Differences in Vascular Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor Bioavailability in Blacks and Whites

Muhiddin A. Ozkor, Ayaz M. Rahman, Jonathan R. Murrow, Nino Kavtaradze, Ji Lin, Amita Manatunga, Salim Hayek, Arshed A. Quyyumi

Objective—Abnormalities in nitric oxide (NO) bioavailability have been reported in blacks. Whether there are differences in endothelium-derived hyperpolarizing factor (EDHF) in addition to NO between blacks and whites and how these affect physiological vasodilation remain unknown. We hypothesized that the bioavailability of vascular NO and EDHF, at rest and with pharmacological and physiological vasodilation, varies between whites and blacks.

Approach and Results—In 74 white and 86 black subjects without known cardiovascular disease risk factors, forearm blood flow was measured using plethysmography at rest and during inhibition of NO with N\textsuperscript{G}-monomethyl-l-arginine and of \textit{K}\textsubscript{Ca} channels (EDHF) with tetraethylammonium. The reduction in resting forearm blood flow was greater with N\textsuperscript{G}-monomethyl-l-arginine (\textit{P}=0.019) and similar with tetraethylammonium in whites compared with blacks. Vasodilation with bradykinin, acetylcholine, and sodium nitroprusside was lower in blacks compared with whites (all \textit{P}<0.0001). Inhibition with N\textsuperscript{G}-monomethyl-l-arginine was greater in whites compared with blacks with bradykinin, acetylcholine, and exercise. Inhibition with tetraethylammonium was lower in blacks with bradykinin, but greater during exercise and with acetylcholine.

Conclusions—The contribution to both resting and stimulus-mediated vasodilator tone of NO is greater in whites compared with blacks. EDHF partly compensates for the reduced NO release in exercise and acetylcholine-mediated vasodilation in blacks. Preserved EDHF but reduced NO bioavailability and sensitivity characterizes the vasculature in healthy blacks.

Clinical Trial Registration—URL: http://clinicaltrials.gov/. Unique identifier: NCT00166166.

(Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: African Americans • exercise • nitric oxide • vasodilation

Endothelial dysfunction predisposes to the development of hypertension and atherosclerosis that together inflict significant morbidity and mortality. Blacks have a higher incidence of premature stroke and myocardial infarction when compared with white counterparts.\textsuperscript{1} Although these disparities are often attributed to a higher prevalence of cardiovascular risk factors and socioeconomic burden, underlying endothelial abnormalities may play a crucial underlying role.\textsuperscript{2-4} Abnormalities in nitric oxide (NO) bioavailability in the microcirculation and conductance vessels, reduced sensitivity of exogenous NO, increased endothelin bioavailability, and structural abnormalities in the vascular wall have all been observed in blacks, but the results have been controversial.\textsuperscript{5-16}

The endothelium contributes to the maintenance of vasodilator tone by releasing NO, prostacyclin, and endothelium-derived hyperpolarizing factors (EDHFs).\textsuperscript{17-20} NO contributes to resting vasodilator tone\textsuperscript{21} and physiological vasodilation during exercise,\textsuperscript{22} and its bioavailability is significantly impaired in individuals with cardiovascular risk factors.\textsuperscript{23,24} We have demonstrated an important contribution of EDHF via activation of \textit{K}\textsubscript{Ca} channels to (1) resting vascular tone, (2) vasodilation in response to the endothelium-dependent agonist, bradykinin, and (3) exercise-induced vasodilation in the healthy human forearm vasculature.\textsuperscript{24,25} Whether racial differences in EDHF exist remains unknown. We hypothesized that there is a differential racial contribution of the 2 endogenous agonists, NO and EDHF to vasodilator tone at rest and vasodilation during pharmacological stimulation and with exercise.

Materials and Methods
Materials and Methods are available in the online-only Supplement (Figure 1).
Results

Baseline Characteristics

Black subjects had a greater body mass index and whites had a higher fasting glucose level, but the remaining characteristics were similar (Table). There were no significant changes in the forearm blood flow (FBF) and forearm vascular resistance (FVR) in the noninfused arm, and mean arterial pressure and heart rate remained unchanged in both groups throughout the study period.

