LITERATURE CITED

1. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System: Generator Mechanisms of Neuropathological Syndromes [in Russian], Moscow (1980).
2. G. N. Kryzhanovskii, A. A. Shandra, L. S. Godlevskii, et al., Byull. Éksp. Biol. Med., No. 11, 582 (1987).
3. D. Sepetliev, Statistical Methods in Scientific Medical Research [Russian translation], Moscow (1968).
4. A. A. Shandra, L. S. Godlevskii, and N. D. Semenyuk, Byull. Éksp. Biol. Med., No. 4, 20 (1983).
5. E. Fifkova and J. Marsala, Electrophysiological Methods in Biological Research, Prague (1968), pp. 653-659.
6. A. M. A. Van Dijk, A. Ernst, and G. C. Schoenenberger, Endogenous Sleep Substances and Sleep Regulation, Tokyo (1985), pp. 167-178.
7. A. M. J. Young and B. J. Key, Neuropharmacology, 23, No. 11, 1347 (1984).

STATE OF LIPID PEROXIDATION AND THE CALCIUM TRANSPORT ENZYME SYSTEM IN THE SARCOPLASMIC RETICULUM OF THE ISCHEMIC MYOCARDIUM

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Ischemia or anoxia of the heart causes considerable impairment of the functional capacity of membranes of the sarcoplasmic reticulum (SR). In total ischemia, for instance, the ability of the membranes of SR to take up Ca^{++} is reduced after only 30 min [12]. This may be one cause of the development of postoperative heart failure after operations on the "dry" heart or after heart transplantation. It has also been shown by direct [3] and indirect [8] methods of investigation that lipid peroxidation (LPO) processes in the myocardium are intensified during ischemia, and that preliminary administration of an antioxidant improves the contractile function of the ischemic heart [9].

However, changes in concentrations of LPO products in membranes of SR and the connection between these changes and disturbance of the ability of SR to accumulate Ca^{++} in total ischemia have not been studied. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Altogether 30 experiments were undertaken on mongrel dogs. The heparinized (3 mg/kg) dogs were anesthetized with thiopental (10 mg/kg), artificial respiration was applied, and the heart was removed and kept in an incubator at 37°C. An area of myocardium of the left ventricle was excised after 15, 30, 60, and 120 min. Fragments of SR membranes were isolated, the state of their Ca^{++} transport enzyme systems was determined, lipids were extracted from the SR membranes and their concentrations of primary LPO products (diene conjugates — DC) were estimated as described previously [4]. Concentrations of total phospholipids (PL) in lipid extract from SR membranes were determined by the method in [14]. Concentrations of secondary LPO products (triene conjugates — TC) were determined spectrophotometrically by measuring the increase in optical density of the solution of lipids at 275 nm. The level of

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TABLE 1. Ca^{++}-Accumulating Capacity and Ca^{++}-ATPase Activity of SR Membranes of Ischemic Myocardium (M ± m)

Parameter	Control	Period of ischemia, min			
		15	30	60	120
Ca^{++} uptake, mmoles/mg protein/10 min	660±210	592±103	274±55*	222±62**	106±38**
Ca^{++}-ATPase activity, μmoles P_i/mg protein/5 min	1,38±0,27	1,38±0,10	1,30±0,31	1,06±0,12	0,37±0,25*

Legend. Here and in Table 2: *p < 0.05, **p < 0.01 compared with control. Values of parameters determined from data of 5-6 experiments.

TABLE 2. Concentrations of Phospholipids and LPO Products in SR Membranes of Ischemic Myocardium (M ± m)

Parameter	Control	Period of ischemia, min			
		15	30	60	120
Total PL, μmoles P_i/mg protein	0,76±0,02	0,80±0,03	0,76±0,03	0,76±0,04	0,79±0,03
DC, conventional units	2,59±0,26	2,17±0,21	2,20±0,19	2,58±0,18	2,10±0,64
TC, conventional units	0,51±0,16	0,50±0,09	0,61±0,03	0,73±0,05	0,72±0,17
MDA, nomoles/mg protein	0,66±0,06	0,71±0,10	0,84±0,03*	0,82±0,06**	0,86±0,10*

malonic dialdehyde (MDA) in the SR membranes was determined by the reaction with 2-thiobarbituric acid [10], the concentration of which was calculated by the use of a molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1}cm^{-1}$.

