Adenosine Causes the Release of Active Renin and Angiotensin II in the Coronary Circulation of Patients With Essential Hypertension

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OBJECTIVES
The aim of the study was to evaluate whether adenosine infusion can induce production of active renin and angiotensin II in human coronary circulation.

BACKGROUND
Adenosine can activate angiotensin production in the forearm vessels of essential hypertensive patients.

METHODS
In six normotensive subjects and 12 essential hypertensive patients adenosine was infused into the left anterior descending coronary artery (1, 10, 100 and 1,000 μg/min × 5 min each) while active renin (radioimmunometric assay) and angiotensin II (radioimmunoassay after high performance liquid chromatography purification) were measured in venous (great cardiac vein) and coronary arterial blood samples. In five out of 12 hypertensive patients adenosine infusion and plasma samples were repeated during intracoronary angiotensin-converting enzyme inhibitor benazeprilat (25 μg/min) administration. Finally, in adjunctive hypertensive patients, the same procedure was applied during intracoronary sodium nitroprusside (n = 4) or acetylcholine (n = 4).

RESULTS
In hypertensive patients, but not in control subjects, despite a similar increment in coronary blood flow, a significant (p < 0.05) transient increase of venous active renin (from 10.7 ± 1.4 [95% confidence interval 9.4 to 11.8] to a maximum of 13.8 ± 2.1 [12.2 to 15.5] with a consequent drop to 10.9 ± 1.8 [9.7 to 12.1] pg/ml), and angiotensin II (from 14.6 ± 2.0 [12.7 to 16.5] to a maximum of 20.4 ± 2.7 [18.7 to 22.2] with a consequent drop to 16.3 ± 1.8 [13.9 to 18.7] pg/ml) was observed under adenosine infusion, whereas arterial values did not change. Calculated venous–arterial active renin and angiotensin II release showed a strong correlation (r = 0.78 and r = 0.71, respectively; p < 0.001) with circulating active renin. This adenosine-induced venous angiotensin II increase was significantly blunted by benazeprilat. Finally, both sodium nitroprusside and acetylcholine did not affect arterial and venous values of active renin and angiotensin II.

CONCLUSIONS
These data indicate that exogenous adenosine stimulates the release of active renin and angiotensin II in the coronary arteries of essential hypertensive patients, and suggest that this phenomenon is probably due to renin release from tissue stores of renally derived renin.

Since the original description of the classical components of the renin–angiotensin system (RAS) in the vessel wall, considerable experimental evidence has suggested that the RAS can act not only as a systemic, but also as a local vascular system (1–5). More recently, the existence of a vascular RAS has been proposed in human hypertension. It has been demonstrated that beta-adrenergic receptor stimulation causes the release of active and inactive renin and angiotensin II in the forearm vessels of hypertensive patients (6–10).

Adenosine is an endogenous substance that induces renin release in in vitro models through the activation of its A2 receptors (11). This evidence has been confirmed in humans by the finding that intrabrachial infusion of exogenous adenosine causes the release of angiotensin II in the forearm of essential hypertensive patients (12). Because adenosine is synthesized in the myocardium, where it plays an important role in the control of coronary circulation (13,14), the present study was designed to evaluate whether exogenous adenosine can stimulate a vascular RAS in human coronary arteries. To investigate this issue, in normotensive subjects and essential hypertensive patients, adenosine was infused into the left anterior descending coronary artery (LAD); simultaneously, sampling for determination of the values of active renin and angiotensin II, the main components of the RAS.
RAS cascade, was collected from the LAD and the great cardiac vein to calculate the net balance, that is, release or uptake, of these substances through the coronary circulation.

