tissue and Plasma Levels**

Aortic and renal artery tissue was snap-frozen in liquid nitrogen and kept at −80°C until determination of tissue ET-1. Measurements were performed in a blinded manner. Vessels were weighed and homogenized in a polytron for 60 s in 2 ml of ice-cold chloroform: methanol 2.1 containing 1 mmol/L N-ethylmaleimide and 0.1% trifluoroacetic acid. Homogenates were left overnight at 4°C, then 0.8 ml of sterile distilled water was added. The mixture was vortexed and centrifuged at 4000 rpm for 15 min, and the supernatant was removed. Aliquots of the extract (1 ml) were diluted with 9 ml of 4% acetic acid and then extracted as described for plasma (40). Eluates were dried in a Speed-Vac (Hettich, Bäch, Switzerland) and reconstituted in working assay buffer for RIA. Overall recovery for ET-1 added to chloroform/methanol homogenates of vessels and taken through all extraction steps was 78 ± 4%, with interassay and intra-assay coefficients of 13.6 and 8.6%, respectively. Plasma ET-1 levels were determined by RIA as described previously (40).

**$\text{ET}_\text{A}$ and $\text{ET}_\text{B}$ Receptor mRNA Expression (Reverse Transcription-PCR)**

Total RNA was isolated from aorta and renal arteries of ARF and sham-operated rats using the acid guanidinium-phenol-chloroform method (41). RNA was quantified by absorbance at 260 nm, and the absence of degradation was determined by electrophoresis of denatured RNA in 1% agarose-formaldehyde gel by ethidium bromide.
staining. One microgram of RNA from four rats of the same group was pooled and used as a single sample.

cDNA for PCR was obtained by transcribing 1 μg of RNA from each sample in a final volume of 20 μl, which contained 5 mM MgCl₂, reverse transcription (RT) buffer (10 mM Tris- HCl, 50 mM KCl, and 0.1% Triton X-100), 1 mM deoxynucleotide mixture (dNTP), 20 U RNAasein (a ribonuclease inhibitor), 15 U of reverse transcriptase of the avian Moloney virus, and 50 ng of random primer. The reaction mixture was incubated at 42°C for 45 min. At the end of the incubation, samples were heated at 95°C to eliminate transcriptase activity and to denature RNA-cDNA hybrids. Four microliters of cDNA templates were used for each PCR of ETα and ETβ receptors, and 2 μl was used for GAPDH.

PCR was performed with rat ETα and ETβ receptor and GAPDH-specific oligonucleotide primers. ETα receptor sense primer 5'-GAAGTCTGCCTGCGCATCA-3' corresponded to nucleotides 495 through 514, and the antisense primer 5'-CTGTGTGCTGCGCCCTTGTA-3' corresponded to nucleotides 691 through 710 of the published sequence (42). ETβ receptor sense primer 5'-TTACAGACGCGCAAAGACT-3' corresponded to nucleotides 801 through 820, and the antisense primer 5'-ACGATGACGAAATGAGATT-3' corresponded to nucleotides 1345 through 1365 of the published sequence (18).

We performed RT-PCR of GAPDH as an internal standard. GAPDH sense primer 5'-AATGCACTCTGCACCACCA-3' corresponded to nucleotides 439 through 458, and the antisense primer 5'-GTAGCCATATTCATTGTCATA-3' corresponded to nucleotides 934 through 954 of the published sequence (43). The cDNA amplification products were predicted to be 216, 565, and 516 bp in length. Ten picomoles of sense and antisense primers, 0.5 μCi dCTP-α-32P (>3000 Ci/mmol; Amersham International, Buckinghamshire, United Kingdom), and 1.5 U Taq DNA polymerase were used per reaction. The reaction mixture (20 μl) was overlaid with mineral oil. Tubes were placed on a Thermal Cycler® (Perkin Elmer Cetus, Emeryville, California), which was programmed as follows: incubation 92°C for 30 s, then 30 cycles of the following sequential steps: (1) ETα receptor: 92°C for 1 min, 60°C for 1 min, 72°C for 1 min; (2) ETβ receptor: 92°C for 1 min, 57°C for 1 min, 72°C for 1 min; (3) GAPDH: 92°C for 1 min, 54°C for 30 s, 73°C for 30 s; and finally incubation was done at 72°C for 7 min. The optimum number of amplification cycles used for quantitative RT-PCR was chosen based on pilot experiments that established the exponential range of each reaction. In all experiments, the presence of possible contaminants was checked by control reactions in which amplification was carried out in the absence of reverse transcriptase. PCR-amplified products were saved and kept at −20°C until analysis. PCR products were then separated by 1% agarose gel electrophoresis, and visualized using the Visionary (R) gel documentation system (Bio Cell Consulting, Reinach, Switzerland). Optical density of mRNA for PCR products on the autoradiograph was quantified using scanning densitometry (Molecular Dynamics, Sunnyvale, CA).

