Adaptation to Hypoxia, Unlike Adaptation to Stress, Fails to Protect the Isolated Heart from Reperfusion After Total Ischemia (an NMR Study)

L. Yu. Golubeva, S. N. Dvoryantsev, A. N. Khatkevich, I. Yu. Malyshev, and F. Z. Meerson

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Effects of adaptation to hypoxia on the contractility of isolated rat hearts and on their levels of ATP and inorganic phosphate after total ischemia were evaluated. This adaptation failed to render the cardiac energy-supplying system more resistant to postischemic reperfusion and thus did not accelerate the restoration of cardiac contractility after ischemia. The results of adaptation to hypoxia were then compared with those of adaptation to stress, which had been shown to bring about a marked increase in cardiac resistance to postischemic reperfusion. It is concluded that the profound differences noted between the cardioprotective effects of these two forms of adaptation are due to a much greater accumulation of stabilizing proteins from the HSP70 family during adaptation to stress.

Key Words: *stress; adaptation; heart*

Adaptation to stress and adaptation to hypoxia are outstanding examples of the organism's long-term adaptation to the environment, producing a wide range of protective effects. As far as the heart is concerned, adaptation to stress increases its resistance to arrhythmias caused by ischemia and reperfusion [7] and mitigates the disturbances of its electrical stability and contractile function occurring in myocardial infarction [4] and postinfarction cardiosclerosis [3]. The cardioprotective effects of such adaptation have been shown to be due to the activation of stress-limiting systems at the central level [7] and to the phenomenon of adaptive stabilization of structures (ASS) at the cardiac level [6]. The ASS phenomenon, which is a cellular component of adaptation, renders the major cellular structures such as the sarcolemma, sarcoplasmic reticulum, mitochondria, and nuclei more resistant to injurious factors. A key role in the development of this phenomenon is played by heat shock proteins from the HSP70 family [6]. In cells of adapted animals, these proteins stabilize cellular structures by breaking down the aggregates composed of damaged proteins [10]. Two striking manifestations of the ASS phenomenon are the much faster return to normal of the ATP concentration and the less marked depression of contractility observed during postischemic reperfusion of hearts isolated from stress-adapted animals [5].

Adaptation to hypoxia, too, activates the central stress-limiting systems [7] and can produce cardioprotective effects *in vivo* similar to those seen with adaptation to stress; thus, it increases the resistance of the heart to the damaging actions of ischemia and reperfusion [1,9]. The question arises as to whether adaptation to hypoxia can also exert protective effects at the level of the iso-

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences; Institute of Experimental Cardiology, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow (Presented by N. R. Paleev, Member of the Russian Academy of Medical Sciences)

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lated heart, or, in other words, whether this form of adaptation can give rise to the ASS phenomenon elicited by adaptation to stress.

Accordingly, the two objectives of the present study were, first, to evaluate the influence of adaptation to hypoxia on the contractility of an isolated heart and on its levels of ATP and inorganic phosphate (P_i) during total ischemia and subsequent reperfusion; and, second, to compare the results with those we obtained previously under similar conditions for hearts isolated from animals adapted to stress.

MATERIALS AND METHODS

Male Wistar rats weighing 350-400 g were used. They were adapted to intermittent hypoxia in a hypobaric chamber at an "altitude" of 4000 m 4 h daily for a total of 30 days. Unadapted rats served as controls. The isolated hearts of all rats were perfused, using the Langendorff method, with Krebs-Henseleit's solution at a constant flow rate (11-12 ml/min) and a constant pressure (90 mm Hg); the solution was aerated with a mixture of 95% O_2 and 5% CO_2. The hearts were contracting spontaneously, and their contractile activity was evaluated by means of a latex balloon placed in the left ventricle and connected via a sensor to a Gould-2 2400S automatic recorder; left ventricular pressure, contraction rate, relaxation rate, and end-diastolic pressure were calculated. Cardiac tissue levels of ATP and P_i were measured by nuclear magnetic resonance (NMR) [2]. ^{31}P-NMR spectra were recorded with a CXP-200 NMR spectrometer (Bruker) after its magnet had been placed in the heart, and ATP and P_i concentrations were then estimated from the areas of the peaks in these spectra.

Postischemic reperfusion was simulated by restoring the solution flow through the heart after 20 min of total ischemia (complete absence of flow). The significance of intergroup differences was estimated by Student's test.

