EFFECT OF CHANGES IN RHEOLOGIC PROPERTIES OF THE BLOOD ON MYOCARDIAL ADAPTATION

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UDC 612.172.1:612.117.7

Changes in the rheologic properties of the blood were induced in rabbits by intravenous injection of high-molecular-weight dextran and of lysine-vasopressin. The rate of uptake of oxygen by the mitochondria of the heart during oxidation of 2.5-10 mmoles succinate was 90-120% greater than in the control. The degree of stimulation of respiration by ADP was reduced by 1.5-2 times. The simultaneous administration of succinate with glutamic acid during disturbance of the microcirculation restored the normal levels of respiration and phosphorylation. The development of inhibition of succinate dehydrogenase (SD) by oxaloacetic acid is postulated. Switching respiration to succinic acid and limitation of SD activity can be regarded as adaptive factors to changes in the rheologic properties of the blood, aimed at maintaining cardiac activity, a view supported by the absence of changes in ATPase activity and in the myosin content in the heart.

Key words: microcirculation and its disturbance; mitochondria of the rabbit heart; oxidative phosphorylation; ATPase; succinate dehydrogenase.

The writers showed previously [6, 8] that disturbances of the microcirculation by a change in the rheologic properties of the blood lowered the ATP concentration in the heart tissue but had no effect on the level of glycolysis in it. Under these conditions a decrease in oxidative phosphorylation was found in the mitochondria of the heart during utilization of NAD-dependent substrates and in the activity of certain enzymes of the Krebs cycle [9]. The ECG showed characteristic findings of hypoxia (sinus bradycardia, arrhythmia, enlargement of the T wave) [7].

In this investigation respiration and oxidative phosphorylation in the mitochondria of the heart were studied by the use of succinate as the oxidation substrate, for in certain states requiring increased energy production there is a shift in the oxidation system [2, 13] to the preferential formation and use of succinic acid. The possibility of controlling succinate dehydrogenase (SD) activity by oxaloacetic acid also was examined and the ATPase activity of myosin, as an indicator of the contractile activity of the myocardium, was studied during a disturbance of the microcirculation.

EXPERIMENTAL METHOD

Experiments were carried out on a model reproducing changes in the rheologic properties of the blood by intravenous injection of high-molecular-weight dextran (mol. wt. 500,000) in a dose of 1 g/kg body weight and of lysine-vasopressin in a dose of 5 pressor units/kg. The animals used were 34 Chinchilla rabbits. Before and after injection of the substances the ECG of all the animals was recorded for 1 h. The heart was perfused with ice-cold 0.15 M KCl solution. The mitochondria were isolated by differential centrifugation in 0.25 M sucrose with 0.01 M EDTA, pH 7.4. Oxidative phosphorylation was measured by a polarographic method, using a stationary platinum electrode. The composition of the incubation medium was 0.03 M K_2HPO_4, 0.05 M KCl, 0.12 M sucrose, and 0.24 μM ADP. The protein content of the sample was

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 3, pp. 29-33, March, 1975. Original article submitted April 2, 1974.

TABLE 1. Oxygen Uptake by Mitochondria of the Heart during Disturbance of the Microcirculation (M ± m)

Concentration of substrate Na succinate (in mM)	V_1	V_2	$\frac{V_2}{V_1}$ (in%)	V_3	$\frac{V_3}{V_2}$ (in%)	V_4	RC	$\frac{ADP}{O}$	DNP ($3 \cdot 10^{-5}$M)	Number of experiments
					Normal					
10	0,54± 0,04	1,10± 0,06	100,0± 3,7	2,93± 0,15	163,0± 1,9	1,4± 0,09	2,08± 0,06	1,78± 0,07	3,16± 0,23	12
5	0,55± 0,02	1,19± 0,04	116,5± 5,3	2,47± 0,21	108,1± 2,4	1,31± 0,015	1,9± 0,07	1,9± 0,1	3,09± 0,4	10
2,5	0,55± 0,04	1,09± 0,025	83,3± 3,1	1,71± 0,17	54,5± 0,9	1,1± 0,01	1,50± 0,035	1,8± 0,07	1,9± 0,25	12
					Disturbance of microcirculation					
10	0,46± 0,02	1,4± 0,05	206,3± 4,2	2,39± 0,12	71,3± 1,6	1,5± 0,023	1,67± 0,03	—	2,3± 0,20	9
P		0,05	0,01	0,05	0,05		0,05		0,02	
5	0,45± 0,013	1,15± 0,03	155,5± 1,21	1,76± 0,09	53,6± 1,7	1,5± 0,024	1,17± 0,11	—	1,98± 0,17	13
P				<0,02	<0,05		<0,05		<0,01	
2,5	0,48± 0,011	1,26± 0,02	162,5± 5,0	1,55± 0,04	19,3± 0,87	1,28± 0,048	1,21± 0,1	—	1,88± 0,22	8
P		<0,5	<0,02	<0,05	<0,01		<0,05			

Legend. Level of significance (P) calculated relative to normal. Here and in Table 2 rate of respiration expressed in microatoms O_2/sec/g mitochondrial protein.

