NEW BIOMEDICAL TECHNOLOGIES

Cardioprotective Effect of Energostim during Occlusion of Coronary Artery

N. V. Karsanov, G. V. Sukoyan, E. A. Chikobava, Z. N. Karsanov, E. V. Yarovaya, D. R. Tatulashvili, and I. I. Gorelishvili

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Experiments on dogs showed that energostim, a directly acting antihypoxant, injected 15 min after occlusion of the upper one-third of the left descending branch of the interventricular coronary artery produced a pronounced cardioprotective effect. The effect was confirmed by electron microscopy (evaluation the necrotic focus), biochemical tests of the heart and blood, and changes in intracardiac hemodynamics (recovery of systolic and diastolic functions). The cardioprotective effect of energostim greatly surpasses that of routine therapy applied during acute myocardium infarction.

Key Words: *ischemia; myocardium; energostim*

Cardiac damage resulting from ischemic stress and acute insufficiency is underlain by dysfunction and dyscoordination of all systems of cardiomyocyte responsible for excitation-relaxation. These disturbances are aggravated by enhanced formation of reactive oxygen forms and activation of peroxidation [2,8,9]. These processes potentiating each other induce irreversible damage in the ischemic zone and maintain hibernation state (adaptive decrease in contractile activity) in extraischemic myocardium including not only the ischemic left ventricle, but also intact right ventricle and both atria [2,5]. An important role under these conditions is played by disturbances in energy supply and metabolic pathways of oxygen utilization and ATP synthesis [2-5,7]. The rate of restoration of ATP synthesis becomes the major factor determining the severity and size of ischemic damage to the heart and reversibility of hibernation state in nonischemic regions [2-5,7,9].

Research Center of Medical Biophysics and Application of New Biomedical Technologies, Georgian Ministry of Health, Tbilisi

MATERIALS AND METHODS

The study was carried out on 38 outbred dogs (body weight 14-18 kg) kept in a vivarium for at least 1 week before experiments. All dogs were randomized to 5 groups. The control group comprised 16 sham-operated dogs (open chest surgery without arterial occlusion). The dogs of the control (n=12) and two experimental groups were subjected to 120-min occlusion of the left descending branch of the interventricular coronary artery (CAO_{120}); the dogs of experimental groups received routine therapy (intravenous atenolol+isoket+heparin, n=7) or energostim (intravenously via dropper in a dose of 110 mg/kg in 15 ml 0.9% NaCl for 15 min starting from the 15th min after CAO, n=5). The dogs of the last experimental group (n=6) were subjected to a 15-min CAO (CAO_{15}).

The procedures of CAO, recording of intracardiac hemodynamics, sacrifice, measuring of adenylic and pyridine nucleotides, cytochrome c, and pyruvate content, isolation of skinned myocardial fibers (SMF), recording of their isometric tension, measuring of changes in free energy of ATP hydrolysis (DG) and

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TABLE 1. Effects of Routine Therapy (RT) and Energostim on Intracardiac Hemodynamics during CAO (*M*±*m*)

Index	Control (*n*=16)	CAO, min			
		15 (*n*=7)	120		
			without therapy (*n*=12)	+RT (*n*=8)	+energostim (*n*=12)
HR, bpm	145±7	176±16**	160±15	160±10**	140±12
P_{syst}, mm Hg	71.2±4.5	63.7±5.5	85.5±9.2^{+ox}	87.0±2.0	75.5±9.2^{+ox}
dP/dt_{max}, mm Hg/sec	1620±238	1248±142	1200±125**	1270±128**	1480±75**
Contraction index, sec^{-1}	20.8±1.0	22.5±2.4	19.6±1.2^{++x}	22.7±1.3	19.6±1.2^{++x}
MFI, mm Hg×bpm/g	184±15	196±13	245±20	314±19	205±20
dP/dt_{min}, mm Hg/sec	1439±118	1057±158**	979±130**	1001±102*	1240±112**
EDP, mm Hg	7.8±0.5	8.6±0.5**	12.6±0.9*^{o}	11.5±0.5*	17.6±0.8*^{o}
Relaxation index, sec^{-1}	12.4±0.7	11.0±0.6^{++}	19.6±1.6**	17.3±1.6*	13.6±1.8*

Note. *p<0.01, **p<0.05 compared to the norm; ^{+}p<0.01, ^{++}p<0.05 compared to 15-min CAO; ^{o}p<0.05 compared to 120-min CAO without treatment; ^{x}p<0.05 compared to 120-min CAO+RT. MFI is maximum functional intensity, and EDP is end diastolic pressure.

inorganic phosphate content, preparation and examination of the specimens for electron microscopy under JEM 100B microscope, and statistical analysis were described in previous papers [1,3,4]. The dogs with atrial or ventricular fibrillation developed within 15 min after CAO were excluded.

