Ethnicity Affects Vasodilation, but Not Endothelial Tissue Plasminogen Activator Release, in Response to Bradykinin

David A. Rosenbaum, Mias Pretorius, James V. Gainer, Daniel Byrne, Laine J. Mumphey, Corrie A. Painter, Douglas E. Vaughan, Nancy J. Brown

Abstract—Previous studies indicate that the vasodilator response to bradykinin (BK) and other endothelium-dependent and -independent agonists is decreased in black Americans compared with white Americans. The purpose of the present study was to determine the effect of ethnicity on fibrinolytic function in humans. Graded doses of BK (100, 200, and 400 ng/min), acetylcholine (15, 30, and 60 μg/min; N=20), or methacholine (3.2, 6.4, 12.8 μg/min; N=20), and sodium nitroprusside (0.8, 1.6, and 3.2 μg/min) were infused via brachial artery in 19 white and 21 black age-matched normotensive subjects. Forearm blood flow (FBF) was measured by plethysmography, and venous and arterial samples were collected for tissue plasminogen activator (tPA) antigen. Compared with whites (increase in FBF from 3.7±0.5 to 23.9±2.5 mL·min⁻¹·100 mL⁻¹), blacks (increase in FBF from 2.8±0.3 to 15.2±1.9 mL·100 mL⁻¹·min⁻¹) exhibited a blunted FBF response to BK (P=0.035). Responses to sodium nitroprusside and methacholine or acetylcholine were similarly decreased. In contrast, there was no effect of ethnicity on net tPA antigen release in response to BK (increase from −0.2±0.4 to 67.3±15.2 ng·min⁻¹·100 mL⁻¹ in blacks; from 0.04±0.9 to 65.9±13.6 ng·min⁻¹·100 mL⁻¹ in whites). Thus, ethnicity significantly influenced the relationship between the flow and tPA release responses to BK (P=0.037). These data suggest that the BK-dependent alterations in vascular fibrinolytic function are preserved in black Americans compared with white Americans. (Arterioscler Thromb Vasc Biol. 2002;22:1023-1028.)

Key Words: bradykinin ■ vasodilator ■ fibrinolysis ■ ethnicity ■ endothelium

The prevalence of hypertension, stroke, congestive heart failure, and diabetic nephropathy is higher in black Americans than in white Americans.1 Black Americans exhibit decreased vasodilation in response to a number of agonists, including bradykinin (BK), isoproterenol, methacholine (MCh), acetylcholine (ACh), and sodium nitroprusside (SNP).2–5 This decreased vasodilation in response to both endothelium-dependent and -independent agonists suggests a generalized impairment in vascular smooth muscle relaxation, the mechanism of which is uncertain.

BK contributes to many of the cardiovascular effects of angiotensin-converting enzyme (ACE) inhibitors.6,7 In addition to causing vasodilation, BK stimulates release of tissue plasminogen activator (tPA) from the vascular endothelium through a BK subtype B₂ receptor-dependent pathway.8 Although BK-mediated vasodilatation reflects both endothelial production of nitric oxide (NO) and other mediators and subsequent vascular smooth muscle relaxation,9 tPA release reflects a direct effect of BK on the endothelium.10

Several lines of evidence suggest that endogenous BK levels are decreased in blacks. Compared with whites, blacks have lower excretion of urinary kallikrein, an index of activity of the kallikrein-kinin system.11 Decreased levels of endogenous BK would be expected to result in upregulation of its major receptor and increased sensitivity to exogenous BK.12,13 Compatible with this hypothesis, blacks exhibit a greater wheal response to intradermally administered bradykinin than do whites.14 However, the effect of ethnicity on the tPA release in response to BK is not known. The purpose of the present study is to compare the effect of ethnicity on the vasodilator and tPA release responses to BK.

