Ethnic Differences in the Vasoconstrictor Activity of Endogenous Endothelin-1 in Hypertensive Patients

Umberto Campia, MD; Carmine Cardillo, MD; Julio A. Panza, MD

**Background**—The pathogenesis of essential hypertension in blacks may differ from that in whites. In particular, black patients usually present with a salt-sensitive, low-renin form, which in animal models is associated with enhanced activity of endothelin-1 (ET-1). This study aimed to assess whether ethnic differences exist in the vascular activity of ET-1 in normotensive and hypertensive blacks and whites.

**Methods and Results**—Forearm blood flow (FBF) responses to intraarterial infusion of an ET<sub>A</sub> receptor blocker (BQ-123) were analyzed by plethysmography in 37 normotensive patients and 27 hypertensive patients according to race. BQ-123 did not affect FBF in normotensive subjects (<i>P</i>=0.30), whereas it produced significant vasodilation in hypertensive subjects (<i>P</i>&lt;0.001). In normotensives, FBF response to BQ-123 was similar in white (<i>n</i>=22) and black (<i>n</i>=15) patients (<i>P</i>=0.85). In contrast, in hypertensive patients, the vasodilator effect of ET<sub>A</sub> receptor blockade was significantly higher in blacks (<i>n</i>=13) than in whites (<i>n</i>=14) (<i>P</i>=0.01). To rule out differences in smooth muscle reactivity, the effects of race on FBF responses to exogenous ET-1 were analyzed in the hypertensive subgroups. Endothelin-1 induced a significant vasoconstriction in both white (<i>n</i>=7) and black patients (<i>n</i>=5) (both <i>P</i>&lt;0.001), without differences between them (<i>P</i>=0.46). In 8 black hypertensives, the response to selective ET<sub>A</sub> blockade was not modified by nonselective blockade of ET-1 receptors by co-infusion of BQ-123 and BQ-788 (<i>P</i>=0.66).

**Conclusions**—Hypertensive blacks have enhanced ET<sub>A</sub>-dependent vasoconstrictor tone, probably related to increased production of ET-1. Given the negative vascular effects of ET-1, this abnormality may contribute to the pathogenesis of hypertension and its complications in black patients. (**Circulation. 2004;109:3191-3195.**)

**Key Words:** hypertension ■ endothelin ■ endothelium

Epidemiological studies indicate that atherosclerosis and its complications, the leading cause of death among adults in the United States, carry considerably higher morbidity and mortality in blacks than in whites.<sup>1</sup>–<sup>3</sup> These observations may be explained in part by an increased risk in blacks of developing traditional atherosclerotic risk factors, such as essential hypertension and diabetes mellitus.<sup>4</sup>–<sup>6</sup> In particular, in black subjects, essential hypertension has higher prevalence, earlier onset, and is associated with more severe end-organ damage, including left ventricular hypertrophy, renal failure, and stroke.<sup>5</sup> Despite intense investigation and the evidence that in blacks essential hypertension usually presents with salt-sensitive, low-renin features,<sup>7</sup> the pathophysiological processes underlying this ethnic predisposition have not been fully elucidated.<sup>8</sup>

Recently, research has focused on possible differences in arterial wall function between blacks and whites, and on the impact of these differences on vascular homeostasis.<sup>9</sup> Studies conducted in our and other laboratories have demonstrated that young, healthy black subjects have reduced nitric oxide (NO)-mediated vasodilation of forearm resistance vessels to mental stress<sup>10</sup> and to endothelium-dependent and -independent pharmacological stimuli.<sup>11</sup>,<sup>12</sup> These results indicate the presence of impaired vascular smooth muscle relaxation, which may lead, in the long term, to increased vascular tone and hypertension. A defect of blood vessels to relax in response to vasodilator stimuli might be related to increased activity of vasoconstrictor substances, as suggested by previous studies in our laboratory showing that enhanced endothelin-1 (ET-1) vasoconstrictor tone importantly contributes to the impaired endothelium-dependent vasodilation in hypertensive patients.<sup>13</sup> Besides its vasoactive actions, ET-1 may exert important mitogenic activity on vascular smooth cells<sup>14</sup> and cardiac myocytes,<sup>15</sup> thereby contributing to the structural changes of the vasculature and the heart associated with hypertension. In addition, ET-1 contributes to the preservation of Na<sup>+</sup> and water balance, and may be involved in the development and maintenance of salt-sensitive hypertension, as indicated by studies in animal models of hypertension.<sup>16</sup>,<sup>17</sup>
A possible involvement of ET-1 in the development of hypertension and its complications in blacks has also been suggested by studies showing that plasma concentrations of ET-1 are increased in hypertensive black patients compared with white patients. Circulating levels of ET-1, however, are likely related to variable spillover of the peptide from the vasculature into the bloodstream, and may not accurately reflect its production or biological effects. Thus, whether an increased ET-1 activity preferentially contributes to the development of hypertension and its complications in blacks has not been elucidated. The present study was therefore designed to test the hypothesis that ethnic differences exist in the activity of ET-1 by the use of antagonists of ET-1 receptors.

