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Research Article

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Angiotensin AT_1 Receptor Blockade Abolishes the Reflex Sympatho-excitatory Response to Adenosine

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Abstract

We tested the hypothesis that endogenous angiotensin II participates in the direct and reflex effects of adenosine on the sympathetic nervous system. Nine healthy men were studied after 1 wk of the angiotensin II type I receptor antagonist losartan (100 mg daily) or placebo, according to a double-blind randomized crossover design. Bilateral forearm blood flows, NE appearance rates, and total body NE spillover were determined before and during graded brachial arterial infusion of adenosine (0.5, 1.5, 5, and 15 μg/100 ml forearm tissue) and nitroprusside. Adenosine increased total body NE spillover ($P < 0.05$) whereas nitroprusside did not. Losartan lowered BP ($P < 0.05$), had no effect on total body NE spillover at rest, or forearm vasodilation during either infusion, but reduced the systemic noradrenergic response to adenosine from 1.0±0.4 nmol/min on the placebo day to 0.2±0.3 nmol/min ($P < 0.01$), and forearm NE appearance rate in response to adenosine was lower in the infused, as compared with the contralateral arm ($P = 0.04$). The sympatho-excitatory reflex elicited by adenosine is mediated through pathways involving the angiotensin II type I receptor. Interactions between adenosine and angiotensin II may assume importance during ischemia or congestive heart failure and could contribute to the benefit of converting enzyme inhibition in these conditions. (*J. Clin. Invest.* 1998. 101:769–776.) Key words: blood pressure • losartan • [^{3}H]-norepinephrine kinetics • norepinephrine spillover • sympathetic nervous system

Introduction

Adenosine has two distinct and opposite actions on the sympathetic nervous system of conscious humans (1). It inhibits NE release from sympathetic nerve endings locally, by a direct action on prejunctional adenosine receptors (2), while it augments sympathetic nerve traffic, plasma NE and epinephrine reflexively by stimulating adenosine-sensitive chemoreceptors in the carotid body (3–5) and sympathetic afferents in skeletal muscle, kidney, and heart (6–10). Animal studies have demonstrated both inhibitory and excitatory brain stem effects of adenosine on sympathetic outflow. These responses are contingent upon the exact site of adenosine application (11). In addition, adenosine inhibits cholinergic signal transduction in rat sympathetic ganglia (12).

The contribution of these direct or local prejunctional and indirect or reflex neural actions to short-term cardiovascular regulation emerges under conditions in which local concentrations of endogenous adenosine increase, such as isometric exercise (13, 14), or myocardial ischemia (10). The indirect actions of this purine may be particularly beneficial during ischemia, when the local accumulation of adenosine will exert a direct vasodilator effect (15) and inhibit local noradrenergic neurotransmission, because reflex increases in cardiac output and peripheral sympathetic vasoconstrictor tone should serve to redistribute nutrient blood flow to hypoperfused yet metabolically active tissue.

In the context of these reported prejunctional actions of adenosine, the results of previous experiments, in which the nucleoside transport inhibitor draflazine was infused directly into the brachial artery to increase interstitial levels of endogenous adenosine, seemed anomalous in that NE appearance rate (NAR)[1] in the experimental arm increased (2). However, no data as to the effects of brachial arterial infusion of adenosine on forearm NAR are available for comparison. Therefore, the first objective of this study was to test the hypotheses that intraarterial infusion of adenosine will increase total body NE spillover (TBS) but decrease forearm NAR.

The mechanism by which stimulation of adenosine-sensitive afferent nerve endings in skeletal muscle and other vascular beds elicits a reflex sympatho-excitatory response is not known. Taddei et al. have demonstrated that infusion of adenosine into the brachial artery increases local angiotensin II production and release (16). Angiotensin II, which in contrast to adenosine has, in general, excitatory effects on sympathetic neurotransmission (17), may participate in this reflex response by acting on angiotensin II type I (AT_1) receptors at one or more central or peripheral sites. Within the brain stem angiotensin II augments sympathetic outflow (17). Local administration of angiotensin II within sympathetic ganglia increases postganglionic nerve firing (17), and stimulation of angiotensin II receptors on sympathetic nerve endings facilitates NE discharge (18, 19). Therefore, the second objective of this study

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1. *Abbreviations used in this paper:* ACE, angiotensin converting enzyme; AT_1, angiotensin II type 1 receptor; AU, arbitrary unit; FBF, forearm blood flow; FPF, forearm plasma flow; FVR, forearm vascular resistance; MAP, mean arterial pressure; NAR, norepinephrine appearance rate; SNP, sodium nitroprusside; TBS, total body norepinephrine spillover.

was to test the hypotheses that blockade of AT_1 receptors by losartan will attenuate the reflex sympatho-excitatory response to intraarterial infusion of adenosine, and potentiate any direct inhibitory effect of infused adenosine on forearm NAR.

In these experiments brachial artery infusion of adenosine increased the systemic appearance rate of NE into plasma. This adenosine-induced systemic sympatho-excitation was greatly reduced by losartan, indicating that angiotensin II, through its AT_1 receptor, is an important component of this reflex response. In addition, there was a significant reduction in the forearm NAR. These observations may have particular relevance for conditions such as congestive heart failure or myocardial infarction, in which tissue ischemia or relative hypoperfusion could stimulate the local production of endogenous adenosine. In both diseases, sympatho-excitation is associated with an adverse prognosis (20, 21), and interruption of angiotensin II production by angiotensin converting enzyme (ACE) inhibitors has clear benefits with respect to survival and disease progression (22, 23).

