Indirect Evidence for Vascular Uptake of Circulating Renin in Hypertensive Patients

Stefano Taddei, Agostino Virdis, Basem Abdel-Haq, Roberto Giovannetti, Piero Duranti, Anna Maria Arena, Stefania Favilla, and Antonio Savietti

To evaluate whether, in the forearm of hypertensive patients with different circulating renin profiles, local β-adrenergic receptor-induced production of active renin, plasma renin activity, angiotensin I (Ang I), and angiotensin II (Ang II) was or was not related to the renin profile, we studied four groups of patients: 1) hypertensive patients with primary aldosteronism and suppressed circulating plasma renin activity values (0.15±0.1 ng Ang I/mL per hour; n=7), 2) essential hypertensive patients with low (0.47±0.1 ng Ang I/mL per hour; n=8) circulating plasma renin activity values, 3) essential hypertensive patients with normal (2.48±0.52 ng Ang I/mL per hour; n=8) circulating plasma renin activity values, and 4) renovascular hypertensive patients with high circulating plasma renin activity values (4.16±2.1 ng Ang I/mL per hour; n=10). Isoproterenol was infused into the brachial artery, and active renin, plasma renin activity, and Ang I and Ang II forearm balance (venous-arterial differences corrected for forearm blood flow by strain-gauge plethysmography) were measured. Despite a comparable vasodilation, β-adrenergic stimulation failed to release active renin, plasma renin activity, and Ang I and Ang II in primary aldosteronism. It slightly increased them (except for Ang I) in low renin patients but determined a local production in normal renin and renovascular hypertensive patients. The individual increments in plasma renin activity and Ang II release induced by isoproterenol showed a correlation with the renin profile. In another group of essential hypertensive patients (n=6), isoproterenol was infused for 60 minutes, and we observed that despite a stable forearm vasodilation, both plasma renin activity and Ang II reached maximum values between 5 and 10 minutes; after that, they immediately started to decline and returned to basal levels. These data suggest the possibility that in hypertensive patients, vascular tissue renin originates from plasma uptake. (Hypertension 1993;21:852-860)

KEY WORDS • renin • angiotensin I • angiotensin II • isoproterenol • receptors, adrenergic, beta

Compelling experimental evidence indicates that renin is present in the vascular wall and that vessels can synthesize and release angiotensin II (Ang II) as the expression of a tissue-vascular renin-angiotensin system (RAS).1-2 Recently, the existence of a vascular RAS was proposed also in humans, because it has been demonstrated that local β-adrenergic receptor stimulation through isoproterenol causes the release of active and inactive renin and Ang II in the forearm vessels of essential hypertensive patients.3 It is still debated whether vascular tissue renin, the enzyme that determines Ang II generation, is locally synthesized or taken up from plasma.1-2 To evaluate this issue, we studied the effect of β-adrenergic receptor stimulation on the vascular RAS in hypertensive patients with different levels of circulating plasma renin activity (PRA). Our working hypothesis was that, if forearm vascular renin derives from plasma renin uptake, the local production of PRA and Ang II should correlate with the circulating renin levels.

Methods

Subjects

Thirty-three inpatients (Table 1) with primary or secondary forms of hypertension who had already been screened in a previous admission to our unit were recruited for the present study. Patients were divided into four groups according to their circulating renin profile: 1) Hypertensive patients with primary aldosteronism (n=7; ALDO) and suppressed levels of circulating renin. 2) Essential hypertensive patients (n=8) classified as low renin (LREH) (Table 1) according to their renin-sodium index. 3) Essential hypertensive patients (n=8) classified as normal renin (NREH) (Table 1) according to their renin-sodium index. 4) Renovascular hypertensive patients (RVH; n=10) with high levels of circulating renin (Table 1). The diagnosis was based on the presence of hypertension, increased production of aldosterone despite low renin levels, and the existence of renal adenoma by computed tomographic scan. After the study, five of these patients underwent surgery, and the presence of an aldosterone-producing adenoma was confirmed by histological examination. 2) Essential hypertensive patients (n=8) classified as low renin (LREH) (Table 1) according to their renin-sodium index. 3) Essential hypertensive patients (n=8) classified as normal renin (NREH) (Table 1) according to their renin-sodium index. 4) Renovascular hypertensive patients (RVH; n=10) with high levels of circulating renin (PRA) (Table 1). The diagnosis was based on the arteriographic demonstration of a significant renal artery stenosis and the suppression of renin secretion in the

From I Clinica Medica, University of Pisa (Italy).
Address for correspondence: Stefano Taddei, MD, I Clinica Medica, University of Pisa, Via Roma, 67, 56100 Pisa, Italy.
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nonstenotic kidney, which was confirmed in eight patients by the normalization of blood pressure after successful revascularization.

