Vascular Renin-Angiotensin System and Neurotransmission in Hypertensive Persons

Stefano Taddei, Stefania Favilla, Piero Duranti, Nadia Simonini, and Antonio Salvetti

The existence of a vascular renin-angiotensin system and its role in modulating sympathetic activity were evaluated in forearm arterioles of hypertensive individuals. Isoproterenol (0.03, 0.01, 0.3 μg/100 ml/min for 5 minutes each; n=5) was infused into the brachial artery, and active and inactive renin, angiotensin II, and norepinephrine forearm balance (venous-arterial differences corrected for forearm blood flow by strain-gauge plethysmography) were measured. Isoproterenol caused vasodilation and a dose-dependent active and inactive renin, angiotensin II, and norepinephrine outflow, an effect blunted by propranolol (10 μg/100 ml/min). To evaluate the role of local angiotensin II on β-mediated norepinephrine overflow, the experiment was repeated with captopril (2.5 μg/100 ml/min for 10 minutes; n=5), which abolished angiotensin II release and significantly reduced norepinephrine overflow. To test whether angiotensin II facilitates both presynaptic norepinephrine release and its postjunctional action, we evaluated the effect of exogenous angiotensin II, infused into the brachial artery at low concentrations (0.001 μg/100 ml/min), on forearm vasoconstriction and norepinephrine release induced by endogenous sympathetic activation (application of a lower body negative pressure: −10 and −20 mm Hg for 5 minutes, n=10) and on the vasoconstrictor effect of local norepinephrine (0.0015, 0.005, 0.015, 0.05, 0.15 μg/100 ml/min for 3 minutes each; n=6). Although angiotensin II increased the vasoconstricting effect and the norepinephrine release induced by lower body negative pressure, it failed to affect norepinephrine-mediated vasoconstriction. Our data indicate the existence in hypertensive individuals of a vascular renin-angiotensin system that seems to modulate sympathetic activity through the presynaptic facilitation of norepinephrine release. (Hypertension 1991;18:266–277)

Experimental data have clearly shown the existence of a tissue vascular renin-angiotensin system (RAS) that can be locally synthesized or taken up from plasma. It has also been shown that locally produced angiotensin II can facilitate the vasoconstrictor response to sympathetic nervous system activation by increasing either the presynaptic release of norepinephrine or the postsynaptic response to this neurotransmitter (or both).2

Although it has been shown that cultured smooth muscle cells from human aorta produce renin, mainly in the inactive form,3 and for its role in modulating sympathetic nervous system activity4,5 in humans is scanty.

Therefore, the purposes of our present study were: first, to provide direct evidence for the existence of a vascular tissue RAS in humans and second, to evaluate whether and how this system can modulate sympathetic nervous system activity. To achieve these goals, we adopted the perfused forearm technique in which simultaneous sampling from the brachial artery and an ipsilateral deep vein allowed us to calculate venoarterial differences and the net balance, that is, venoarterial differences multiplied by forearm blood flow (FBF).8 The experimental idea originated from the work of Nakamaru et al,9 which has shown that in isolated perfused rat mesenteric arteries, α-adrenergic receptor stimulation can release angiotensin II. In a group of hypertensive patients, we tested whether isoproterenol, a selective agonist of β-adrenergic receptors,10 infused into the brachial artery at systematically ineffective doses can activate tissue vascular RAS and whether any locally produced angiotensin II can modulate isoproterenol-mediated norepinephrine overflow.

Moreover, to elucidate the mechanism by which angiotensin II modulates sympathetic vasoconstriction, whether a presynaptically mediated norepinephrine overflow or a postsynaptic facilitating effect on muscle cell contraction or both, we investigated the effect of exogenous angiotensin II on forearm
vasoconstriction induced both by the endogenous sympathetic activation obtained through the application of a lower body negative pressure and by local administration of exogenous norepinephrine.

Methods

Subjects

Thirty-one inpatients (17 men and 14 women aged 43.7±7.4 years, mean±SD) with mild-to-moderate, uncomplicated essential hypertension (166.7±9.8/102.4±7.3 mm Hg) were recruited for the studies. According to institutional guidelines, all patients were aware of the investigational nature of the study and informed consent was obtained. The subjects were without any treatment for at least 1 week before they were admitted to the ward where they were maintained on a normocaloric diet with constant sodium (80–100 mmol/day) and potassium chloride (60–80 mmol/day) intake.

Experimental Procedure

All studies were performed at 8:00 AM after an overnight fast with the subjects lying supine in a quiet, air-conditioned room (22–24°C). A polyethylene cannula (21 gauge, Abbott, Sligo, Ireland) was inserted into the brachial artery under local anesthesia (2% lidocaine) and was connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, Colo.) for systemic mean blood pressure (one third pulse pressure+diastolic pressure) and heart rate monitoring (model VSM1, Physiocontrol, Redmond, Wash.) and for intra-arterial infusions. The electronic beat-to-beat signal was digitized on-line through an AT-IBM-compatible personal computer by using a customized software program (Saniware SNC, Casalecchio sul Reno, Bologna, Italy). Another cannula (6 cm long) was inserted into the brachial artery at the rate of 2.5 jtg/100 ml forearm tissue/min for 10 minutes. Arteriovenous samples of enzyme (ACE) inhibitor, infused through the brachial artery at the rate of 0.001 jtg/100 ml forearm tissue/min for 10 minutes. Arteriovenous samples for active and inactive renin, angiotensin II, and catecholamines were again collected during the experiment at the same time intervals.

