Bradykinin Stimulates Tissue Plasminogen Activator Release in Human Vasculature

Nancy J. Brown, James V. Gainer, C. Michael Stein, Douglas E. Vaughan

Abstract—Bradykinin stimulates tissue plasminogen activator (tPA) release in isolated perfused animal tissues. The present study tests the hypothesis that bradykinin increases tPA release in humans through local effects on the vasculature. Graded doses of sodium nitroprusside (0.8 to 3.2 μg/min), acetylcholine (ACh) (7.5 to 60 μg/min), and bradykinin (100 to 400 ng/min) were administered intra-arterially in random order in 10 salt-depleted (10 mmol/d of Na) normotensive volunteers. None of the drugs altered mean arterial pressure or heart rate. Forearm blood flow (FBF) was measured by strain-gauge plethysmography. All 3 drugs caused a dose-dependent increase in FBF, although ACh was less potent than either nitroprusside or bradykinin (maximum FBF 7.5 ± 2.4 versus 10.0 ± 1.5 and 11.9 ± 2.1 mL ⋅ 100 mL⁻¹ ⋅ min⁻¹, respectively). Bradykinin caused a significant, dose-dependent increase in venous (effect of dose F = 9.9, P = 0.028 by ANOVA), but not arterial (F = 0.154, P = 0.92) tPA antigen in the infused arm. Thus, net tPA release increased significantly in response to bradykinin (50.6 ± 13.3 at the highest dose versus 0.9 ± 0.4 ng ⋅ 100 mL⁻¹ ⋅ min⁻¹ at baseline, P = 0.014). In contrast, bradykinin did not affect plasminogen activator inhibitor antigen. Neither nitroprusside nor ACh altered plasma levels of tPA or plasminogen activator inhibitor antigen. Bradykinin increased tPA release across the forearm in the absence of systemic effects. This effect could not be attributed to changes in blood flow because doses of equivalent potency of the vasodilator nitroprusside did not increase tPA. These data demonstrate that bradykinin stimulates tPA release in the human vasculature. (Hypertension. 1999;33:1431-1435.)

Key Words: bradykinin ■ plasminogen, tissue activator ■ endothelium ■ angiotensin-converting enzyme inhibitors

Bradykinin is a vasoactive polypeptide that has cardioprotective effects.1 Bradykinin promotes vasodilation by stimulating the production of arachidonic acid metabolites, nitric oxide, and endothelium-derived hyperpolarizing factor in vascular endothelium.2 Bradykinin inhibits thrombin-induced platelet activation.3 In addition, bradykinin is a potent stimulus for release of tissue plasminogen activator (tPA) in endothelial cells4 and perfused tissue preparations, such as the pig ear5 or rat hind limb.6 The effect of bradykinin on tPA release in human vasculature has not been extensively studied. A previous study demonstrated that systemic administration of bradykinin increases venous tPA antigen concentrations in angiotensin-converting enzyme (ACE) inhibitor-pre-treated subjects.7 However, because the systemic administration of the vasodilator bradykinin resulted in activation of the sympathetic nervous system, adrenergic stimulation of tPA release, a well-recognized phenomenon,8 could not be excluded. Moreover, changes in venous tPA concentrations in response to systemically administered bradykinin and consequent hemodynamic alterations may have resulted from changes in hepatic blood flow and tPA clearance.9

The purpose of the present study was to test the hypothesis that bradykinin directly stimulates endothelial release of tPA in human vasculature by measuring the effect of direct intra-arterial administration of bradykinin on tPA release across the forearm. This model allows determination of local, vascular tPA release while avoiding confounding systemic effects or changes in hepatic clearance10 and has been used to measure the effect of a number of agonists on endothelial tPA release, including desmopressin,11 methacholine,10,12 and isoproterenol.12 The forearm vasodilator and tPA responses to bradykinin were compared with the responses to acetylcholine (ACh) (another endothelium-dependent vasodilator)13 and nitroprusside (a direct-acting vasodilator).14

Methods

Subjects

The study group was comprised of 10 normotensive subjects. Informed consent was obtained. The protocol was approved by the Vanderbilt University Institutional Review Board, and the study was conducted according to the Declaration of Helsinki. Each subject underwent a history and physical examination, ECG, and routine laboratory analysis. None had any evidence of hypertension, hyperlipidemia, cardiovascular disease, or other systemic condition. All but 1 of the subjects were nonsmokers. A total of 6 men and 4 women were studied. Of the 10 subjects, 5 were black and 5 were white. The mean age was 32.5 ± 2.8 years; mean body mass index was 25.1 ± 1.1 kg/m². The mean plasma renin activity measured during 10 mmol sodium intake per day and after
Experimental Protocol

