Attenuation of Cyclic Nucleotide–Mediated Smooth Muscle Relaxation in Blacks as a Cause of Racial Differences in Vasodilator Function

Carmine Cardillo, MD; Crescence M. Kilcoyne, RN, MS; Richard O. Cannon III, MD; Julio A. Panza, MD

Background—Vasodilator reactivity is attenuated in normotensive blacks, and this may contribute to their enhanced susceptibility to hypertension and its complications. However, the mechanisms responsible for this phenomenon are unknown. We therefore studied nitric oxide (NO)–dependent and –independent vasorelaxation in healthy blacks and whites to investigate the nature of racial differences in vasodilator function.

Methods and Results—Forearm flow responses to intra-arterial infusion of increasing doses of acetylcholine (a vasodilator that stimulates endothelial release of NO), sodium nitroprusside (an exogenous NO donor), and isoproterenol (a β-adrenergic agonist whose vasodilator effect stems from the combination of direct smooth muscle stimulation and endothelial NO release) were studied in 18 normotensive whites and 18 blacks by use of strain-gauge plethysmography. A blunted vasodilator response to acetylcholine (7.2±1.1 versus 14.4±1.8 mL·min⁻¹·dL⁻¹; P<0.001) and sodium nitroprusside (8.2±1.1 versus 12.1±1.3 mL·min⁻¹·dL⁻¹; P<0.001) was observed in blacks compared with whites, suggesting decreased cGMP-mediated smooth muscle relaxation. The vasodilator effect of isoproterenol was lower in blacks than in whites both before (10.9±1.7 versus 14.9±1.5 mL·min⁻¹·dL⁻¹; P=0.006) and after N⁶-monomethyl-L-arginine (6.1±1.2 versus 10.1±0.8 mL·min⁻¹·dL⁻¹; P<0.001), implying that cAMP-dependent vasodilator response to isoproterenol is diminished in blacks. No significant difference was observed in the hyperemic response to forearm ischemia.

Conclusions— Compared with whites, healthy blacks have reduced vasodilation in response to NO-dependent and –independent stimuli. This difference seems to be related to an attenuation in cyclic nucleotide–mediated vascular smooth muscle relaxation and may play a role in the increased prevalence of hypertension and its complications in blacks. (Circulation. 1999;99:90-95.)

Key Words: race ■ nitric oxide ■ receptors, adrenergic, beta ■ nucleotides, cyclic ■ vasodilation

The prevalence of essential hypertension and the severity of its cardiovascular complications are considerably higher in blacks compared with whites.¹ ² This greater susceptibility to end-organ involvement leads, in turn, to higher rates of morbidity and mortality from those diseases that are directly related to hypertension, such as cerebrovascular accident and renal failure.³ Various genetic and environmental factors have been postulated to explain these racial differences in the clinical presentation of hypertension.⁴–⁶ One hypothesis is that the development and subsequent course of hypertension in blacks are related to enhanced vasoconstrictor reactivity to environmental stressors,⁷–¹⁰ resulting from decreased vasodilator capacity of resistance arteries, as suggested by the results of a recent study demonstrating that the vasodilator response to β-adrenergic receptor stimulation is attenuated in normotensive blacks.¹¹ Among several vasorelaxing substances that participate in the regulation of vascular tone, endothelium-derived nitric oxide (NO) plays a major physiological role, as demonstrated by studies showing that NO synthesis inhibition in normal subjects results in marked vasoconstriction.¹²,¹³ Importantly, endothelial release of NO has recently been shown to contribute to the vasodilator effect of β-adrenergic stimulation¹⁴,¹⁵; thus, it is possible that decreased NO activity could also explain the blunted vasodilator response to isoproterenol previously demonstrated in blacks.¹¹ Furthermore, a reduction in both basal and receptor-stimulated NO activity has been widely demonstrated in hypertensive patients⁶–⁹ and, more recently, even in normotensive offspring of hypertensive parents,²⁰ supporting the concept that decreased NO activity might play a pathophysiological role in the development of hypertension. It is therefore reasonable to hypothesize that decreased vascular activity of NO could also be...
involved in the reduced vasodilator capacity and the increased susceptibility to develop high blood pressure observed in normotensive blacks.

