Effect of Adaptation to Repeated Stress on Cardiac Function Repair and Creatine Phosphate Level Restoration Following Total Ischemia (^{31}P-NMR Investigation)

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Adaptation to repeated short-term immobilization stress in animals is known to bring about the development of a protective effect, manifested in increased resistance of the body to cold [12], direct chemical damage [18], ischemic cardiac lesions [3], and even cardiac radiation injuries [15]. A study of the mechanism involved in this phenomenon revealed that the isolated hearts of these animals show increased resistance to ischemia, reperfusion, a high Ca^{2+} concentration, toxic catecholamine concentration [2], and to heat shock [16]. Later it was demonstrated that organelles isolated from such a heart (sarcoplasmic reticulum elements [1] and mitochondria [6]) exhibit increased resistance to autolysis for long-term storage. A suspension of nuclei isolated from the same hearts is characterized by a greatly increased resistance to single-strand DNA, which is known to activate nucleoproteases [5]. This complex of shifts is referred to as the structure adaptational stabilization phenomenon (SASP). Moreover, it is not in the heart alone that SASP has been demonstrated; the effect extends to the whole body and is manifested in an enhanced resistance of the animals to sublethal hypoxia [7]. This observation led us to conclude that SASP might be to a high degree involved in the cardiac energy supply. It is known [9,10,17], that the major process involved in myocardial energy supply is the interaction between the mitochondrial system of oxidative phosphorylation, ATP-translocase, and creatine kinase, providing for macroergic group transport from the mitochondria to myofibril contractile apparatus.

The aim of this study was to verify the hypothesis that in animals exposed earlier to repeated stress the energy supply system can remain largely intact, promoting a fundamentally more rapid myocardial creatine phosphate restoration and repair of cardiac contractile function in reperfusion after long-term ischemia.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 250-300 g. Half of the animals were used as controls (the first series) and the rest were exposed to repeated immobilization stress by fixing the four paws (the head was not fixed) in the supine position for 8 sessions every other day (the second series). The first three sessions lasted 15 min, 30 min, and 45 min and the other 5 sessions lasted 60 min each. At the end of the experiments none of the animals showed limb edema, stress-induced damage to the gastric mucosa, or identifiable changes in behavior.