Methods

Subjects

Forty healthy, normotensive volunteers were studied. Nineteen subjects were white and 21 subjects were black. Race was self-defined by the ethnicity of the subjects’ maternal and paternal grandparents. For example, subjects were defined as black if all four of the subject’s grandparents were African American; Africans or Caribbeans of African descent were excluded. All subjects gave written, informed consent, gave a medical history, and underwent a physical examination, laboratory screening, and ECG. All subjects weighed within 25% of ideal body weight, and none was taking medication. Smokers and individuals with a fasting serum cholesterol ≥220 mg/dL were excluded. All subjects abstained from caffeine ingestion for 5 days before the study day and were studied under salt-replete conditions, as dietary sodium intake does not affect the vasodilator response to BK.8 The protocol was approved by the
Vanderbilt University Institutional Review Board and conducted according to institutional guidelines.

**Experimental Protocol**

Studies were performed in the morning, in a temperature-controlled room in the General Clinical Research Center. Subjects were studied in the supine position and in the fasting state. A 20-gauge polyurethane catheter (Cook, Inc) was inserted into the brachial artery of the nondominant arm, and an intravenous catheter was placed in the antecubital vein. Before the infusion of drugs, arterial catheter patency was maintained by infusion of 5% dextrose in water at a rate of 1 mL/min, and subjects were allowed to rest 30 minutes before baseline measurements were made and between drug infusions.

Blood pressure was monitored in the contralateral arm with an automated blood pressure cuff throughout the study. After measurement of basal forearm blood flow (FBF) and blood sampling, graded doses of SNP (Gensia Siccor Pharmaceuticals), ACh (Mibebol-E, Ciba Vision), and BK (Calbiochem) were infused in random order. After the first 20 subjects were studied (12 black and 8 white), the muscarinic agonist was changed to MCh (Pharmaceutical Compound Center of America) because of concerns about instability of ACh. SNP was infused at 0.8, 1.6, and 3.2 μg/min; ACh at 15, 30, and 60 μg/min; MCh at 3.2, 6.4, and 12.8 μg/min; and BK at 100, 200, and 400 ng/min. Each dose was infused for 5 minutes, and FBF was measured during the last 2 minutes of infusion. Drug concentrations in the infused were adjusted to maintain infusion volumes at 1 mL/min. FBF was measured by stratic-in-mucropy-systain-gauge plethysmography, as previously described. Forearm vascular resistance (FVR) was calculated as mean arterial pressure (MAP)/FBF.

**Blood Sampling and Biochemical Assays**

After measurement of FBF, arterial and venous samples were obtained from the infused arm before and after each dose of study drug. All samples were obtained after the first 3 mL of blood was discarded. Blood samples for fibrinolytic assays were collected on ice and centrifuged immediately, and plasma was stored at −80 °C until the time of assay. Blood for measurement of plasminogen activator inhibitor-1 (PAI-1) and tPA was collected in tubes containing 0.105 mol/L acidified sodium citrate, and antigen levels were determined by using a 2-site ELISA (Biopool AB). tPA activity was measured in 15 black subjects (9 female, 6 male) and 16 white subjects (7 female, 9 male) by using a commercially available chromogenic assay (Biopool). Forearm plasma flow (FPF) was calculated from the FBF and arterial hematocrit corrected for 1% trapped plasma. Thus, net release was calculated as the arterio-venous concentration gradient across the forearm times FPF by using the formula: net release=(Cv−Ca)/(FBF×(101−hematocrit)/100)), in which Cv and Ca represent the plasma concentrations measured in simultaneously collected venous and arterial blood. For tPA activity, the term net increment is used to emphasize the fact that changes may not only reflect tissue release/uptake but also possible shifts between the complex-bound and free forms during passage through the forearm. In 14 white and 11 black subjects, venous blood was collected immediately into chilled ethanol (1:3 vol:vol) at the end of the 400-ng/min BK infusion, and BK and its stable metabolite, BK 1-5, were measured with liquid chromatography mass spectrometry, as previously described.

**Statistics**

Data are expressed as mean values ± SEM. Subject characteristics were analyzed by unpaired Student t test or χ² analysis, as appropriate. Comparisons between ethnic groups were made with the Mann-Whitney test. An α value of less than 0.05 was considered statistically significant.

**Results**

There were no significant differences between the blacks and whites in age, heart rate, diastolic blood pressure, sex, cholesterol, or baseline tPA and PAI-1 antigen (Table 1). BMI and systolic blood pressure were significantly higher in blacks compared with whites. There were no adverse effects of BK, SNP, or ACh. Three of the 20 subjects who received MCh complained of transient headache, which resolved after discontinuation of the MCh.

