The Course of Experimental Myocardial Infarction under Conditions of Suppressed and Enhanced NO Synthesis

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Experimental myocardial infarction (coronary ligation without drug infusion) reduced heart rate, stroke volume, cardiac output, and blood pressure. Under conditions of blocked NO synthesis, experimental myocardial infarction was accompanied by a transient increase in heart rate, stroke volume, cardiac output, and blood pressure, followed by their decrease throughout the experiment. Experimental myocardial infarction against the background of L-arginine treatment increased all these parameters with their subsequent stabilization at the attained level. Animal survival after administration of L-arginine was 80% *vs.* 33 in the control, and 22% after administration of L-NAME. It can be hypothesized that L-arginine possesses an intrinsic NO-independent cardioprotective effect.

Key Words: *experimental myocardial infarction; nitric oxide; L-arginine; L-NAME*

Great recent interest to the cardiac NO system is explained by its involvement into ischemic damages accompanying myocardial infarction (MI). Experimental MI (EMI) is associated with enhanced production of inducible and endothelial NO synthase in the heart and aortal wall and accumulation of oxidized NO products (nitrites and nitrates) [1,6,7,9]. Expression of inducible NO synthase in the heart of patients with MI also increases [13]. However, not only mechanisms, but even effects of NO under normal and pathological conditions are poorly understood, while the opinions about the role of NO in ischemia are extremely controversial.

Enhanced generation of NO induces sustained hypotension, decreases myocardial contractility due to increased content of cGMP and decreased concentration of intracellular Ca^{2+}, which leads to the development of heart failure [2,8]. For instance, blockade of inducible NO synthase inhibits progression of MI, decreases the level of nitrites and nitrates, and improves heart function [13]. Experiments on isolated rabbit heart showed that NO synthase blocker L-NAME decreases the severity of MI after coronary occlusion and reperfusion [12]. Moreover, L-arginine (substrate for NO synthesis) impairs myocardial function under ischemic conditions due to the formation of peroxynitrites [11]. Despite the known fact the MI is associated with enhanced NO production, patients with MI are usually prescribed nitroglycerin and sodium nitroprusside.

On the other hand, NO induces vasodilation and improves myocardial contractility by increasing cAMP concentration (due to cGMP-dependent inhibition of phosphodiesterase) and enhancing ionic current through L-type Ca^{2+}-channels [15]. NO produces an antioxidant effect, inhibits platelet adhesion and aggregation, increases parasympathetic and decreases sympathetic tone of autonomic nerves, which can play a positive role in MI development [7]. There are data that administration of L-arginine decreases the necrotic zone in MI. But other investigators did not confirm these findings [14].

The aim of the present study was to evaluate the effect of NO synthase blocker L-NAME and NO precursor L-arginine on the dynamics of cardiovascular parameters in EMI.

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MATERIALS AND METHODS

Experiments were carried out on mature albino male rats weighing 180-200 g. The animals were intraperitoneally narcotized with chloral hydrate (0.4 g/kg). The femoral vein was catheterized for continuous recording of systolic and diastolic blood pressure (SBP and DBP, MLT0698 transducer, ADInstruments). The test agents were infused through a catheter inserted into the femoral vein. ECG and volume and differential rheograms were recorded simultaneously. ECG was recorded in standard lead II using an EK1T-03M2 electrocardiograph. Stroke volume (SV) was measured by the method of tetrapolar rheography [5]. Cardiac output (CO) was calculated as SV×HR. EMI was modeled by ligation of the posterior branch of the right coronary artery [3]. The time from ligation to the start of recordings was 3-4 min. Three experimental series were performed: EMI without drug infusion (n=9, series I, control), EMI after 4-day treatment with L-NAME (Sigma, 100 mg/kg/day, n=9, series II), and EMI after preliminary (20 min) single intravenous injection of L-arginine (Sigma, 100 mg/kg, n=10, series III).

