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
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. Among youths with a positive family history of EH, blacks have been shown to exhibit greater vasoconstrictor-mediated BP levels at rest or during acute stress than whites. 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 pathophysiology of EH. Based on previous findings indicating that ET-1 mediates vasoconstrictive tone and that blacks exhibit greater vasoconstrictor-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 vasoconstrictor-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. 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. 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 measured and 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 venous access during the remainder of the protocol. The subject was then instructed to relax, and 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. 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.
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 levels of ET-1 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).

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Mean±SEM TPRI, DBP, and ET-1 levels at each evaluation point by race.
of stress leads to early vascular remodeling and increased chronic vasoconstrictive tone is unknown but is being examined in longitudinal studies in our laboratory.

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References
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