Dexamethasone prevents the specific effect of exogenous PGE_2 on the circulating adrenalin level, probably due to its direct intervention at the synaptic level, and also with compensatory processes developing in the dogs with inhibited activity of their HHAS. Processes of temporary adaptation in old animals compared with middle-aged [9] are accompanied by predominance of the cholinergic phase over the adrenergic in all extremal situations.

LITERATURE CITED

1. A. I. Balakleevskii, Lab. Delo, No. 3, 151 (1976).
2. V. V. Men'shikov, Methods of Clinical Biochemistry of Hormones and Mediators, 3rd edition [in Russian], Moscow (1974).
3. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981).
4. S. M. Ksents, S. A. Khoreva, T. G. Ol'shanskaya, et al., in: Endocrine Mechanisms of Regulation of Adaptation to Muscular Activity [in Russian], Tartu (1977), pp. 63-67.
5. M. N. Ostroumova, Probl. Éndokrinol., No. 5, 57 (1978).
6. V. M. Rozental', Probl. Éndokrinol., No. 6, 69 (1969).
7. V. V. Frol'kis, in: Age Physiology [in Russian], Leningrad (1975), pp. 375-404.
8. S. A. Khoreva, T. G. Ol'shanskaya, and T. I. Shustova, in: Proceedings of an All-Union Symposium on Prostaglandins and the Circulation [in Russian], Erevan (1980), pp. 74-76.
9. S. A. Khoreva and T. G. Ol'shanskaya, Fiziol. Zh. SSSR, No. 10, 1356 (1982).
10. I. R. Chellis, G. D. Carson, and F. Najtolin, J. Endocrinol., 76, 177 (1978).
11. S. Feldman, N. Conforti, and I. Chorvers, Acta Endocrinol. (Copenhagen), 73, 660 (1973).
12. J. Gutman, in: Neurotransmitters, Paris (1978), pp. 217-229.
13. P. O. Hedgvist, Biochem. Soc. Trans., 6, 714 (1978).
14. K. V. Honn and W. Chavin, Life Sci., 22, 543 (1978).
15. B. Koch, B. Bucher, and C. Mialhe, Neuroendocrinology, 15, 365 (1974).

SPIN-LABEL STUDY OF THE STRUCTURE OF SKELETAL MUSCLE SARCOPLASMIC RETICULUM Ca-ATPase OF RABBITS WITH HYPERCHOLESTEROLEMIA

A. A. Timofeev, L. V. Stoida, O. A. Azizova, A. G. Maksina, and G. V. Chernysheva

UDC 06.153.922-008.61-07:616.74-008.931-092.9

KEY WORDS: Ca-ATPase; sarcoplasmic reticulum; skeletal muscles; cholesterol; spin label.

Alimentary hypercholesterolemia (HCh) in rabbits, leading to a persistently high plasma cholesterol (Ch) concentration, is known to cause pathological changes in the membranes of various organs and tissues [1, 8, 9]. It has been shown, for instance, that in HCh there is a substantial disturbance of membrane-bound enzyme function [7, 8]. In particular, in a study of the Ca-pump of rabbit skeletal muscles during HCh, enzyme activity of Ca-ATPase was found to be lowered and passive outflow of Ca^{++} from vesicles of the sarcoplasmic reticulum (SR) was increased [7, 10]. Accordingly the study of the molecular mechanisms of these disturbances is very interesting.

The object of this investigation, conducted by the spin-label method, was to study correlation between structural changes and disturbances of Ca-ATPase function in skeletal muscle SR of rabbits with HCh.

Laboratory of Molecular Pathology and Biochemistry, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Department of Biophysics, Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 11, pp. 59-62, November, 1983. Original article submitted March 9, 1983.

