Brain Natriuretic Hormone Predicts Stress-Induced Alterations in Diastolic Function

Pratik Choksy, MD, Harry C. Davis, MS, James Januzzi, MD, Julian Thayer, PhD, Gregory Harshfield, PhD, Vincent J.B. Robinson, MD and Gaston K. Kapuku, MD, PhD

Abstract: *Background:* Mental stress (MS) reduces diastolic function (DF) and may lead to congestive heart failure with preserved systolic function. Whether brain natriuretic hormone (brain natriuretic peptide [BNP]) mediates the relationship of MS with DF is unknown. *Methods:* One hundred sixty individuals aged 30 to 50 years underwent 2-hour protocol of 40-minute rest, videogame stressor and recovery. Hemodynamics, pro-BNP samples and DF indices were obtained throughout the protocol. Separate regression analyses were conducted using rest and stress E/A, E′ and E/E′ as dependent variables. Predictor variables were entered into the stepwise regression models in a hierarchical fashion. At the first level, age, sex, race, height, body mass index, pro-BNP and left ventricular mass (LVM) were permitted to enter the models. The second level consisted of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR). The final level contained cross-product terms of race by SBP, DBP and HR. *Results:* E/A ratio was lower during stress compared to rest and recovery ($P < 0.01$). Resting E/A ratio was predicted by a regression model of age (−0.31), pro-BNP (0.16), HR (−0.40) and DBP (−0.23) with an $R^2 = 0.33$. Stress E/A ratio was predicted by age (−0.24), pro-BNP (0.08), HR (−0.38) and SBP (−0.21) with total $R^2 = 0.22$. Resting E′ model consisted of age (−0.22), pro-BNP (0.26), DBP (−0.27) and LVM (−0.15) with an $R^2 = 0.29$. Stress E′ was predicted by age (−0.18), pro-BNP (0.35) and LVM (−0.18) with an $R^2 = 0.18$. Resting E/E′ was predicted by race (0.17, B > W) and DBP (0.24) with an $R^2 = 0.10$. Stress E/E′ consisted of pro-BNP (−0.36), height (−0.26) and HR (−0.21) with an $R^2 = 0.15$. *Conclusions:* pro-BNP predicts both resting and stress DF, suggesting that lower BNP during MS may be a marker of diastolic dysfunction in apparently healthy individuals.

Key Indexing Terms: Brain natriuretic hormone; Mental stress; Diastolic function. **[Am J Med Sci 2014;348(5):366–370.]**

Mental stress (MS) induces cardiac malfunction due to increased cardiac load deriving from hemodynamics arousal expressed as increased blood pressure, heart rate (HR) and total peripheral resistance.[1–8] This MS stimulation of the cardiovascular system may translate into increased vascular tone, reduced myocardial perfusion, decreased ratio of early to late filling (E/A) velocities and reduced myocardial relaxation (E′).[3,6,7] The reduction of diastolic function (DF) in response to MS suggests that repetitive biobehavioral stress of modern life may induce diastolic dysfunction in at-risk individuals such as blacks and women who are more likely to develop premature congestive heart failure than whites and males.[9,10]

Pump function deterioration is associated with increased blood levels of brain natriuretic peptide (BNP), which plays an important role in body fluids (ie, salt handling) and vascular tone regulation.[11–13] As diastolic dysfunction is also a putative mechanism of congestive heart failure, it becomes necessary to determine whether MS-induced alteration in DF is linked to secretion of cardioprotective hormone such as BNP.[14,15] BNP is a marker of wall tension that is determined by chamber size and pressure. In general, increased blood levels of BNP are observed in reduced systolic function of hypertensive and coronary artery disease patients but not in normotensive individuals.[16–18] This raises the concern of whether BNP levels may represent changes in DF of healthy individuals.

We hypothesize that pro-BNP, which is a precursor of BNP, would be a predictor for DF in healthy subjects especially during stressful circumstances. To address this, we conduct this study to probe the predictive value of pro-BNP on DF at rest and during stress.