As shown in Table 1, the four groups of hypertensive patients were matched for age, body weight, blood pressure, and other clinical variables. According to institutional guidelines, all patients were aware of the investigational nature of the study and consented to it. Patients discontinued any treatment for at least 3 weeks before admittance to the clinic. In this period, they were seen once a week so that blood pressure values could be checked, as well as plasma potassium in the ALDO group. Once admitted to the ward, they were maintained on a normocaloric diet with constant sodium intake so that a constant sodium excretion rate could be obtained; usually, it was reached at the fifth day of hospitalization. When the state of sodium equilibrium was reached, peripheral venous samples for PRA measurement were obtained after patients had been standing for 1 hour. The circulating renin profile was defined as the relation between circulating PRA and 24-hour urinary sodium excretion.

**Experimental Procedure**

All studies were performed at 8 AM after an overnight fast with patients lying supine in a quiet air-conditioned room (22°–24°C). A polyethylene cannula (21 gauge, Abbot, Sligo, Ireland) was inserted into the brachial artery with patients under local anesthesia (2% lidocaine) and was connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, Colo.) for monitoring of systemic mean blood pressure (½ pulse pressure+diastolic pressure) and heart rate (model VSM1, Physiocontrol, Redmond, Wash.) and for intra-arterial infusions. Another cannula (6 cm long) was advanced into an ipsilateral deep forearm vein retrogradely. Forearm blood flow (FBF) was measured in both forearms (experimental and contralateral) by strain-gauge venous plethysmography (LOOSCO, GL LOOS, Amsterdam, The Netherlands). Arterial and deep venous blood were sampled simultaneously at timed intervals. Circulation to the hand was excluded 1 minute before each sampling or FBF measurement by inflating a pediatric cuff around the wrist at suprasystolic blood pressure. Details concerning the sensitivity and reproducibility of the method as performed in our laboratory have already been published.

Drug infusion rates were normalized for 1 dL of tissue by adjusting the speed of infusion to the desired infusion rates. Drugs were infused at systemically ineffective rates through separate ports via three-way stopcocks.

**Experimental Design**

Local vascular renin-angiotensin system activation and circulating renin profile. After an equilibrium period of 20 minutes in the presence of saline administration (0.206 mL/min), patients were given intrabrachial isoproterenol at cumulative increasing doses (0.03, 0.1, and 0.3 µg per 100 mL forearm tissue per minute, 5 minutes each) as already described while arteriovenous samples for active renin, PRA, angiotensin I (Ang I), and Ang II were collected basally and at the end of each infusion period (fifth minute for each isoproterenol dose and 20th minute for saline).

Time course of the β-adrenergic receptor-mediated effect on vascular renin-angiotensin system. This experiment was designed to evaluate the time course of β-adrenergic receptor–induced release of PRA and Ang II. In another group of essential hypertensive patients (n=6; four men; mean age, 44.5±7.3 years; blood pressure, 168.4±9.3/104.8±5.4 mm Hg), after a 20-minute period of equilibration with saline (0.206 mL/min), intrabrachial isoproterenol was infused at the rate of 0.03 µg per 100 mL forearm tissue per minute for 60 minutes. Arteriovenous samples for PRA and Ang II were collected basally, during saline, and at the fifth, 10th, 20th, 30th, 40th, 50th, and 60th minutes of isoproterenol infusion.

**Exogenous angiotensin II–mediated vasoconstriction.** To investigate the role of local Ang II in affecting vascular tone, in another group of essential hypertensive patients (n=5; three men; mean age, 48.3±8.4 years; blood pressure, 171.3±11.2/107.5±8.4 mm Hg), we evaluated the vascular effect of a dose–response curve to exogenous Ang II infused into the brachial artery at cumulative increasing doses (0.003, 0.009, and 0.03 µg per 100 mL forearm tissue per minute, 5 minutes each).

