THE TRANSFERRIN–CERULOPLASMIN ANTIOXIDANT SYSTEM IN EXPERIMENTAL HYPERCHOLESTEROLEMIA

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Keeping animals on a high cholesterol diet is a model of atherosclerosis. However, the presence of cholesterol in food is not the only condition for the development of this disease. Besides hypercholesterolemia, an important role in the development of atherosclerosis also is ascribed to processes of lipid peroxidation (LPO) [1, 3, 9].

An investigation [11] involving fractionation of blood serum showed that two serum proteins possess antioxidant activity (AOA): ceruloplasmin and transferrin. Unlike other serum proteins, the concentrations of ceruloplasmin and transferrin can be estimated by the electron paramagnetic resonance (EPR) method [6].

The aim of this investigation was to use the EPR method to study serum ceruloplasmin and transferrin concentrations in rabbits with experimental hypercholesterolemia and compare the data with serum levels of cholesterol and LPO products.

EXPERIMENTAL METHOD

Male chinchilla rabbits weighing 3-4 kg and aged 9-12 months were used. The rabbits were fed with cholesterol (0.25 g/kg body weight daily) for 3 months. Blood for analysis was taken from the marginal vein of the ear. The serum cholesterol concentration was measured on a "Centrifichem" analyzer. Preparations for EPR-spectrometry were made in the form of tablets, frozen in liquid nitrogen [2]. EPR spectra were recorded on a "Varian E-4" spectrometer under the following conditions: frequency of klystron generator 9.03 MHz, power 10 mW, amplitude of modulation 6.3 G, field scanning speed 250 G/min, time constant of instrument 3.0 sec.

The typical EPR spectrum of rabbit blood serum is shown in Fig. 1. Two signals are illustrated in the spectrum. One, with g = 2.05, belongs to ceruloplasmin, the other, with g = 4.3, belongs to transferrin. The amplitude of the EPR signals was measured, assuming it to be proportional to the concentration of paramagnetic centers.

Concentrations of hydroperoxides in the blood serum were determined by the method in [10]. According to this method, hydroperoxides are decomposed by Fe^{+++} to malonic dialdehyde (MDA) in the presence of ionol. The quantity of MDA was determined in the reaction with 2-thiobarbituric acid, based on measurement of absorption of light at 532 nm. In this investigation the ratios between reagents suggested in [10] were modified, namely the quan-

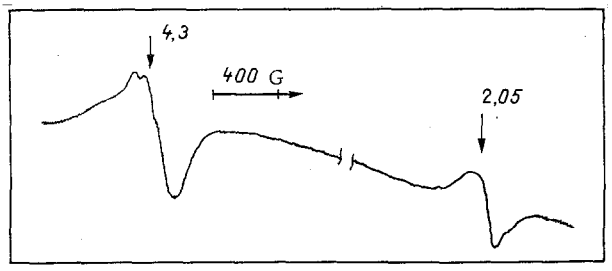

Fig. 1. EPR spectrum of rabbit serum.

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TABLE 1. Intensity of EPR Signals of Transferrin (Tf) and Ceruloplasmin (Cp), Cp/Tf Ratio, and Concentrations of Cholesterol and Hydroperoxides in Blood Serum of Control Rabbits and of Rabbits Fed with Cholesterol ($M \pm m$; $n = 12$)

Parameter studied	Exptl. conditions	
	control	feeding
Transferrin, rel. units	10,3±3,5	7,6±1,9*
Ceruloplasmin, rel. units	1,6±0,4	2,6±1,4**
Cp/Tf	0,17±0,07	0,38±0,2***
Cholesterol, mg %	72,4±25,1	657±281***
Hydroperoxide, μM of MDA	0,114±0,007	0,121±0,007***

Legend. *P < 0.05, **P < 0.025, ***P < 0.001 compared with control.