Measurement of ET-1 Clearance

125I-ET-1 with specific activities of approximately 2000 Ci/mmol and purity of >95% were used for plasma clearance studies. In previous studies, radiolabeled endothelins have been considered to reflect in vivo uptake of physiologic levels of endothelin peptides more closely than pharmacologic pressor doses, which may selectively influence hemodynamics in different vascular beds. On the day of the clearance experiments, animals were anesthetized with sodium pentobarbital (50 mg/kg), and cannulae were inserted into the left jugular vein and right carotid artery. Bolus 0.3 ml injections of nonpressor doses of approximately 200,000 dpm (0.13 pmol) of radiolabeled human [3-125I]-endothelin-1 (ET-1; 125I-ET-1; Amersham, United Kingdom) were given into the jugular vein, and 50 μl of blood was sampled from the carotid artery at varying times after injection. Fractions were collected over a 2-min period for 125I-ET-1. Preliminary studies established these times to be sufficient to allow virtually complete removal of radiolabeled endothelins from the circulation. Radioactivity in each blood sample was determined with a gamma counter (Canberra Packard, Zürich) and measured as counts/ min versus time fitted to the monoeponential decay curve (Biosoft, Cambridge, United Kingdom). Over the time of the collections, radioactivity in plasma remained as intact peptide as assessed by extraction and reversed-phase HPLC. Hence, during the course of the studies, radiolabel taken up into tissues was not degraded and released back into the circulation as free iodine or peptide fragments. Moreover, during this time, endothelin peptides were not detectably degraded by plasma.

Measurement of 125I-ET-1 Tissue Uptake

At the end of the clearance measurements, representative weighted tissue samples were taken from kidney, liver, lung, and heart for determination of tissue radioactivity content. Weights of the remaining tissues were measured, and total radioactive counts per organ were calculated. Radioactivity in each organ was then expressed as a percentage of the sum of the total radioactivity recovered from all organs investigated.

Statistical Analyses

Data are given as mean ± SEM, and n equals the number of animals. Relaxations were expressed as percent relaxation of preconstriction to norepinephrine. Contractions exhibiting 50% of the response to KCl, EC₅₀ values (as negative logarithm: pD₂), and maximal responses were calculated by nonlinear regression analysis. The area under the concentration–response curve (AUC) was calculated in arbitrary units ranging from 0 to 1000 with higher values representing stronger contractions and lower values reflecting weaker contractions. For multiple comparisons, data were analyzed by two-way ANOVA followed by Bonferroni correction, and for simple comparison between two values, unpaired t test was applied when appropriate. ET-1 clearance data were analyzed using the nonparametric Mann-Whitney U test. P < 0.05 was considered statistically significant.

Results

Animals and Tissues

Creatinine clearance was reduced from 2.8 ± 0.3 ml/min to 0.2 ± 0.2 ml/min in ARF, but was unchanged in sham-operated animals (sham: 2.7 ± 0.3 ml/min to 2.6 ± 0.3 ml/min). BP (134 ± 5 mmHg versus 0.132 ± 4 mmHg, NS) and body weight (237 ± 11 g versus 235 ± 16 g, NS) did not change significantly within 24 h after clamp release. In ARF, tissue weight of rings of the aorta (12.4 ± 2.1 versus 11.0 ± 1.9 mg, NS) and the renal artery (3.2 ± 0.8 mg versus 3.4 ± 1.1 mg, NS) did not differ from the controls.

