**Endothelin and active renin levels in essential hypertension and hypertension with renal artery stenosis before and after percutaneous transluminal renal angioplasty**

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**Objective** To determine whether active renin and endothelin levels in venous plasma differ between patients with renal artery stenosis and patients with primary hypertension. Among the patients with renal artery stenosis we also compared active renin and endothelin levels between subjects who had been cured or whose blood pressure had improved after treatment of the stenosis and those without a beneficial reaction after such treatment.

**Methods** We measured immunoreactive endothelin and active renin levels in peripheral venous plasma before and 1 h after angiotensin converting enzyme inhibition in 25 patients with primary hypertension and in 27 patients with hypertension and renal artery stenosis. Percutaneous transluminal angioplasty was performed in 21 patients of the latter group. For 11 patients of this group, hypertension was cured or there was an improvement, whereas 10 other patients did not respond to this treatment. Baseline active renin and endothelin levels were compared between these groups, as were the clinical characteristics of the patients.

**Results** Baseline endothelin levels were similar in members of the renal artery stenosis [median 3.6 pg/ml (range 1.4–11.7)] and in members of the no stenosis group [5.0 pg/ml (1.5–8.0)]. Also baseline endothelin levels did not differ between members of the successfully treated [3.6 pg/ml (1.8–8.9)] and unsuccessfully treated groups [3.75 pg/ml (1.4–8.3)]. Angiotensin converting enzyme (ACE) inhibition failed to cause a significant change in endothelin level in members of any of the patient groups. Although baseline renin levels differed significantly between members of the renal artery stenosis and no stenosis groups [40.2 μU/ml (0.9–543) versus 13.4 μU/ml (2.5–931), (P<0.05)], there was no difference in baseline renin levels between the members of successful and unsuccessful groups [25.7 μU/ml (9.2–475.6) versus 65.3 μU/ml (12.3–542.6)]. ACE inhibition caused a significant increase in renin level in members of all groups except the unsuccessfully treated group.

**Conclusions** Circulating endothelin levels did not differ significantly among patients with essential hypertension, hypertension with renal artery stenosis and proven renovascular hypertension and, although the renin–angiotensin system was clearly activated in members of the renovascular hypertension group, ACE inhibition did not affect their endothelin levels. These results suggest that endothelin does not play a direct role in the pathophysiology of renovascular hypertension.

**Introduction**

The endothelins are vasoactive peptides of which up to now three different isopeptides have been identified. Endothelin-1 (ET-1) is a potent vasoconstrictor produced by endothelial cells [1]. In the kidney it is produced both in endothelial and in non-endothelial cells [2]. No storage granules of ET-1 have been discovered thus far and approximately 25% of ET-1 synthesized is secreted into the general circulation [3]. Endothelins have been thought to have a considerable impact on the vascular tone both in normal physiological and in diseased states. However, plasma endothelin levels have not been found to be elevated in essential hypertension [4,5]. Endothelin levels are elevated in pulmonary hypertension and renal failure [5,6]. Because vasoactive substances take such an important part in the pathophysiology of renovascular hypertension (RVH), it is worth considering whether endothelins are involved in at least some of the vascular reactivity mechanisms operating in this form of high blood pressure.

Specific information about the role of endothelin in RVH is, however, incomplete. Several findings suggest that endothelin could be involved in RVH through involve-
ment with the renin–angiotensin system. First, endothe-
lial cell culture studies have shown that hypoxia [7] and 
angiotensin II (Ang II) induce synthesis and release of 
endothelin [8]. Angiotensin converting enzyme (ACE) 
inhibition attenuates this release [9]. Second, Ang II stim-
ulates endothelial production of endothelin and thereby 
potentiates contractions in response to noradrenaline in 
mesenteric resistance arteries of spontaneously hyper-
tensive rats [10]. Furthermore, Sventek et al. [11] found 
vacular overexpression of ET-1 gene in one-kidney, one 
clip hypertensive rats but only during the late phase of 
two-kidney, one clip hypertensive rats. Also several 
clinical studies have been reported but they produced 
contradictory results in which endothelin levels were 
found to be either normal or elevated in patients with 
renal artery stenosis (RAS) [12–15].