### Table 1. Characteristics of the Study Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>ALDO (n=7)</th>
<th>LREH (n=8)</th>
<th>NREH (n=8)</th>
<th>RVH (n=10)</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>45.8±9.5</td>
<td>43.6±6.4</td>
<td>44.8±7.3</td>
<td>46.3±9.6</td>
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<tr>
<td>Sex (male/female)</td>
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<td>5/3</td>
<td>5/3</td>
<td>7/3</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>121.8±12.1</td>
<td>118.6±9.4</td>
<td>119.4±9.1</td>
<td>121.8±9.4</td>
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<tr>
<td>HR (bpm)</td>
<td>72.1±7.4</td>
<td>68.3±7.8</td>
<td>70.5±8.4</td>
<td>73.8±9.2</td>
</tr>
<tr>
<td>FBF (mL/100 mL/min)</td>
<td>4.1±0.9</td>
<td>3.4±0.9</td>
<td>3.8±0.9</td>
<td>3.7±0.9</td>
</tr>
<tr>
<td>PRA ([ng Ang I/mL]/hr)</td>
<td>0.15±0.1*</td>
<td>0.47±0.1*</td>
<td>2.48±0.5*</td>
<td>4.16±2.1*</td>
</tr>
<tr>
<td>Ang II (pg/mL)</td>
<td>6.3±1.4*</td>
<td>13.6±1.7*</td>
<td>32.6±5.1*</td>
<td>39.4±6.4*</td>
</tr>
<tr>
<td>UNa (mEq/24 hr)</td>
<td>94.2±7.8</td>
<td>89.6±8.6</td>
<td>98.3±9.2</td>
<td>91.3±8.8</td>
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<tr>
<td>Forearm volume (mL)</td>
<td>1,104±123</td>
<td>995.8±117</td>
<td>924.1±105</td>
<td>1,053±113</td>
</tr>
</tbody>
</table>

ALDO, primary hyperaldosteronism patients; LREH, low renin essential hypertensive patients; NREH, normal renin essential hypertensive patients; RVH, renovascular essential hypertensive patients; MBP, mean blood pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II; UNa, urinary sodium excretion. Values are mean±SEM.

*p<0.05.
These rates were chosen on the basis of a previous experiment in which exogenous Ang II, intra-arterially infused at the dose of 0.001 µg per 100 mL forearm tissue per minute (which caused an increment in forearm plasma venous Ang II from 9.7±3.1 to 19.8±4.8 pg/mL), did not affect FBF but increased the vasoconstrictor effect and norepinephrine release induced by an endogenous sympathetic nervous system activation.3

Analytical Procedures

Active renin (picograms per milliliter) was measured by radioimmunometric assay using a kit from IRMA Pasteur (ERIA Diagnostics, Pasteur, Marnes La Coquette, France). In our laboratory, the intra-assay and interassay variation coefficients were 9.69% (n=20) and 14.5% (n=6), respectively.

PRA (nanograms Ang I per milliliter per hour) was measured by radioimmunooassay after plasma had been incubated at pH 5.7 for 1.5 hours. Plasma Ang I (nanograms per milliliter) was measured by the same radioimmunooassay and calculated from the unincubated plasma (blank values). Plasma Ang II (picograms per milliliter) was determined by radioimmunooassay after extraction of the peptide from plasma with a Sep-Pak C18 column.8

In one representative experiment with the intrabrachial isoproterenol dose–response curve, arteriovenous values of Ang II were measured by radioimmunooassay after reversed-phase high performance liquid chromatographic (HPLC) separation modified as follows: We used a liquid chromatograph (model 486 with a tunable absorbance detector, Waters Chromatography Division, Millipore Corp., Milford, Mass.) with a model 7125 manual injection valve (Rheodyne Inc., Berkeley, Calif.). A nucleosil C18 steel column (200x4 mm and 5-µm particle size) (Macherey-Nagel, Duren, FRG) served as the stationary phase. An isocratic mixture of methanol/0.085% phosphoric acid (33.5:66.5) was used as the mobile phase, with a flow rate of 1 mL/min. One hundred microliters of 140 µL extracted solution in water/mobile phase (50:950) of 2 mL plasma was analyzed by HPLC. Fractions (500 µL) of the peptide were collected and dried in a vacuum centrifuge (Hetovac and Hetotrap CT60, Heto Lab Equipment, A/S, Birkerod, Denmark). Samples were redissovled in Tris buffer and subjected to radioimmunoassay as described above. Recoveries were calculated for each measurement (n=10) by addition of 200 pg/mL Ang II (Peninsula Laboratories, Inc., San Carlos, Calif.) to the plasma pool and by measurement of the corresponding peptide concentration while endogenous levels of immunoreactive Ang II were subtracted. The mean value of recovery was 78.4±9.2%. Each measured value of Ang II was corrected for its own recovery. The solvent blank, 100 µL water/mobile phase mixture, was extracted, analyzed by HPLC and subsequent radioimmunooassay, and resulted in a detection limit below that of the radioimmunoassay.