RESULTS

In contrast to routine therapy, energostim increased the maximum rate of pressure rise and drop during the first two hours after ligation of coronary artery (Table 1). The maximum intensity of structures decreased: the same pressure developed at lower load per mass unit, hibernating structures are less abundant, lower number of structures was involved to achieve the same ventricular pressure. Energostim decreased the end diastolic pressure and relaxation index (Table 1). Thus, this drug improved systolic and diastolic cardiac function. Of particular importance is the fact that energostim restored coordination of these functions: the correlation coefficient (r) dP/dt_{max} and dP/dt_{min} in the control group was 0.69 ($p<0.001$), while in CAO_{15} and CAO_{120} groups it was 0.63 ($p<0.05$) and 0.45, respectively. These data indicate that energostim induced transition of destructive and necrotic alterations into mild and reversible damage or even adaptive (functional) depression of the myocardium.

This favorable effect of energostim on hemodynamics is underlain by a decrease in the size of focus of irreversible myocardial alterations 2.8-fold from 34.7 to 12.4% of left ventricle mass compared to the control (Fig. 1), while routine therapy decreased it only by 2.5 times. Energostim significantly decreased

TABLE 2. Effect of Energostim on Degree of Necrotic Damage in Cardiomyocytes and Mitochondrial Energy Efficiency Coefficient (*M*±*m*)

Index	Norm	CAO_{15}	CAO_{120}		
			control	+RT	+energostim
S_{MC}/S_E	0.21±0.03	0.28±0.08	0.11±0.02*$^{++}$	0.13±0.03**$^{+++}$	0.19±0.08^{oooxxx}
S_{MF}/S_E	0.45±0.04	0.30±0.04***	0.13±0.05*$^{+}$	0.22±0.04*^{oo}	0.29±0.03*^{ooo}
S_{MC+MF}/S_E	0.64±0.06	0.58±0.08	0.24±0.07*$^{+}$	0.26±0.04*$^{++}$	0.42±0.02*$^{+++ooxx}$
S_{MC}/S_{MF}	0.45±0.04	0.88±0.14*	0.85±0.25**	0.78±0.14*	0.63±0.04^{oooxxx}
$S_{necrosis}/S_E$	—	0.16±0.05	0.68±0.03^{+}	0.56±0.05^{+}	0.21±0.03^{ox}
$S_{MC+MF}/S_{necrosis}$	—	3.6±0.4	0.40±0.09^{+}	0.6±0.4^{+ooo}	2.0±0.1*^{ox}
MEEC, %	100	22.8±8.3*	10.0±2.0*$^{+}$	29.8±8.3^{+++}	78.0±15.2*$^{++ox}$
Number of intact sarcomeres	100	40±5*	5.0±0.3^{+}	12±5^{+ooo}	37±6^{+x}

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the norm; ^{+}p<0.001, ^{++}p<0.01, ^{+++}p<0.05 compared to 15-min CAO; ^{o}p<0.001, ^{oo}p<0.01, ^{ooo}p<0.05 compared to control; ^{x}p<0.001, ^{xx}p<0.01, ^{xxx}p<0.05 compared to 120-min CAO+RT.