RESULTS

Variations in the parameters of cardiac contractility (developed pressure, end-diastolic pressure, and contraction and relaxation rates) during the ischemia and 30-minute reperfusion are shown in Fig. 1. It can be seen that the two groups did not differ in any of these parameters either initially or during the 20-minute period of ischemia; by the end of this period, the developed pressure and contraction and relaxation rates had fallen to zero, while the end-diastolic pressure had risen as a result of cardiac muscle contracture to approximately the same level (≈50 mm Hg) in both groups.

The two groups also showed similar variations in the parameters of cardiac contractility during the 30-minute reperfusion period. Although the developed pressure, contraction rate, and relaxation rate had risen by the end of this period to 42-44% of their initial levels in the control group and to 60-62% in the hypoxia-adapted group, these differences were insignificant; the intergroup difference in end-diastolic pressure by minute 30 of reperfusion was also insignificant (55 and 48 mm Hg in the control and adapted groups, respectively, *vs.* the initial 11 mm Hg in both groups).

Nor did the two groups differ significantly in ATP or P_i levels before or during the periods of ischemia and reperfusion (Table 1). Thus, we see that ATP levels in the cardiac myocytes of both groups were similar before ischemia (18-20 μmol/g dry tissue weight), dropped approximately threefold during ischemia, and then rose to about 8-9 μmol/g dry tissue weight over the 30-minute reperfusion period. Accumulation of P_i in the myocardium dur-

TABLE 1. Effects of Adaptation to Intermittent Hypoxia on ATP and P_i Levels (μmol/g Dry Weight) in Isolated Rat Hearts during Ischemia and Reperfusion

Time	ATP		P_i	
	control hearts (n=8)	adapted hearts (n=9)	control hearts (n=8)	adapted hearts (n=9)
Before ischemia	21.3±3.7	17.6±1.7	13.3±1.4	11.5±1.7
Ischemia, minutes:				
5	18.7±3.6	13.2±1.3	21.9±4.9	19.7±2.2
10	14.2±2.3	11.1±1.4	43.6±3.0	41.6±2.9
15	9.7±2.6	4.8±0.8	55.4±2.7	57.2±3.5
20	6.3±1.7	5.2±0.7	72.6±5.6	65.3±5.5
Reperfusion, minutes:				
5	9.3±2.1	7.5±0.7	20.2±2.4	18.3±3.4
10	7.7±1.4	7.4±0.9	25.4±2.3	19.7±2.4
20	9.2±1.7	8.0±0.9	18.5±1.9	17.6±0.9
30	8.2±1.7	9.1±1.3	14.2±0.9	11.3±1.1