Table 1 illustrates FBF, FVR, net tPA antigen release, and net tPA activity increment in response to intra-arterial infusion of BK. A significant dose-dependent increase in FBF was found in response to BK in both whites (P<0.001) and blacks (P<0.001). BMI (P=0.009 for FBF and P=0.001 for FVR) and cholesterol (P=0.011 for FBF and P=0.031 for FVR), both included as covariates in the ANOVA, significantly affected the vasodilator response to BK. There was no effect of sex on the vasodilator response to BK (P=0.232 for FBF and P=0.621 for FVR). The FBF response to BK was significantly greater in whites than in blacks (P=0.035). There was no significant effect of ACE I/D genotype on FBF (P=0.926). Ethnicity alone did not significantly affect FVR during BK infusion (P=0.534). However, as reported previ-
ously, there was a significant interactive effect of ACE I/D genotype and ethnicity on the FVR response to BK (P=0.038). ACE I/D genotype alone also predicted the FVR to BK (P=0.021).

There was no effect of ethnicity on venous BK (435±52 and 725±159 fmol/mL in blacks and white, respectively; P=0.094) or BK 1-5 (1661±33 and 1440±270 fmol/mL in blacks and whites, respectively; P=0.68) concentrations achieved during the highest dose of BK. However, the molar ratio of BK1-5 to BK was significantly higher in blacks compared with whites, after controlling for ACE I/D genotype (3.97 vs 2.39, P=0.009). There was no effect of sex on BK (P=0.397) or BK1-5 (P=0.514) concentrations or on the molar ratio of BK1-5 to BK (P=0.254).

### TABLE 2. FBF, tPA Antigen, and tPA Activity During Intra-arterial BK (mean±SEM)

<table>
<thead>
<tr>
<th>BK Dose (ng/min)</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FBF, mL·min⁻¹·100 mL⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>2.8±0.3</td>
<td>10.8±1.4§</td>
<td>11.6±1.3¶</td>
<td>15.2±1.9¶</td>
</tr>
<tr>
<td>White</td>
<td>3.7±0.5</td>
<td>16.9±7.4 ¶</td>
<td>21.0±1.9 ¶</td>
<td>23.9±2.5 ¶</td>
</tr>
<tr>
<td>All</td>
<td>3.2±0.3</td>
<td>13.6±1.2 ¶</td>
<td>16.1±1.3 ¶</td>
<td>19.3±1.7 ¶</td>
</tr>
<tr>
<td></td>
<td>a-v gradient tPA antigen, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>−0.1±0.2</td>
<td>4.2±1.3 ¶</td>
<td>5.7±1.0 ¶</td>
<td>7.3±1.2 ¶</td>
</tr>
<tr>
<td>White</td>
<td>0.0±0.4</td>
<td>2.4±0.6 ¶</td>
<td>3.6±0.9 ¶</td>
<td>4.9±0.8 ¶</td>
</tr>
<tr>
<td>All</td>
<td>−0.1±0.2</td>
<td>3.3±0.8 ¶</td>
<td>4.7±0.7 ¶</td>
<td>6.2±0.7 ¶</td>
</tr>
<tr>
<td></td>
<td>a-v gradient tPA activity, IU/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>0.3±0.2</td>
<td>1.2±0.4</td>
<td>2.4±0.8*</td>
<td>2.4±0.8*</td>
</tr>
<tr>
<td>White</td>
<td>0.0±0.1</td>
<td>0.2±0.5</td>
<td>0.9±0.5</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td>All</td>
<td>0.2±0.1</td>
<td>0.7±0.3</td>
<td>1.6±0.5 ¶</td>
<td>1.8±0.4 ¶</td>
</tr>
<tr>
<td></td>
<td>Net release tPA antigen, ng·min⁻¹·100 mL⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>−0.2±0.4</td>
<td>31.6±10.6 ¶</td>
<td>47.3±11.5 ¶</td>
<td>67.3±15.2 ¶</td>
</tr>
<tr>
<td>White</td>
<td>0.2±0.9</td>
<td>24.4±4.9 ¶</td>
<td>46.7±10.2 ¶</td>
<td>65.9±13.6 ¶</td>
</tr>
<tr>
<td>All</td>
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<td>47.0±7.6 ¶</td>
<td>66.6±10.1 ¶</td>
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<tr>
<td></td>
<td>Net increment tPA activity, IU·min⁻¹·100 mL⁻¹</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>0.7±0.4</td>
<td>12.0±6.5 ¶</td>
<td>21.3±7.6 ¶</td>
<td>31.7±15.8*</td>
</tr>
<tr>
<td>White</td>
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<td>5.1±3.5</td>
<td>13.5±5.8*</td>
<td>16.5±7.8*</td>
</tr>
<tr>
<td>All</td>
<td>0.4±0.2</td>
<td>8.5±3.6*</td>
<td>17.3±4.7 ¶</td>
<td>23.9±8.6*</td>
</tr>
</tbody>
</table>