Contribution of NO and K\textsuperscript{+}-Ca Channel Activation to Resting Vasodilator Tone

Effect of NG-Monomethyl-l-Arginine on Resting Forearm Vascular Tone

Resting FBF and FVR were similar in black and white subjects. NO blockade with NG-monomethyl-l-arginine (l-NMMA) reduced resting FBF and increased FVR in both groups, but the 21.3% reduction in FBF (P=0.019) and 35% increase in FVR (P=0.023) in blacks were both lower than in whites (32.1% decrease in FBF and 54% increase in FVR), indicating reduced contribution of NO to resting vasodilator tone in blacks (Figure 2A).

Effect of Tetraethylammonium on Resting Forearm Vascular Tone

Blockade of K\textsuperscript{+}-Ca channels with tetraethylammonium (TEA) decreased resting FBF and increased FVR in both subsets, and the magnitude of change was similar in whites and blacks, indicating similar contribution of K\textsuperscript{+}-Ca channel activation to resting vasodilator tone in blacks and whites (Figure 2B).

Effect of l-NMMA Combined With TEA on Resting Forearm Vascular Tone

Combined blockade of NO and K\textsuperscript{+}-Ca channels with l-NMMA and TEA had an additive effect in both subsets and resulted in significantly greater reduction in FBF and increase in FVR than with either antagonist alone (Figure 2C). The reduction in FBF (P=0.003) and increase in FVR (P=0.006) was greater in whites than in blacks, indicating that there is increased contribution to resting vasodilator tone of NO and EDHF together in whites compared with blacks.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDHF</td>
<td>endothelium-derived hyperpolarizing factor</td>
</tr>
<tr>
<td>FBF</td>
<td>forearm blood flow</td>
</tr>
<tr>
<td>FVR</td>
<td>forearm vascular resistance</td>
</tr>
<tr>
<td>l-NMMA</td>
<td>N\textsuperscript{6}-monomethyl-l-arginine</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
</tbody>
</table>

Figure 1. Aspirin (975 mg) was administered 1 hour before commencement of the study. In separate protocols, forearm blood flow measurements were performed after either intra-arterial infusions of bradykinin (100, 200, and 400 ng/min), or intra-arterial acetylcholine (7.5, 15, and 30 μg/min), or handgrip exercise (15%, 30%, and 45% of maximum grip strength) followed by endothelium-independent vasodilation with sodium nitroprusside (1.6, 3.2 μg/min). Measurements were repeated after nitric oxide blockade with N\textsuperscript{6}-monomethyl-l-arginine (l-NMMA), K\textsuperscript{+}-Ca channel blockade with tetraethylammonium (TEA), and after combined blockade with l-NMMA and TEA.
Bradykinin-Stimulated Vasodilation

Bradykinin infusion resulted in dose-dependent vasodilation in both ethnic subsets; however, the response was attenuated in black compared with white subjects, with a 27.3% ($P<0.0001$) lower mean FBF and 32% ($P<0.0001$) greater mean FVR in blacks (Figure 3A).

Effects of L-NMMA on Bradykinin-Mediated Vascular Responses

Bradykinin-induced dilation was attenuated with L-NMMA in both ethnic subsets; however, the reduction in FBF ($-32\%$ versus $-26\%$; $P=0.012$) and the increase in FVR ($78\%$ versus $45\%$; $P=0.002$) were greater in whites than in blacks, indicating reduced contribution of NO to bradykinin-mediated microvascular dilation in black subjects.

Effects of TEA on Bradykinin-Mediated Vascular Responses

Bradykinin-induced dilation was attenuated with TEA in both ethnic subsets; however, the reduction in FBF ($-31\%$ versus $-22\%$; $P<0.0001$) and the increase in FVR ($40\%$ versus $32\%$; $P=0.008$) were greater in whites than in blacks (Figure 4B), indicating a decreased contribution of K$^+$/Ca$^{2+}$ channel activation to bradykinin-stimulated vasodilation in blacks compared with whites.

Effects of Combined Infusions of L-NMMA and TEA on Bradykinin-Mediated Vascular Responses

After combined infusion of L-NMMA and TEA, bradykinin-induced vasodilation was inhibited additively and equally in both subsets; in blacks, $45\%$ ($P<0.0001$) mean reduction in FBF and $94\%$ ($P<0.0001$) increase in mean FVR; and in whites, $57\%$ ($P<0.0001$) lower FBF and $155\%$ ($P<0.0001$) higher FVR.

Acetylcholine-Stimulated Vasodilation

Acetylcholine infusion produced dose-dependent vasodilation in both ethnic subsets; however, the response was attenuated in blacks compared with whites with a 34% ($P<0.0001$) lower mean FBF and 66% ($P<0.0001$) greater mean FVR in blacks (Figure 3B).

