Hemodynamic Effects of Endothelin-1 and Big Endothelin-1 in Chronic Hemodialysis Patients

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Abstract. Increased plasma concentrations of endothelin-1 (ET-1) and big endothelin-1 (big ET-1) have been reported in patients with end-stage renal failure (ESRD). In the present study, which included hemodialysis (HD) patients with \((n = 21)\) and without \((n = 32)\) ischemic heart disease, the putative association between plasma levels of ET-1 and big ET-1 and ischemic heart disease and the influence of the dialysis procedure on ET concentrations was investigated. This study also examined in an additional five HD patients without cardiac disease whether intravenously infused ET-1 and big ET-1 \((0.2, 1, \text{and } 4 \text{ pmol/kg per min, each dose for } 20 \text{ min})\) preserve their vasoactive potency and whether circulating big ET-1 is still converted to ET-1 in HD patients than in healthy humans. Thus, ET-1 and big ET-1 preserve their vasoactive potency, and circulating big ET-1 is still converted to active ET-1 in ESRD. Consequently, the increased plasma levels of ET-1 and big ET-1 noted in HD patients, especially in patients with ischemic heart disease, might play a role in the development of uremic cardiovascular complications.

The vascular endothelium has been shown to play a fundamental role in the regulation of cardiovascular function. In healthy subjects, a delicate balance exists between endothelium-derived relaxing factors (EDRF) and endothelium-derived constricting factors (EDCF). When the balance is disturbed, EDCF may predominate, causing endothelial dysfunction and cardiovascular remodeling (1), which will result in cardiovascular disease. The most potent EDCF known thus far is endothelin-1 (ET-1), which in addition to its vasoconstrictor effects has been demonstrated to be involved in the regulation of cell growth and proliferation and in the modulation of inflammatory responses (2). Haynes \textit{et al.} have elegantly demonstrated that physiologically ET-1 plays a fundamental role in the maintenance of BP in humans (3). Recent clinical research using ET-receptor antagonists has shown that pathophysiologically, in the presence of endothelial dysfunction, ET-1 contributes to elevated BP in patients with mild-to-moderate essential hypertension (4) and to increased systemic and pulmonary vascular resistance and decreased cardiac function in patients with chronic heart failure (5). In addition, evidence is accumulating that ET-1 might be a pathophysiologic factor in other diseases characterized by endothelial dysfunction such as angina pectoris (6), acute myocardial infarction (7), atherosclerosis (8), and acute (9) and chronic renal failure (10). In these diseases, increased plasma concentrations of ET-1 have been reported (5–10).

In hemodialysis (HD) patients, several studies have demonstrated a two- to sixfold increase in plasma concentrations of ET-1 compared with healthy control subjects (11,12). In addition, Miyauchi \textit{et al.} also found a fivefold increase in plasma levels of the propeptide big ET-1 in ESRD (11). It is unknown whether the augmented plasma levels of ET-1 and big ET-1 are caused by increased production or decreased degradation or both. In addition, it is unclear whether dialysis reduces or increases plasma concentrations of ET-1 and big ET-1, because previous studies, which only studied HD-induced changes in ET-1, have demonstrated conflicting results (10,13).

It is also not known whether the elevated plasma concentrations of ET-1 and big ET-1 have any pathophysiologic significance in end-stage renal failure (ESRD), which is a syndrome associated with a high cardiovascular morbidity and mortality. Indeed, cardiovascular diseases including ischemic heart disease and heart failure account for more than 50\% of deaths in dialysis patients, and the incidence of cardiac death is...
five- to 10-fold greater in ESRD patients than in an age-matched general population (14). Although ET-1 has been shown to be involved in cardiovascular diseases in nonuremic patients (4–8), the increased endothelin levels in ESRD patients do not imperatively suggest an increased endothelin activity, since the responsiveness of target tissues to hormones and other active substances may be altered in uremia and since continuously increased plasma levels of endothelin may cause endothelin receptor downregulation or tachyphylaxis. Therefore, it is important to determine whether ET-1 and big ET-1 preserve their vasoactive potency in humans in the presence of ESRD.

