LIPID PEROXIDATION AND CALCIUM PUMP FUNCTION OF THE SARCOPLASMIC RETICULUM OF SKELETAL MUSLCES IN HYPERCHOLESTEROLEMIA

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Alimentary hypercholesterolemia (HCh) is an experimental model with which to study atherosclerosis. In HCh, besides accumulation of cholesterol, an increase in the content of lipid peroxidation (LPO) products also is observed in the blood and tissues [3, 4]. The reduced efificiency of operation of the Ca-pump of the sarcoplasmic reticulum (SR) of skeletal muscles observed previously in HCh [7, 8] may be caused by the action of any of these factors, for in experiments *in vitro* both accumulation of cholesterol [11, 12] and induction of LPO in SR membranes [4] lead to disturbances of this kind. It is not known which factor — excess of eholesterol in the membranes or elevation of the LPO level therein — makes the main contribution to disturbance of the functioning of the Ca-pump of SR in NCh.

The aim of this investigation was to study the connection between LPO and disturbance of functioning of the Ca-pump of SR during HCh.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male Chinchilla rabbits weighing 2.5-3 kg. The animals were divided into four groups with six rabbits in each group: 1) control rabbits kept on the standard animal house diet, 2) animals whose diet was supplemented with cholesterol in a dose of 1 g/kg daily, 3) besides cholesterol the rabbits received α-tocopherol acetate in the form of a 10% solution in oil in a dose of 30 mg/kg, 4) the animals' diet was supplemented with α-tocopherol acetate alone. All the animals took part in the experiments for 30 days. Preparations of SR of skeletal muscles were obtained as described in [6], and Ca-ATPase activity of SR and efficiency of Ca^{++} transport were measured pH-metrically [1] at 25°C. Cholesterol in the blood serum was determined with a "Trace III System" automatic biochemical analyzer using a standard kit of reagents from Beckman (USA). The cholesterol concentration in the SR preparations was measured by two methods: enzymically, with a kit of reagents from Boehringer (West Germany) and by a fluorescence method [10]; the latter gave exaggerated values of the cholesterol concentration in the membranes, for not only cholesterol, but its derivatives, also are determined by this method, unlike by the strictly specific enzymic method. The α-tocopherol concentration in the SR membranes was measured by the method in [13], Schiff's bases by the method in [9], and the intensity of LPO induced by Fe with ascorbate in the SR membranes was recorded on the basis of malonic dialdehyde (MDA) formation [2].

EXPERIMENTAL RESULTS

With the development of HCh by the rabbits their serum cholesterol level rose by more than tenfold, but in SR membranes (estimated by two independent methods) it was increased by 1.5-2 times (Table 1). The LPO level is SR membranes rose parallel with it (Table 2). The antioxidative activity (AOA) of the system was judged by the intensity of induced LPO. In HCh AOA of the SR membranes was reduced, since the level of induced LPO was higher than in the control. Under these circumstances the α-tocopherol concentrations was reduced by half.

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TABLE 1. Cholesterol Concentration in Blood Serum and SR Membranes Following Administration of α-Tocopherol during HCh (M ± m)

Exptl. conditions	Cholesterol concn.		
	in blood serum, mg %	in SR, μg/mg protein	
		fluorescence method	enzymic method
Control	63,5±7,3	31,1±2,0	19,8±1,4
Cholesterol	731,3±85,3*	57,6±2,7*	35,1±2,6*
Cholesterol + α-tocopherol	676,0±90,7	52,0±3,5*	25,6±0,9†
α-Tocopherol	43,2±6,4	42,7±2,4	19,1±2,5

Legend. *P < 0.02 compared with control, †P < 0.02 compared with high cholesterol diet.