Data Analysis

Net forearm balance of active renin (picograms per minute), PRA (nanograms Ang I per milliliter per hour per minute), Ang I (nanograms per minute), and Ang II (picograms per minute) was obtained as the product of

the venous-arterial plasma concentration gradient and forearm plasma flow [FBF×1-(hematocrit)].10

Because arterial pressure did not significantly change during the study, all data were analyzed in terms of FBF, and FBF increments or decrements were taken as evidence of local vasodilation and vasoconstriction, respectively. Raw data were analyzed by two- or three-way analysis of variance, and Duncan's test was applied for multiple-comparison testing. Correlation coefficients were calculated using Pearson's test. Results are expressed as mean±SEM.

Drugs

Isoproterenol HCl (Isuprel, Winthrop-Breon, N.Y.), sodium nitroprusside (Malesci, Milan, Italy), and Ang II (Hypertensina Ciba,Origgio [VA], Italy) were obtained from commercially available sources and diluted in fresh solutions to the desired concentrations by addition of normal saline. Sodium nitroprusside was dissolved in glucosate solution and protected from light by aluminum foil.

Results

Local Vascular Renin-Angiotensin System Activation and Circulating Renin Profile

At the time of the study, the four groups had statistically different circulating PRA values (Table 1), despite a similar urinary sodium excretion. As expected, circulating PRA values were suppressed in the ALDO group and were highest in the RVH group.

Forearm Blood Flow

Isoproterenol caused a dose-dependent increment in FBF that was not statistically different in the four patient groups (ALDO: from 4.1±0.6 to 7.8±2.3, 11.9±3.5, and 17.5±6.7; LREH: from 3.4±0.9 to 6.7±2.4, 12.8±4.4, and 16.4±6.5; NREH: from 3.8±0.9 to 7.2±2.0, 10.7±3.6, and 17.8±5.1; RVH: from 3.7±0.9 to 7.5±2.6, 11.5±2.9, and 17.2±5.2 mL per 100 cm² tissue per minute). Drug infusion did not affect contralateral FBF.

Active Renin Release

In all patients, isoproterenol administration did not change arteriovenous values; it failed to increase venous active renin in the ALDO group, but increased venous active renin in the other groups of patients studied (Figure 1). If data are expressed as forearm net balance, it is evident that, despite a comparable vasodilating effect, β-adrenergic receptor stimulation failed to cause a slight active renin release in the LREH group (from −1.5±0.4 to 4.2±0.6, 4.3±0.7, and 8.2±1.4 pg/min) and caused a slight renin secretion in the LREH group only at the highest dose (p<0.05 versus basal and versus ALDO, from 0.7±0.02 to 0.8±0.3, 25.2±5.3, and 43.7±8.4 pg/min); however, there was a significant (p<0.01 versus basal and versus LREH and ALDO) dose-dependent renin secretion in NREH patients (from −8.8±0.2 to 12.6±2.1, 32.5±4.8, and 67.3±8.4 pg/min) and RVH patients (from 0.5±0.1 to 38.8±4.3, 52.1±7.3, and 87.8±9.4 pg/min; p<0.001 versus basal and versus the other groups).