### Methods

#### Study Subjects

The study population included 27 hypertensive patients and 37 healthy controls who participated in prospective studies designed to investigate the in vivo vasoconstrictor activity of ET-1 in patients with essential hypertension and conducted in the Cardiology Branch of the National Heart, Lung, and Blood Institute (NHLBI) (Table). Hypertensive patients were followed-up at the outpatient clinic and had well-documented histories of chronically elevated blood pressure (≥140/90 mm Hg) without any apparent underlying cause. Treated hypertensive patients were not taking any medication other than their antihypertensive drugs, which were discontinued at least 2 weeks before the study. None of the patients had a history of dyslipidemia, diabetes mellitus, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon. Normal volunteers selected as a control group were matched with the patients for approximate race, gender, and age. Each subject was screened by clinical history, physical examination, ECG, chest x-ray, and routine chemical analyses. None had evidence of present or past hypertension, dyslipidemia, cardiovascular disease, or any other systemic condition, and none of them was taking medications at the time of the study. None of the subjects or patients participating in this study smoked. The study protocols were approved by the Investigational Review Board of the NHLBI and all participants gave written informed consent.

#### Protocols

Studies were performed in the morning in a quiet room with a temperature of approximately 22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine for at least 24 hours before the studies. Each study consisted of infusion of drugs into the brachial artery and measurement of the response of the forearm vasculature by means of strain-gauge venous occlusion plethysmography, as previously described in detail. Forearm blood flow (FBF) measurements were recorded for approximately 7 seconds every 15 seconds; 7 readings were obtained for each mean value. Blood pressure was recorded directly from the intraarterial catheter, and heart rate was recorded from an electrocardiographic lead. All drugs were approved for human use by the Food and Drug Administration in the form of investigational new drug and were prepared following specific procedures to ensure accurate bioavailability and sterility of the solutions. Throughout all studies, volumes infused were matched by administration of variable amounts of normal saline solution.

#### Assessment of the Effects of Race on Vascular Responses to ET<sub>A</sub> Receptor Blockade in Normal Subjects and in Hypertensive Patients

Basal FBF measurements were obtained after a 15-minute infusion of saline at 1 mL/min. Normal subjects and hypertensive patients then received intraarterial infusion of BQ-123. BQ-123 (Peninsula Laboratories), a synthetic peptide with high potency of antagonism for the ET<sub>A</sub> receptor, was infused at 100 nmol/min (100 nmol/mL solution), a dose that effectively counteracts the vasconstrictor effect of ET-1 infusion in the human forearm. BQ-123 was given for 60 minutes (1 mL/min infusion rate) and FBF was measured every 10 minutes.

#### Assessment of Vascular Responses to ET-1 in Black and White Hypertensive Patients

To determine whether differences exist in the vascular sensitivity to the hemodynamic effects of ET-1 between black and white hypertensives, experiments were performed, on a separate day, to compare the vasomotor responses to exogenous ET-1 in the 2 groups. To this end, after basal measurements were obtained, 5 black and 7 white hypertensives received intraarterial infusion of ET-1. ET-1 ( Bachem Inc.; 5 pmol/mL solution) was given at 5 pmol/min (1 mL/min infusion rate) for 60 minutes, and FBF was measured at 10-minute intervals.

#### Assessment of Vascular Responses to Selective ET<sub>A</sub> Blockade and Nonselective ET<sub>A/B</sub> Blockade in Black Hypertensive Patients

To investigate whether differences exist between vascular responses to selective ET<sub>A</sub> blockade and nonselective ET<sub>A/B</sub> blockade in black hypertensives, on a different occasion, 8 black patients received a co-infusion of BQ-123 and BQ-788. BQ-788 (Peninsula Laboratories; 50 nmol/mL solution), a highly selective synthetic antagonist of the ET<sub>A</sub> receptor, was given at 50 nmol/min (1 mL/min infusion rate), a dose that allows a local concentration in the forearm more than 10-fold higher than the pA<sub>2</sub> (negative logarithm of the molar concentration of antagonist that causes a 2-fold parallel shift to the right of the concentration-response curve) at the ET<sub>A</sub> receptor. The combination of BQ-123 (at the same dose as before) and BQ-788 was infused for 60 minutes. FBF measurements were obtained every 10 minutes and the response to the combination of BQ-123 and BQ-788 was compared with that observed in the same patients during the administration of BQ-123 alone.

