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Enhancement of Nitric Oxide Synthesis in the Aorta Wall in Experimental Myocardial Infarction

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It is well known that the post-infarction drop of arterial pressure (AP) may be a function not only of the reduction of the minute heart volume, but also of the decrease of the vascular tone [6]. The progressive drop of the vascular tone may play an important role in the development of cardiogenic shock accompanying extensive myocardial infarctions [6]. Another clinical situation, which is also accompanied by a decrease of the vascular tone, frequently takes place in myocardial infarction in hypertensive patients, in whom the AP may plunge from hypertensive to a subnormal level for several weeks or months [4]. In a study of the regulatory mechanisms of the post-infarction drop of AP, a certain role in this phenomenon was ascribed to the increase of acetylcholine-induced endothelium-dependent relaxation of the vascular wall [3]. This led us to assume that the actual factor participating in the post-infarction decrease of the vascular tone might be an enhanced generation of NO by the endothelial wall.

The present study aimed to verify of this assumption by directly measuring the NO production by the aorta wall after experimental myocardial infarction.

MATERIALS AND METHODS

The experiments were conducted on Wistar male rats weighing 220-250 g. Experimental myocardial infarction was produced after Selye [7] by ligating the left coronary artery. Intact animals served as the control. Three hours after infarction the animals were killed by decapitation, because this is the time of the maximal drop of arterial pressure [3]. The thoracic aorta was removed and freed of fatty and connective tissue. An aorta ring 3 mm wide was placed in a thermostatically controlled (37°C) working bath containing an oxygenated (95% O_2+5% CO_2) Krebs solution, the initial rest-

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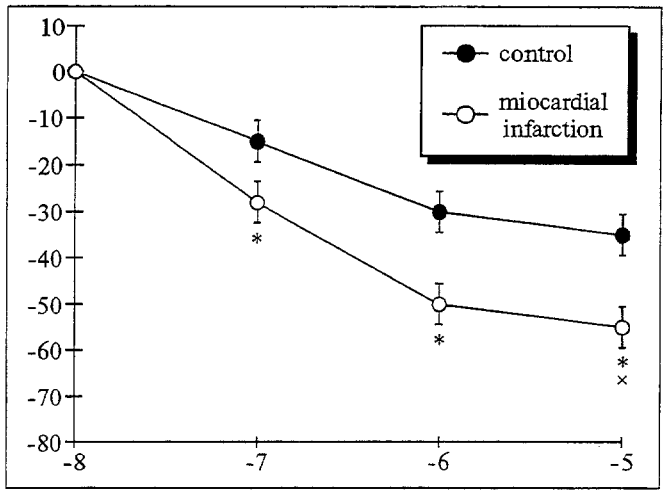

Fig. 1. Curves of endothelium-dependent relaxation of NE-precontracted (5×10^{-7} M) isolated aorta induced with AC ($10^{-8}-10^{-5}$ M). *: $p<0.05$ in comparison with the control.

ing tension being 1200 mg. The preparations were allowed to equilibrate under these conditions for one hour before recordings were begun (stabilization period). Endothelium-dependent relaxation was induced by cumulative addition of acetylcholine (AC) in a concentration of 10^{-8}-10^{-5} M to the incubation bath against the background of precontraction induced by 5×10^{-7} M norepinephrine (NE). The extent of endothelium-dependent relaxation was expressed as a percentage of maximal epinephrine-induced precontraction. Following the washing of the preparation with physiological saline and the restoration of the tone to the initial value, Na-DETC (sodium diethyldithiocarbonate, $C_5H_{10}NS_2Na$) was introduced in an effective concentration of 10^{-3} M. Fifteen minutes after the addition of Na-DETC, contraction of the preparation was induced with NE and, as the contractile response reached a plateau, the endothelium-dependent relaxation was produced. Ten minutes after the relaxation reached a plateau, the preparation was removed from the bath without washing, frozen, and stored in liquid nitrogen. Registration of isometric contractions was performed with a Gemini dual-channel unit (Ugo Basile, Italy). Statistical processing of the results was performed using the Student t test.