Our research group has demonstrated previously that circulating ET-1 as well as circulating big ET-1 induce an increase in BP and a decrease in coronary, renal, and splanchnic blood flow in healthy humans (15–17). However, big ET-1 did not appear to be vasoactive by itself, but it is converted to the mature peptide ET-1, which alone seems to be able to elicit potent and long-lasting hemodynamic effects (18). In healthy humans, we found that an intravenous infusion of big ET-1 resulted in an increased release of ET-1 only from the renal vascular bed, indicating renal conversion of big ET-1, but not from the splanchnic, pulmonary, cerebral, or skeletal muscle vasculature (19). Nevertheless, since circulating big ET-1 elicited potent vasoactive effects not only in the human kidney but also in the human splanchnic region (16), we cannot exclude the possibility of splanchnic conversion of big ET-1. Whether the failing kidney still has the capacity to convert circulating big ET-1 to ET-1 is not known, and there are no data available that show whether big ET-1 or ET-1 is still able to induce a rise in BP and a fall in splanchnic blood flow in ESRD patients.

The purpose of the present study was to investigate: (1) whether ischemic heart disease is associated with increased plasma levels of ET-1 and big ET-1 in HD patients as has been shown in nonuremic patients; (2) whether ET-1 preserves its vasoactive potency in ESRD, which is a prerequisite for ET-1 being able to act as a pathophysiologic factor in uremic cardiovascular disease; (3) whether big ET-1 is still converted to ET-1, thereby being able to elicit vasoactive effects in ESRD; (4) whether the increased plasma concentrations of ET-1 and big ET-1 in ESRD are due to a decreased plasma clearance; and (5) whether hemodialysis, which might stimulate ET-1 synthesis by inducing shear stress, hypoxia, and cytokine activation, elevates or reduces plasma levels of ET-1.

Materials and Methods

All patients were informed of the purpose of the study and, in the second part of the study, also of the risks before giving voluntary consent. Approval was obtained from the local ethics committee.

First Part of the Study

Fifty-three chronic HD patients (34 men, 19 women; mean age 66 ± 1.8 yr; range, 32 to 90 yr) were studied on two occasions in connection with a routine bicarbonate hemodialysis. Twenty-one patients suffered from ischemic heart disease defined as angina pectoris and/or previous myocardial infarction. In addition, 11 of these 21 patients had claudicatio intermittents, which was confirmed by echo-doppler. None of the patients without ischemic heart disease suffered from claudicatio intermittens. None of the patients had acute symptoms of myocardial ischemia during the study. Eight patients had diabetes mellitus, and six of them were also affected by ischemic heart disease and claudicatio intermittents. All patients with ischemic heart disease were treated with nitroglycerin, eight patients were treated with angiotensin-converting enzyme inhibitors, 12 patients with calcium antagonists, and 48 patients with erythropoietin (Table 1).

Fifty-one patients were treated with hemodiablisation, whereas two patients received hemodiablisation. In 43 HD patients, low-flow cel-lulosynthetic hemophane dialyzers (GF5 + 20) were used. Eight HD patients received high-flux polycrylonitrile or cellulose triacetate dialyzers (Filtral 16 or CT 190 G). In the patients treated with hemodiablisation, polycrylonitrile dialyzers (Filtral 16) were used. All patients were treated three times per week. The mean treatment time was 3.7 ± 0.1 h. The mean blood flow was 297 ± 4 ml/min and the dialysate flow 500 ml/min.

Immediately before the start and after the end of a routine hemodiablisation (HD 1) and before the start of the following hemodiablisation (HD 2), weight and BP were measured and blood samples were taken from the venous side of the arteriovenous fistula to determine plasma levels of ET-1 and big ET-1, as well as standard serum parameters such as creatinine, urea, sodium, and potassium. Blood samples for analyses of serum carbonate, calcium, phosphate, albumin, alanine aminotransferase (ALT), hemoglobin, and C-reactive protein (CRP) were taken immediately before the start of HD 1. BP, which was measured using standard sphygmomanometry, was also checked during the dialysis treatment when symptoms indicating hypotension appeared. The dialysis-free interval was longer before HD 1 than before HD 2 (3 d compared to 2 d). KUVurea and protein catabolic rate were calculated by urea kinetic modeling.