FOREARM VOLUME, MEASURED BY WATER DISPLACEMENT, was increased to 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.

Endogenous sympathetic activation was obtained by a −10 and −20 mm Hg lower body negative pressure (for 5 minutes), a maneuver that causes sympathetic discharge and peripheral vasoconstriction without changing systemic blood pressure and heart rate. Lower limbs were placed up to the waist into an air-tight Plexiglas container that was sealed by rubber flaps around the waist, and negative pressures, quantified by a U-shaped mercury manometer connected to the inside of the apparatus, were generated through a vacuum cleaner (Bosch, Milan, Italy).

Experimental Design

β-Adrenergic receptor–mediated vascular tissue renin-angiotensin system activation and norepinephrine overflow. In a group of five patients, a dose–response curve to intra-arterial isoproterenol (cumulative infusion, 0.03, 0.1, 0.3 μg/100 ml forearm tissue/min for 5 minutes each) was generated in combination with either saline (0.2 ml/min for 10 minutes) or β-adrenergic receptor blockade (intra-arterial propranolol, 10 μg/100 ml forearm tissue/min). Arteriovenous samples for active and inactive renin, angiotensin II, and catecholamines were collected basally and at the end of each infusion period (fifth minute for isoproterenol and 10th minute for saline and propranolol).

The possibility that repeated isoproterenol administration might cause β-adrenergic receptor desensitization was excluded in a preliminary experiment (n=4 adjunctive patients) in which we found that the dose–response curve to the β-adrenergic receptor agonist, repeated twice, produced the same increment in FBF (first infusion from 3.7±0.5 to 6.9±1.4, 10.3±3.8, and 14.7±5.4; second infusion from 3.6±0.5 to 6.5±1.3, 10.8±4, and 14.9±5.8 ml/100 ml forearm/min; NS).

Vascular angiotensin II–induced modulation of β-adrenergic receptor–mediated norepinephrine overflow. In another group of five patients, the dose–response curve to isoproterenol, at the same previous rates, was obtained under basal conditions and then in presence of captopril, an angiotensin converting enzyme (ACE) inhibitor, infused through the brachial artery at the rate of 2.5 μg/100 ml forearm tissue/min for 10 minutes. Arteriovenous samples of active and inactive renin, angiotensin II, and catecholamines were again collected during the experiment at the same time intervals.

Local modulation of sympathetic-mediated vasoconstriction by exogenous angiotensin II. To test the effect of exogenous angiotensin II on sympathetic vasoconstriction, lower body negative pressure (−10 and −20 mm Hg for 5 minutes each; n=10 patients) was applied in basal condition (during saline infusion) and in the presence of exogenous angiotensin II infused into the brachial artery at a dose of 0.001 μg/100 ml forearm tissue/min for 10 minutes. This rate was titrated to obtain plasma concentration increments comparable with those reached during a physiological stimulus such as upright position, which in a group of 14 hypertensive patients, caused an increment in plasma venous angiotensin II from 8.3±2.4 (supine) to 22.4±6.8 (standing) pg/ml. In five of the 10 patients, the following humoral param-
eters were evaluated: forearm arteriovenous catecholamines (to determine the effect of exogenous angiotensin II on norepinephrine outflow) and forearm venous angiotensin II (to confirm that angiotensin II infusion produced levels within the physiological range). Arterial and venous samples were obtained and stored at -20 °C in the absence and the presence of angiotensin II infusion.

Finally, to study the effect of angiotensin II on α-mediated forearm vasoconstriction in another group of six patients, the vasoconstricting effect of exogenous norepinephrine, infused into the brachial artery at increasing and cumulative doses (0.0015, 0.005, 0.015, 0.05, 0.15 µg/100 ml forearm tissue/min for 3 minutes each) was evaluated during both saline and exogenous angiotensin II infused previously.

**Analytical Procedures**

Active renin (plasma renin activity in ng angiotensin I/ml/hr) was measured by radioimmuno assay after plasma had been incubated at pH 5.7 for 1.5 hours. Total renin was measured by the same radioimmunoassay after sepharose-bound trypsin activation. Inactive renin was calculated as the difference between total and active renin.

Catecholamine concentrations (pg/ml) were measured by high-performance liquid chromatography. Serum ACTE activity (nmol/min/ml) was measured by a radioenzymatic method and hematocrit by a micromethod.