For evaluating the amount of nitric oxide generated in the isolated aorta rings in response to AC, use was made of the ability of NO to complexate with Fe^{2+}-diethyldithiocarbomate (Fe^{2+}-DETC), yielding paramagnetic mononitrosyl iron complexes (MNIC) with DETC). These complexes are characterized by an EPR signal with the g-factor being $g_{\perp}=2.035$ and $g_{\parallel}=2.012$ (Fig. 2) and triplet superfine structure at $g_{\perp}$. The amount of MNIC-DETC in the sample and, consequently,

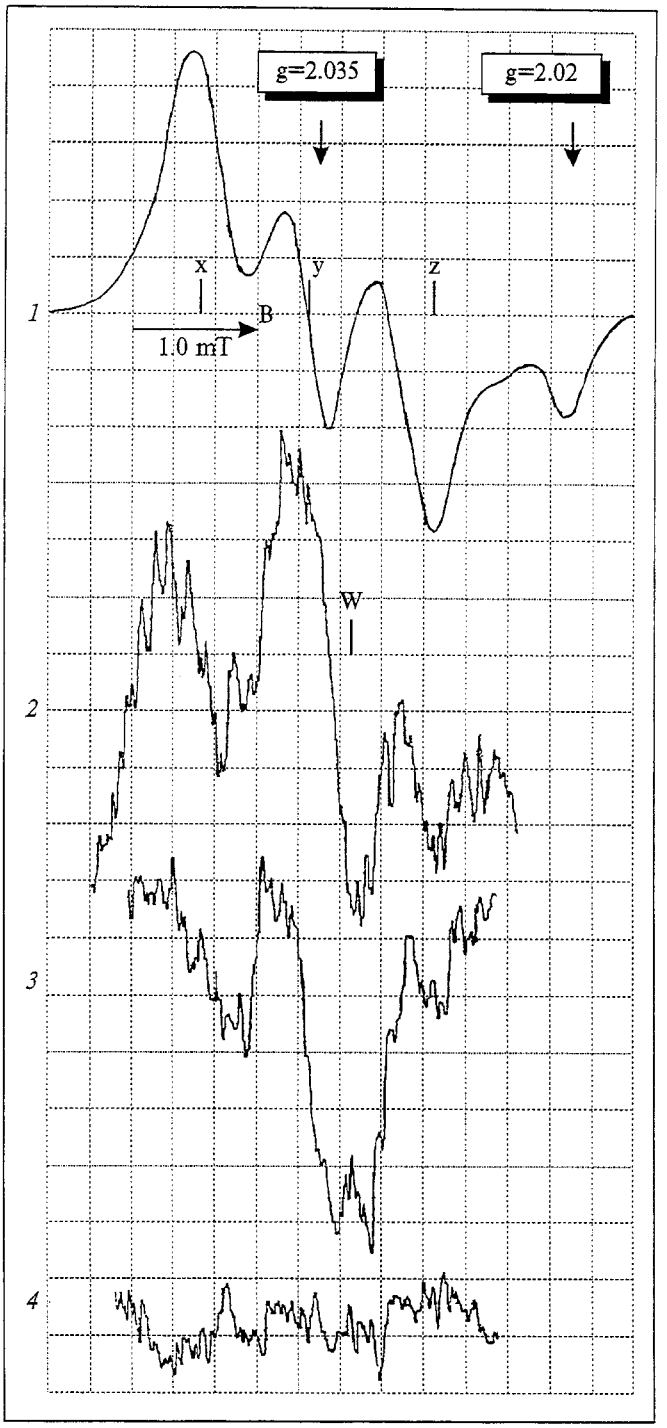

Fig. 2. EPR signals of MNIC-DETC in DMSO (*1*) and of aorta preparations from infarction survivors (*2*) and control animals (*3*). *x*, *y*, and *z* are components of SFS triplet of MNIC-DETC; *w*, second low-field component of quartet SFS of Cu^{2+}-DETC signal overlapped with MNIC-DETC signal; *4*) EPR signal from quartz Dewar flask. Operation mode: 9330 MHz frequency, 100 kHz frequency modulation, 5 E modulation amplitude, 77K temperature. Calibration: 1 μG.