METHODS

The study population included 6 normotensive control subjects and 20 matched mild to moderate uncomplicated essential hypertensive patients. Subjects with diabetes mellitus, hypercholesterolemia (total cholesterol greater than 5.2 mmol/liter), cerebral ischemic vascular disease, impaired renal function or a smoking history of more than five cigarettes per day were excluded from the study. Control subjects were defined as normotensive according to the absence of a familial history of essential hypertension and blood pressure values below 140/90 mm Hg. All essential hypertensive patients recruited reported the presence of a chest pain syndrome. Inclusion criteria were the positivity on a Virdis et al. Adenosine and Renin–Angiotensin System

METHODS

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Experimental procedure. Diagnostic left heart catheterization and coronary angiography were performed by a standard percutaneous femoral approach, in the angiographic laboratory of the Institute of Clinical Physiology of Pisa. After completion of diagnostic catheterization, 10,000 U of heparin were given intravenously and a temporary pacing wire was advanced in the right ventricle from the femoral vein. A 7-F catheter was inserted in a left arm vein and positioned in the great cardiac vein. An 8-F guiding catheter was positioned in the left coronary ostium. A 20-MHz pulsed Doppler crystal mounted on the tip of a 3-F infusion catheter (Millar Instruments, Houston, Texas) was advanced through the guiding catheter into the proximal segment of the LAD. The Doppler catheter was connected to a photographic multichannel oscillographic recorder (Ote-Biomedica, Firenze, Italy) to display phasic and mean velocity waveforms. Before beginning the experimental protocol, the position of the Doppler flow velocity catheter and the range gate control were adjusted to optimize the audio flow velocity signal and the phasic flow velocity waveform. The Doppler catheter position and range gate control were not changed thereafter.

Experimental design. To evaluate the possibility that adenosine could activate a vascular RAS in 12 essential hypertensive patients and six normotensive subjects, adenosine was infused into the LAD at increasing cumulative doses (1, 10, 100 and 1,000 μg/min × 5 min each) in the presence of intracoronary saline (0.4 ml/min), started 10 min earlier. Simultaneous arterial and venous samples for active renin and angiotensin II were collected basally, at the end of saline infusion and at the end of each adenosine dose. To confirm the local origin of angiotensin II, in the final five out of the 12 hypertensive patients adenosine was repeated in the presence of benazepril, an angiotensin-converting enzyme (ACE) inhibitor, infused into the LAD at the rate of 25 μg/min, started 15 min before adenosine and continued throughout. Arterial and venous samples for active renin, ACE and angiotensin II were again simultaneously collected at the same time intervals as previously.

Finally, in two adjunctive groups of essential hypertensive patients (n = 4 each), the effect of sodium nitroprusside (10, 20 and 30 μg/min × 5 min each) or acetylcholine (15, 45 and 150 μg/min × 5 min each) was also evaluated. Patients were allocated to the different treatment groups in a sequential order.

Analytical procedures. Active renin (pg/ml) was measured by radioimmunometric assay using a kit from IRMA Pasteur (ERIA Diagnostic, Pasteur, Marnes La Coquette, France) (15). In our laboratory the intra-assay and interassay variation coefficient is 9.69% (n = 20) and 14.5% (n = 6), respectively.

