HIGH MYOCARDIAL MITOCHONDRIAL CREATINE KINASE ACTIVITY DURING ISCHEMIA IS COMBINED WITH IMPAIRED RECOVERY OF CONTRACTILITY AFTER REPERFUSION

V. I. Veksler, A. N. Khatkevich, and V. I. Kapel'ko

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Restoration of the coronary blood flow after a period of ischemia, in the case of operations on the heart, for example, is often accompanied by incomplete recovery of myocardial contractility [8, 10]. The incomplete recovery of the tissue ATP level observed under these circumstances has suggested that the cause may be a disturbance of mitochondrial function [6, 7].

It is difficult to interpret data on the state of the mitochondria after ischemia because the process of isolation of these organelles may itself have some effect on them. By means of a method recently developed [2, 13] the function of mitochondria can be studied without isolating them from the tissue in saponin-treated biopsy specimens of the myocardium, and this also enables the dynamics of mitochondrial function from each heart to be studied during ischemia and reperfusion. By means of this method it is also possible to evaluate the functional state of mitochondrial creatine kinase, an enzyme to which an important role has been ascribed in energy transport in heart cells [5, 12]. In the investigation described below the dynamics of mitochondrial function was compared with changes in contractility of the heart.

EXPERIMENTAL METHOD

Isolated hearts of guinea pigs anesthetized with urethane (1.6 g/kg) were perfused through the aorta at 37°C and with a constant coronary flow of 17 ml/min. The perfusion fluid consisted of Krebs — Henseleit solution, containing 118 mM NaCl, 4.7 mM KCl, 3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 0.5 mM EDTA, and 11 mM glucose, pH 7.36, after saturation with carbogen. The isovolumic pressure in the left ventricle was measured by means of a latex balloon introduced into its lumen and connected to a "Gould Statham P23 Db" pressure transducer. The results were recorded on a "Gould 2400" automatic writer. The end-diastolic pressure was set at 12-14 mm Hg. Cardiac function was assessed by the developed pressure (the difference between the systolic and diastolic pressures).

Cardiac activity was arrested after perfusion with Krebs—Henseleit solution for 45-50 min by perfusing for 3 min with cardioplegic solution. The perfusion was then stopped for 45 min, after which reperfusion was carried out with ordinary Krebs—Henseleit solution. In the control group (n = 14) a cardioplegic solution of the following composition was used: 135 mM NaCl, 20 mM KCl, 1.5 mM $CaCl_2$, 2.4 mM $MgSO_4$, 15 mM Tris, pH 7.5. The cardioplegic solution in the other group also contained substances used in clinical practice to protect the myocardium [9, 11]: 10 mM disodium salt of creatine phosphate and 15 mM glutamic acid (the PG group). The NaCl concentration in this solution was lowered to 115 mM so that $[Na^+]$ was the same as in the control.

Biopsy specimens from the myocardium, weighing 6-8 mg, were taken from the subepicardium of the left ventricle immediately before cardioplegic arrest, at the end of the ischemic period, and 30 min after the beginning of reperfusion. Parameters of mitochondrial respiration in the biopsy material were determined by the method described previously [2, 13].

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TABLE 1. Parameters of Rate of Mitochondrial Respiration (ng-atoms O_2/min/mg dry weight) in Saponin-Treated Myocardial Biopsy Specimens

Group	V_0	V_{ADP}	V_{cr}	V_{max}	V_{max}/V_0
Before ischemia					
Control	4,59±0,22	6,90±0,30	9,82±0,59	21,3±1,2	4,75±0,33
PG	4,22±0,43	7,23±0,59	10,06±0,92	22,6±1,8	5,53±0,38
After ischemia					
Control	4,46±0,22	6,44±0,32	9,34±0,66	20,7±1,0	4,74±0,26
PG	4,17±0,38	6,46±0,50	9,32±0,54	21,2±1,7	5,17±0,27
After reperfusion					
Control	3,93±0,35	5,65±0,41	7,99±0,59	17,3±1,3	4,54±0,21
PG	3,25±0,24	5,59±0,31	8,15±0,49	18,8±1,2	5,87±0,32

Legend. Here and in Table 2 V_0 denotes rate of mitochondrial respiration in medium without ADP, V_{ADP} — in the presence of 100 μM ADP, V_{cr} — in the presence of 100 μA ADP + 20 mM creatine, V_{max} — in the presence of 1 mM ADP + 20 mM creatine. *$p < 0.05$ compared with control.