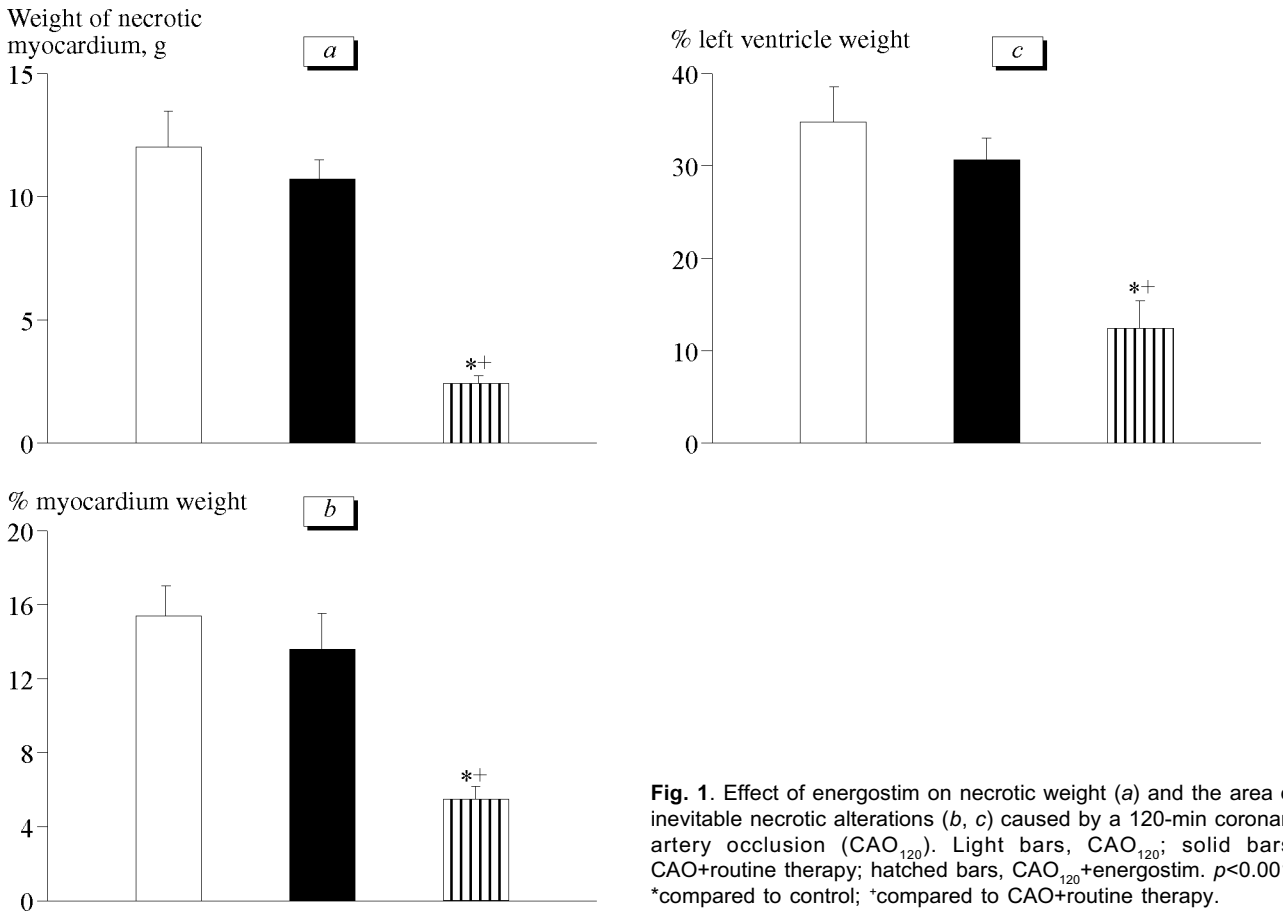

Fig. 1. Effect of energostim on necrotic weight (*a*) and the area of inevitable necrotic alterations (*b*, *c*) caused by a 120-min coronary artery occlusion (CAO_{120}). Light bars, CAO_{120}; solid bars, CAO+routine therapy; hatched bars, CAO_{120}+energostim. $p<0.001$: *compared to control; +compared to CAO+routine therapy.

the area of enhanced risk of irreversible changes in ischemic myocardium — by 47% compared to CAO_{120} and by 45% compared to CAO_{120}+ routine therapy. The results achieved with routine therapy (nitrates and β-blockers) coincided with published data [7,9].