RESULTS

In series I all recorded parameters were stable within several hours (Fig. 1). EMI induced characteristic changes in ECG (Fig. 2, *2*): ST segment elevation with T wave inversion and decrease in R wave amplitude. In some cases rhythm disturbances (solitary ventricular extrasystoles) and conduction disturbances (I-III degree atrioventricular block) were noted. HR decreased by 14.20±1.52% (p<0.001) after 5 min and remained at this level for 15-20 min (Fig. 1, *a*). Initial sharp decrease in SV (by 39.80±10.84% on minute 7) was followed by its gradual decrease (Fig. 1, *b*). By the 25th min SV decreased by 54.60±10.62% (p<0.01). The decrease in HR and SV resulted in CO drop (by 52.59±9.68 and 66.69±7.47% by minutes 7 and 25, respectively, Fig. 1, *c*). Parameters SBP and DBP also decreased (Fig. 1, *d*, *e*). Animal death was usually observed on minutes 25-30 against the background of decreased BP, HR, and SV. Survival in the control group was 33.3%.

Chronic administration of L-NAME reduced HR, SV, and CO by 28.80±3.58, 18.8±5.8, and 39.90±7.58%, respectively, and increased SBP and DBP (p<0.05). Under these conditions EMI was associated with a sharp transient HR rise to practically normal values followed by its progressive decrease to 280-320 bpm (Fig. 1, *a*). SV gradually decreased (Fig. 1, *b*), this decrease attained 2.70±10.03% (p<0.05) by minute 25. It should be noted that SV on minutes 5-9 was higher than in the control (series I). The dynamics of CO traced the dynamics of SV (Fig. 1, *c*). SBP and DBP gradually decreased (Fig. 1, *d*, *e*), but remained higher than in the control series. Changes in ECG were similar to those in the control series (Fig. 2, *3*). Animal survival in this group was 22.2%.

Single injection of L-arginine reduced HR by 18.70±4.24%, increased SV by 20.90±8.74%, and decreased SBP and DBP (p<0.05). CO did not differ from the normal (Fig. 1, *c*). Preliminary administration of L-arginine improved the dynamics of cardiovascular parameters during EMI development (Fig. 2, *4*). Thus, ST segment elevation was less pronounced, and during the development of EMI this parameter often gradually decreased. HR increased by 18.47±5.99% and then remained at this level (Fig. 1, *a*). SV insignificantly decreased and also stabilized (Fig. 1, *b*). It should be noted that SV and CO in animals receiving L-arginine were higher than in the control and animals receiving L-NAME. CO in series III remained unchanged. SBP and DBP decreased insignificantly (p<0.05). Blood pressure parameters were practically stable in this group, in contrast to other groups. Animal survival in series III was 80%.

The development of EMI was accompanied by a decrease in BP, SV, and HR (Fig. 1). These changes are probably mediated by the NO system. The changes observed in the heart can be associated with activation of inducible NO synthase and enhanced production of NO and peroxynitrites [11]. Impaired myocardial contractility and decreased SV of the left ventricle are responsible for the decrease in intraaortal pressure and perfusion pressure in coronary arteries, aggravation of ischemia, and further impairment of the pumping function. This leads to pronounced cardiovascular insufficiency and determine high mortality of experimental animals (~70%).

If the assumption about the negative effect of NO on the development of MI is true, the blockade of NO synthesis should produce a positive effect on the course of MI [12]. Against the background of the blockade of NO synthase and NO formation (for 5-10 min) we observed high SV (0.23-0.25 ml) and HR (350-380 bpm) surpassing the corresponding values in the control. This suggests that under conditions of NO-synthesis blockade CO considerably increased. These positive but transient changes were followed by a progressive decrease in SV and HR: the dynamics of these parameters on minutes 10-40 of EMI under conditions of NO synthase blockade and in the control practically coincided (Fig. 1). This is probably related to vascular effects of NO synthesis blockade: constriction of blood vessels and high SBP and DBP values. The blockade of NO synthesis induces spasm of coronary vessels, impairs collateral circulation, and increases vascular resistance. Peripheral vasoconstriction and inhibition

of the vasodilator effects of acetylcholine and bradykinin against the background of NO synthesis blockade [4] resulted in BP rise and increased afterload. That is why survival of animals receiving NO synthesis blocker little differed from the control (22.2 and 33.3%, respectively).