Although most studies of endothelial dysfunction in essential hypertension have been limited to Caucasian populations of patients and control subjects, a recent report has shown that NO-dependent vasodilation is decreased in normotensive blacks, strengthening the view that this defect might be related to the increased prevalence of hypertension and its vascular complications in blacks. The precise mechanism underlying the abnormality in vasodilator function in blacks, however, has not been fully elucidated. Thus, the present study was designed to investigate NO-dependent and -independent vasorelaxation in blacks and whites to better characterize the nature of racial differences in vasodilator function.

Methods

Study Population
A population of 36 normal volunteers (18 whites, 18 blacks) with no evidence of present or past hypertension or hypercholesterolemia (plasma cholesterol \( \leq 200 \) mg/dL) was selected for this study. The clinical characteristics of the 2 groups are reported in the Table. In each study subject, clinic blood pressure was defined as the average of 3 sitting blood pressure readings taken after 5 minutes of rest on 3 different occasions during a 3- to 4-week period.

Before admission, subjects of each group were screened by clinical history, physical examination, routine chemical analyses, ECG, and chest radiography. Exclusion criteria were history or evidence of present or past diabetes mellitus, cardiac disease, peripheral vascular disease, coagulopathy, or any other disease predisposing them to vasculitis or Raynaud’s phenomenon. None of the patients was taking any medication at the time of the study.

The study protocol was approved by the National Heart, Lung and Blood Institute Investigational Review Board, and all participants gave written informed consent for all procedures.

Protocol
All studies were performed in the morning in a quiet room with a temperature of \( \sim 22^\circ \)C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before studies.

Clinical Characteristics of the Study Population

<table>
<thead>
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<th></th>
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<tr>
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<tr>
<td>Age, y</td>
<td>52±2</td>
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<td>Weight, kg</td>
<td>78±4</td>
<td>78±4</td>
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<td>Body mass index, kg/m²</td>
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<td>Smoking, Y/N</td>
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<td>1/17</td>
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<td>Mean arterial pressure, mm Hg</td>
<td>84±2</td>
<td>86±2</td>
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<td>8/10</td>
<td>6/12</td>
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<tr>
<td>Forearm blood flow, mL · min⁻¹ · dL⁻¹</td>
<td>3.1±0.1</td>
<td>3±0.3</td>
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<td>Plasma glucose, mg/dL</td>
<td>91±2</td>
<td>93±3</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
<td>180±6</td>
<td>165±6</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
<td>49±5</td>
<td>46±4</td>
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</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>104±15</td>
<td>96±22</td>
<td>0.77</td>
</tr>
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</table>

Data are mean±SEM.

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. All drugs used in this study were approved for human use by the Food and Drug Administration in the form of investigational new drugs and were prepared by the Pharmaceutical Development Service of the National Institutes of Health following specific procedures to ensure accurate bioavailability and sterility of the solutions.

While the participants were supine, a 20-gauge Teflon catheter (Arrow Inc) was inserted into the brachial artery of the nondominant arm (left in most cases). This arm was slightly elevated above the level of the right atrium, and a mercury-filled Silastic strain gauge was placed in the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, DE 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. A wrist cuff was inflated to suprasystolic pressures 1 minute before each measurement to exclude the hand circulation. Flow measurements were recorded for 7 seconds every 15 seconds; 7 readings were obtained for each mean value.