Microscopy showed edema of cardiomyocytes developed 15 min after CAO and focal necrotic alterations occupied 16% area of electron micrograph (Fig. 2). The disturbances in mitochondria (MC) were mosaic ranging from partial clarification and disappearance of cristae to complete vacuolation of MC. The destructive processes in myofibrils became more pronounced: irregular orientation and partial lysis of sarcomeres, which usually began from *I*-disks (melting of *Z*-lines). The mean length of sarcomeres was 1.8 μ. Damage to myofibrils developed more rapidly than changes in MC. This was confirmed by the analysis of functional activity: the amplitude and rate of force generation drastically decreased 15 min after CAO, while the content of ATP and creatine phosphate and phosphorylation potential of MC did not significantly change; mitochondrial energy efficiency coefficient (MEEC) decreased to 22.8% (Table 2). The number of glycogen granules decreased. Sarcoplasmic reticulum was damaged, sometimes dilated, and detached from the sarcolemma. After 2-h ischemia, the structure of organelles could not be detected. Only clusters of small vacuolated MC could be found. Pronounced edema of cardiomyocytes developed.

Energostim 3.2-fold decreased the intensity of necrotic process in the infarction zone (imminent necrotic area), although the degree of myofibril destruction in cardiomyocytes on minute 120 after CAO did not differ from that observed at the moment of drug injection (15 min after CAO). On minute 120, most vacuolated MC were filled with cristae. This filling started from the sites adjacent to outer membrane. MC looked condensed, small clarified foci produced an appearance of spotted and speckled MC, which reliably attested to application of energostim. MEEC increased from 10% in control to 78% 1.75 h postinjection. As a rule, the myofibrils retained normal orientation. *A*- and *I*-disks as well as *Z*-lines were clearly seen. However, there were also the areas with partial lysis of myofibrils. The sarcomere length was no less than 1.5 μ. MC were small and compact. Clusters of MC were found in the area of partial lysis of myofibrils. The number of glycogen granules increased. The edematous sites of cardiomyocytes in the micrographs decreased more than 3-fold, which was accompanied by normalization of the structure of sarcoplasmic reticulum. These data suggest that energostim not

