Racial Differences in Nitric Oxide–Mediated Vasodilator Response to Mental Stress in the Forearm Circulation

Carmine Cardillo, Crescence M. Kilcoyne, Richard O. Cannon III, Julio A. Panza

Abstract—An abnormal hemodynamic response to stressful stimuli has been proposed as a mechanism involved in the higher prevalence of hypertension in blacks. Given the important role of nitric oxide (NO) in the regulation of cardiovascular homeostasis, we investigated the possibility of racial differences in vascular NO activity during mental stress. To test this hypothesis, we compared the forearm blood flow (FBF) response to mental stress in 14 white and 12 black healthy subjects during intra-arterial infusion of either saline or NO synthesis inhibitor Nω-monomethyl-L-arginine (L-NMMA; 4 μmol/min). We also examined vascular responses of the two groups to intra-arterial infusion of sodium nitroprusside (0.8 to 3.2 μg/min), an exogenous NO donor. During saline infusion, the increase in FBF from baseline induced by mental stress was significantly higher in whites than in blacks (109±20% versus 58±8%; P = 0.03). L-NMMA significantly reduced stress-induced increase in FBF in whites (from 109±20% to 54±11%; P = 0.004) but not in blacks (from 58±8% to 42±10%; P = 0.24); thus, the vasodilator effect of stress testing during L-NMMA was similar in whites and blacks (54±11% versus 42±10%; P = 0.44). The vasodilator response to sodium nitroprusside was also lower in blacks than in whites (maximum flow, 6.9±2 versus 11.6±3.5 mL·min⁻¹·dL⁻¹; P = 0.001) and was not significantly modified by L-NMMA in either group. Our findings indicate that blacks have a reduced NO-dependent vasodilator activity during mental stress. This difference seems related to reduced sensitivity of smooth muscle to the vasodilator effect of NO and may play some role in the increased prevalence of hypertension and its complications in blacks. (Hypertension. 1998;31:1235-1239.)

Key Words: race ■ nitric oxide ■ stress ■ vasodilation

The prevalence of essential hypertension is considerably higher and the severity of its cardiovascular complications greater in blacks than in 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 accidents and renal failure.³ Various genetic and environmental factors have been postulated to explain these racial differences in the clinical presentation of hypertension.¹⁻⁶ One hypothesis is that the development and subsequent course of hypertension in blacks are related to an abnormal pattern of hemodynamic reactivity, characterized by increase in peripheral vascular resistance after exposure to environmental stimuli such as cold or mental stress.⁷⁻¹² The mechanism responsible for this hemodynamic abnormality, however, has not been clearly defined.

Among the vasoactive substances that physiologically participate in the regulation of vascular adaptation to mental stress, endothelium-derived NO plays an important role, as demonstrated by recent studies showing that NO synthesis inhibition in normal subjects results in marked blunting of forearm vasodilator response to mental tasks.¹³,¹⁴ Importantly, a reduction in NO activity has been widely demonstrated in hypertensive patients¹⁵⁻²⁰ and, more recently, even in normotensive offspring of hypertensive parents²¹; these findings support the concept that decreased action of NO might play a pathophysiological role in the development of hypertension. It is therefore reasonable to hypothesize that a decreased vascular activity of NO also could be involved in the abnormal hemodynamic reactivity pattern to stress and the increased susceptibility to developing high blood pressure over repeated exposures to stressful situations that are observed in African Americans. Thus, the present study was designed to investigate the possibility of racial differences in NO-dependent vasodilator response to mental stress in the forearm circulation of healthy subjects.

Methods

Study Population
A population of 26 normal volunteers (14 whites and 12 blacks) with no evidence of present or past hypertension or hypercholesterolemia (plasma cholesterol ≤200 mg/dL) was selected for this study. The clinical characteristics of the 2 groups are reported in Table 1. The distribution of premenopausal and postmenopausal women was similar between the white (2 and 5, respectively) and black (3 and 3, respectively) populations (P = 0.53).

Before admission, subjects of each group were screened by clinical history, physical examination, routine chemical analyses, electrocardiography, 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.

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 approximately 22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before studies.

Each study consisted of the measurement of the response of the forearm vasculature by means of strain-gauge venous occlusion plethysmography under different experimental conditions. All drugs used in this study were approved for human use by the Food and Drug Administration in the form of Investigational New Drug (IND) 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 polytetrafluoroethylene (Teflon) catheter (Arrow Inc) was inserted into the brachial artery of the left arm. This arm was slightly elevated above the level of the right atrium and a mercury-filled silicone elastomer (Silastic) strain gauge was placed in the widest part of the forearm.22 The strain gauge was connected in turn to a chart recorder to record the flow measurements.