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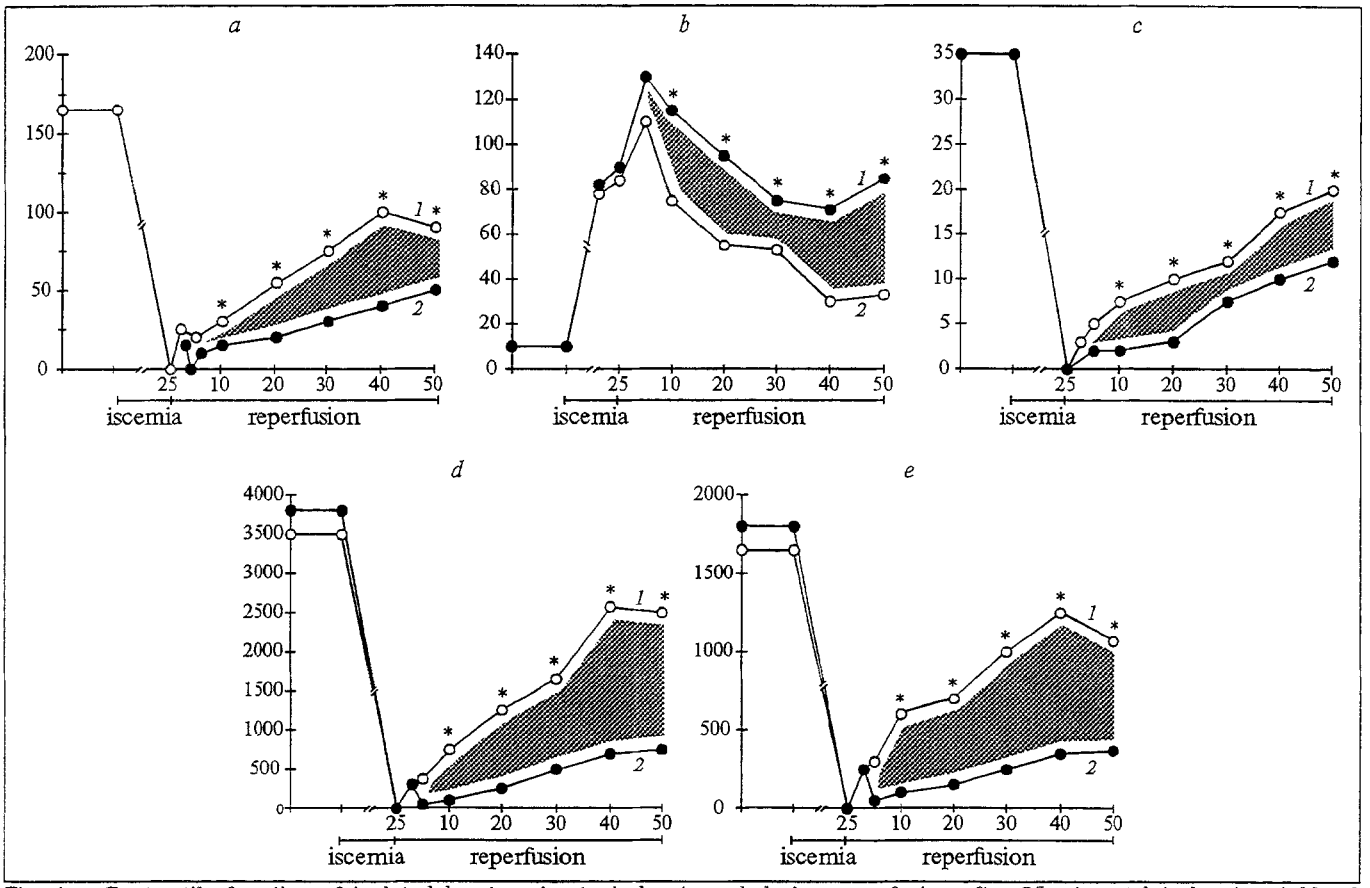

Fig. 1. Contractile function of isolated hearts prior to ischemia and during reperfusion after 25 min total ischemia, *a*) blood pressure, mm Hg; *b*) end dyastolic pressure mm Hg; *c*) contractile function mm Hg≥contraction/min; *d*) contraction rate, mm Hg/sec; *e*) relaxation rate, mm Hg/ sec.

For perfusion of the isolated hearts the rats were narcotized with urethane in a dose of 1.8 to 2.1 g per kg body weight and heparinized (500 U/ kg). The heart was promptly removed from the chest and suspended for 1 to 2 min by the aorta to a cannula. Perfusion was performed by the method of Langendorf with a specified coronary flow rate (about 11-12 ml/min/g of wet cardiac tissue weight using Krebs-Henseleit solution with the following composition in mM: NaCl 118, KCl 5.9, $CaCl_2$ 3.0, $MgSO_4$ 1.2, Na-EDTA 0.5, $NaHCO_3$ 25, glucose 11. The solution did not contain orthophosphate, since it would have interfered with monitoring the intracellular inorganic phosphate level in nuclear magnetic resonance phosphate spectra. The solution was saturated with carbogen (95% O_2 and 5 % CO_2) at 37°C (pH 7.4). A catheter with a latex balloon filled with water was introduced into the left ventricle for intraventricular pressure measurement. With the head of Statham P23Db electromanometer, Gould Co., USA, and a Gould 2400S 4-channel recorder, (USA) the following physiological parameters were recorded: left ventricular pressure, its first derivative to determine the contraction ($+dp/dt$) and relaxation ($-dp/dt$) rates, end-systolic pressure, and heart rate. The heart contracted spontaneously. The integral index of cardiac function was calculated as the product of the pressure (the difference between the systolic and the end-diastolic pressure) and heart rate. The heart was placed in a latex bag filled with perfusion solution and then immersed into an ampoule with a diameter of 20 mm containing an aliquot of D_2O for better adjustment of the transducer. The entire system was then set in the magnet of an CXP-200 NMR spectrometer (Bruker Co, Germany). Next to the heart a sealed ampoule was positioned with 100 mM sodium methylene diphosphonate solution serving as a standard solution for quantitation of the phosphorus-containing compounds in each heart.