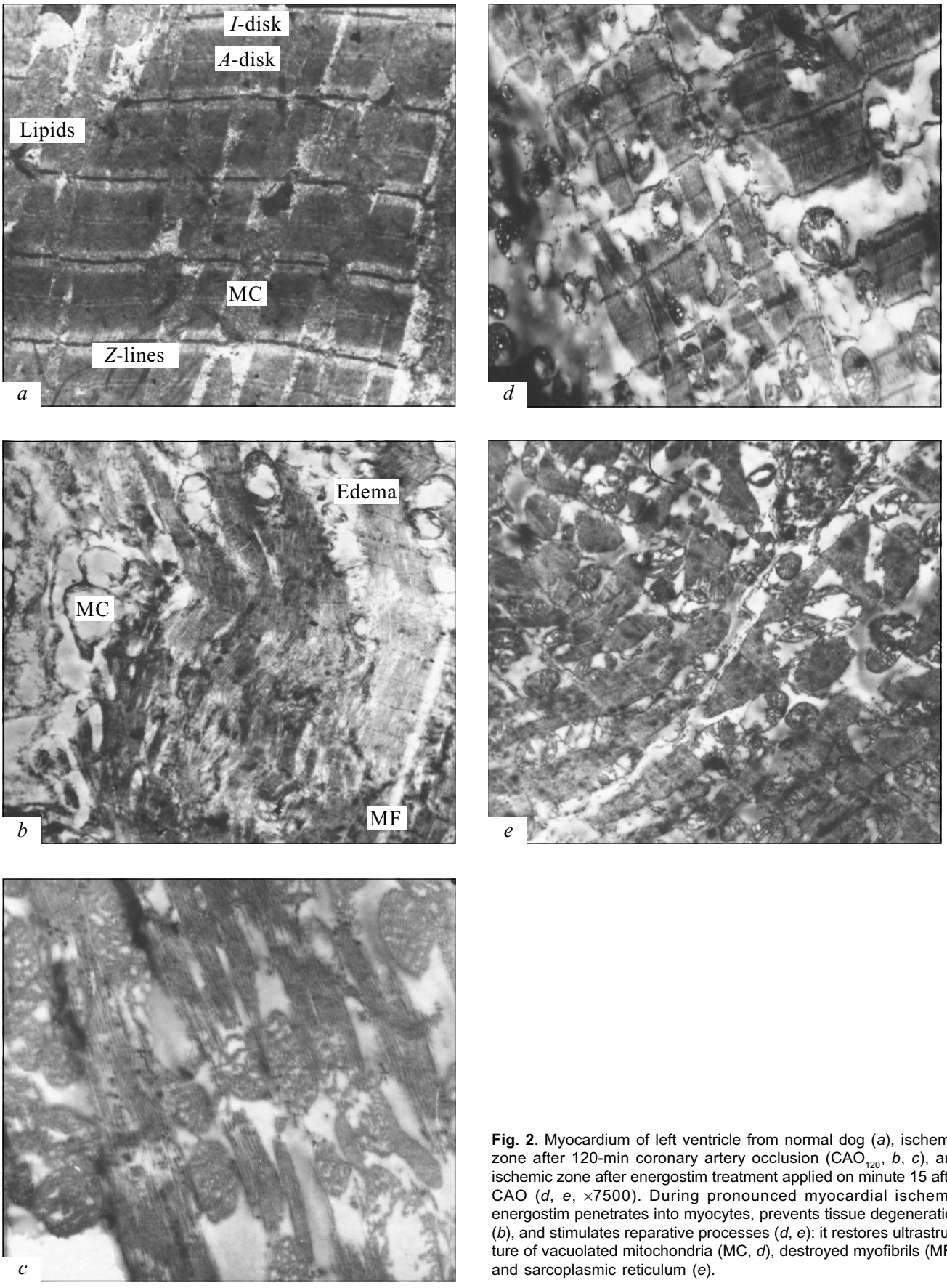

Fig. 2. Myocardium of left ventricle from normal dog (*a*), ischemic zone after 120-min coronary artery occlusion (CAO_{120}, *b*, *c*), and ischemic zone after energostim treatment applied on minute 15 after CAO (*d*, *e*, ×7500). During pronounced myocardial ischemia energostim penetrates into myocytes, prevents tissue degeneration (*b*), and stimulates reparative processes (*d*, *e*): it restores ultrastructure of vacuolated mitochondria (MC, *d*), destroyed myofibrils (MF), and sarcoplasmic reticulum (*e*).

TABLE 3. Effect of Energostim on Adenylic Nucleotides and Creatine Phosphate (CP) Content (μmol/g wet tissue), Energy Charge (EC), and Phosphorylation Potential (PP) in Myocardium during 120-min CAO (*M*±*m*)

