

# Endothelin-1 urinary excretion, but not endothelin-1 plasma concentration, is increased in renovascular hypertension

ILARIA CECIONI, PIETRO AMEDEO MODESTI, LOREDANA POGGESI, FEDERICA ROCCHI, LUIGI REGA, and GIAN GASTONE NERI SERNERI

FLORENCE, ITALY

Animal experiments have shown an increase in prepro-endothelin-1 (prepro-ET-1) mRNA expression in the clipped kidney but none in the aortic and mesenteric arteries in 2-kidney, 1-clip Goldblatt hypertensive rats. The present study was aimed at investigating whether plasma and renal endothelin-1 (ET-1) systems are differently activated in patients with renovascular hypertension (RH). The plasma concentration and urinary excretion of ET-1 were measured in 5 patients with RH (before and after successful renal angioplasty), in 7 patients with essential hypertension (EH), and in 8 normotensive control subjects. Immediately before renal angioplasty, plasma samples for ET-1 and plasma renin activity (PRA) measurements were withdrawn from the aorta and both renal veins. Unlike the PRA, the plasma ET-1 concentration did not significantly differ between the involved and the uninvolved sides. The urinary ET-1 excretion level (Fig 1) was markedly increased in patients with RH ( $30 \pm 4$  ng/g urinary creatinine (UC) vs  $2.5 \pm 0.2$  ng/g UC and  $2.6 \pm 0.5$  ng/g UC in control subjects and patients with EH, respectively;  $P < .001$ ), whereas the plasma ET-1 concentration was normal ( $0.8 \pm 0.2$  pg/mL vs  $0.65 \pm 0.3$  pg/mL and  $0.8 \pm 0.2$  pg/mL in control subjects and EH, respectively, not significant). Renal angioplasty was followed in all patients by normalization of blood pressure and PRA. One week after angioplasty, urinary ET-1 decreased to one fourth of baseline ( $8.04 \pm 5.23$  ng/g UC,  $P < .001$  vs values before angioplasty and  $P < .04$  vs control subjects) and normalized 1 month thereafter ( $3.13 \pm 1.62$  ng/g UC, not significant vs control subjects), whereas plasma ET-1 remained steady. The present findings clearly indicate that in patients with RH, urinary ET-1 excretion is increased, whereas plasma ET-1 concentration remains normal. Successful percutaneous transluminal renal angioplasty induced a notable reduction in ET-1 urinary excretion, whereas it did not affect ET-1 plasma concentration. (J Lab Clin Med 1999;134:386-91)

**Abbreviations:** ACE = angiotensin-converting enzyme; EH = essential hypertension; ERPF = effective renal plasma flow; ET-1 = endothelin-1; GFR = glomerular filtration rate; PRA = plasma renin activity; prepro-ET-1 = prepro-endothelin-1; RAS = renin angiotensin system; RH = renovascular hypertension; UC = urinary creatinine

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Reprint requests: Pietro Amedeo Modesti, MD, PhD, Clinica Medica Generale e Cardiologia, University of Florence, Viale Morgagni 85, 50134 Florence, Italy.

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**E**ndothelin-1 is a vasoconstrictor peptide that is also present in urine, where its concentration is about 10 times higher than that in plasma.<sup>1</sup> Urinary ET-1 appears to be mainly derived from the amount of ET-1 locally produced in the kidney<sup>2,3</sup> by different cell types, including peritubular vascular endothelial cells and tubular epithelial cells.<sup>4</sup>

Experimental evidence seems to indicate that ET-1 acts in the kidney as a local autocrine paracrine factor rather than as a circulating hormone for the following reasons: (1) ET-1 secretion by endothelial cells in culture is polar, being directed toward the basolateral

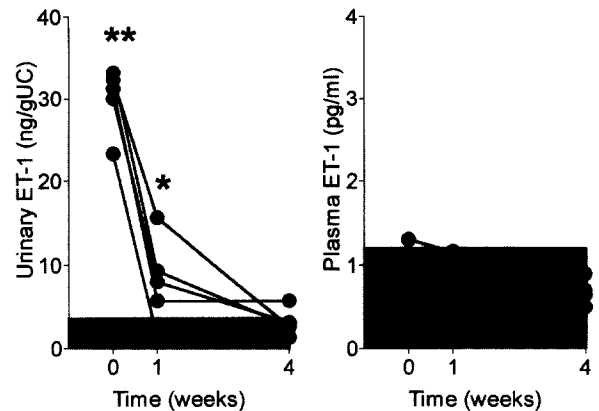
rather than the luminal side;<sup>5</sup> (2) ET-1 and the mRNA of its precursor, the prepro-ET-1, are present in high concentrations in endothelial cells and in the inner medullary collecting ducts in both tubules and the interstitium<sup>4,6-8</sup>; (3) in the absence of changes in circulating ET-1, changes occur in the renal synthesis and urinary excretion of ET-1 in close relationship to fluid handling.<sup>9,10</sup>

