Racial Differences in Endothelin-1 at Rest and in Response to Acute Stress in Adolescent Males

Frank A. Treiber, Robert W. Jackson, Harry Davis, Jennifer S. Pollock, Gaston Kapuku, George A. Mensah, David M. Pollock

Abstract—Blacks exhibit greater vasoconstriction-mediated blood pressure (BP) increases in response to stress than do whites. Endothelin-1 (ET-1), a potent vasoconstrictive peptide, has been proposed as having a role in racial differences in stress reactivity. We evaluated the hemodynamic and plasma ET-1 levels of 41 (23 whites, 18 blacks, mean age 18.6 years) normotensive adolescent males at rest and in response to a video game challenge and forehead cold stimulation. Measurements were performed at catheter insertion and before and immediately after the 2 stressors, which were separated by 20-minute rest periods. Blacks exhibited higher absolute levels of diastolic blood pressure, total peripheral resistance index, or both in response to catheter insertion and to the video game challenge and during recovery from video game challenge and cold stimulation (P<0.05 for all). Blacks exhibited higher absolute levels of ET-1 at every evaluation point (P<0.05 for all) and greater increases in ET-1 in response to both stressors (ps<0.05). These findings suggest that altered endothelial function may be involved in racial differences in hemodynamic reactivity to stress and possibly in the development of essential hypertension. (Hypertension. 2000;35:722-725.)

Key Words: endothelin ■ stress ■ blood pressure ■ race ■ young adults ■ blacks ■ hypertension, essential

Blacks exhibit a higher prevalence and earlier onset of essential hypertension (EH) and more frequent associated target-organ damage than do whites.1 Although a few race-specific genetic factors have been identified,2,3 race-shared physiological mechanisms that contribute to the development of EH (eg, increased sympathetic nervous system [SNS] arousal in response to stress) are believed to be differentially activated within different environmental contexts. It has been postulated that blacks experience greater chronic SNS arousal due to more frequent exposure to social and environmental stressors (eg, aversive social interactions related to socioeconomic status inequality, racism).4 Although not entirely consistent, exaggerated blood pressure (BP) reactivity to brief laboratory stressors has been shown to be an independent predictor of future BP levels in youths and of EH in adults (see reviews5–7). Studies that involve normotensive adults8,9 and youths10,11 have found that blacks exhibit greater BP increases in response to acute stress than do whites and that these differences are frequently mediated by greater increases in total peripheral resistance (TPR).

The underlying physiological mechanisms responsible for enhanced vasoconstrictive reactivity (ie, increased TPR) to stress in blacks are unknown. Endothelial cell–derived vasoconstrictive and vasodilator substances are central to the regulation of vascular tone. Endothelin-1 (ET-1) is a 21-residue peptide with potent vasoconstrictive activity.12 ET-1 is expressed by endothelial cells and is primarily released basolaterally to elicit smooth muscle cell contractions.13 Therefore, circulating ET-1 may reflect only a minor portion of total ET-1 synthesis such that elevations in venous ET-1 represent spillover from large increases in ET-1 production. The systemic infusion of ET-1 in humans causes increases in BP and TPR for both normotensives and hypertensives through the potentiation of SNS activity.14 Some studies in adults, but not all,15,16 have reported higher levels of ET-1 in hypertensives compared with normotensives.17,18 Recently, adult black hypertensives were found to have considerably higher basal ET-1 levels than normotensive blacks and whites regardless of hypertension status.19 A second study that involved normotensive adults found that black men exhibited higher basal ET-1 levels than white men.20 Although the pathogenesis of EH has its origins in childhood,21 whether race differences in ET-1 levels at rest are present in youths has not been evaluated.

Several studies have found that ET-1 is rapidly released in response to acute physical (eg, cold pressor) and mental (eg, mental arithmetic) stress.22,23 Letizia et al24 found that borderline hypertensive adults exhibited a greater release of ET-1 in response to cold stimulation than did normotensives. Interestingly, Noll et al25 observed that ET-1 levels increased significantly more in response to mental arithmetic in adults with a positive family history of EH than in those with a negative family history. To date, the potential differential impact of race on ET-1 release during acute stress has not been examined.
The present study provided an exploratory examination of possible racial differences in vasoconstrictive function and plasma ET-1 levels at rest and in response to 2 brief laboratory stressors in normotensive males with positive family histories of EH. Individuals with positive family histories of EH are at an increased risk for the development of EH.28 Among youths with a positive family history of EH, blacks have been shown to exhibit greater vasoconstriction-mediated BP levels at rest or during acute stress than whites.10,11 Thus, an examination of ET-1 levels in multiethnic samples of youths with family histories of EH may be particularly informative regarding possible racial differences in the underlying pathophysiologic basis of EH. Based on previous findings indicating that ET-1 mediates vasoconstrictive tone and that blacks exhibit greater vasoconstriction-mediated BP at rest and during acute stress, it was hypothesized that blacks would experience greater vascular tone concomitant with increased plasma ET-1 levels at baseline and in response to 2 acute stressors known to elicit vasoconstriction-mediated BP increases.