Second Part of the Study

Five male chronic HD patients (age 37 ± 4 yr; body mass index [BMI] 23.9 ± 2.0 kg/m²) without cardiac or extracardiac

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ischemic Heart Disease</th>
<th>No Ischemic Heart Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>70 ± 2.2 (range, 50 to 86)</td>
<td>63 ± 2.5 (range, 32 to 90)</td>
</tr>
<tr>
<td>Treated with nitrates</td>
<td>21/21</td>
<td>0/32</td>
</tr>
<tr>
<td>Treated with ACEI</td>
<td>6/21</td>
<td>2/32</td>
</tr>
<tr>
<td>Treated with CA</td>
<td>3/21</td>
<td>9/32</td>
</tr>
<tr>
<td>Treated with ACEI + CA</td>
<td>2/21</td>
<td>2/32</td>
</tr>
<tr>
<td>Treated with erythropoietin</td>
<td>19/21</td>
<td>29/32</td>
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</tbody>
</table>

ACEI, angiotensin-converting enzyme inhibitor; CA, calcium antagonist.
atherosclerotic manifestations (as evaluated by physical examination, electrocardiogram, exercise testing, and chest radiograph) were studied in the supine position after an overnight fast on two different occasions in a randomized order. The weight of the patients did not differ significantly between the two study times. For comparison of basal arterial plasma concentrations of ET-1 and big ET-1, blood samples were taken from eight healthy gender-, age-, and BMI-matched control subjects (age 36 ± 4 yr; BMI 23.2 ± 1.0 kg/m²).

Hemodialysis treatment was performed 1 d before the study times. Four patients were treated with furosemide, which was temporarily stopped 1 d before the study. Two of these four patients were on additional antihypertensive treatment (calcium antagonist, β-receptor blocker), which was temporarily stopped 4 d before the study. Both received a low dose of felodipine (5 mg daily), which in one patient was combined with metoprolol (100 mg daily). The ordinary medication was restarted immediately after the end of the study.

Thin Teflon catheters were inserted percutaneously into a femoral vein for infusions and into a femoral artery for blood sampling and intra-arterial BP measurements. After an equilibration period of 60 min, synthetic human ET-1 (Peninsula Laboratory, Belmont, CA) or synthetic human big ET-1 (Peptide Institute, Scientific Marketing Association, Barnet, Hertfordshire, United Kingdom) dissolved in 0.9% NaCl was administered in increasing doses (0.2, 1, and 4 pmol/kg per min, each dose for 20 min) followed by a recovery period of 120 min. One hour before the start of the ET-1 or big ET-1 infusion, a continuous infusion of indocyanine green dye (0.7 mg/ml; rate 0.8 ml/min) was given to enable estimates of changes in splanchic blood flow, assuming a fractional splanchic dye extraction of 0.8 (20). Blood samples were taken before the start of the dye infusion, immediately before the start of the ET infusions, at 20, 40, 50, 55, and 60 min during the ET infusions and at 1, 2, 3, 4, 5, 10, 15, 30, 45, 60, 90, and 120 min after the ET infusions. Heart rate was measured from continuous electrocardiographic recordings, and intra-arterial BP was monitored continuously during the experiment using a pressure transducer Gould Statham and a polygraph Gould ES 1000 (Gould Instrument Systems Limited, Hainault, Ilford, Essex, United Kingdom).

Analyses
For the determination of plasma big ET-1 and ET-1 concentrations, RIA was used. Plasma aliquots (1 ml) were extracted with acid ethanol and dried under a nitrogen stream. For determination of ET-1, rabbit ET-1 antiserum (E1), which was diluted to give a specific binding of 35 to 40%, and 125I-labeled ET-1 (Amersham, United Kingdom) were used. The detection limit for the assay was 0.39 fmol per tube. The cross reactivity for the E-1 antiserum when the ET-1 (1–21) value is expressed as 100% was: ET-1(16–21), 16%; ET-2, 27%; ET-3, 8%; and big ET-1, 0.4%. No cross reactivity with big ET-1 (22–38) was observed at concentrations up to 1 nM. The intra- and interassay variations were 6 and 14%, respectively (19). For determination of big ET-1, big ET-1 antiserum (B6) and 125I-labeled big ET-1 (Amersham) were used. The detection limit was 0.78 fmol per tube. Expressing the big ET-1 value as 100%, the cross reactivity of the used antiserum (B6) was <0.007% for ET-1 (1–21), <0.03% for ET-2, <0.03% for ET-3, and 35% for the big ET-1 fragment (22–38). The intra-assay variation was 5.6% at a plasma concentration of 50 pmol/L (19). Determination of serum creatinine, urea, sodium, potassium, carbonate, calcium, phosphate, albumin, ALT, hemoglobin, and CRP was carried out in the Department of Clinical Chemistry, Huddinge Hospital, using routine methods.