EXPERIMENTAL RESULTS

The results of estimation of the state of the Ca^{++} transport enzyme system in SR membranes of the ischemic myocardium are given in Table 1 and Fig. 1. After 15 min of ischemia the ability of the SR membranes to take up Ca^{++} was the same as initially. After 30 min of ischemia marked worsening of the Ca^{++}-accumulating capacity of SR was observed, and at this time its value was 2.4 times below that observed initially. A similar (threefold) fall in the Ca^{++}-accumulating capacity of SR of the ischemic myocardium also was demonstrated by other workers [12]. Later this parameter continued to fall progressively, and after 2 h of ischemia it was only 16% of the initial value.

Ca^{++}-ATPase activity of SR membranes after 15 and 30 min of ischemia was virtually unchanged, although Ca^{++} uptake by this time was already reduced. After 1 h of ischemia Ca^{++}-ATPase activity was reduced to 77% of its initial value, but this change was not significant. Only after 2 h of ischemia did this parameter fall in value, namely to 27% of the initial level.

Correlation analysis was carried out separately at the early stages of ischemia (up to 30 min) and after 1 and 2 h of ischemia, between parameters reflecting the state of the Ca^{++} transport enzyme system. It was shown that until 30 min the fall in Ca^{++}-accumulating capacity of the SR membranes did not depend significantly on Ca^{++}-ATPase activity ($r = 0.20$), whereas after 1 and 2 h of ischemia, marked correlation was found between the parameters ($r = 0.67$; $p < 0.05$).

Data on the concentrations of total PL and of LPO products in SR membranes are given in Table 2 and Fig. 1. The PL and DC concentrations at these times of observation were unchanged, the TC level showed a tendency to rise starting with 30 min of ischemia, and the MDA concentration was increased after 30 min of ischemia and remained high throughout the subsequent period. About the same MDA accumulation (125% after 2 h) was found in homogenate of the dog myocardium in the presence of focal ischemia [13].

The experiments thus showed that even in the early stages of total myocardial ischemia (the first 30 min) there is a marked decline in the ability of SR membranes to take up Ca^{++} and an increase in their MDA concentration. At the later stages of ischemia (after 60 and

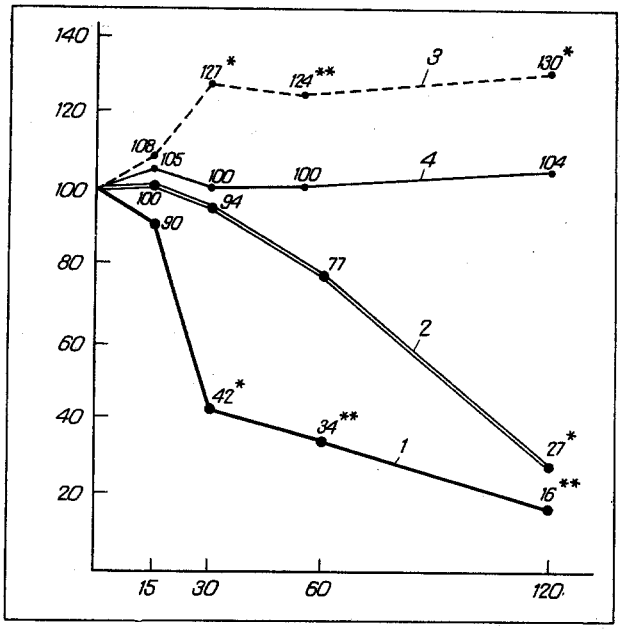

Fig. 1. State of calcium transport enzyme system, and concentrations of MDA and total PL in SR membranes of ischemic myocardium (in % of initial level). 1) Ca^{++} uptake; 2) Ca^{++}-ATPase activity; 3) MDA concentration; 4) total PL concentration. Asterisk indicates significance of differences compared with initially: *$p < 0.05$, **$p < 0.01$.

120 min) a further decline of the Ca^{++}-accumulating function of SR and significant inhibition of Ca^{++}-ATPase activity were observed, whereas the raised MDA concentration in the SR membranes still remained.

The sharp worsening of the Ca^{++}-accumulating capacity of SR in the early stages of ischemia, whereas Ca^{++}-ATPase activity remained intact, evidently indicates an increase in the passive permeability of SR membranes for Ca^{++}. The earlier increase in passive permeability of SR membranes for Ca^{++} and the later impairment of Ca^{++}-ATPase function were demonstrated by the writers previously in ischemia of skeletal muscles [1].