Methods

Subjects. Nine healthy nonsmoking male volunteers completed this study. Their average age, weight, and height were 32.6±9.4 (SD) yr, 78.9±6.2 kg, and 179.2±7.5 cm, respectively. Mean volumes of the experimental and contralateral forearms were 1,156±151 (SD) ml and 1,109±152 ml, respectively. All subjects provided written informed consent before their participation. This study protocol was approved by our institutional ethics committee.

Study design. These volunteers participated in a double-blind, randomized crossover trial, comparing the effect on BP, forearm vascular tone, and local and systemic NE spillover, of 1 wk of treatment with losartan (100 mg, once per day), in the absence of dietary sodium restriction, to 1 wk of treatment with placebo.

Each volunteer visited the laboratory four times, at 8:00 A.M., in the fasting state. The aim of the first and third visits was to ensure that the first dose of placebo or losartan was well tolerated. Blood pressure and heart rate were recorded in the supine position at 5-min intervals by an automatic cuff recorder (model Lifestat 200; Physio-Control, Redmond, WA). After a 15-min baseline period, the first dose of either placebo or losartan was given. Subjects were then provided a light breakfast. BP and heart rate were recorded over the next 2 h.

The second and fourth visits occurred at the end of each week of treatment and after 24 h of caffeine abstinence. The last dose of placebo or losartan was given under supervision. The purpose of these visits was to characterize the local and systemic responses to an intraarterial infusion of adenosine. Because vasodilation caused by adenosine might affect forearm NE spillover nonspecifically, sodium nitroprusside (SNP) was infused as a vasodilator control (24). Lead II of the electrocardiogram was recorded continuously. Deep antecubital veins of both arms were cannulated retrogradely (2 inch, 20 gauge catheter) (Angiocath; Becton Dickinson, Sandy, UT), to prevent contamination of skeletal muscle venous effluent by blood originating from the cutaneous vascular bed. Successful retrograde cannulation of skeletal muscle vascular beds was confirmed by blood gas analysis of oxygen saturation $< 65\%$ (model Oxicom-3000; Waters Instruments, Inc., Rochester, MN).

After local anesthesia, the brachial artery of the nondominant arm was cannulated (2 inch, 20 gauge catheter) for the continuous recording of intraarterial BP (Novakit™ pressure monitoring line connected via Novotrans™ pressure transducer; Medex, Inc., Hilliard, OH, to a blood pressure recorder, model Horizon 2000™; Mennen Medical, Inc., Clarence, NY), infusion of adenosine or SNP by a syringe pump (model 11; Harvard Apparatus, Inc., South Natick, MA), and for blood sampling. A calf or foot superficial vein was cannulated anterogradely for infusion of tritiated NE by an anesthesia syringe pump (model P3000; IVAC Canada, Inc., Richmond Hill, Ontario, Canada).

Blood flow was measured in both forearms simultaneously using venous occlusion strain gauge plethysmography. For this purpose, a straight segmental cuff was applied to each upper arm and inflated to a venous occlusion pressure of 40 mmHg for 6–10 s (cuff model SC10, rapid deflate bladder model SC10DRB; D.E. Hokanson, Inc., Bellevue, WA). Changes in forearm volume were detected by strain gauge plethysmography (model EC4SB; D.E. Hokanson, Inc.). During measurement of forearm blood flow and blood sampling, circulation to the hand was occluded bilaterally by inflation of wrist cuffs to suprasystolic pressures (model TMC7; D.E. Hokanson, Inc.). This was done to exclude, as much as possible, the cutaneous vascular bed from the experimental preparation (25). Both forearms were supported by slings above the level of the right atrium throughout these experiments to assure rapid recovery of forearm volume after deflation of the upper arm cuffs.

2 h after oral drug intake and at least 30 min after these cannulations, venous blood was sampled for the measurement of plasma renin activity, plasma caffeine, and hematocrit. Tritiated NE (Levo-[ring-2,5,6-^{3}H]-NE, sp act 30–60 Ci/mmol) (New England Nuclear, Boston, MA) was then infused at 1 μCi/min. 15 min later, the wrist cuffs were inflated to 200 mmHg and saline was infused into the brachial artery for 5 min. This was followed immediately by sequential infusions of 0.5 and 1.5 μg adenosine/100 ml forearm/min, each for 5 min. After blood sampling was completed (see below), the wrist cuffs were deflated to prevent discomfort. 10 min later, wrist cuffs were reinflated and adenosine was reinfused at 5 and 15 μg/100 ml forearm/min, each for 5 min. After blood sampling was completed, the wrist cuffs were deflated and the NE infusion was interrupted. This was done to minimize the uptake and release of tritiated NE by sympathetic nerve endings and to reduce the total dose of radioactivity administered. 15 min later, the NE infusion was restarted (1 μCi/min). After 15 min, the wrist cuffs were inflated and glucose (5%), 0.02 μg SNP, and 0.2 μg SNP/100 ml forearm/min were infused sequentially (5 min per dose). The wrist cuffs were again deflated after blood sampling was complete (see below). 10 min later, the wrist cuffs were reinflated and the highest dose of SNP (0.6 μg/100 ml forearm/min) was infused for 5 min.