Angiotensin II (pg/ml) was determined by radioimmu-
Table 1. Characteristics of Normotensive Subjects and Essential Hypertensive Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotensive Subjects (n = 6)</th>
<th>Hypertensive Patients (n = 20)</th>
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<tr>
<td>Age (yr)</td>
<td>45.9 ± 6.3 (41.7 to 48.3)</td>
<td>47.8 ± 5.2 (45.4 to 50.2)</td>
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<td>Gender (male/female)</td>
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<td>15/5</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7 ± 1.3 (21.4 to 24.1)</td>
<td>23.1 ± 1.9 (22.2 to 24.1)</td>
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<td>Systolic blood pressure (mm Hg)</td>
<td>123.9 ± 4.6 (117.7 to 130.3)</td>
<td>158.9 ± 8.2 (154.9 to 162.9)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>81.3 ± 2.5 (78.5 to 83.9)</td>
<td>102.8 ± 3.7 (101.1 to 104.6)</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>61.5 ± 4.7 (56.4 to 66.9)</td>
<td>63.9 ± 4.1 (62.1 to 65.9)</td>
</tr>
<tr>
<td>Active renin (pg/ml)</td>
<td>5.9 ± 1.1* (4.4 to 7.2)</td>
<td>10.5 ± 2.2 (9.5 to 11.6)</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>8.2 ± 2.9* (4.6 to 11.9)</td>
<td>14.4 ± 2.4 (13.1 to 15.4)</td>
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<tr>
<td>Urinary Na excretion (mEq/24 h)</td>
<td>75.3 ± 9.8 (63.5 to 87.3)</td>
<td>78.5 ± 8.6 (74.3 to 82.4)</td>
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<td>Plasma total cholesterol (mmol/liter)</td>
<td>4.56 ± 0.5 (3.98 to 5.13)</td>
<td>4.62 ± 0.4 (4.38 to 4.73)</td>
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<tr>
<td>Plasma HDL cholesterol (mmol/liter)</td>
<td>1.24 ± 0.3 (0.96 to 1.65)</td>
<td>1.16 ± 0.1 (1.03 to 1.18)</td>
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<tr>
<td>Plasma LDL cholesterol (mmol/liter)</td>
<td>2.64 ± 0.3 (2.28 to 2.91)</td>
<td>2.81 ± 0.3 (2.63 to 2.90)</td>
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<tr>
<td>Plasma glucose (mmol/liter)</td>
<td>4.82 ± 0.2 (4.6 to 5.1)</td>
<td>4.91 ± 0.3 (4.77 to 5.05)</td>
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*p < 0.05 or less vs. essential hypertensive patients. Data are reported as mean ± SEM with 95% confidence interval in parentheses.

HDL = high density lipoprotein; LDL = low density lipoprotein; Na = sodium.

no assay after extraction of the peptide from plasma by Sep-Pak C18. This procedure was previously described (6) and validated by measurement with high performance liquid chromatography of purified angiotensin II (8). In our laboratory, the plasma level of purified angiotensin II in healthy subjects is 13.4 ± 7.9 pg/ml (range, 3.3 to 34.8 pg/ml). Serum ACE activity (nmol/min/ml) was measured by a radioenzymatic method (16) and hematocrit by a micromethod. Samples from each patient were measured in the same assay for active renin and angiotensin II, respectively.

**Quantitative coronary angiography.** Quantitative coronary angiography was performed to convert flow velocity to an estimate of coronary blood flow (CBF). The proximal LAD was filmed in right anterior oblique projection injecting 6 to 12 ml of nonionic contrast medium at a rate of 5 to 10 ml/s. Quantitative angiographic analysis was performed with a MIPRON medical image processing unit (Kontron Instruments, Munich, Germany) to obtain the cross-sectional area of the arterial segment 2 to 4 mm distal to the Doppler tip. The tip of the guiding catheter filmed in the same projection at the center of the screen, free from contrast medium and before Doppler probe insertion, was used as calibration. An estimate of CBF was obtained by multiplying the mean coronary flow velocity, as measured directly by the Doppler catheter, by the vessel cross-sectional area.

**Data analysis.** Net balance of active renin (pg/min) and angiotensin II (pg/min) was obtained as the product of the venous–arterial plasma concentration gradient and coronary plasma flow (CBF × 1-hematocrit) (17). Raw data were analyzed by analysis of variance for repeated measures, and Scheffé test was applied for multiple comparison testing. Correlation coefficients were calculated using Pearson test. Results are expressed as mean ± SEM and 95% confidence interval.

**Drugs.** Adenosine (Sigma, Milan, Italy), sodium nitroprusside (Malesci, Milan, Italy), acetylcholine HCl (Farmigea S.p.A., Pisa, Italy) and benazeprilat (Novartis, Origgio [VA], Italy) were obtained from commercially available sources. Adenosine, acetylcholine and benazeprilat were diluted in saline to the desired concentrations; sodium nitroprusside, diluted in glucosate solution, was protected from light by aluminum foil.