Plasma Renin Activity Release

In the present study, we found a strict correlation between PRA and active renin values (r=0.95, p<0.001).
Thus, parallel to active renin, PRA net balance under β-adrenergic receptor stimulation was not affected in the ALDO group (from 0.04±0.02 to -0.12±0.3, -0.07±0.03, and -0.47±0.12 ng Ang I/mL per hour per minute) (Figure 2), was slightly increased in LREH patients (from 0.04±0.02 to 0.05±0.02, 0.04±0.02, and 0.9±0.3 ng Ang I/mL per hour per minute; p<0.05 versus basal and versus ALDO at the highest dose), and was greatly augmented in NREH patients (from -0.07±0.03 to 0.37±0.1, 1.19±0.2, and 2.7±0.6 ng Ang I/mL per hour per minute; p<0.01 versus basal and versus LREH and ALDO) and RVH patients (from 0.1±0.04 to 0.9±0.25, 3.4±1.12, and 7.12±1.9 ng Ang I/mL per hour per minute; p<0.001 versus basal and versus the other groups). PRA concentration in artery and vein are shown in Figure 2.

**Angiotensin I Release**

β-Stimulation did not affect Ang I net balance in the ALDO group (from -0.02±0.02 to 0.09±0.1, 0.14±0.08, and -0.31±0.14 ng/min) and LREH patients (from -0.04±0.03 to -0.04±0.03, 0.15±0.08, and 0.1±0.1 ng/min). It caused a significant (p<0.01) Ang I secretion in NREH patients only at the highest dose (from 0.14±0.06 to 0.13±0.1, 0.38±0.1, and 4.3±1.6 ng/min), and an evident dose-dependent release in RVH patients (from 0.09±0.04 to 1.62±0.45, 2.62±1.23, and 5.26±2.3 ng/min; p<0.001 versus basal and versus the other groups). Arterial and venous concentrations of Ang I are reported in Table 2.

**Angiotensin II Release**

In the four groups of patients, basal arterial Ang II values were significantly higher than venous values in NREH and RVH patients. Isoproterenol infusion did not modify arterial Ang II, whereas it was increased dose dependently in LREH, NREH, and RVH patients, but not in the ALDO group (Figure 3). The calculated net balance shows that β-stimulation did not release Ang II in the ALDO group (from -0.6±0.2 to 3.5±0.9, 4.8±1.4, and 5.5±1.2 pg/min) but caused a dose-dependent release of the peptide in the other three groups that was lowest in LREH patients (from -3.1±1.5 to 11.9±4.6, 19.3±7.5, and 29.3±10.9 pg/min; p<0.05 versus basal and versus ALDO at the second and third doses), intermediate in NREH patients (from -8.6±3.0 to 16.7±5.3, 44.5±9.1,
TABLE 2. Arterial and Venous Concentrations of Angiotensin I During Increasing Doses of Isoproterenol in Study Patients

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Saline</th>
<th>Isoproterenol [(μg/100 mL)/min]</th>
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<th>0.1</th>
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<td>ALDO (n=7)</td>
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<tr>
<td>Artery</td>
<td>0.05±0.01</td>
<td>0.04±0.02</td>
<td>0.04±0.02</td>
<td>0.03±0.01</td>
<td>0.06±0.02</td>
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</tr>
<tr>
<td>Vein</td>
<td>0.04±0.02</td>
<td>0.06±0.02</td>
<td>0.06±0.03</td>
<td>0.05±0.02</td>
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<td>LREH (n=8)</td>
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<tr>
<td>Artery</td>
<td>0.25±0.09</td>
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<td>Vein</td>
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<td>0.21±0.09</td>
<td>0.22±0.09</td>
<td>0.25±0.09</td>
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<td>Artery</td>
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<td>0.53±0.18</td>
<td>0.59±0.09</td>
<td>0.65±0.08</td>
<td>1.03±0.15*†</td>
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<tr>
<td>Artery</td>
<td>1.07±0.49</td>
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<td>0.92±0.51</td>
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<tr>
<td>Vein</td>
<td>1.03±0.40</td>
<td>0.96±0.49</td>
<td>1.28±0.40*†</td>
<td>1.29±0.48*†</td>
<td>1.56±0.56*†</td>
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ALDO, hypertensive patients with primary aldosteronism; LREH, low renin essential hypertensive patients; NREH, normal renin essential hypertensive patients; RVH, renovascular hypertensive patients.

Data are mean±SEM.

*p<0.05 vs. arterial values.

and 88.1±20.3 pg/min; p<0.01 versus basal and versus LREH and ALDO), and greatest in RVH patients (from -11.1±2.7 to 86.4±38.1, 134.4±37.5, and 152.3±45.1 pg/min; p<0.001 versus basal and versus the other groups).