Blood flow in the experimental and contralateral forearms was measured during the 5-min saline and glucose (5%) infusion and during the last 2 min of each drug infusion. At the end of each drug infusion, forearm venous (bilaterally) and arterial blood was sampled for measurement of plasma NE and for the calculation of NE kinetics. To prevent any interruption of the intraarterial drug infusions, these arterial samples were collected only after bilateral venous sampling was complete.

In five subjects (randomly allocated), adenosine was infused before nitroprusside. In the other four volunteers, the order of infusion was reversed. The recovery period between the adenosine and SNP infusions was 30 min.

Drugs and solutions. Tritium-labeled NE (*L*-[2,5,6-^{3}H]-NE) was diluted in sterile 0.2 mol/liter acetic acid in saline solution with 10 mmol/liter ascorbate, sterilized by filtration through a 0.22-μm Millex-GV filter, packaged in 4-ml aliquots ($\sim$ 50 μCi/ml), and stored at −70°C until use. Before each experiment, solutions were prepared by dissolving the original aliquot in 0.9% saline, obtaining a preinfusion activity of $\sim$ 1 μCi/ml. To calculate TBS (see below) the actual activity was measured afterwards.

Adenosine was purchased from Fujisawa (Markham, Ontario, Canada). Final solutions were prepared on the morning of the study day using 0.9% NaCl as a solvent. SNP (Nipride) (Hoffman-La Roche, Ltd., Mississauga, Ontario, Canada) was dissolved before infusion in 5% glucose and protected from light.

Opaque No. 2 gelatin capsules (TUB Enterprises, North Augusta, Ontario, Canada) containing either 100 mg of losartan (Cozaar®) (Merck Frosst, Pointe-Claire, Quebec, Canada) or lactulose as the

placebo were prepared by our Department of Pharmacy Services. The mean weight of 200 losartan 50-mg tablets was determined. Tablets were triturated to a fine powder. A weight equivalent to two tablets of losartan was transferred to the opaque gelatin capsules. Patients were randomized by the Department of Pharmacy Services to receive first either losartan or placebo in a crossover design. The randomization code was not revealed until all data were collected, analyzed, and compiled.

Analytical methods. Plasma caffeine concentrations were determined by a homogeneous enzyme immunoassay technique using an Emit Caffeine Assay kit (Syva Co., Kanata, ON, Canada). This method detects total serum caffeine concentration (both protein bound and unbound) with a limit of detection of 5 μmol/liter (1 μg/ml). In our institution, the batch-to-batch coefficient of variation is 4.2% at a concentration of 77.2 μmol/liter.

Blood samples for determination of plasma NE were collected in prechilled tubes containing EDTA as an anticoagulant. The tubes were centrifuged at 4°C and the plasma was separated and stored at −70°C. Measurements for concentrations of NE and [^{3}H]-NE in all plasma samples and infusates were performed within 2 mo of sampling according to methods published previously by our group (26). For endogenous NE, the intraassay coefficient of variation was 1.9% (n = 10) and the interassay coefficient of variation was 3.0%. The detection limit of the method was ∼ 0.1 nmol/liter and the peak area was linear from 0.1 to 50 nmol/liter (n = 8). Postcolumn radiolabeled NE was fraction collected with a Gilson fraction collector (model FC 204). The fraction was quantitated by liquid scintillation spectrophotometry.

Plasma renin activity was measured by the quantitation of generated angiotensin I, using a RIANEN angiotensin I [^{125}I] radioimmunoassay kit (DuPont, Boston, MA).

Data collection and statistical analysis. During the intraarterial experiments, all signals (BP, electrocardiogram, and forearm plethysmography) were recorded continuously onto paper (model RS 3800; Gould Instrument Systems, Inc., Valley View, OH). In addition, these signals were digitized using model AT-MIO-16XE-50 multifunction analog and digital I/O for IBM PC/AT and EISA bus PC (National Instruments, Austin, TX) at a sampling rate of 200 Hz for BP and plethysmography and 1,000 Hz for the electrocardiogram, and stored onto a computer. Computerized data acquisition and analysis were performed using the LabVIEW software package version 3.1 (National Instruments, Austin, TX). The systolic, mean arterial (MAP), and diastolic BPs of all cardiac cycles occurring over the course of each forearm blood flow (FBF) measurement were averaged to obtain one mean value for each variable. Forearm vascular resistance (FVR = MAP/FBF, expressed as arbitrary units [AU]) was calculated for each flow measurement. Baseline values were defined as the average of all measurements obtained during saline (for comparison with values during adenosine infusion) or 5% glucose (for comparison with values during SNP infusion). The response to these during infusions was calculated as the difference between the average values obtained over the last 2 min of each dose and baseline.

