DISTURBANCE OF Ca^{++}-TRANSPORT FUNCTION AND LIPID COMPONENT OF SARCOPLASMIC RETICULUM MEMBRANES DURING TOTAL ISCHEMIA OF THE RAT MYOCARDIUM

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In myocardial ischemia the contractile function of the heart is quickly disturbed. This is due to several factors, including injury to membranes of the sarcoplasmic reticulum (SR) [13]. Among the basic mechanisms of membrane damage an essential role is ascribed to activation of lipid peroxidation (LPO) and of phospholipolysis. Experiments in vitro have shown that phospholipase A_2 [10] and the xanthine–xanthine oxidase oxygen radical generation system [12] inhibit calcium uptake by SR. Some investigators consider that the dominant role in damage to the Ca^{++}-transport function of SR in ischemia is played by activation of LPO [4]. Activation of LPO in the liver microsomes during ischemia was observed much earlier than accumulation of lysophospholipids [2]. The determinant role of phospholipases in ischemic damage to the liver mitochondria [3] and an increase in the content of lysophospholipids even in the initial period of cardiac ischemia [8] have been demonstrated.

The aim of this investigation was to study the role of LPO and of phospholipolysis in the disturbance of the Ca^{++}- transport function of SR, depending on the duration of myocardial ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170-200 g. Total ischemia was created by keeping the animals' heart in 0.9% NaCl at 37°C for 15, 30, and 60 min. The control and ischemic hearts were forced through a press and homogenized in 10 volumes of isolated medium, containing 100 mM KCl, 5 mM sodium azide, and 40 mM Tris-HCl (pH 7.4), with the aid of a Potter's homogenizer at 2000 rpm for 1 min. One part of the homogenates, intended for determination of the intensity of LPO and concentrations of phospholipids, was frozen in liquid nitrogen, while the other was centrifuged at 8000g for 20 min. The supernatant was then centrifuged at 52,000g for 50 min. The residue was suspended in 20 volumes (compared with the initial weight of the heart tissue sample) of rinsing medium, containing 600 mM KCl, 5 mM sodium azide, and 20 mM Tris-HCl (pH 7.2), and centrifuged for 50 min at 52,000g. The residue of SR was suspended in keeping medium, containing 20% sucrose, 100 mM KCl, 5 mM sodium azide, and 40 mM Tris-HCl (pH 7.0) and kept at the temperature of liquid nitrogen. The homogenate and SR were heated in a water bath at 37°C for 1 min. The rate of Ca^{++} uptake was measured by means of a Ca^{++}-selective electrode (model 9320, from Orion, France). The reaction medium contained 100 mM KCl, 6 mM $MgCl_2$, 5 mM sodium azide, 5 mM ATP, 25 μM $CaCl_2$, 15 mM potassium oxalate, and 25 mM imidazole (pH 7.0). Phospholipids were extracted by the method in [10] and the extract was partially evaporated in a current of oxygen. The phospholipids were separated on Silufol plates with a mixture of chloroform–methanol–water (65:25:4). The lipids were identified by reference to their Rf values. Phospholipids were determined quantitatively by the method in [5]. LPO in the homogenate and SR were studied by measuring the rate of malonic dialdehyde (MDA) accumulation and the intensity of chemiluminescence (ChL) in medium containing 0.5 mM ascorbate, 0.012 mM Mohr's salt, and 100 mM Tris-HCl (pH 7.4) at 37°C with continuous bubbling through of air. The protein concentration in SR was 0.5-0.8 mg/ml. The MDA level and intensity of ChL were determined after incubation for 20 min as described previously [6]. Protein was determined by Lowry's method in Miller's modification [13].

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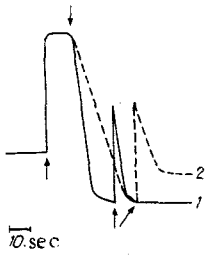

Fig. 1

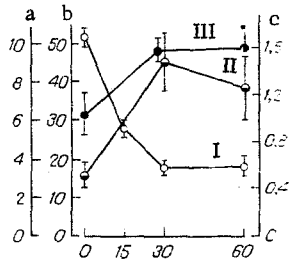

Fig. 2

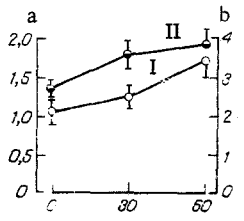

Fig. 3

Fig. 1. Uptake of Ca^{++} by SR vesicles recorded in homogenate from normal (1) and ischemic (2) myocardium. Arrow pointing downward — homogenate (100 mg tissue), arrow pointing upward — 70 mM Ca^{++}.

Fig. 2. Rate of uptake of Ca^{++} (I), intensity of ChL (II), and lysophosphatidylcholine content (III) in rat myocardial SR under normal and total ischemic conditions. Abscissa, duration of ischemia (in min); ordinate: a) rate of uptake of Ca^{++} (μmoles/min/g tissue, n = 3); b) intensity of ChL (in cpm × 10^3/mg protein, mean results of five or six experiments); c) lysophosphatidylcholine content (in % of total phospholipid content, n = 4).