Statistical Analyses
All data are presented as means ± SEM. In the first part of the study, nonparametric tests were used. A multivariate regression analysis using multiple regression was performed. For pairwise comparisons, the Wilcoxon matched-paired signed-rank test was carried out. To compare two independent samples, the Mann–Whitney U test was used. Correlations between variables were tested by the Spearman rank correlation coefficient. In the second part of the study, ANOVA for repeated measurements was used followed by—if significance was obtained—Fisher protected least significant difference. Skewed distributions were assessed with the F test, and differences between means of samples with skewed distribution were determined using the Wilcoxon matched-paired signed-rank test. A P value <0.05 was considered statistically significant.

Results
First Part of the Study
Plasma concentrations of ET-1 decreased significantly during hemodialysis from 7.81 ± 0.29 to 6.80 ± 0.28 pmol/L (P = 0.002). Plasma levels of big ET-1 were also reduced from 18.40 ± 0.80 to 15.45 ± 0.82 pmol/L (P = 0.0001). Excluding the two patients treated with hemodiafiltration, the HD-induced reduction in plasma ET-1 levels did not differ between low-flux and high-flux membranes. However, a greater decrease in plasma concentrations of big ET-1 was noted during HD when high-flux rather than low-flux membranes were used (1.40 ± 0.34 versus 0.63 ± 0.16 pmol/L per h, P = 0.04). No difference was found between plasma levels of ET-1 before HD 1 and HD 2 (7.81 ± 0.29 versus 7.33 ± 0.26 pmol/L). In contrast, plasma concentrations of big ET-1 differed significantly between HD 1 and HD 2 (18.40 ± 0.80 versus 17.00 ± 0.72 pmol/L, P = 0.02).

A weight reduction of 2.5 ± 0.2 kg was obtained during HD 1. A significant positive correlation was noted between weight reduction during HD 1 and plasma concentrations of ET-1 before HD 2 (P = 0.01). A weight gain of 1.88 ± 0.17 kg was found between HD 1 and HD 2, which showed a positive correlation to ET-1 levels before HD 2 (P = 0.008).

Mean arterial BP (MAP) was stable during hemodialysis (before HD 100 ± 2 mm, after HD 98 ± 2 mmHg). No significant correlation was detected between MAP, systolic or diastolic BP, and plasma ET-1 or big ET-1 or between changes of these parameters during dialysis. In nine patients, symptomatic hypotension occurred during dialysis. However, plasma ET-1 and big ET-1 or intradialytic changes of the endothelins did not differ between patients with and without symptomatic hypotension.

Studying putative correlations between the endothelins and the measured serum parameters, a significant correlation was found between ET-1 and albumin-corrected calcium (P = 0.004).

Patients with ischemic heart disease demonstrated higher predialytic plasma concentrations of ET-1 than patients without ischemic disease (pre-HD: 8.34 ± 0.36 versus 7.06 ± 0.26 pmol/L, P = 0.005) and higher postdialytic plasma levels of big ET-1 (17.89 ± 1.73 versus 13.85 ± 0.65 pmol/L, P = 0.04). Multiple regression analysis showed that these findings were independent of age, gender, and diabetes. Predialytic
concentrations of big ET-1 did not differ between patients with and without ischemic disease (19.5 ± 1.3 versus 16.5 ± 0.7 pmol/L; \( P = 0.09 \)). No difference was noted in the rate of reduction of plasma big ET-1 levels during hemodialysis, in hemodialysis time, in Kt/V, in residual urea clearance, in the reduction in weight, systolic, diastolic, and mean arterial BP, or in age between patients with and without ischemic heart disease. Furthermore, in patients with ischemic heart disease higher serum levels of calcium corrected for albumin were noted than in patients without ischemic heart disease (2.54 ± 0.05 mmol/L versus 2.43 ± 0.04 mmol/L; \( P = 0.05 \)). Pre- and postdialytic concentrations of ET-1 and big ET-1 did not differ between patients with ischemic heart disease and claudicatio intermittens and patients with ischemic heart disease without claudicatio intermittens.

