Renin-Angiotensin System

Angiotensin-(1–7)–Induced Renal Vasodilation in Hypertensive Humans Is Attenuated by Low Sodium Intake and Angiotensin II Co-Infusion


Abstract—Current evidence suggests that angiotensin-(1–7) plays an important role in the regulation of tissue blood flow. This evidence, however, is restricted to studies in animals and human forearm. Therefore, we studied the effects of intrarenal angiotensin-(1–7) infusion on renal blood flow in hypertensive humans. To assess the influence of renin–angiotensin-system activity, sodium intake was varied and co-infusion with angiotensin II was performed in a subgroup. In 57 hypertensive patients who were scheduled for renal angiography, renal blood flow was measured (131Xenon washout method) before and during intrarenal infusion of angiotensin-(1–7) (3 incremental doses: 0.27, 0.9, and 2.7 ng/kg per minute). Patients were randomized into low or high sodium intake. These 2 groups of patients received angiotensin-(1–7), with or without intrarenal co-infusion of angiotensin II (0.3 ng/kg per minute). Angiotensin-(1–7) infusion resulted in intrarenal vasodilation in patients adhering to a sodium-rich diet. This vasodilatory effect of angiotensin-(1–7) was clearly attenuated by low sodium intake, angiotensin II co-infusion, or both. Regression analyses showed that the prevailing renin concentration was the only independent predictor of angiotensin-(1–7)–induced renal vasodilation. In conclusion, angiotensin-(1–7) induces renal vasodilation in hypertensive humans, but the effect of angiotensin-(1–7) is clearly attenuated by low sodium intake and co-infusion of angiotensin II. This supports the hypothesis that angiotensin-(1–7) induced renal vasodilation depends on the degree of renin-angiotensin-system activation. (Hypertension. 2013;62:789-793.)

Key Words: angiotensin-(1–7) ■ angiotensin II ■ hypertension ■ kidney ■ renin-angiotensin system ■ sodium

Angiotensin-(1–7) (Ang-(1–7)) is a vasoactive peptide that plays an important role in the regulation of tissue blood flow.1 It is closely linked to the classical renin–angiotensin system (RAS) because the enzymes producing Ang-(1–7) use angiotensin I and angiotensin II (Ang II) as its substrate. Available data, limited to studies using animal models and human forearm vasculature, predominantly demonstrate a vasodilatory effect of Ang-(1–7).2,3 Human data on the effect of Ang-(1–7) in clinically important vascular beds, such as that of the kidney, are lacking.4 As the kidney and RAS activation play a key role in blood pressure regulation and the development of hypertension, studying the intrarenal flow effects of Ang-(1–7) in hypertensive patients may lead to new insights in the pathophysiology of hypertension. We hypothesized that, in correspondence to the data currently available, Ang-(1–7) increases renal blood flow in hypertensive humans. For that reason we studied the effect of intrarenal Ang-(1–7) infusion on renal blood flow in hypertensive patients. To assess the influence of the RAS, various states of RAS stimulation were simulated by restriction of dietary sodium intake (inducing endogenous RAS activation) and co-infusion of Ang II (increasing Ang II availability).

Methods

Participants and Protocol

This study was performed in 85 hypertensive outpatients (all whites) who were angiographically evaluated for the presence of renovascular abnormalities. Inclusion criteria were difficult-to-treat hypertension (mostly blood pressure remaining above goal in spite of the use of ≥3 full-dose antihypertensive drugs) or clinical suspicion on renovascular abnormalities based on clinical clues (eg, the presence of an abdominal bruit, peripheral vascular disease, or a rise in serum creatinine after treatment with a RAS inhibitor). Patients previously diagnosed with renovascular abnormalities or other secondary causes of hypertension were excluded. Three weeks before the angiography all antihypertensive medication was discontinued to avoid interference with the experiments. Patients in whom interruption of the medication was not possible because of a high risk for acute cardiovascular events (a recent cardiovascular event or [expected] blood pressure >180/110 mm Hg) were excluded from the study. Patients were randomly allocated to adhere to either a sodium-restricted diet (<55 mmol sodium/24 hours) or a sodium-rich diet (>200 mmol sodium/24 hours) during the week preceding the study. The day preceding the angiography study, patients underwent a noninvasive 24-hour ambulatory blood pressure measurement (using SpaceLabs ambulatory blood pressure monitor type 90207 or 90217-b) and collected a 24-hour urine specimen to measure urinary sodium excetration to monitor dietary compliance. All patients gave written consent to participate in the study.