Arterial and venous concentrations of [^{3}H]-NE and NE were used for calculations of NE kinetics, as previously described (24, 27, 28). Total body NE spillover, i.e., the estimated rate of appearance of endogenous NE in arterial plasma, was derived from the arterial plasma NE concentration (NE_a), the arterial steady-state plasma concentration of [^{3}H]-NE ([^{3}H]-NE_a), and the infusion rate of [^{3}H]-NE, according to the equation: *total body NE spillover (pmol/min)* = NE_a (*pmol/ml*) × {*Infusion rate (dpm·min*$^{-1}$)}/[^{3}H]-NE_a (*dpm/ml).* The appearance rate for NE in the experimental and control arms was calculated using the following formula, which assumes that the forearm extraction of arterial NE equals the extraction of locally spilled NE (24, 28): *Forearm NE appearance rate (pmol*/100 *ml forearm per min)* = {(*FPF* × NE_a × f_{NE}) + [*FPF* × (NE_v − NE_a)]}/(*1* −f_{NE}), where f_{NE} is the fractional extraction of the tracer across the forearm, calculated as ([^{3}H]NE_a − [^{3}H]NE_v)/[^{3}H]NE_a, where [^{3}H]NE_v, and [^{3}H]NE_a are the venous and arterial plasma concentration of [^{3}H]-NE, respectively, and FPF is the forearm plasma flow (ml/100 ml forearm/min), calculated from the FBF and the hematocrit as FBF × (1 − hematocrit).

All results are presented as mean±SEM. Effects on BP, heart rate, FBF, and FVR were assessed by ANOVA for repeated measurements using oral treatment and intraarterial infusions as within-subject factors. Since data on NE kinetics (TBS and forearm NAR) were not normally distributed ($P < 0.1$, Shapiro-Wilk test for normality), the Wilcoxon–matched-pairs signed-ranks test was used to assess the effects of these intraarterial infusions on baseline values and of losartan on these variables. To avoid multiple comparisons within this nonparametric analysis, for each study day the overall response to intraarterial adenosine or SNP was quantified, for each volunteer, by generating areas under the infusion–response curves standardized for the duration of infusions, for adenosine (four doses) or SNP (three doses) for each study day (29). A two-sided P value of 0.05 was selected for statistical significance.

Results

This study was completed in all nine volunteers. Plasma caffeine levels were consistently below the level of detection, indicating at least 24 h of caffeine abstinence in all subjects. Plasma renin activity was 0.3±0.1 ng/ml/h during placebo and 2.3±1.0 ng/ml/h on losartan treatment ($P < 0.05$; n = 9), demonstrating successful interruption of the negative feedback of angiotensin II on renin release, and indicating that the drug was effectively absorbed in all subjects. In the infused arm, oxygen saturation in deep antecubital venous blood was 50.0±4.2 and 51.4±3.3% (P = NS) during placebo and losartan treatment, respectively. In the noninfused arm, these figures were 50.7±2.3 and 54.6±2.8% (P = NS), respectively. These values demonstrate that the relative contribution of blood originating from skin and muscle did not differ between the two arms or study days.

During treatment with placebo, the specific activity of tritiated NE in the arterial blood did not change significantly over the course of these experiments. Values were 525±50 dpm at the end of intraarterial saline infusion and 503±42, 558±48, 513±44, and 510±48 dpm at the end of the subsequent adenosine infusions; 489±40 dpm at the end of intraarterial dextrose infusion and 501±33, 522±55, and 566±72 dpm at the end of the subsequent SNP infusions. Similar arterial activities of tritiated NE were obtained over the course of the experiments conducted on the losartan day. The stable course of arterial specific activity on tritiated NE indicates that a 15-min infusion of the tracer without a loading dose was sufficient to reach steady-state arterial tracer concentrations.

The hemodynamic and neurohumoral effects of losartan

1-wk treatment with losartan significantly reduced BP, by ∼ 5 mmHg, but had no effect on heart rate (Table 1). In the noninfused arm, losartan tended to lower FVR at baseline (44.2±7.4 to 37.6±6.7 AU before adenosine and from 43.7±8.1 to 31.1±4.2 AU before SNP [P = 0.06 for losartan effect]).

At baseline, forearm NAR in the contralateral arm was not affected significantly by losartan treatment: 4.8±1.8 vs. 5.5± 1.6 pmol/100 ml forearm tissue/min before adenosine infusion (P = NS) and 4.2±1.1 vs. 5.4±0.9 pmol/100 ml forearm tissue/min before SNP infusion (P = NS) on placebo and losartan treatment days, respectively. Likewise, losartan had no effect on TBS: 2.5±0.7 vs. 2.5±0.5 nmol/min before adenosine (P = NS) and 3.0±0.8 vs. 3.3±1.0 nmol/min before SNP (P = NS).

Table I. Effect of Losartan on BP and Heart Rate

	MAP		Heart rate	
	Placebo	Losartan	Placebo	Losartan
Saline 0.9%	86.5±5.1	81.0±4.3*	58.3±3.1	57.0±2.7
Adenosine dose (μg/100 ml forearm tissue/min)				
0.5	+0.5±0.6	+1.3±0.4	+0.6±1.2	+1.3±1.2
1.5	+2.1±0.4	+3.3±0.7	+0.6±0.7	+3.2±1.0
5	+2.7±0.5	+2.8±0.8	+3.3±1.3	+0.7±1.5
15	+3.5±0.8	+3.4±1.2	+3.4±1.1	+3.4±1.6
5% Glucose	87.6±4.4	81.2±4.7‡	58.6±3.1	57.7±2.6
SNP dose (μg/100 ml forearm tissue/min)				
0.02	+1.1±0.5	−0.1±0.6	+0.7±1.2	+1.0±0.7
0.2	+0.7±0.7	−0.0±1.1	+2.9±1.4	+3.0±1.5
0.6	−1.1±0.6	−1.8±1.2	+0.7±1.0	+2.5±1.2

Baseline BP and heart rate and changes from baseline during intraarterial adenosine or SNP infusions (mean±SE). $*P < 0.05$, $‡P < 0.01$ for comparison between placebo and losartan treatment.