Regarding pre- and postdialytic plasma concentrations of ET-1 and big ET-1, no difference could be noted between patients with and without diabetes mellitus and between diabetic and nondiabetic patients with ischemic heart disease. Patients treated with angiotensin-converting enzyme inhibitors, calcium antagonists, and erythropoietin did not differ from untreated patients with regard to pre- and postdialytic plasma levels of ET-1 and big ET-1.

\( \text{Kt/V}_{\text{urea}} \) was >1.2 and protein catabolic rate 0.99 ± 0.03 g/kg body wt. No correlation was noted between these parameters and ET-1 or big ET-1. Nor was there any correlation found between ET-1 or big ET-1 and serum creatinine, urea, sodium, potassium, albumin, phosphate, carbonate, ALT, hemoglobin, and CRP (Table 2).

**Second Part of the Study**

In the control subjects, plasma levels of ET-1 (1.30 ± 0.10 pmol/L) and of big ET-1 (1.75 ± 0.38 pmol/L) were significantly lower (\( P = 0.003 \)) than basal concentrations of big ET-1 and ET-1 in the HD patients. In the HD patients during the infusion of ET-1, arterial plasma levels of ET-1 increased from 5.29 ± 0.51 pmol/L basally to 134 ± 5.1 pmol/L at the end of the infusion (\( P = 0.04 \)) (Table 3). During the infusion of big ET-1, arterial plasma levels of big ET-1 rose from 19.9 ± 5.9 to 546 ± 70 pmol/L at the end of the infusion (\( P = 0.04 \)). In addition, the big ET-1 infusion increased plasma arterial concentrations of ET-1 from 4.58 ± 0.50 pmol/L basally to a maximum level of 6.47 ± 1.01 pmol/L at 10 min post infusion (\( P = 0.03 \)) (Table 3).

The disappearance curve for arterial plasma concentrations of big ET-1 and ET-1 upon the infusion of big ET-1 or ET-1 showed a curvilinear appearance when logarithmic concentrations were plotted against time. The half-life of ET-1 was 1.6 ± 0.2 min for the initial phase and 101 ± 10 min for the late phase (Figure 1A). The half-life of big ET-1 was 3.8 ± 0.4 min for the initial phase and 54 ± 8.6 min for the late phase (Figure 1B).

During the ET-1 infusion, MAP rose only during the highest dose of ET-1, from 97.8 ± 8.3 mmHg basally to 109.4 ± 8.0 mmHg at the end of the infusion (\( P < 0.001 \)). Fifteen minutes after the end of the infusion, MAP had returned to the basal level. Already during the low dose of big ET-1, MAP increased from 93.6 ± 4.7 mmHg basally to 98.0 ± 5.2 mmHg (\( P < 0.05 \)). After the intermediate dose of big ET-1, MAP showed a value of 99.0 ± 4.6 mmHg (\( P < 0.05 \) versus basal). When the high dose of big ET-1 was given, MAP increased to a maximum of 112.2 ± 5.2 mmHg at 15 min after the end of the infusion (\( P < 0.001 \) versus basal). MAP was still elevated 2 h after the end of the infusion (100.8 ± 5.6 mmHg; \( P < 0.01 \) versus basal). Basal MAP values did not differ significantly between the study occasions, but a more marked response was noted during the big ET-1 infusion compared with ET-1 (\( P < 0.0001 \)) (Figure 2). Systolic and diastolic BP are shown in Table 4 (ET-1 infusion) and Table 5 (big ET-1 infusion).

During the ET-1 infusion, regardless of the dose administered, heart rate remained unchanged. During the infusion of the low and the intermediate dose of big ET-1, heart rate did not change significantly. When the high dose was given, heart rate fell from a basal value of 72 ± 5 bpm to a minimum of 65 ± 5 bpm at the end of the infusion (\( P < 0.01 \)). At 15 min postinfusion, heart rate had returned to the basal level. No statistically significant difference could be detected between the results from the big ET-1 and the ET-1 infusion (Figure 3).