Recording of ^{31}P-NMR spectra. Usually the spectra were recorded every 5 min at a standard frequency of 80.98 MHz, using 90° recording pulses (37 μsec) repeated every 2 sec. The spectrum was obtained by adding in the memory unit with a volume of 4 kB 150 individual attenuation signals of free induction. Following exponential multiplication with an amplification factor of 10-20 Hz and Fourier transform, a frequency spectrum

TABLE 1. Effect of Adaptation to Immobilization Stress on Metabolism of Rat Isolated Heart in Ischemia and Reperfusion

Index	Before ischemia	Ischemia, min				Reperfusion, min				
		5	10	15	25	5	10	20	30	45
Creatine phosphate, mM/g dry weight										
Control	24.3±2.5	4.8±0.5	3.2±0.2	3.4±0.1	2.8±0.2	18.6±4.0	7.9±0.4	10.2±1.3	11.7±1.2	13.0±0.45
Adaptation to stress	23.5±1.6	3.8±0.6	3.1±0.4	3.5±0.3	2.1±0.1	21.6±2.0	16.7±2.8*	19.1±3.4*	21.6±2.5**	23.8±2.4**
ATP, mM/g dry weight										
Control	19.7±1.3	14.8±1.9	9.2±2.0	4.5±0.4	3.1±0.5	8.7±1.0	5.1±0.1	4.5±0.04	4.4±1.3	5.8±0.05
Adaptation to stress	17.5±1.03	13.7±1.4	8.5±1.2	4.2±0.4	4.02±0.2	7.8±0.7	5.9±0.6	6.3±1.1	7.4±0.8	6.8±0.7
Inorganic phosphorus mM/g dry weight										
Control	12.4±1.3	19.9±4.9	41.1±3.0	59.1±2.8	70.9±6.4	19.7±2.4	25.4±2.5	18.6±2.2	14.2±0.9	17.5±2.9
Adaptation to stress	11.1±1.2	18.8±2.7	40.8±5.8	55.6±8.1	64.1±8.5	17.3±3.5	18.8±4.1	16.0±3.3	11.4±2.9	13.7±3.5

Note. In the control series n=10, in the adaptation to stress series n=9; confidence level: * – $p<0.05$, ** – $p<0.01$.

was obtained in the range of 4000 Hz (50 m.d.) [11]. Both at the beginning and at the end of each experiment "quantitative" spectra were accumulated, pulse repetition rate being 10 sec for greater relaxation of the phosphates of each peak. The peak areas cut out of these spectra were used to assess PCr, P_i and ATP tissue levels by correlating the peak areas with those of the standard solution containing 10 mM of ^{31}P and with the dry weight of the tissue used for extraction. Weak saturation of the peak with PCr was estimated by multiplying its area by 1.1.

The plasma concentration of the free ADP was calculated according to creatine kinase equilibrium by the formula:

$$[ADP] = [ATP]\frac{[EC]}{[FEC]\cdot[EC]},$$

assuming that the equilibrium constant (EC) is 104 for pH 7.2, the free Mg^{2+} concentration is 1 mM [14] and the cytoplasmic ATP is 85% of the total level [13]. The cytoplasmic water content will be 2.5 ml/g dry weight.

Total creatine was determined by the specific color reaction with diacetyl and a-naphthol using the method described in [8]. During the first 40 to 50 min the hearts were perfused using Langendorf's method for stabilization of the baseline spectra. Further, total normothermic ischemia was induced by coronary blood flow arrest for 25 min. Inside the magnet the temperature was maintained by air heating and a temperature-sensitive element. For 55-65 min perfusion was performed with the coronary flow used before ischemia induction.

