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Endothelin-1 response to mental stress in early ischemic lesions of the extremities due to systemic sclerosis

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Abstract

We studied circulating levels of endothelin-1, catecholamines and nitric oxide after a mental arithmetic test in 14 patients with early ischemic lesions of the extremities due to systemic sclerosis and slightly impaired peripheral vascular flow. The test induced an increase ($P < 0.01$) in blood pressure, heart rate, endothelin-1 and catecholamine levels, whereas it did not change the low basal levels of nitric oxide. In healthy subjects ($n = 20$) the test significantly ($P < 0.01$) decreased endothelin-1 without affecting nitric oxide. The low basal levels of nitric oxide and the high plasma concentration of endothelin-1 after psychological stress cannot be explained by an impaired release from the limited ischemic lesions alone. This suggests a diffuse microvascular derangement that aggravates the course of peripheral microvascular ischemic lesions.

Keywords: Limb ischemia; Systemic sclerosis; Mental arithmetic test; Endothelin-1; Nitric oxide; Norepinephrine

1. Introduction

The mental arithmetic test (MAT) is one of the most useful procedures for eliciting measurable stress-induced cardiovascular responses in humans [5,16] and investigating the effects of the psychological stressor on the neuroendocrine system [5,17]. Plasma endothelin-1 levels are decreased by MAT in healthy subjects [5] but increased in dilated cardiomyopathy patients with congestive heart failure [7] with a concomitant increase in sympatho-adrenergic tone [5,7].

In peripheral ischemic conditions an imbalance in the endothelial system is believed to impair the release of vascular factors whose interaction maintains basal vascular tone [2,12]. In diffuse systemic sclerosis an excess of the vasoconstrictor endothelin-1 and a deficiency in the vasodilator nitric oxide were described [9,19,20]. Whether endothelial dysfunction is primary or secondary to tissue ischemic damage remains unsettled.

In order to address this problem, we investigated patients with recent ischemic lesions of the extremities and slightly impaired peripheral vascular flow. The homeostasis of the vascular tone modulators was evaluated under basal conditions and in response to MAT in an attempt to unmask latent defects in the release of the factors controlling vascular resistance.

2. Materials and methods

2.1. Patients

We studied 14 patients (five men aged 32–50 years and nine women aged 40–65 years) with progressive systemic sclerosis and microvascular involvement of the fingers. All patients had Raynaud's phenomenon and painful limited

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necrosis of recent onset (<1 year). Local rest blood flow varied between 16.5 and 23.4 perfusion units (PU), whereas peak flow ranged between 40.5 and 80.0 PU (basal values obtained in 80 healthy subjects varied between 20.1 and 35.1 PU and peak flow values between 100.2 and 150.5 PU). None had renal failure and/or coronary artery disease. The pharmacological treatment, which included vasodilator and hemorrheologic drugs, was discontinued 24 h before the study.

Twenty healthy subjects (10 men aged 40–60 years and 10 women aged 35–63 years) served as controls. Written informed consent was obtained from all subjects.

2.2. Experimental procedure

The patients and healthy subjects underwent MAT between 9:00 and 11:00 a.m. after an overnight fast. MAT was performed by the subjects in supine position following a 30 min period of rest. MAT consisted of asking the subjects to subtract the number 17 serially from 1013 down to 10 or the number 7 serially starting from the number 251 down to 6, according to their level of schooling. Testing was repeated for 3 min. Subjects were urged to perform as quickly and accurately as possible, and reproached for lack of effort if the answer was not correct. Periodically, the subjects were urged to improve their performance. During the MAT none of the patients complained of increased pain at the level of vascular lesions.

In the study population, electrocardiogram leads were placed on the chest and a sphygmomanometer cuff was placed on the right arm. Blood samples for the determination of endothelin-1, nitric oxide and catecholamines were taken from the left forearm vein before the beginning and immediately after the MAT.

Heart rate was monitored continuously by electrocardiography and blood pressure determinations were made at 1 min intervals. Electrocardiogram was recorded by a Cardiostat 3 Siemens electrocardiograph; blood pressure was measured by an automatic Hewlett-Packard 78354A sphygmomanometer.

Local blood flow was measured by Laser Doppler flowmetry. We applied the output from a 780 nm diode laser that produced a divergent continuous wave light with a maximum accessible emission of 1 mW (PeriFlux 4001 Master, Perimed, Jarfalla, Sweden) and a standard angle probe. The laser Doppler value was expressed in arbitrary perfusion units; this measure was calibrated using a latex suspension (Perimed) that produces a standard deflection of 2.5 V or 250 PU. The study was performed after 30 min of acclimatization in a quiet room at 25 °C. The following parameters were assessed by laser Doppler flowmetry: (1) rest flow over a 3 min period, (2) peak flow (the maximum flow during reactive hyperemia after the release of a 3 min arterial occlusion).

2.3. Hormone assays

Endothelin-1 was radioimmunoassayed after chromatographic pre-extraction validated by HPLC analysis as previously detailed [8]. The detection limit was 0.1 pg/tube and an IC_{50} value of 1.28 ± 0.08 pg/tube. The intra-assay and inter-assay coefficients of variation were 5.5 and 13.6%, respectively. Average recovery of standard endothelin-1 was $72 \pm 2\%$.

Circulating NO_2^- levels were determined upon diazotization (Griess reaction) after conversion of NO_3^- to NO_2^- by nitrate reductase (EC 1.9.6.1) as previously described [8].

Norepinephrine and epinephrine were determined by column switching HPLC with electrochemical detection as detailed elsewhere [10]. The detection limit for norepinephrine and epinephrine were 11.8 and 16.3 fmol/ml, respectively. The intra-assay variations were 3.0 and 4.2% and the interassay variations were 5.1 and 3.5% for norepinephrine and epinephrine, respectively. The recovery of radiolabeled norepinephrine and epinephrine added to blood samples was over 90%.

2.4. Statistical analysis

Two-way ANOVA was used to compare neurohormonal and hemodynamic values before and after MAT in patients with microvascular disorders and in healthy subjects. Individual means were compared using post hoc Duncan's test. The degree of association between hemodynamic parameters and endothelin-1, hemodynamic parameters and catecholamines, as well as endothelin-1 and catecholamines after MAT was analyzed by Pearson's r correlation coefficient and regression analysis. Values are expressed as mean ± S.E.M., and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Hemodynamic and neuro-hormonal parameters in patients and healthy subjects under basal conditions

The patients exhibited significantly ($P < 0.01$) lower nitric oxide values than healthy subjects [$F(1, 65) = 155.43$], whereas they did not significantly differ in any of the other measured variables (Table 1).

3.2. Hemodynamic and neuro-hormonal parameters in patients and healthy subjects in response to MAT

MAT produced the same expected significant ($P < 0.01$) increase in systolic blood pressure [$F(1, 65) = 155.42$], diastolic blood pressure [$F(1, 65) = 136.83$] and heart rate [$F(1, 65) = 165.81$] both in patients and in healthy subjects. This stress-induced activation was paralleled by similar significant ($P < 0.01$) increase in epinephrine [$F(1, 65) = 104.30$] and norepinephrine levels [$F(1, 65) = 77.23$]. Endothelin-1 levels showed a divergent effect of MAT with a 60% increase in patients and 42% decrease in healthy subjects [interaction between factors: $F(1, 65) = 7.89$, $P < 0.05$], whereas nitric