**RESULTS**

Baseline demographic, hemodynamic and humoral characteristics for normotensive subjects and essential hypertensive patients are summarized in Table 1. The two groups were closely matched for age, body mass index, heart rate, urinary sodium excretion, plasma cholesterol and glucose. Normotensive subjects showed circulating values of active renin and angiotensin II significantly lower as compared with those of hypertensive patients. At the time of the study all patients had reached a constant sodium excretion rate (Table 1).

**Adenosine-mediated vascular RAS activation.** In normotensive control subjects the infusion of adenosine caused a dose-dependent increment in CBF, plateauing at the last dose (from 24.7 ± 2.6 [21.5 to 28] to 25 ± 3.2 [19.9 to 28.1], 53.1 ± 19.1* [29.2 to 76.8], 94.3 ± 24.1* [64.3 to 124.3] and 104.3 ± 13.1* [88.1 to 120.7] ml/min; *p < 0.001 vs. basal). During adenosine administration blood pressure and heart rate did not change significantly (Table
2). Throughout the experiment, adenosine infusion did not affect either arterial or venous values of active renin (artery: from 6.1 ± 1.0 [4.7 to 7.3] to 5.8 ± 1.2 [4.7 to 7.4], 5.9 ± 0.8 [4.9 to 6.9], 5.5 ± 0.8 [4.4 to 6.6] and 6.08 ± 0.6 [5.3 to 6.9] pg/ml; p = NS; vein: from 5.9 ± 1.1 [4.6 to 7.4] to 6.0 ± 0.9 [4.7 to 7.7], 5.6 ± 0.9 [4.4 to 6.8], 5.7 ± 1.0 [4.4 to 7.1] and 6.0 ± 0.4 [5.4 to 6.6] pg/ml; p = NS). When data were expressed as net balance, it was confirmed that adenosine failed to cause local active renin release (from −2.7 ± 2.7 [−4.1 to −0.7] to 5.28 ± 5.2 [−5.6 to −15.8], −16.9 ± 6.4 [−42.1 to 28.6], −22.6 ± 6.8 [−77.5 to 44.6] and −20.8 ± 8.9 [−66.3 to 45.6] pg/min). During adenosine infusion, arterial and venous values of angiotensin II did not significantly change (artery: from 10.9 ± 2.1 [7.5 to 13.6] to 10.1 ± 1.8 [7.9 to 12.4], 11.3 ± 2.9 [7.7 to 15.2], 10.8 ± 2.3 [7.9 to 13.7] and 10.3 ± 3.5 [5.9 to 14.7] pg/ml; vein: from 11.0 ± 2.5 [6.7 to 15.5] to 10.1 ± 3.1 [6.2 to 13.9], 11.2 ± 2.9 [7.6 to 14.8], 10.3 ± 2.2 [7.5 to 13.1] and 9.8 ± 3.0 [6.0 to 13.6] pg/ml). Similar to active renin, angiotensin II net balance was unaffected by adenosine (from −4.8 ± 3.7 [−9.3 to −0.5] to −2.4 ± 3.2 [−6.3 to 1.2], −8.4 ± 4.4 [−17.3 to −2.9] and −22.6 ± 7.8 [−54.2 to 29.8] pg/min).

In essential hypertensive patients the infusion of adenosine caused a dose-dependent increment in CBF which was similar to that observed in normotensive control subjects (from 22.0 ± 4.5 [18.5 to 25.9] to 28.9 ± 6.1 [21.5 to 37.9], 50.8 ± 15.5* [35.4 to 69.5], 107.4 ± 20.2* [85.4 to 121.4] and 115.1 ± 19.8* [91.2 to 127.6] ml/min; *p < 0.001 vs. basal). During adenosine administration blood pressure and heart rate did not change significantly (Table 2). Intracoronary adenosine infusion did not affect arterial values of active renin. In contrast, intracoronary adenosine significantly (p < 0.05) increased venous active renin at 1 and 10 μg/min; its effect started to decline at 100 μg/min and was exhausted at 1,000 μg/min (Fig. 1). Data expressed as net balance indicate that adenosine caused a dose-dependent release of active renin at the first two doses (from −0.65 ± 1.3 [−2.5 to 1.4] to 64.7 ± 9.3* [42.5 to 83.5] and 146.9 ± 23.8* [108.4 to 199.2] pg/min; *p < 0.001 vs. basal) which decreased at the greater infusion rates (86.1 ± 18.8* [51.5 to 117.4] and 2.3 ± 3.2 [−2.6 to 4.9] pg/min; p < 0.001 vs. basal) (Fig. 2), suggesting that over three 4-min periods, approximately 1.90 pg of active renin was worked out from the myocardium by the effects of adenosine.

