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Handgrip Increases Endothelin-1 Secretion in Normotensive Young Male Offspring of Hypertensive Parents

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Objectives. **We tested the hypothesis that an abnormal response of plasma endothelin-1 (ET-1) is elicited by handgrip exercise (HG) in young normotensive offspring of hypertensive parents.**

Background. **It has been hypothesized that ET-1 is involved in blood pressure control and plays a pathophysiologic role in the development of clinical hypertension.**

Methods. **Two groups of healthy male subjects, 11 with hypertensive parents (group A) and 10 without a family history of hypertension (group B), underwent 4 min of HG at 50% maximal capacity. Heart rate and blood pressure and plasma levels of ET-1, epinephrine and norepinephrine were measured at baseline, peak HG, and after 2 (R2) and 10 (R10) min of recovery.**

Results. **Group A had higher norepinephrine levels than group B throughout the test (baseline 181 ± 32 [SEM] vs. 96 ± 12 pg/ml, $p < 0.05$; peak HG 467 ± 45 vs. 158 ± 12 pg/ml, $p < 0.000001$; R2 293 ± 46 vs. 134 ± 8 pg/ml, $p < 0.01$; R10 214 ± 27 vs. 129 ± 10 pg/ml, $p < 0.0005$); no significant difference in epinephrine levels was detected. Compared with group B subjects, group A had higher baseline ET-1 levels (1.07 ± 0.14 vs. 0.59 ± 0.11 pg/ml, $p < 0.02$), which increased to a greater extent at peak HG (1.88 ± 0.31 vs. 0.76 ± 0.09 pg/ml, $p < 0.005$) and R2 (2.46 ± 0.57 vs. 1.31 ± 0.23 pg/ml, $p < 0.05$) and remained elevated at R10 (3.16 ± 0.78 vs. 0.52 ± 0.09 pg/ml, $p < 0.002$). Multivariate analysis demonstrated that only a family history of hypertension (chi-square = 7.59, $p = 0.0059$) and ET-1 changes during HG (chi-square = 4.23, $p = 0.0398$) were predictive of blood pressure response to HG and that epinephrine and norepinephrine were not.**

Conclusions. **The response to HG in offspring of hypertensive parents produced increased ET-1 plasma levels and resulted in a sustained ET-1 release into the bloodstream during recovery compared with offspring of normotensive parents. This may be an important marker for future clinical hypertension.**

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A body of evidence from experimental and clinical studies suggests that endothelium plays an important role in the control of vascular tone, in both normal physiology and disease states (1,2). A complex interaction between endothelium-derived vasodilating factors and endothelium-derived contracting factors mediates the regulation of basal vascular tone and modulates long-term structural responses of the smooth muscle cells in the vascular wall (3). Among endothelium-derived contracting factors, endothelin-1 (ET-1) is involved in arterial pressure control by local actions (contraction of vascular smooth muscle and activation of angiotensin-converting enzyme) and by systemic and central effects (resetting of baroceptors, increase in sympathetic tone and modulation of synaptic transmission) (4). Accordingly, it has been hypothesized that ET-1 is involved in blood pressure elevation and plays a pathophysiologic role in essential hypertension (1,2).

Previous studies have shown that postural changes and acute volume expansion induce a transient but marked elevation of plasma ET-1 levels, suggesting that ET-1 acts as a stress hormone (5,6). Recently, Noll et al. (7) reported that ET-1 levels are increased during mental stress only in normotensive offspring of hypertensive parents. This finding indicates that functional changes in endothelial regulation might be genetically determined and possibly contribute to the abnormal circulatory response to mental stress in these subjects.