Basal measurements were obtained after a 3-minute infusion of 5% dextrose solution at 1 mL/min. Forearm blood flow was then measured after the infusion of acetylcholine, sodium nitroprusside, and isoproterenol. Acetylcholine induces vasodilation by stimulating the release of relaxing factors from the vascular endothelium. Sodium nitroprusside, in contrast, was used as an endothelium-independent vasodilator because its vasodilator effect is largely due to its direct action on smooth muscle cells. Isoproterenol is a \( \beta \)-adrenoceptor agonist whose vasodilator effect is partially mediated by endothelial release of NO. Acetylcholine chloride (Sigma Chemical Co) was infused at 7.5, 15, and 30 \( \mu \)g/min, sodium nitroprusside at 0.8, 1.6, and 3.2 \( \mu \)g/min, and isoproterenol at 50, 100, and 200 ng/min (infusion rates were 0.25, 0.5, and 1 mL/min, respectively, for each drug). Each dose was infused for 5 minutes, and forearm flow was measured during the last 2 minutes. A 30-minute rest period was allowed, and another basal measurement was obtained between infusions of the 3 drugs.

Because of the combined nature (NO-dependent and -independent) of the vasodilator effect of isoproterenol, NO synthase inhibitor \( N^\text{G} \)-monomethyl-L-arginine (L-NMMA; Sigma) was used to investigate potential differences between the 2 groups in the different components of isoproterenol-mediated vasodilation. L-NMMA was infused at 4 \( \mu \)mol/min (infusion rate, 1 mL/min) for 15 minutes, and baseline flow measurements were obtained. This dose of L-NMMA has previously been shown to effectively blunt in vivo the synthesis of NO and thereby reduce the vasodilator effect of acetylcholine in the human forearm. Subsequently, the cumulative dose-response curve for isoproterenol was repeated during the concomitant infusion of L-NMMA with the same doses, infusion rates, and resting interval reported above.

The sequence of infusion of acetylcholine, sodium nitroprusside, and isoproterenol before the infusion of L-NMMA was randomized to avoid any bias related to the order of drug infusion. During the studies, participants were unaware of the drug being infused.

To test the possibility of generalized interethnic differences in vasodilator function, reactive hyperemic blood flow responsiveness to forearm ischemia was also measured on a different occasion at least 4 weeks apart. Ischemia is a nonspecific stimulus thought to induce vasodilation through involvement of different biochemical mediators and structural mechanisms. The peak reactive hyperemic response to ischemia in the forearm circulation, however, is not significantly determined by NO bioavailability, as demonstrated in a previous study showing that the peak flow response to ischemia is not modified by NO synthesis inhibition with L-NMMA. Ischemia was induced by inflation of a сфимпометрический cuff on the upper arm to suprasystolic pressure (200 mm Hg) for 5 minutes and was aimed at producing a vasodilator response of a magnitude at least similar to that induced by the pharmacological stimuli used in this study.
study. Peak reactive hyperemic flow was measured 5 seconds after release of the cuff.

All blood pressures were recorded directly from the intra-arterial catheter after each flow measurement. Forearm vascular resistance was calculated as mean arterial pressure divided by forearm blood flow.

**Statistical Analysis**

Differences between the 2 groups were analyzed by unpaired Student’s t-test, χ² test, and 2-way ANOVA as appropriate. ANCOVA was used to adjust the vasodilator response to each drug for the age differences between the 2 groups. L-NMMA effects on baseline hemodynamic variables were analyzed by paired Student’s t-test. Within-group responses before and after L-NMMA were compared by ANOVA for repeated measures. Factors potentially affecting forearm blood flow responses to acetylcholine, sodium nitroprusside, and isoproterenol were identified by multiple regression analysis. The covariates considered were race, age, sex, mean arterial pressure, history of hypertension in a first-degree relative, and plasma cholesterol. All covariates were examined as predictors of forearm blood flow response to acetylcholine, sodium nitroprusside, and isoproterenol as a group in 1 multivariate regression model. All calculated probability values are 2-tailed, and P<0.05 was considered to indicate statistical significance. All group data are reported as mean±SEM.