Effects of L-NMMA on Acetylcholine-Mediated Vascular Responses

Acetylcholine-induced vasodilation was significantly inhibited by L-NMMA in both ethnic subsets; however, the reduction in FBF ($-36\%$ versus $-20\%$; $P=0.042$) and the increase

<table>
<thead>
<tr>
<th>Table. Characteristics of Study Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacks (n=86)</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL/min</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
</tr>
<tr>
<td>High-density lipoprotein, mg/dL</td>
</tr>
<tr>
<td>Low-density lipoprotein, mg/dL</td>
</tr>
</tbody>
</table>

Glomerular filtration rate calculated using Cockcroft-Gault equation. Data are means±SD. BP indicates blood pressure.

*Statistically significant ($P<0.05$; t test) difference between groups.

Figure 2. Change in resting forearm blood flow and forearm vascular resistance in response to (A) L-$\text{NMMA}$ in 42 healthy black and 46 white subjects, (B) tetraethylammonium (TEA) in 41 healthy black and 45 white subjects, and (C) combined L-$\text{NMMA}$ and TEA infusions in 38 healthy black and 46 white subjects. Data are shown as mean±SEM.
in FVR (81% versus 39%; \(P=0.004\)) were greater in whites than in blacks, indicating greater contribution of NO to acetylcholine-mediated dilation in white compared with black subjects (Figure 5A).

Effects of TEA on Acetylcholine-Mediated Vascular Responses

Acetylcholine-induced vasodilation was inhibited in blacks with TEA (−15% \([P=0.06]\) reduction in FBF and 14%...
NO and EDHF in Blacks

Exercise-Induced Vasodilation

Intermittent handgrip exercise resulted in progressive vasodilation that was not significantly different between the 2 ethnic groups \( (P=0.15) \), although at the 2 higher exercise levels, there was a trend toward a lower increase in FBF in blacks \(-11\%; P<0.06\; \text{Figure 3C})\.

Effects of l-NMMA on Exercise-Induced Vasodilation

Exercise-induced vasodilation was significantly inhibited by l-NMMA in both ethnic subsets; however, the reduction in FBF was greater in whites than in blacks \(-21\% \text{ versus } -9\%; P<0.0001\), indicating decreased contribution of NO to exercise-induced vasodilation in blacks (Figure 6A).

Effects of TEA on Exercise-Induced Vasodilation

Exercise-induced vasodilation was significantly inhibited by TEA in both ethnic subsets; however, the reduction in FBF was greater in blacks than in whites \(-20\% \text{ versus } -16\%; P=0.013\), indicating greater contribution of \( K^+_{\text{Ca}} \) channel activation to exercise-stimulated vasodilation in blacks (Figure 6B). Moreover, in black subjects, exercise-induced vasodilation was attenuated to a greater extent with TEA than with l-NMMA \(-20\% \text{ versus } -9\% \; [P<0.0001] \) reduction in FBF, respectively), indicating that \( K^+_{\text{Ca}} \) channel activation and not NO is the predominant mechanism of exercise-induced vasodilation in blacks.

Sodium Nitroprusside-Induced Vasodilation

Sodium nitroprusside produced vasodilation in both ethnic subsets; however, the response was attenuated in blacks compared with whites \(-22\% \; [P=0.0002] \) lower mean FBF and 12\% \; [P=0.0015] \) greater mean FVR; Figure 3D). This indicates reduced sensitivity of the smooth muscle cells to NO in blacks. Importantly, l-NMMA and TEA did not alter the vasodilator responses to sodium nitroprusside in either group.

Discussion

In the most comprehensive comparative investigation of microvascular function in a healthy biracial population to date, we investigated the relative contributions of NO and EDHF to basal vasodilator tone, agonist-stimulated endothelium-dependent and -independent vasodilation, and physiological vasodilation. Important novel findings are as follows: (1) there is greater contribution of NO than of EDHF to resting vasodilator tone, agonist-stimulated endothelium-dependent and -independent vasodilation, and physiological vasodilation. Important novel findings are as follows: (2) there is reduced endothelium-dependent vasodilation with both acetylcholine and bradykinin in black compared with white subjects, and this is secondary to reduced contribution of both NO and EDHF to bradykinin-mediated dilatation and to reduced contribution...
of NO to acetylcholine-mediated dilation in blacks; EDHF partly compensates for the reduced NO release with acetylcholine; (3) there is reduced contribution of NO and relatively increased contribution of EDHF to exercise-induced vasodilation in black compared with white subjects; and (4) there is reduced sensitivity of the vascular smooth muscle to NO, demonstrable as a reduced responses to sodium nitroprusside in blacks compared with whites.