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informed consent, and the study was approved by the Medical Ethics Committee of the Maastricht University Medical Center.

Experimental Procedures

Patients were admitted to our ward 1 day before the angiography. After an overnight fast, the aorta and both renal veins were cannu-
lated via the femoral route. Blood samples were drawn from the aorta and both renal veins. Subsequently, mean renal blood flow (MRBF) was measured using the 133Xenon washout technique, as described previously. In short, after infusion of a bolus of 133Xenon directly into the renal artery, 133Xenon washout curves were recorded using an extracorporeal scintillation counter. After subtraction of background radiation, the disappearance of 133Xenon from the kidney was analyzed mathematically using either a 2-phase or a 1-phase exponential decay model, depending on the best fit. From this model we calculated MRBF (expressed as mL/100 g kidney per minute). In our hands, 133Xenon washout technique has a coefficient of variation of 8% for repeated measurements. Intrarenal placebo infusions have no measurable effects on MRBF.

After obtaining baseline data, infusion studies were started (see below). For reasons of standardization, we performed all infusion studies in the right kidney. During each MRBF measurement, heart rate and blood pressure (intra-arterially) were measured continuously. After the renal blood flow measurements, we performed subtraction angiography of the renal arteries. All radiographic films were reviewed by 2 independent and experienced radiologists. No contrast agents were administered before the flow measurements had been completed.

Infusion Studies

Patients were randomly allocated to 1 of the 2 infusion studies:

Study 1: Infusion of Ang-(1–7) (n=45)

We infused Ang-(1–7) into the renal artery in 3 incremental doses (0.27, 0.9, and 2.7 ng/kg per minute, respectively) for 5 minutes each. After each dose, MRBF was measured as described above.

Study 2: Pre-Constriction With Ang II, Combined With Infusion of Ang-(1–7) (n=40)

Ang II was infused into the renal artery at a continuous rate of 0.3 ng/kg per minute. After 5 minutes, MRBF was measured. Thereafter, similar to study 1, we infused 3 incremental doses of Ang-(1–7) (0.27, 0.9, and 2.7 ng/kg per minute) into the renal artery, additionally to the continuous infusion of Ang II. Again, MRBF was measured after each dose of Ang-(1–7).

Doses of Ang-(1–7) were derived from studies using animal models and human forearm vasculature, demonstrating a local vasodilatory response without systemic effects. We conducted a pilot study in 6 patients with the aforementioned doses. As a clear dose–response relationship was found, we used these doses in all study patients.

Analyses

Each of the 2 study groups was divided into 2 subgroups according to their prescribed dietary sodium intake: a high sodium group and a low sodium group (abbreviated as HS and LS in study 1 and as HS-Ang II and LS-Ang II group in study 2). As we aimed to study the effects of Ang-(1–7) in essentially hypertensive patients, patients with atheroembolic renal artery stenosis (defined as a reduction of the luminal diameter of ≥50%) or fibromuscular dysplasia were excluded from the present analyses. Because these patients could only be excluded after the renal angiography (thus after randomization and the flow measurements), study groups in the present analyses are of unequal size. Based on the normal variability in renal blood flow, we considered a change in MRBF by ≥25% to be significant. To demonstrate such a difference at the 5% level with a power of 80%, 28 patients were needed in each group. As mentioned before, patients with renal artery abnormalities could only be excluded after finishing the study protocol; therefore, a larger group of patients was studied to provide sufficient power.

