Effect of NAD on Recovery of Adenine Nucleotide Pool, Phosphorylation Potential, and Stimulation of Apoptosis during Late Period of Reperfusion Damage to Myocardium

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The system of energy supply in the myocardium of the left and right ventricles did not recover after short-term circulatory disturbances. ATP synthesis decreased in parallel with activation of poly-(ADP-ribose)-polymerase in the ischemic region of the right ventricle, extra-ischemic region, and in the left ventricle by 5.85, 5.4, and 2.2 times, respectively. Intravenous injection of NAD immediately after blood flow resumption in the subacute period of ischemia-reperfusion damage virtually completely normalized the pool of adenine nucleotides, energy change of the adenine nucleotide system, and phosphorylation potential. Exogenous NAD inhibited activity of poly-(ADP-ribose)-polymerase in the ischemic region of the right ventricle, extra-ischemic region, and in the ischemic region of the left ventricle by 2.4, 2.9, and 1.52 times, respectively. We hypothesize that NAD acts as a regulator of signal mechanism of apoptosis induction during ischemia-reperfusion damages to the myocardium.

Key Words: *ischemia; reperfusion; NAD; energy supply system; apoptosis*

Irreversible morphological alterations in ischemic myocardium during hibernation are preceded by a series of interdependent biochemical events triggering the mechanism of cardiomyocyte death [8]: decrease in ATP and creatine phosphate contents, depletion of glycogen stores, accumulation of H^+, lactate, CO_2, and NADH, and activation of proapoptotic enzyme poly-(ADP-ribose)-polymerase. It was demonstrated that NAD content in the myocardium decreases 10-15 min after coronary occlusion [3], and after 2 h this content was only 25% of the initial value or 70% of normal level [4]. It was hypothesized that NAD degradation play a role in necrotic damage to cells and in transition from reversible to irreversible damage. Our aim was to study the effect of exogenous NAD on the energy supply system in the myocardium and on activation of poly-(ADP-ribose)-polymerase during the development of chronic ischemic-reperfusion damage.

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MATERIALS AND METHODS

The study was carried out on outbred dogs (n=21, body weight 15-25 kg) kept in a vivarium at least for one week before the experiments. The animals were randomized into two groups. Group 1 (control, n=6) comprised sham-operated dogs, in group 2 dogs (n=18) the posterior descending branch of the coronary artery was stenosed for 20 min a day for 4 days. This second group was subdivided into two subgroups: stenosis without treatment (n=10) and stenosis+intravenous injection of NAD (0.5 mg/kg, 2 ml water solution) immediately after 20-min artery clamping (n=8). The details of surgery and hemodynamic recording were described elsewhere [2]. Coronary artery stenosis was modeled as follows: a nylon thread (0.3-0.4 mm) was passed under the artery and attached to a mechanical driver mounted on the myocardial surface, the chest was closed, and a stationary venous catheter was fixed. After 7-day period of adaptation and wound healing (the wound was daily dressed and the dogs were

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given antibiotics and vitamins) the dogs were subjected to repeated graduated stenosis (transitory ischemia sessions) followed by complete resumption of the blood flow. With the help of a scaled device, the artery was ligated (blood flow reduction by 70-80%) for 20 min. During this period, the epicardiogram was presented by a typical monophasic curve, and blood oxygen dropped by 18%. After loosing the ligature ECG returned to normal, rhythm disturbances (tachycardia) disappeared. Lactate dehydrogenase activity did not increase. Sham-operated dogs subjected to all manipulations except artery clamping demonstrated no changes in ECG and blood oxygen. Both control and experimental animals were euthanized on day 14 after termination of occlusion sessions.

The methods for measuring the content of adenine nucleotides (AN), pyridine nucleotides, creatine phosphate, creatine, inorganic phosphates, AN system energy charge, phosphorylation potential PPI and PPII, tension developed by skinned cardiac muscle fibers (SCMF), and statistical methods were described elsewhere [2-4]. Poly-(ADP-ribose)-polymerase activity was measured using Amersham Pharmacia Biotech kits [11].