RESULTS

The data on the contractile function of isolated hearts before ischemia and in the course of reperfusion after 25 min of total ischemia are presented in Fig. 1. The data suggest that under aerobic conditions the cardiac performance index of the animals which had been exposed to repeated stress was no different from that of control animals. However, in the course of reperfusion when induction of ischemia was completed, the differences were found to be highly significant. The blood pressure of the adapted animals was found to be higher than in the control animals virtually throughout the reperfusion period (Fig. 1, *a*) and after 40 to 50 min it was twice as high as shown in Fig. 1 (hatched area). The diastolic pressure curves in Fig. 1, *b* show that the observed differences can be attributed to a slight increase in myocardial contracture rate found by the end of ischemia induction period followed by a rapid decrease during reperfusion of adapted animals, and 20 min after the beginning of reperfusion the myocardial contracture time decreased twofold. Thirty to 40 min following the beginning of reperfusion these differences proved to be still higher. The hatched area in Fig. 1 shows the anticontracture effect of the previous adaptation to stress that was responsible for the higher blood pressure in the adapted animals. Despite the slightly lower heart rate observed in adapted animals, the higher blood pressure resulted in a marked increase of the integral function index (blood pressure × heart rate) in comparison with the control value at all stages of reperfusion (Fig. 1, *c*). In the early stages the cardiac index in adapted animals was 2 to 3 times higher, and 30-50 min after the reperfusion it was 90% higher in comparison with the control animals. Significant differences were observed in the cardiac contraction and relaxation rate (Fig. 1, *d, e*). These two indexes returned to normal much faster in animals previously adapted to stress than in control animals. At the 5th-10th min of reper-

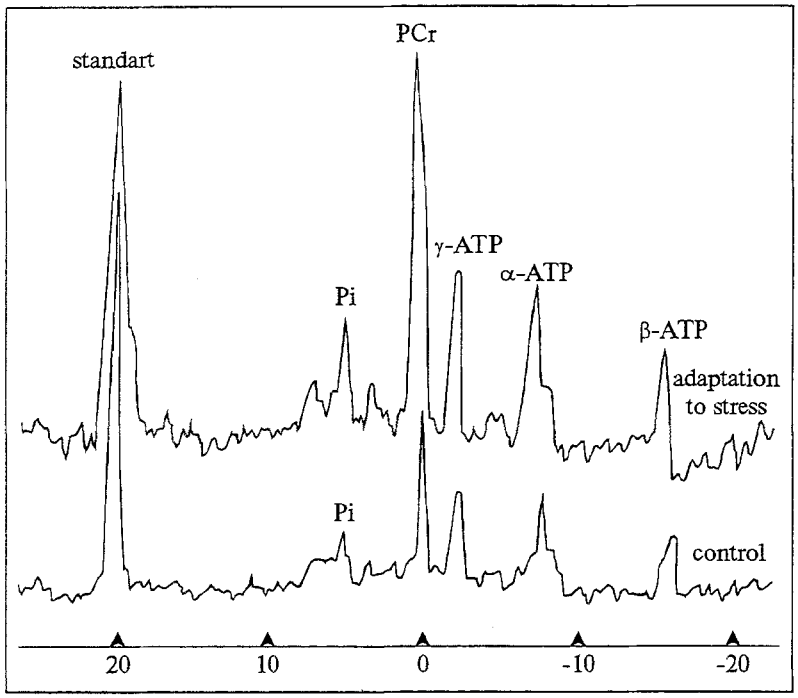

Fig. 2. NMR spectra between 15th and 20th min of reperfusion

fusion they exceeded the control 5 times, and at the 20th-50th min, 2.5-3 times. Thus, exposure to repeated stress provides for a faster restoration of the cardiac contraction and relaxation rate, which in turn results in a more effective diastole. It should be borne in mind that contracture in ischemia is brought about by a local ATP decrease in the myofibrils due to both limited transport of ATP from the myoplasm and limited CP transport from the mitochondria [8,10], which results in some actomyosin bonds generating force being unbroken and hence in the formation of contracture [12]. Reversal of ischemia involves restoration of oxidative phosphorylation and CP transport from mitochondria to myofibrils. The data in Table 1 obtained by NMR method characteristic for this process show that ischemia resulting in a dramatic fall in CP and ATP and P_i accumulation is followed by a relatively rapid CP level restoration and P_i drop in reperfusion, whereas the ATP level returns to normal rather slowly. This observation is well known and is consistent with the concept that creatine kinase system is generally a system of emergency energy transport and, in particular, is involved in contracture reversal and repair of cardiac contractile function [9,10,17]. In view of the above-described facts, the major and essentially new finding is the fact that previous adaptation to stress by exposure to repeated stress considerably increases the rate of restoration of the myocardial CP level during postischemic perfusion. In the interval between 10 and 45 min of reperfusion the myocardial CP level of the adapted animals was about twice as high as in the control animals. The curves presented in Fig. 2 make it possible to compare the NMR spectra recorded for the hearts of control and adapted animals in the interval between 15 and 20 min of reperfusion. Note that for equal standard signals the peak signal found in adapted animals reflecting myocardial CP is considerably higher than in the control, as shown in Table 1. The curves in Fig. 3 *a, b* allow us to compare the dynamics of myocardial CP

