tive form of cytochrome P-420 fell in microsomes from the liver of the poisoned animals (Fig. 3c). At the same time the coefficient of inactivation of cytochrome P-450 fell (Table 1). However, the concentration of microsomal cytochrome P-450 did not return to normal during phospholipid therapy. An increase in the degree of unsaturation of the phosphatidylcholine did not increase the effectiveness of phospholipid therapy. Activity of the other microsomal marker enzyme — G-6-P — was restored to the control level by means of phosphatidylcholine (Table 1).

The results demonstrate the possibility of repair of damaged liver cell membranes by injection of phospholipids in the form of multilayered liposomes. The reparative action may be based on their interaction with injured biological membranes by the principle of fusion or exchange of lipids.

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LIPID PEROXIDATION AS THE MAIN CAUSE OF MODIFICATION OF THE CATALYTIC FUNCTION OF SARCOPLASMIC RETICULUM Ca-ATPase IN HYPERCHOLESTEROLEMIA

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In some pathological states such as atherosclerosis, ischemia, myopathy, and malignant degeneration of tissue cholesterol (ChS) metabolism is disturbed and, in particular, an excess of ChS accumulates in the tissues. This process can be simulated by experimental hypercholesterolemia (HChE) [6]. It has been shown that in experimental HChE additional accumulation of ChS takes place in the plasma membranes, which normally contain about 30% of the total content of lipids. Elevation of the ChS level in the plasma membranes is evidently the primary cause of the change in activity of membrane-bound enzymes and of membrane permeability [11]. In HChE the absolute ChS content in the intracellular membrane is not significantly

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increased [10], but nevertheless a disturbance of the structure of the intracellular membranes and functions of membrane-bound enzymes has been described. In particular, it has been shown [4] that the development of HChE in rabbits is accompanied by disturbance of functioning of the Ca pump of the membranes of the sarcoplasmic reticulum (SR) of rabbit skeletal muscle. Under normal conditions membranes of SR contain virtually no ChS but contain large quantities of unsaturated phospholipids, which account for the high "flowability" of the lipid phase of these membranes [4, 12]. The very small rise of the ChS level in membranes of SR during HChE is evidently not the immediate cause of disturbances of function of the Ca pump observed under these circumstances. At the same time, we know that HChE leads to activation of lipid peroxidation (LPO) in various tissues [5], and also in SR membranes [10]. It has also been found that the addition of the natural antioxidant α-tocopherol (TP) together with ChS to the diet of rabbits sharply lowers the level of LPO products in SR membranes and prevents disturbance of functional activity of the Ca pump of SR [4]. This suggests that activation of LPO in HChE makes an important contribution to the disturbance of the Ca transporting function of SR membranes.

The aim of this investigation was to compare the modifying action of LPO, induced in vitro, and the harmful effect of HChE on SR Ca-ATPase.

EXPERIMENTAL METHOD

Male Chinchilla rabbits weighing 2.5-3 kg were used. Experimental HChE was induced by keeping the rabbits on a diet with the addition of commercial ChS in a dose of 1 g/kg daily (the animals were fed once a day) for 3 months. The development of HChE in the rabbits was monitored by periodic assay of ChS in the blood. The serum ChS level was determined on an automatic analyzer (Frace III System) with a standard kit of reagents (Beckman, West Germany).

Fragments of SR from white skeletal muscles of rabbits were isolated by the method described previously [7]. The SR preparations were delipidized by gel-filtration on a column (1.1 × 25 cm) packed with ultragel AcA 34 [8]. After application of 2.5 ml of the SR preparation (10 mg protein), solubilized in equilibration medium, to the column, elution was carried out with the same medium at 4°C. The volume of the fractions collected was 1 ml. To determine the degree of delipidation and the ability of ATPase to be reactivated by ovolecithin, the fractions were analyzed for protein and activity of the enzymes in them was determined in the absence of phospholipid and after addition of ovolecithin. Proteoliposomes containing Ca-ATPase were prepared from the delipidized SR preparation and ovolecithin by chromatography on an anionic exchange resin [8].