The intermediate dose of ET-1 was able to decrease estimated splanchnic blood flow (ESBF), from 1.25 ± 0.15 ml/min basally to 1.05 ± 0.16 ml/min (\( P < 0.05 \)). During the high dose of ET-1, ESBF continued to decrease to 0.71 ± 0.11 ml/min at the end of the ET-1 infusion (\( P < 0.001 \) versus basal). ESBF had returned to the basal level 30 min after the end of the infusion. The low dose of big ET-1 infusion did not change ESBF, whereas the intermediate dose lowered ESBF from 1.31 ± 0.14 ml/min basally to 1.01 ± 0.14 ml/min (\( P < 0.001 \)). ESBF fell further during the high dose of big ET-1 to 0.84 ± 0.12 ml/min noted at the end of the infusion (\( P < 0.001 \) versus basal value). A significant reduction of ESBF was still noted 45 min after the end of the infusion when measurements of splanchnic blood flow were finished (1.02 ± 0.10 ml/min; \( P < 0.001 \) versus basal value). No difference was detected

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before HD</th>
<th>After HD</th>
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<tbody>
<tr>
<td>Creatinine (μmol/L)</td>
<td>829 ± 33</td>
<td>346 ± 15</td>
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<tr>
<td>Urea (mmol/L)</td>
<td>22.5 ± 0.8</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>139 ± 0.4</td>
<td>142 ± 0.3</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.36 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Albumin-corrected calcium (mmol/L)</td>
<td>2.47 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>35.4 ± 0.6</td>
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</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.8 ± 0.1</td>
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</tr>
<tr>
<td>Carbonate (mmol/L)</td>
<td>24.1 ± 0.5</td>
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</tr>
<tr>
<td>Alanine aminotransferase (μkat/L)</td>
<td>0.44 ± 0.02</td>
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<tr>
<td>Hemoglobin (g/L)</td>
<td>120 ± 2</td>
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</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>21.0 ± 5.3</td>
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</tbody>
</table>

Table 2. Biochemical data on 53 chronic dialysis patients before and after hemodialysis (HD)
between the basal values of ESBF on the two study occasions, but a more marked response was seen after the ET-1 infusion compared to the big ET-1 infusion ($P < 0.02$) (Figure 4).

### Discussion

The major finding in the present study was that both exogenous ET-1 and big ET-1, which in healthy humans have been shown to elicit potent cardiovascular responses (15–18), were still able to induce vasoactive effects in the presence of ESRD. Since the design of the present study differed from previous studies, when intravenous ET-1 and big ET-1 were infused into healthy volunteers (15,16,18), it was not possible to carry out direct statistical comparisons between HD patients and healthy control subjects. However, a similar pattern was perceptible: Big ET-1 gave rise to more prolonged and, with regard to MAP, more potent effects than ET-1 in HD patients as well as in healthy individuals (16,18).

This may be due to a continuous conversion of circulating big ET-1 to vasoactive ET-1, which was indicated by increasing plasma levels of ET-1 during the big ET-1 infusion. Big ET-1 seems to be converted to ET-1 predominantly abluminally (21), resulting in high interstitial levels of ET-1 but relatively low plasma levels of ET-1. Interstitial ET-1 in turn appears to bind rapidly to vasoconstrictive ETA- and ETB2-receptors, which are located abluminally on the vascular smooth muscle cells (22). Since exogenous big ET-1 was able to induce similar pressor effects as exogenous ET-1 in HD patients and in healthy subjects, interstitial concentrations of ET-1 resulting from the big ET-1 infusion should not be lower than those attained during the ET-1 infusion. In HD patients, maximum plasma concentrations of ET-1 after the big ET-1 infusion corresponded to only 5% of the maximum levels after the ET-1 infusion, which clearly demonstrates that the intensity of the vasoconstrictor effects is not determined by the plasma levels of ET-1.

Unfortunately, our study did not allow us to determine in which vascular bed big ET-1 is converted in ESRD patients. In healthy humans, intravenously administered big ET-1 gave rise to a significant increase in ET-1 release from the renal vascular bed, indicating renal conversion of big ET-1, but not from the splanchnic, pulmonary, cerebral, or skeletal muscle vasculature (19). However, circulating big ET-1, which was shown to be extracted from plasma in the renal and splanchnic vasculature,
caused a potent vasoconstriction not only in the kidney but also in the splanchnic area (16). This finding may indicate that conversion of big ET-1 actually takes place in both regions, since we and other investigators have shown that big ET-1 itself lacks any major intrinsic vasoactive potency. Thus, it is conceivable that if all ET-1, which is produced by local conversion of extracted circulating big ET-1, is bound to local ET receptors, a local vasoactive effect will be seen without an increase in ET-1 release, which would explain our previous results. An additional argument for extrarenal conversion of big ET-1 is our observation that in HD patients, exogenous big ET-1 caused a fall in ESBF by approximately 35% at plasma ET-1 levels of 6.5 pmol/L, whereas exogenous ET-1 elicited a decrease in ESBF by only approximately 10% at plasma ET-1 levels of 13 pmol/L. A putative splanchnic conversion of big ET-1 is also in agreement with findings in healthy subjects in whom an infusion of big ET-1 (16) elicited a more pronounced splanchnic vasoconstrictor response than an ET-1 infusion (23), although plasma ET-1 levels were similar.

