Adenosine Activates a Vascular Renin-Angiotensin System in Hypertensive Subjects

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In vitro data indicate that the activation of $\Lambda_2$ adenosine receptors increases renin release by the accumulation of cyclic AMP. Because in human forearm vessels $\beta$-adrenergic receptor stimulation causes the local release of renin and angiotensin II through the increase of cyclic AMP, we evaluated in six essential hypertensive subjects whether adenosine can release vascular angiotensin II. Adenosine was infused into the brachial artery at cumulatively increasing doses (0.5, 1.5, and 5 $\mu$g/100 ml forearm tissue per minute for 5 minutes each) during saline infusion and in the presence of the adenosine antagonist theophylline (100 $\mu$g/100 ml forearm tissue per minute for 15 minutes), while venous (ipsilateral deep forearm vein) and arterial (brachial artery) angiotensin II (picograms per milliliter) were measured at the end of each infusion period, and forearm angiotensin II net balance (picograms per minute) was calculated by venous-arterial differences corrected for forearm blood flow (strain-gauge plethysmography) and hematocrit. In control conditions, adenosine, at higher doses, caused a dose-dependent vasodilation and increased venous angiotensin II without affecting arterial values; therefore, the calculated angiotensin II net balance showed an adenosine-mediated dose-dependent release. Theophylline pretreatment blunted adenosine-mediated forearm blood flow increments and angiotensin II release. The local origin of angiotensin II was further confirmed in another group of six hypertensive subjects in whom the angiotensin converting enzyme inhibitor captopril, locally infused at the rate of 2.5 $\mu$g/100 ml forearm tissue per minute for 15 minutes, abolished the adenosine-mediated venous angiotensin II increments. Our data indicate that exogenous adenosine can stimulate the production of angiotensin II in the forearm vessels of hypertensive patients.

(Hypertension 1992;19:672-675)

KEY WORDS • adenosine • angiotensin II • theophylline • captopril

We recently demonstrated the existence of a tissue vascular renin-angiotensin system (RAS) in humans by showing that $\beta$-adrenergic receptor stimulation causes the release of active and inactive renin and of angiotensin II in the forearm arterioles of hypertensive patients. This effect of $\beta$-adrenergic receptors on the tissue RAS is probably related to the intracellular accumulation of cyclic AMP (cAMP), a well-known stimulus for active renin release.

Adenosine is an endogenous substance that, in in vitro models, can activate intracellular cAMP and therefore induce renin release through the stimulation of its specific $\Lambda_2$-receptors coupled to adenylate cyclase. Therefore, we decided to evaluate whether exogenous adenosine can stimulate the release of angiotensin II in the forearm of hypertensive patients.

Methods

Subjects

Eighteen inpatients (10 men and eight women; age, 46.3±9.2 years [mean±SD]) with mild-to-moderate, uncomplicated essential hypertension (171.4±12.4/106.1±9.6 mm Hg) took part in the studies. In accordance with institutional guidelines, all subjects were aware of the investigational nature of the study and consented to it. The subjects were admitted to the ward and maintained on a constant sodium (80-100 mmol/day) and potassium chloride (60-80 mmol/day) intake. Patients abstained from any treatment for at least 1 week and from methylxanthines and alcohol for 48 hours before the study.

Experimental Procedure

All studies were performed at 8 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 connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, Colo.) for systemic mean blood pressure (pulse pressure+diastolic pressure) and heart rate monitoring (model VSM1, Physiocor, Redmond, Wash.) for intra-arterial infusions. Another cannula (6 cm long) was advanced into a deep forearm vein and connected through stopcocks to a pressure transducer (model MS20, Electromedics, Englewood, Colo.) for systemic mean blood pressure (pulse pressure+diastolic pressure) and heart rate monitoring (model VSM1, Physiocor, Redmond, Wash.) for intra-arterial infusions. Another cannula (6 cm long) was advanced into an ipsilateral deep forearm vein retrogradely. Forearm blood flow (FFB) was measured in both forearms (experimental and contralateral) by strain-gauge venous plethysmography (LOOSCO, GLOOS, Amsterdam, The Netherlands). Arterial and deep venous blood were sampled simultaneously at time intervals (1) basal; 2) after 10 minutes of saline infusion; 3, 4, 5) after 5 minutes of each adenosine dose infusion; 6) basal; 7) after 15 minutes of...
Adenosine-mediated vascular tissue angiotensin II activation. In two different groups of six hypertensive patients, two cumulative dose–response curves to adenosine (lower curve, 0.01, 0.03, and 0.1 μg/100 ml forearm tissue per minute for 5 minutes each; higher curve, 0.5, 1.5, and 5 μg/100 ml forearm tissue per minute for 5 minutes each) were generated in combination with either saline (0.2 ml/min for 15 minutes) or adenosine receptor blockade through the specific antagonist theophylline (100 μg/100 ml forearm tissue per minute for 15 minutes). Arterial and venous samples for angiotensin II were collected at basal conditions and at the end of each infusion period.