Group		ATP	ADP	AMP	ATP/ADP	EC	CP	PP
Control (*n*=14)	LV	4.6±0.2	2.2±0.2	1.35±0.10	2.10±0.06	0.70±0.02	4.8±0.3	1.53±0.12
	RV	4.3±0.2	2.0±0.3	1.45±0.10	2.15±0.08	0.68±0.01	4.3±0.4	1.53±0.10
	LA	5.2±0.3	2.8±0.2	0.95±0.06*	1.86±0.04	0.73±0.02	4.8±0.3	1.60±0.08
	RA	4.5±0.3	2.7±0.3	0.90±0.05*	1.67±0.05	0.73±0.02	4.2±0.3	1.60±0.08
CAO								
without treatment (*n*=8)	IZ	2.8±0.8*	1.6±0.2*	0.95±0.12**	1.75±0.08***	0.65±0.03***	1.5±0.3*	0.95±0.02*
	EIZ	3.1±0.6**	1.9±0.2	0.80±0.06**	1.63±0.05***	0.63±0.04	2.4±0.5*$^{++}$	1.21±0.08^{+}
	RV	3.1±0.5**	2.0±0.2	0.80±0.06**	1.55±0.04***	0.63±0.04	2.5±0.3*	1.20±0.06**
	LA	2.2±0.3*	1.7±0.3***	1.15±0.06	1.42±0.08***	0.60±0.03***	2.9±0.3*	1.35±0.05***
	RA	3.3±0.2*	1.6±0.2***	0.98±0.05	2.10±0.06***	0.69±0.03	3.2±0.3*	1.45±0.05***
+RT (*n*=8)	IZ	3.0±0.3**	2.2±0.2***	0.99±0.19**	1.36±0.10*^{oo}	0.65±0.05***	2.0±0.2*^{x}	0.95±0.02^{o}
	EIZ	3.2±0.6**	1.35±0.19***	1.05±0.19**	2.37±0.08**	0.64±0.01	2.1±0.3*	1.24±0.03**$^{+}$
	RV	3.4±0.4**	2.1±0.3	0.87±0.13**	1.62±0.08*^{o}	0.64±0.01^{ooo}	2.3±0.2*	1.20±0.03*
	LA	3.8±0.3^{oo}	2.8±0.2**^{oo}	1.05±0.04***	1.86±0.08*^{oo}	0.55±0.02**	3.6±0.3*^{o}	1.34±0.08*
	RA	3.5±0.3**	2.7±0.3**^{oo}	1.08±0.05***	1.67±0.03^{xx}	0.68±0.03	3.4±0.2***	1.40±0.08*^{ox}
+energostim (*n*=8)	IZ	3.8±0.3***	2.1±0.2	1.15±0.12	1.81±0.05	0.69±0.02*	3.4±0.3***^{x}	1.75±0.02***
	EIZ	4.2±0.4**	2.3±0.5	1.20±0.19	1.83±0.04	0.69±0.01	3.6±0.4***	1.21±0.08***$^{+++}$
	RV	4.4±0.5**	2.10±0.3	1.32±0.13	2.10±0.04	0.70±0.01	3.2±0.2***^{xx}	1.20±0.06***$^{++}$
	LA	4.5±0.3	2.2±0.2	0.85±0.04*	2.05±0.05	0.74±0.02	4.8±0.3***^{x}	1.60±0.08^{ooxx}
	RA	4.1±0.4	2.3±0.3	0.95±0.06	1.78±0.06	0.71±0.03	4.2±0.3***^{x}	1.60±0.08^{ooxx}

Note. Here and in Table 4. *p<0.001, **p<0.01, ***p<0.05 compared to the norm; ^{+}p<0.001, ^{++}p<0.01, ^{+++}p<0.05 compared to the indices in infarction zone; ^{o}p<0.001, ^{oo}p<0.01, ^{ooo}p<0.05 compared to CAO; ^{x}p<0.001, ^{xx}p<0.01, ^{xxx}p<0.05 compared to CAO+RT. IZ – infarction zone; EIZ — extra infraction zone; RV — right ventricle; LV — left ventricle; RA — right atrium; LA — left atrium.

only arrested the destructive processes in the myocardium from the moment of its injection, but also activated repair processes, protein synthesis, and the development of new structures. These properties explain pronounced decrease in the area of inevitable necrotic damage (almost 3-fold) and marked moderation in the degree of this damage in ischemic area (more than 3-fold).

The combined effect of isosorbide dinitrate and atenolol on contractile and relaxation capacity of myofibrils increased generated force of SMF in ischemic area by 54%, that is to the level, which does not significantly differ from that observed after CAO_{15}. This attests to some beneficial effect to the moment of injection. However, the increase in generated force of SMF in ischemic area was not accompanied by an increase in force generation rate or the rate of relaxation, as well as efficiency of contractile process in all region of myocardium in comparison with these parameters after CAO_{120}. The increase in contractile ability and the capacity of SMF to relax under the conditions of strictly isometric contraction were observed only under the action of energostim. Moreover, while energostim completely restored efficiency of contraction, it produced only partial increase in the rate and amplitude of SMF force in the ischemic and nonischemic areas, which were still smaller than the normal values by 19.5 and 14.0%, respectively. The rate of relaxation in these areas also increased, but remained below the normal by 19 and 10%, respectively. These results and the data on capacity of SMF both in ischemic and nonischemic areas to restore under the effect of cardiac glycosides *in vitro* indicate, on the one side, that increase of phosphorylation potential, content of adenylic nucleotides, and elimination of energy deficiency produce a certain increase in contractile and relaxation capacity of myofibrils. On the other hand, the contractile capacity of myofibrils is not stimulated via increase in the contraction force of remaining intact sarcomeres as takes place under the action of strophanthin K or β-acetyldigoxin *in vitro* provided the content of ATP is optimal [5]. Energostim acts on SMF mainly due to elimination of energy deficiency, while the effect of cytochrome c (a positive inotropic agent) is probably counterbalanced by the negative inotropic effect of NAD. However, it is also possible that these effects do not develop *in vivo* after administration of therapeutic doses of energostim. The obtained data cast doubt on advisability to include cardiac glycosides (especially β-acetyldigoxin) producing a pronounced positive effect on myofibril relaxation into the therapy of acute myocardial infarction in cases when signs cardiac insufficiency appeared or when myocardial infarction developed against the background decreased contractile activity.