Plasma angiotensin II (pg/ml) was determined by radio immunoassay after extraction of the peptide from plasma. Five milliliters blood was rapidly collected in prechilled plastic tubes containing 60 µl of a 10% solution of Na2 ethylenediaminetetraacetic acid plus 300 µl of a 0.5% solution of phenanthroline (Sigma Chemical Co., St. Louis, Mo.). Blood samples, kept on ice, were immediately centrifuged at 3,000g for 10 minutes at 4°C, stored at -70°C, extracted within 2 days, and assayed within 1 week. We previously showed that the low temperature (2°C-4°C) is effective in inhibiting renin activity from the moment of blood collection throughout all the operative steps. Plasma, usually 2 ml, was applied to preactivated C8 octadecysilane cartridges (Sep-Pak, Waters Associates, Millford, Mass.), moistened with methanol 100% (3 ml), and washed with 1% trifluoroacetic acid (TFA) (10 ml) in a water solution. Cartridges were coated with 1% polypeptide solution (Polyprop, Sigma) to minimize nonspecific absorption and were washed again in a mixture of methanol/water/TFA (80/19/1%, vol/vol/vol). The sample was applied, and the gel was washed with a mixture of 5 ml 1% TFA/1% NaCl (1:1 vol/vol). The absorbed angiotensin II was eluted with 2 ml methanol and evaporated to dryness (Hetovac and Hototrap CT60, Heto Lab Equipment, A/S, Birkerod, Denmark). The extraction procedure was performed at 4°C using the cartridges once, and the extraction efficiency (recovery rate 86.4±5.6%, mean±SD, n=206) was estimated through radiolabeled 125I-angiotensin II (New England Nuclear, Boston, Mass.) added to individual plasma aliquots before extraction. The extracts were dried (Hetovac and Hototrap CT60, Heto Lab Equipment) and then redissolved in 1 ml phosphate-buffered saline, pH 7.4. Duplicate 400-µl aliquots of these reconstituted extracts were incubated for 6 hours with 100 µl rabbit antiserum (Immuno Technology Service, Wychen, The Netherlands) at 4°C. 125I-Angiotensin II (100 µl) was added, and the incubation was prolonged up to 18 hours at 4°C. Bound and free angiotensin II were separated by the addition of 100 µl goat anti-rabbit γ-globulin bound on solid phase and centrifuged (3,000g for 15 minutes at 4°C) after 30 minutes of incubation at room temperature. Precipitate and supernant were counted in a gamma counter (LKB 1275, Turku, Finland). The final values of immunoreactive angiotensin II-like material were calculated after correction for the extraction losses from a standard curve (Immuno Technology Service). The cross-reactivity of our antiserum is 0.1% with angiotensin I and 30% with angiotensin III; the sensitivity of the assay is 1.5 pg/ml with 50% displacement by 37-40 pg/ml. The intra-assay and interassay variation coefficient is 11.7% (n=20) and 14.5% (n=6), respectively. No nonspecific interferences from compounds used in the steps before radioimmunoassay were found. Angiotensin II concentrations in venous plasma from healthy subjects on a constant sodium intake (80-100 mmol/day) in supine position were 13.4±7.9 pg/ml, range 3.3–34.8 (n=22).

**Data Analysis**

Net forearm balance of active and inactive renin, angiotensin II, and norepinephrine was obtained as the product of the respective venous-arterial plasma concentration gradient and forearm plasma flow. Plasma flow rates were calculated as the product of FBF by 1 minus hematocrit.

To determine a more accurate measurement of local norepinephrine production, the forearm extraction of norepinephrine in arterial plasma was estimated through epinephrine forearm extraction according to the following equation:

\[ VeNE = \frac{VeE}{ArE} \times FBF \times 1 - Htc \]

where VeNE is venous norepinephrine, ArNE is arterial norepinephrine, VeE is venous epinephrine, ArE is arterial epinephrine and Htc is hematocrit.

Since systolic, diastolic, and mean arterial pressure did not significantly change during the study, all data were analyzed in terms of FBF, and FBF increments were taken as an evidence of local vasodilation. Raw data were analyzed by two- or three-way analysis of variance, accounting for time, phases (i.e., either artery or vein), and subjects. The interaction times×phases were taken as the relevant statistics. Duncan's test was applied for multiple comparison testing.
TABLE 1. Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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<tr>
<td>Age (yr)</td>
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<tr>
<td>Sex (m/f)</td>
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<tr>
<td>MBP (mm Hg)</td>
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<td>HR (beat/min)</td>
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<tr>
<td>FBF (ml/100 ml/min)</td>
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<td>FVR (mm Hg/ml/100 ml/min)</td>
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<tr>
<td>Forearm volume (ml)</td>
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<td>Plasma</td>
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<td>aPRA (ng Ang I/ml/hr)</td>
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</tr>
<tr>
<td>iPRA (ng Ang I/ml/hr)</td>
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</tr>
<tr>
<td>Ang II (pg/ml)</td>
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</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
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<tr>
<td>Epinephrine (pg/ml)</td>
<td>22.2±6.8</td>
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</table>

Most values are mean±SEM. MBP, mean blood pressure; HR, heart rate; FBF, forearm blood flow; FVR, forearm vascular resistance; aPRA, active plasma renin activity; iPRA, inactive plasma renin activity; Ang, angiotensin.

test was used to check the statistical significance of the difference between nonparametric values. Correlation coefficients were calculated using Pearson's test. Results were expressed as mean±SEM.