#### Statistical Analyses

Two means were compared by Student’s t-test. Within each group, changes in FBF from baseline in response to the infused drugs were

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**Clinical Characteristics of the Study Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls</th>
<th>Hypertensive Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>24/13</td>
<td>15/12</td>
<td>0.54</td>
</tr>
<tr>
<td>Age, y</td>
<td>43 ± 1</td>
<td>52 ± 1</td>
<td>0.11</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79 ± 2</td>
<td>85 ± 3</td>
<td>0.14</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 2</td>
<td>170 ± 2</td>
<td>0.10</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>76 ± 1</td>
<td>112 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race, white/black</td>
<td>22/15</td>
<td>14/13</td>
<td>0.61</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.43 ± 0.10</td>
<td>4.57 ± 0.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mmol/L</td>
<td>2.77 ± 0.13</td>
<td>2.87 ± 0.016</td>
<td>0.59</td>
</tr>
<tr>
<td>mg/dL</td>
<td>171 ± 4</td>
<td>177 ± 5</td>
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</tr>
<tr>
<td>mg/dL</td>
<td>107 ± 5</td>
<td>111 ± 6</td>
<td></td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mmol/L</td>
<td>1.40 ± 0.07</td>
<td>1.35 ± 0.16</td>
<td>0.80</td>
</tr>
<tr>
<td>mg/dL</td>
<td>54 ± 3</td>
<td>52 ± 6</td>
<td></td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.05 ± 0.1</td>
<td>1.26 ± 0.16</td>
<td>0.27</td>
</tr>
<tr>
<td>mg/dL</td>
<td>93 ± 10</td>
<td>112 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. MAP indicates mean arterial pressure.
assessed by 1-way ANOVA for repeated measures. Group comparisons of the responses to selective ET<sub>A</sub> blockade and to exogenous ET-1 infusion were performed by 2-way ANOVA. Comparison of the effects of selective ET<sub>A</sub> blockade and nonselective ET<sub>A/B</sub> blockade in black hypertensives was performed by 2-way ANOVA for repeated measures. When significant differences were found by ANOVA, post-hoc analyses for multiple comparisons were performed by Dunnett’s or Student-Newman-Keuls’ test, as appropriate.

All calculated probability values are 2-tailed, and a probability value /H110210.05/ was considered to indicate statistical significance. All group data are reported as mean±SEM.

Results

Mean arterial pressure and heart rate did not significantly change after infusion of any of the drugs used in the study, thus indicating that drug effects were limited to the infused forearm. Baseline FBF was not significantly different between groups at all times (all P>0.05).

Effects of ET<sub>A</sub> Receptor Blockade in Normal Subjects and in Hypertensive Patients

As previously reported, in control subjects, infusion of BQ-123 did not result in significant changes of FBF from baseline (6±3% increase; P=0.30). On the contrary, in hypertensive patients, BQ-123 administration resulted in a significant vasodilator response (19±4% increment in FBF; P<0.001). As a result, FBF values during selective ET<sub>A</sub> blockade were significantly higher in hypertensive patients than in normotensive subjects (Figure 1).

Effects of Race on the Vascular Responses to ET<sub>A</sub> Receptor Blockade in Normal Subjects and in Hypertensive Patients

In the normotensive group, FBF response to BQ-123 was similar between black (n=15) and white (n=22) subjects (5±2% versus 6±2%, respectively; P=0.85). In contrast, in the hypertensive population, the vasodilator effect of ET<sub>A</sub> receptor blockade was significantly higher in the black (n=13) compared with the white (n=14) patients (24±3% versus 15±3%, respectively; P=0.01) (Figure 2).

Effects of Race on the Vascular Responses to ET-1 in Hypertensive Patients

Infusion of ET-1 induced a significant vasoconstrictor response in both black (n=5) and white (n=7) hypertensives (both P<0.001 versus baseline), without significant differences between the 2 groups (35±2% versus 37±2% decrease in FBF, respectively; P=0.46) (Figure 3).

Vascular Responses to Selective ET<sub>A</sub> and Nonselective ET<sub>A/B</sub> Blockade in Black Hypertensive Patients

In a subgroup of black hypertensive patients (n=8), nonselective ET<sub>A/B</sub> receptor blockade induced a vasodilator re-

Figure 1. Graph showing FBF responses to BQ-123 (100 nmol/min) in normotensive subjects and hypertensive patients. Values represent mean±SEM. The probability value refers to the comparison between the 2 groups in FBF response from baseline during selective ET<sub>A</sub> blockade by 2-way ANOVA.