Selected Abbreviations and Acronyms

FBF = forearm blood flow
L-NMMA = Nω-monomethyl-L-arginine
NO = nitric oxide
SNP = sodium nitroprusside

TABLE 1. Clinical Characteristics of Study Population

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<thead>
<tr>
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<tbody>
<tr>
<td>Sex, M/F</td>
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<td>6/7</td>
<td>0.98</td>
</tr>
<tr>
<td>Age, y</td>
<td>49±2</td>
<td>42±2</td>
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<tr>
<td>Weight, kg</td>
<td>77±5</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<td>Smoking, Y/N</td>
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<td>1/11</td>
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<tr>
<td>Basal FBF, mL · min⁻¹ · DL⁻¹</td>
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<td>Mean arterial pressure, mm Hg</td>
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<tr>
<td>Family history of hypertension, Y/N</td>
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<td>0.98</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
<td>175±8</td>
<td>163±7</td>
<td>0.29</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>74±7</td>
<td>88±19</td>
<td>0.45</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>50±5</td>
<td>49±7</td>
<td>0.96</td>
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Data are mean±SEM.

Results

Baseline Measurements

The clinical characteristics of the subjects in the two study groups are shown in Table 1. There was no significant difference between the two groups in gender, age, weight, body mass index, smoking habit, baseline FBF, mean arterial pressure, family history of hypertension, plasma glucose, and plasma lipids.

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<td>0.96</td>
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</table>

Data are mean±SEM.

During infusion of saline, mental stress testing induced a significant increase in FBF from baseline in both groups, but its vasodilator effect was significantly reduced in blacks compared with whites (Figure 1).

Mental stress testing resulted in a significant increase in mean arterial pressure from baseline in both whites and blacks.
blacks (both $P<0.001$) (Table 2), without any significant difference between the two groups ($P=0.69$). Similarly, the performance of mental arithmetic determined a significant increase in heart rate over baseline in both groups (both $P<0.001$) (Table 2), without any significant difference between them ($P=0.66$).

**Effects of L-NMMA on Vascular Responses to Mental Stress**

During L-NMMA administration, the performance of mental stress testing resulted in a significant increase in FBF from baseline in both whites (from 2.5 to 3.5 mL min$^{-1}$ dL$^{-1}$; $P<0.001$) and blacks (from 2.1 to 2.9 mL min$^{-1}$ dL$^{-1}$; $P=0.003$). In whites, however, the percent increase in FBF from baseline during the performance of mental arithmetic testing was significantly lower during NO synthase inhibition than during saline infusion (Figure 2), whereas in blacks it was not significantly different during saline and L-NMMA infusions (Figure 2). The vasodilator effect of mental stress testing was significantly higher in whites than in blacks during saline infusion but was not significantly different between the 2 groups during NO synthesis inhibition by L-NMMA (Figure 2).

During L-NMMA administration, mean arterial pressure and heart rate values during mental stress testing (Table 2) were not significantly different in blacks and whites ($P=0.70$ and $P=0.95$ for mean arterial pressure and heart rate, respectively), and in both groups they were similar to those observed during saline infusion (all $P>0.05$).

**FBF Responses to SNP and Effects of L-NMMA**

During saline infusion, infusion of increasing doses of SNP resulted in a progressive increase in FBF from baseline in both groups; the vasodilator response to SNP, however, was significantly greater in whites than in blacks (Figure 3). L-NMMA administration did not significantly modify the FBF response to the three doses of SNP either in whites (6.3±0.7, 8.4±0.9, 10.9±1 mL min$^{-1}$ dL$^{-1}$; $P=0.43$ versus saline) or in blacks (4.1±0.3, 5.4±0.4, and 7.1±0.6 mL min$^{-1}$ dL$^{-1}$; $P=0.84$ versus saline).

**Vascular Response to Reactive Hyperemia**

After 5 minutes of forearm ischemia, a marked increase in FBF from baseline was observed in both whites (from 3±0.4

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**TABLE 2. Systemic Hemodynamic Variables at Baseline and During Mental Stress Before and After NO Synthesis Inhibition in Whites and Blacks**

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>L-NMMA, 4 μmol/min</th>
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<tr>
<td></td>
<td>Whites</td>
<td>Blacks</td>
</tr>
<tr>
<td>Mean arterial pressure, mL min$^{-1}$ dL$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>86±3</td>
<td>83±3</td>
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<tr>
<td>Mental Stress</td>
<td>101±3</td>
<td>99±3</td>
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<td>Heart rate, bpm</td>
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</tr>
<tr>
<td>Baseline</td>
<td>63±3</td>
<td>66±3</td>
</tr>
<tr>
<td>Mental Stress</td>
<td>81±3</td>
<td>83±3</td>
</tr>
</tbody>
</table>

Data are mean±SEM. No significant group difference was observed in mean arterial pressure and heart rate at baseline and in response to mental stress during either saline or L-NMMA administration.
to 20.6±2.5 mL·min⁻¹·dL⁻¹) and blacks (from 3.3±0.2 to 22.4±2.3 mL·min⁻¹·dL⁻¹), without any significant difference between the two groups in the peak reactive hyperemic response (P=0.30).

