Functional Importance of Angiotensin-Converting Enzyme–Dependent In Situ Angiotensin II Generation in the Human Forearm
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Functional Importance of Angiotensin-Converting Enzyme–Dependent In Situ Angiotensin II Generation in the Human Forearm


Abstract—To assess the importance for vasoconstriction of in situ angiotensin (Ang) II generation, as opposed to Ang II delivery via the circulation, we determined forearm vasoconstriction in response to Ang I (0.1 to 10 ng · kg⁻¹ · min⁻¹) and Ang II (0.1 to 5 ng · kg⁻¹ · min⁻¹) in 14 normotensive male volunteers (age 18 to 67 years). Changes in forearm blood flow (FBF) were registered with venous occlusion plethysmography. Arterial and venous blood samples were collected under steady-state conditions to quantify forearm fractional Ang I-to-II conversion. Ang I and II exerted the same maximal effect (mean ± SEM 71 ± 4% and 75 ± 4% decrease in FBF, respectively), with similar potencies (mean EC₅₀ [range] 5.6 [0.30 to 12.0] nmol/L for Ang I and 3.6 [0.37 to 7.1] nmol/L for Ang II). Forearm fractional Ang I-to-II conversion was 36% (range 18% to 57%). The angiotensin-converting enzyme (ACE) inhibitor enalaprilat (80 ng · kg⁻¹ · min⁻¹) inhibited the contractile effects of Ang I and reduced fractional conversion to 1% (0.1% to 8%), thereby excluding a role for Ang I-to-II converting enzymes other than ACE (eg, chymase). The Ang II type 1 receptor antagonist losartan (3 mg · kg⁻¹ · min⁻¹) inhibited the vasoconstrictor effects of Ang II. In conclusion, the similar potencies of Ang I and II in the forearm, combined with the fact that only one third of arterially delivered Ang I is converted to Ang II, suggest that in situ–generated Ang II is more important for vasoconstriction than circulating Ang II. Local Ang II generation in the forearm depends on ACE exclusively and results in vasoconstriction via Ang II type 1 receptors. (Hypertension. 2000;35:764-768.)

Key Words: angiotensin ■ angiotensin-converting enzyme inhibitors ■ receptors, angiotensin II ■ blood flow

Circulating angiotensin (Ang) I is converted to Ang II in many vascular beds.¹⁻⁵ The functional importance of this locally generated Ang II compared with arterially delivered Ang II is currently unknown. Organ bath experiments in which the contractile responses of isolated human or porcine coronary arteries were recorded have shown that Ang I and Ang II display similar vasoconstrictor potencies,⁶⁻⁷ despite the fact that the levels of Ang II in the bath fluid during exposure to Ang I are <1% of those during exposure to Ang II.⁶ These in vitro experiments therefore suggest that locally generated rather than circulating Ang II mediates vasoconstriction. A study of the local generation of Ang II and its vasoconstrictor effects in perfused rat hindquarters, in which the venous Ang II levels after the infusion of renin were compared with those after the infusion of Ang I or II, also indicated that vasoconstriction was caused by local Ang II rather than by Ang II in the perfusion buffer.⁸

Two enzymes have been reported to contribute to Ang I-to-II conversion: ACE and chymase. ACE is present both in circulating blood plasma and on the membrane of vascular endothelial cells, whereas chymase is located in the adventitia, in the cytosol of mast cells.⁹¹⁰ Although the results of in vitro studies in isolated human vessels⁶⁷ and tissue homogenates¹¹¹² support the contribution of chymase to Ang I-to-II conversion, in vivo studies do not support this view, because ACE inhibition suppressed Ang I-to-II conversion in the human and porcine coronary vascular beds by >90%.³¹³ However, coronary Ang I-to-II conversion in these latter studies was quantified with systemic or intracoronary infusions of¹²⁵I-labeled Ang I, an approach that does not allow the detection of Ang II generation with chymase in the adventitia if such generation does not result in Ang II overflow into the blood compartment. Moreover, contractile effects were not quantified in these studies.

It was the aim of the present study to compare the in vivo potencies of Ang I and II to assess the functional importance of locally generated Ang II. Ang I and Ang II were infused into the brachial artery, and forearm vasoconstriction was recorded under steady state conditions. Forearm Ang I-to-II conversion was quantified with measurement of the venous Ang I and II levels at steady state. Infusions were made in the presence and absence of the ACE inhibitor enalaprilat and the...
Ang II type 1 (AT₁) receptor antagonist losartan to investigate (1) whether enzymes other than ACE contribute to the local generation of Ang II and (2) whether Ang II mediates vascular effects through receptors other than the AT₁ receptor.