Contribution of NO and EDHF to Resting Vasodilator Tone

We have demonstrated an important contribution of both NO and EDHF, measured as TEA-inhibitable K⁺Ca channel activation, to resting forearm microvascular dilator tone in both ethnic subsets.26–28 There were no differences in the contribution of EDHF whether TEA was administered before or after l-NMMA was given. Although resting FBF was similar in the 2 groups, blacks had reduced contribution of NO, demonstrated by the decreased response to l-NMMA, and preserved contribution of EDHF in comparison with white subjects. Conductance vessel NO bioavailability has been reported to be either lower5,10,14 or not different in blacks compared with whites.9 This is the first demonstration of reduced tonic basal NO activity in the microcirculation of healthy black subjects and may provide an explanation for the proportionally higher risk and prevalence of hypertension and cardiovascular disease in this group.1 Whether other endogenous vasodilators, such as prostacyclin, adenosine, carbon monoxide, are differentially bioavailable in black compared with white subjects needs to be studied.

Contribution of NO and EDHF to Bradykinin-Stimulated Vasodilation

Not only did we confirm that bradykinin-mediated vasodilation is attenuated in blacks, we also show that this is because of lower contribution of NO and EDHF.8,15 Bradykinin is a potent endogenous vasodilator that stimulates NO, prostaglandins, and EDHF release by stimulating endothelial B2 receptors. We confirmed that bradykinin-mediated vasodilation is both NO and EDHF mediated,22,24,26,29–31 but whether their reduced contribution in the black vasculature is because of enhanced metabolism needs further study.15

Contribution of NO and EDHF to Acetylcholine-Stimulated Vasodilation

Previous studies have reported either impaired15,16 or intact11 endothelial responses to muscarinic receptor stimulation with methacholine or acetylcholine in healthy blacks compared with whites. We recently demonstrated that acetylcholine-mediated vasodilation is largely attributable to stimulation of release of NO, and unlike bradykinin, acetylcholine does not stimulate EDHF release, a finding consistent with previous reports.7,22 Now we demonstrate not only that is acetylcholine-mediated vasodilation depressed in blacks compared with whites, but also that there is some compensatory increase in release of EDHF with acetylcholine from the black microvasculature, a finding also observed in experimental models.33,34 These findings in healthy blacks are similar to those observed in patients with multiple risk factors.24
Contribution of NO and EDHF to Exercise-Induced Vasodilation

We have recently shown that both NO and K$_{Ca}$ channel activation (EDHF) contribute individually and in concert to exercise-induced microvascular vasodilation. Here, we demonstrate that the contribution to exercise-induced forearm microvascular dilation of NO is lower, and of EDHF is greater, in blacks compared with white subjects. Thus, even in healthy blacks, EDHF is the predominant mechanism responsible for exercise-induced forearm vasodilation, whereas NO is the predominant endothelium-dependent agonist in white subjects. These abnormalities are similar to the reduced mental stress–induced vasodilation we previously reported in normotensive blacks.

Mechanisms Underlying Vascular Dysfunction in Blacks

Oxidative stress from upregulation of superoxide anions and its effects on NO metabolism and endothelial NO synthase uncoupling and inhibition play a central role in risk factor–mediated vascular pathophysiology. Decreased NO bioavailability and increased production of peroxynitrite have been demonstrated in isolated human umbilical vein endothelial cells obtained from blacks, and we have also demonstrated increased systemic oxidative stress measured as higher oxidized aminothiol levels and increased endothelin activity in blacks compared with whites, which provides an explanation for the observed reduced NO bioavailability. Treatment with nebivolol seems to normalize the increased free radical production in cells from blacks and restores NO bioavailability to that observed in cells from whites. Other studies have demonstrated higher asymmetrical dimethylarginine in blacks compared with whites.

Differences in Endothelium-Independent Vasodilation

We confirmed previous findings of reduced microvascular dilatory responses to sodium nitroprusside, an NO donor, in blacks, likely attributable to decreased responsiveness of the vascular smooth muscle to NO. Our previous findings of reduced cyclic nucleotide–mediated responses in black forearm microcirculation involving both the adenylate cyclase and guanylate cyclase systems suggest either a generalized decrease in responsiveness of multiple vasodilator systems or involvement of a common distal step in the vasodilating pathways. Modulation of cytosolic calcium concentration is one potential final pathway for regulating smooth muscle tone, and racial differences in its regulation may be one mechanism. Paradoxically, an enhanced response of conductance vessels to sublingual nitroglycerin was observed in blacks.