On the basis of genetic factors and environmental components, first-degree relatives of essential hypertensive patients are known to be at risk of developing hypertension (8). In addition, specific neurohumoral and hemodynamic hyperreactivity to various stresses, such as isometric handgrip exercise (HG), has been recognized in normotensive offspring of hypertensive parents, and thus linked to the hereditability of hypertension (7,9). In healthy humans, HG causes: 1) a rapid heart rate increase in the initial seconds of exercise, and a subsequent slow further increase during the work period; and 2) a rise in arterial blood pressure, which increases slightly throughout the contraction period. Both heart rate and blood pressure return to basal levels soon after the end of the

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Abbreviations and Acronyms

ET-1 = endothelin-1
HG = handgrip exercise
R = recovery phase

voluntary contraction. Such circulatory response has been explained by the combined action of vagal withdrawal and sympathoadrenomedullar activation (10,11). We hypothesized that in normotensive offspring of hypertensive parents isometric HG would cause ET-1 release in the bloodstream that would differ from young healthy subjects without a family history of hypertension.

Methods

Patients. Twenty-one young men were enrolled in this study. All were normotensive and free of cardiovascular diseases, as evidenced by clinical examination and laboratory findings. Smokers and physically trained and overweight individuals (weight >10% than ideal) were excluded. Group A included 11 healthy sons (mean age 25.4 ± 1.5 years) of patients followed-up at our Institution because of medically treated essential hypertension. All group A subjects underwent 24-h blood pressure monitoring. Only those with a normal profile were entered into the protocol. Group B included 10 healthy men (mean age 27.3 ± 0.5 years), who were sons of normotensive outpatients. The study protocol was approved by the local Medical Ethics Committee, and informed consent was obtained from each subject.

Study protocol. The young men were studied in a laboratory (room temperature 22° to 24°C) during the morning hours (9 to 12 AM) after an overnight fast. They were in the supine position for at least 1 h before the start of HG exercise and were instructed to avoid Valsalva-like maneuvers. HG was performed with a calibrated handgrip dynamometer (Vigorimeter Martin, Germany). The men performed the maximal compressive force with the dominant arm three times, at 3 min intervals. After 30 min rest, 50% of the average maximal voluntary contraction was performed for 4 min by each subject. Heart rate and arterial blood pressure were obtained by electrocardiogram and cuff sphygmomanometer at baseline, at peak exercise (peak HG), and every 2 min (R2) throughout an average recovery period of 10 min (R10).

A short 18-gauge polyethylene cannula was inserted into an antecubital vein of the nondominant arm 30 min before the beginning of the study, and kept patent by slow infusion of 5% dextrose. Blood samples for ET-1 and catecholamine levels were drawn immediately before the beginning of the test (basal), at peak HG, and after R2 and R10.

Biochemical assays. Blood samples were collected into prechilled tubes (Becton Dickinson Vacutainer Systems) containing EDTA-K3 (15%) and aprotinin (1,000 KIU/ml of blood) and promptly centrifuged at 1,600*g* for 15 min at 4°C. Plasma was pipetted into polypropylene tubes and stored at −80°C until assayed. At the time of analysis, plasma samples were acidified with an equal volume of 0.1% trifluoroacetic acid and centrifuged at 2,500*g* at 4°C for 30 min to remove proteolytic activity. Plasma compounds tested were first concentrated by extraction through C18 Sep-PaK cartridges (Millipore Corporation).

For catecholamine assay, 2 ml of plasma were mixed with 8 ml of a 0.1 mol/liter phosphate buffer (pH 7.0) and 20 μl of internal standard solution (224 ng/ml DHBA) and applied to a small polypropylene column filled with 2 ml of preswollen ion-exchanger Sephadex CM. Before use, the packed column was washed with 10 ml of 0.1 mol/liter phosphate (pH 7.0). After the samples had been tested, the column was rinsed with 10 ml of distilled water and 4 ml of 0.1 perchloric acid. Catecholamines were eluted with 3 ml of 0.5 mol/liter perchloric acid, and the eluate was collected in 15-ml conical tubes. Two milliliters of Tris buffer (1.5 mol/liter, pH 9.3), containing 0.06 mol/liter EDTA and 20 mg of activated alumina, were added. The tube was vortex mixed for 2 min. The supernatant was removed by aspiration, and the alumina was washed twice with 2 ml of water, centrifuging between each wash (3000*g*, 3 min). After the second washing, as much water as possible was removed by aspiration and by drying the wall tube with little strips of filter paper. Catecholamines were eluted from the alumina with 100 (1 of 0.1 mol/liter acetic acid after vortex mixing for 2 min. After centrifugation, the supernatant was removed, and 25 μl were injected into the high performance liquid chromatographic system (12).

