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DISTURBANCES OF THE Ca^{++} TRANSPORT ENZYME SYSTEM IN MEMBRANES OF THE SARCOPLASMIC RETICULUM CAUSED BY HYDROPEROXIDES OF PHOSPHOLIPIDS AND OF FATTY ACIDS*

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The hydroperoxide (HP) of phosphatidylethanolamine, if added to a suspension of vesicles of the sarcoplasmic reticulum (SR), was shown to have a weak activating effect on Ca-dependent ATPase and to increase the permeability of SR membranes for Ca^{++}, measured during activity of the enzyme. HP of linoleic acid did not affect the parameters of the Ca^{++} transport enzyme system, the activity of Ca^{++}-dependent ATPase, the Ca/ATP ratio, or the rate of outflow of Ca^{++} in SR membranes on account of the low level of its incorporation into SR fragments. It is concluded that among the primary molecular peroxidation products (HP of free fatty acids, HP of phospholipids), induced both in vitro (by the Fe^{++} + ascorbate system) and in vivo (ischemia, avitaminosis-E), only phospholipid HP is an effective modifier of Ca^{++} transport in SR membranes.

KEY WORDS: sarcoplasmic reticulum, hydroperoxides of lipids, membrane permeability, Ca-dependent ATPase.

The development of ischemia is accompanied by accumulation of lipid peroxidation products (LPP) in the membranous structures of the cell [1] and by hydrolysis of phospholipids with the formation of monoacylglycerophosphatides and free polyene fatty acids [8] which, in turn, can undergo autooxidation. Accumulation of LPP is known to lead to an increase in the permeability of artificial and biological membranes for ions and nonelectrolytes [4, 8, 15] and to inhibition of membrane-bound enzymes [2]. Among the many different compounds produced by autooxidation of lipids of biological membranes, the HP of phospholipids in fatty acids, which are primary molecular LPP, occupy a special place: Their ability to intrude into the network of metabolic conversions (through peroxidase reactions) [2] means that the effects of modification of biomembranes by hydroperoxides can be regarded as reversible, whereas in the later stages of lipid peroxidation "irreversible" secondary products (for example, intermolecular polymer cross-linkages), which cannot be utilized by the cell enzyme systems, accumulate. It was shown previously that a consequence of modification of membranes of the sarcoplasmic reticulum (SR) by active forms of oxygen during ischemia of skeletal muscles is an increase in permeability of the SR vesicles for Ca^{++} ions (a decrease in the Ca/ATP ratio) and partial inhibition of Ca^{++}-dependent ATPase [1]; both effects, moreover, are due to the accumulation of LPP. Accordingly, in the investigation described below the action of primary molecular LPP, namely exogenous hydroperoxides of phospholipids and hydroperoxides of free fatty acids, and also unmodified phospholipids and fatty acids, on the parameters of the Ca transport enzyme system, located in SR of skeletal muscles, was studied.

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Laboratory of Physical Chemistry of Biomembranes, M. V. Lomonosov Moscow State University. Laboratory for Transplantation of Organs and Tissues, Academy of Medical Sciences of the USSR, and Department of Operative Surgery and Topographic Anatomy, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 2, pp. 145-149, February, 1979. Original article submitted December 13, 1977.

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TABLE 1. Effect of Compounds Tested on Activity of Ca^{++}-Dependent ATPase (V_{ATP}, nmoles P_i/min · mg protein) and Rate of Outflow of Ca^{++} ($V_{ATP,out.Ca}$, in nmoles Ca^{++}/min · mg protein) in Course of Transport*