© 1984 Plenum Publishing Corporation

TABLE 1. Functional Parameters of SR Ca-ATPase during HCh (M ± m)

Experimental conditions	Ca-ATPase activity	Rate of accumulation of Ca^{++}	Ca/ATP	Ch concentration, μg/mg protein
Control	4,84±0,50	4,92±0,46	1,10±0,06	31,1±4,1
Cholesterol				
1 month	2,77±0,13**	1,55±0,06*	0,57±0,03*	58,9±1,7*
3 months	2,27±0,10*	1,39±0,05**	0,66±0,03**	60,9±2,3**

Legend. Activity of SR Ca-ATPase expressed in micromoles PI/min/mg protein, accumulation of Ca^{++} in micromoles Ca^{++}/min/mg protein. Ca/ATP denotes coefficient of efficiency of SR Ca-pump. *$P < 0.001$, **$P < 0.01$.

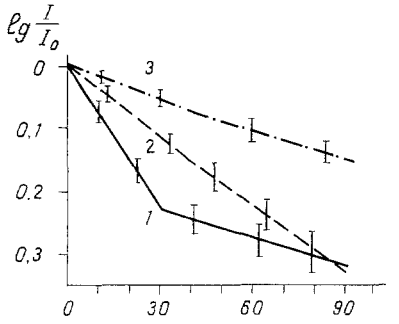

Fig. 1. Kinetics of binding of maleimide spin label with thiol groups of SR Ca-ATPase. Temperature 22°C, pH 7.0, ratio of concentrations of label to protein 2:1. Abscissa, time (in min); ordinate, log of amplitude of low-field component of EPR spectrum of spin label not bound with protein, normalized for amplitude in initial time period. 1) control, 2) Ch (1 month), 3) Ch (3 months).

EXPERIMENTAL METHOD

Sixteen male chinchilla rabbits weighing 2.5-3 kg were used. HCh was induced in 11 rabbits by keeping them on a diet with the addition of Ch (1 g/kg body weight) for 1 month (5 rabbits) and 3 months (6 rabbits). The SR fraction was obtained by differential centrifugation [6]. The rate of Ca^{++} transport and the ATPase activity of SR were recorded pH-metrically [2]. The Ch concentration in the SR membranes was determined by a fluorescence method [13], and the protein concentration by Lowry's method. The spin label used was 4-maleimido-2,2,6, 6-tetramethylpiperidine-1-oxyl, which reacts with thiol groups of proteins. Modification of SR Ca-ATPase by the spin label was carried out with constant mixing in medium containing 1 M sucrose, 25 mM imidazole-HCl, pH 7.0, at 4-5°C for 12-14 h, and the ratio of label to protein was 5:1. Binding of label with protein was judged by the reduction in amplitude of the low-field component of the SPR spectrum of the free (not bound with protein) label. EPR spectra were recorded on the Varian E4 EPR Spectrometer (USA). The spin correlation time (τ) and the hydrophobicity (h) of the microenvironment of the spin label were measured in medium containing 1 M sucrose and 25 mM imidazole-HCl, pH 7.0, at 22°C. Values of τ were estimated by the method described in [4], and those of h were calculated by the method in [3, 5].

EXPERIMENTAL RESULTS

Development of HCh was shown to be accompanied by accumulation of Ch in the SR membranes. A decrease in the rate of hydrolysis of ATP, the rate of accumulation of Ca^{++}, and the efficiency of operation of the Ca-pump, with a decrease in the Ca/ATP ratio were observed (Table 1).

TABLE 2. Spin Correlation Time (τ) and Parameter of Hydrophobicity (h) of Spin Labels Bound with Thiol Groups of SR Ca-ATPase (M ± m)

Experimental conditions	τ, $\times 10^{-7}$	h, relative units
Control	3,11±0,18	0,62±0,04
Cholesterol		
1 month	9,75±0,66*	0,80±0,02*
3 months	6,37—0,31*	0,72—0,01**

Legend. *$P < 0.001$, **$P < 0.01$.