For ET-1 assay, Sep-Pak columns were first activated with 0.1% trifluoroacetic acid buffer, loaded with 2 ml plasma and then washed with 0.1% trifluoroacetic acid buffer. The retained material was eluted with 3 ml of a buffer containing acetonitril (60%) in 0.1% trifluoroacetic acid and dried under vacuum by a centrifugal evaporator system (Gyrovap, Howe and Co., London, UK). The radioimmunoassay of the reconstituted pellet was performed by a commercial ET-1 radioimmunoassay kit (Peninsula Laboratories). Each measurement was performed twice, and the average value of the two measurements was generally considered, although differences between the two measurements were <5%. In two cases that had one of the baseline measurements far above normal ranges, only the other measurement was taken into account. Cross-reactivity of the system for ET-1 was 100%; it was lower than 7% for both endothelin-2 and endothelin-3, according to the supplier. Intraassay and interassay variations in our laboratory were <10%. Recovery was 80%. Plasma ET-1 levels are expressed as picograms per milliliter.

Statistical analysis. All data are expressed as mean value ± 1 SEM. Statistical analysis was performed with BMDP statistical software (13). Analysis of variance and *t* tests were performed with the Bonferroni correction (BMDP-7D). Because data represent time (protocol)-related repetitions, a repeated measures model with structured covariance matrices (BMDP-5V: type = compound symmetry) was used as a means of comparing multivariately the studied groups (14). Models

Table 1. Average Values of Evaluated Variables in Groups A and B

	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)	HR (beats/min)	ET-1 (pg/ml)	EPI (pg/ml)	NEPI (pg/ml)
Group A							
Basal	120 ± 4	77 ± 2	91 ± 7	64 ± 7	1.07 ± 0.14	35.73 ± 5.06	181 ± 31
Peak HG	163 ± 4‡	106 ± 3‡	125 ± 9‡	96 ± 4‡	1.88 ± 0.31†	68.89 ± 12.94*	466 ± 45‡
R2	131 ± 3	82 ± 2	99 ± 7	76 ± 4	2.46 ± 0.57†	49.01 ± 14.91	293 ± 46
R10	122 ± 3	78 ± 2	93 ± 8	71 ± 3	3.16 ± 0.78†	33.15 ± 4.10	214 ± 27
Group B							
Basal	121 ± 3	77 ± 1	91 ± 4	71 ± 1	0.59 ± 0.11	28.16 ± 6.50	95 ± 11
Peak HG	157 ± 5‡	103 ± 4‡	121 ± 14‡	83 ± 3†	0.76 ± 0.09*	50.13 ± 8.06*	158 ± 12‡
R2	122 ± 2	79 ± 2	93 ± 6	68 ± 1	1.31 ± 0.23*	36.29 ± 4.06*	134 ± 8‡
R10	115 ± 2*	76 ± 2	89 ± 4	66 ± 1*	0.52 ± 0.09	28.80 ± 5.41	129 ± 10*

*$p < 0.05$, †$p < 0.02$ and ‡$p < 0.001$ versus basal values. Data presented are mean value ± SD. DBP = diastolic blood pressure; EPI = epinephrine; ET-1 = endothelin-1; HG = handgrip; HR = heart rate; MAP = mean arterial blood pressure; NEPI = norepinephrine; R2 = 2 min of recovery; R10 = 10 min of recovery; SBP = systolic blood pressure.