Adenosine administration did not modify arterial values of angiotensin II, whereas it dose-dependently increased venous angiotensin II at the first three doses. At the fourth dose, venous angiotensin II declined but was still significantly greater as compared with baseline and with the corresponding arterial values (Fig. 1). Thus, in terms of net balance, adenosine caused a dose-dependent release of angiotensin II at the 1, 10 and 100 μg/min infusion rates,
which decreased at 1,000 μg/min (from $-7.8 \pm 2.1$ to $13.2$) to 50.8 ± 4.7* [36.4 to 71.9], 139.7 ± 24.7* [97.4 to 172.6] 616 ± 47.9* [312.5 to 944.8] and 198.1 ± 27.5* [101.6 to 291.4] pg/min; *p < 0.001 vs. basal) (Fig. 2). Active renin and angiotensin II release induced by exogenous adenosine infusion in coronary vessels (calculated as the area under the curve of venous–arterial differences) showed a positive correlation (r = 0.73; p < 0.01). Moreover, when maximal individual increments of active renin and angiotensin II induced by adenosine were tested versus the basal circulating renin profile, we found a significant correlation between local vascular active renin (r = 0.78; p < 0.001), angiotensin II (r = 0.71; p < 0.001) release and circulating active renin.

**Effect of ACE inhibition on adenosine-mediated vascular RAS activation.** As in the previous experiment, in this subgroup (n = 5) of essential hypertensive patients adenosine also caused a similar dose-dependent increment in CBF, plateauing at the last dose (from 21.4 ± 4.5 [15.7 to 27.1] to 30.2 ± 7 [21.4 to 38.9], 53.3 ± 19.3* [29.3 to 77.3], 105.1 ± 12.1* [89.9 to 120.2] and 111.7 ± 8.0* [101.7 to 121.6] ml/min; *p < 0.001 vs. basal), without affecting blood pressure and heart rate (data not shown). Likewise, adenosine infusion did not modify arterial levels of active renin, whereas it increased venous values at the first two doses, an effect that declined at 100 and 1,000 μg/min (Fig. 3A, left). Thus, similarly to the previous experiment, the net balance showed that adenosine induced a dose-dependent release of active renin at the first two doses, which decreased during the higher infusion rates (from $-15.9 \pm 2.1$ [−30.6 to 16.9] to 44.4 ± 5.9* [27.4 to 68.6], 196 ± 32.7* [127.3 to 251.4], 60.9 ± 18.9* [21.5 to 99.3] and 7.8 ± 3.5 [1.5 to 14.4] pg/min; *p < 0.001 vs. basal). Arterial angiotensin II values did not change under adenosine infusion, whereas venous angiotensin II significantly increased at the first three doses and declined at the highest rates (Fig. 3B, left). Again, in terms of net balance, adenosine caused a dose-dependent release of angiotensin II at the 1-, 10-, and 100-μg/min infusion rates, which decreased at 1,000 μg/min (from $-7.7 \pm 2.5$ [−15.3 to 3.9] to 79.4 ± 6.3* [34.5 to 131.6], 150.3 ± 29.7* [91.4 to 201.6], 630 ± 54.1* [374.5 to 922.3] and 127.1 ± 19.5* [83.7 to 189.3] pg/min; *p < 0.001 vs. basal).