Fig. 3. Intensity of ChL (I) and MDA accumulation (II) during ascorbate-dependent LPO in rat heart homogenate under conditions of total ischemia. Abscissa, duration of ischemia (in min); ordinate: a) intensity of ChL (in cpm × 10^3/mg protein); b) MDA accumulation (in nmoles/20 min/mg protein). Mean results of five-six experiments shown.

EXPERIMENTAL RESULTS

Addition of potassium oxalate and sodium azide to the medium for measuring the Ca^{++}-transport function of SR enabled the rate of Ca^{++} uptake exclusively by vesicles of SR to be recorded [14]. As Fig. 1 shows, after ischemia of the heart for 1 h the Ca^{++}-transport activity of SR was reduced by about two-thirds compared with the control. The first addition of calcium was completely absorbed by SR from the normal and ischemic heart. Addition of more Ca^{++} was not followed by complete absorption of calcium by SR from the ischemic heart, unlike in the control, evidence of a reduction of the Ca^{++}-oxalate capacity of SR. The disturbance of the Ca^{++}-transport system of SR demonstrated after total ischemia of the heart for 1 h is in agreement with data in the literature [1].

Investigation of the Ca^{++}-transport activity of SR as a function of the duration of ischemia showed that the rate of Ca^{++} uptake by SR in the homogenate after ischemia of the heart for 30 min was reduced by 75%, and thereafter remained virtually unchanged until after 60 min of ischemia (Fig. 2a). Other workers also have demonstrated a marked decrease in the rate of Ca^{++} transport by SR after 30 min of ischemia [4, 12]. However, unlike in our own investigation, the authors of [4] observed a further decrease in the rate of Ca^{++} uptake with an increase in the duration of ischemia to 60 min, which was explained by progressive accumulation of LPO products in SR.

In the present investigation the intensity of ChL during ascorbate-dependent LPO in SR increased sharply until the 30th minute and remained at the same level during 60 min of myocardial ischemia (Fig. 2b). The increase in the intensity of ChL under these circumstances correlated with the decrease in the rate of Ca^{++} uptake by SR. The coefficient of correlation between these parameters was 0.68. In the heart homogenate the intensity of ascorbate-dependent LPO, measured as the rate of MDA accumulation and the intensity of ChL, increased steadily throughout the 60-min period of ischemia (Fig. 3); this can perhaps be explained by a gradual decrease in activity of the antioxidant systems in the cytosol of the ischemic heart [7].

Comparison of the phospholipid composition of the homogenates and SR from normal hearts and hearts rendered ischemic for 60 min showed (Table 1) an increase in the lyosphosphatidylcholine content in both cases without any significant change in the levels of the other phospholipids, in agreement with data in the literature [8]. Principal accumulation of lysophos-

TABLE 1. Phospholipid Spectrum of Homogenates and SR from Normal and Ischemic Rat Hearts

Phospholipid	Homogenate		SR	
	control	ischemia (60 min)	control	ischemia (60 min)
Lysophosphatidylcholine	0,75±0,06	1,07±0,11*	1,06±0,18	1,62±0,17**
Sphingomyelin	2,21±0,21	1,98±0,54	4,24±0,16	4,22±0,50
Phosphatidylcholine	28,08±0,72	27,72±0,43	38,96±0,59	39,40±0,43
Phosphatidylinositol	14,10±0,90	12,52±0,44	8,69±0,63	8,47±0,31
Phosphatidylethanolamine	22,01±1,09	24,06±0,78	27,26±0,38	27,99±0,66
Phosphatidylserine	14,32±0,90	14,77±0,38	9,92±0,75	9,62±0,72
Cardiolipin	17,74±0,61	18,80±0,50	12,40±0,20	14,12±0,88

Legend. *$p < 0.02$, **$p < 0.01$. Mean results of 4-6 experiments shown.

phatidylcholine in the isolated SR fraction took place during the first 30 min of ischemia of the heart (Fig. 2c), which correlated with the disturbance of the Ca^{++}-transport function of SR. The coefficient of correlation in this case was 0.83.

The results are evidence that disturbance of the Ca^{++}-transport function of SR, an increase in the intensity of lipoperoxide ChL, and accumulation of lysophosphatidylcholine take place during the first 30 min of ischemia of the rat myocardium, i.e., in the period preceding the transition from reversible ischemic damage to irreversible LPO, by "loosening" the membranes, evidently increases the accessibility of phospholipids for phospholipases. Meanwhile the free fatty acids formed during activation of phospholipases are the chief substrates for the LPO process. For this reason, and also bearing in mind the similar time course of the increase in intensity of ChL and accumulation of lysophospholipids, it can be tentatively suggested that both free-radical LPO and phospholipase activation are directly related to disturbance of the Ca^{++}-transport function of SR during myocardial ischemia.

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