Methods

Subjects

Fourteen white male volunteers (mean age 39 years, range 18 to 67 years; mean weight 83 kg, range 64 to 107 kg) were recruited via advertisement after the Medical Ethics Committee of the Leiden University Medical Center approved the protocol of the study. All participants gave their informed consent. Medical history, physical examination, and routine laboratory tests did not reveal any abnormalities. All subjects were on a normal-sodium diet (180 mmol/d), and none of them received medication. Subjects did not smoke, and they refrained from the consumption of alcohol or caffeine-containing substances for ≥12 hours before the experiment.

Experimental Set-Up

Each experiment was performed with the subject in the supine position in a quiet room at a constant temperature of 22° to 24°C. Forearm and hand volumes were measured with water displacement. One-lead ECG was monitored continuously. After local anesthesia with 1% lidocaine, the brachial artery of the nondominant arm was cannulated. The cannula (1.0×45 mm) was connected to a Statham P23Id pressure transducer ( Gould Inc). Drugs were infused into the brachial artery with Harvard Apparatus volumetric precision pumps (model 22). Both forearms were instrumented with mercury-in-Silastic strain gauges, which were connected to a Hokanson EC-2 plethysmograph. Both upper arms were connected to a Hokanson E-10 rapid cuff inflator. For the measurement of forearm blood flow (FBF), R wave–triggered cuff inflation (at 40 mm Hg) for venous occlusion plethysmography was controlled with a personal computer (model DT 2801; Data Translation Inc).

Study Protocol

The infusion studies were started ≥60 minutes after the cannulation of the brachial artery. Between the various infusion experiments, the wrist cuffs were deflated, and sufficient time (minimum of 45 minutes) was taken to allow the subjects to recover from hand ischemia and to allow FBF to return to baseline levels. The protocol is summarized in Figure 1. Baseline arterial and venous blood samples were taken before the start of the infusions. Steady-state venous blood samples were obtained at the end of each Ang infusion. Sodium nitroprusside was used to predilate the vascular bed of the forearm to ≈5 mL × 100 mL⁻¹ · min⁻¹ because measurements of vasoconstrictor effects are more accurate when flow levels remain at >1 mL × 100 mL⁻¹ · min⁻¹.16

Blood Sampling

Blood for Ang measurements was rapidly drawn with a plastic syringe containing the following inhibitors (0.25 mL inhibitor solution in 5 mL blood): 6.25 mmol/L disodium EDTA, 1.25 mmol/L 1.10-phenanthroline, and 0.01 mmol/L concentration of the renin inhibitor remikiren (final concentrations in blood). The blood was transferred into prechilled polystyrene tubes and centrifuged at 3000 g for 10 minutes at 4°C. Plasma was stored at −70°C.

Measurement of Ang I and II

Baseline arterial and venous Ang I and II concentrations were measured with radioimmunoassay, after SepPak extraction and high-performance liquid chromatographic separation, as described previously.2,3 The high Ang concentrations in the venous samples collected under steady state conditions at the end of each infusion were measured without prior high-performance liquid chromatographic separation.6

Data Analysis

Data were normally distributed and are expressed as mean±SEM. The Ang-induced effects are expressed as percentage change in FBF of the infused forearm. The percentage change was calculated relative to the values measured at baseline (ie, at the beginning of infusion 3) (Figure 1). The steady state arterial Ang plasma concentrations (in pmol/L) during the infusions were calculated as follows: [Ang]_arterial, steady state = [IR] × [BW] × 10⁷/[((1−Ht) × FBF × FAV × MW]) + [Ang]_arterial, baseline, where IR is Ang I or II infusion rate (in ng · kg⁻¹ · min⁻¹), BW is body weight (in kg), Ht is hematocrit, FAV is forearm volume, MW is molecular weight of Ang I or II, and [Ang]_arterial, baseline is arterial Ang I or II concentration at baseline.

Fractional conversion and degradation of Ang I (ie, the percentage of arterially delivered Ang I that is converted to Ang II or degraded into other metabolites) and fractional degradation of Ang II (ie, the
Software). 15, 17

4-parameter logistic regression analysis (InPlot 2.0; GraphPAD) of plasma concentrations and the corresponding FBF values with

of the maximal effect is achieved) were calculated from the arterial experiments.

significant.