Alterations in the ET System

ET-1 Plasma and Tissue Concentrations. ARF caused a 2.7-fold increase in ET-1 plasma levels (n = 6, P < 0.01 versus control) (Figure 1, left panel), a 1.8-fold increase in ET-1 kidney content (n = 6, P < 0.01 versus control) (Figure
1, right panel), a 1.9-fold increase in the renal artery ET-1 tissue content (n = 6, *P < 0.01 versus control) and a 2.3-fold increase in the aortic ET-1 tissue content (n = 7, *P < 0.01 versus control). Renal artery ET-1 tissue content was significantly higher than that of the aorta (n = 7, *P < 0.05 versus control). Twenty-four-hour clamping did not change ET-1 tissue content of the renal artery and aorta (NS versus ARF/30-min clamping).

ET-1 Clearance. ARF markedly prolonged the clearance of radiolabeled 125I-ET-1. In control rats, 125I-ET-1 half-life averaged 7.2 ± 0.3 s compared with 23.3 ± 1.9 s in ARF (n = 6, P < 0.001 versus control).

125I-ET-1 Tissue Uptake. ARF caused a marked decrease of 125I-ET-1 tissue uptake in the kidney (n = 6, *P < 0.05 versus control) (Figure 4). In contrast, tissue uptake increased in the liver to a similar extent (P < 0.05 versus control), but remained unchanged in the heart and lung.

Contractions to ET-1. In ARF, maximal contractions, AUC, and sensitivity (pD2 values) to ET-1 (10−11−3 × 10−7 mol/L) were markedly augmented in the renal artery (Figure 5, right panel) (n = 7, P < 0.05 to 0.0001) as well as in the aorta (Figure 5, left panel) (n = 7, P < 0.001 to 0.004). Values of maximal contractions, AUC, and pD2 are shown in Table 1. Contractions are expressed as percentage of the increase in tension to KCl (100 mmol/L).

Contractions to Big ET-1. Vascular responses to big ET-1 (10−9−3 × 10−7 mol/L) were enhanced in ARF in the renal artery (Table 1) (n = 7; P < 0.05 to 0.001) and the aorta (Table 1) (n = 7; P < 0.05 to 0.002). Values of maximal contractions, AUC, and pD2 are shown in Table 1.
Vascular Expression of ET<sub>A</sub> and ET<sub>B</sub> Receptor mRNA.

When compared with that of controls, ET<sub>A</sub> and ET<sub>B</sub> receptor mRNA expression markedly increased in the renal artery and aorta in ARF (Figure 6). As assessed by densitometric analysis, ET<sub>A</sub> receptor mRNA increased 2.3-fold in the renal artery and 2.5-fold in the aorta in ARF (P < 0.05 versus controls; n = 4 animals per group). Correspondingly, ET<sub>B</sub> receptor mRNA expression was enhanced in the aorta and in the renal artery (2.4-fold and 2.2-fold, respectively, P < 0.05 versus controls; n = 4 animals per group).

Table 1. Contractions to ET-1 and big ET-1<sup>a</sup>

<table>
<thead>
<tr>
<th>Category</th>
<th>Maximal Responses</th>
<th>AUC</th>
<th>pD&lt;sub&gt;2&lt;/sub&gt;</th>
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<tr>
<td>ET-1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>control/aorta</td>
<td>92 ± 4</td>
<td>121 ± 8</td>
<td>7.7 ± 0.2</td>
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<tr>
<td>ARF/aorta</td>
<td>122 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>218 ± 13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>control/renal artery</td>
<td>94 ± 4</td>
<td>124 ± 19</td>
<td>7.9 ± 0.1</td>
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<tr>
<td>ARF/renal artery</td>
<td>133 ± 12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>232 ± 16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.2 ± 0.1&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>Big ET-1</td>
<td></td>
<td></td>
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<tr>
<td>control/aorta</td>
<td>77 ± 5</td>
<td>74 ± 5</td>
<td>7.3 ± 0.1</td>
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<tr>
<td>ARF/aorta</td>
<td>102 ± 3&lt;sup&gt;g&lt;/sup&gt;</td>
<td>115 ± 10&lt;sup&gt;h&lt;/sup&gt;</td>
<td>7.5 ± 0.2&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td>control/renal artery</td>
<td>82 ± 4.1</td>
<td>89 ± 4</td>
<td>7.4 ± 0.1</td>
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<tr>
<td>ARF/renal artery</td>
<td>124 ± 5.7&lt;sup&gt;j&lt;/sup&gt;</td>
<td>154 ± 3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>7.6 ± 0.1&lt;sup&gt;f&lt;/sup&gt;</td>
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</table>

<sup>a</sup>Data are given as mean ± SEM (expressed as % of response to 100 mmol/L KCl). ET-1, endothelin-1; AUC, area under the concentration–response curve; ARF, acute renal failure.