All studies were performed in the morning in a temperature-controlled room. Subjects were studied in the supine position and in the fasting state. An intravenous catheter was placed in the antecubital vein in both arms. After subdermal administration of 1% lidocaine, an 18 gauge polyurethane catheter (Cook, Inc) was inserted into the brachial artery of the nondominant arm allowing direct intra-arterial administration of drugs. Before the infusion of vasoactive drugs, arterial catheter patency was maintained by infusion of 5% dextrose in water at rate of 1 mL/min. After intravenous and intra-arterial catheters were placed, subjects were allowed to rest for 30 minutes before baseline measurements were made. After measurement of basal forearm blood flow (FBF) and blood sampling, graded doses of sodium nitroprusside, ACh, and bradykinin were infused in random order. Sodium nitroprusside was infused at 0.8, 1.6, and 3.2 μg/min; ACh was infused at 7.5, 15, and 30 μg/min in the first 3 subjects and 15, 30, and 60 μg/min in the remaining 7 subjects, and bradykinin was infused at 100, 200, and 400 ng/min. Each dose was infused for 5 minutes and FBF was measured during the last 2 minutes of the infusion. Drug concentrations in the infusate were adjusted to maintain infusion volumes at 1 mL/min. Before infusion of each drug, a 30-minute rest period was allowed and basal measurements were repeated.

Forearm Perfusion Measurements

FBF was measured using mercury-in-Silastic strain gauge plethysmography. The wrist was supported in a sling to raise the level of the forearm to above the level of the atrium, and the strain gauge was placed in the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson), calibrated to measure the percent change in volume and connected to a chart recorder to record the flow measurements. For each measurement, a cuff placed around the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10, Hokanson) to occlude venous outflow from the extremity. The hand was excluded from the measurement of blood flow by inflation of a pediatric sphygmomanometer cuff to 200 mm Hg around the wrist before and during measurement of FBF. Flow measurements were recorded for ~7 of 15 seconds, and the slope was derived from the first 3 to 4 pulses; 7 readings were obtained for each mean value.

Effect of Vasodilators on Hemodynamic and Fibrinolytic Parameters

![Figure 1. Effect of sodium nitroprusside, ACh, and bradykinin on FBF (n=10, except as noted). All 3 vasodilators caused a significant, dose-dependent increase in FBF. The vasodilator response to ACh was significantly less than the response to the other 2 drugs. *P<0.05, **P<0.01, ***P<0.005 vs baseline. +P<0.05, ++P<0.01, +++P<0.005 vs ACh.](http://hyper.ahajournals.org/content/33/6/1432)

### Effect of Vasodilators on Hemodynamic and Fibrinolytic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sodium Nitroprusside, μg/min</th>
<th>ACh, μg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>84.4±2.1</td>
<td>83.4±2.8</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>62.2±3.2</td>
<td>62.6±3.8</td>
</tr>
<tr>
<td>FBF, mL·100 mL⁻¹·min⁻¹</td>
<td>2.1±0.2</td>
<td>6±0.8†</td>
</tr>
<tr>
<td>tPA antigen–infused arm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial, ng/mL</td>
<td>3.23±1.00</td>
<td>3.18±0.99</td>
</tr>
<tr>
<td>Venous, ng/mL</td>
<td>3.97±1.00</td>
<td>3.90±1.1</td>
</tr>
<tr>
<td>A-V gradient, ng/mL</td>
<td>0.73±0.21</td>
<td>0.72±0.52</td>
</tr>
<tr>
<td>Net release, ng·100 mL⁻¹·min⁻¹</td>
<td>1.12±0.31</td>
<td>2.50±1.41</td>
</tr>
<tr>
<td>PAI-1 antigen–infused arm</td>
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<td></td>
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<tr>
<td>Arterial, ng/mL</td>
<td>5.2±0.60</td>
<td>7.0±2.0</td>
</tr>
<tr>
<td>Venous, ng/mL</td>
<td>5.0±0.8</td>
<td>3.3±0.6</td>
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<tr>
<td>A-V gradient, ng/mL</td>
<td>-1.4±0.5</td>
<td>-2.5±8.8</td>
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<tr>
<td>Net release, ng·100 mL⁻¹·min⁻¹</td>
<td>-2.0±0.7</td>
<td>-10.5±3.8</td>
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<tr>
<td>Norepinephrine+epinephrine–infused arm</td>
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<td></td>
</tr>
<tr>
<td>Venous, pg/mL</td>
<td>276±45</td>
<td>301±46</td>
</tr>
</tbody>
</table>

n=10 for hemodynamic measurements and venous sampling before and after drug; n=6 for arterial sampling, venous sampling at intermediate doses, and net release calculations unless noted otherwise in parentheses.