Renal ET-1 production is affected by volemic changes because it is inhibited by the increased kidney blood perfusion during acute volume expansion<sup>11</sup> and conversely is enhanced by prolonged standing<sup>9</sup> or blood volume contraction,<sup>12</sup> both of which reduce renal blood flow. Renal ET-1 in turn appears to contribute to renal fluid handling by causing antinatriuresis and an increase in free water clearance.<sup>10,13</sup> Animal experiments showed an increased renal expression of mRNA for prepro-ET-1<sup>14</sup> and normal vascular expression of the ET-1 gene in two-kidney, one-clip Goldblatt hypertensive rats.<sup>15</sup> In renovascular hypertensive patients, either normal<sup>16-18</sup> or increased<sup>19</sup> plasma ET-1 concentration has been reported, but no study has assessed urinary ET-1. The aim of the present study was therefore to investigate whether plasma and renal ET-1 systems are differently activated in patients with RH.

## METHODS

**Subjects investigated.** Five patients with atherosclerotic RH, confirmed by renal angiography with renal vein PRA sampling, and 7 patients with EH were investigated. Eight healthy normotensive subjects served as control subjects. Informed consent was obtained from all of the subjects investigated. The clinical and echocardiographic characteristics of the subjects investigated are shown in Table I. Antihypertensive medications, except clonidine, were gradually withdrawn before the study so that none of the patients used any other antihypertensive drugs for at least 2 weeks before experimental procedures. Subjects were excluded from entry if they had experienced myocardial infarction or any cerebrovascular accident within the previous 6 months or if there was any evidence in the history, physical examinations, or routine laboratory evaluations of other secondary forms of hypertension. Subjects were also excluded if they had diabetes mellitus, kidney failure, abnormal liver function tests, heart failure, significant valvular heart disease, or atrial fibrillation. RH was diagnosed or excluded on the basis of radionuclide scintigraphy with technetium 99m-labeled diethylenetriamine-pentaacetic acid to assess the GFR and iodine 131-labeled hippurate to evaluate ERPF,<sup>20</sup> renal arteriography, and selective PRA assay in the renal veins. Fifty percent or more luminal narrowing of a main renal artery with a renal vein PRA ratio of 1.5 or above, calculated by dividing the highest renal vein PRA value by the lowest, was considered to be indicative of RH.<sup>21</sup>

**Experimental procedure.** All of the subjects with hypertension were inpatients of our institution. They underwent 24-



**Fig 1.** Daily urinary ET-1 excretion (*left panel*) and plasma ET-1 concentration (*right panel*) in patients with RH before (0) and 1 and 4 weeks after renal angioplasty. The shaded area indicates 95% confidence limits for healthy controls. \* $P < .05$ , \*\* $P < .0001$  vs control subjects.

hour blood pressure monitoring, assay of peripheral PRA in the supine and standing positions, radionuclide scintigraphy for measurement of GFR and ERPF, renal arteriography, and assay of selective PRA and ET-1 concentration in the renal veins. All procedures were performed in the morning, with the patients supine and fasting after at least 1 week of normal sodium diet (108 mEq Na/d). The day before renal catheterization, 24-hour urine samples were collected and diuresis, urinary ET-1, aldosterone, creatinine, sodium excretion, and free water clearance were measured. At the same time, venous samples were obtained for measurement of plasma ET-1, sodium, and creatinine. In subjects with a diagnosis of RH, renal angioplasty was performed, and all investigations, except renal catheterization, were repeated 1 week and 1 month thereafter.