Drugs
Isoproterenol HCl (Isuprel, Winthrop-Breon, New York), propranolol HCl (Inderal, ICI, Milan, Italy), captopril HCl (Capoten, Bristol-Myers Squibb, Rome, Italy), angiotensin II (Hypertensina Ciba, Origgio [VA], Italy), and (-)-norepinephrine bitartrate (Levophed, Breon, New York) were obtained from commercially available sources and diluted in fresh solutions to the desired concentrations by adding normal saline.

Results
At the time of the study, all patients had reached a constant sodium excretion rate (92.4±9.7 mmol/24 hr). Their demographic, hemodynamic, and humoral parameters are reported in Table 1.

Experiments
β-Adrenergic receptor-mediated vascular tissue renin angiotensin system activation and norepinephrine overflow. Isoproterenol caused a dose-dependent increment in FBF in the presence of saline (from 3.6±0.8 to 6.3±1.7, 11.2±3.4, and 17.7±5.9 ml/100 ml forearm tissue/min; p<0.001 versus basal) (Figure 1A) that was significantly blunted by β-adrenergic receptor blockade (from 3.7±0.5 to 3.6±0.5, 4.2±0.8, and 5.5±1.1 ml/100 ml forearm tissue/min; p<0.001 versus saline) (Figure 1A). Contralateral FBF was not affected by drug infusion throughout the whole experiment (Figure 1A).

Under basal conditions, arterial active renin was 1.32±0.3 ng angiotensin I/ml/hr and venous renin was 1.18±0.3 ng angiotensin I/ml/hr (Figure 2A). When isoproterenol was infused, arterial active renin...
did not significantly change, but venous active renin increased in a dose-dependent way (Table 2 and Figure 2A). When isoproterenol infusion was repeated in the presence of propranolol, β-adrenergic receptor–induced venous active renin increment was significantly (*p<0.01) blunted, whereas arterial active renin remained unchanged (Table 2 and Figure 2A). If these data are expressed as net balance, β-adrenergic receptor stimulation caused a dose-dependent net forearm release of active renin (active renin from −0.28±0.15 to 0.46±0.18, 1.12±0.34, and 3.65±1.12 ng angiotensin I/ml/hr/min; *p<0.001 versus basal) (Figure 2B), an effect opposed by propranolol (active renin from −0.18±0.14 to −0.32±0.24, −0.09±0.12, and 0.11±0.09 ng angiotensin I/ml/hr/min; *p<0.01 versus saline) (Figure 2B). Inactive renin showed a similar behavior: although isoproterenol failed to affect arterial values, venous inactive renin showed a significant (*p<0.01) dose-dependent increment, an effect blunted by propranolol (Table 2). In terms of net balance, β-adrenergic receptor–mediated inactive renin release (from −1.2±0.4 to −0.4±0.3, 2.7±1.3, and 10.3±3.4 ng angiotensin I/ml/hr/min; *p<0.001 versus basal) was evident at the second isoproterenol infusion rate, whereas active renin was already released at the first dose. In the presence of propranolol, inactive renin release was antagonized (from −1.3±0.5 to −1.1±0.4, −1.2±0.4, and −0.3±0.3 ng angiotensin I/ml/hr/min; *p<0.01 versus saline).

Under basal conditions, venous angiotensin II concentrations were significantly lower than the arterial levels (7.19±1.5 versus 9.46±1.9 pg/ml; *p<0.01) (Figure 3A). Isoproterenol infusion reversed this gradient in a dose-dependent manner, an effect significantly blunted by propranolol (Table 2 and Figure 3A), as shown throughout venous-arterial differences calculation (saline from −1.9±0.4 to 2.3±0.3, 3.3±0.5, and 8.2±1.1; propranolol from −1.1±0.3 to −1.8±0.3, 1.3±0.3, and 6.3±0.9; *p<0.001 versus saline). Angiotensin II showed in basal conditions a negative venous-arterial balance, an index of net tissue uptake that was reversed by β-adrenergic receptor stimulation (from −5.2±1.1 to 9.4±3.1, 22.8±7.3, and 89.2±24.7 pg/ml/min; *p<0.001 versus basal) (Figure 3B), an effect that was antagonized by propranolol pretreatment (from −4.9±3.6 to −4.3±3.4, 5.8±0.9, and 23.8±5.6 pg/ml/min; *p<0.01 versus saline) (Figure 3B).