<sup>b</sup>P = 0.001 versus control.
<sup>c</sup>P < 0.0001 versus control.
<sup>d</sup>P < 0.001 versus control.
<sup>e</sup>P = 0.001 versus control.
<sup>f</sup>P < 0.05 versus control.
<sup>g</sup>P < 0.004 versus control.
<sup>h</sup>P = 0.003 versus control.
<sup>i</sup>P = 0.002 versus control.

Endothelial-Dependent Relaxations

Relaxations to Sodium Nitroprusside. Endothelium-independent function as assessed by responses to sodium nitroprusside (10<sup>−10</sup>–3 × 10<sup>−5</sup> mol/L) remained unchanged in ARF (Table 2).

Relaxations to Acetylcholine. Endothelium-dependent relaxations to acetylcholine (10<sup>−10</sup>–3 × 10<sup>−5</sup> mol/L) in the renal artery (n = 7) (Figure 7, left panel) and the aorta (n = 7) (Figure 7, right panel) were significantly blunted in ARF (P < 0.05 to 0.001), indicating decreased NO bioavailability. Values of maximal responses, AUC, and pD<sub>2</sub> are shown in Table 2.

Most interestingly, endothelial function was highly and inversely correlated with tissue ET-1 levels in the renal artery (n = 6; r = −0.827, P < 0.001) (Figure 8, right panel) and the aorta (n = 6; r = −0.812, P < 0.001) (Figure 8, left panel).

Other Vasconstrictors

Contractions to AngI and AngII. Contractions to AngI and AngII (10<sup>−7</sup> mol/L) were attenuated in ARF in the renal artery (data not shown, n = 7, P < 0.05 versus control) and the aorta (n = 7, P < 0.05 versus control).

Contractions to KCl. In the renal artery and the aorta, contractions to KCl (100 mmol/L) did not differ significantly in ARF and sham-operated animals (aorta: 1.1 ± 0.2 g versus 1.0 ± 0.2 g, NS; renal artery: 0.8 ± 0.1 g versus 0.8 ± 0.1 g, NS).
The present study demonstrates that in ARF, an increase of circulating and vascular ET-1 protein levels and ET A and Et B receptor gene expression induces an impairment of endothelial function, in particular an enhanced vascular reactivity to ET-1 and a decreased bioavailability of NO, which was inversely correlated with vascular ET-1 content. Interestingly, endothelial dysfunction was not confined to the renal artery, but was also evident in the aorta, indicating that impairment of endothelial function occurs systemically within the first 24 h after induction of ARF.

The integrity of the endothelium has recently been recognized to control intrarenal hemodynamics by releasing endothelium-derived vasoactive substances (7). Thus, it is conceivable that changes in endothelial function subsequently may modulate vascular resistance and kidney function. The potent endothelium-derived vasoconstrictor and mitogen ET-1 has been implicated a local vascular factor in the pathogenesis of ARF (20). At low concentrations, the effects of ET-1 on glomerular microcirculation may help to maintain perfusion pressure. However, sustained reductions of glomerular filtration and renal blood flow were observed during administration of ET-1 in humans (34). Such effects may contribute to renal injury in clinical conditions characterized by elevated plasma levels of ET-1, which have been reported to be highest in patients with various causes of ARF (44).