P values for ANOVA are provided in the text. For post hoc comparisons, *P<0.05, †P<0.01, ‡P<0.005 vs baseline. §P<0.01, ||P<0.005 vs whites.
FBF increased significantly and FVR decreased significantly in response to SNP and either ACh or MCh in both blacks and whites (data not shown). BMI (P=0.023), but not cholesterol (P=0.090), significantly affected FVR during SNP administration. Cholesterol (P=0.033), but not BMI (P=0.150), significantly affected FVR during infusion of either muscarinic agonist. There was no effect of sex on FVR during infusion of either SNP (P=0.670) or the muscarinic agonists (P=0.256). Ethnicity affected FVR during MCh or ACh (P=0.049) and SNP (P=0.051), such that FVR was greater in blacks than in whites. There was no effect, however, of the ACE I/D polymorphism alone or in interaction with ethnicity on FVR, during infusion of the muscarinic agonists or SNP (all P>0.45).

Net tPA antigen release increased significantly in a dose-dependent manner in response to BK in both blacks (P=0.005) and whites (P<0.001). As reported previously, PAI-1 antigen did not increase. The increase in net tPA antigen release reflected a dose-dependent increase in the arterio-venous (a-v) concentration gradient for tPA antigen (P<0.001), as well as an increase in FBF during BK infusion (Table 2). The increase in the a-v concentration gradient for tPA antigen was greater in blacks compared with whites (P=0.048), reflecting the decreased FBF response to BK and consequent decreased dilutional effect. There was no effect of BMI (P=0.548), cholesterol (P=0.064), or ACE I/D genotype (P=0.999) on net tPA antigen release in response to BK. There was a borderline significant effect of sex (P=0.054) on net tPA antigen release in response to BK, with women tending to have a higher net tPA antigen release during BK. In contrast to the effect of ethnicity on the FBF response to BK, there was no effect of ethnicity on net tPA antigen release in response to BK (P=0.734), even after controlling for sex. For example, net tPA antigen release in response to 400 ng/min BK was 31.7±15.8 IU · min⁻¹ · 100 mL⁻¹ in blacks and 16.5±7.8 IU · min⁻¹ · 100 mL⁻¹ in whites (95% confidence interval of the difference -12.2, 57.3 IU · min⁻¹ · 100 mL⁻¹).

As reported previously, there was no effect of either SNP or ACh on net tPA antigen release (data not shown). Net tPA antigen release increased in a dose-dependent manner during MCh (P=0.009); there was no effect of either sex (P=0.225) or ethnicity (P=0.296) on net tPA antigen release in the 20 subjects who received MCh.

**Discussion**

Numerous previous studies indicate that the vasodilatation response to both endothelium-dependent and -independent agonists is decreased in black Americans compared with white Americans. The present study confirms these findings, but indicates that tPA release in response to BK is preserved in black Americans compared with white Americans.