Because we could demonstrate that the vascular reactivity of ET-1 and big ET-1 is preserved in the presence of ESRD, it is not inconceivable that the increased plasma levels of ET-1 and big ET-1 noted in HD patients could play a role in the development of cardiovascular disease, which is found so frequently in these patients. Our finding that dialysis patients with ischemic heart disease and claudicatio intermittens have higher pre- or postdialytic plasma concentrations of ET-1 and big ET-1 than patients without these disorders lends some support to this hypothesis. In addition, Demuth et al. found a positive correlation between plasma levels of ET-1 and signs of cardiovascular remodeling in HD patients (24). In contrast to our findings and those of Demuth, Mallamaci et al., who used a less specific ET-1 antiserum than we did, found in a smaller group of uremic patients treated with either diet or hemodialysis or peritoneal dialysis, that plasma ET did not differ between patients with and without severe cardiovascular damage (25). On the other hand, Pernow et al. recently demonstrated that exogenous ET-1, which gave rise to only slightly higher

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**Table 4.** Systolic (SBP) and diastolic blood pressure (DBP) in five chronic hemodialysis patients receiving an infusion of ET-1 in increasing doses, each dose for 20 min

<table>
<thead>
<tr>
<th>Dose</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>128 ± 9.3</td>
<td>79 ± 6.7</td>
</tr>
<tr>
<td>0.2 pmol/kg per min</td>
<td>128 ± 9.1</td>
<td>77 ± 5.7</td>
</tr>
<tr>
<td>1 pmol/kg per min</td>
<td>131 ± 11.3</td>
<td>80 ± 6.8</td>
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<tr>
<td>4 pmol/kg per min</td>
<td>143 ± 9.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 min postinfusion</td>
<td>134 ± 11.1</td>
<td>83 ± 6.7</td>
</tr>
<tr>
<td>60 min postinfusion</td>
<td>129 ± 10.7</td>
<td>83 ± 6.3</td>
</tr>
<tr>
<td>120 min postinfusion</td>
<td>132 ± 10.9</td>
<td>81 ± 7.3</td>
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</tbody>
</table>

<sup>a</sup>P < 0.001 versus basal.

**Table 5.** Systolic (SBP) and diastolic blood pressure (DBP) in five chronic hemodialysis patients receiving an infusion of big ET-1 in increasing doses, each dose for 20 min

<table>
<thead>
<tr>
<th>Dose</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>125 ± 4.8</td>
<td>76 ± 3.9</td>
</tr>
<tr>
<td>0.2 pmol/kg per min</td>
<td>131 ± 5.4</td>
<td>78 ± 4.5</td>
</tr>
<tr>
<td>1 pmol/kg per min</td>
<td>132 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 pmol/kg per min</td>
<td>142 ± 4.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 min postinfusion</td>
<td>149 ± 6.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92 ± 4.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 min postinfusion</td>
<td>138 ± 4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>120 min postinfusion</td>
<td>133 ± 5.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82 ± 4.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>P < 0.05 versus basal.<br><sup>b</sup>P < 0.001 versus basal.<br><sup>c</sup>P < 0.01 versus basal.

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**Figure 2.** Mean arterial blood pressure (MAP) in five hemodialysis patients before, during, and after an intravenous infusion of ET-1 or big ET-1 in increasing doses (0.2, 1, and 4 pmol/kg per min), each dose for 20 min.

**Figure 3.** Heart rate in five hemodialysis patients before, during, and after an intravenous infusion of ET-1 or big ET-1 in increasing doses (0.2, 1, and 4 pmol/kg per min), each dose for 20 min.
plasma levels of ET-1 than those reported in patients with cardiovascular disorders, elicited coronary vasoconstriction in healthy humans (26). However, the increased plasma ET levels in patients with ischemic heart disease could also be a result of the disease since the induction of myocardial ischemia has been shown to lead to a significant increase in ET-1 in the coronary sinus and in peripheral venous blood (27). Additional studies using ET receptor antagonists will hopefully help to answer the question of whether the elevated plasma ET levels are cause or consequence of cardiovascular disease.