When benazeprilat was infused, arterial ACE activity did not change (from 88.6 ± 4.1 to 89.4 ± 3.8 nmol/ml/min; p = NS), whereas venous ACE activity was suppressed (from 89.3 ± 3.4 to 13.7 ± 2.8 nmol/ml/min; p < 0.05 vs. basal). Benazeprilat infusion did not significantly modify either basal CBF (from 18.0 ± 3.0 [14.2 to 21.8] to 17.6 ± 2.3 [14.7 to 20.5] ml/min; p = NS) or the increment in CBF induced by adenosine (from 17.6 ± 2.3 to 26.9 ± 5.2 [20.3 to 33.4], 60.2 ± 15.7 [40.7 to 79.7], 98.0 ± 19.8 [73.4 to 122.6] and 116.8 ± 14.5 [98.7 to 134.8] ml/min).

Figure 2. Bars show net balance of active renin (A.R.) and angiotensin II (Ang II) in 12 essential hypertensive patients. Data are shown as mean ± SEM. *p < 0.05 or less versus basal.

Figure 3. (A) Line graphs show arterial (open circles) and venous (solid circles) concentrations of active renin (A.R.) in basal conditions (left) and in the presence of benazeprilat infusion (BEN, 25 μg/min, right) in five essential hypertensive patients. Data are shown as mean ± SEM. *p < 0.05 or less. (B) Line graphs show arterial (open circles) and venous (solid circles) concentrations of angiotensin II (Ang II) in basal conditions (left) and in the presence of benazeprilat infusion (right) in five essential hypertensive patients. Data are shown as mean ± SEM. *p < 0.05 or less.
Arterial active renin values were unaffected, either when benazeprilat was infused alone or when it was confounded with adenosine. In contrast, venous active renin values were increased by benazeprilat infusion, without further modification during adenosine coinfusion (Fig. 3A, right). Finally, benazeprilat significantly (p < 0.005) reduced basal angiotensin II venous values and abolished the adenosine-mediated venous angiotensin II increments, without changing arterial concentrations (Fig. 3B, right).

Effect of sodium nitroprusside and acetylcholine on vascular RAS activation. In four adjunctive essential hypertensive patients the infusion of sodium nitroprusside caused a dose-dependent increment in CBF comparable to that induced by adenosine (from 25.4 ± 9.1 [16.5 to 31.4] to 42.5 ± 11* [31.5 to 55.7], 72.5 ± 17.4* [49.5 to 87.2] and 103.8 ± 24.2* [92.5 to 112.6] ml/min; *p < 0.001 vs. basal) without affecting either arterial or venous values of active renin (artery: from 9.5 ± 2.3 [6.4 to 13.2] to 10.2 ± 1.8 [6.9 to 14.1], 9.7 ± 2.4 [7.1 to 12.9] and 9.8 ± 2.1 [7.5 to 13.3] pg/ml; p = NS; vein: from 9.5 ± 2.2 [7.5 to 13.1] to 10.2 ± 1.8 [8.2 to 13.5], 9.7 ± 2.4 [6.8 to 12.8] and 9.3 ± 2.4 [6.5 to 13.1] pg/ml; p = NS) or angiotensin II (artery: from 13.1 ± 1.8 [10.1 to 15.1] to 13.5 ± 2.8 [9.8 to 15.7], 12.6 ± 3.0 [9.8 to 14.2] and 13.1 ± 4.2 [10.5 to 17.9] pg/ml; p = NS; vein: from 13.5 ± 1.3 [9.5 to 15.8] to 13.3 ± 2.5 [10.5 to 15.9], 13.2 ± 2.3 [9.8 to 15.7] and 13.2 ± 1.6 [10.1 to 15.4] pg/ml; p = NS). Similarly, in one adjunctive subgroup (n = 4) of essential hypertensive patients the infusion of acetylcholine induced a dose-dependent increment in CBF comparable to that induced by adenosine (from 18.3 ± 2.4 [18.3 to 25.4] to 37.3 ± 9.7* [21.5 to 49.8], 64.6 ± 13.7* [50.1 to 75.9] and 108.1 ± 23.5* [93.5 to 117.2] ml/min; *p < 0.001 vs. basal). Throughout the experiment, acetylcholine administration did not affect either arterial or venous values of active renin (artery: from 11.1 ± 1.2 [9.1 to 12.7] to 10.9 ± 0.5 [9.5 to 12.9], 10.5 ± 1.3 [9.1 to 13.1] and 11.3 ± 1.6 [9.5 to 12.8] pg/ml; p = NS; vein: from 12.5 ± 2.9 [10.1 to 14.5] to 13.4 ± 3.0 [11.4 to 15.8], 12.5 ± 3.3 [10.4 to 15.2] and 13.0 ± 2.9 [10.7 ± 16.1] pg/ml; p = NS) or angiotensin II (artery: from 13.2 ± 1.3 [11.4 to 15.1] to 13.0 ± 1.2 [11.5 to 14.9], 13.4 ± 1.3 [10.9 to 14.8] and 13.1 ± 1.5 [10.9 to 15.9] pg/ml; p = NS; vein: from 13.9 ± 1.2 [11.5 to 16.5] to 14.9 ± 2.8 [12.5 to 17.1], 13.9 ± 3.6 [11.3 to 16.2] and 14.2 ± 2.9 [12.5 to 17.1] pg/ml; p = NS). Aortic blood pressure and heart rate were not modified by sodium nitroprusside and acetylcholine infusion (data not shown).