In the present study, ET-1 plasma levels significantly increased 24 h after induction of ARF. This strongly supports the functional importance of endogenous ET-1 in the regulation of vascular tone in ARF. Activation of the endothelin system has been shown in different forms of ARF (11,24,45), and thus is most likely independent of the mode of how it is induced. In humans, long-lasting vasoconstriction of the renal vascular bed has been demonstrated after administration of low dosages of ET-1 leading to a twofold increase of ET-1 plasma levels (34). However, the relevance of ET-1 plasma levels is questionable, since at least two-thirds of ET-1 are released abluminally toward the vascular smooth muscle cells (8,46). It is widely accepted that ET-1 tissue content more accurately represents the autocrine/paracrine mode of action of the endogenous endothelin system (47). Indeed, in this study we report for the first time that ET-1 tissue levels of the renal artery, the aorta, and the kidney are significantly augmented in ARF. The increase in endothelin levels in ARF may be due to impaired ET-1 clearance by the lung and kidney, in particular by neutral endopeptidases in the brush border of the proximal tubule (48). This study for the first time demonstrates that 125I-ET-1 circulating half-life is indeed markedly prolonged in ARF. Moreover, ET-1 tissue uptake was diminished in the kidney, whereas no changes occurred in the heart and lung. Interestingly, ET-1 tissue uptake increased in the liver most likely as a compensatory mechanism for the observed reduction in the

**Figure 6.** Vascular expression of ET A and ET B receptor mRNA. Receptor mRNA levels of ET A receptors (n = 4; left panel) and of ET B receptors (n = 4, right panel) increased up to 2.5-fold in the renal artery and aorta in ARF. The figure shows a representative experiment of each group of animals. The amplification product of GAPDH served as an internal standard for reverse transcription-PCR.

<table>
<thead>
<tr>
<th>Table 2. Relaxations to acetylcholine and sodium nitroprusside a</th>
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<td>Category</td>
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<tr>
<td>Acetylcholine</td>
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<tr>
<td>control/aorta</td>
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<tr>
<td>ARF/aorta</td>
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<td>control/renal artery</td>
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<tr>
<td>ARF/renal artery</td>
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<tr>
<td>Sodium nitroprusside</td>
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<tr>
<td>control/aorta</td>
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<tr>
<td>ARF/aorta</td>
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<tr>
<td>control/renal artery</td>
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<tr>
<td>ARF/renal artery</td>
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</tbody>
</table>

a Data are given as mean ± SEM (expressed in % of preconstriction with norepinephrine). ET-1, endothelin-1; AUC, area under the concentration–response curve; ARF, acute renal failure.
b P < 0.001 versus control.
c P < 0.05 versus control.

**Discussion**

The present study demonstrates that in ARF, an increase of circulating and vascular ET-1 protein levels and ET A and Et B receptor gene expression induces an impairment of endothelial function, in particular an enhanced vascular reactivity to ET-1 and a decreased bioavailability of NO, which was inversely correlated with vascular ET-1 content. Interestingly, endothelial dysfunction was not confined to the renal artery, but was also evident in the aorta, indicating that impairment of endothelial function occurs systemically within the first 24 h after induction of ARF.

The integrity of the endothelium has recently been recognized to control intrarenal hemodynamics by releasing endothelium-derived vasoactive substances (7). Thus, it is conceivable that changes in endothelial function subsequent...
renal uptake of the peptide. Increased availability of autocrine ET-1 may in turn result in competitive antagonism of $^{125}$I-ET-1 uptake. However, the distinct tissue and receptor-dependent, as well as nonreceptor-linked, mechanisms involved in the shift of ET-1 tissue uptake from the kidney to the liver in ARF remain to be determined.

It is intriguing to propose that activation of the plasma and tissue endothelin systems may alter endothelial function in the early phase of ARF. Indeed, contractions to ET-1 and its precursor big ET-1 were markedly enhanced in the renal artery and also in the aorta in ARF. The effects of ARF on contractions to ET-1 are specific and rule out an increased net basal vascular tone, since responses to the angiotensins decreased and those to potassium chloride remained unchanged in the present experiments. Such a hyper-responsiveness to ET-1 in ARF is indicative of an upregulation of endothelin receptors in ARF. Indeed, we report here for the first time an up to 2.5-fold increase of ETA and ETB receptor mRNA expression both in the renal artery and the aorta within the first 24 h of ARF. Although both receptor subtypes are known to mediate the vasoconstrictive effects of ET-1 \textit{in vivo} (49), their expression and function may vary considerably between species. For example, the effects of ET-1 in the rat kidney are largely mediated by ETB receptors, whereas in the dog they are dependent on activation of ETA receptors (50). In the human kidney, ETB receptors predominate, but effects of ET-1 in the

Figure 7. Endothelium-dependent relaxations to acetylcholine. Endothelium-dependent relaxations to acetylcholine ($10^{-10} - 3 \times 10^{-5}$ mol/L) in the aorta ($n = 7$, left panel) and the renal artery ($n = 7$, right panel) were significantly blunted in ARF. $pD_2: ^*P < 0.05$ versus control.