Isoproterenol increased venous norepinephrine but did not significantly change its arterial values (Table 2). When propranolol was infused, basal venous-arterial values significantly decreased, but the increasing effect of isoproterenol on venous norepinephrine was reduced (Table 2). When norepinephrine net balance, corrected for epinephrine extraction, was calculated, it was evident that isoproterenol induced an overflow of norepinephrine (from 400.4±15.8 to 671.8±92.5, 1,327.9±148.7, and 1,730.4±250.8 pg/ml/min; *p<0.01 versus basal) (Figure 4A). In the presence of propranolol infusion, basal norepinephrine output significantly decreased (from 396.3±19.4 to 259.8±16.5 pg/ml/min; *p<0.05) (Figure 4A), and the dose–response curve to isopro-
TABLE 2. Isoproterenol infusion increased (from 3.6 ± 0.6 to 4.0 ± 1.0 ml/min; *p < 0.001 versus basal) brachial artery flow: The effect of angiotensin converting enzyme inhibition.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Angiotensin II (ng/ml/hr)</th>
<th>Angiotensin II (ng/ml/hr)</th>
<th>Angiotensin II (ng/ml/hr)</th>
<th>Angiotensin II (ng/ml/hr)</th>
<th>Angiotensin II (ng/ml/hr)</th>
<th>Angiotensin II (ng/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active renin (ng Ang I/ml/hr)</td>
<td>Inactive renin (ng Ang I/ml/hr)</td>
<td>Active and inactive renin (ng Ang I/ml/hr)</td>
<td>Angiotensin II (ng Ang I/ml/hr)</td>
<td>Angiotensin II (ng Ang I/ml/hr)</td>
<td>Angiotensin II (ng Ang I/ml/hr)</td>
</tr>
<tr>
<td>Basal</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>11.2 ± 4.9</td>
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</tr>
<tr>
<td>Propranolol</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>11.2 ± 4.9</td>
<td>11.3 ± 4.9</td>
<td>11.3 ± 4.9</td>
</tr>
</tbody>
</table>

terenol was blunted (from 259.8 ± 16.5 to 268.4 ± 34.8, 305.8 ± 63.6, and 430.5 ± 89.4 pg/ml/min; p < 0.01 versus saline) (Figure 4A).

In one additional patient, we evaluated the effect of two nonspecific (non-β-adrenergic receptor correlated) vasodilator drugs, histamine and sodium nitroprusside, on active and inactive renin and on angiotensin II venous-arterial values. The two drugs were infused at the following doses: histamine 0.015, 0.05, and 0.15 μg/100 ml forearm tissue/min for 5 minutes each; sodium nitroprusside 1.5, 5, and 15 μg/100 ml forearm tissue/min for 5 minutes each. Despite a vasodilating effect comparable with that of isoproterenol (FBF from 3.8 to 5.4, 11.6, and 17.3 ml/100 ml forearm tissue/min), histamine failed to increase venous-arterial differences for active renin (vein from 0.82 to 0.80, 0.76, and 0.72; artery from 0.88 to 0.90, 0.92, and 0.86ng angiotensin I/ml/hr), inactive renin (vein from 11.33 to 11.30, 11.24, and 11.21; artery from 11.28 to 11.34, 11.36, and 11.33 ng angiotensin I/ml/hr) and angiotensin II (vein from 6.42 to 5.32, 5.11, and 5.85; artery from 8.1 to 8.0, 7.8, and 8.2 pg/ml). The same results were obtained when sodium nitroprusside was infused: FBF increased from 4.0 to 6.9, 14.1, and 19.8 ml/100 ml forearm tissue/min. However, active renin (vein from 0.88 to 0.74, 0.81, and 0.70; artery from 0.82 to 0.84, 0.86, and 0.80 ng angiotensin I/ml/hr) inactive renin (vein from 11.21 to 11.13, 11.04, and 11.07; artery from 11.36 to 11.31, 11.28, and 11.37ng angiotensin I/ml/hr) and angiotensin II (vein from 6.9 to 6.4, 5.3, and 5.9; artery from 8.5 to 8.3, 8.7, and 8.8 pg/ml) did not show venous increments.

Vascular angiotensin II–induced modulation of β-adrenergic receptor–mediated norepinephrine overflow: The effect of angiotensin converting enzyme inhibition. As in the previous experiment, isoproterenol in the presence of saline induced a similar dose-dependent increment in FBF (from 3.5 ± 0.7 to 6.0 ± 1.6, 10.8 ± 4.6, and 16.8 ± 6.3 ml/100 ml forearm/min; p < 0.001 versus basal) (Figure 1B) and increased to a similar extent venous active and inactive renin and angiotensin II without changing arterial active and inactive renin and angiotensin II (Table 3). Calculated net balance of both renin forms (active renin from −0.4 ± 0.2 to 0.8 ± 0.3, 1.1 ± 0.4, and 3.3 ± 1.1 ng angiotensin I/ml/hr/min; p < 0.001 versus basal; inactive renin from −1.1 ± 0.5 to −0.8 ± 0.3, 3.5 ± 1.2, and 12.3 ± 2.7 ng angiotensin I/ml/hr/min; p < 0.001 versus basal) and angiotensin II (from −5.1 ± 1.1 to 7.9 ± 2.3, 33.2 ± 8.4, and 73.2 ± 14.6 pg/ml/min; p < 0.001 versus basal) showed dose-dependent increments similar to those of the previous experiment.