One possible explanation for the diminished FBF response to BK in blacks compared with whites is an effect of ethnicity on the pharmacokinetics of BK. Although there was no effect of ethnicity on BK or BK 1-5 concentrations during BK infusion, the finding that the molar ratio of BK1-5 to BK was increased in blacks compared with whites suggests that BK degradation occurs more rapidly in blacks compared with whites. However, even though enhanced degradation of BK could account for the decreased FBF response to BK, it could not account for the diminished vasodilator response to other agonists. In addition, the preserved tPA release in response to BK in blacks, in the setting of enhanced degradation of BK, may indicate increased sensitivity at the receptor level.

Several possibilities may account for the differential effect of ethnicity on the vasodilator and tPA release responses to BK. First, whereas the vasodilatation response to BK depends on both endothelial production of NO and other mediators and subsequent vascular smooth muscle relaxation, net tPA release reflects a direct endothelial effect of BK. Thus, the finding that vasodilation but not tPA release in response to BK is decreased in black Americans compared with white Americans is consistent with an effect of ethnicity on vascular smooth muscle relaxation rather than endothelial function. A limitation of the present study is the difference in blood pressure between the blacks and whites studied. In this regard, the effect of ethnicity on the vasodilator response to BK may reflect an effect of blood pressure.

In previous studies, comparison of the vasodilator response to endothelium-dependent agonists to the vasodilator response to NO donors, along with measurement of the vasodilator response to endothelium-dependent agonists before and after administration of a NO synthase (NOS) inhibitor, has been used to differentiate endothelial versus vascular smooth muscle function. The present study suggests that...
comparison of the vasodilator and tPA release responses to a single agonist, BK, may be used to distinguish between endothelial and vascular smooth muscle function.

A second possible explanation for the differential effect of ethnicity on the tPA release and vasodilator responses to BK involves differences in the mechanisms of these two effects. In vitro, BK has been observed to cause vasodilation through NO, through prostaglandins, and through endothelium-derived hyperpolarizing factor (EDHF).9 In humans, administration of the NOS inhibitor N'–monomethyl-L-arginine attenuates the vasodilator response to BK, indicating the NO contributes to BK-induced vasodilation.19 In contrast, N’–monomethyl-L-arginine alone or in combination with indomethacin does not affect the increase in net tPA release in response to BK,9 suggesting that tPA release is mediated through a NOS- and cyclooxygenase-independent pathway, such as through EDHF. Thus, it is possible that ethnicity could affect either the production of or response to NO, but not the production of or the response to EDHF.

In addition to elucidating the effect of ethnicity on the vasodilator and net tPA release responses to BK, the present study provides preliminary data regarding a possible effect of sex on net tPA antigen release in response to BK. Although there was no effect of sex on the vasodilator response to either endothelium-dependent or -independent agonists in this healthy subject population, female sex was marginally associated with an increased tPA antigen release but not tPA activity increment in response to BK. Estrogen agonists induce tPA expression,20 and increased estrogen concentrations are associated with an increased fibrinolitic potential. While estrogen induces rapid vasodilation through increased nitric oxide,21,22 BK stimulates tPA through a NOS-independent pathway, as outlined above. Interestingly, a recent report in ovarectomized rats indicates that estrogen increases the contribution of EDHF to flow-mediated vasodilation.23 Because the present study was not specifically designed to assess the effect of sex on BK-stimulated tPA release, further investigation is needed. Similarly, effects of the menstrual cycle and hormone replacement therapy on BK-stimulated tPA release warrant study.

The finding that ethnicity affects the vasodilator response but not net tPA release during BK infusion may have important clinical implications. Endogenous BK contributes to the acute blood pressure–lowering effects of ACE inhibitors,7 which improve fibrinolitic balance24 and reduce cardiovascular mortality.25 Previous studies indicate that stimulated tPA release from the forearm vasculature provides a good model of stimulated coronary tPA release and that ACE inhibition potentiates the effect of BK on coronary tPA release.26–28 Black Americans, as a population, exhibit decreased sensitivity to the antihypertensive effects of ACE inhibitors.29 While this results in part from the prevalence of low renin hypertension among black Americans,30 decreased sensitivity to BK-mediated vasodilation may also play a role. Given the finding that BK-stimulated tPA release is preserved in blacks, further studies are needed to examine the effect of ACE inhibition on net tPA release in black and white patients at risk for cardiovascular and renal disease.

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References


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