TABLE 2. LPO in SR membranes Following Administration of α-Tocopherol *in vitro* during HCh (M ± m)

Exptl. conditions	Schiff's bases, relative units	Induced LPO, nanomoles MDA/mg protein		Concn. of α-tocopherol, μg/mg protein
		I	II	
Control	44,7±3,9	0,92±0,06	14,3±1,32	0,285±0,014
Cholesterol	202,1±28,8*	1,12±0,34	20,6±4,32*	0,130±0,016*
Cholesterol + α-tocopherol	21,4±1,9*	0,51±0,19†	0,90±0,20†	0,248±0,067†
α-Tocopherol	18,0±2,2*	0,38±0,21*	0,61±0,25*	0,315±0,041

Legend. *P < 0.01 compared with control, †P < 0.01 compared with high cholesterol diet. LPO was induced by addition of Fe + ascorbate to incubation medium of SR membranes up to final concentrations of 10^{-5} and $2 \cdot 10^{-4}$ M respectively (37°C). I) Preincubation at 37°C for 2 min in absence of Fe^{++}, II) 15 min after addition of Fe^{++}.

In order to increase AOA of the SR membranes, α-tocopherol was added to the diet simultaneously with cholesterol (group 3).

Addition of α-tocopherol to the animals' diet prevented the fall in its concentration in the SR membranes, sharply reduced the level of products of both endogenous (Schiff's bases) and induced LPO (Table 2), but had virtually no effect on the total cholesterol concentration (fluorescence method) in the blood serum and SR membranes (Table 1). However, the small decrease in the free cholesterol concentration (enzymic method) in the animals of group 3 compared with that in the rabbits of group 2 is evidence that it is perhaps the accumulation of free cholesterol (enzymic method) in SR membranes in HCh that is the pathogenic factor for the the Ca-pump of SR, more especially becuase this reduction in the free cholesterol concentration in samples of SR correlates with the powerful inhibition of LPO following addition of α-tocopherol to the high cholesterol diet (Tables 1 and 2).

The results may be evidence that α-tocopherol evidently has no direct effect on the level of cholesterol metabolism in rabbits, or no difference was observed in the total cholesterol concentration in the blood serum and SR membranes in the animals of groups 2 and 3. Meanwhile the fall in the free cholesterol concentration is SR membranes in the animals of group 3 compared with the rabbits of group 2 shows that addition of α-tocopherol to the diet potentiates the formation of cholesterol derivatives (probably esters), for the total cholesterol concentration in SR membranes was unchanged. This is clearly apparent in the animals of group 4, in which the total cholesterol level was twice as high as the free cholesterol level in SR membranes (Table 1). The action of α-tocopherol may be due to the fact that the sharp reduction in LPO after administration of α-tocopherol promotes more intensive cholesterol metabolism *in vivo*.

TABLE 3. Effect of α-Tocopherol on Function of Ca-Pump during HCh (M ± m)

Exptl. conditions	V_{ATP}	V_{Ca}	Ca/ATP
Control	2,98±0,10	3,09±0.09	1,19±0,05
Cholesterol	2,23±0,14*	1,62±0,06*	0,67±0,03*
Cholesterol + α-tocopherol	2,68±0,08*	2,66±0,11*	1,03±0,06**
α-Tocopherol	2,53±0,02*	2,65±0,18*	1,04±0,03

Legend. *P < 0.02 compared with control, †P < 0.05 compared with group 2. V_{ATP}) Ca-ATPase activity of SR (in μmoles P_i/mg protein/min), V_{Ca}) rate of Ca^{++} transport in SR vesicles (in μmoles Ca^{++}/mg protein/min), Ca/ATP) efficiency of function of Ca-pump of SR [1].

Investigation of functional parameters of the Ca-pump of SR showed that the development of HCh leads to a decrease in Ca-ATPase activity and Ca^{++} transport in SR membranes (Table 3), in agreement with data in the literature [7]. Addition of α-tocopherol to the diet increased the efficiency of working of the SR Ca-pump in HCh.

In the animals of group 4, addition of α-tocopherol to the standard diet had virtually no effect on the rate of ATP hydrolysis or of Ca^{++} accumulation by SR membranes. As might be expected, AOA of the system in the animals of this group was higher than in the control (Table 2).