**Results**

**Baseline Measurements**

The clinical characteristics of the subjects in the two study groups are indicated in the Table. There was no significant difference between the 2 groups in sex, weight, body mass index, smoking habit, mean arterial pressure, family history of hypertension, baseline forearm blood flow, plasma glucose, and plasma lipids. Black subjects were, on average, 9 years younger than whites (P=0.002).

**Vascular Responses to Acetylcholine, Sodium Nitroprusside, and Isoproterenol**

Acetylcholine infusion induced a dose-dependent increase in forearm blood flow in both groups, but the vasodilator effect of this substance was significantly blunted in blacks compared with whites (Figure 1).

During isoproterenol infusion, there was a dose-dependent increase in forearm blood flow in both groups, and similar to acetylcholine and sodium nitroprusside results, the vasodilator effect of this substance was significantly blunted in blacks compared with whites (Figure 1).

A significant decrease in the vasodilator response to acetylcholine (P<0.001), sodium nitroprusside (P<0.001), and isoproterenol (P=0.002) was present in blacks compared with whites even after adjustment for age differences between the 2 groups. Results of multivariate regression analysis showed that race was the only independent linear predictor of the forearm blood flow responses to acetylcholine (P=0.001), sodium nitroprusside (P=0.01), and isoproterenol (P=0.009), whereas age, sex, mean arterial pressure, family history of hypertension, and plasma cholesterol were not significant predictors. Moreover, in the overall study population, subjects with positive family histories of hypertension (n=14) did not have reduced vasorelaxing response to acetylcholine (P=0.48), sodium nitroprusside (P=0.83), and isoproterenol (P=0.92) compared with those without family histories (n=22).

**Effects of L-NMMA on Vascular Responses to Isoproterenol**

No significant change in systemic blood pressure or heart rate was observed with infusion of L-NMMA in either whites or blacks. Baseline forearm blood flow was significantly lower during L-NMMA than during saline administration in both whites (P=0.006) and blacks (P=0.003).

Compared with saline, L-NMMA administration significantly blunted the forearm blood flow response to isoproterenol in both whites and blacks (Figure 2). The vasodilator response to isoproterenol after L-NMMA administration was significantly lower in blacks than in whites (P<0.001) (Figure 2).

**Vascular Response to Reactive Hyperemia**

After 5 minutes of forearm ischemia, a marked increase in forearm blood flow was observed in both groups. Peak
reactive hyperemic blood flow tended to be higher in blacks than in whites, although the difference was not statistically significant (Figure 3).

**Discussion**

The results of the present study demonstrate that compared with whites, normotensive blacks have a decreased responsiveness to pharmacological agents that produce vascular smooth muscle relaxation through both cGMP- and cAMP-dependent pathways. Thus, the blunted vasodilator response to acetylcholine and sodium nitroprusside indicates that NO-mediated vasorelaxation is impaired in black subjects because both these drugs induce vasodilation by stimulating soluble guanylyl cyclase and increasing vascular smooth muscle cGMP content through either stimulation of endogenous release of NO (acetylcholine) or direct formation of NO (sodium nitroprusside). The attenuation in vasodilator function of normotensive blacks differs from that previously reported in mostly white populations of hypertensive patients, in whom blunted vasodilation to acetylcholine is generally associated with preserved vascular response to sodium nitroprusside, indicating a specific defect in endothelium-dependent vasodilator function. In contrast, the decreased vasodilator responsiveness to both endogenous and exogenous NO observed in normotensive blacks suggests an endothelium-independent defect. This finding is in agreement with the results of a recent study showing that NO-dependent vasodilation is impaired in normotensive blacks. One possible explanation of the blunted vasodilator action of NO in healthy blacks is a selective resistance of vascular smooth muscle to vasorelaxation induced by cGMP. Another possibility that should be considered is that the diffusion of NO to vascular smooth muscle could be affected by early changes in the thickness of the vessel wall, as recently described in normotensive blacks. However, this mechanism seems unlikely because in patients with essential hypertension, who commonly have vascular wall hypertrophy, the vasodilator response to sodium nitroprusside is preserved, indicating that the diffusion capacity of NO is not affected by structural changes in the arterial wall.