Limitations

Although we found that the vasoconstrictor response to l-NMMA was lower in blacks, we did not examine effects of other nonspecific vasoconstrictors to investigate whether this is a reflection of reduced sensitivity of the vascular smooth muscle to vasoconstrictors. However, the fact that the constrictor response to TEA was similar to whites suggests that the response to l-NMMA is specific for reduced NO bioavailability. The reduced sensitivity to exogenous NO (sodium nitroprusside) complicates the interpretation of the reduced dilator responses observed with acetylcholine and bradykinin in blacks. However, because basal NO and the contribution of NO during exercise is lower in blacks, it is likely that in addition to reduced sensitivity, there is also an endothelial defect in NO release in blacks.

l-NMMA and TEA are competitive inhibitors, and thus our results may underestimate the physiological contribution of both NO and K$_{Ca}$ channels to vasodilation. Our investigation was conducted on a background of cyclooxygenase inhibition because of its negligible contribution in the healthy human circulation. Although we used intermittent isometric handgrip exercise, other muscle beds and different intensities of exercise may need to be explored in future studies. Using plethysmography, we have assessed the microcirculatory responses and not the conductance vessel changes in this study; however, it is known that the contribution of EDHF is less in conductance vessels than in the microvessels.

Sources of Funding

This work was supported by National Institutes of Health Research Foundation and the National Blood Foundation.

Disclosures

None.

References

25.
24.
22.
21.
20.
19.
18.
17.
16.
15.
14.
13.
12.
11.
10.
9.
8.
7.
6.
5.
4.
3.
2.
1.

Although nitric oxide and endothelium-derived hyperpolarizing factor contribute to resting blood flow, agonist-mediated vasodilation, and vasodilation during exercise, the contribution of nitric oxide is reduced and of endothelium-derived hyperpolarizing factor is preserved or increased in the microcirculation of healthy blacks with these stimuli. Preserved endothelium-derived hyperpolarizing factor but reduced nitric oxide bioavailability in blacks may underlie their increased risk of hypertension and cardiovascular disease and have crucial therapeutic implications that need to be explored.
Differences in Vascular Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor Bioavailability in Blacks and Whites
Muhiddin A. Ozkor, Ayaz M. Rahman, Jonathan R. Murrow, Nino Kavtaradze, Ji Lin, Amita Manatunga, Salim Hayek and Arshed A. Quyyumi

Arterioscler Thromb Vasc Biol. published online March 27, 2014;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2014/03/27/ATVBAHA.113.303136

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2014/03/27/ATVBAHA.113.303136.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
METHODS

Materials and Methods are available in the online-only Data Supplement

Subjects
Eighty six self-reported AAs and 74 white healthy volunteers, aged between 21 and 60 years, were enrolled into 4 separate protocols that explored the contribution of NO and EDHF to resting, agonist-stimulated, and exercise-induced vasodilation (Table 1). Subjects were recruited via information flyers placed around hospitals of the Emory Healthcare system, advertisements in local newspaper and radio station. Enrolled subjects were compensated financially for their time. Any current smoker, pregnant woman, subject on any medication, or with known hypertension, diabetes, cardiovascular disease or other systemic disorders was excluded. The study was approved by the Emory University Institutional Review Board and all subjects signed an informed consent.

Measurement of forearm blood flow
Measurements were performed after an overnight fast in a quiet temperature-controlled (22 to 24°C) room. Subjects refrained from exercise, alcohol and caffeine for at least 24 hours. After insertion of an intra-brachial arterial cannula for arterial pressure monitoring and delivery of drug infusions, subjects received oral aspirin (975mg) to inhibit prostacyclin synthesis at least 1 hour prior to the study. Simultaneous forearm blood flow (FBF) measurements were obtained in both arms using a dual-channel venous occlusion strain gauge plethysmograph (model EC6, DE Hokanson, Bellevue, WA) as previously described.1, 2 Flow measurements were recorded for approximately 7 seconds, every 15 seconds up to eight times and a mean FBF value in mL·min⁻¹·100 mL⁻¹ was computed. Forearm vascular resistance (FVR) was calculated as the mean arterial pressure ÷ FBF and expressed as mmHg·mL·min⁻¹·100 mL⁻¹.