To study the effect of Ch accumulation on the structure of the Ca-ATPase proteolipid complex the kinetics of binding of the spin label with protein SH-groups was studied. The results showed (Fig. 1) that the kinetic curves have two regions that correspond in their reactivity with thiol reagents to the two types of SH-groups of Ca-ATPase (reacting quickly and slowly with the spin label). These results agree with data in the literature [11, 12]. The rate of binding of the spin label in SR preparations from animals with HCh with slowly reacting groups was practically the same as normally. For quickly reacting groups (the initial region of the curves) the rate of binding of the spin label with SH-groups of Ca-ATPase fell substantially below normal during the development of HCh. With an increase in the Ch concentration in the SR membranes, the structure of the membranes evidently changes so that either the total number of thiol groups on the surface of the Ca-ATPase is reduced or the accessibility of the SH-groups for the spin labels is reduced. A change in the actual appearance of the kinetic curves must also be noted: during the development of HCh the curves become straighter, and differences between quickly and slowly reacting SH-groups disappear. The reason for this may be structural changes affecting the proteolipid Ca-ATPase complex.

Functioning of the Ca-pump is known to depend on mobility of the Ca-ATPase molecule and its definite position in the membrane. To study how mobility and position of definite regions of the enzyme change in the SR membrane, changes in the spin correlation time (τ) and the parameter of hydrophobicity (h) of the maleimide spin label, attached to the protein SH-group, were studied. As Table 2 shows, mobility of the spin label rigidly bound to SH-groups of Ca-ATPase decreases in HCh (τ increases), and the hydrophobicity of its microenvironment also increases. These changes may be evidence that, besides a decrease in mobility of the protein fragment to which the label is bound, changes also take place in its position in the membrane, so that the thiol groups are in a less polar environment.

For a more detailed investigation of the localization and position of the spin-labeled thiol groups, accessibility of the spin label to paramagnetic ions of different sign, namely, potassium ferricyanide and $NiCl_2$, and also to sodium ascorbate was studied. In HCh the accessibility of the spin labels to the negatively charged ferricyanide ion and ascorbate in the SR preparations was lower than normally (Figs. 2b and 3). These findings confirm the conclusion that the position of the fragment of the Ca-ATPase molecule in the membrane changes. The Ni^{++} ion widens the EPR spectrum by an equal degree under normal conditions and during HCh (Fig. 2a). During HCh developing by the first month of feeding rabbits with Ch, the absolute Ch content in the SR membranes was twice the normal level and it remained at practically the same level until the end of the third month of feeding (Table 1). However, the structural characteristics recorded by spin labels differ significantly at different stages of HCh. The differences in the structural parameters observed after 1 and 3 months of HCh may perhaps be due not only to accumulation of Ch, but also to the development of lipid peroxidation in the SR membranes.

The experiments thus showed that HCh in rabbits is accompanied by accumulation of Ch in SR membranes, by disturbance of functioning of the Ca-pump, and by changes in the structure of the proteolipid Ca-ATPase complex. What is the mechanism of the processes causing a change in structure of the membranes, and how are changes in the structural organization of the SR membranes connected with disturbance of the functioning of the Ca-pump at the molecular level? As was shown above, during HCh the mobility of some fragments of Ca-ATPase is reduced. Since