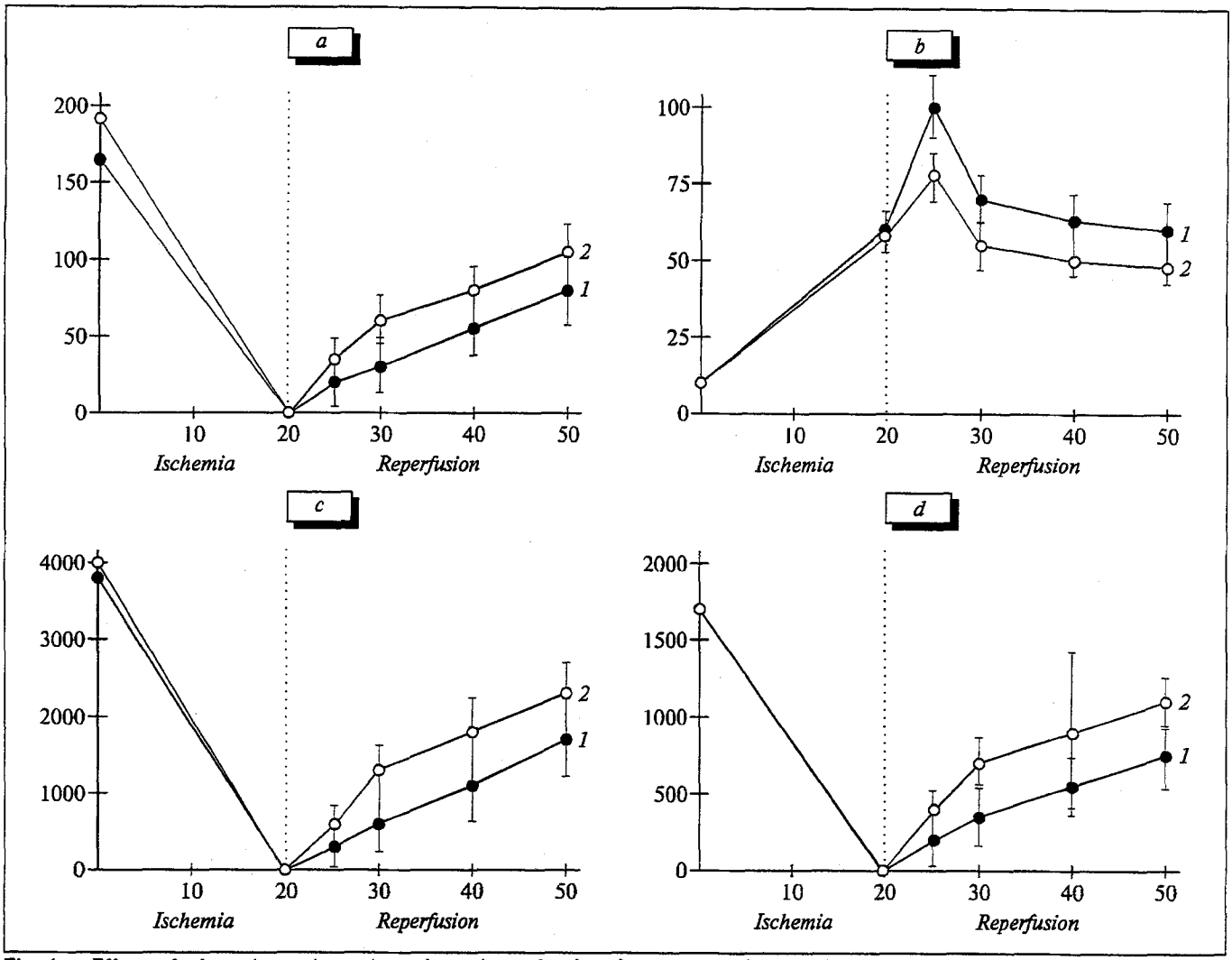

Fig. 1. Effects of adaptation to intermittent hypoxia on developed pressure, end–diastolic pressure, contraction rate, and relaxation rate in isolated rat hearts. Abscissa: time, min. Ordinate: *a*) developed pressure, mm Hg; *b*) end–diastolic pressure, mm Hg; *c*) contraction rate, mm Hg/sec; *d*) relaxation rate, mm Hg/sec. *1*) control hearts ($n=8$); *2*) adapted hearts ($n=9$).

ing ischemia points to intensified breakdown of adenine nucleotides. In our study, the 20-minute ischemia resulted in an approximately fivefold rise of P_i in the cardiac myocytes of the control and adapted groups, indicating that the pool of adenine nucleotides was considerably depleted in the absence of oxygen. When reperfusion was started, P_i dropped precipitously during the first 5 min and reached its initial level by minute 30 in both groups.

The adaptation to intermittent hypoxia thus failed to render the energy-supplying system of the myocytes of an isolated heart more resistant to ischemia and, consequently, did not speed up the restoration of cardiac contractile function during postischemic reperfusion.

To better understand the reasons for the failure of such adaptation to protect the isolated heart, it is useful to compare the present results with those obtained in our previous study with isolated hearts, where adaptation to stress afforded good protection against reperfusion-induced damage [5]. It is important to note that the protective effect of adaptation to stress was achieved in hearts containing higher ATP levels than the control (unadapted) hearts. Hence a plausible explanation for the failure of adaptation to hypoxia to protect the heart is that this form of adaptation, unlike adaptation to stress, does not lead to the formation of a mechanism by which the energy-supplying system is protected against the injurious factors acting during ischemia and reperfusion. In evaluating this adaptive mechanism, one should bear in mind the key role of intracellular calcium excess in the depletion of ATP stores in the course of ischemia and reperfusion. The ATP level may by lowered by at least three mechanisms that are activated by Ca^{2+} excess. These are: 1) excessive activation of Ca^{2+}-dependent ATPases; 2) activation of proteases whose targets may