Although systemic venous ACE activity did not change (from 89.3 ± 9.7 to 85.3 ± 8.6 mmol/ml/min; NS) when captopril was infused, forearm venous ACE activity was highly reduced (from 84.3 ± 8.4 to 7.7 ± 4.2 mmol/ml/min; p < 0.001). FBF slightly, but not significantly, increased (from 3.6 ± 0.6 to 4.0 ± 1.0 ml/100 ml forearm/min) (Figure 1B). However, captopril
infusion significantly increased the vasodilating effect of isoproterenol (from 4.0±1.0 to 12.4±4.9, 18.8±7.1, and 24.3±9.3 ml/100 ml forearm tissue/min; p<0.001 versus saline) (Figure 1B) and as expected strongly reduced basal angiotensin II venous values (from 8.6±2.0 to 2.3±0.6 pg/ml; p<0.001). Captopril did not change the isoproterenol-mediated increase of venous and arterial active and inactive renin (Table 3). However, in terms of net balance, captopril shifted to the left the dose–response curve of β-stimulated active and inactive renin release when compared with saline (active renin from -0.1±0.3 to 0.7±0.4, 1.9±0.8, and 6.2±3.1; p<0.01 versus saline; inactive renin from -0.2±0.4 to 6.3±2.9, 11.4±4.8, and 16.4±5.1 ng angiotensin I/ml/hr/min; p<0.01 versus saline). In the presence of captopril, venous angiotensin II values failed to increase under isoproterenol infusion, and its arterial concentrations did not change (Table 3), which indicates that the local infusion of captopril did not cause any systemic humoral effect. Moreover, calculated net balance of angiotensin II indicates that captopril increased angiotensin II vascular extraction both before (basal -5.5±8.8; captopril -21.7±8.3 pg/ml/min; p<0.001) and during (-55.5±8.6, -96.3±17.2, and -111.7±25.3 pg/ml/min; p<0.001 versus basal) isoproterenol infusion.

As in the previous experiment, isoproterenol caused similar increments of venous norepinephrine without changing its arterial concentrations (Table 3) and caused a similar norepinephrine overflow (from 460.8±54.3 to 850.8±167.4, 1,521.9±237.8, and 1,967.8±543.7 pg/ml/min; p<0.001 versus basal) (Figure 4B). In the presence of captopril, isoproterenol failed to induce venous norepinephrine increment (Table 3), but in terms of net balance, β-adrenergic receptor stimulation still caused a norepinephrine outflow, which, however, was significantly reduced when compared with control (saline) (from 435.7±48.4 to 530.6±112.3, 902.6±189.4, and 1,345.8±412.5 pg/ml/min; p<0.001 versus basal and p<0.01 versus saline) (Figure 4B).

Local modulation of sympathetic-mediated vasoconstriction by exogenous angiotensin II. In basal conditions, lower body negative pressure caused a significant vasoconstriction, either when applied at -10 mm Hg (FBF from 3.5±0.7 to 2.3±0.4 ml/100 ml forearm tissue/min; -30.5±8.9%; p<0.001 versus basal) or at -20 mm Hg (FBF to 1.9±0.3 ml/100 ml forearm tissue/min; -44.8±8.3%, p<0.001 versus basal and p<0.01 versus lower body negative pressure -10 mm Hg) (Figure 5). Venous norepinephrine at lower body negative pressure -20 mm Hg significantly increased compared with saline (from 248.3±41.6 to 373.7±79.2 pg/ml; p<0.01) without changes in arterial values (from 194.6±33.1 to 223.8±38.7 pg/ml). However, calculated outflow tended to increase but not to a significant extent (from

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**Figure 3.** Arterial (○) and venous (●) concentrations (panel A) and net balance (panel B) of angiotensin II across forearm during isoproterenol infusion (0.03, 0.1, 0.3 μg/100 ml forearm tissue/min for 5 minutes each) both in presence of saline (0.2 ml/min for 10 minutes) and in presence of propranolol (10 μg/100 ml forearm tissue/min for 10 minutes). Data are mean±SEM (n=5). *p<0.01 or less between arterial and venous concentrations.
4. Bar graphs show norepinephrine outflow across forearm during isoproterenol infusion (0.03, 0.1, 0.3 μg/100 ml forearm tissue/min for 5 minutes each) both in presence of saline and in presence of either propranolol (10 μg/100 ml forearm tissue/min for 10 minutes) (panel A) or captopril (2.5 μg/100 ml forearm tissue/min for 10 minutes) (panel B). Data are mean±SEM (n=5 for panels A and B, respectively). *p<0.01 or less.

On the contrary, arteriolar vasoconstriction induced by exogenous norepinephrine was not affected by local angiotensin II pretreatment (saline from 3.8±0.6 to 3.2±0.5, 2.7±0.4, 2.0±0.3, 1.1±0.2, and 0.6±0.2; angiotensin II from 3.8±0.7 to 3.1±0.5, 2.5±0.4, 1.2±0.4, 1.3±0.3, and 0.7±0.2 ml/100 ml forearm tissue/min; NS).

Correlations among active renin, inactive renin, and angiotensin II. If we pool together the dose–response curves to isoproterenol performed in the two studies, we can calculate the following data in 10 patients.

1) There was a positive correlation between basal renin and local renin release, considered at the greatest isoproterenol dose, either in the active (r=0.84, p<0.001) or the inactive (r=0.78, p<0.001) forms. Similarly basal active renin significantly correlated with basal angiotensin II (r=0.79, p<0.001), and there was a positive correlation between active renin and angiotensin II increments (r=0.71, p<0.001) induced by isoproterenol infusion at the three doses.