TABLE 2. Coefficients of Correlation between Preischemic Respiratory Parameters of Mitochondria and also between Relative Changes in these Parameters and Degree of Recovery of Developed Pressure (DRDP)

	Respiratory parameters					
	V_{cr}	V_{max}	V_{max}/V_0	$V_{cr}-V_0$	$V_{cr}-V_{ADP}$	$V_{max}-V_0$
			Preischemic			
DRDP	—0,54**	—0,48*	—0,34	—0,57**	—0,61**	—0,50**
			Postischemic/preischemic			
DRDP	0,70***	0,65***	0,52**	0,82***	0,65***	0,69***
			Post reperfusion/preischemic			
DRDP	0,44*	0,45*	0,61**	0,61**	0,59**	0.52**

Legend. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ — significant correlations.

EXPERIMENTAL RESULTS

Values of the developed pressure (91-113 mm Hg) and heart rate (3.0-2.8 Hz) before cardioplegic arrest in both groups agreed with data published previously for the same object [4]. There were likewise no significant differences between the groups as regards the velocity of mitochondrial respiration, measured successively in the absence of adenine nucleotides (V_0), in the presence of a low (100 μM) ADP concentration (VADp), after addition of 20 mM creatine (V_{cr}), and after maximal stimulation of respiration by the addition of 1 mM ADP (V_{max}) (Table 1). Values of the acceptor control likewise did not differ significantly (V_{max}/V_0).

Contractions of the heart ceased a few seconds after the beginning of perfusion with the cardioplegic solution. Toward the end of the ischemic period the left ventricular pressure was rather higher in the control group than in the PG group (20 ± 4 and 14 ± 3 mm Hg respectively); the absolute values corresponded to observations indicating development of weak ischemic contracture in guinea pigs' hearts [4]. The parameters of mitochondrial respiration at the end of ischemia did not differ significantly from their initial values (Table 1).

After reperfusion for 30 min recovery of contractility in both groups was incomplete. Thus the degree of recovery of developed pressure (DRDP) amounted to 40 ± 5 and 45 ± 4% in the control and PG groups respectively. Meanwhile parameters of mitochondrial respiration did not change significantly (Table 1). There were no significant differences between the groups with the exception of higher acceptor control in the PG group.