The development of pronounced energy deficiency during CAO_{120} manifested in *1*) decrease in ATP content in ischemic area by 40% and in nonischemic area by 33%; 2) decrease in total content of adenylic nucleotides and in their energy potential; 3) decrease of ATP/ADP ratio in ischemic and nonischemic area by 1.25 and 1.2 times, respectively (Table 3), caused by disturbance in ATP production in the processes of oxidative phosphorylation and glycolysis caused by drastic decrease in myocardial NAD and release of cytochrome c from MC (Table 4). The content of cytochrome c and NAD in the homogenate of ischemic myocardium decreased after CAO_{15} by 17 and 14.5%, respectively, although these parameters in other myocardial regions remained unchanged (Table 4). The destructive processes developed to the end of CAO_{120} were characterized by a decrease in cytochrome c content in ischemic and nonischemic areas by 34 and 17%, respectively. Its content decreased to the same degree in suspensions of MC isolated from the same areas. The content of NAD decreased in ischemic and nonischemic areas by 19 and 24%, respectively, while the decrease in nonischemic right ventricle was 15%. The content of NADP also decreased, although to a lesser degree (Table 3). Energostim increased the content of pyridine nucleotides in the myocardium and impeded the progressing decrease in NADP. As a result, the NADP/NADPH ratio markedly increased, which was probably related to energostim-induced activation of pentose phosphate metabolic pathway of glucose utilization. This is of particular importance in erythrocytes lacking MC. Activation of glycolysis in erythrocytes can be the main mechanism of the increase in ATP content in erythrocytes and hence, normalization of their elastic properties and osmotic resistance [1]. The concomitant increase in the total content of pyridine nucleotides is the basis of activation of synthetic processes. The capacity of energostim to moderate histotoxic hypoxia and produce antioxidant effect [1] together with its ability to improve oxygen transport to the cells and to decrease the oxygen requirement of the myocardium create necessary requirements to limit the focus of ischemic damage.

Therefore, in contrast to all known anti-ischemic preparations [10], energostim, a direct action antihypoxant, can affect myocardial metabolism disturbed during acute infarction, normalize the processes of ATP synthesis, and produce a more pronounced protective effect on the myocardium compared to routine therapy.

REFERENCES

1. N. A. Andriadze, G. V. Sukoyan, N. O. Otarishvili, *et al., Ros. Med. Vesti,* No. 2, 31-42 (2001).

TABLE 4. Effect of Energostim on Pyridine Nucleotides, Cytochrome *C*, Lactate, and Pyruvate Content in Myocardium and Blood during 120-min CAO (*M*±*m*)