2) The proportion of inactive renin (percentage of total renin) in basal conditions was similar to that in

192.4±38.1 to 234.7±48.7 pg/ml/min) (Figure 5). Angiotensin II infusion significantly increased its forearm venous plasma concentrations (from 9.7±3.1 to 19.8±4.8 pg/ml; p<0.01) similar to those observed during standing but did not change FBF and local venous norepinephrine concentrations. When lower body negative pressure application was repeated in the presence of angiotensin II infusion, its vasoconstrictor effect was increased compared with control conditions (−10 mm Hg from 3.3±0.6 to 1.8±0.4 ml/100 ml forearm tissue/min, −45.9±7.2%, p<0.001 versus saline; −20 mm Hg to 1.3±0.4 ml/100 ml forearm tissue/min, −60.2±9.3%, p<0.001 versus saline) (Figure 5). Venous norepinephrine at lower body negative pressure −20 mm Hg showed a greater increment (from 274.6±47.3 to 624.5±94.8 pg/ml; p<0.001) compared with that observed during saline, and when the outflow was calculated, it was evident that in the presence of exogenous angiotensin II, lower body negative pressure–induced norepinephrine overflow (from 203.7±34.2 to 299.3±94.1 pg/ml/min) was significantly greater as compared with both basal (p<0.01) and saline (p<0.001) (Figure 5).
Evidence of a Tissue Vascular Renin-Angiotensin System in Human Forearm

The measurement of venous-arterial gradient of active and inactive renin and of angiotensin II and the calculation of their net balance allowed us to evaluate whether these three components of the RAS are locally released under a stimulus acting only on the vasculature of the experimental forearm. In the present study, the selective stimulation of vascular β-adrenergic receptors induced a clear dose-dependent increment of both active and inactive renin and of angiotensin II in venous blood drawn from human forearm vasculature in the absence of any change in arterial blood, a finding that indicates a local release. Moreover, this is a highly reproducible phenomenon considering that similar data were obtained by using the same protocol in two different groups of patients. However, our calculation of net forearm balance of angiotensin II and renin does not take into account the percent extraction of these substances in the vascular bed, and therefore it cannot be used to calculate the amount of local production but is only a qualitative index of this phenomenon.

β-Adrenergic receptor stimulation is a well-known stimulus for active renin release, at least at the renal level, and its specific effect on forearm renin release and angiotensin II generation is supported by the following observations. First, propranolol, a β-adrenergic receptor antagonist, blunted the renin as well as the angiotensin II stimulating effect of isoproterenol. Second, the possibility that FBF increments per se might decrease vascular degradation of angiotensin II is excluded by our findings that vasodilation, induced by histamine and sodium nitroprusside, did not change venous renin and angiotensin II concentrations. This last hypothesis is further reinforced by the fact that under isoproterenol infusion in the presence of captopril, venous angiotensin II did not change despite a dose increment in FBF.

In our experimental conditions, isoproterenol caused an overflow of active and inactive renin and of angiotensin II, and a direct correlation was found between basal values of active renin and of angiotensin II (a finding already reported) and between their increments under isoproterenol infusion. These findings indicate that β-adrenergic receptor stimulation released both active and inactive renin and that active renin stimulation might generate angiotensin

the presence of the highest isoproterenol dose (74.6±14.8 versus 79.9±15.2%).

Discussion

The present data fulfill our working hypothesis and demonstrate, for the first time to our knowledge, that a vascular tissue RAS is present in the forearm of essential hypertensive persons and that locally released angiotensin II increases sympathetic vasoconstriction mainly through the presynaptic modulation of norepinephrine release.
II locally. These data, together with the finding that renin overflow induced by isoproterenol is increased when angiotensin II generation is blunted by captopril, support experimental data showing that local RAS is present in the vascular tissue as an independent and autonomous system with its own regulatory feedback mechanisms.

The local angiotensin II generation under β-adrenergic receptor stimulation is in agreement with in vitro data in mesenteric artery preparations, but it is apparently at variance with those obtained in the hind limb vasculature. In fact, Mizuno et al. found that although isoproterenol did not cause an angiotensin II overflow in rat hind limb, propranolol reduces the basal release. Li and Zimmerman found that isoproterenol infused in the femoral artery at a dose that increased arterial and systemic angiotensin I and II, caused a local production of these substances in rabbit hind limb, an effect abolished by nephrectomy. This last finding might suggest that the RAS, locally activated by isoproterenol, is mainly taken up from plasma.

Our finding that isoproterenol released inactive renin is apparently at variance with the data of Derkx et al. showing that systemic isoprenaline administration reduced inactive renin in renal veins of renovascular patients. However, others have shown that systemic sympathetic activation causes a slight but significant increment in inactive renin. What is important to consider is that in our experimental model, the local isoproterenol plasma concentrations that we reached are high and their effect is not confused by reflexogenic adjustments caused by a systemic route of administration. For these reasons, it is quite difficult to compare results obtained in such different experimental conditions.