TABLE 2. Oxygen Uptake by Mitochondria of the Heart during a Disturbance of the Microcirculation (M ± m)

Concentration of substrate (succinate + glutamate) (in mM)	V_1	$\frac{V_2}{V_1}$ (Table 1) (in%)	V_3	$\frac{V_3}{V_2}$ (Table 1) (in%)	$\frac{V_3}{V_2}$ (in%)	V_4	RC	Number of experiments
				Normal				
Succinate 10 + glutamate 10	1,13± 0,053	0	2,85± 0,09	162,5± 3,1	162,7± 1,7	1,4± 0,09	2,03± 0,05	12
Succinate 5 + glutamate 5	1,20± 0,07	0	2,50± 0,12	108,2± 1,5	103,0± 1,8	1,3± 0,05	1,85± 0,04	10
Succinate 2.5 + glutamate 10	1,23± 0,02	12,8± 1,0	1,66± 0,04	45,4± 2,0	33,3±2,4	1,1± 0,02	1,5± 0,03	12
				Disturbance of microcirculation				
Succinate 10 + glutamate 10	1,94± 0,04	38,5± 1,3	3,4± 0,09	142,8± 0,5	78,8± 0,9	1,5± 0,02	2,2± 0,07	9
P_1	<0,01	<0,02	<0,05					
P_2				<0,05			<0,05	
Succinate 5 + glutamate 5	1,48± 0,055	36,3± 1,8	2,58± 0,07	136,2± 0,85	44,0± 1,2	1,5± 0,06	1,7± 0,03	13
P_1	0,05	0,05						
P_2			<0,02	<0,05			<0,05	
Succinate 2.5 + glutamate 10	1,4± 0,02	12,6± 0,8	2,02± 0,1	66,6± 2,1	42,8± 2,7	1,28± 0,02	1,56± 2,2	
P_1			<0,5					
P_2			<0,05	<0,05			<0,05	

Legend: P_1) level of significance calculated relative to normal; P_2) level of significance calculated relative to experiments with succinate alone during a disturbance of the microcirculation.

1.5-3 mg. Protein was determined by Lowry's method [11, 12]. Sodium succinate (2.5, 5, or 10 mM) with rotenone (4 μM) and glutamic acid (5 or 10 mM) was used as the substrate. The characteristics of the metabolic state of the mitochondria corresponded to those given in the literature: endogenous respiration of the mitochondria (V_1), rate of uptake of oxygen in the presence of exogenous substrate (V_2), rate of respiration in the actively phosphorylating state in the presence of substrate and ADP (V_3), and rate of respiration in the controlled state after exhaustion of the added ADP (V_4). The ratio V_3/V_4 expresses the degree of respiratory control (RC) and reflects the degree of phosphorylation [14].

Myosin was isolated and its ATPase activity determined from the increase in inorganic phosphorus during incubation (37°C, 10 min) [11]. The numerical results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS

The rate of endogenous respiration in the mitochondria obtained from the heart 1 h after intravenous injection of high-molecular-weight dextran and vasopressin was 18.5% below normal (Table 1). The rate of oxygen uptake on the addition of 10, 5, and 2.5 mM succinate was increased by 120, 30, and 96% respectively (Table 1). This high level of respiration was evidently connected both with the increase in the succinate concentration and with an increase in SD activity. Both these factors, as previous investigations [2, 4, 13] showed, are considerably enhanced under the conditions of hypoxia that developed in these experiments in association with a change in the rheologic properties of the blood and disturbance of the microcirculation [6, 7].

However, despite the high level of respiration of the mitochondria with succinate, the degree of stimulation of respiration by ADP in a succinate concentration of 2.5-10 mM was 35-45% below normal (Table 1). RC also fell correspondingly. The results suggest that oxaloacetic acid has an inhibitory action on SDH activity [1, 2, 10].

To verify the results and to evaluate them quantitatively a series of experiments was carried out to determine the rate of oxygen uptake on the addition of glutamate to the succinate. Glutamate abolishes the inhibitory action of oxaloacetic acid of SD and participates in succinic acid formation. Under normal conditions the addition of glutamic acid (5-10 mM) to the succinate (5-10 mM) did not affect the rate of oxygen uptake by the mitochondria of the heart either in state V_2 (Tables 1 and 2) or in the actively phosphorylating state V_3 (Tables 1 and 2). With a change in the rheologic properties of the blood and a consequent disturbance of the microcirculation, the addition of glutamic acid led to an increase in the rate of oxygen uptake in the V_2 state by 38.5 and 36.3% when 10 and 5 mM succinate respectively were used as the oxidation substrate (Table 2). The stimulant effect of ADP increased under these conditions (relative to the rate of oxygen uptake with succinate alone) up to normal limits with all succinate concentrations used (Table 2). RC also was increased. Addition of 10 mM malonate, inhibiting SD, completely inhibited oxidation of the substrate.

Determination of the ATPase activity of the myosin isolated from the heart after administration of vasopressin and dextran caused no deviation from the normal level whether activated by Ca^{++} (from 1 to 10 mM) or by Mg^{++}.

The results showed that a brief (1 h) disturbance of the microcirculation through a change in the rheologic properties of the blood leads to increased utilization of succinate. The change to oxidation predominantly of succinate may provide the metabolic basis for repair processes by maintaining cardiac activity. This conclusion is confirmed by the unchanged level of the ATPase activity of the myosin. The decrease in the stimulant action of ADP on mitochondrial respiration discovered during a disturbance of the rheologic properties of the blood and the change of this effect into inhibitory are evidence of the inhibition of SD activity by oxaloacetic acid. This inhibition by oxaloacetic acid is regarded [1, 2, 3, 10] as a factor controlling the respiration rate in the presence of an energy deficiency in the mitochondrial respiratory chain.

During a short disturbance of the microcirculation in the mitochondria of the heart adaptive processes thus arise and are manifested as a switch of respiration from NAD-dependent substrates to the FAD-dependent substrate succinic acid, as "protective" inhibition of SD activity, and as ability to synthesize succinate from glutamate.

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