The results are evidence that disturbance of function of the Ca-pump of SR in HCh is due both to cholesterol accumulation and to intensification of LPO in SR membranes. The rise in the LPO level in HCh is perhaps the result of a fall of AOA of the SR membranes. Since α-tocopherol effectively prevents the disturbance of working of the SR Ca-pump during HCh *in vivo*, whereas the total cholesterol concentration is unchanged under these circumstances, and since the free cholesterol level in SR membranes does not change significantly, it can be tentatively suggested that it is LPO which plays the decisive role in the reduction of Ca-ATPase activity of SR in HCh. However, this does not mean that intensification of LPO in SR membranes in HCh is the primary cause of disturbance of the structural and functional organization of the Ca-pump of SR. Whereas at the whole body level the pathological process is triggered by the raised blood cholesterol concentration, and intensification of LPO in the various tissues [3, 5] is a secondary factor in this case, i.e., it follows initiation of HCh, at the skeletal muscle SR level this sequence of events strictly speaking does not apply, for SR membanes are not in direct contact with the blood stream, and the raised levels of cholesterol and LPO products in the blood can have no direct action on them. Accumulation of cholesterol and intensification of LPO in SR membranes, in particular, are the distinctive response of the muscle cell to the state of HCh in the body as a whole. The question of which of these pathogenic factors is the primary cause of disturbed function of the Ca-pump of SR in HCh still awaits an answer.

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PROTEIN-SYNTHESIZING FUNCTION OF THE LIVER IN RABBITS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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Myocardial infarction gives rise to profound functional and metabolic changes both in heart muscle and in other tissues [4]. Histochemical and biochemical investigations have shown that the most marked changes in the liver during myocardial infarction are observed in the first hours and days of the disease [2]. Disturbance of the protein-synthesizing function in this period is particularly interesting, because the liver plays an important role in the supply of necessary proteins to other organs and tissues and, in particular, to the blood serum. Meanwhile, the molecular mechanisms of the changes in protein biosynthesis observed in the liver in myocardial infarction have virtually not been studied.

The aim of the present investigation was to study the function of the cell-free protein-synthesizing system obtained from the liver of intact rabbits (control) and 6, 12, and 24 h after production of experimental myocardial infarction (EMI).

EXPERIMENTAL METHOD

Rabbits weighing 2.5-3.0 kg were used. The model of EMI consisted of occlusion of the left descending coronary artery [10]. Thoractomy was performed under thiopental anesthesia (40 mg/kg), using sterile instruments. The degree of ischemia was monitored electrocardiographically [10]. The serum albumin level was determined by polyacrylamide gel (PAG) disc electrophoresis [8]. The total serum protein level was determined by the method in [7]. To prepare the cell-free protein-synthesizing system the liver was homogenized in 2.5 volumes of 30 mM HEPES (pH 7.5), containing 0.25 M sucrose, 70 mM KCl, 5 mM magnesium acetate, 0.25 mM EDTA, and 2 mM dithiothreitol. The homogenate was centrifuged for 15 min at 30,000g (unpurified S-30 fraction). Part of this fraction was filtered through Sephadex G-25 gel to remove as much as possible of the endogenous low-molecular-weight compounds (purified S-30 fraction). The standard incubation mixture, in a volume of 100 µl, contained 30 mM HEPES, 0.5 mM ATP, 0.02 mM GTP, 10 mM creatine phosphate (CP), and 2 µg creatine phosphokinase (CPK), each of the unlabeled amino acids (except leucine) in a concentration of 0.02 mM, 0.02 mM [^{14}C]leucine, 5 mM magnesium acetate, 120 mM KCl, 2 mM dithiothreitol, and 1 optical unit (A_{260}-A_{320}) of the purified S-30 fraction. In the case of unpurified S-30 the incubation mixture contained the same components except ATP, GTP, CP, CPK, and unlabeled amino acids.

The kinetic tests showed that to obtain the maximal level of translation, the essential incubation time is 30 min at 37°C. In the case of determination of the velocity of this pro-

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