were set to explain the dependent variable mean blood pressure reading (from baseline to peak HG, R2 and R10) based on both time-constant (age, weight and height) and time-varying (respectively from baseline to peak HG, R2 and R10: heart rate, ET-1, epinephrine and norepinephrine) covariates (13,14). The analysis consists of a multivariate analysis of variance model used to assess the power of the single covariates (norepinephrine, epinephrine and ET-1) in determining the event (changes in mean arterial pressure during all steps of the HG test). To construct the dependent variable, the formula diastolic blood pressure + (systolic blood pressure − diastolic blood pressure)/3 was used. The Akaike information criterion was used to select the most successful model (13). As a means of approaching possible cause and effect relations between time-varying variables (x*i* independent variables) and mean blood pressure (y dependent variable), linear regression (BMDP-1R) was used, and several models were compared, entering different delta values from baseline to peak HG, R2 and R10 periods, respectively, for both x*i* and y variables. Mean squared residuals and r^2 values were used to test significance. A p value < 0.05 was considered significant.

Results

The average values of the evaluated variables are presented in Table 1.

Healthy offspring of hypertensive parents (group A). Starting from an average value of 64 ± 7 beats/min at baseline, heart rate increased to 96 ± 4 beats/min at peak HG ($p < 0.002$) and decreased to 76 ± 4 beats/min (p = NS) and 70 ± 3 beats/min (p = NS) at R2 and R10, respectively. Systolic blood pressure increased from 120 ± 3 mm Hg to 163 ± 4 mm Hg ($p < 0.00001$) at peak HG, returned to basal values at R2 and persisted unchanged until R10 (131 ± 3 mm Hg, p = NS and 122 ± 3 mm Hg, p = NS, respectively). A similar trend was observed for diastolic blood pressure, with an increase from 77 ± 2 mm Hg to 106 ± 3 mm Hg ($p < 0.000005$) and a persistent decrease to basal values at R2 and R10 (82 ± 2 mm Hg, p = NS and 78 ± 2 mm Hg, p = NS, respectively).

The average basal ET-1 levels (1.07 ± 0.14 pg/ml) were within the normal ranges reported in the literature (7) and showed a progressive and significant increase from 1.88 ± 0.31 pg/ml ($p < 0.02$) at peak HG to 2.46 ± 0.66 pg/ml ($p < 0.02$) at R2 and to 3.16 ± 0.78 pg/ml ($p < 0.02$) at R10. Norepinephrine values showed a significant increase at peak HG compared with baseline (181 ± 31 pg/ml vs. 466 ± 45 pg/ml, $p < 0.001$), with a decrease to 293 ± 45 pg/ml (p = NS) and 214 ± 26.55 pg/ml (p = NS) at R2 and R10, respectively. Epinephrine also changed from 35.73 ± 5.06 pg/ml to 68.89 ± 12.94 pg/ml ($p < 0.05$) at peak HG, whereas R2 and R10 values showed no significant differences in comparison with basal values (49.01 ± 14.91 pg/ml, p = NS, and 33.15 ± 4.10 pg/ml, p = NS).

Healthy offspring without family history of hypertension (group B). Starting from an average value of 71 ± 1 beats/min at baseline, heart rate increased to 83 ± 3 beats/min at peak HG ($p < 0.01$) and decreased to 68 ± 1 beats/min (p = NS) and 66 ± 1 beats/min ($p < 0.05$) at R2 and R10, respectively. Systolic blood pressure increased from 121 ± 2 mm Hg to 157 ± 5 mm Hg ($p < 0.0001$) at peak HG and returned to basal values at R2 and R10 (121 ± 2 mm Hg, p = NS and 115 ± 2 mm Hg, $p < 0.05$, respectively). A similar trend was observed for diastolic blood pressure, with an increase from 77 ± 1 mm Hg to 103 ± 5 mm Hg ($p < 0.00001$) and a persistent decrease to basal values at R2 and R10 (79 ± 2 mm Hg, p = NS and 76 ± 2 mm Hg, p = NS, respectively).