The effect of losartan on adenosine- and SNP-induced hemodynamic and neurohumoral changes

FBF. Neither adenosine nor SNP-induced forearm vasodilation were affected by losartan (Fig. 1). Adenosine increased blood flow in the infused forearm dose-dependently, from 3.3±0.7 ml/100 ml forearm tissue/min at baseline to 5.0±1.2, 7.7±1.6, 13.2±3.6, and 20.1±7.1 ml/100 ml forearm tissue/min during treatment with placebo ($P < 0.01$), and from 3.0±0.5 ml/100 ml forearm tissue/min at baseline to 3.8±0.7, 7.4±1.2, 8.5±1.5, and 15.9±3.2 ml/100 ml forearm tissue/min during treatment with losartan (placebo versus losartan, P = NS). The three incremental SNP doses increased blood flow in the infused forearm from 3.2±0.7 ml/100 ml forearm tissue/min at baseline to 4.9±1.0, 9.9±2.2, and 14.0±3.5 ml/100 ml forearm tissue/min during treatment with placebo ($P < 0.01$) and from 3.4±0.5 ml/100 ml forearm tissue/min at baseline to 5.2±0.8, 10.4±1.5, and 14.0±2.3 ml/100 ml forearm tissue/min during treatment with losartan (placebo versus losartan, P = NS). In the contralateral forearm, blood flow did not change significantly during these intraarterial infusions on either study day. Similar results were obtained when these data were expressed as percent changes in FVR (data not shown).

Forearm NAR. In the infused arm, on the placebo day, NAR was 6.8±3.0 pmol/100 ml forearm tissue/min at baseline and 2.8±1.5, 3.8±1.2, 4.7±1.3, and 4.2±1.7 pmol/100 ml forearm tissue/min during the four incremental adenosine doses. On the losartan day NAR was 4.6±0.9 pmol/100 ml forearm tissue/min at baseline and 3.2±0.8, 3.1±1.0, 3.4±0.8, and 3.1± 0.8 pmol/100 ml forearm tissue/min during the four incremental adenosine doses. In the opposite or noninfused arm, forearm NAR was not affected significantly by adenosine on either the placebo day (4.8±1.8 pmol/100 ml forearm tissue/min at baseline and 5.1±1.7, 4.1±1.3, 4.3±1.8, and 5.5±1.6 pmol/100 ml forearm tissue/min during the four adenosine doses) or the losartan day (5.5±1.6 pmol/100 ml forearm tissue/min at baseline and 5.5±1.6, 5.3±1.0, 5.6±0.9, and 6.6±1.2 pmol/100 ml forearm tissue/min).

There was a trend for lower NE appearance rates in the infused arm during adenosine (−2.6±2.1 vs −0.1±0.9 pmol/100 ml forearm tissue/min), but the area under the infusion–response curve for the experimental arm was not significantly different from the contralateral arm due to the variability in this effect between subjects. On the losartan day the area under the infusion–response curve for NE appearance rate was significantly lower in the experimental forearm than in the contralateral forearm (−1.2±0.5 vs +0.1±0.7 pmol/100 ml forearm tissue/min, $P = 0.02$).

SNP had no effect on forearm NAR in either arm on either day (Fig. 2). In the infused arm on the placebo day NAR was 3.5±0.8 pmol/100 ml forearm tissue/min at baseline and 5.0± 1.2, 4.7±1.3, and 4.2±1.8 pmol/100 ml forearm tissue/min for

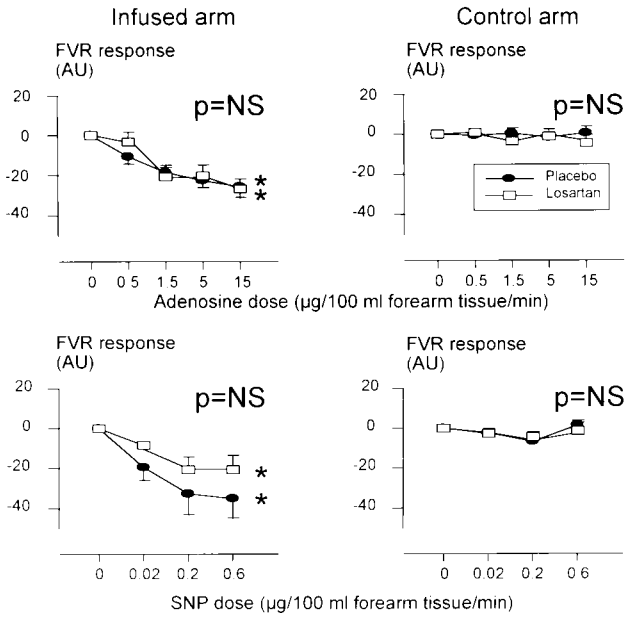

Figure 1. Effect of intraarterially infused adenosine and SNP on FVR (AU) during placebo and losartan treatment. Changes from baseline in absolute values are expressed as means±SEM (n = 9). *Responses that differ significantly from baseline. P values indicate level of significance for comparison between placebo and losartan.