RESULTS

Two weeks after the short-term decreases in cardiac circulation, the subacute period was characterized by multiple disturbances of blood flow in the posterior descending coronary artery by 70-80%. In this period, no functional recovery of cardiac energy supply system was observed in the right or left ventricle (Table 1). ATP content increased, but the total content of AN, ATP/ADP and ADP/AMP ratios, and AN system energy charge were below the control values. Thus, even during short-term clamping of coronary artery, the development of energy deficiency was accompanied by a persistent decrease in AN system energy charge (by 13%) and energy level in the ATP—ADP—AMP system, as well as by imbalance between accumulation and utilization of high-energy bonds [1]. PPI decreased by 20%, the decrease of PPII was more pronounced, which reflected not only energy deficiency, but also the state of energy stores (accumulation and utilization of creatine phosphate). After 2 weeks, creatine phosphate and PPII decreased 3-fold below the control values. In other words, free energy of the system, which could be utilized to perform work, decreased 3-fold. The decrease in ATP synthesis is inversely related to activation of poly-(ADP-ribose)-polymerase (r=0.87, p<0.001). Activity of this enzyme in the ischemized region of the right ventricle and in the extraischemic zone increased by 5.85 and 5.4 times, respectively (Table 1). Similar phenomenon was observed in the myocardium of the left ventricle, although in this case the system energy charge and ATP/ADP ratio also remained low. The content of creatine phosphate was 2.5 time below the control, PPII decreased 2-fold, and activity of poly-(ADP-ribose)-polymerase increased by 2.2 times (Table 1).

Thus, the examined phenomenon, which is characteristic of patients with unstable angina, could hardly be related to the pre-conditioning effect improving tolerance to long-term ischemia. This phenomenon should be considered as repetitive transitory ischemia-reperfusion damages similar to the disturbances observed after elimination of the spasm by therapeutic means. This viewpoint agrees with published data [7,8], which by contrast to our study were obtained during a single ischemic episode (one cycle of artery clamping and reperfusion). We showed that even after a short-term ischemia (15-min), complete recovery of the energy supply system takes from 36 h [8] to a week and even to a month [10] (judging from the data on contractile activity even longer [5]). In our case, on day 14 after termination of short-term circulatory disturbances caused by artery clamping, the contractile capacity of myocardial myofibrils, which was observed even during isotonic (not isometric) contractions remained below the control by 40, 24.4, and 18.6% in the ischemic region of the right ventricle, the extraischemic zone, and the left ventricle, respectively. Thus, even 2 weeks after termination of repeated short-term ischemia, energy potency and contractile activity of the myocardium in both ventricles did not recover.

Injection of NAD immediately after blood flow recovery practically completely restored AN pool, ATP/ADP ratio, energy charge of the AN system, and PPI=([ATP])/([ADP]×[P]) in the subacute period of ischemia-reperfusion damage ([P] means inorganic phosphate). The existence of a close relationship between PPI reflecting changes in free energy of cardiomyocyte and [NAD]/[NADH] redox potential [1-3] seems to underlie the pronounced normalizing effect of NAD on the recovery of regulatory interrelations in the AN system. Moreover, under conditions of PPI normalization the decreased content of creatine phosphate can be adaptive and transitory and can result from accelerated utilization of ATP store in the form of creatine phosphate and its effect on anabolic processes. This hypothesis is corroborated by the fact that PPII under the effect of NAD also increased almost 2-fold compared to the untreated group, but remained 1.5-fold below the control (Table 1). Exogenous NAD inhibited poly-(ADP-ribose)-polymerase in the ischemic region of the right ventricle, extraischemic zone, and in the ischemic region of the left ventricle by 2.4, 2,9, and 1.52 times, respectively (Table 1). This indi-

TABLE 1. Effect Exogenous NAD on AN Pool, Creatine Phosphate Content, and Activity of Poly-(ADP-Ribose)-Polymerase during 4-Fold 20-Min Stenosis and 2-Week Reperfusion (*M*±*m*)