*P<0.005 vs baseline; †P<0.05 vs Ach; ‡P<0.05, §P<0.01 vs baseline; ||P<0.01, ¶P<0.05 vs Ach; #P<0.05, **P<0.01 vs sodium nitroprusside.
Blood Sampling and Biochemical Assays

After FBF was measured, simultaneous venous samples were obtained from the infused and noninfused arms before drugs were administered and at the highest dose of each drug. In 6 subjects (subjects 5 through 10), samples were obtained at each dose of drug and simultaneous arterial samples were obtained from the infusion arm to allow calculation of the net release or uptake rate (see below). Drug infusion was interrupted during arterial sampling. All samples were obtained after the first 3 mL of blood were discarded.

Fibrinolytic Parameters

No significant effect of either nitroprusside or ACh was seen on venous tPA antigen concentration in the infused or opposite arm, arterial tPA antigen, or net tPA release (Table 2 and Figure 2). In contrast, bradykinin caused a significant and dose-dependent increase in venous tPA from the infused arm (effect of dose F=9.9, P=0.007) and bradykinin (F=7.3, P=0.015) (Figure 1). The increase in FBF in response to ACh was significantly less than the increase in FBF in response to either nitroprusside (effect of drug F=14.4, P=0.004) or bradykinin (F=15.7, P=0.003). However, there was not a significant difference between the FBF response to nitroprusside or to bradykinin.

Statistics

Data are presented as mean±SEM. Comparisons between drugs were made using ANOVA for repeated measures in which the within-subject variables were drug and dose increments. Post hoc comparisons were made using the paired t test or Wilcoxon signed rank test, as appropriate. A 2-tailed value of P<0.05 was the criterion for statistical significance.

Results

Hemodynamics and FBF

There was no significant effect of sodium nitroprusside, ACh, or bradykinin on either mean arterial pressure or heart rate (Table). FBF increased in a dose-dependent fashion in response to nitroprusside (F=8.9, P=0.009 for dose effect by ANOVA), ACh (F=9.8, P=0.007), and bradykinin (F=7.3, P=0.015) (Figure 1). The increase in FBF in response to ACh was significantly less than the increase in FBF in response to either nitroprusside (effect of drug F=14.4, P=0.004) or bradykinin (F=15.7, P=0.003). However, there was not a significant difference between the FBF response to nitroprusside or to bradykinin.

Blood samples were collected on ice and centrifuged immediately and plasma was stored at −70°C until the time of assay. Blood for measurement of plasminogen activator inhibitor (PAI-1) and tPA was collected in tubes containing 0.105 mol/L sodium citrate. Antigen levels were determined using a 2-site ELISA (Biopool AB) as previously described.16 Catecholamines were collected in tubes containing reduced glutathione and measured by high-performance liquid chromatography, as previously described.17

Arteriovenous concentration gradients were calculated by subtracting the plasma level measured in simultaneously collected venous and arterial blood. Forearm plasma flow was calculated from the FBF and arterial hematocrit corrected for 1% trapped plasma. Thus, individual net release or uptake rates at each time point were calculated with the following formula:

\[
\text{Net Release} = (C_v - C_a) \times \left\{ \frac{\text{FBF} \times [(100 - \text{Hematocrit})/100]}{100} \right\}
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\]
ACh (3.1±0.4 infused versus 3.6±0.7 ng/mL, *P*=0.4), or bradykinin (5.3±1.4 infused versus 3.9±0.6 ng/mL noninfused, *P*=0.5).

**Catecholamines**

No effect of nitroprusside, ACh, or bradykinin was found on venous norepinephrine or epinephrine concentrations (Table).

**Discussion**

tPA, the key enzyme in the initiation of fibrinolysis, is synthesized in endothelial cells; stored in small, dense vesicles; and secreted by endothelial cells in response to a number of vasoactive agents. Studies of vascular endothelial cells isolated perfused tissue preparations, and in whole rats suggest that bradykinin is a potent stimulant of endothelial tPA release. For example, bradykinin was 1000-fold more potent than other agonists, such as histamine, norepinephrine, vasopressin, and ACh in stimulating release of circulating tPA in a rodent model. The present study provides evidence that bradykinin is a dose-dependent stimulant of endothelial tPA release in humans.

In an earlier study, systemic administration of bradykinin resulted in increased venous tPA concentrations in the presence of ACE inhibition; however, interpretation of that study was confounded by activation of the sympathetic nervous system and by potential changes in hepatic blood flow resulting from systemic hemodynamic effects of bradykinin. The use of local intra-arterial infusion of bradykinin in the present study allowed us to exclude such confounding factors. Thus, intra-arterial infusion of vasodilators had no systemic effects as measured by mean arterial pressure and heart rate. Although venous tPA increased slightly in the noninfused arm during the highest dose of bradykinin, venous tPA was significantly higher in the infused arm compared with the noninfused arm. This finding and the lack of change in arterial tPA concentrations suggest a local effect of bradykinin on tPA release.