Methods

Subjects

Subjects were randomly selected from male participants in a longitudinal study of the biobehavioral antecedents of cardiovascular disease in youths.10 A total of 41 adolescent males (23 whites, 18 blacks; mean age 18.6 years) participated in the study. All subjects had a positive family history of EH, defined as having at least 1 parent and 1 grandparent with EH, as verified by the individuals’ physicians. Subjects were normotensive for age and gender and apparently healthy on the basis of parental report of medical history.

Hemodynamic Measurements

The study was approved by the institutional review committee. After informed consent was obtained, anthropometric measurements were performed according to established protocols.28 Subjects were then escorted to a private, temperature-regulated room (20° to 22°C), and spot electrodes were placed for the measurement of cardiac output with a thoracic bioimpedance system (NCCOM-3 model 6; BoMed Medical Manufacturing, Ltd). Subjects were fitted with an appropriately sized BP cuff on the right arm for use with a Dinamap model 1846 SX automated BP monitor (Critikon). Cardiac output was measured concomitantly with BP readings. TPR index (TPRI) was calculated as TPRI=(1/5 systolic BP+1/5 diastolic BP [DBP]/cardiac output/body surface area).

Blood Collection and Stress Protocol

After attachment of the electrodes and BP cuff, subjects were asked to lie on a bed in the supine position. After the left elbow was stabilized with an armboard, a 21-gauge butterfly needle (4492; Abbott Laboratories) was inserted into the antecubital vein, and a 3-way plastic stopcock was attached. Immediately after needle placement, a 5-mL blood sample was drawn, transferred to a 10-mL prechilled EDTA tube (Vacutainer), and maintained on ice. Then, 1 mL of 0.9% saline was infused at 1- to 3-minute intervals to maintain prechilled EDTA tube (Vacutainer), and maintained on ice. Then, 1 mL of 0.9% saline was infused at 1- to 3-minute intervals to maintain venous access during the remainder of the protocol. The subject was then instructed to relax, and 5-mL blood samples were drawn 15 and 20 minutes later during the initial rest period. Next, a 10-minute video game (Breakout; Atari, Inc) was presented under a monetary incentive challenge as described previously.11 After a 20-minute recovery period, forehead cold stimulation was conducted by placing a plastic bag containing 1.5 cups of water and 6 cups of crushed ice on the subject’s forehead for 1 minute. Then, 5-mL blood samples were drawn, and hemodynamics were concomitantly measured immediately on completion of each stressor and at minutes 15 and 20 during the recovery period that followed each stressor. The forehead cold stimulation task was presented last due to significant variability in hemodynamic recovery rates.10 At the end of the session, tubes were centrifuged at 3000 rpm for 15 minutes, and plasma was stored at −80°C.

ET-1 Measurements

Plasma ET-1 levels were determined with ELISA (QuantiGlo; R&D Systems) according to the manufacturer’s instructions. The reported cross-reactivity of the antibody was <0.02% for all big ETs, 7.8% for ET-3, and 27.4% for ET-2. Samples were thawed at room temperature, inverted 3 times, and centrifuged for 5 minutes at 1500g at 4°C. All samples and standards were processed in duplicate. Unknown sample data were fitted to a standard curve with commercially available software (Prism 2.0; GraphPad Software). At the end of the assay, plates were covered with a yellow plastic cover (R&D Systems), and the luminol substrate was detected with total photon counting (TopCount; Packard). The intra-assay variability was 4.2%.

Data Analyses

Initial analyses of possible racial differences in anthropometric and resting hemodynamics were made with univariate ANOVAs. A series of 2×9 (race×time) repeated measures ANOVAs were conducted with the hemodynamic parameters and ET-1 across the evaluation periods from catheter insert through completion of the final recovery reading. An α level of 0.05 was used. Possible race differences in reactivity to the stressors were also examined with a series of follow-up t tests in which change scores were computed through subtraction of the previous resting measure from the stressor response. One-tailed tests were used to interpret the latter findings.

Results

The mean and SEM values of the anthropometric and resting hemodynamic data by race are presented in the Table. There were no significant racial differences for any of the anthropometric parameters (P>0.12 for all). Likewise, no racial differences were noted for resting hemodynamic parameters (P>0.08 for all).