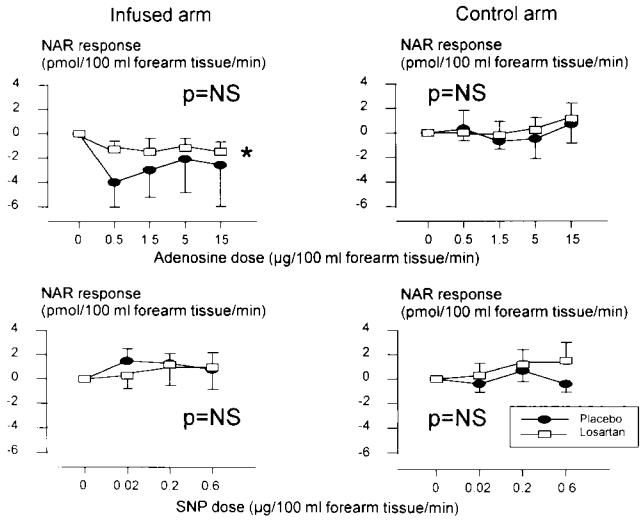

Figure 2. Effect of intraarterially infused adenosine and SNP on forearm NAR during placebo and losartan treatment. Changes from baseline in absolute values are expressed as means±SEM ($n = 9$). *$P < 0.05$ for the reduction in NAR during intraarterial adenosine infusion during losartan treatment. P values indicate level of significance for comparison between placebo and losartan.

each incremental dose of SNP. In the infused arm on the losartan day, NAR was 4.3±0.7 pmol/100 ml forearm tissue/min at baseline and 4.6±1.6, 5.6±1.8, and 5.3±2.0 pmol/100 ml forearm tissue/min for the three SNP doses. Corresponding values for NAR in the noninfused arm were 4.2±1.1, 3.8±0.7, 4.9±1.4, and 3.9±1.0 pmol/100 ml forearm tissue/min on the placebo day, and 5.4±0.9, 5.8±1.7, 6.6±1.9, and 7.0±1.9 pmol/100 ml forearm tissue/min on the losartan day.

TBS. Intraarterial infusions of adenosine and SNP had no significant effects on heart rate, systolic, or diastolic BP on either study day (Table I). Adenosine increased TBS on the placebo day, from 2.5±0.7 nmol/min at baseline to 3.9±1.2, 3.3±1.1, 3.4±1.1, and 4.0±1.3 nmol/min, respectively ($P <$ 0.05) (Fig. 3). Intraarterial SNP infusions did not affect this variable: 3.0±0.8 nmol/min at baseline and 3.6±1.1, 3.1±0.8, and 2.9±0.8 nmol/min for the three increasing SNP doses, respectively (Fig. 3).

The increase in TBS in response to intraarterial adenosine infusion was markedly attenuated by losartan: +1.4±0.5, +0.9±0.4, +0.9±0.6, and +1.4±0.8 nmol/min for the four sequential adenosine doses during placebo, and −0.2±0.1, +0.5± 0.5, +0.4±0.6, and +0.4±0.4 nmol/min (from a baseline of 2.5±0.5 nmol/min) during losartan treatment. TBS during adenosine infusion rose 1.0±0.4 nmol/min above baseline on the placebo day and 0.2±0.3 nmol/min above baseline on the losartan day ($P < 0.01$ for comparison of areas under these two infusion–response curves). The average percentage increase in TBS in these subjects was +34±12% on the placebo day and 0±7% on the losartan day ($P < 0.05$).

Corresponding values for TBS in response to intraarterial SNP were +0.6±0.4, +0.05±0.4, and −0.2±0.1 nmol/min from a baseline value of 3.0±0.8 nmol/min during placebo (P = NS) and −0.4±0.4, +0.1±0.5, and +0.04±0.7 nmol/min from a baseline value of 3.3±1.0 nmol/min during losartan treatment (P = NS), representing, overall, a 0.2±0.3 nmol/min increase and a 0.1±0.4 nmol/min decrease during placebo and losartan treatment, respectively (P = NS) (Fig. 3).

Discussion

The purpose of this study was to test the hypotheses that endogenous angiotensin II, through its AT_1 receptor, mediates the indirect, or reflex, excitatory actions of adenosine on the sympathetic nervous system of normal humans, and counters the local inhibitory effect of adenosine on neural NE release through its facilitatory prejunctional action. Our objectives were, first, to characterize the effect of brachial artery infusion of adenosine on forearm and total body NARs and, second, to determine the effect of angiotensin II AT_1 receptor blockade with losartan on sympathetic neurotransmission and the forearm vasodilator response to this purine. The radiotracer technique, which permits simultaneous estimation of forearm and total body NE release, was used to quantify local and systemic sympathoneural responses to adenosine, and to SNP, which was administered to control for any nonspecific effects of forearm vasodilation. The bilateral forearm model was chosen, since measurements in the contralateral noninfused arm enabled us to discriminate between local and reflex responses to intraarterial infusions of these agents at doses without systemic effects.