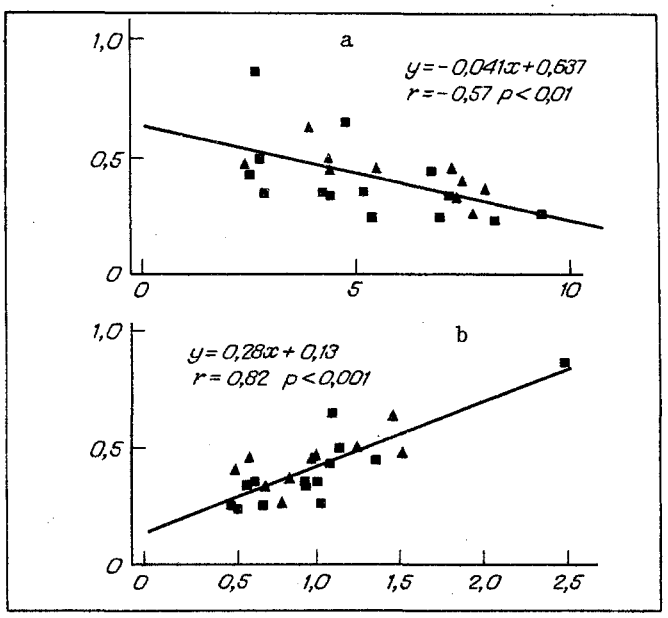

Fig. 1. Correlation between preischemic (creatine + 100 μM ADP)-stimulated mitochondrial respiration and DRDP (a) and correlation between relative changes (creatine + 100 μM ADP)-stimulated respiration during period of ischemia and DRDP (b). Abscissa: a) degree of stimulation of respiration by creatine + 100 μM ADP ($V_{cr} - V_0$) (in ng-atoms O/min/mg dry weight), b) ratio of postischemic value ($V_{cr} - V_0$) to initial value; ordinate, DRDP. Squares indicate control, triangles — PG group.

The absence of any marked effect in the PG group by contrast with results obtained previously [1, 3] could be due both to the longer duration of the ischemia and to species differences. Meanwhile, this enabled the data for both groups to be pooled in order to study correlation between the preischemic mitochondrial parameters and DRDP after reperfusion (Table 2). Nearly all the respiratory parameters correlated negatively with DRDP. Particularly close correlation was demonstrated by parameters of creatine stimulated respiration (Fig. 1), reflecting functional activity of mitochondrial creatine kinase.

Relative changes in the mitochondrial respiratory characteristics after ischemia (the ratio of values measured before reperfusion and the initial values) were very closely correlated with DRDP (Table 2). Here also the highest coefficients of correlation were demonstrated by parameters connected with mitochondrial creatine kinase function (Fig. 1). Positive correlation, although less close, also was observed between relative changes in respiratory parameters after reperfusion and DRDP (Table 2).

The results of the investigation showed that incomplete recovery of contractile activity of the heart in the postischemic period may be observed under conditions of almost total preservation of mitochondrial respiration and of its regulation. This is evidence against the hypothesis of the leading role of mitochondrial lesions in disturbances of contractility after ischemia. Moreover, other conditions being the same, hearts with a high initial level of mitochondrial function (and in particular, of mitochondrial creatine kinase) were found to be more sensitive to ischemia. Investigation of mitochondrial function in myocardial biopsy specimens before ischemia can evidently give useful information on the prognosis of recovery of contractility of the heart after reperfusion.

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EFFECT OF ADAPTATION TO INTERMITTENT HYPOXIA ON ELECTRICAL ACTIVITY OF CARDIOMYOCYTES OF THE ISOLATED HEART DURING ISCHEMIA AND REPERFUSION

V. I. Vovk and F. Z. Meerson

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Adaptation to hypoxia not only makes the energy supply to the heart more efficient through an increase in the coronary blood flow [8], an increase in the myoglobin concentration [12], and other metabolic shifts, but also limits adrenergic stressor influences on the heart [1]. As a result of these effects, adaptation to hypoxia has proved to be a powerful antiarrhythmic factor in acute ischemia [5], myocardial infarction [6], and postinfarction cardiosclerosis [4], and also in neurocirculatory dystonia [3]. However, this antiarrhythmic effect of adaptation has not been sufficiently fully explained until very recently, since the effect of adaptation to intermittent hypoxia on electrical activity of the cardiomyocytes has not been studied during ischemia and reperfusion.

The aim of this investigation was to assess the effect of adaptation to intermittent hypoxia on electrical activity of the cardiomyocytes of the isolated heart successively under aerobic conditions during total ischemia, and during subsequent reperfusion.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated heart of male Wistar rats weighing 320-380 g. Adaptation to intermittent hypobaric hypoxia was carried out in a pressure chamber at an altitude of 4000 m for 5 h daily. The course of adaptation consisted of 40 sessions of hypoxia. Contractility and parameters of electrical activity were studied on the isolated heart, perfused by Langendorff's method, as described previously [2], using a TD-112S isotonic transducer and specialized modules of the PM-6000 polygraph (Nihon Kohden, Japan). The transmembrane potential of the cardiomyocytes on the subepicardial surface of the left ventricle was recorded by means of floating microelectrodes [9, 14], filled with

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