ATPase activity was measured by pH-metry [8]. Temperature dependence of Ca-ATPase activity in the range 0-40°C was studied as described previously [8]. LPO was induced by the addition of Fe^{++} (10 nmoles/mg protein) and ascorbate (0.2 mM) [1].

EXPERIMENTAL RESULTS

It was shown previously [3] that experimental HChE in rabbits modifies the temperature dependence of SR Ca-ATPase activity. If Ca-ATPase activity is measured in the absence of Ca^{++} ionophores and the rate of ATP hydrolysis is controlled by the calcium permeability of the membranes, the discontinuity on the graph of temperature dependence of Ca-ATPase activity between Arrhenius coordinates will disappear in HChE. If the true catalytic activity of Ca-ATPase is measured (in the presence of alamethicin), the graph of temperature dependence of Ca-ATPase activity in HChE is characterized by a discontiuity in the region of 30°C, i.e., in the region of higher temperature than in the control (20°C) [3]. It has been shown [1] that induction of LPO in vitro in SR membranes leads to some "straightening" of the graph of temperature dependence of Ca-ATPase activity measured in the absence of calcium ionophores.

To compare the modifying action of HChE and LPO, induced in vitro, on temperature dependence of Ca-ATPase activity, it was necessary to determine the effect of LPO on the true catalytic activity of Ca-ATPase. The effect of LPO induced in SR membranes by an Fe^{++}–ascorbate system [1] on temperature dependence of the true catalytic activity of Ca-ATPase, measured in the presence of alamethicin was investigated. When the true catalytic activity of Ca-ATPase was measured, the same character of modification of temperature dependence of Ca-ATPase activity was observed under the influence of LPO and of HChE (Fig. 1). In both cases the change in direction took place in the region of higher temperatures, with no change in the apparent activation energies. It was shown previously [3] by the method of Ca-ATPase reconstruction into proteoliposomes that the change in character of the temperature dependence of Ca-ATPase activ-

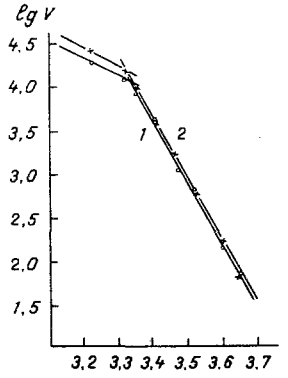

Fig. 1

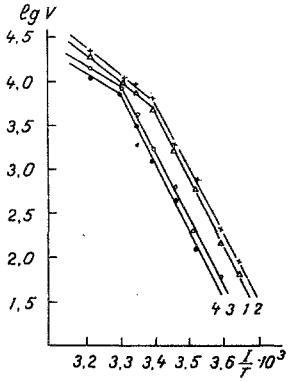

Fig. 2

Fig. 1. Temperature dependence of Ca-ATPase activity of SR during HChE (1) and activation of LPO in vitro (2). Here and in Fig. 2: incubation medium contained 3 μg/ml of alamethicin.

Fig. 2. Temperature dependence of Ca-ATPase activity of SR. 1) Control; 2) reconstruction by ovolecithin; 3) during induction of LPO in vitro (30 min); 4) reconstitution with ovolecithin after induction by LPO in vitro (30 min).

ity in HChE is the result of modification of the catalytic polypeptide of Ca-ATPase. It has in fact been found [3] that replacement of the lipid environment of Ca-ATPase by ovolecithin does not lead to recovery of the normal catalytic function of the enzyme, modified by HChE. If it is assumed that LPO is the cause of the change in the properties of Ca-ATPase in HChE, it may be expected that the shift of the discontinuity of the graph of temperature dependence of Ca-ATPase activity during LPO induction in vitro is also a reflection of damage to the polypeptide performing a catalytic function. To test this hypothesis control preparations of SR membranes and preparations of SR membranes in which LPO had been induced in vitro were delipidized with choleate on a column with AcA 34 ultragel. ATPase activity was restored by reconstruction into ovolecithin proteoliposomes.