TABLE 2. Intensity of EPR Signals of Tf and Cp, Ct/Tf Ratio, and Concentrations of Cholesterol and Hydroperoxides in Blood Serum of Conditionally Resistant and Nonresistant Rabbits ($M \pm \sigma$)

Parameter studied	Group of animals	
	resistant ($n=5$)	nonresistant ($n=7$)
Transferrin, rel. units	6,9±2,2	8,3±1,7
Ceruloplasmin, rel. units	3,7±1,2[a]	1,5±0,4[d]
Cp/Tf	0,56±0,18[a]	0,19±0,1[c]
Cholesterol, mg %	380±112[a]	855±108[c]
Hydroperoxide, μM of MDA	0,002±0,007[b]	0,129±0,007[a, c]

Legend. a) P < 0.01, b) P < 0.01 relative to control; c) P < 0.001, d) P < 0.002 relative to resistant group.

tity of the specimen added to each sample was increased fivefold. With this modification it was possible to record a small quantity of LPO products, contained in fresh blood serum, sufficiently accurately. To allow for the effect of scattering of light on the optical density at 532 nm, the absorption spectrum of specimens was recorded in the 400-700 nm region in the chamber for turbid specimens of a "Specord" (East Germany) spectrophotometer.

EXPERIMENTAL RESULTS

The experiments showed that the intensity of the EPR signal of transferrin was reduced in the experimental animals, whereas the intensity of the ceruloplasmin signal, on the other hand, was increased (Table 1). The ratio between the signals of ceruloplasmin and transferrin (Cp/Tf) was changed to the greatest degree in the EPR spectrum. The question naturally arises: What does this ratio reflect, and is it connected with AOA of the proteins studied. It was shown in [8] that AOA of ceruloplasmin is proportional to the concentration of that form of it in which the copper atom is in the oxidized state, and it is in precisely that state that ceruloplasmin possesses paramagnetic properties and is characterized by g = 2.05. Consequently, the intensity of the EPR signal of blood serum with g = 2.05 is proportional to the AOA of ceruloplasmin. The same workers showed [7] that the apotransferrin molecule also possesses AOA. With saturation of apotransferrin with iron, i.e., with an increase in the transferrin concentration, AOA of this protein falls. The transferrin molecule possesses paramagnetic properties. Consequently, the intensity of the EPR signal of blood serum with g = 4.3 is inversely proportional to AOA of the transferrin system. In the presence of physiological concentrations of ceruloplasmin and apotransferrin, each of them in-

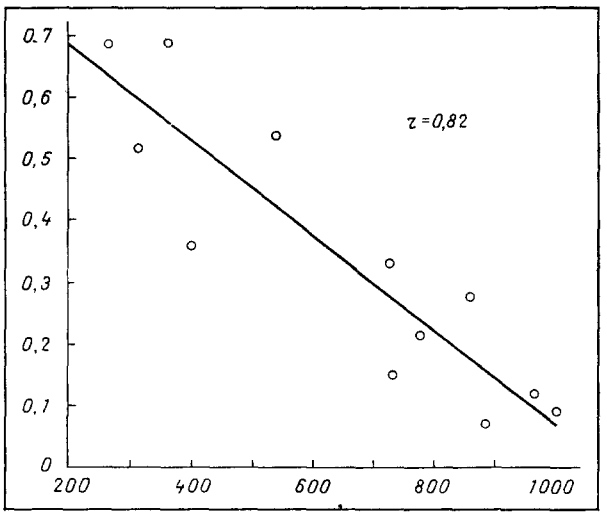

Fig. 2. EPR signal ratio (Cp/Tf) as a function of serum cholesterol concentration of rabbits fed with cholesterol for 3 months. Abscissa, cholesterol concentration (in mg %); ordinate, Cp/Tf (in relative units).

hibits LPO by about 50% [7, 8]. Hence it can be concluded that the Cp/Tf ratio reflects total AOA of these proteins, and an increase in this ratio is evidence of an increase in AOA. It follows from the results that the Cp/Tf ratio was increased in the experimental rabbits to twice its value in the control (Table 1).

Measurement of the serum concentration of hydroperoxides showed that it was increased, although only slightly, in the experimental animals (Table 1). This results is in agreement with data obtained by other workers [5, 12], according to whom LPO processes in the blood serum are intensified during feeding of rabbits with cholesterol.