Compound added (C)	SR phospholipids/C	V_{ATP}	$V_{ATP,out.Ca^{++}}$
Control		2,81±0,17	3,18±0,36
HP of PEA	320	3,32±0,22	4,84±0,79
	100	3,74±0,28	5,53±0,75
	10	4,07±0,33	7,54±0,94
HP of LA	10	2,76±0,22	3,17±0,47
	1,0	2,79±0,28	3,40±0,61
PEA	10	2,80±0,15	3,16±0,34
	1,0	2,84±0,23	3,24±0,46
LA	100	2,89±0,25	3,27±0,54
	10	3,12±0,23	4,21±0,62
	1,0	3,67±0,28	6,94±0,79
HP of lipids †	320	2,98±0,25	4,62±0,63
	100	3,20±0,28	6,27±0,64

*Incubation medium: 100 mM NaCl + 5 mM Na oxalate + 2 mM ATP + 2 mM $MgCl_2$ + 5 mM Tris-HCl, pH 7.0, 37°C; SR protein concentration 0.3 mg/ml.

†On induction of lipid peroxidation in SR suspension by system of of Fe^{++} (10 nmoles/mg protein) + ascorbate (0.3 mM).

EXPERIMENTAL METHOD

Fragments of SR from white skeletal muscles of rabbits were isolated by the method in [14]. The velocity of Ca-dependent hydrolysis of ATP (V_{ATP}) was measured by a pH-metric method and the parameter Ca/ATP was calculated as described in [6]. The rate of outflow of Ca^{++} from the SR vesicles in the course of its active transport ($V_{ATP,out.Ca}$) was calculated by the equation:

$$V_{ATP,\,out.\,Ca} = (2 - \frac{Ca^{++}}{ATP})V_{ATP},$$

assuming that Ca^{++}/ATP for native membranes in 2 [7]. The rate of passive outflow of Ca from SR, loaded beforehand with Ca oxalate ($V_{out.Ca}$) was measured by a pH-metric method based on the reaction of binding of Ca^{++} in the external medium with EGTA, a specific complex of ethylene-glycol (aminoethyl ester) and sodium N,N'-tetraacetate [6] or by means of a Ca-selective electrode [5]. The protein concentration was determined by the biuret reaction.

Phosphatidylethanolamine (PEA) was isolated from egg yolk [13]. Linoleic acid (LA, from Nutr. Biochem. Corp.) or PEA was subjected to peroxidation by means of lipoxygenase (from Reakhim, 20 μg/mg lipids) in an aqueous suspension of 0.6 mg lipids/ml medium of the composition: 50 mM Tris-HCl + 100 mM NaCl, pH 8.0, 37°C. The hydroperoxide (HP) of LA was isolated by the method in [11, 9], suitably modified. The HP of PEA was isolated by preparative thin-layer chromatography on silica gel (from Ferrak, Germany) in a system of chloroform — methanol — 28% NH_4OH (13 : 7 : 1). After fractionation, the chromatographic plate was developed in a chamber saturated with I_2 vapor and the stain containing HP of PEA was eluted with a methanol — hexane (3:1) mixture. LA and PEA were dissolved in ethanol and their hydroperoxide derivatives in methanol and added to a suspension of SR fragments by means of a Hamilton microsyringe.

EXPERIMENTAL RESULTS

The formation and maintenance of a transmembrane Ca^{++} gradient in the SR system, which is the mechanism of electromechanical coupling in the muscle fiber, are possible because of two factors: the presence of a calcium pump (Ca-dependent ATPase) and the low permeability of the SR membranes for Ca^{++} [7]. It will be clear from the data given in Table 1 that the addition of HP of LA and also of unmodified PEA to the SR suspension did not affect the Ca-dependent ATPase activity. The probable reason why HP of LA had no action on