Discussion
The results of the present study demonstrate that compared with white subjects, normotensive blacks have a blunted vasodilator response to mental stress. NO synthase inhibition by L-NMMA significantly reduced the mental stress–induced vasodilation in whites but did not result in any significant change in blacks. As a result, during NO synthesis inhibition, the vasomotor response to mental stress was similar in both groups. These findings indicate that the defect in vasodilator responsiveness to mental stress testing observed in blacks was likely related to decreased NO-mediated vasorelaxation.

NO is known to produce its vasorelaxing effect through activation of the soluble guanylyl cyclase within vascular smooth muscle, leading to increased intracellular content of cGMP and subsequent vasodilation. A decreased NO-dependent vasodilator capacity could be potentially related to either reduced availability of NO at the level of smooth muscle cell (as a consequence of decreased NO production and/or increased NO breakdown) or to reduced smooth muscle sensitivity to the dilatory effect of NO.

To investigate the potential mechanism underlying the decreased NO-dependent dilatory response to mental stress observed in healthy blacks compared with whites, we assessed the vasodilator effect of an exogenous NO donor, SNP. We observed that the vasodilator effect of SNP was lower in blacks than in whites, suggesting a decreased smooth muscle responsiveness to nitrovasodilators as a mechanism to explain the decreased NO-dependent vasodilation during mental stress testing observed in African Americans. This mechanism seems different from that underlying the decreased vasodilator response to mental stress in patients with essential hypertension recently observed in our laboratory. Thus, in hypertensive patients, a decreased vascular relaxation to mental stress is associated with preserved responsiveness to SNP. This suggests that the vasodilation defect in these patients is related to decreased availability of NO at the smooth muscle level rather than to blunted responsiveness to NO. The precise intracellular mechanism involved in the decreased vascular smooth muscle responsiveness to endogenous and exogenous NO in African Americans cannot be determined from our data. It is known that cGMP-mediated smooth muscle relaxation is dependent on stimulation of cGMP-dependent protein kinase and phosphorylation of cellular substrates that modulate cytosolic Ca²⁺ concentration and/or Ca²⁺ sensitivity of the contractile apparatus. Thus, it is reasonable to speculate that racial differences in one or more sites along this intracellular pathway might be involved in the decreased vascular smooth muscle responsiveness to NO observed in African Americans. Among the molecules involved in this pathway are phospholamban (which modulates sarcoplasmic reticulum Ca²⁺ uptake), sarcosomal Ca²⁺ pumps, myosin light chain kinase, and myosin phosphatase. Moreover, because both cGMP-dependent protein kinase and NO directly can activate K⁺ channels, it is also possible that racial differences in intracellular regulation of this ion might affect Ca²⁺ handling. This mechanism could in turn lead to decreased NO-dependent smooth muscle vasodilation in African Americans, as suggested by a recent study showing that changes in dietary potassium may modulate the vasodilator responsiveness to mental stress.

To rule out the possibility that the decreased vascular smooth muscle dilator capacity in response to both endogenous and exogenous NO observed in African Americans could be related to a generalized defect in vasodilator function, we compared the vasodilator response to forearm ischemia in blacks and whites. Ischemia is a stimulus that induces vasodilation through involvement of different biochemical mediators, as well as structural mechanisms. The peak reactive hyperemic response to ischemia in the forearm microcirculation, however, seems independent of NO availability, as demonstrated in a previous study showing that the peak flow response to ischemia is not different before and after NO synthesis inhibition with L-NMMA. We observed that blacks and whites have a similar peak reactive hyperemic response to ischemia, thereby suggesting that the reduction in NO-mediated vasodilation of African Americans is not related to a generalized defect in vasodilator function. In our study, we cannot exclude the presence of early vascular structure abnormalities in healthy blacks as previously reported by other investigators, because the existence of vascular hypertrophy can be detected only when ischemia is applied for 10 minutes or more in conjunction with hand exercise. It is important to consider, however, that 5 minutes of forearm ischemia in our study population resulted in a reactive vasodilator response of a magnitude greater than those observed during mental stress and SNP infusion. Thus, this observation supports the view that the racial differences in the vasodilator responsiveness to endogenous and exogenous NO observed in our study could not be accounted for by structural changes of the vessel wall.

Interestingly, in our study, despite the presence of reduced NO-dependent vasodilator responsiveness to mental stress in healthy blacks, the increase in systemic arterial pressure during the psychological challenge was not different between the two groups. Because blood pressure response to mental stress may be achieved through variable combinations of changes in cardiac output and peripheral resistance, our findings are likely explained by a different pattern of hemodynamic reactivity in the two groups, with higher peripheral resistance and lower cardiac output responses in blacks, in keeping with previous observations by other investigators.

In conclusion, our study indicates that healthy blacks have reduced NO-dependent vasodilator responsiveness to mental stress compared with whites. This difference appears to be related to an attenuation of vascular smooth muscle responsiveness to NO, a mechanism that might play a role in the increased prevalence of hypertension and its complications in blacks.

References


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