the amount of nitric oxide incorporated into this complex were evaluated by the intensity of the EPR signal, which was calculated by double integration using a solution of paramagnetic dinitrosyl

iron complex with thiosulfate of a determined concentration as a standard. The method used for evaluating the nitric oxide content in animal tissues has been described at length elsewhere [1,2,5,8], where it is noted that the EPR signal of MNIC, if weak, may be partially masked by the second (low-field) component (*w*) SFS of the EPR signal from DETC complexes with endogenous copper ions (Cu^{2+}). This component overlaps with two low-field components of the triplet EPR signal (*x* and *y*, Fig. 2) of MNIC-DETC without masking the third high-field component of this signal (*z*). From its amplitude, calibrated by the double integral of the MNIC-DETC signal, it is possible to evaluate the number of these complexes in the sample. Isolated segments of the rat aorta, weighing 2.5 mg, incubated with DETC and AC as described above, were frozen in liquid nitrogen 10 min after the last dose of AC (10^{-5} M). Twenty analogous preparations were then pressed together in a glass tube in liquid nitrogen. The cumulative sample 4 mm in diameter and 2 mm high was used for EPR registration with a Radiopam EPR spectrometer at 77 K, modulation amplitude 5 E and SHF-power 10 mW. The measurements were repeated 6-8 times for every sample, after which the mean intensity of the signal and then the number of MNIC-DETC complexes were evaluated. The MNIC-DETC complexes in dimethylsulfoxide were synthesized as described elsewhere [2]. In order to evaluate the total concentration of NO traps of the Fe^{2+}-DETC complexes the preparations were treated with NO at a pressure of 200 mm Hg for 20 min in closed tvacnasid vials.

RESULTS

At the first stage of the experiment it was found that the aorta from animals which had survived myocardial infarction responded to the addition of AC to the incubation medium by a more pronounced relaxation than did the aorta from the controls. For example, the epinephrine-induced tone of the aorta decreased by 41±4% in the controls and by 61±2% after infarction ($p<0.05$), i.e., 1.5 times more (Fig. 1).

Figure 2 shows the characteristic EPR spectra of the aorta preparations from control rats and survivors of infarction and the EPR spectrum of MNIC-DETC in dimethylsulfoxide. In the aorta preparations the first and second components (*x* and *y*) of the triplet SFS of the EPR signal from the MNIC-DETC complex are masked by the component of the EPR signal (*w*) from the Cu^{2+}-DETC complexes. Only the third component of SFS (*z*) of the MNIC-DETC signal is not overlapped. From its amplitude we evaluate the intensity of the EPR signal from MNIC-DETC in the aorta preparations and thereby the number of these complexes. In the control preparations there was 0.12±0.03 nmol NO/g wet tissue, and in the aorta preparations from infarction survivors 0.3±0.1 nmol NO/g wet tissue.

Thus, NO generation in the aorta wall in animals which had survived myocardial infarction was 2.4-fold higher than in the control. Evaluation of the total concentration of NO traps of Fe^{2+}-DETC revealed their equal amount in both preparations, corresponding to an incorporation into their complexes of 50 nmol NO per g wet tissue.

When evaluating this result, it should be borne in mind that myocardial infarction is accompanied by a more or less pronounced decrease of cardiac output [6]. On the other hand, hypovolemia induced by blood loss leads to a drop of arterial pressure preventable by administration of inhibitors of NO synthesis [9]. This leads us to the conclusion that in our case the enhanced generation of NO in the aorta wall was provoked by a decrease in cardiac output and consequently produced transitory hypovolemia. On the whole, the data obtained suggest the importance of developing methods for NO measurements adapted for clinical practice.

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