Chromatographic Separation of Angiotensin II

In one representative experiment, when Ang II was measured after HPLC separation, isoproterenol still caused a dose-dependent increment in venous Ang II without affecting arterial values (Table 3). Ang II values after HPLC separation accounted for 64.5±5.7% of immunoreactive Ang II. The coefficient of correlation between immunoreactive and HPLC-separated Ang II was 0.96.

With isoproterenol infusion, a positive correlation was found between venous-arterial differences of active renin or PRA and Ang II (active renin versus Ang II: r=0.77, p<0.001; PRA versus Ang II: r=0.81, p<0.001), between venous-arterial differences of active renin or PRA and Ang I (active renin versus Ang I: r=0.69, p<0.001; PRA versus Ang I: r=0.71, p<0.001), and between venous-arterial differences of Ang I and Ang II (r=0.71, p<0.001). Moreover, if data concerning the individual increments of PRA and Ang II induced by isoproterenol at the three doses are tested versus the basal circulating levels of PRA (Figure 4), we can observe a significant correlation between the local PRA and Ang II release and the circulating renin profile.

To exclude the possibility that isoproterenol-mediated FBF increment could eventually affect PRA or Ang II reuptake from vascular tissue through the reduced forearm transit time, we tested the effect of sodium nitroprusside (a non-β-receptor-related vasodilator drug) (at 1.5, 5, and 15 μg per 100 mL forearm tissue per minute, 5 minutes each) on PRA and Ang II arteriovenous values in adjunctive hypertensive patients (n=3) with high circulating PRA values (3.8±0.6 ng Ang I/mL per hour). Despite an FBF increment comparable to that previously shown by isoproterenol (from
TABLE 3. Arterial and Venous Concentrations of Immunoreactive and HPLC-Separated Angiotensin II During Increasing Doses of Isoproterenol in One Patient With Essential Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Saline</th>
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<tr>
<td></td>
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<td>Immunoreactive angiotensin II (pg/mL)</td>
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<td>Artery</td>
<td>16.4</td>
<td>16.3</td>
<td>22.5</td>
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<tr>
<td>Vein</td>
<td>10.1</td>
<td>13.1</td>
<td>10.7</td>
</tr>
<tr>
<td>Separated angiotensin II (pg/mL)</td>
<td></td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>Artery</td>
<td>13.1</td>
<td>29.3</td>
<td>12.9</td>
</tr>
<tr>
<td>Vein</td>
<td>11.3</td>
<td>22.8</td>
<td>11.3</td>
</tr>
</tbody>
</table>

HPLC, high performance liquid chromatography. Angiotensin II was separated by HPLC from cross-reacting metabolites.

Time Course of the β-Adrenergic Receptor-Mediated Effect on Vascular Renin-Angiotensin System

Isoproterenol infusion caused the expected increment in FBF, which reached a plateau at the fifth minute and remained constant throughout the infusion period (Table 4, Figure 5). Both arterial PRA and Ang II were not affected by drug infusion, confirming the absence of systemic effects (Table 4, Figure 5). On the contrary, venous values immediately increased to reach the maximum value within the fifth to 10th minutes and then slightly but constantly decreased so that at the 50th minute they were not statistically different from the arterial levels (Table 4, Figure 4).

Exogenous Angiotensin II–Mediated Vasconstriction

Exogenous Ang II caused a dose-dependent FBF decrement in the infused forearm (from 3.6±0.9 to 2.7±0.8, 1.5±0.5, and 0.8±0.3 mL per 100 mL forearm tissue per minute), which is an index of direct vasoconstriction because contralateral FBF (from 3.8±0.8 to 3.6±0.7, 3.8±0.9, and 3.9±0.9 mL per 100 mL forearm tissue per minute), blood pressure, and heart rate did not significantly change during the infusion.