Increased shear has been reported to stimulate tPA release; however, the present study does not support the hypothesis that increased blood flow alone is responsible for the observed effect of bradykinin on tPA release. Administration of nitroprusside, a nitric-oxide synthase-independent vasodilator, did not increase tPA release, despite its having a vasodilator effect similar to that of bradykinin. A lack of effect of nitroprusside on tPA release has been observed in several other studies. Furthermore, ACh, a muscarinic agonist, failed to induce an increase in net tPA release in this study. This contrasts with data from Jern et al and from a previous study by our group that show that methacholine increases net tPA release across the forearm. However, Pedrinelli et al have also observed a lack of effect of intra-arterial ACh on tPA concentration across the forearm. One possible explanation for the finding of the present study is that doses of ACh inadequate to stimulate tPA release were administered. The FBF response to ACh was significantly less than the FBF response to either of the other 2 vasodilators administered, despite the fact that the doses of ACh administered (0 to 60 μg/min) were higher than those generally used to assess endothelial vasodilation in humans (0 to 30 μg/min). The inclusion of black subjects, who have been shown to have blunted vasodilator responses to muscarinic agonists, may have contributed to this effect. Further studies are needed to assess the effect of race on the tPA response to bradykinin and other vasoactive substances. A second explanation for the lack of effect of ACh on tPA release is that ACh and methacholine may differ in their potency for stimulating endothelial tPA release. Previous investigators have shown a 10-fold greater vasodilator potency of methacholine versus ACh and have attributed this to more rapid degradation of ACh by cholinesterases. Nevertheless, given the blunted vasodilator response to ACh observed in the present study versus previous studies in healthy volunteers and our previous observation that methacholine increases tPA release, we cannot exclude the possibility that ACh would stimulate tPA release under different experimental conditions.

Although this is the first study to delineate the effects of intra-arterial administration of bradykinin on local vascular release of tPA in humans, the subject population studied was heterogeneous with respect to characteristics such as race and gender. Numerous studies have demonstrated that factors such as race, smoking, lipid level, estrogen level, hypertension, and glucose and insulin levels affect endothelial function, as measured by vasodilator responses to intra-arterial agonists or reactive hyperemia. Jern et al have also demonstrated that tPA release across the forearm in response to methacholine is attenuated in hypertensive subjects versus normotensive subjects. The extent to which individual characteristics influence endothelial function, as measured by the tPA response to bradykinin, remains to be studied.

The mechanism through which bradykinin induces endothelial tPA release is not clear from the present study. Like most substances that have been shown to induce acute tPA release, bradykinin stimulates prostacyclin and nitric oxide synthesis. The vasodilator effects of bradykinin are attenuated by bradykinin subtype B-specific receptor antagonists and by the nitric oxide synthase inhibitor, Nω-monomethyl-L-arginine, but not by cyclo-oxygenase inhibitors in humans. Nω-monomethyl-L-arginine has been shown to attenuate the tPA response to substance P which suggests that nitric oxide plays a role in stimulation of endothelial tPA release. Further studies are needed to delineate the pathways involved in bradykinin-stimulated endothelial tPA release.

Decreased bradykinin degradation appears to contribute to the vasodilator effects of ACE inhibitors in humans. Thus, coadministration of an ACE inhibitor potentiates the effects of intravenous and intra-arterial bradykinin. Conversely, bradykinin receptor blockade attenuates the effects of ACE inhibition on endothelium-mediated vasodilation and on blood pressure. The present finding that bradykinin increases endothelial tPA release suggests that bradykinin may also contribute to a favorable effect of ACE inhibitors on fibrinolytic balance. In this regard, Hornig et al recently reported a significant increase in forearm venous tPA antigen during intra-brachial administration of an ACE inhibitor. In addition, during chronic ACE inhibition, plasma tPA antigen concentrations (which reflect both active tPA and inactive tPA complexes with PAI-1) are preserved, despite the fact that PAI-1 antigen concentrations decrease. Further studies are needed to determine if coadmin-
istration of a bradykinin antagonist will abolish this effect of ACE inhibition on endothelial tPA release.

In summary, the present study demonstrates that intra-arterial administration of bradykinin results in a substantial local release of tPA. This release occurred in a dose-dependent fashion, in the absence of systemic effects, and was independent of changes in FBF. Thus, these data suggest that bradykinin is a flow-independent stimulus for tPA release in the human vasculature.

Acknowledgments
This work was supported by NIH grants HL 51387, HL 60906, HL 56251, HL 56963, and M01 RR 0095; by a Clinical Investigator Award from the Veterans Affairs Research Administration (D.E.V.); and by a Pharmaceutical Research and Manufacturers Association Foundation Career Development Award (C.M.S.).

References
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Hypertension. 1999;33:1431-1435
doi: 10.1161/01.HYP.33.6.1431

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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