The repeated measures ANOVA yielded significant time and race main effects for ET-1, DBP, and TPRI (P<0.03 for all) and a significant time effect for systolic BP (P<0.001). The time effect indicated that regardless of race, subjects exhibited significant increases in ET-1, systolic BP, DBP, and TPRI in response to the 2 stressors. As can be seen in the Figure, the main effect for race was such that blacks exhibited higher ET-1, DBP, and TPRI values throughout the protocol.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Black Males (n=18)</th>
<th>White Males (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>18.4±0.6</td>
<td>18.8±0.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175.5±2.1</td>
<td>176.9±1.7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.8±4.6</td>
<td>73.9±3.0</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.81±0.01</td>
<td>0.83±0.01</td>
</tr>
<tr>
<td>Sum of skinfolds, cm</td>
<td>44.0±6.6</td>
<td>41.3±4.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.9±1.4</td>
<td>23.5±0.7</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>114.3±2.5</td>
<td>112.9±2.2</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>61.4±1.6</td>
<td>58.4±1.2</td>
</tr>
<tr>
<td>TPR, mm Hg · L · min⁻¹ · m⁻²</td>
<td>30.1±1.5</td>
<td>26.1±1.1</td>
</tr>
</tbody>
</table>

Resting SBP, DBP, and TPR are the mean of measurements taken at minutes 15 and 20 during the initial rest period. Values are mean±SEM.
compared with whites. Post-hoc univariate ANOVAs were conducted on these parameters at each time point. Findings revealed that blacks exhibited higher ET-1 and TPRI levels at every time point during the protocol (P<0.05 for all). Blacks exhibited significantly higher levels of DBP during the video game and the subsequent initial recovery, as well as during both recovery periods after cold stimulation (P<0.04 for all).

Finally, the analyses involving change scores revealed that blacks exhibited significantly greater increases in ET-1 in response to both stressors (P<0.02 for both). Similarly, blacks showed trends toward greater increases in DBP, TPRI, or both in response to both stressors (P<0.07 for all).

Discussion

This study is the first to examine race differences in ET-1 levels at rest and in response to brief vasoconstrictive stress in normotensive youth. Black youth exhibited significantly higher plasma ET-1 levels from catheter insertion throughout all sampling points in the study. This finding replicates and extends that of Evans et al, in which blacks exhibited higher “basal” ET-1 levels than whites among normotensive adults. Interestingly, the highest average ET-1 levels for whites and blacks were observed at catheter insertion. It is unclear whether higher levels at insertion were due to local stimulation of the vein, SNS arousal associated with anticipatory anxiety, or both. In many previous studies, a single venipuncture was used, and a resting period was not allowed before a blood sample was obtained. The lack of consistency of findings in previous studies with respect to the magnitude of group differences (eg, race, gender, hypertensive status) may be due in part to variability in the duration of time before sampling.

The significant increases in ET-1 in response to forehead cold stimulation corroborate previous studies in adults that used forearm cold stimulation. To our knowledge, this is the second study to indicate that ET-1 increases rapidly in response to an active behavioral challenge (ie, video game). Although the ET-1 responses to the stressors mirrored the increases in DBP and TPR, it is unclear whether changes in ET-1 contributed to the hemodynamic changes. Indirect support for a role of ET-1 as a mediator of BP and TPR changes is provided by the observations that ET-1 infusion in normotensive and hypertensive adults results in immediate and dose-dependent increases in DBP and TPR.

Similar to the race differences in ET-1 levels, blacks exhibited higher levels of vasoconstrictive tone at every evaluation point. Trends were observed in which blacks exhibited greater increases in BP, vasoconstrictive tone, or both in response to the stressors, which is consistent with previous youth reactivity studies. Blacks did exhibit greater increases in ET-1 in response to the 2 stressors; reasons for the racial differences in the hemodynamic and ET-1 responses are unclear. The groups did not differ in age, height, weight, adiposity, resting BP, or family history of EH, factors that might affect SNS activity and plasma ET-1 levels. However, the nitric oxide pathway has also been shown to differ between normotensive blacks and whites. Lang et al identified a blunted vasodilatory response to isoproterenol in healthy blacks. Cardillo et al confirmed this observation and extended the differences in drug responsiveness in the context of mental stress in blacks. Further evidence for an upregulation of the ET system in blacks comes from a recent finding of Ergul et al indicating that blacks possess a higher ratio of vasoconstriction-promoting ET receptors in saphenous vein preparations. Thus, the synergistic effects of increased receptor bound ET-1 and reduced vasodilator responsiveness to classic vasodilators may explain the race differences in vasoconstrictive tone at rest or during acute physical or mental stress, particularly in studies involving healthy youths and young adults.

Although the findings are provocative, they should be interpreted cautiously for several reasons. First, the sample consisted of youths with family histories of EH. Whether such race differences would be evident in youths without family histories of EH is unknown. Second, the sample involved only males, who have been shown to exhibit higher basal levels of ET-1 than women in adult studies. Again, whether similar patterns of results would be obtained with normotensive women remains to be determined. Third, whether an exaggerated release of ET-1 is responsible for greater vasoconstriction-mediated BP reactivity in blacks is unclear. Studies with ET receptor antagonists are necessary to determine whether there is a causal link between ET-1 release and BP responsivity during stress. In addition, whether exaggerated ET-1 release in response to repeated acute bouts...
of stress leads to early vascular remodeling and increased chronic vasoconstrictive tone is unknown but is being examined in longitudinal studies in our laboratory.

Acknowledgments
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References
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