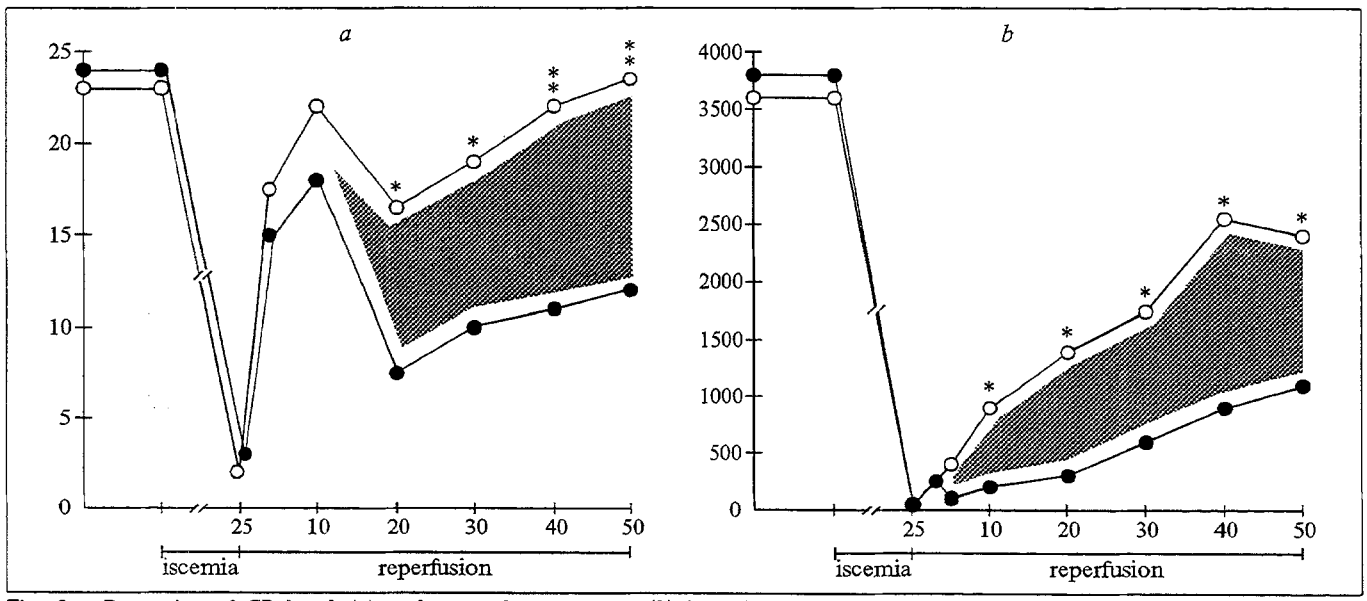

Fig. 3. Dynamics of CP level (*a*) and rate of contraction (*b*) in ischemia and subsequent reperfusion.

(*a*) and heart rate (*b*) in ischemia with subsequent reperfusion. It can be seen that adaptation to stress markedly increases the myocardial CP restoration rate and, to a still higher degree, the heart rate, that is, the key changes responsible for cardiac function repair as a whole. In light of the above-described concept of the structure adaptational stabilization phenomenon, adaptation to repeated stress is presumably involved in the accelerated return to normal of the myocardial CP level during postischemic reperfusion due to a greater structural stability or in other words, a greater resistance of the oxidative phosphorylation system in the mitochondria - ATP-translocase and creatine kinase - to the ischemic insult. In reperfusion this results in a faster reactivation of these enzymes and hence in a more rapid normalization of the myocardial CP level.

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