Three parameters thus undergo a parallel increase in the blood serum of rabbits fed with cholesterol: the cholesterol concentration, AOA, and intensity of LPO. A more detailed analysis of the results, however, shows that there is even closer correlation between the cholesterol level and the Cp/Tf ratio. It will be clear from Table 1 that two parameters — the Cp/Tf ratio and the cholesterol concentration — vary greatly in the group of experimental animals. Despite the fact that all animals were fed with equal doses of cholesterol, by the end of the same period of feeding the plasma cholesterol concentration of some rabbits was 200-400 mg %, whereas in others it was 800-1000 mg %. The same may also be said about the EPR signals, which varied from 0.1 to 0.69; the lower the serum cholesterol, moreover, the higher the ratio between the EPR signals. This may be evidence of negative correlation between these parameters. To discover correlation between them the following graph was constructed: The ratio of the EPR signals was plotted along the ordinate and the serum cholesterol concentration for each experimental rabbit along the abscissa (Fig. 2). A straight line, calculated by the method of least squares for the experimental points, is drawn in Fig. 2. Correlation between the parameters of the graph was found to be negative: with an increase in the EPR signal ratio the cholesterol concentration falls. Depending on the magnitude of these parameters the experimental rabbits as a whole could be divided into two groups. Group 1 included animals with a blood serum cholesterol of between 200 and 600 mg %. These animals had the highest Cp/Tf ratio and, consequently, the highest AOA. Group 2 included animals whose serum cholesterol concentration exceeded 600 mg %. Animals of this group had a low Cp/Tf ratio. Rabbits of group 1 were conventionally described as resistant to cholesterol accumulation, whereas the rabbits of group 2 were nonresistant.

The EPR signal ratio in the animals of group 1 was found to be increased mainly on account of an increase in intensity of the ceruloplasmin signal (Table 2). It can be tentatively suggested that fewer LPO products will accumulate in resistant rabbits, due to elevation of the AOA level, than in nonresistant animals. In fact, the level of LPO products was significantly lower in the resistant than in the nonresistant rabbits (Table 2). The content of LPO products in the resistant rabbits was actually a little lower than in the control (Table 1). Thus LPO processes were not intensified in the resistant rabbits. Hence it follows that elevation of the serum level of LPO products is a phenomenon accompanying hyper-

cholesterolemia and a tendency toward atherosclerosis, as has frequently been demonstrated previously [1, 3, 4, 9]. Elevation of the Cp/Tf ratio and, correspondingly, of the blood serum AOA, however, is evidence of resistance to cholesterol accumulation and to the development of atherosclerosis.

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EFFECT OF CHOLESTEROL ON COOPERATIVENESS OF Ca-ATPase OF THE SARCOPLASMIC RETICULUM OF RABBIT SKELETAL MUSCLES

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Ca-ATPase of the sarcoplasmic reticulum (SR) is an oligomeric allosteric enzyme consisting of several functional units or protomers, Ca-ATPase function is not governed by the traditional Michaelis–Menten kinetics; in particular, an abnormal curve of enzyme activity versus substrate concentration is observed with additional activation at high ATP concentrations [3-5]. According to one hypothesis, the nature of the allosteric effect of ATP can be explained by the presence of a special allosteric center [14, 15]. More recently, however, data have been published to show that cooperative interactions existing between the protomers of Ca-ATPase may be modified in the course of function so that the hydrolytic center of one protomer becomes the allosteric center of the other [1]. It is quite possible that hydrolytic and transport functions of this enzyme are controlled by a change in cooperative interactions between the protomers of Ca-ATPase. It is accordingly interesting to study factors influencing the cooperativeness of interaction between Ca-ATPase protomers and, in particular, modifiers of the phase state of the phospholipid environment of Ca-ATPase [2].

The aim of this investigation was to study the effect of cholesterol, one regulator of the phase state of membrane lipids, on cooperativeness of interaction between Ca-ATPase protomers, using ATP and UTP as substrate.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male chinci-la rabbits weighing 2.5-3 kg. To increase the cholesterol concentration in SR membranes nine experimental animals were kept on a diet to which cholesterol was added in a dose of 1 g/kg body weight for 1, 3, and 6 months [8, 11]. The SR fraction was isolated from white muscles of the hind limbs of the rabbits

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