The aim of the present study was to investigate a poss-
ible role of endothelin in the pathophysiology of RVH in 
humans. We studied endothelin and renin levels in per-
ipheral venous blood before and after ACE inhibition 
in members of the following groups of hypertensive 
patients: subjects with essential hypertension, persons 
with hypertension and RAS, patients whose hypertension 
was cured or alleviated by percutaneous transluminal renal 
angioplasty (PTRA) treatment of RAS and patients whose 
hypertension was not lessened by PTRA treatment of 
RAS.

In studying these specific patient groups, we supposed 
that we would be able to employ ACE inhibition and 
responses to PTRA as tools to select out RAS patients 
with true RVH, allowing us to compare endothelin levels 
of these renovascular hypertensives with those of other 
hypertensives. Given the evidence on endothelin summa-
rized above, we hypothesized that ACE inhibition would 
cause not only an increase in renin levels but also, via a 
decrease in Ang II levels, a decrease in circulating 
endothelin levels in patients with RVH. Furthermore, the 
renin : endothelin level ratio before and after ACE inhibi-
tion could provide a reliable marker for distinguishing 
true RVH from essential hypertension cases with and 
without RAS.

Patients and methods
For 52 hypertensive patients newly referred to the out-
patient clinic, the physicians in charge deemed it neces-
sary to perform intra-arterial digital subtraction angi-
ography. Indications for angiography were treatment-resis-
tant hypertension, defined as blood pressure values above 
160/90 mmHg despite two-drug treatment, and other 
clinical clues suggestive of RAS, such as an abdominal 
bruit and a sudden rise of a known blood pressure.

Clinical and biochemical data of these patients were col-
lected. Blood pressure was measured by using a standard 
mercury sphygmomanometer with the patient supine. The 
mean of three consecutive readings was used. The possi-
bility that these patients had other forms of second-
ary hypertension was assessed by determination of serum 
electrolyte levels, plasma renin activity, aldosterone levels 
and catecholamine levels. Urinary excretions of creatinine, 
metanephrines and protein were also measured. Endo-
genous creatinine clearance was calculated from serum 
creatinine level measurements [16]. The urine screenings, 
together with microscopy of urine sediments were used to 
exclude the possibility that these patients had parenchymatous renal disease. Patients with other forms of 
secondary hypertension and patients with renal insuf-
iciency were excluded from this study. For determi-
nation of plasma concentrations of active renin and 
endothelin, venous blood was collected into tubes 
containing EDTA. Samples were centrifuged immedi-
ately at 0°C and stored at −20°C until assay. Active renin 
and endothelin-1 levels were measured by radioim-
munoassays, as described previously [17,18]. The anti-
scrum for endothelin-1 exhibited 67% cross-reactivity 
with endothelin-2 and 84% with endothelin-3. PTRA was 
performed as a standard procedure via the femoral 
approach under direct high-resolution fluoroscopy. The 
reactions of blood pressure to the procedure were deter-
mined according to the internationally accepted criteria. 
A patient with blood pressure below 90 mmHg without 
medication was considered cured. Improvement was 
defined as a decline in blood pressure by at least 15% 
with the same medication or a decline by 10% with 
discontinuation of administration of at least one anti-
hypertensive drug. Patients who had been cured and 
patients who had improved were placed in the success-
fully treated group and all patients with other outcomes 
in the unsuccessfully treated group. A stenosis was consid-
ered significant if the vascular lumen was found by angiog-
raphy to have narrowed by more than 50%. PTRA was 
performed in patients with RAS for whom there was no 
contra-indication to the treatment and who consented to 
be subjected to the procedure. Patients were adminis-
tered 300 mg acetylsalicylic acid a day starting 1 day 
before the procedure and continuing for 3 months after-
wards.

Statistical analysis
Values are expressed as medians (ranges) unless indicated 
otherwise. Comparisons of the data within groups were 
analysed by Wilcoxon’s paired rank test for para-
eters with non Gaussian distributions. Student’s paired 
t test was used for parameters with normal distributions. 
Differences between groups were assessed by unpaired 
tests. When appropriate the Bonferroni correction for 
multiple comparisons was applied. The correlations of 
endothelin levels in members of the RAS, the primary 
hypertension, the successfully treated and the unsuc-
cessfully treated groups either with renin level or with
endogenous creatinine clearance were analysed by Spearman's rank correlation test. Statistical significance was assumed at a 5% level, two-sided.