The dose of theophylline was chosen on the basis of previous data showing its effectiveness in antagonizing the vascular effect of adenosine in human forearm. Effect of angiotensin converting enzyme inhibition on adenosine-mediated vascular angiotensin II generation. To further demonstrate the local vascular origin of angiotensin II, in another group of six patients, the dose–response curve to adenosine, at the higher previous rates, was obtained under basal conditions and then in the presence of captopril, an angiotensin converting enzyme (ACE) inhibitor, infused into the brachial artery at the rate of 2.5 μg/100 ml forearm tissue per minute for 15 minutes. Arterial and venous samples for angiotensin II were again collected during the experiment at the same previous time intervals.

Analytical procedures. Plasma angiotensin II (picograms per milliliter) was determined by radioimmunoassay after extraction of the peptide from plasma by Sep-Pak C18. Details concerning the method have already been described. Serum ACE activity (nanomoles per minute per milliliter) was measured by a radioenzymatic method and hematocrit by a micromethod.

Data analysis. Net forearm balance of angiotensin II was obtained as the product of the venous-arterial plasma concentration gradient and forearm plasma flow (FBF x 1–hematocrit). Because arterial pressure did not significantly change during the study, all data were analyzed in terms of FBF, and FBF increments were taken as evidence of local vasodilation. Raw data were analyzed by two- or three-way analysis of variance (ANOVA), and Duncan's test was applied for multiple comparison testing. Wilcoxon's test was used to check the statistical significance of the difference between nonparametric values. Results were expressed as mean±SEM.

Drugs

Adenosine (Sigma, Milan, Italy), theophylline ethylendiamine (Aminomal, Recordati, Milan, Italy), and captopril HCl (Capoten, Bristol Meyer-Squibb, Rome, Italy) were obtained from commercially available sources and diluted in fresh solutions to the desired concentrations by the addition of normal saline.

Results

At the time of the study, all patients had reached a constant sodium excretion rate (82.8±13.4 mmol/24 hr).

Adenosine-Mediated Vascular Tissue Angiotensin II Activation

At lower rates, in control conditions, adenosine caused a dose-dependent vasodilation (FBF from 2.8±0.5 to 3.5±0.8, 4.4±1.2, and 6.6±1.8 ml/100 ml forearm tissue per minute; p<0.01 versus basal values), an effect significantly (p<0.001) blunted by theophylline (from 2.9±0.5 to 2.9±0.5, 2.7±0.4, and 3.3±0.6 ml/100 ml forearm tissue per minute). At these doses adenosine did not significantly change angiotensin II (artery, from 9.7±3.2 to 9.5±3.3, 9.3±3.2, and 8.9±3.2 pg/ml; vein, from 9.9±3.3 to 9.6±3.3, 9.5±3.3, and 9.4±3.5 pg/ml).

At the higher infusion rates, the vasodilating effect of adenosine was obviously greater (from 3.8±0.9 to 7.7±1.4, 11.9±2.5, and 13.1±3.6 ml/100 ml forearm tissue per minute; p<0.001 versus basal values), an effect again significantly (p<0.001) blunted by theophylline pretreatment (from 4.0±1.0 to 3.9±1.0, 4.1±1.1, and 5.8±1.9 ml/100 ml forearm tissue per minute). In both experiments, contralateral FBF was not affected by drug infusion.

During basal conditions, arterial levels of angiotensin II (14.7±3.2 pg/ml) were higher than venous ones (13.8±2.9 pg/ml) (Figure 1A). When adenosine was infused, arterial angiotensin II did not change (15.2±3.8, 15.9±3.4, and 15.3±3.7 pg/ml) (Figure 1A), whereas venous values significantly (p<0.001 versus basal values) increased (20.3±4.1, 24.7±4.6, and 24±4.1 pg/ml) (Figure 1A), an effect that was clearly blunted by theophylline (artery, from 15.5±3.9 to 15.3±3.8, 15.0±3.8, and 15.2±3.7 pg/ml; vein, from 14.7±3.6 to 15.1±3.8, 14.8±3.6, and 18±3.6 pg/ml) (Figure 1A). When net forearm balance is calculated, it is evident that adenosine reversed the basal negative arteriovenous balance of angiotensin II (−1.4±0.6 pg/min) (Figure 1B), an index of local uptake, and caused a significant (p<0.001) output (23.5±5.2, 62.8±15.8, and 69.2±17.9 pg/min; p<0.001 versus basal values) (Figure 1B), an effect clearly blunted by theophylline (from −1.9±0.5 to −2.3±0.9, −0.4±0.5, and 11.1±2.3 pg/min; p<0.001 versus saline values) (Figure 1B).