Figure 2. Bars showing the average FBF responses to 60 minutes’ infusion of BQ-123 (100 nmol/min) in normotensive subjects and hypertensive patients according to race. Values represent mean±SEM. The probability values refer to the comparison in the FBF responses from baseline during selective ET<sub>A</sub> blockade by 2-way ANOVA.

Figure 3. Graph showing FBF responses to ET-1 (5 pmol/min) in hypertensive patients according to race. Values represent mean±SEM. The probability value refers to the comparison between the 2 groups in FBF response from baseline during ET-1 infusion by 2-way ANOVA.
The main finding of the present study is that ET_A receptor antagonism (20±10% versus 25±10%, respectively; P=0.66) (Figure 4).

**Discussion**

The main finding of the present study is that ET_A receptor blockade with BQ-123 results in a significantly higher forearm vasodilator response in black than in white hypertensive patients. In contrast, BQ-123 does not significantly affect forearm blood flow in either black or white healthy controls.

Diverse mechanisms may underlie the greater ET_A-dependent vasoconstriction observed in black hypertensive patients compared with white hypertensive patients, such as an increased availability of ET-1 at the level of ET_A receptors or an enhanced vascular sensitivity to the constrictor effects of ET-1. To better define the pathophysiological abnormalities underlying these ethnic differences, we compared forearm vascular responsiveness to exogenous ET-1 between black and white hypertensives. ET-1 infusion induced an equivalent vasoconstrictor response in both ethnic groups, suggesting that an increased production and/or a decreased clearance of ET-1 are the most likely mechanisms in play and arguing against the presence of an enhanced vascular reactivity to the peptide in blacks. Our results are in keeping with the evidence of an augmented ability for vascular ET-1 biosynthesis in hypertensive blacks, consistent with increased tissue endothelin converting enzyme-1a expression and activity and significantly higher plasma ET-1 concentrations.

Several mechanisms may potentially account for a selectively enhanced vascular production of ET-1 in blacks. Physiologically, ET-1 gene expression is stimulated by numerous vasoconstrictor factors, including catecholamines and angiotensin II, and is inhibited by vasodilators such as nitric oxide (NO) and prostacyclin. It is possible that the amplified sympathetic reactivity to stressful environmental stimuli observed in blacks, and the concomitant decreased sensitivity to NO-dependent and adrenergic vasodilation, may lead to enhanced ET-1 gene transcription and ET-1 synthesis. This hypothesis is supported by recent data showing higher baseline levels of plasma ET-1 and exaggerated plasma ET-1 responses to behavioral and physical challenges in adolescent blacks with a family history of essential hypertension. Further, ET-1 may amplify the contractile response to other vasoactive agents, thus enhancing hemodynamic reactivity and promoting a self-maintained vasoconstrictor cycle.

Another potential mechanism that may contribute to the higher ET-1 plasma levels and activity observed in hypertensive black patients is a decreased clearance of ET-1. A significant proportion of ET-1 clearance seems to occur through endothelial cell ET_B receptor binding and internalization. In vivo animal studies have shown that ET_B receptor antagonism increases plasma ET-1 concentrations and prolongs its biological half-life. Recent data demonstrate that hypertensive black subjects have a decreased endothelial cell expression of ET_B receptors, which may hinder ET-1 clearance, thereby increasing bioavailability of the peptide at the ET_B receptors. This possibility is consistent with the results obtained in the present study in the subgroup of hypertensive blacks in whom the hemodynamic effects of selective ET_A and nonselective ET_A/B blockade were compared. In those patients, the vasodilator response was similar under both experimental conditions, in contrast to our previous observations in a prevalent white population of hypertensive patients, in whom the combined infusion of BQ-123 and BQ-788 induced a greater vasodilation than the administration of BQ-123 alone. These findings suggest a “neutral” effect of endothelial and smooth muscle ET_B receptors in determining the hemodynamic response to ET-1 in hypertensive blacks and are compatible with the possibility of a consensual down-regulation of both ET_B receptors in these patients. However, the design of our investigation does not allow more definitive conclusions in this regard. Further studies will be necessary to better characterize the contribution of different ET receptor subtypes in the pathophysiology of the ET system in blacks with essential hypertension.

The results of this investigation may have important pathophysiological and clinical implications and may explain, at least in part, the increased cardiovascular risk present in black hypertensives. Thus, the multiple actions of ET-1, by altering renal function and promoting vascular remodeling, atherosclerotic plaque formation, and left ventricular hypertrophy, may contribute to the genesis and maintenance of essential hypertension and to the development its complications in these patients. Based on our observations, ET-1 receptor blockade might potentially exert beneficial effect in the treatment of essential hypertension and its cardiovascular consequences in black subjects.

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References

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