METHODS

Study Population

The subjects were 80 blacks (B; 40 males) and 80 white (W; 40 females) healthy normotensive adults aged 30 to 50 years (mean ± SD = 39.5 ± 5.9), not on any medications and without a history of any medical diagnosis.

Inclusion criteria were as follows: normal blood pressure (systolic <140 mm Hg and diastolic <90 mm Hg), no history of coronary artery disease, no chest pain syndrome, a normal resting electrocardiogram, normal ejection fraction, normal kidney function (creatinine <1 mg/L, no microalbuminuria), no hypercholesterolemia (total cholesterol ≤250 mg/dL and low-density lipoprotein cholesterol ≤160 mg/dL), no food allergies by self-report and ability to complete the necessary protocols and questionnaires.

Exclusion criteria were as follows: pregnancy, smoking, endocrine systemic disease (eg, thyroid disorders, diabetes mellitus), chronic pulmonary disease, abnormal echocardiography findings (ejection fraction <50%), regional wall motion abnormalities, peripheral vascular disease and anything that would impede the subject from complying with the diet.

The protocol was approved by the Human Assurance Committee of the Georgia Health Sciences University. Written informed consent was obtained before testing.

Laboratory Evaluation

Participants were placed on a controlled, normal sodium (4000 ± 200 mg/d) diet for 3 days before testing. On the fourth

From the Georgia Prevention Center/Institute of Public and Preventive Health (HCD, GH, VJBR, GKK), Department of Pediatrics, Medical College of Georgia, Georgia Regents University, Augusta, Georgia; Department of Medicine (Cardiology) (PC, GKK), Medical College of Georgia, Augusta, Georgia; Division of Cardiology (JJ), Division of Cardiology, Massachusetts General Hospital, Boston, Massachusetts; and Department of Psychology, The Ohio State University (JT), Columbus, Ohio.

Submitted November 22, 2013; accepted in revised form February 18, 2014.

Supported by the National Institutes of Health Grant HL076696 and Cardiovascular Discovery Institute/Georgia Regents University.

The authors have no financial or other conflicts of interest to disclose.

Correspondence: Gaston K. Kapuku, MD, PhD, Department of Pediatrics, Medical College of Georgia, Georgia Regents University, MCG Annex H.S. 1640, Augusta, GA 30912-4534 (E-mail: gkapuku@gru.edu).

day, the participants were brought to the laboratory and given breakfast. Blood samples were then drawn and urine was collected. During the 40-minute pretest "rest" phase, the subjects watched movies of their own choosing from our video library. During the experimental visit, this was followed by a 40-minute stress phase during which the subjects played a competitive video game task for a monetary reward (Snowboard; Sony Corp, Foster City, CA). Subjects improving their scores in the final stages of the game were given an additional $20. Finally, there was a 40-minute posttest "recovery" phase that was the same as the pretest phase. During each of the 40-minute phases, subjects consumed one 12-oz bottle of water, and after each phase, blood and urine samples were taken. Hemodynamic measurements were obtained during the 2 hours at 5-minute intervals using the Dinamap monitor (Dinamap Compact Monitor, Tampa, FL) for systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR.

For DF, pulsed Doppler echocardiography (Hewlett–Packard Sono 7500; Hewlett–Packard, Andover, MA) was used to record the mitral inflow to derive indices of left ventricular filling. The sample volume was placed at the tips of mitral leaflets to record the highest velocity of diastolic inflow. The tracing of 5 consecutive cardiac cycles having the highest velocity in early filling were analyzed as previously described.[19,20] A number of parameters were examined including the following: peak velocity of early filling (E), peak velocity of late filling (A) and the ratio of early to late filling peak velocities (E/A).

Tissue Doppler

Tissue Doppler measurements were obtained by using apical 4-chamber view for evaluating the septum portion of the mitral valve annulus. The sample volume was placed at the basal portion of the referred walls. The lowest possible wall filter settings and the minimum optimal gain were used as recommended by the manufacturer. Initial (E′) and final (A′) diastolic velocities for 5 consecutive beats were analyzed; the E/E′ ratio was calculated.