In keeping with the results of a previous report, healthy blacks in our study also had decreased vasodilator response to isoproterenol, an agonist of β-adrenergic receptors. β-Adrenergic–mediated vasodilation involves a combination of endothelial and smooth muscle mechanisms because isoproterenol induces direct smooth muscle relaxation and NO release from endothelial cells. We therefore used L-NMMA to investigate potential ethnic differences in the NO-independent component of isoproterenol-induced vasodilation. We observed that the vasodilator response to isoproterenol after NO synthesis inhibition by L-NMMA was significantly lower in blacks than in whites, indicating that the direct, NO-independent component of isoproterenol-induced vasorelaxation, which is determined by stimulation of adenyl cyclase and increased smooth muscle content of cAMP, is attenuated in blacks.

To rule out the possibility that the reduction in vascular smooth muscle dilator capacity observed in blacks could be related to a generalized defect in vasodilator function, we also analyzed the peak hyperemic vasodilator response to forearm ischemia, which involves several biochemical mediators in conjunction with structural mechanisms, but is not heavily dependent on NO availability. Blacks and whites had similar peak reactive hyperemic responses to ischemia, thereby suggesting that their reduction in cyclic nucleotide–mediated vasodilation is unlikely related to nonspecific abnormalities in vascular reactivity. These results are in contrast with those of previous studies reporting that the forearm vasodilator capacity in response to ischemia is reduced in normotensive blacks and suggesting the presence of early vascular remodeling in blacks. It must be noted, however, that both studies reporting racial differences in reactive hyperemia were designed to assess minimal forearm vascular resistance, so the ischemic stimulus was applied for 10 minutes in conjunction with isometric exercise, and hyperemic blood flow responses were generally higher than that observed in our study. In contrast, ischemia was applied for only 5 minutes in our study because it was aimed at producing a vasodilator response of a magnitude at least similar to that observed with the pharmacological stimuli.
Taken together, the results of our study indicate that the decreased vasodilator capacity observed in normotensive blacks is related to an attenuation in cyclic nucleotide–mediated smooth muscle relaxation because the responses to both cGMP- (endogenous and exogenous NO) and cAMP-dependent (isoproterenol) are impaired. A common mechanism might be involved in the genesis of these blunted vasodilator responses. For example, both cGMP and cAMP stimulate kinases (G- and A-kinase, respectively) that, in turn, induce smooth muscle relaxation through phosphorylation of unknown cellular substrates. Among the possible downstream targets of cyclic nucleotide–mediated phosphorylation are (1) phospholamban, which modulates Ca2+ uptake by sarcoplasmic reticulum Ca2+-ATPase or possibly the Ca2+ leak from this organelle; (2) plasmalemmal Ca2+ pumps or K+ channels, leading to hyperpolarization and vasodilation; and (3) kinases and/or phosphatases upstream of myosin light-chain phosphorylation, leading to a decreased sensitivity of contractile proteins to Ca2+ and resulting in vasodilatation. It is possible that racial differences may exist at one or more of these sites that contribute to the blunted vasodilator responses in normotensive blacks. Another possible explanation is that the effect of local intra-arterial NO is blunted in blacks because of decreased sensitivity of the endothelium-dependent NO/cGMP pathway.

The results of our study may help to explain the decreased vasodilator capacity observed in normotensive blacks. Thus, a defective responsiveness to vasodilator stimuli in these subjects may result in an abnormal pattern of hemodynamic reactivity to environmental stimuli, thereby leading, in the long term, to increased vascular tone and vascular hypertrophy. Our observations may also be relevant to the mechanisms of coronary heart disease in blacks. Previous reports have indicated that blacks have a higher prevalence of hypertension and its complications in blacks.

References


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