Incorporation of Ca-ATPase of the control membranes into ovolecithin did not significantly change the character of temperature dependence of its activity, or at least the position of the disconvinuity remained within the 20°C region characteristic of ATPase in its native environment (Fig. 2). Replacement of the "peroxidized" lipids in SR membranes by ovolecithin did not restore the normal character of temperature dependence of Ca-ATPase activity (Fig. 2). In other words, the graph of temperature dependence of activity of Ca-ATPase reconstructed with ovolecithin from "peroxidized" SR membranes was characterized by a discontinuity in the high-temperature region (30°C). The cause of the shift on the graph of temperature dependence of Ca-ATPase activity during LPO and, correspondingly, in HChE is not yet clear. Temperature dependence of Ca-ATPase activity has been discussed in the literature in connection with the problem of conformational mobility of the enzyme [9]. Comparison of activity of Ca-ATPase reconstructed into proteoliposomes from SR membranes of warm and cold blooded animals showed that reduction of the conformational mobility of Ca-ATPase shifts the discontinuity on the Arrhenius plot into the high-temperature region [8]. On the basis of these data, and also on the fact that Ca-ATPase undergoes oligomerization through the action of LPO, it can be postulated that under the influence of LPO the conformational mobility of protein is reduced as a result of the formation of intra- and intermolecular cross-linkages through the action of dialdehydes formed during LPO. Reduction of conformational mobility under the influence of bifunctional cross-linking reagents has been used to increase their thermostability [2]. Reduction of the conformational mobility of Ca-ATPase as a result of cross-linkage may lead to a shift of the discontinuity of the Arrhenius plot into the high temperature region.

The data currently available thus indicate that the main cause of damage to function of the Ca pump in HChE is not the higher ChS level in SR membranes, but activation of LPO in them. This follows both from the data mentioned above, indicating that the effect of HChE on temperature dependence of Ca-ATPase activity can be simulated by LPO induced in vitro and also from previous investigations [3] which showed that total replacement of the lipid environment of Ca-ATPase by ovolecithin does not abolish disturbance of function of that enzyme induced by HChE. Under these circumstances both HChE- and in vitro-induced LPO not only have

a similar damaging effect on Ca-ATPase but also have similar mechanisms of action: in both cases polypeptide is damaged, fulfilling the catalytic function (Fig. 2) [3].

Earlier studies on the influence of HChE and LPO, induced in vitro, on the structure and function of the SR membrane [1, 4] also point out the principle role of LPO in the process of molecular pathogenesis during HChE in SR membranes. In both cases the efficiency of operation of the Ca-pump is reduced, viscosity of the membranes increased, the fatty acid composition of the phospholipids modified, membrane proteins undergo oligomerization, and the number of free SH-groups is reduced. Finally, it was shown that addition of the natural antioxidant TP to the diet simultaneously with ChS sharply lowers the level of LPO products and prevents disturbance of functional activity of the Ca-pump of SR.

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PHARMACOLOGICAL EFFECT OF EXOGENOUS HISTONES ON MUSCLES

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UDC 615.31:547.962.2].015.4:612.741

KEY WORDS: histones; myocardium; small intestine.

Histones are interesting as a test object because not only are they components of the eukaryotic chromosomal apparatus, but they also give rise to various pharmacological effects [1]. They attract even closer attention because histones have certain of the features which distinguish the primary structure of many regulatory peptides: a high prevalence of arginine, lysine, and proline and a correspondingly more frequent proximity of these amino acids in the primary structure.

Comparison of the amino-acid sequences of individual fractions of histones and of known regulatory peptides, polypeptide growth factor and hormones, undertaken by the writers with the aid of a computer, showed that histones contain tetrapeptide sequences identical to regulatory peptides and other protein regulators. Histones also contain longer fragments which are homologous with various peptides. The COOH-end of histone H4, for example, consists of

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