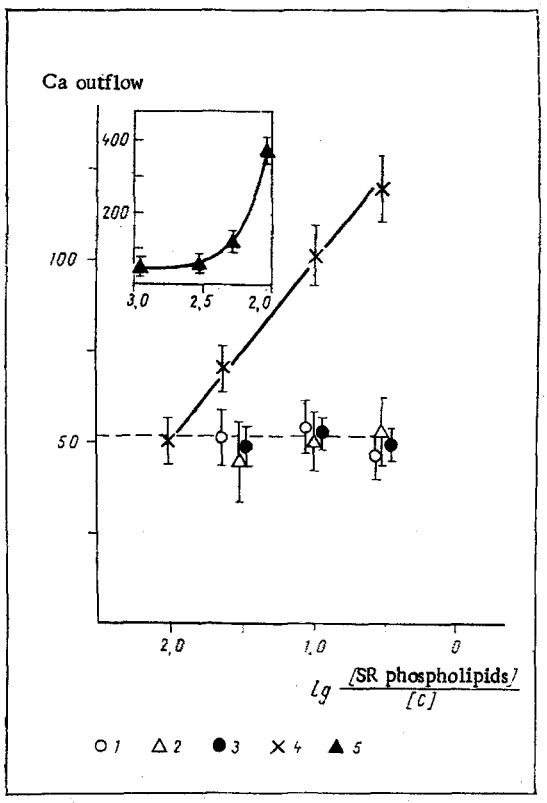

Fig. 1.

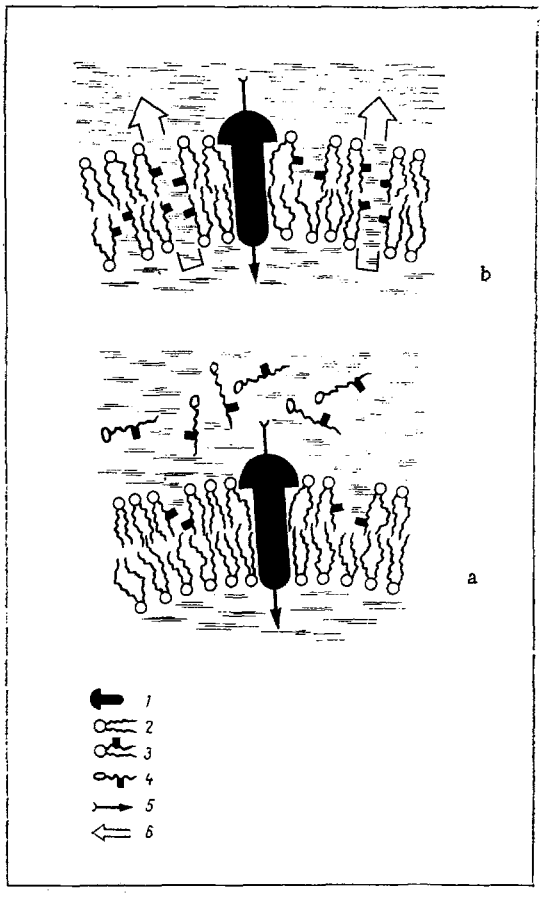

Fig. 2.

Fig. 1. Velocity of passive outflow of Ca ions ($V_{out.Ca^{++}}$) from SR vesicles loaded beforehand with Ca oxalate (200 nmoles/mg protein) under influence of HP of PEA (1), HP of LA (2), PEA (3), and LA (4) and during induction of lipid peroxidation by system consisting of Fe^{++} (10 nmoles/mg protein) + ascorbate (0.3 mM) (5). [C] — concentration of compound added or of induced hydroperoxides of phospholipids. Incubation medium: 100 mM NaCl + 0.5 mM EGTA + 0.5 mM imidazole or (for measurements with Ca^{++}-selective electrode) 100 mM NaCl + 5 mM imidazole; pH 7.0, 37°C, SR protein concentration 0.5 mg/ml.

Fig. 2. Scheme of action of HP of fatty acids and HP of phospholipids added from exogenous sources (a) and arising during induction of lipid peroxidation (b). 1) CA^{++}-dependent ATPase, 2) phospholipid, 3) HP of phospholipid, 4) HP of fatty acid, 5) active Ca^{++} transport, 6) passive Ca^{++} outflow.