**ET-1 assay.** Blood and urine samples for ET-1 measurement were collected and extracted as previously described.<sup>11</sup> The extraction recovery rate, calculated by the addition of four concentrations of cold ET-1 (Peninsula Lab Inc, Belmont, CA) was  $70\% \pm 9\%$  for plasma and  $90\% \pm 5\%$  for urine. ET-1 was assayed by radioimmunoassay with polyclonal specific rabbit antibody (Peninsula) at a final dilution of 1:24,000 for urine and 1:72,000 for plasma.<sup>11</sup> Antibody cross-reactivities were 7% with ET-2 (human), 7% with ET-3 (rat, human), and 17% with big-endothelin (human). There was no cross-reactivity with big-endothelin 22-38 (human). Intra- and inter-assay variability values averaged 4% and 10% for plasma and 3% and 12% for urine, respectively. The minimum detectable concentration was 0.1 pg/tube. Results were expressed as picograms per milliliter for plasma and nanograms per gram of UC for urine.

Blood samples for PRA were collected in ice-cold tubes, immediately centrifuged, and stored at  $-20^{\circ}\text{C}$ . PRA was then measured by radioimmunoassay and results were expressed as the hourly rate of angiotensin I generation (ng/mL/h) (sensitivity of 50 pg/tube angiotensin I, intra-assay and inter-assay variability coefficients 6% and 10%, respectively).

**Table 1.** Clinical and echocardiographic characteristics of investigated subjects

	Controls	Essential hypertension	Renovascular hypertension		
			Before angioplasty	1 Week after angioplasty	1 Month after angioplasty
Age (y)	61 ± 6	58 ± 7	60 ± 5	—	—
Sex (M/F)	6/2	6/1	4/1	—	—
Blood pressure (mm Hg)					
Systolic					
Basal	123 ± 7	164 ± 15	197 ± 8	135 ± 3	130 ± 6
24-Hour	118 ± 4	160 ± 10	183 ± 10	130 ± 5	127 ± 5
Daytime	120 ± 5	166 ± 8	186 ± 10	136 ± 5	134 ± 4
Night-time	110 ± 4	157 ± 7	180 ± 8	125 ± 5	120 ± 8
Diastolic					
Basal	76 ± 4	101 ± 8	104 ± 5	81 ± 4	73 ± 4
24-Hour	75 ± 5	96 ± 6	99 ± 4	78 ± 3	77 ± 1
Daytime	78 ± 4	98 ± 8	103 ± 6	81 ± 3	83 ± 3
Night-time	71 ± 6	94 ± 7	95 ± 2	72 ± 3	70 ± 1
Echocardiography					
LVEF (%)	64 ± 4	59 ± 5	62 ± 9	—	—
S (mm)	8.9 ± 1.0	11.4 ± 1.4	11.3 ± 1.2	—	—
PW (mm)	9.2 ± 0.8	11.1 ± 1.5	11.3 ± 1.2	—	—
LVDDI (cm/m <sup>2</sup> )	2.7 ± 0.1	2.7 ± 0.3	3.0 ± 0.2	—	—
LVMI (g/m <sup>2</sup> )	110 ± 16	148 ± 20	166 ± 39	—	—

LVEF, Left ventricular ejection fraction; S, septum; PW, posterior wall; LVDDI, left ventricular end-diastolic dimension index; LVMI, left ventricular mass index.

**Statistical analysis.** If not otherwise indicated, data are presented as mean ± SD. Comparisons of a single observation between groups (control subjects, patients with EH, and patients with RH) were performed with analysis of variance. All statistical analyses were performed with BMDP statistical software (BMDP Statistical Software, Los Angeles, CA).

## RESULTS

The clinical and echocardiographic characteristics of the subjects investigated are given in Table I. In patients with EH, plasma ET-1 concentration in the antecubital vein ( $0.79 \pm 0.20$  pg/mL) was non-significantly different from that in control subjects ( $0.65 \pm 0.30$  pg/mL). In patients with EH, the ET-1 concentration in blood from renal veins did not significantly differ between the two sides (on average  $0.64 \pm 0.23$  pg/mL, with a mean ET-1 ratio between the two kidneys of  $1.00 \pm 0.10$  pg/mL and a mean ET-1 aorta-renal vein gradient of  $0.002 \pm 0.18$  pg/mL). Urinary ET-1 excretion was non-significantly different from that in control subjects ( $2.62 \pm 0.53$  ng/g UC vs  $2.46 \pm 0.18$  ng/g UC, not significant).