Our key findings were that brachial arterial administration of adenosine elicited an increase in TBS and that angiotensin II AT_1 blockade markedly attenuated this indirect or reflex sympatho-excitatory response without affecting the forearm vasodilator response to adenosine. We also observed a reduction in forearm NAR at the site of adenosine infusion. This was significant on the losartan day.

The effect of adenosine on TBS and forearm NAR. TBS increased significantly during the brachial arterial infusion of adenosine. This response was not simply secondary to its vasodilator action, since SNP did not affect TBS at doses that exerted similar effects on FBF. As our method does not allow for calculation of organ-specific spillover of NE, we cannot determine which vascular bed contributed most to this increase in

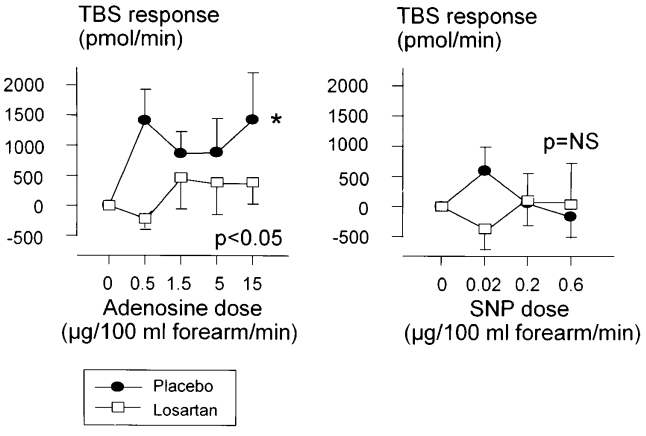

Figure 3. Effect of intraarterially infused adenosine and SNP on TBS, during placebo and losartan treatment. Changes from baseline in absolute values are expressed as means±SEM ($n = 9$). *Response that differs significantly from baseline. P values indicate level of significance for comparison between placebo and losartan.

TBS. The kidney, skeletal muscle, heart, brain, and lungs are all potential sources, whereas NE originating from the gut is rapidly metabolized by the liver, and therefore has little effect on TBS as quantified by this method (30).

This study was not intended to address the question whether this reflex is initiated within the infused forearm, or elsewhere by adenosine that spills into the systemic circulation. This issue has been dealt with by previous investigators. Using an isolated forearm technique, Costa and Biaggioni demonstrated that the increase in sympathetic nerve firing rate in the leg in response to a brachial artery injection of adenosine is due to a local action of infused adenosine and is not a response to increased circulating levels of this purine (13). This sympatho-excitation was attributed to a reflex arising from adenosine-sensitive afferent nerves in the infused forearm. Relatively high doses (20 μg/kg/min or more) of intravenous adenosine infusion are needed to increase venous plasma NE concentrations or muscle sympathetic nerve firing rate in the leg (3, 31). Therefore, even if some or all the intraarterially infused adenosine had spilled into the systemic circulation, the amount involved would be insufficient to activate the sympathetic nervous system.

The lack of increase in NAR in the experimental, or infused forearm can be explained by the prejunctional inhibitory action of this purine on neurotransmitter release (32). Indeed, our comparison of the experimental and contralateral arms identified a trend to lower NARs in the infused arm on the placebo day, and a smaller yet significant reduction, with less variance between subjects, on the losartan day. These findings are consistent with the concept that blockade of facilitatory prejunctional angiotensin AT_1 receptors on sympathetic nerve endings unmasked an inhibitory prejunctional action of adenosine on NE release in some of these subjects. However, it would appear that the overall reduction in forearm NAR was due primarily to a local effect of adenosine, because this response was qualitatively and quantitatively similar (P = NS) on the two study days.

The effect of local infusion of adenosine on muscle sympathetic nerve activity in the opposite forearm has not been reported. Differences in sympathetic vasoconstrictor outflow to the arm and the leg in response to intraarterial adenosine might account for the dissociation between TBS, which increased, and NAR in the contralateral noninfused forearm, which did not. Similar discordance has been observed during mental stress (33), or on stimulation of chemosensitive cardiac afferents (34). However, Wallin and colleagues demonstrated that circulatory arrest, after handgrip, increases muscle sympathetic traffic in both the opposite radial, and in the peroneal nerve (35), leading us to anticipate an increase in NAR in the contralateral arm in these experiments. Our findings may relate to our less intense stimulus. Indeed, these relatively low infusion rates appear to have elicited a more moderate sympatho-excitatory response than the intraarterial bolus injections of 0.5–4 mg of adenosine administered by Costa and Biaggioni (6, 13).

The effect of losartan on adenosine-induced forearm vasodilation and sympatho-excitation. Losartan markedly attenuated the increase in TBS, indicating that angiotensin II is a necessary component of the reflex arc that is stimulated by adenosine. This reflex can be divided into its afferent neural, central (brain stem, spinal cord), and efferent components (pre- and postganglionic sympathetic nerves). Peripherally administered losartan binds to angiotensin AT_1 receptors at sites involved in cardiovascular regulation by the autonomic nervous system both within and outside the blood brain barrier (36). Thus, the observed interaction between adenosine and losartan might have occurred at any or all three levels.