The average basal ET-1 level (0.59 ± 0.11 pg/ml) rose progressively to 0.76 ± 0.09 pg/ml ($p < 0.05$) at peak HG and to 1.31 ± 0.23 pg/ml ($p < 0.02$) at R2 and decreased to 0.52 ± 0.08 pg/ml (p = NS) at R10. Norepinephrine and epinephrine plasma levels showed a significant increase at peak HG compared with baseline, from 95 ± 11 pg/ml to 158 ± 11 pg/ml ($p < 0.000001$) and from 28.16 ± 6.50 pg/ml to 50.13 ± 8.06 pg/ml ($p < 0.02$), respectively. During recovery there was a progressive reduction, although average values remained slightly higher than baseline for norepinephrine (134 ± 8 pg/ml, $p < 0.001$ and 129 ± 10 pg/ml, $p < 0.05$) and

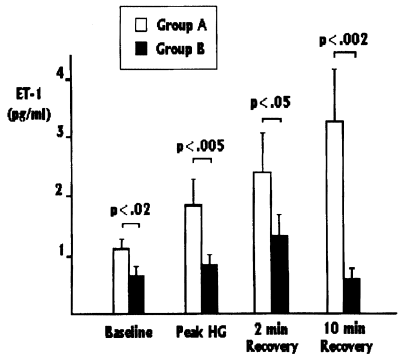

Figure 1. Endothelin-1 levels throughout handgrip isometric exercise test in the two groups with (Group A) and without (Group B) family history of hypertension.

epinephrine values did not differ from baseline (36.29 ± 4.06 pg/ml, $p < 0.05$, and 28.8 ± 5.41 pg/ml, p = NS).

Univariate comparisons between groups. Systolic and diastolic blood pressure levels, as well as heart rate, did not differ significantly between groups during all evaluated times.

Baseline ET-1 plasma levels, although always within normal ranges, were higher in group A than in group B ($p < 0.02$). At peak HG, the ET-1 response increased in group B only, although average values remained higher in group A ($p < 0.005$). At R2, a significantly higher increase in group A was found ($p < 0.05$), whereas at R10 a statistically significant ($p < 0.002$) opposite behavior was evident, toward a further increase in group A and a decrease to basal values in group B (Fig. 1).

Norepinephrine levels were significantly higher at baseline ($p < 0.05$) and there was a greater increase at peak HG ($p < 0.001$) in group A than in group B. A similar behavior between the groups was found at R2 and R10; however, average plasma levels were significantly higher in group A at both times ($p < 0.01$ and $p < 0.001$, respectively). Average epinephrine values did not differ significantly between groups during all evaluated times.

Multivariate comparisons between groups. The highest Akaike information criterion (−274.82) was found in the model where mean blood pressure response to HG (dependent variable) did not include age among the explanatory covariates. Age did not contribute significantly enough (chi-square = 0.62, p = NS) to explain the dependent variable mean blood pressure in the models in which it was considered. None of the remaining time-constant covariates were contributory (weight: chi-square = 0.29, p = NS; height: chi-square = 0.68, p = NS). Among time-varying covariates, neither epinephrine (chi-square = 0.69, p = NS) nor norepinephrine (chi-square = 1.43, p = NS) contributed significantly to explain the dependent variable, and heart rate did so with only borderline significance (chi-square = 3.27, p = 0.0704). By contrast, ET-1 was a significant time-varying covariate (chi-square = 4.23, p = 0.0398), as was the grouping covariate (presence vs. absence of family history of hypertension: chi-square = 7.59, p = 0.0059). Interestingly, the dependent variable was explained by time (chi-square = 196.13, p = 0.00005) but not by the interaction term grouping*time (chi-square = 1.58, p = 0.66). Of the several linear regression models tested in an attempt to explain the delta changes (from baseline) of mean blood pressure at different time points of the protocol, none was statistically significant.