Discussion

Despite general agreement on the existence of a tissue RAS in vessels, the origin of vascular renin, whether locally produced or taken up from plasma, is still strongly debated.\(^1\,2\) We approached this problem using the perfused forearm model, based on the assumption that forearm arteriovenous differences corrected for FBF allow the calculation of the net balance.
TABLE 4. Forearm Blood Flow and Arterial and Venous Concentrations of Plasma Renin Activity and Angiotensin II During Prolonged Isoproterenol Infusion

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Saline</th>
<th>Isoproterenol infusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm blood flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[mL/(100 mL)/min] Infused forearm</td>
<td>3.7±1.2</td>
<td>3.6±1.1</td>
<td></td>
</tr>
<tr>
<td>Contralateral forearm</td>
<td>3.7±1.3</td>
<td>3.6±1.1</td>
<td>5.7±1.2*</td>
</tr>
<tr>
<td>Plasma renin activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ng Ang I/mL]/hr Arterial values</td>
<td>1.24±0.4</td>
<td>1.15±0.4</td>
<td></td>
</tr>
<tr>
<td>Venous values</td>
<td>1.24±0.4</td>
<td>1.15±0.4</td>
<td>5.7±1.2*</td>
</tr>
<tr>
<td>Angiotensin II (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial values</td>
<td>16.9±3.5</td>
<td>15.5±3.3</td>
<td></td>
</tr>
<tr>
<td>Venous values</td>
<td>15.5±3.3*</td>
<td>16.8±3.8</td>
<td></td>
</tr>
</tbody>
</table>

Ang I, angiotensin I. Sixty-minute isoproterenol was at a rate of 0.03 μg per 100 mL forearm tissue per minute. Data are mean±SEM; n=6.

* p<0.05 vs. contralateral forearm or arterial values.

of substances through the forearm vasculature. With the use of this technique, it was already shown that β-adrenergic receptor stimulation by isoproterenol is an adequate stimulus to cause a local release of renin and Ang II, because this effect is blunted by propranolol and is not reproduced by non-β-adrenergic receptor-related vasodilators such as sodium nitroprusside or histamine. In the present study, this experimental approach was further validated by Ang II measurements after HPLC separation. Even after this procedure, β-adrenergic stimulation still caused an increment of venous values of Ang II without affecting arterial values, an index of local production. The lower values of separated Ang II compared with immunoreactive values confirm the expected cross-reactivity of the antibody for the peptide but do not limit the observation that the local β-adrenergic receptor stimulation can significantly activate the local production of Ang II, which, as already discussed, probably originates from the activity of a vascular tissue RAS.

Using this experimental approach, in this study we compared vascular RAS activity in patients with different forms of hypertension and different levels of circulating PRA. The present results indicate that despite a comparable effectiveness of β-adrenergic receptor stimulation, as shown by the equivalent vasodilation induced by isoproterenol, the behavior of forearm vascular released active renin, PRA, Ang I, and Ang II was completely different in the four groups, ranging from the absence of release in the ALDO group, a very low and intermediate release in LREH and NREH patients, respectively, and a greater production in RVH patients. This evidence seems to support the concept that renin uptake from plasma plays an important role in β-adrenergic receptor-induced vascular RAS activation. However, several points deserve further comment.

Because our measurements of renin, Ang I, and Ang II were performed in plasma and not directly in tissues, we can only indirectly infer that the local renin and Ang II plasma modifications, which we observed with isoproterenol administration, might originate from forearm vessel wall. The absence of systemic effects induced by intrabrachial isoproterenol (no modification of blood pressure, heart rate, contralateral FBF, and arterial values of active renin, PRA, Ang I, and Ang II) seems to indicate an effect of β-adrenergic receptor stimulation limited to the vasculature of the infused forearm.

Another problem could originate from an eventual modification of Ang II vascular reuptake. On the basis of experimental literature (reviewed by Campbell), it can be calculated that 60–90% of circulating Ang II is...
metabolized in one passage through the femoral vascular bed. Similar data were obtained by Li and Zimmerman\(^\text{12}\) in rabbit hind limb, using the extraction ratio of \(^{125}\text{I}-\text{Ang II}\). Moreover, Admiraal et al\(^\text{13}\) showed that 47.4% of Ang I is degraded across the human forearm vessels. We are not aware of comparable studies for active renin or PRA, but the possibility exists that the decrement of the transit time through the forearm vasculature due to the increase of FBF induced by isoproterenol could decrease not only the extraction rate of Ang II but also that of active renin or PRA. Therefore, it could have been possible that the extraction of the RAS components was reduced by the increase in FBF mainly in patients, such as NREH and RVH patients, with high levels of circulating renin and Ang II. However, this possibility can be reasonably excluded by our finding that in hypertensive patients with high circulating renin and Ang II levels, sodium nitroprusside, a non-\(\beta\)-adrenergic vasodilator, did not change arteriovenous gradients for active renin, PRA, and Ang II, despite a vasodilating effect comparable to isoproterenol.