Analysis of the possible mechanisms of the increase in passive permeability of SR membranes in total ischemia is of great interest. We know that prolonged focal myocardial ischemia (8-12 h) leads to a significant fall in the total PL concentration in SR membranes, and this correlates with the marked increase of their passive permeability for Ca^{++} [11]. However, in the early stages of ischemia (until 2 h), no decrease in the total PL concentration in SR membranes could be found either in the investigation cited or in the present investigation during total ischemia of the heart.

The data now described, namely a marked increase in the MDA concentration in SR membranes, coinciding in time with beginning of decline of their Ca^{++}-accumulating capacity, are evidence that LPO processes are involved in the increase in passive permeability of SR membranes during total ischemia. Previously the present writers showed in vitro that induction of LPO processes in vesicles of SR in skeletal muscles previously loaded with Ca^{++} oxalate, or their treatment with PL hydroperoxides, led to an increase in membrane permeability for Ca^{++} ions and to their release from vesicles [6]. It has also been found that induction of LPO in fragments of SR membranes from the myocardium, accompanied by accumulation of MDA in them, leads to a rapid decrease in the ability of the membranes to take up Ca^{++} [7].

The absence of an increase in concentration of primary LPO products (DC) in SR membranes does not rule out the possibility that LPO processes may be involved in the membrane damage during ischemia. The more marked rise in the concentration of secondary, but not of primary products was found previously in a study of ischemia of the limb muscles [5], and may be due to rapid breakdown of unstable primary products. LPO processes also evidently participate in the lowering of Ca^{++}-ATPase activity observed in the late stages of total cardiac ischemia, for we know that their development in SR membranes, together with increased permeability of the latter for Ca^{++}, is accompanied by a decrease in Ca^{++}-ATPase activity [1, 2]. This

inhibition may be due both to a decrease in the concentration of polyene lipids, required for normal functioning of the enzyme, and burned up during development of LPO, and also to interaction of LPO products of dialdehyde nature (MDA) with Ca^{++}-ATPase, with the formation of intermolecular cross-linkages of the Schiff base type [2].

Thus an increased concentration of MDA in SR membranes may be an important cause of the progressive decline of Ca^{++}-ATPase activity which, together with the increase in passive permeability for Ca^{++}, leads to the virtually total loss of Ca^{++}-accumulating capacity of SR membranes of the ischemic myocardium.

LITERATURE CITED

1. Yu. V. Arkhipenko, M. V. Bilenko, S. K. Dobrina, et al., Byull. Éksp. Biol. Med., No. 6, 683 (1977).
2. Yu. V. Arkhipenko, V. E. Kagan, and Yu. P. Kozlov, Biokhimiya, No. 3, 433 (1983).
3. M. V. Bilenko, E. B. Burlakova, A. V. Alesenko, et al., Transplantation of Organs and Tissues [in Russian], Rostov-on-Don (1976), p. 117.
4. M. V. Bilenko, V. N. Otverchenko, and V. V. Astaf'ev, Grudnaya Khir., No. 6, 20 (1983).
5. M. V. Bilenko, T. D. Churakova, S. L. Arkhangel'skaya, et al., Vest. Akad. Med. Nauk SSSR, No. 4, 24 (1985).
6. V. E. Kagan, T. D. Churakova, and V. P. Karagodin, Byull. Éksp. Biol. Med., No. 2, 145 (1979).
7. V. E. Kagan, V. M. Savov, and V. V. Didenko, Byull. Éksp. Biol. Med., No. 4, 46 (1983).
8. A. Kh. Kogan, A. N. Kudrin, and S. M. Nikolaev, Free-Radical Oxidation of Lipids under Normal and Pathological Conditions [in Russian], Moscow (1976), p. 68.
9. V. N. Otverchenko, M. V. Bilenko, Ya. Styk, et al., Transplantation of Organs and Tissues [in Russian], Tbilisi (1982), p. 212.
10. W. R. Bidlack and A. L. Tappel, Lipids, 8, 177 (1973).
11. K. R. Chien, R. G. Pfau, and J. H. Farber, Am. J. Path., 97, 505 (1979).
12. M. L. Hess, S. M. Krause, and L. J. Greenfield, J. Throac. Cardiovasc. Surg., 80, 293 (1980).
13. B. Török, E. Röth, and B. Matkovics, Acta Physiol. Acad. Sci. Hung., 68, 25 (1986).
14. V. E. Vaskovsky, E. J. Kostetsky, and I. M. Vasendin, J. Chromatogr., 114, 129 (1975).