Index		Norm	CAO		
			control	+RT	+energostim
NAD	myocardium, μmol/g	5.4±0.3	4.55±0.52*** (3.9±0.2*)	4.0±0.4** (4.1±0.3*)	4.8±0.4^{xxx} (5.0±0.3^{xxx})
	blood, nmol/ml	18.2±0.4	8.50±0.95	8.70±0.75*^{o}	16.5±0.5^{ox}
NADH	myocardium, μmol/g	3.9±0.5	4.5±0.3** (5.25±0.85)	6.34±0.72**^{ooo} (5.10±0.35**$^{+++}$)	4.14±0.12^{xx} (4.25±0.25^{xx})
	blood, nmol/ml	19.9±0.4	31.5±2.3*	25.7±0.5*	20.5±0.6^{ox}
NAD/NADH	myocardium	1.2±0.4	0.74±0.11*** (0.63±0.10***$^{+++}$)	0.63±0.10* (0.80±0.11*)	1.17±0.10^{ox} (1.18±0.12^{ox})
	blood	0.95±0.10	0.27±0.04*	0.34±0.05*^{o}	0.85±0.05^{ox}
NAD+NADH	myocardium, μmol/g	9.3±0.5	9.2±0.6 (10.3±1.0)	10.4±0.4 (9.2±0.3)	9.0±0.4 (9.25±0.15)
	blood, nmol/ml	38.2±2.4	40.0±1.0	41.5±1.3*^{o}	41.0±1.0
NADP	myocardium, μmol/g	5.3±0.5	4.38±0.23*** (5.15±0.27)	4.05±0.37** (5.38±0.6)	5.15±0.25 (5.18±0.30)
	blood, nmol/ml	22.4±0.5	15.3±1.2	13.70±0.35*	18.7±0.4^{ox}
NADPH	myocardium, μmol/g	4.9±0.6	6.6±0.8 (6.1±0.7*$^{+++}$)	6.1±0.7* (6.6±0.8^{+++})	6.1±0.7 (6.6±0.8)
	blood, nmol/ml	24.9±0.6	20.8±1.9	25.7±0.5*	22.5±0.5^{ox}
NADP+NADPH					
	myocardium, μmol/g	10.1±0.6	10.5±0.9 (11.2±0.3)	10.2±0.9 (12.0±0.7)	11.2±0.7 (11.8±0.7)
	blood, nmol/ml	47.4±0.6	36.0±2.0**	39.4±0.4**	41.8±0.5**^{oo}
NADP/NADPH					
	myocardium	1.3±0.4	0.71±0.14* (0.84±0.14*)	0.65±0.12* (0.82±0.11*)	0.85±0.10 (0.79±0.09)
	blood	1.4±0.3	0.73±0.07*	0.53±0.05*^{o}	0.87±0.50^{ox}
Cytochrome *c*					
	myocardium, nmol/g	24.6±2.2	15.1±1.0* (18.8±1.7*$^{+++}$)	16.7±1.3* (18.1±1.0*)	20.2±1.3^{ooxx} (23.1±1.0$^{+++ooxx}$)
	blood, nmol/ml	0.62±0.12	0.80±0.06***	0.78±0.06	0.60±0.06^{ooxx}
Lactate	myocardium, μmol/g	2.7±0.1	6.10±1.25* (3.69±0.14)	4.7±0.2 (3.10±0.25)	2.8±0.4 (2.4±0.3)
	blood, nmol/ml	1.38±0.12	4.3±0.4	3.4±0.2*^{ooo}	1.32±0.34^{ox}
Pyruvate	myocardium, μmol/g	0.95±0.10	0.87±0.09 (0.55±0.10*$^{++}$)	0.78±0.10 (0.57±0.10*$^{+++}$)	0.80±0.10 (0.85±0.10)
	blood, nmol/ml	0.14±0.02	0.18±0.02	0.16±0.02	0.18±0.02
Lactate/pyruvate					
	myocardium	3.0±0.6	11.1±0.1* (4.73±0.13*$^{++}$)	6.0±0.40*^{oo} (5.4±0.30*$^{+++}$)	3.5±0.1^{oxx} (2.8±0.10^{oxx})
	blood	10.0±1.2	23.8±2.8*	21.3±2.2***	7.3±0.8^{ox}

Note. Myocardial indices outside infarction zone are given in brackets.

2. N. V. Karsanov, *Byull. Eksp. Biol. Med.,* **128**, No. 8, 124-140 (1999).
3. N. V. Karsanov, L. E. Gogiashvili, and E. V. Selikhova, *Vopr. Med. Khimii,* No. 2, 50-55 (1993).
4. N. V. Karsanov, G. V. Sukoyan, D. R. Tatulashvili, *et al., Pat. Fiziol.,* No. 3, 3-8 (1999).
5. N. V. Karsanov, G. V. Sukoyan, D. R. Tatulashvili, *et al., Ros. Kardiol. Zh.,* No. 6, 26-34 (2000).
6. P. F. Cohn, *Clin. Cardiol.,* **17**, 415-421 (1994).
7. M. F. DiCarli, M. Davidson, and R. Little, *J. Am. Coll. Cardiol.,* **73**, 527-533 (1994).
8. L. H. Opie, *Medicographia,* **18**, 3-9 (1996).
9. D. M. Yellon and G. F. Baxter, *Heart,* **83**, 381-387 (2000).