Moreover, the possibility that isoproterenol per se could influence transformation of prorenin to renin has been already tested and excluded,\(^\text{3,14}\) and aspecific effects of this drug on local Ang II conversion or assay can be excluded by the measurements performed after HPLC separation. Furthermore, a positive correlation between the venous-arterial differences of the various components of the RAS was maintained in the presence of isoproterenol, suggesting that Ang II measured under \(\beta\)-adrenergic receptor stimulation is the final product of the classic RAS cascade.

In the present paper, we measured active renin and PRA, because this last value might be influenced by angiotensinogen concentration. However, these two parameters showed a highly significant direct correlation \((r=0.95, p<0.001)\), both basally and under \(\beta\)-adrenergic receptor stimulation, a finding which indicates that, in these experimental conditions, similar information can be determined with either active renin or PRA.

As concerns the origin of vascular renin, experimental data support either local generation or uptake from plasma. The synthesis of the essential components of the RAS\(^\text{15-18}\) and the presence of renin messenger RNA\(^\text{19,20}\) have been demonstrated in vascular smooth muscle and endothelium, but most in vivo studies show that, after bilateral nephrectomy, renin activity disappears from vascular wall.\(^\text{21,22}\) This has been recently reinforced by Li and Zimmerman,\(^\text{12}\) who showed in the rabbit femoral artery that the local Ang I and Ang II generation induced by \(\beta\)-adrenergic receptor stimulation is blunted by bilateral nephrectomy, and by Danser et al,\(^\text{23}\) who demonstrated that in intact pigs, the local production of Ang I was closely correlated with renal-derived PRA. Our present data seem to reinforce also in human vessels the possibility that local Ang II production induced by \(\beta\)-adrenergic receptor activation depends on the presence of circulating PRA.

In agreement with this possibility is the experiment with the prolonged isoproterenol infusion, which showed that, despite a constant \(\beta\)-adrenergic stimulation for the entire infusion period, as shown by FBF behavior, PRA and Ang II release is a time-related phenomenon, with a rapid maximum effect and a slight but constant decrease. Because PRA half-life is approximately 30 minutes,\(^\text{24}\) the rapid onset of its release (5 minutes) supports the possibility that isoproterenol acts on a preformed tissue renin pool that, as previously discussed, might derive at least partially from the uptake of circulating renin.

However, our data provide only slight information on the relevance and physiological role of tissue renin, because the present results can be referred only to blood vessels, and forearm vasculature is a minor tissue contributor in the forearm.

The experiment with the infusion of exogenous Ang II was designed to evaluate the direct effect of locally produced Ang II on vascular tone. We previously showed that intrabrachial Ang II infused at a very low dose, titrated in order to double forearm venous concentrations (and therefore to cause a plasma increment comparable to that induced by a physiological stimulus such as standing), did not affect basal FBF but increased the vasoconstrictor effect and norepinephrine release induced by an endogenous sympathetic nervous system stimulation.\(^\text{3}\) In the present study, we infused exogenous Ang II at doses three, 10, and 30 times greater than the ones used in the above-mentioned experiments. The first approximates Ang II plasma concentrations obtained with \(\beta\)-adrenergic receptor stimulation, and the others are in a pharmacological range. Exogenous Ang II caused a dose-dependent vasoconstriction, which was already significant at the first infusion rate. Therefore, these data seem to indicate that vascular Ang II, at least at the concentration released during \(\beta\)-adrenergic receptor stimulation, can directly affect basal FBF. Moreover, the present data can probably underestimate the real effect of vascular tissue Ang II on vessel reactivity, because the activity on smooth muscle cells from the peptide generated inside the vessel wall might be more potent than that induced by an endogenous infusion.

In conclusion, our data confirm that \(\beta\)-adrenergic receptor stimulation can activate a tissue RAS in the forearm vessels of hypertensive patients and demonstrate that its local release is related to the level of circulating renin. This latter finding supports the possibility that plasma uptake can be a source of vascular renin; although the existence and role of a local synthesis cannot be excluded.

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Indirect evidence for vascular uptake of circulating renin in hypertensive patients.
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