DISCUSSION

The present data indicate that in patients with essential hypertension, but not in normotensive subjects, intracoronary infusion of adenosine causes an increase in active renin and angiotensin II in the coronary circulation, an effect which is blocked by intracoronary administration of the ACE inhibitor benazeprilat. These results suggest that adenosine could activate angiotensin II production in the coronary circulation of patients with essential hypertension. This finding is in line with previous observations obtained in the forearm vasculature of essential hypertensive patients (12) indicating that intrabrachial adenosine caused local release of angiotensin II, an effect blunted by either theophylline, an adenosine receptor antagonist, or the ACE inhibitor captopril.

Adenosine-induced vascular RAS activation. In the coronary circulation the effect of adenosine on the release of active renin and angiotensin II seems to be specific, since two chemically unrelated vasodilators such as sodium nitroprusside, acting directly on smooth muscle cells, or acetylcholine, a muscarinic agonist, did not modify coronary active renin and angiotensin II net balance despite a vasodilation comparable to that induced by adenosine. Moreover, activation of systemic RAS induced by our experimental intervention can be reasonably excluded by the lack of modifications in blood pressure, heart rate and arterial active renin and angiotensin II. It is therefore conceivable that the increase in active renin and angiotensin II observed in the coronary LAD during adenosine infusion could be determined by a direct effect of the purine compound on the vessel wall. This possibility is reinforced by the series with benazeprilat. Thus, the ACE inhibitor, when directly infused into the LAD, caused local but not systemic blockade of both basal and adenosine-mediated production of angiotensin II, further suggesting that the peptide is synthesized inside the coronary circulation.

Adenosine-stimulated active renin release depends upon the subtype of adenosine receptor involved, since stimulation of A₁- or A₂-adenosine receptors leads to inhibition and increase of renin release, respectively (11,18). In agreement with this possibility, the vasodilator response induced in anesthetized rats by selective activation of A₂-adenosine receptors was potentiated by losartan, an angiotensin II receptor antagonist, suggesting that A₂-mediated RAS stimulation participates in vascular control (19).

Concerning the mechanism through which adenosine could activate the RAS, available evidence indicates that compounds that increase cyclic adenosine monophosphate, such as adenosine or beta-adrenoceptor agonists, can stimulate the release of active renin in various experimental models (20). However, this mechanism seems to operate exclusively on renally derived renin (20).