To decrease the influence of intraindividual variation in response to the various doses of Ang-(1–7), we calculated mean and maximal change in MRBF (defined as the patient’s average and, respectively, highest MRBF during infusion of the 3 doses of Ang-(1–7) minus baseline MRBF). For the patients in the second study protocol (pre-constriction by infusion of Ang II), MRBF during Ang II infusion was used as the baseline MRBF in these formulas. Arterial active plasma renin concentrations were measured using an immunoradiometric assay. Estimated glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

For the statistical analyses we used Graphpad (Graphpad Software Inc, version 5.01, San Diego, CA) and SPSS software (SPSS Inc, version 17.0, Chicago, IL). Within-group comparisons (expressed as mean±SEM; all data were distributed normally) were evaluated using paired t test or 1-way repeated-measures ANOVA with Dunnett’s post hoc test. For between-group comparisons we used 1-way ANOVA or Student t test in case of normally distributed data and Kruskal–Wallis test in case of non-normally distributed data (expressed as medians with interquartile ranges). The χ2 test was used in case of categorical data. We applied regression analyses to study the determinants of the change in MRBF during Ang-(1–7) infusion (only in the data of study 1, to avoid distortion of the results by Ang II co-infusion). Correlations were tested using Pearson’s R. A P value of <0.05 was considered statistically significant.

Results

Patient Characteristics

Eighty-five patients were studied, of whom 28 were excluded because of renovascular abnormalities (19 had atherosclerotic renal artery stenosis, 8 had fibromuscular dysplasia, and 1 had a combination of both atherosclerotic renal artery stenosis and fibromuscular dysplasia). Fifty-seven essential hypertensive patients were eligible for inclusion, 32 in study 1 and 25 in study 2. As mentioned before, study groups were of unequal size as patients with renovascular abnormalities could only be excluded after the renal angiography and the flow measurements. Baseline characteristics are listed in Table 1. No statistically significant differences were found between the study groups, except for 24-hour urinary sodium excretion which was, as expected, lower in the low sodium groups (P<0.001; versus HS and HS-Ang II group). The demographic data of the excluded patients did not significantly differ from the total group (Table 1).

Flow Studies

Results of the flow studies are presented in Figure 1 and Table 2. Infusion of Ang-(1–7) resulted in a clear dose–response relationship in the HS group, but not in the LS group (P is not significant [P=NS]; Figure 1). In the second study group, Ang II infusion resulted in a fall in MRBF (P<0.001 versus baseline). Infusion of the various doses of Ang-(1–7) combined with continuous Ang II infusion did not result in a clear dose–effect relationship in the LS-Ang II group (P=NS). In the HS-Ang II group, however, a small but statistically significant change in MRBF during infusion of Ang-(1–7) was found (Figure 1). As shown in Table 2, Ang-(1–7) induced renal vasodilation in all groups because maximal MRBF was higher than baseline in each group (P<0.001). In addition, maximal change in MRBF was significantly higher in the HS group as compared with the LS-Ang II group (group with double Ang II stimulation, P=0.035 for between-group comparison; Table 2). Although maximal MRBF was higher than baseline in each group, a significant change in
mean MRBF was found in the HS and HS-Ang II group only ($P=0.002$ and 0.005, respectively, for mean MRBF versus baseline; Figure 2), and not in the LS and LS-Ang II group (similar to the trends demonstrated in Figure 1). In the second study group, the infused doses of Ang-(1–7) could only partially counteract the Ang II–induced decrease in MRBF ($P<0.001$ versus baseline before Ang II co-infusion). Blood pressure and heart rate did not change during any of the infusions, indicating that no systemic effects of either Ang-(1–7) or Ang II, or the combination of both occurred (data not shown).

Determinants of Ang-(1–7) Effect
Mean change in MRBF during Ang-(1–7) infusion correlated inversely with renin levels (Pearson’s $R=-0.457$; $P=0.010$). A step-wise regression analysis showed that the active plasma renin concentration was the only independent determinant of mean change in MRBF ($\beta=-0.551$; $P=0.010$). No relation was found with age, body mass index, sex, 24-hour urinary sodium excretion, estimated glomerular filtration rate, or 24-hour blood pressure.

Discussion
The present study demonstrates that intrarenal Ang-(1–7) infusion induces renal vasodilation in essentially hypertensive humans. Co-infusion of Ang II, stimulation of RAS by a sodium-restricted diet, or both clearly attenuates this effect.