Baseline arterial Ang I and II levels (6.9 ± 6 and 10.8 ± 2.3 fmol/mL, respectively) were comparable to baseline venous Ang I and II levels (10.8 ± 2.3 and 3.0 ± 0.2 fmol/mL, respectively). Baseline Ang levels were unrelated to age or weight.

Discussion
The results of the present study provide in vivo evidence in humans that in situ–generated Ang II is more important for vasoconstriction than circulating Ang II. This conclusion is based on 2 findings. First, Ang I and Ang II induced forearm vasoconstriction with similar potencies, despite the fact that only one third of Ang I was converted to Ang II in the forearm circulation. Second, the venous Ang II levels at the highest Ang I infusion rate in the presence of enalaprilat were

FBF did not change in the noninfused control arm during the infusions, nor did the Ang infusions affect heart rate and blood pressure (data not shown).

Ang I and II reduced FBF by a maximum of 71 ± 4% and 75 ± 4%, respectively, with comparable potencies (EC50 5.6 ± 1.0 and 3.6 ± 0.5 nmol/L, respectively; p = NS) (Figure 2). Enalaprilat virtually completely blocked the constrictor effects of Ang I. Losartan blocked the vasoconstrictor effects of Ang II; in fact, a tendency for a vasodilator effect (p = NS) was observed at the 2 lowest doses of Ang II in the presence of this drug.

Fractional Ang I conversion was similar at all Ang I doses and was reduced to very low values in the presence of enalaprilat (Table). Fractional Ang I and II degradations were higher at the high doses than at the low doses of these peptides, most likely because of the reduced FBF at these high doses. In support of this assumption, FBF correlated negatively with fractional Ang I and II degradation (fractional Ang I degradation = −0.06 × FBF+0.69 [r = 0.56, p < 0.05] and fractional Ang II degradation = −0.06 × FBF+0.97 [r = 0.76, p < 0.01]). The relationship between FBF and Ang degradation was unaltered with enalaprilat and losartan (data not shown).

Venous Angiotensin Levels, Fractional Ang I Conversion, and Fractional Ang I and II Degradation During Infusion of Ang I or II With or Without Concomitant Infusion of ACE Inhibitor Enalaprilat (80 ng · kg−1 · min−1) or AT1 Receptor Antagonist Losartan (3000 ng · kg−1 · min−1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infusion Rate, ng · kg−1 · min−1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ang I</td>
</tr>
<tr>
<td>Ang I, pmol/L</td>
<td>0.1 1.0 10</td>
</tr>
<tr>
<td>43±6</td>
<td>304±56 1691±334</td>
</tr>
<tr>
<td>Ang II, pmol/L</td>
<td>70±5 544±96 5307±1037</td>
</tr>
<tr>
<td>Fractional Ang I conversion, %</td>
<td>41±3 32±3 34±2</td>
</tr>
<tr>
<td>Fractional Ang I degradation, %</td>
<td>48±2 62±2* 64±2*</td>
</tr>
<tr>
<td>Ang II, pmol/L</td>
<td>0.1 0.5 5.0</td>
</tr>
<tr>
<td>97±10</td>
<td>458±66 4283±627</td>
</tr>
<tr>
<td>Fractional Ang II degradation, %</td>
<td>64±4 78±3* 88±3*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (n = 14).

*P < 0.01 vs lowest infusion rate.

†P < 0.001 vs without enalaprilat.
Ang I infusion rate without enalaprilat, yet vasoconstriction was calculated during the infusion of 125 I-labeled Ang I,\(^{2} \) which will result in chymase concentrations in vitro that are far above those in vivo.\(^{18,19} \) This latter finding is not surprising, because at lower flow rates and (2) conversion most likely occurred at steady state were too low to induce vasoconstriction. Remarkably, despite the clear dose-dependent vasoconstriction that occurred in the present study, fractional forearm Ang I-to-II conversion remained constant at all FBF values. In contrast, fractional forearm Ang I and II degradation correlated inversely with FBF. This latter finding is not surprising, because at lower flow rates, more time is available for metabolism. The fact that Ang I-to-II conversion was not related to FBF suggests that (1) it is a highly efficient process with a maximal result even at high flow rates and (2) conversion most likely precedes degradation (ie, that ACE might be located predominantly in the arterioles).

In the present study, losartan, a competitive AT\(_{1}\) receptor antagonist,\(^{29} \) fully prevented vasoconstriction at the 2 lowest Ang II doses and in large part (>70%) inhibited vasoconstriction at the highest dose of Ang II. These data are in agreement with the contention that Ang II induces vasoconstriction in the human forearm through the activation of AT\(_{1}\) receptors.

References