ATPase activity was the low level of incorporation of polar hydroperoxides of fatty acids into the hydrophobic phase of the membrane, for their partition coefficient in a heptane — water system is 1:19 [3], and unmodified fatty acids are virtually completely distributed in the hydrocarbon. Meanwhile HP of PEA and LA had a weak activating effect. A similar effect of activation of Ca-dependent ATPase also was observed in the initial stages of lipid peroxidation (up to 10 nmoles HP/mg lipids) in SR membranes with a catalytic system of Fe^{++} + ascorbate which, however, was replaced by inhibition of the enzyme on the accumulation of over 20 nmoles HP/mg lipids, and complete inactivation of CA-dependent ATPase occurred after the accumulation of more than 26 nmoles HP/mg lipids. It follows from these findings that whereas exogenous hydroperoxides of phospholipids provide a good model of the effect of activation of Ca-dependent ATPase during lipid peroxidation, inhibition of the enzyme in the late stages of lipid peroxidation could hardly be due to the action of primary molecular products of free-radical oxidation of lipids (hydroperoxides) and it is connected with oligomerization of molecules of the enzyme and the scarcity of polyene lipids in the microenvironment of the ATPase. The mechanism of inhibition of Ca-dependent ATPase during lipid peroxidation is examined in greater detail elsewhere [2].

Investigation of the action of LA, PEA, and their hydroperoxides on passive permeability of the SR membranes showed that an increase in $V_{out.Ca}$ was observed only in the presence of unmodified fatty acids, whereas

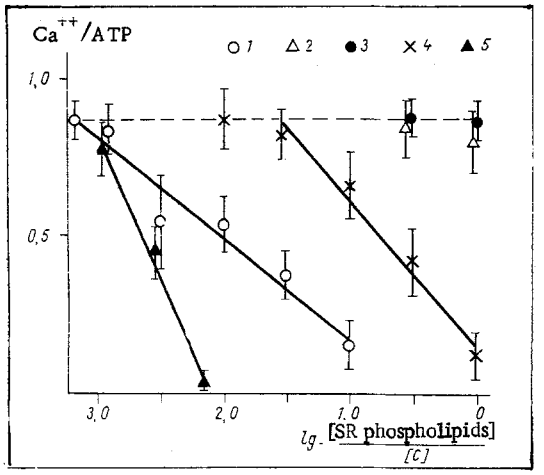

Fig. 3. Parameter Ca^{++}/ATP in SR suspension during action of HP of PEA (1), HP of LA (2), PEA (3), and LA (4) during induction of lipid peroxidation (5). Conditions of incubation stated in legend to Table 1.

PEA itself and the two hydroperoxide derivatives did not give this effect (Fig. 1). Meanwhile, on induction of lipid peroxidation in the SR membranes by the Fe^{++} + ascorbate system, accumulation of HP of lipids was accompanied by an increase in $V_{out.Ca}$ from SR vesicles (a twofold increase in $V_{out.Ca}$ on the appearance of an additional 7 nmoles HP/mg lipids; Fig. 1). It can be concluded from a comparison of these results that the increase in $V_{out.Ca}$ from the vesicles observed during accumulation of LPP in SR could not be due to the action of HP of PEA or HP of LA as carriers of Ca^{++} through the SR membrane. As was pointed out above, the absence of any effect of HP of LA was probably the result of the low level of its incorporation into the SR membrane.