Previous research on this subject is limited. Animal studies reported an increase in renal blood flow in response to Ang-(1–7) infusion (using doses comparable with this study). This effect is thought to be the result of Mas-receptor (Ang-(1–7)’s target receptor) stimulation, which induces endothelial nitric oxide (NO) release and inhibition of intracellular Ang II/AT1 receptor (type-1 Ang II receptor) signaling, both resulting in vasodilation. Other animal studies using higher doses of Ang-(1–7) reported a minor vasoconstrictor effect.

Table 1. Baseline Characteristics of the Study Patients

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Included Patients</th>
<th>Excluded Patients</th>
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<tbody>
<tr>
<td></td>
<td>High Sodium</td>
<td>Low Sodium</td>
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<tr>
<td>n</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Sex (men/women)</td>
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<td>12/6</td>
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<tr>
<td>Age, y</td>
<td>54.0±2.6</td>
<td>58.0±2.9</td>
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<td>Body mass index, kg/m²</td>
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<tr>
<td>24-h systolic blood pressure, mmHg</td>
<td>166±7</td>
<td>162±5</td>
</tr>
<tr>
<td>24-h diastolic blood pressure, mmHg</td>
<td>96±5</td>
<td>94±3</td>
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<tr>
<td>eGFR, mL/min per 1.73 m²</td>
<td>76±6</td>
<td>81±4</td>
</tr>
<tr>
<td>Urinary sodium excretion, mmol/24 h</td>
<td>204±25</td>
<td>64±10*</td>
</tr>
</tbody>
</table>

Values expressed as mean±SEM. Ang II indicates angiotensin II; eGFR, estimated glomerular filtration rate using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula; HS, high sodium; and LS, low sodium. No statistically significant differences were found between the study groups, except for urinary sodium excretion which was lower in the low sodium groups (*$P<0.001$ for LS vs HS and LS-Ang II vs HS-Ang II).

Figure 1. Mean renal blood flow (MRBF) for incremental doses of intrarenal angiotensin-(1–7) (Ang-(1–7)) infusion. Data presented as mean and SEM and divided into 4 groups according to sodium intake and presence or absence of continuous angiotensin II (Ang II) infusion (0.3 ng/kg per minute). $P<0.05$ vs baseline (repeated-measures ANOVA with Dunnett’s post hoc test).
probably as a result of weak AT$_1$ receptor stimulation by high doses of Ang-(1–7).$^{12}$ Human data on Ang-(1–7) flow effects are limited to studies in the forearm. Results of those studies are not uniform. This may be caused by a dose–response effect with an increased in blood flow with low/norrmal doses of Ang-(1–7),$^\dagger$ but a decrease in blood flow higher doses.$^{13}$

In the present study, the Ang-(1–7)–induced flow response was clearly affected by RAS activation after sodium restriction. Whereas the Ang-(1–7)–induced vasodilatory effect was only marginally significant in the sodium-restricted groups (a significant response was found after selection of the maximal change in MRBF), but no significant effect was found for the mean change in MRBF), a clear dose–response effect of Ang-(1–7) was found in patients with a sodium-rich diet (suppressing endogenous RAS activity). In combination with the regression analysis which demonstrated that renin was the only independent determinant of Ang-(1–7)–induced vasodilation, these findings support the previously suggested hypothesis$^{14}$ that RAS activation (because of low sodium intake) results in a diminished effect of Ang-(1–7). These findings are in agreement with in vitro studies using rat aortas that showed that Ang-(1–7) induces vasodilation in rats with normal or high sodium diet, but not in sodium-depleted rats.$^{15}$ Three possible mechanisms could explain this phenomenon: first, a low sodium state results in high endogenous Ang-(1–7) levels as RAS is activated.$^{16,17}$ Possibly, the Ang-(1–7)–induced vasodilatory effect is already on its maximum, so that additional Ang-(1–7) infusion would hardly be able to produce an additional effect. Previous research showing that blocking of Ang-(1–7) only exerts a hemodynamic effect in sodium-depleted rats but not in sodium-repleted rats supports this hypothesis.$^{18,19}$ Second, a low sodium state may decrease Ang-(1–7) effects by Mas-receptor desensitization, as continuous stimulation by high Ang-(1–7) levels leads to Mas-receptor internalization.$^{20}$ Third, an activated RAS leads to higher levels of several vasoconstrictor peptides, such as Ang II.$^{16,17}$, which may reverse the Ang-(1–7) effect. Presumably, each of the proposed mechanisms contributes to this phenomenon to some extent.