Relationship between circulating and vascular RAS. Although a recent review of experimental evidence raised some doubts concerning the local synthesis of renin in heart and extrarenal blood vessels, favoring a renal origin of the tissue enzyme (21), at the present time this question is still debated (22–24). It has however been demonstrated in the heart of intact pigs that vascular active renin is taken up from the circulation into the tissue interstitium (25) and in the forearm of essential hypertensive patients that release of vascular active renin is strictly correlated to the profile of
Behavior of coronary blood flow and vascular RAS. In the present study, the maximal individual local active renin and angiotensin II release showed a significant positive correlation with the circulating renin profile. Thus it can be speculated that even in the coronary vessels of patients with essential hypertension, vascular active renin is taken up from circulating blood. In line with this possibility is the finding that adenosine does not activate the RAS in normotensive subjects, who show a lower circulating RAS profile as compared with essential hypertensive patients. Nevertheless, whether locally synthesized renin also contributes to adenosine-mediated angiotensin II production cannot be ascertained by the present experimental design.

In the present report active renin was evaluated by radioimmunoassay (15). This is at variance with the common knowledge that the most popular method for assessing RAS activity is the enzymatic assay, based on the quantification of angiotensin I generated by the reaction between renin and its substrate (26). However, this classical method, although very convenient for estimating the action of the renin system, has several methodologic difficulties. The recently developed immunometric assay of renin potentially overcomes the limitations of the enzymatic assay because the use of monoclonal antibodies allows the direct quantification of the active form of the enzyme. This possibility is substantiated by the demonstration of a greater reproducibility of the immunometric as compared with the enzymatic assay. Finally, a very high correlation exists between the two methods in different experimental conditions (8,15).

Whatever the origin of vascular active renin, the present observation that adenosine increases active renin and angiotensin II concentrations only in the coronary circulation, without changing arterial values, indicates that even if vascular renin is taken up from plasma, it can be activated to produce angiotensin II independently of the circulating RAS. This in turn suggests that the cardiac renin–angiotensin pathway can be considered a local vascular system, at least from a functional point of view.

Behavior of coronary blood flow and vascular RAS. A discrepancy exists between the behavior of CBF, active renin and angiotensin II during adenosine infusion. Whereas CBF increases in a dose-dependent manner to reach a plateau at the last infusion rate of adenosine, the release increments of both active renin and angiotensin II are transient. This finding seems to indicate that despite a maximal stimulation of adenosine receptors, activation of the local RAS is a short-lasting event that rapidly declines. Such an observation is in agreement with previous data from our laboratory (8), which indicate that a prolonged (60 min) infusion of isoproterenol, despite causing a stable increase in forearm blood flow, leads to a rapid but short-lasting release of plasma renin activity and angiotensin II in the forearm of patients with essential hypertension. The present data therefore seem to reinforce the hypothesis that renin is stored in vessel walls as a pool that can be easily activated to produce angiotensin II, but is then immediately exhausted. Finally the delay in production of angiotensin II as compared with active renin is likely due to a time lag in increasing local concentrations of angiotensinogen or ACE.

Conclusions. In conclusion, the present data indicate that exogenous adenosine causes the release of active renin and angiotensin II from the coronary vessels of patients with essential hypertension. This finding may have potential clinical relevance, considering the beneficial effect of ACE inhibitors in experimental myocardial ischemia (27–29) and in patients with acute myocardial infarction (30–36). It is tempting to speculate that adenosine produced by hypoxic tissue during myocardial ischemia can reach coronary blood (37) and activate angiotensin II production. Moreover, vascular angiotensin II could also be important in the pathogenesis of cardiac remodeling, a pathologic condition that can be reversed by ACE inhibitors (38–42). However, further investigations are needed to demonstrate this hypothesis.

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REFERENCES
13. McKenzie JE, McCoy FP, Bockman EL. Myocardial adenosine and


40. Devereux RB, Dahlof B, Levy D, Pfeffer MA. Comparison of enalapril vs nifedipine to decrease left ventricular hypertrophy in systemic hypertension (the PRESERVE trial). Am J Cardiol 1996;78:61–5.


42. Devereux RB. Do antihypertensive drugs differ in their ability to regress left ventricular hypertrophy? Circulation 1997;95:1983–5.