Based on published data [14] we expected a sharp aggravation of MI against the background of L-argi-

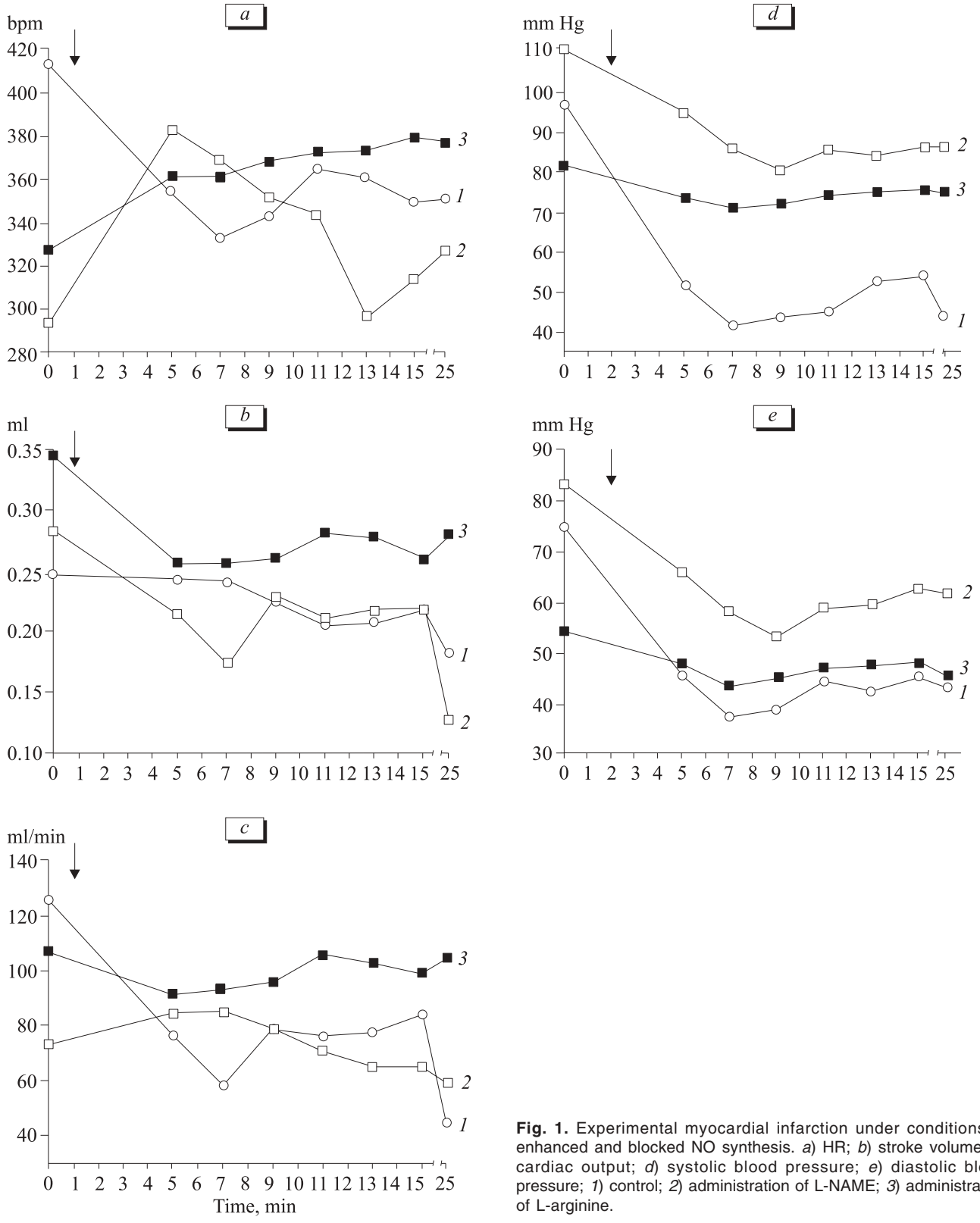

Fig. 1. Experimental myocardial infarction under conditions of enhanced and blocked NO synthesis. *a*) HR; *b*) stroke volume; *c*) cardiac output; *d*) systolic blood pressure; *e*) diastolic blood pressure; *1*) control; *2*) administration of L-NAME; *3*) administration of L-arginine.

nine treatment. However, MI under these conditions runs a milder course and animal mortality does not exceed 20%. These findings suggest that in EMI against the background of L-arginine treatment the balance between oxygen demand and oxygen supply to the myocardium is not disturbed and that L-arginine possesses cardioprotective properties. The rise of SV and HR preventing BP drop also attests to preserved contractile function of the heart essential for the favorable course of EMI (Fig. 1). There were no pronounced shifts in cardiovascular parameters (but these shifts were noted in the control group and in animals receiving NO synthesis blocker) and the dynamics of ECG was positive. Some effects of L-arginine can be explained by accelerated synthesis of NO inducing dilation of coronary vessels and improving collateral circulation [15].