Physiologic forearm vasodilation was investigated by employing intermittent handgrip exercise where the instrumented forearm was exercised by squeezing an inflated pneumatic bag as previously described.1, 3 Exercise was performed at 15%, 30%, and 45% of the subject’s maximum voluntary grip strength. Each contraction lasted for 5 seconds followed by relaxation for 10 seconds and was repeated for 5 minutes at each workload. FBF was measured in the final 2 minutes of each workload. We have shown that FBF remained constant after 2 minutes of exercise.1

Protocol 1: Contribution of NO and K⁺Ca channel activation to resting microvasculature tone.
All agents were administered intra-arterially after resting measurements were made during saline infusion (2.5 ml min⁻¹). In 42 AA and 46 white subjects, FBF was measured during a 5-minute infusion of L-NMMA (Clinalfa, Switzerland) given at 8µmol/min to inhibit NO synthesis (Figure 1). While continuing L-NMMA, intra-arterial tetraethylammonium chloride (TEA; Clinalfa, Switzerland) at 1 mg/min was infused for 5 minutes to investigate the effect of combined blockade of NO and K⁺Ca channels on resting vessel tone. The L-NMMA dose has been previously shown to be effective in attenuating resting and agonist-stimulated FBF.2 When given at 0.25, 0.5 and 1 mg/min, TEA is known to selectively inhibit K⁺Ca channels and also inhibits bradykinin-mediated vasodilation at the highest dose.2, 4, 7

In separate experiments, in 41 AA and 45 white subjects, TEA was infused first in order to investigate the effects of blockade of K⁺Ca channels without prior NO inhibition. This was followed by combined infusions of TEA and L-NMMA. Arterial blood pressure and FBF were measured in the last 2 minutes of each intervention.
**Protocol 2: Contribution of NO and $K^+_{Ca}$ channel activation to bradykinin-stimulated vasodilation.** In 36 AA and 38 white subjects, FBF was measured at rest, after infusion of the endothelium-dependent vasodilator bradykinin (Clinalfa, Switzerland) at 100, 200 and 400ng/min, and after infusion of the endothelium-independent vasodilator, sodium nitroprusside (SNP) at 1.6 and 3.2 mg/min for 8 minutes each (Figure 1). This was followed by infusion of L-NMMA at 8µmol/min, followed by re-infusion of bradykinin. After recovery, TEA and L-NMMA were infused followed by repeat infusion of bradykinin. To investigate the effect of inhibition of $K^+_{Ca}$ channels on bradykinin-mediated vasodilation without prior blockade of NO, we also studied another 32 AA and 28 white subjects in whom TEA was infused before infusion of L-NMMA (Figure 1). This protocol enabled assessment of the contribution of NO and $K^+_{Ca}$ channel activation alone and in combination to bradykinin-stimulated endothelium-dependent and SNP-mediated endothelium-independent vasodilation in healthy AA and whites.

**Protocol 3: Contribution of NO and $K^+_{Ca}$ channel activation to acetylcholine-stimulated vasodilation.** In 26 AA and 17 white subjects, Protocol 2 was repeated with the use of an intra-arterial infusion of the endothelium-dependent vasodilator, acetylcholine (ACH, Novartis, East Hanover, NJ) at 7.5, 15 and 30 µg/min instead of bradykinin, (Figure 1). This enabled measurement of the contribution of NO and $K^+_{Ca}$ channel (EDHF) activation to ACH-mediated vasodilation.

**Protocol 4: Contribution of NO and $K^+_{Ca}$ channel activation to exercise-mediated vasodilation.** To investigate the differential contribution of NO and EDHF to physiologic vasodilation, we studied 24 AA and 19 white subjects in whom FBF was measured at rest, after intermittent handgrip exercise, and after intra-arterial infusion of SNP (1.6 and 3.2 mg/min), (Figure 1). Measurements were repeated after infusion of L-NMMA at 16µmol/min for 8 minutes, a dose that was effective in attenuating exercise-induced vasodilation in our previous study. After a 30-minute rest period, intra-arterial TEA (1mg/min) was given in addition to L-NMMA and FBF measurements repeated with exercise.

In separate experiments, to investigate the sole effect of inhibition of K+Ca channels on exercise-induced vasodilation, the protocol was repeated with the infusion of TEA alone followed by exercise. Finally, combined intra-arterial infusions of TEA and L-NMMA were given and the exercise and sodium nitroprusside infusions repeated.

**References**