Patients with RH had a significant stenosis of one main renal artery at angiography ( $85\% \pm 8\%$ ). The mean size of the affected kidney ( $104 \pm 11$  mm) was not significantly lower than the unaffected one ( $122 \pm 4$  mm). ERPF and GFR were significantly reduced in the affected kidney in comparison with the unaffected one ( $106 \pm 50$  mL/min/1.73 m<sup>2</sup> vs  $263 \pm 80$  mL/min/1.73 m<sup>2</sup> and

$27 \pm 9$  mL/min/1.73 m<sup>2</sup> vs  $51 \pm 16$  mL/min/1.73 m<sup>2</sup>, respectively,  $P < .05$  for both). PRA values were higher in renal veins of the affected kidney ( $5.3 \pm 1.4$  ng/mL/h) than in those of the unaffected kidney ( $1.8 \pm 0.2$  ng/mL/h,  $P < .001$ ), with a renal vein PRA ratio between the involved and the uninvolved side of  $2.94 \pm 0.89$ .

Plasma ET-1 concentration in the antecubital vein ( $0.80 \pm 0.29$  pg/mL) was non-significantly different from that in control subjects and in patients with EH (not significant for both). ET-1 concentration was similar in the renal veins of the involved ( $0.55 \pm 0.27$  pg/mL) and uninvolved kidneys ( $0.57 \pm 0.27$  pg/mL, not significant between the two sides), with an average ratio of  $1.10 \pm 0.21$ , and was non-significantly different in comparison with patients with EH (not significant). Conversely, in patients with RH the daily urinary ET-1 excretion ( $30.02 \pm 3.52$  ng/g UC) was markedly higher than that in control subjects ( $2.46 \pm 0.18$  ng/g UC,  $P < .0001$ ) and in patients with EH ( $2.62 \pm 0.53$  ng/g UC,  $P < .0001$ ).

Renal angioplasty was technically successful, and after 1 week, 24-hour urinary aldosterone and blood pressure were normalized. PRA in the supine and upright postures was significantly reduced (from  $4.3 \pm 2.0$  ng/mL/h and  $13.4 \pm 8.2$  ng/mL/h at baseline to  $0.6 \pm 0.4$  ng/mL/h and  $1.8 \pm 0.4$  ng/mL/h 1 week after angioplasty). After 1 month, the ERPF and GFR values in the affected kidney were significantly increased ( $180 \pm 41$  mL/min/1.73 m<sup>2</sup> and  $40 \pm 8$  mL/min/1.73 m<sup>2</sup>,

respectively,  $P < .05$  vs baseline), with a mild reduction in values of the unaffected side ( $200 \pm 48$  mL/min/1.73 m<sup>2</sup> and  $42 \pm 7$  mL/min/1.73 m<sup>2</sup>).

One week after angioplasty, urinary ET-1 excretion was reduced to one fourth ( $8.04 \pm 4.68$  ng/g UC,  $P < .001$  vs baseline, and  $P < .05$  vs control subjects) and normalized 1 month thereafter ( $3.13 \pm 1.45$  ng/g UC, not significant vs control subjects). The reduction in urinary ET-1 excretion 1 week after angioplasty was paralleled in all subjects by an increase in 24-hour urinary volume (from  $1587 \pm 502$  mL to  $1975 \pm 466$  mL,  $P < .05$ ) and sodium excretion (from  $110 \pm 5$  mEq/24 h to  $156 \pm 5$  mEq/24 h,  $P < .05$ ). Free water clearance decreased from  $-0.54 \pm 0.23$  mL/min to  $-0.85 \pm 0.23$  mL/min ( $P < .05$ ), and fractional sodium excretion increased from  $0.69\% \pm 0.20\%$  to  $1.34\% \pm 0.26\%$  ( $P < .05$ ).

## DISCUSSION

The present results showed that urinary ET-1 excretion, but not plasma ET-1 concentration, was increased in patients with RH and was restored to normal values after successful renal angioplasty.