Results

Of the 52 patients with hypertension who had undergone diagnostic digital subtraction angiography, 25 had no RAS and were diagnosed as having essential hypertension. The remaining 27 patients proved to have RAS. There were 20 unilateral and seven bilateral cases. The clinical characteristics of the patients with and without RAS are summarized in Table 1. The groups differed only in baseline systolic blood pressure, that of the RAS group being significantly higher than that of the group without stenosis [186 mmHg (140–246) versus 168 mmHg (120–215), \( P < 0.05 \)]. Furthermore, atherosclerosis was the major aetiological factor for RAS in our study rather than fibromuscular dysplasia (FMD, 20 versus seven patients).

Twenty-one of the 27 patients with RAS were treated by PTRA of the stenosis. Of the 21 patients treated, 11 exhibited decreases in blood pressure in response to this treatment. The remaining 10 patients did not respond to this treatment. The clinical characteristics of these patients are summarized in Table 1. Bilateral RAS was found in patients in the unsuccessfully treated group more often than it was in patients in the successfully treated group (four versus one patient). Blood pressures of all patients with FMD-related RAS responded to PTRA treatment. Conversely, the unsuccessfully treated group was comprised entirely of patients with RAS as a result of atherosclerosis. The five patients with FMD RAS were also the five women in the successfully treated group. Thus, although male patients with atherosclerotic RAS were distributed evenly between the successfully and unsuccessfully treated groups, the female patients with atherosclerotic RAS did not respond to treatment by PTRA.

The baseline renin level in members of the RAS group was significantly higher than that in members of the essential hypertension group [40.2 \( \mu \)u/ml (0.9–543) and 13.4 \( \mu \)u/ml (2.5–931), respectively, \( P = 0.049 \)]. The baseline level of endothelin did not differ significantly between RAS [3.6 pg/ml (1.4–11.7)] and essential hypertension [5.0 pg/ml (1.5–8.0)] cases.

The baseline renin level in members of the successfully treated group [25.7 \( \mu \)u/ml (9.2–475)] was not significantly different from that in members of the unsuccessfully treated group [65.3 \( \mu \)u/ml (12.3–542.6)] and neither were the baseline endothelin levels [3.6 pg/ml (1.8–8.9) and 3.75 pg/ml (1.4–8.3), respectively].

ACE inhibition caused significant rises in active renin levels in members both of the RAS and of the essential hypertension group, from 40.2 \( \mu \)u/ml (1–543) to 98.6 \( \mu \)u/ml (3.2–2004); \( P = 0.001 \) and from 13.4 \( \mu \)u/ml (2.5–931) to 20.4 \( \mu \)u/ml (2.9–1432), \( P = 0.03 \), respectively. In addition, the RAS patients who responded to treatment by PTRA displayed a significant rise in renin levels after ACE inhibition [from 25.7 \( \mu \)u/ml (9.2–475) to 72.8 \( \mu \)u/ml (10.4–2004), \( P = 0.001 \)], whereas members of the unsuccessfully treated group failed to respond to ACE inhibition in terms of a significant rise in renin levels from baseline [from 65.3 \( \mu \)u/ml (12.3–542.6) to 145 \( \mu \)u/ml (11.3–1298)].

The differences (i.e. after–before values) in renin levels before and after ACE inhibition did not differ significantly between members of the RAS group [47.6 \( \mu \)u/ml (–28.5 to 1528) and members of the group with essential hypertension [3.1 \( \mu \)u/ml (–106 to 500)]. This also held true for the successfully treated group [47 \( \mu \)u/ml (–2 to 1528) and the unsuccessfully treated group [50 \( \mu \)u/ml (–28 to 847)]. The relative differences between baseline values and those after ACE inhibition were not significant for any of the groups.