However, it is difficult to explain the increase in SV, HR, CO, and the absence of BP drop in EMI against the background of L-arginine treatment. We can assume the existence of an intrinsic NO-independent mechanism underlying the effect of L-arginine on the course of MI. It can be hypothesized that L-arginine binds free radicals [10] or interacts with an unknown cell target.

Thus, NO system participates in the formation of MI and the severity of this condition is determined by the complex of interrelated cardiac and vascular effects of NO.

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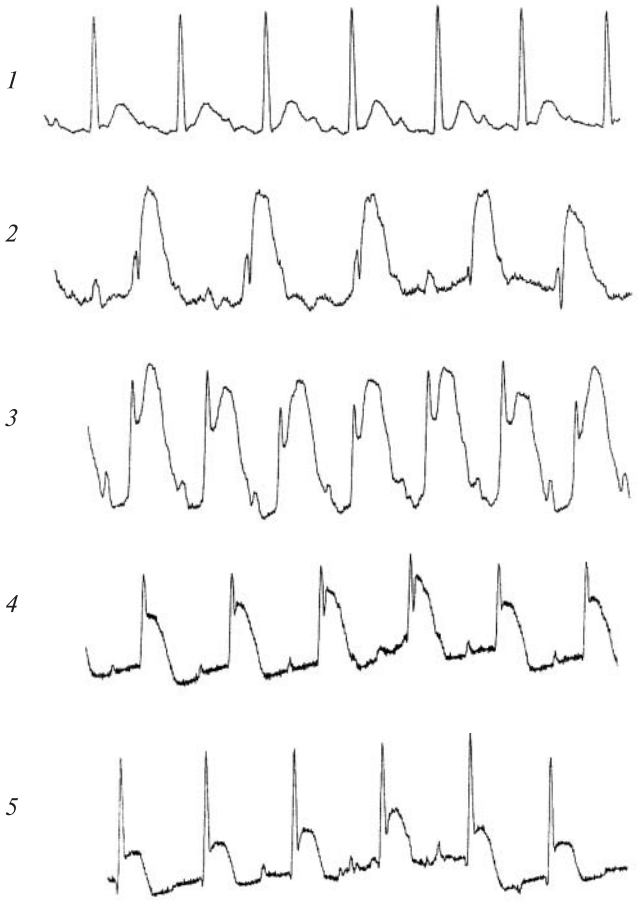

Fig. 2. ECG changes in experimental myocardial infarction. *1*) initial parameters; *2*) control group on minute 5 after ligation; *3*) group with NO synthesis blockade on minute 5 after ligation; *4,5*) group treated with L-arginine on minutes 5 (*4*) and 20 (*5*) after ligation.

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