Discussion

The present findings confirm earlier observations of 1) higher basal levels of norepinephrine, 2) a more pronounced responsiveness of blood pressure and heart rate during HG, and 3) an exaggerated response to sympathoadrenal stimulation by HG in persons with a family history of hypertension. The novel finding of the current investigation is the response of plasma ET-1 levels during HG exercise in young normotensive subjects with family history of hypertension. We found that ET-1 plasma levels were higher throughout the four assessed steps of the HG test and that the ET-1 release into the bloodstream was found to progressively increase throughout recovery. The results of the multivariate analysis demonstrated that the change in plasma ET-1 levels during HG exercise and recovery was the only variable that differed between offspring of hypertensive versus normotensive parents.

Physiologic and pathophysiologic effects of ET-1. ET-1, the most important endothelin known so far, is produced in the endothelium and by smooth muscle cells (9,15). In addition to powerful vasoconstricting properties that exceed those of other cardiovascular hormones, ET-1 potentiates at very low concentrations the contraction induced by norepinephrine and serotonin in human arteries (16). It may also induce hypertrophy of smooth muscle cells and is mitogenic to various cell lines, including vascular smooth muscle cells (17). ET-1 could contribute to blood pressure increases if production of this peptide was increased (18,19). However, only in rare cases has a pathogenetic role of ET-1 in raising blood pressure been reported (20). Different reports have shown plasma ET-1 to be slightly increased or normal in hypertensive humans (21–24). As ET-1 acts mainly as a paracrine agent, rather than as a circulating hormone, ET-1 secretion is mostly abluminal, toward the vascular wall, and circulating levels are the result of spillover into the blood stream (25). Thus, it is not surprising that ET-1 plasma levels are similar in normotensive and hypertensive individuals.

Noll et al. (7) reported for the first time an increase in plasma endothelin levels during mental stress only in offspring of hypertensive parents. They measured sympathetic nerve activity and plasma levels of norepinephrine and endothelin under resting conditions, during hypoxia, and during a mental stress test in 10 normotensive offspring of hypertensive parents and in 8 offspring of normotensive parents. During hypoxia, norepinephrine and endothelin levels increased to the same extent in both groups, whereas mental stress induced an increase of norepinephrine and endothelin plasma levels and of blood pressure only in offspring of hypertensive parents. Their results support the concept that an early paracrine

endothelial dysfunction is present in individuals genetically prone to develop hypertension.

Results of previous studies. The finding of progressive increases in ET-1 throughout recovery was an unexpected finding in the current investigation. We have previously reported that plasma ET-1 levels increased at peak HG but returned to elevated baseline values at recovery in patients with congestive heart failure (26). In addition, previous studies have reported the association of increased circulating and local endothelin with endothelial abnormalities and generalized arteriolar morphologic changes in patients with congestive heart failure and atherosclerosis (27–30). It is known that ET-1 is localized within internal storage vesicles of endothelial cells, and that its constitutive secretion is a rapid process, with the formation and transit time of a vesicle to the plasma taking approximately 10 min (31). This could explain the abnormal ET-1 synthesis and release during peak HG and recovery in our patients with a family history of hypertension. Whether the increases in ET-1 were independent or secondary to increased catecholamine levels is an open question. However, the sustained increase in ET-1 levels 10 min into recovery as norepinephrine levels were decreasing suggests that the increase in ET-1 may result from an independent mechanism. This possible mechanism requires further investigation.

Conclusions. To our knowledge, this is the first study analyzing the behavior of hemodynamic variables and neurohumoral hormones throughout the recovery period of isometric HG exercise. The results provide new information showing a progressive increase in ET-1 plasma levels during the recovery period of the test only in the offspring of hypertensive parents. These early functional changes of local cardiovascular regulation may be important in the pathophysiology of essential hypertension.

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