The present study also shows that the Ang-(1–7)–induced flow response was diminished in patients with Ang II infusion compared with those without, indicating that Ang II decreases the effects of Ang-(1–7). Conversely, we also demonstrated that Ang-(1–7) is able to at least partially counteract the vasoconstrictor effect of Ang II in human renal circulation, as MRBF during combined infusion of Ang-(1–7) and Ang II was higher than with infusion of Ang II alone (HS-Ang II group). This finding is in line with previous studies in human forearm and rat renal arteries.$^{2,13}$ The combination of both exogenous Ang II infusion and endogenous RAS activation (a double Ang II stimulation in the LS-Ang II group) almost completely blocked the Ang-(1–7)–induced increase in renal blood flow as compared with the effect in patients without any Ang II/RAS stimulation (HS group without Ang II co-infusion). Again, one can discuss the pathophysiological mechanisms involved: either diminished vasodilatory effects of Ang-(1–7) or increased vasoconstriction mediated by Ang II (see above). Further research is required to unravel the exact pathway. Nevertheless, we may conclude that in the human hypertensive kidney there is a delicate balance between vasoconstrictor peptides, such as Ang II, on one side and vasodilator peptides, such as Ang-(1–7), on the other. This balance seems to be influenced by dietary sodium intake.

The present study is limited by the fact that we were not able to perform a crossover study (ie, studying the same patient during both a high and a low sodium diet), as this would require a second invasive procedure with additional risks for the patients. Therefore, we do not have information on the individual changes in response to the shift in sodium intake. Also, because of the relatively small number of included patients we cannot definitively rule out the possibility that the outcomes were influenced by small (not statistically significant) differences in patient characteristics, such as age and estimated glomerular filtration rate. However, the fact that no
correlations were found between the outcome and any of these characteristics reduces the risk of such bias. Furthermore, this study was performed in a highly selected population of difficult-to-treat hypertensive subjects who were off medication, which limits extrapolation of the results to the general hypertensive population.

Perspectives
The present study demonstrates that intrarenal infusion of exogenous Ang-(1–7) decreases renal vasodilation and partially counteracts Ang II–induced vasoconstriction in hypertensive humans. Both endogenous (low dietary sodium intake) and exogenous (Ang II co-infusion) RAS stimulation clearly attenuate Ang-(1–7)–induced vasodilation, indicating that the effects of Ang-(1–7) depend on the degree of RAS activation. The vasodilatory effects of Ang-(1–7) could provide an interesting pathway for new pharmacological interventions in hypertension and the prevention of chronic kidney disease.

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We thank Dr W. van Zwam for reviewing the radiographic films and M. Fuss-Lejeune for collecting and handling the blood samples.

Disclosures
None.

References

Novelty and Significance

What Is New?
- The present study is the first one to assess the influence of angiotensin-(1–7) on renal blood flow in hypertensive humans.

What Is Relevant?
- Angiotensin-(1–7) induces vasodilation in the hypertensive kidney.
- Stimulation of the renin–angiotensin system (by a low sodium diet or co-infusion) attenuates angiotensin-(1–7)–induced vasodilation.

Summary
Intrarenal infusion of angiotensin-(1–7) induces renal vasodilation in hypertensive humans, but this effect is attenuated by both endogenous (low sodium intake) and exogenous (angiotensin II co-infusion) renin–angiotensin system stimulation.
Angiotensin-(1–7)–Induced Renal Vasodilation in Hypertensive Humans Is Attenuated by Low Sodium Intake and Angiotensin II Co-Infusion

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