Table 1 Clinical characteristics of hypertensive patients with or without renal artery stenosis, successfully or unsuccessfully treated by percutaneous transluminal renal angioplasty

<table>
<thead>
<tr>
<th></th>
<th>No stenosis (n = 25)</th>
<th>Renal artery stenosis (n = 27)</th>
<th>Treatment successful (n = 11)</th>
<th>Treatment unsuccessful (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>13/12</td>
<td>18/12</td>
<td>6/5</td>
<td>6/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 (24–68)</td>
<td>52 (29–79)</td>
<td>48 (29–71)</td>
<td>58 (44–76)</td>
</tr>
<tr>
<td>Unilateral/bilateral stenosis</td>
<td>20/7</td>
<td>10/1</td>
<td>6/4</td>
<td>10/0</td>
</tr>
<tr>
<td>Aetiology: atherosclerosis/FMD</td>
<td>2/0/7</td>
<td>6/0/1</td>
<td>10/0</td>
<td></td>
</tr>
<tr>
<td>Baseline SBP (mmHg)</td>
<td>168 (120–215)</td>
<td>186 (140–248)*</td>
<td>180 (140–210)</td>
<td>197 (158–246)</td>
</tr>
<tr>
<td>Baseline DBP (mmHg)</td>
<td>101 (70–120)</td>
<td>108 (90–145)</td>
<td>108 (90–130)</td>
<td>109 (96–145)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 (17.8–43.0)</td>
<td>25.1 (17.6–39.6)</td>
<td>26.2 (19.1–30.5)</td>
<td>25.2 (21.0–29.6)</td>
</tr>
<tr>
<td>ECC (ml/min)</td>
<td>88.3 (264–269.2)</td>
<td>82.3 (22.2–193.9)</td>
<td>86.3 (145–153)</td>
<td>74.8 (22–134)</td>
</tr>
<tr>
<td>Smokers/non-smokers</td>
<td>12/13</td>
<td>14/13</td>
<td>7/4</td>
<td>5/5</td>
</tr>
<tr>
<td>With/without diabetes mellitus</td>
<td>1/1</td>
<td>1/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
</tbody>
</table>

Values are expressed as medians (ranges). FMD, fibromuscular dysplasia; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ECC, endogenous creatinine clearance. *No relation to the seven bilateral RAS patients. All FMD patients were women. \*0.05, versus no stenosis.
The patients with essential hypertension had a baseline endothelin level of 5.0 pg/ml (1.5–8.0) and the subjects with RAS had a baseline endothelin level of 3.6 pg/ml (1.4–1.7), which values were not significantly different (Fig. 1). In members of the successful treatment group baseline endothelin level was 3.6 pg/ml (1.8–8.9) and in members of the unsuccessful group it was 3.75 pg/ml (1.4–8.3); also these values were not significantly different (Fig. 2).

ACE inhibition caused no significant change in endothelin levels in members of any of the four study groups. Furthermore, the differences and relative differences in endothelin levels before and after ACE inhibition did not differ significantly between members of the RAS group and the essential hypertension group and between members of the successfully and the unsuccessfully treated groups.

The renin : endothelin level ratio at baseline was significantly higher for the RAS group in comparison with that for the essential hypertension group [9.4 μu/pg (0.1–131.5) versus 2.5 μu/pg (0.4–221.7), respectively, \( P = 0.020 \)]. ACE inhibition further increased the significant difference between renin : endothelin level ratios for the RAS and essential hypertension groups [26.5 μu/pg (0.4–554.2) versus 3.6 μu/pg (0.5–227.3), respectively \( P = 0.005 \)].

The renin : endothelin level ratio at baseline was the same for the group that responded as it was for the group that did not respond to treatment by PTRA. Furthermore, ACE inhibition failed to cause a significant difference between values of this ratio for these patient groups. The difference in ratios before and after ACE inhibition did not differ significantly between the successfully and unsuccessfully treated groups.

Endothelin levels in peripheral venous blood were not correlated either to renin levels or to endogenous creatinine clearances for all four groups.

**Discussion**

The results of the present study indicate that endothelin levels in peripheral venous blood under baseline resting conditions are comparable among patients with essential hypertension, hypertension combined with RAS and RVH. In addition, ACE inhibition and thus a decrease in Ang II levels did not affect endothelin levels in patients with essential hypertension, patients with essential hypertension combined with RAS and patients with RVH. Although the renin : endothelin level ratio differed signif-
Comparison of circulating endothelin levels in patients for whom percutaneous transluminal renal angioplasty had been successful and unsuccessful at lowering blood pressure before and after angiotensin converting enzyme (ACE). Horizontal bars, medians.