It must be remembered that during induction of lipid peroxidation the formation of HP of phospholipids takes place both in the external and in the internal monolayer of the membrane, as is shown by the identical rates of accumulation of LPP in intact SR membranes (16.4 ± 4.5 nmoles MDA* /mg protein after 15 min) and in SR preparations treated with ultrasound (3 × 20 sec; 22 kHz, 0-4°C) in the presence of inducers of lipid peroxidation — the Fe^{++} + ascorbate system (18.0 ± 2.6 nmoles MDA/mg protein after 15 min). On addition of exogenous HP of PEA, it was invariably found chiefly in the outer monolayer (Fig. 2). The appearance of permeability channels for Ca^{++} requires the presence of polar hydroperoxide groups in both lipid monolayers of the membrane, i.e., it must depend on the translation mobility of molecules of HP of phospholipids and, above all, on their transmembrane transport, the velocity of which is low (the half-turnover time of phospholipids between the two monolayers of the membrane is measured in hundreds of minutes for liposomes and tens of minutes for biomembranes [10]). It can therefore be postulated that factors capable of increasing the rate of turnover of lipids between the inner and outer monolayers must lead to an increase in permeability of the membrane for Ca^{++}. The permeability of the SR membranes is known to depend on the functional state of Ca-transporting ATPase, and rises sharply on activation of the latter [7]. Correspondingly, $V_{ATP,out.Ca}$ (the rate of outflow of Ca^{++}, measured during functioning of Ca-dependent ATPase) rises not only on the addition of free LA to the SR suspension, but also on addition of HP of PEA (Table 1). It can tentatively be suggested that in this case the activity of the "calcium pump" is a factor contributing to the increase in the turnover rate of HP of PEA between the monolayers of the SR membrane and the consequent formation of "peroxide channels" for leakage of Ca ions [2]. Differences in the effectiveness of action of HP of PEA on the permeability of SR membranes in two functional states of the Ca^{++} transport enzyme system (no effect of HP of PEA when ATPase is inactive and marked induction of permeability for Ca^{++} in the presence of HP of PEA during activity of the Ca^{++}-transporting enzyme) can hardly be explained by essentially different levels of incorporation of HP of PEA into the SR membranes in these states. A separate series of experiments showed that the quantity of HP of PEA bound with the membranes of SR fragments in these two cases did not differ by more than 30%. The physiological importance of this result will be clear if it is remembered that each contraction-relaxation cycle of the muscle fiber is invariably accompanied by the liberation and accumulation of Ca ions in the SR system; these processes take place on account of activation of Ca-dependent ATPase. When Ca-dependent ATPase was active, HP of PEA was found to be even more effective than LA, i.e., the "uncoupling" action of HP of PEA on

*Malonic dialdehyde.

the enzyme system for Ca transport in SR was stronger (Fig. 3). The presence of exogenous HP of LA in this case also had no effect on the permeability of SR membranes for Ca ions (Fig. 3, Table 1).

It can be concluded from these results that among the primary molecular products of lipid peroxidation, namely hydroperoxides of free fatty acids and hydroperoxides of phospholipids, only the latter are effective modifiers of permeability of SR membranes for Ca ions.

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TISSUE-SPECIFIC UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION OF MITOCHONDRIA FROM THE RAT HEART, KIDNEY, THYMUS, AND LUNG

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The effect of microsome-free cytoplasmic fractions from the rat heart, kidney, thymus, and lung on oxidative phosphorylation (OP) of mitochondria (MCH) from these tissues and also of MCH from liver and brain was investigated. Crossed experiments showed the existence of tissue-specific uncouplers of OP in these fractions, similar in their properties to that found previously in rat liver. The possible role of these regulators in the initiation of enzymic degradation of MCH through activation of phospholipase A during the outflow of calcium ions from MCH is discussed. Activation of DNase I associated with the mitochondrial membrane is postulated under these conditions.

KEY WORDS: tissue-specific uncouplers; calcium; phospholipase A; DNase I.

The writer showed previously that rat liver tissue contains a factor whose action on homologous mitochondria (MCH) has been identified as uncoupling [2]. On the addition of the fraction containing this factor to MCH from the kidney, heart, brain, and lung its action on oxidative phosphorylation (OP) was found to be tissue-specific [3]. Tissue-specificity was expressed as a selective increase in the uptake of oxygen by MCH in state 3 (ΔO_{act}) and an increase in the duration of phosphorylation (t_p) in homologous MCH. The addition of bovine albumin, which inhibits the uncoupling action of free fatty acids on OP, did not affect the amplitude of tissue-specific uncoupling.

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