Index	Right ventricle					Left ventricle		
	control	ischemic region	ischemic region+NAD	extraischemic region	extraischemic region+NAD	control	ischemia	ischemia+NAD
ATP, μmol/g	5.43±0.15	3.52±0.12*	4.53±0.13*+	3.63±0.11*	5.25±0.15*+	5.55±0.13	4.13±0.16*	5.63±0.23+
ADP, μmol/g	1.66±0.07	1.67±0.11	1.50±0.06	1.70±0.09	1.66±0.07	1.60±0.06	1.63±0.07	1.64±0.08
AMP, μmol/g	0.58±0.05	0.84±0.06*	0.67±0.05	0.82±0.06*	0.69±0.05+	0.65±0.03	0.70±0.05	0.45±0.05*+
Total AN, μmol/g	7.66±0.18	6.03±0.11*	6.80±0.10*+	6.15±0.08*	7.60±0.21+	7.80±0.25	6.46±0.28*	7.72±0.18+
ATP/ADP	3.27±0.07	2.10±0.09*	2.75±0.07*+	2.13±0.05*	3.16±0.06*+	3.47±0.10	2.53±0.09*	3.43±0.07+
ADP/AMP	2.86±0.12	1.98±0.09*	2.38±0.14*+	2.1±0.1*	2.4±0.1*+	2.46±0.10	2.32±0.09	3.6±0.1*+
Energy charge of AN system	0.75±0.02	0.66±0.02*	0.75±0.02+	0.69±0.02*	0.70±0.02	0.73±0.02	0.69±0.02*	0.77±0.02
PPI	1.49±0.11	1.19±0.07*	1.92±0.11*+	1.54±0.08*	1.62±0.09	1.67±0.09	1.80±0.06*	1.60±0.10
Creatine phosphate, μmol/g	5.83±0.23	2.03±0.13*	3.76±0.17*+	2.76±0.13*	3.99±0.15*+	6.49±0.17	2.83±0.12*	4.16±0.13*+
Creatine, μmol/liter	11.6±0.3	14.7±0.4*	13.6±0.3*+	16.0±0.4*	13.3±0.3*+	11.1±0.4	13.0±0.2*	12.9±0.4*
Creatinine, μmol/g	0.42±0.06	0.48±0.05	0.43±0.04	0.48±0.05	0.45±0.05	0.38±0.05	0.43±0.03	0.44±0.04
PPII	0.76±0.08	0.27±0.03*	0.47±0.04*+	0.44±0.03*	0.59±0.03+	0.47±0.05	0.40±0.04	0.62±0.04*+
Poly-(ADP-ribose)-polymerase, pmol/mg protein	0.035±0.006	0.205±0.016*	0.085±0.009*+	0.189±0.010*	0.064±0.008*+	0.043±0.008	0.099±0.009*	0.065±0.007*+

Note. $p<0.05$ compared to *control and +corresponding index in the subgroup not treated with NAD.

cates direct inhibition of apoptosis under the action of exogenous NAD and corroborates the hypothesis on the role of NAD not only as the key factor of glycolysis and tricarboxylic acid cycle, but also as a regulator of signal mechanism of apoptosis induction during ischemia-reperfusion damages [9].

NAD increased the contractile capacity of SCMF in the ischemic region of the right ventricle by 18% in comparison with untreated control, but remained 20% below the corresponding value in intact dogs (22.3±1.4, 18.5±1.3, and 28.9±1.8 mN/mm^2, respectively). In addition, NAD increased SCMF tension in the extraischemic region of the right and left ventricle to normal. Thus, NAD markedly accelerates recovery of the energy supply system and improves the contractile reserve of myofibrils, which results mostly from the supply of necessary amount of ATP, because NAD exerts no direct positive inotropic effect on the contractile protein system. The effect of NAD is probably associated with activation of glycolytic processes and glycolytic synthesis of ATP, and with recovery of the proton transport capacity of the mitochondrial respiratory chain caused by restoration of the redox potential [NAD]/[NADH] or PPI.

Administration of NAD is necessary and probably sufficient during single disturbance of the coronary circulation (<70-80%) and for the prevention of ischemic lesions. However, during severe damages and multiple parallel episodes of ischemic and reperfusion stresses, essential recovery of mitochondrial respiratory chain (first of all, cytochrome c) is required. The absence of adequate response of this chain is the major cause of slow rise of creatine phosphate [1-3].

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