Fig. 2

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Significantly between the groups with and without RAS, this was largely, if not completely, due to the significant difference in renin levels. This argument also applies to the renin : endothelin level ratio after ACE inhibition, which displayed an even more significant difference.

In dividing the group of patients with RAS into those who responded to PTRA and those who did not, we attempted to distinguish between RAS leading to RVH and RAS occurring concomitantly with essential hypertension. Interestingly, among the patients with RAS, administration of the ACE inhibitor caused renin levels to rise only in members of the group who responded to treatment by PTRA. Therefore, the fact that endothelin levels did not differ between these groups and were not affected differently by ACE inhibition makes the hypothesis that endothelin plays a role in the pathophysiology of RVH less plausible.

It is possible that the production and release of endothelin were not affected during the relatively short 60 min sampling period used in this study. However, an oral dose of captopril reaches its maximum plasma level 1 h after ingestion and the maximum blood-pressure-lowering affect occurs 60–90 min after ingestion. Thus it can be concluded that, 60 min after ingestion, while captopril is affecting the renin–angiotensin system and other mechanisms involved in regulation of blood pressure, regulation of endothelin production seems not to be altered.

Plasma levels of endothelin both in essential hypertension and in RVH patients both with and without ACE inhibition have been investigated by several other groups. Results of most studies [12–15] concur with the results of the present study. Two groups who studied endothelin levels in blood samples taken directly from renal arteries and veins found endothelin levels in healthy controls, patients with essential hypertension and patients with RAS to be within the normal range [14,15]. Januszewicz et al. [19] reported that the degree of activation of the renin–angiotensin system had no effect on the endothelin levels, which were also not elevated in their patients with RAS. In contrast, Giussani et al. [12] did find an increase in plasma endothelin levels in patients with RAS and elevated Ang II levels.

Several in-vitro studies with isolated arteries from rats and humans have shown that the vasoconstricting effect of infusion of endothelin-1 on vascular smooth muscle cells is blunted in subjects with mild forms of hypertension [19–21]. Furthermore, vascular smooth muscle cells from spontaneous hypertensive rats display lower than normal responses of intracellular calcium levels to infusion of endothelin-1 but exhibit higher than normal responses to...
infusion of Ang II [22,23]. These results suggest that endothelin does not affect vasoreactivity once hypertension has been established and thus possibly this could explain why endothelin levels are not elevated in most hypertensive (patients with essential hypertension and patients with RVH) subjects studied. Possibly endothelin plays a role in vasoreactive events leading to chronic hypertension. However, Sventek et al. [11] recently showed that, although the one-kidney one clip Goldblatt hypertensive rat exhibits vascular overexpression of the endothelin-1 gene, the two-kidney, one clip Goldblatt hypertensive rat only does so during a late phase. This late expression could also be linked to renal failure or to an inability of the lungs or kidneys to clear the endothelin. Thus, although many studies, including the present one, could not show that circulating endothelin plays a role in maintaining RVH, endothelin’s role in the early phase of RVH has yet to be sufficiently studied.

With the exception of baseline systolic blood pressure, there were no significant differences among the clinical characteristics of the groups studied. Male and female patients were distributed equally among groups. In a recent study [24] it was found that plasma endothelin levels are modulated by the menstrual cycle. Although this new knowledge does not directly affect the results of the present study, all future studies of this type should take into consideration the menstrual cycle on endothelin levels in order to evaluate sex differences. Of the 27 patients with RAS, seven were diagnosed as having bilateral stenosis. Furthermore, four of the unsuccessfully operated and one of the successfully operated patients had bilateral RAS. In such cases, both renal arteries were treated by PTRA. It was, however, beyond the scope of this study to evaluate the difference between unilateral and bilateral stenosis with regard to hypertension, renin levels and endothelin levels.

In conclusion, the present study showed that circulating levels of endothelin in patients with essential hypertension, patients with hypertension with RVH and patients with true RVH are similar. Although the renin–angiotensin system in patients with RVH was activated, as shown by a significant increase in plasma renin activity after ACE inhibition, endothelin levels were not correlated to the renin levels and were not affected by ACE inhibition. These results do not support the hypothesis that production of endothelin in RVH patients is dependent on Ang II.

References