Both endothelial and adventitial smooth muscle cells produce angiotensin II (37). Consequently, one could postulate that it was angiotensin II, produced in response to the local adenosine infusion, rather than adenosine itself, that stimulated sympathetic afferents in the forearm, causing reflex activation of the sympathetic nervous system. However, this hypothesis is not supported by experimental evidence in humans: brachial artery infusion of angiotensin II does not increase TBS (19). Nor can this action be attributed to spillover of angiotensin II into the systemic circulation. The amount detected by Taddei et al. (16) in venous effluent was insufficient to increase its arterial concentrations, and systemic sympatho-excitation was observed in the setting of forearm venous occlusion, which would prevent any locally released angiotensin II from reaching the systemic circulation (13). Thus, the reflex increase in efferent sympathetic nerve activity and TBS elicited by brachial artery infusion of adenosine would appear to be due to an action of angiotensin II as a neurotransmitter or neuromodulator within the central or efferent components of the reflex arc, rather than at its afferent site.

Angiotensin II AT_1 receptors are present within the central nervous system, sympathetic ganglia, and sympathetic nerve endings (17). Depending on the experimental preparation, and species studied, the central nervous system administration of angiotensin II will increase sympathetic outflow (17). Application of angiotensin II into sympathetic ganglia also increases the firing rate of postganglionic nerves (38, 39). Stimulation of prejunctional angiotensin II receptors on sympathetic nerve endings facilitates NE release (19). Observations on the losartan day indicate that the reflex sympatho-excitatory response to locally infused adenosine is mediated through reflex pathways involving the angiotensin II AT_1 receptor in neurotransmission at its central or efferent sites, or both.

Blockade of angiotensin II AT_1 receptors did not augment forearm vasodilation when adenosine was infused, indicating that if this purine did induce local angiotensin II production, as postulated by Taddei et al. (16), adenosine-induced vasodilation may have overwhelmed any potential local vasoconstrictor action of increased angiotensin II in the infused forearm. This conclusion is consistent with their study, in which the brachial artery infusion of captopril markedly attenuated increases in forearm venous angiotensin II concentrations caused by adenosine, but did not affect adenosine-induced vasodilation (16). In a study of intravenously infused adenosine type II receptor agonists in rats, augmented vasodilation after losartan was attributed to potentiation of the response to stimulation of adenosine type II receptors (40). However, in light of our present findings, those observations could have equally been due to an inhibitory effect of losartan on adenosine-induced sympatho-excitation, which was not measured in those experiments.

These dual actions of losartan, in the presence of adenosine, namely, abolition of generalized sympathetic vasoconstrictor responses, but preservation of local vasodilator responses, may have important and beneficial implications for a number of physiological and pathological states.

Clinical implications. In vitro and animal in vivo studies indicate that stimulation of adenosine receptors on cardiomyo-

cytes and skeletal muscle cells can protect these cells against the consequences of ischemia and reperfusion (41–44). However, in conscious humans suffering acute myocardial infarction, where acute administration of beta-adrenoceptor blockade exerts a significant, but modest, survival benefit (45), reflex activation of the sympathetic nervous system, in general, and sympathetic neural drive to the heart, in particular, could offset these direct beneficial effects of adenosine (46, 47). The present experiment indicates that angiotensin II receptor antagonists abolish reflex sympathetic activation evoked by adenosine, but preserve the local vasodilator response to this purine. These properties of AT_1 receptor antagonism favor the use of agents which inhibit the production of angiotensin II, or block its direct actions, in conjunction with adenosine receptor agonists, or nucleoside transport inhibitors to exploit the protective actions of exogenous and endogenous adenosine. This may be one mechanism by which ACE inhibition improves the outcome of patients suffering acute myocardial infarction (48).

This mechanism of benefit may also extend to improvements in survival and disease progression observed when patients with congestive heart failure are treated with this class of drugs (22). Results of experiments in dogs (49) suggest that relative cardiac or peripheral hypoperfusion in patients with end-stage congestive heart failure may stimulate the production of endogenous adenosine and increase local tissue concentrations, even under resting conditions. Plasma adenosine concentrations increase in heart failure, relative to symptom class (50), and increased plasma hypoxanthine concentrations (a major product of adenosine metabolism) during treadmill exercise have been reported in a subgroup of heart failure patients who displayed concomitant increases in plasma lactate and NE (51). Mechanisms responsible for the generalized and cardiac-specific sympathetic nervous system activation characteristic of heart failure are not completely understood (20, 52, 53), but the adverse impact of such activation on survival has been well documented (21). Although the effects of endogenous adenosine were not addressed in this study, we hypothesize that adenosine-mediated stimulation of afferent nerves in skeletal muscle may contribute to such activation by eliciting the sympatho-excitatory reflex described in this paper.

Conclusions. In healthy volunteers, a brachial artery infusion of adenosine elicited a sympatho-excitatory response, as reflected by an increase in TBS, without increasing the local forearm NAR. This reflex increase in TBS was greatly reduced by pretreatment with losartan, a selective angiotensin II AT_1 receptor antagonist. In addition there was a significant reduction in the NAR in the infused arm on the losartan day. The pathophysiological significance of this adenosine-angiotensin II interaction merits further investigation, as it may underlie some of the sympatho-excitation of myocardial ischemia and heart failure. Interruption of this action of endogenous angiotensin II may contribute to the beneficial effects of ACE inhibitors on survival and disease progression in these conditions.

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