Figure 8. Correlation of acetylcholine-induced relaxations and ET-1 tissue content of the aorta and the renal artery. Endothelial function (as assessed by $pD_2$ values of acetylcholine-induced relaxations in vessels that were preconstricted with norepinephrine) was inversely correlated with tissue ET-1 levels (aorta: $r = -0.812$, $P < 0.001$; renal artery: $r = -0.827$, $P < 0.001$).
renal circulation are mediated by both receptor subtypes (33). Whereas ET-1 produced by cells in culture downregulates ET-1 receptors by an autocrine mechanism (51), upregulation of endothelin receptors associated with elevated preproET-1 mRNA levels has been observed in various animal models of chronic renal failure (52,53). In line with that, receptor mRNA content increased to a similar extent as plasma and tissue ET-1 protein levels and vascular response to ET-1 in the present experiments of ARF.

Interestingly, upregulation of the circulating and tissue ET-1 protein and receptor mRNA levels were not confined to the renal artery (at the site of clamping), but were also evident upstream in the aorta in ARF. This could be explained in part by a reduced endothelin clearance and stimulatory effects of yet unknown uremic toxins. In addition, loss of inhibitory mechanisms could account for the systemic activation of the ET-1 axis levels within the first 24 h after induction of ARF. It is of note that effects of ET-1 are counterbalanced by other endothelin-derived factors, particularly by NO (54). NO is constitutively released by endothelial cells and continuously modulates vascular tone, thus maintaining renal blood flow (55). Indeed, in the present study an impairment of endothelial-dependent vasodilation in response to acetylcholine indicating decreased NO bioavailability was observed both in the renal artery and the aorta in ARF. This must be due to endothelial dysfunction, since endothelium-independent vasodilation to sodium nitroprusside remained unchanged, indicating preserved vascular smooth muscle responsiveness to NO. We report here for the first time that impaired endothelium-dependent relaxations to acetylcholine (and loss of NO) were inversely correlated with the increase of tissue ET-1 content in ARF. Hence, local vascular ET-1 may inhibit the effects of NO. Alternatively, as NO inhibits ET-1 production in vitro (54), and a decrease in vascular ET-1 production facilitates NO synthesis (56,57), a reduced NO production may cause increased vascular ET-1 tissue content. This is in line with a decreased activity of endothelial NO synthase (eNOS), as recently shown in heart, lung, and brain in ARF (58). However, impaired production of NO could only partly be invoked for the specifically increased sensitivity to ET-1 in ARF, since larger responses to other vasoconstrictors should have been noted as well. Finally, oxygen radical generation may be increased in ARF, which may account for decreased bioavailability of NO due to an enhanced breakdown. However, this interpretation appears to be unlikely at least under the present experimental conditions, because vascular responses did not change when the clamps were left in situ for 24 h to prevent reperfusion. This is in line with recent data suggesting that renal ischemia rather than reperfusion injury is mediating endothelial dysfunction in 2-kidney, 2-clip-induced ARF (59). It is of note that accumulation of an endogenous inhibitor of NO synthesis has been recently discovered in chronic renal failure, i.e., asymmetrical dimethyl-arginine (ADMA) (60). However, this hypothesis has recently been questioned by Anderstam et al., who showed that plasma levels of ADMA in patients with renal insufficiency are below the levels that hitherto have been thought to exert clinical relevance (61).

In conclusion, the present study demonstrates that in ARF an increase of circulating and vascular ET-1 protein levels and ET$_A$ and ET$_B$ receptor gene expression occurs, which in turn induces endothelial dysfunction and enhanced vasoconstriction in the renal as well as the systemic vasculature. Because endothelial dysfunction is related to severe renal and cardiovascular complications, these findings of an ET-1-induced systemic vascular dysfunction could provide the basis for a therapeutic approach of improving endothelial dysfunction and the course of ARF.

Acknowledgments

This work was supported by grants from the Swiss National Foundation (No. 32-325 41.91/2) and the Deutsche Forschungsgemeinschaft (RU 612/1-1). The authors thank Ms. Jane Boden for expert technical assistance.

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