Effect of Angiotensin Converting Enzyme Inhibition on Adenosine-Mediated Vascular Angiotensin II Generation

As in the previous experiment, adenosine in the presence of saline induced a similar dose-dependent increment in FBF (from 3.1±0.7 to 6.1±2.6, 7.9±3.9, and 12.4±2.9 ml/100 ml forearm tissue per minute; p<0.001 versus basal values) and increased to a similar extent venous angiotensin II (from 7.2±1.6 to 11.1±2.2, 12.9±2.1, and 13.9±4.9 pg/ml; p<0.01 versus basal values) without changing arterial values (from 9.3±2.2 to 9.2±2.3, 8.8±2.1, and 9.1±2.1 pg/ml; NS).
When captopril was infused, systemic ACE activity did not change (from 82.8±15.7 to 86.8±17.1 nmol/ml/min; NS), whereas forearm ACE activity was highly reduced (from 86.4±14.7 to 12.5±3.9 nmol/ml/min; p<0.001). FBF slightly, but not significantly, increased (from 3.2±0.8 to 3.8±1.0 ml/100 ml forearm tissue per minute). Captopril infusion did not significantly affect the vasodilating effect of adenosine (from 3.8±1.0 to 5.1±1.5, 6.5±2.5, and 10.8±2.5 ml/100 ml forearm tissue per minute; NS), but it strongly reduced basal angiotensin II venous values (from 7.8±1.6 to 2.2±0.5 pg/ml; p<0.001) and antagonized adenosine-mediated venous angiotensin II increments (from 2.2±0.5 to 2.4±0.6, 2.1±0.4, and 2.5±0.5 pg/ml; NS) without changing arterial concentrations (from 9.4±2.4 to 9.1±2.2, 9.5±2.5, and 9.3±2.4 pg/ml). This last effect confirms the absence of systemic humoral effects during captopril administration.

Discussion

The present data indicate that exogenous adenosine infused into the brachial artery at systemically ineffective rates caused a venous angiotensin II increment without affecting arterial values, a finding that, in agreement with our previous results obtained with isoproterenol, further confirms a release of angiotensin II from human vascular tissue.

This effect of adenosine on angiotensin II seems to be specific since it is antagonized by theophylline, a specific adenosine antagonist. A possibility exists that vasodilation per se could cause a flow-dependent decrease in angiotensin II uptake through the forearm vasculature: this has already been excluded in our previous work in which we showed that vasodilators such as sodium nitroprusside or histamine failed to increase venous angiotensin II values, despite an effective vasodilation. Moreover, the experiment with low adenosine doses, which significantly increased FBF without changing venous angiotensin II, seems to confirm that vasodilation per se is not the mechanism causing venous angiotensin II increments.

Thus in agreement with experimental data, our findings seem to indicate that adenosine is able to activate the RAS in human vessels, an effect that might be mediated by the accumulation of intracellular cAMP linked to A2-adenosine receptor activation. This hypothesis is in agreement with the knowledge that the cAMP increment is the same mechanism involved in β-adrenergic receptor stimulation, a well-known system also capable of stimulating either the circulating or the tissue vascular RAS in humans. Otherwise, the absence of renin measurements does not allow us to be conclusive in our affirmation, and therefore other mechanisms different from the classical RAS cascade could be involved in the local angiotensin II production, such as an increment in the release of preformed angiotensin I, angiotensin II, or both, from vascular cells.

Our finding that adenosine, at low doses, failed to stimulate angiotensin II production despite an evident vasodilating effect could be explained not only by an insufficient A2-receptor stimulation, but also by mechanisms involved in adenosine-mediated vasodilation such as calcium antagonism or hyperpolarization of the presynaptic nerve terminal, which cannot be involved in angiotensin II production. In every case, the doses used to obtain the activation of the local RAS seem to

FIGURE 1. Graphs show arterial-venous concentrations (panel A) and net balance (panel B) of angiotensin II (Ang II) across forearm during adenosine infusion (0.5, 1.5, and 5 µg/100 ml forearm tissue per minute for 5 minutes each) both in the presence of saline (0.2 ml/min for 10 minutes) and in the presence of theophylline (100 µg/100 ml forearm tissue per minute for 15 minutes). Data are mean±SEM (n=6). *p<0.01 or less vs. basal.
be in a physiological range since, for example, much more exogenous adenosine is required to obtain a forearm vasodilation comparable to that induced by ischemia, a pathophysiological condition in which endogenous adenosine is mainly involved. 13

Finally, the experiment with captopril confirms the local origin of adenosine-mediated angiotensin II release. It is interesting that captopril did not potentiate the vasodilating effect of adenosine, a finding that is at variance with our previous data showing that the local ACE inhibitor increased the vasodilating effect of β-adrenergic receptor activation induced by isoproterenol. 1 The most likely explanation of this different hemodynamic effect could be that angiotensin II causes vasoconstriction also indirectly through a potentiating effect on norepinephrine release, an effect that might be less important in the presence of adenosine, which inhibits norepinephrine release. 11

In conclusion, these data show that adenosine can stimulate the production of angiotensin II in the forearm vasculature of hypertensive patients, a finding that confirms the existence of a tissue vascular RAS in humans. The pathophysiological relevance of this effect must still be evaluated, mainly in those organs such as the heart in which high endogenous adenosine levels can be reached.

Acknowledgments

Captopril was a gift from Bristol-Myers Squibb, Rome, Italy. Artwork was done by Moreno Rocchi.

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Adenosine activates a vascular renin-angiotensin system in hypertensive subjects.
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Hypertension. 1992;19:672-675
doi: 10.1161/01.HYP.19.6.672

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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