Three other points deserve further consideration. First, although propranolol abolishes isoproterenol-induced FBF increment, it exerts only a blunting effect on β-receptor-mediated angiotensin II overflow when venous-arterial differences are considered. This discrepancy might be due to a weaker link between β-receptor stimulation and local angiotensin II generation compared with the action on FBF but also might be due to the different diluting effect on the venous levels of angiotensin II caused by the different expansion of the forearm vascular bed caused by isoproterenol-induced vasodilation, which is greater during saline than during propranolol.

**FIGURE 5.** Bar graphs show potentiating effect of exogenous angiotensin II (0.001 μg/100 ml forearm tissue/min for 5 minutes) on the vasoconstriction (lower panel) and norepinephrine (NA) outflow (upper panel) induced by lower body negative pressure (LBNP) application. Data are mean±SEM (n=10 and n=5, respectively). p<0.05 or less compared with saline.
Second, our finding of a significant angiotensin II extraction in forearm vasculature (about 20% fall in the venous-arterial ratio) in basal conditions is in agreement with previous results obtained in animals and humans. However, a more recent paper of Campbell and Kladis did not find a significant venous-arterial gradient for either angiotensin I or II, a finding confirmed by Admiraal et al. for angiotensin I. This discrepancy could be attributed to the fact that in our study, venous samples were collected from a deep vein of the forearm that essentially draws blood from muscle tissue, which is at variance with superficial veins (used in the two previously mentioned studies) that receive blood also from the skin. Therefore, it might be possible that different angiotensin II uptake exists in skin and muscle tissues. Third, as far as the relation between active and inactive renin is concerned, two main hypotheses have been proposed. The first is that inactive renin might be a precursor of the active form and, in the presence of an increased demand, the proportion converted to active renin is augmented. The second hypothesis is that the two forms of renin originate from two different and separate pools, that of active renin being more accessible and responsive to stimuli. Our data seem to support the second hypothesis since we have found that a higher isoproterenol dose is needed to stimulate inactive renin release and that the proportion of the two forms of renin was not modified by β-adrenergic receptor stimulation. Certainly a third recently proposed hypothesis suggesting that active and inactive renin (this last also called prorenin) represent two distinct substances, the first one operating as a circulating system and the second one as a tissue substance, seems to be unsupported by our results showing that active renin also is released in human forearm vasculature. Finally, although we have shown that active and inactive renin and angiotensin II are released from human forearm vasculature, we cannot assess whether they are locally synthesized or taken up from plasma.

Vascular Angiotensin II Modulation and Sympathetic Nervous System Activity: An Effect Mediated by the Facilitation of Presynaptic Release of Norepinephrine

In agreement with experimental data, our present study indicates that locally generated angiotensin II can increase sympathetic vasoconstriction by modulating presynaptic norepinephrine release. In fact, in our experimental conditions, isoproterenol causes a parallel increment of angiotensin II and norepinephrine release, an effect blunted by propranolol. When angiotensin II production was inhibited by captopril, the norepinephrine overflow induced by isoproterenol decreased, a finding that suggests a direct and potentiating effect of local angiotensin II on sympathetic-mediated neurotransmitter release. As hemodynamic consequence, the vasodilating effect of isoproterenol was increased in the presence of captopril, an effect probably due to the decreased local amount of angiotensin II and norepinephrine.

To confirm the facilitating effect of vascular angiotensin II on sympathetic neurotransmission, we reversed the experimental design: we infused exogenous angiotensin II at a dose titrated to increase plasma concentrations to values comparable with those obtained by the application of a physiological stimulus such as standing, and we evaluated its effect on endogenous sympathetic-mediated vasoconstriction and norepinephrine release obtained through the application of lower body negative pressure. Exogenous angiotensin II, which did not affect basal FBF, increased the vasoconstrictor response to lower body negative pressure compared with control conditions (saline), confirming previous data of Webb et al. What is original in our present study is the evidence that angiotensin II increased norepinephrine release during endogenous sympathetic activation, a fact that until now had only been inferred in humans.

Finally our data, showing that the vasoconstrictor effect of exogenous norepinephrine is unmodified by angiotensin II infusion, seem to exclude a postsynaptic potentiating action of angiotensin II on norepinephrine. Similar results were obtained by Webb et al., who infused only one norepinephrine dose; others claimed that angiotensin II exerts its facilitating action at the postsynaptic site. This discrepancy may originate from the fact that in these two studies, the facilitating postsynaptic effect of angiotensin II was indirectly inferred and directly shown after the systemic administration of captopril. In these experimental conditions, the results observed might have been influenced by either reflexogenic adjustments or central sympathetic nervous system effects.

In conclusion, our data show the existence of a tissue vascular RAS in human forearm arterioles and that one of the functions of this tissue RAS is the modulation of sympathetic nervous system activity, an effect exerted mainly through the facilitation of norepinephrine release at the presynaptic site. However, this evidence has been obtained in essential hypertensive persons; whether similar data can be found in normotensive individuals and in patients with secondary forms of hypertension is still to be evaluated.

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