Interestingly, patients with ischemic heart disease demonstrated increased serum levels of albumin-corrected calcium compared to patients without this disorder. Elevated serum calcium could accelerate the development of coronary and vascular calcification, which is a common finding in dialysis patients (28). We also noted a positive correlation between albumin-corrected calcium and ET-1. Since ET-1 exerts its activity by raising intracellular free calcium levels (2), serum calcium as well as cytosolic calcium may be increased in these patients, which could result in an increase in vascular tone and coronary and vascular remodeling accompanied by medial calcification.

We also showed that compared with gender-, age-, and BMI-matched healthy control subjects, HD patients demonstrated higher plasma levels of ET-1 and big ET-1, which may be due to a decreased removal of the endothelins from the plasma. This assumption is confirmed by our findings demonstrating prolonged plasma half-lives of ET-1 and big ET-1 in the presence of ESRD. The initial half-lives of ET-1 and big ET-1 in HD patients were in accordance with those noted in healthy humans (ET-1: 1.6 min HD versus 1.4 min control; Big ET-1: 3.8 min HD versus 6.6 min control) (15,19). However, the half-lives for the late phase were two to three times longer in HD patients than in healthy humans (ET-1: 101 min HD versus 35 min control; Big ET-1: 54 min HD versus 23 min control) (15,19). Our results are not unexpected because previous studies have demonstrated that circulating ET-1 and big ET-1 are efficiently extracted in the human kidney (15,19). Kohno et al. also demonstrated in a rat model that bilateral nephrectomy results in delayed disappearance of 125I-labeled ET-1 (29). However, our findings do not exclude the possibility that the increased plasma levels of ET-1 and big ET-1 in ESRD are not only due to impaired plasma clearance, but also to a stimulated synthesis since many known stimulators of ET-1 synthesis such as hypoxia, shear stress, and cytokines (2) are active in uremia. Furthermore, dimethylarginine, an endogenous inhibitor of the L-arginine/nitric oxide pathway, has been shown to accumulate in patients with renal failure, which may result in a decreased nitric oxide-mediated inhibition of endothelin synthesis (30).

In addition, our finding that plasma concentrations of ET-1 and big ET-1 are lowered during HD is in accordance with some reports (10) but in contrast to others (13). However, previous studies regarding ET levels during hemodialysis are difficult to evaluate because most of the articles lack data concerning the dialysis procedure and/or the cross reactivity of the ET antibody used in the actual RIA. Different dialysis procedures may stimulate ET-1 synthesis to a varying degree by dialysis-induced hypoxemia, shear stress, and cytokine production. Interestingly, Lundblad et al. (31) recently reported in a study using cardiopulmonary bypass that the interaction between blood and the synthetic surface of the extracorporeal circuit caused a rise in plasma ET-1. Consequently, dialysis-induced ET-1 production may counteract and perhaps sometimes even exceed the fall in plasma ET achieved by dialysis.

We also found that more biocompatible high-flux membranes caused a greater reduction in plasma ET-1 levels than low-flux membranes. This could be due to a higher clearance rate of the high-flux membranes attributable to an enhanced capacity of removing larger molecules, but might also depend on a decreased cytokine-stimulated synthesis of big ET-1 (32). Niwa et al., who used ET-1 antibodies showing an 80% cross reactivity with big ET-1, demonstrated that high-flux membranes lowered plasma levels of ET-1 efficiently, whereas low-flux membranes did not change plasma ET-1 (33).

In summary, we found that: (1) Plasma levels of ET-1 and big ET-1 were more elevated in HD patients with ischemic heart disease than in HD patients without this disorder. (2) ET-1 preserves its vasoactive potency in the presence of ESRD. (3) Circulating big ET-1 is converted to vasoactive ET-1 in ESRD. (4) The increased plasma concentrations of ET-1 and big ET-1 in dialysis patients are at least partially due to a decreased plasma clearance. (5) Hemodialysis decreases plasma levels of ET-1 and big ET-1.

In conclusion, since ET-1 and big ET-1 maintain their vasoconstrictor properties in ESRD, they may play a pathophysiological role in the development of endothelial dysfunction and coronary and vascular remodeling in uremia.

Acknowledgments

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References