then become the mitochondrial enzymes of oxidative phosphorylation; and 3) abnormal functioning of mitochondria as a result of Ca^{2+} absorption by these organelles [8]. In view of this, it should be appreciated that adaptation to stress, unlike adaptation to hypoxia, almost completely prevents the exit of Ca^{2+} to the myocardial homogenate from the sarcoplasmic reticulum and mitochondria in the process of its prolonged storage [7], effectively precludes falls in the rates of oxygen consumption by and phosphorylation in mitochondria during autolysis [6], and enables the Ca^{2+}-transporting systems of the sarcoplasmic reticulum to function more reliably in the face of injurious agents [7]. This implies that the adaptation to stress leads, on the one hand, to increased stability of membrane structures and of mitochondria where ATP is synthesized, and, on the other hand, to a greater reliability of the Ca^{2+}-transporting systems of the sarcoplasmic reticulum, which provides the main mechanism limiting the intracellular calcium overload. Clearly, these adaptive shifts can both play an important role in the accelerated replenishment of ATP stores and restoration of contractile function during reperfusion.

The effects just mentioned are not produced by adaptation to hypoxia, which by itself accounts for the failure of such adaptation to ensure accelerated restoration of ATP levels and contractile activity during reperfusion. Of considerable interest in this context is the question of why this form of adaptation is not accompanied by the increase in the stability of cellular structures that occurs during adaptation to stress. One possible answer lies in the ASS phenomenon and the important role played by the protective effects of HSP70 in its mechanisms. As shown previously, five isoforms of these proteins accumulate in large quantities in the myocardium during adaptation to stress, whereas only two isoforms accumulate there, and in small amounts, during adaptation to hypoxia [6]. The adaptation to stress presumably results in accelerated restoration of ATP stores during postischemic reperfusion because the membranes and calcium pump of the sarcoplasmic reticulum as well as the mitochondrial membranes and oxidative phosphorylation enzymes involved in ATP synthesis (ATP translocase and creatine kinase) are rendered, as a result of HSP70 accumulation, more resistant to the reperfusion shock. In contrast, in the course of adaptation to hypoxia these proteins accumulate in very minor amounts and thus cannot afford protection to the energy-supplying system and contractile function. In this form of adaptation, therefore, the ASS phenomenon is virtually absent and the cardioprotective effects observed at the organismic level are attributable to activation of the central stress-limiting systems, enlargement of the coronary vascular bed, and increased delivery of oxygen and oxidation substrates [7]. Obviously, none of these mechanisms can operate in a totally ischemic isolated heart, as was used in the present study.

It may be concluded that, due to the profound differences between the cardioprotective mechanisms exerted by the two forms of adaptation considered here, the combined use of these forms may prove an extremely effective means of protecting the heart.

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REFERENCES

1. Yu. N. Kopylov and L. Yu. Golubeva, *Byull. Eksp. Biol. Med.*, **111**, № 1, 27-30 (1991).
2. V. V. Kupriyanov, A. Ya. Shteinshneider, V. L. Lakomkin, *et al.*, in: *Myocardial Metabolism* [in Russian], Moscow (1988), pp. 81-118.
3. F. Z. Meerson, L. M. Belkina, and S. S. Dyusenov, *Byull. Eksp. Biol. Med.*, **102**, № 11, 536-538 (1986).
4. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, *et al.*, *Kardiologiya*, № 8, 19-24 (1986).
5. F. Z. Meerson, L. Yu. Golubeva, V. L. Lakomkin, *et al.*, *Byull. Eksp. Biol. Med.*, **115**, № 6, 569-572 (1993).
6. F. Z. Meerson and I. Yu. Malyshev, in: *The Phenomenon of Adaptive Stabilization of Structures, and Protection of the Heart* [in Russian], Moscow (1993), p. 158.
7. F. Z. Meerson, *Adaptive Protection of the Heart: Protecting against Stress and Ischemic Damage*, Boca Raton (1991).
8. W. G. Nayler and M. J. Daly, in: *Physiology and Pathophysiology of the Heart*, Boston (1984), pp. 240-257.
9. B. Ostadal, F. Kolar, V. Pelouch, *et al.*, *Biomed. Biochim. Acta*, **48**, 558-562 (1989).
10. H. R. B. Pelham, *Cell*, **30**, 517-528 (1986).