Previous studies have shown that severe renal ischemia, after abdominal aortic cross-clamping proximal to the renal arteries in dogs,<sup>22</sup> or total renal pedicle occlusion in rats,<sup>23</sup> increased renal tissue ET-1 content. Similarly, renal hypoperfusion in a two-kidney, one-clip rat model caused a 2-fold to 3-fold increase in the ET-1 mRNA content in the clipped kidney.<sup>14</sup> Under these conditions a powerful activation of the RAS occurs, and angiotensin II was in fact reported to enhance the ET-1 synthesis in cultured endothelial cells.<sup>24-27</sup> However, the inhibition of ACE with ramipril (7.5 mg/kg daily) halved but did not normalize ET-1 mRNA in the clipped rat kidney, thus suggesting that ET-1 synthesis is only partially controlled by RAS.<sup>14</sup> Human studies also showed that ACE inhibition only partially reduced (by about 30%) the increased ET-1 renal production in healthy subjects during standing<sup>9</sup> or a low sodium diet.<sup>10</sup> The present study does not allow us to speculate on the net effect of angiotensin II, because we did not administer ACE inhibitors to our renovascular hypertensive patients. Furthermore, increased angiotensin II levels in the circulation were not found to be correlated with increased expression of mRNA for prepro-ET-1 in the aorta and mesenteric artery of two-kidney, one-clip hypertensive rats.<sup>15</sup> Thus, additional factors seem to be involved, because according to the present findings, only urinary ET-1 excretion is markedly increased in patients with RH, with plasma ET-1 concentration being unaffected. Normal ET-1 concentrations in plasma were also reported by other groups,<sup>16-18</sup> but no study has yet investigated the pattern of urinary ET-1. Thus, in agreement with previous studies by our group,<sup>11</sup> the factors that enhance

circulating and renal endothelins seem to be physiologically independent.

Apart from angiotensin II, other stimuli such as hypoxia and increased osmolality of the renal medulla, caused by the reduced renal blood flow, may be responsible for the enhanced renal ET-1 production in RH. Hypoxia significantly increases ET-1 synthesis in endothelial calf<sup>28</sup> and human<sup>29,30</sup> cultured cells and in the epithelial cells of isolated rat inner medullary collecting ducts.<sup>31</sup> Animal studies assessing the effects of hyperosmolality by NaCl gave conflicting results that showed either a decrease<sup>32</sup> or an enhancement<sup>33,34</sup> of ET-1 production by the epithelial cells of inner medullary collecting ducts. However, in human subjects, low sodium intake, which is characterized by increased osmolality of the renal medulla, was found to be associated with increased prepro-ET-1 mRNA expression in the epithelial cells of the inner medullary collecting ducts and tubules and urinary ET-1 excretion, even in the presence of angiotensin II blockade.<sup>10</sup>

The stimulating effect of all these factors (angiotensin II, hypoxia, and osmolality) is transient in experimental studies, being reversed on removal of the stimulus so that the normalization of urinary ET-1 excretion after successful renal angioplasty sheds no further light on the pathophysiologic mechanism that is responsible for the enhanced urinary ET-1 excretion in renovascular patients.

The significance of increased renal production of ET-1 in patients with renal artery stenosis is only a speculation, because the functional role of endothelin in the kidney is still unclear. In vitro experiments showed that ET-1 inhibits sodium reabsorption in epithelial cells of the collecting ducts.<sup>35,36</sup> However, the physiologic response to ET-1 in vivo seems to be more complex. Clavell et al,<sup>37</sup> using selective ETA and ETB antagonists, elegantly showed in dogs that the natriuretic effect of ET-1 at the level of epithelial cells became evident only when ETA vasoconstrictor receptors were selectively blocked. Thus the natriuretic effect seems to be mediated by ETB receptor stimulation, whereas ETA receptor subtype stimulation seems to cause anti-natriuresis. In human subjects, low-dose (1 ng/kg/min) ET-1 infusion caused sodium retention, with a fall in the fractional excretion of lithium, in the absence of any significant changes in renal blood flow.<sup>38</sup> Furthermore, in human subjects an increased prepro-ET-1 mRNA expression in the endothelial cells of the post-glomerular vascular capillary network, circumventing the proximal and distal tubules and vasa recta, was associated with enhanced sodium retention.<sup>10</sup> Therefore renal ET-1 in human subjects seems to cause sodium retention,<sup>10,13</sup> so that its activation in the stenosis of the renal artery could contribute to the blood pressure increase.

Although urinary ET-1 excretion was increased in all patients with RH as compared with control subjects, the number of patients investigated in the present study is too low for us to conclude that increased urinary ET-1 excretion may be a marker for the diagnosis of RH.

In conclusion, the present findings indicate that (1) RH is associated with increased urinary ET-1 excretion; (2) RAS activation does not cause an increase in plasma ET-1 concentration; (3) successful percutaneous transluminal renal angioplasty induces a notable reduction in ET-1 urinary excretion, whereas it does not affect ET-1 plasma concentration.

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