The reproducibility of both acquiring and measuring E′ and A′ were determined in recordings obtained from 10 subjects. The intraobserver and interobserver differences in parameter estimates were less than 10%. Doppler measurements were obtained at 20 and 40 minutes in each of the 3 phases.

Brain Natriuretic Peptide

Pro-BNP concentrations in plasma samples were determined using commercially available kits purchased from Biomedica-Gruppe (American Research Products, Belmont, MA). Two hundred microliters of standards, controls and diluted samples (1:2 in assay buffer) and 100 μL of detection antibody were added to a 96-well microtiter plate and incubated for 2.5 hours at 37°C. Contents of wells were discarded and washed. One hundred microliters of conjugate were added to each well, and samples were incubated for 1 hour at room temperature. Contents of wells were discarded again and washed. One hundred microliters of substrate were added to all wells, and samples were incubated for 20 minutes at room temperature in the dark; at which point, 50 μL of stop solution were added to each well. Concentrations of pro-BNP in samples were determined by measuring absorbance at 450 nm and comparing with a calibration curve generated from the standards.

Pro-BNP and Left Ventricular Mass

For 39 subjects, we were unable to obtain values for pro-BNP and/or left ventricular mass (LVM). We compared those 39 subjects with the 121 for which we had complete data on the variables shown in Table 1. There were no significant differences for any of those variables (ie, all Ps > 0.05). Additionally, the groups did not differ significantly by race or sex (Fisher's exact test P-values $= 0.27$ and 1.00, respectively).

Statistical Analyses

The distribution of pro-BNP and body mass index (BMI) was skewed, so we used log values of pro-BNP and BMI. Initially repeated measures analyses of variance were conducted to test if the stress protocol produced changes in E, A, E/A, E′, A′, and E/E′. Protocol phase (rest, stress and recovery) were used as the trial effects in these analyses. Following the repeated measures analyses of variance, separate regression analyses were conducted using the E/A ratio, E′, and E/E′ during the rest phase and during the stress phase as dependent variables. The predictor variables were entered into the stepwise regression models in a hierarchical fashion. At the first level, age, sex, race, height, BMI, pro-BNP and LVM were permitted to enter the models. The second level consisted of SBP, DBP and HR. The third (final) level contained cross-product terms of race by SBP, DBP and HR.

TABLE 1. Demographic, hemodynamic and ultrasound characteristics

	EA		AA		
Variable	**Male**	**Female**	**Male**	**Female**	**Significant differences[a]**
Age (yr)	39.5 ± 6.3	40.8 ± 5.2	39.8 ± 5.8	40.4 ± 6.1	
Height (cm)	178.5 ± 8.0	165.8 ± 6.0	177.3 ± 5.8	163.3 ± 6.0	M > F
BMI ln (kg/m^2)	3.31 ± 0.15	3.28 ± 0.18	3.33 ± 0.22	3.42 ± 0.20	F AA > M AA, M EA, F EA
Pro-BNP ln (pg/mL)	2.73 ± 0.69	3.37 ± 0.56	2.36 ± 1.03	2.89 ± 0.78	F > M; EA > AA
SBP (mm Hg)	118.9 ± 11.2	109.0 ± 12.3	126.4 ± 14.3	114.9 ± 12.5	M > F; AA > EA
DBP (mm Hg)	75.9 ± 6.5	69.2 ± 8.1	77.7 ± 7.6	71.6 ± 8.1	M > F
HR (beats/min)	70.5 ± 10.7	74.2 ± 6.5	67.9 ± 7.9	75.4 ± 8.2	F > M
LVM (g/ht$^{2.7}$)	27.7 ± 6.3	23.7 ± 5.1	29.4 ± 6.7	28.2 ± 6.5	M > F; AA > EA

[a] $P < 0.05$.

Cell entries contain mean ± SD. The significance column uses results from analysis of variance for main effects and Tukey's honestly significant difference post hoc test for interaction effects.

AA, African American; BMI, body mass index; BNP, brain natriuretic peptide; DBP, diastolic blood pressure; EA, European American; F, female; HR, heart rate; LVM, left ventricular mass; M, male; SBP, systolic blood pressure.