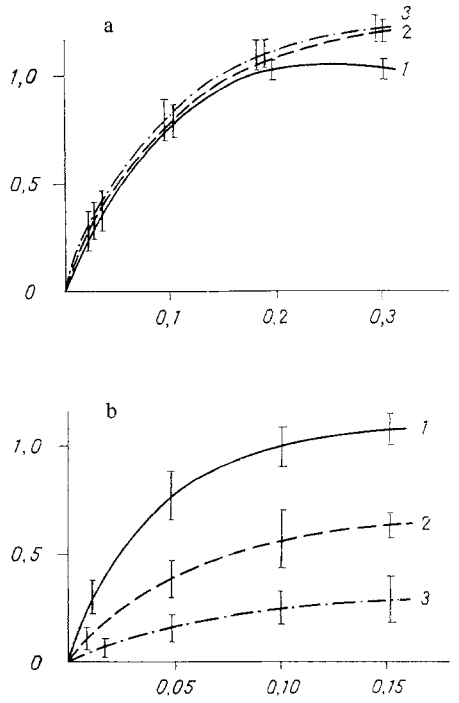

Fig. 2

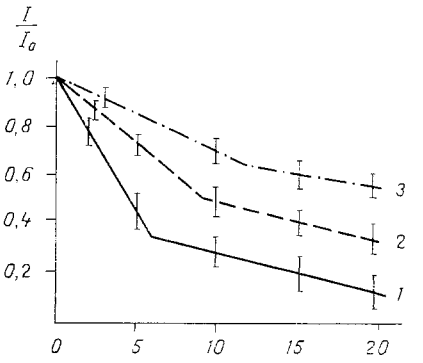

Fig. 3

Fig. 2. Changes in width of low-field component of EPR spectrum of spin labels bound with SR Ca-ATPase under the influence of paramagnetic ions. Temperature 22°C. Abscissa, concentration of paramagnetic ion in test: a) nickel chloride, b) potassium ferricyanide (in moles); ordinate, ½ width at ½ height of low-field component of EPR spectrum (in sec). Remainder of legend as to Fig. 1.

Fig. 3. Kinetics of quenching of low-field component of EPR spectrum of spin labels bound with SR Ca-ATPase and with sodium ascorbate. Temperature 4°C, concentration of sodium ascorbate 20 times higher than protein concentration. Ordinate, amplitude of low-field component of EPR spectrum normalized for amplitude in initial time period. Remainder of legend the same as to Fig. 1.

Ca-ATPase is an integral protein and is surrounded by membrane lipids, the decrease in its mobility may be due to an increase in the viscosity of the lipid bilayer as a result of Ch accumulation. In addition the position of the spin-labeled fragment of the Ca-ATPase molecule in the membrane changes. Probably as a result of the change in conformation of the enzyme molecule during HCh the spin-labeled fragment is buried in the bilayer and, as a result, the mobility of the label is reduced and the hydrophobicity of its environment is increased. Aggregation of individual ATPase molecules in the plain of the membrane may lead to similar consequences. The change in the position of the enzyme in the molecule may lead to a disturbance of protein–lipid interactions and, as a result, to a reduction in the efficiency of the Ca-pump.

LITERATURE CITED

1. P. G. Bogach, L. K. Finagin, and Z. N. Miroshnichenko, in: Biochemistry of Animals and Man [in Russian], Vol. 3, Kiev (1979), p. 39.
2. A. A. Boldyrev, A. V. Lebedev, and V. B. Ritov, Biokhimiya, 34, 119 (1969).
3. G. V. Gyul'khandanyan, "A study of globular proteins and their interactions with biologically active substances by the spin probe method," Author's Abstract of Candidate's Dissertation, Moscow (1976).
4. V. A. Livshits, Teor. Éksp. Khim., 13, 863 (1977).
5. A. N. Kuznetsov, The Spin Probe Method [in Russian], Moscow (1976).
6. V. B. Ritov, V. I. Mel'gunov, P. G. Komarov, et al., Dokl. Akad. Nauk SSSR, 233, 730 (1977).
7. L. V. Stoida and A. A. Boldyrev, Byull. Éksp. Biol. Med., No. 7, 32 (1978).
8. T. I. Torkhovskaya, L. G. Artemova, B. G. Khodzhakuliev, et al., Byull. Éksp. Biol. Med., No. 6, 675 (1980).

9. G. V. Chernysheva, G. G. Amarantova, et al., in: Current Problems in General Pathology and Pathological Physiology [in Russian], Moscow (1978), p. 43.
10. G. V. Chernysheva, L. V. Stoida, and I. L. Kuz'mina, Byull. Éksp. Biol. Med., No. 3, 292 (1980).
11. H. Yoshida and Y. Tonomura, J. Biochem. (Tokyo), 79, 649 (1976).
12. B. P. Yu, E. J. Masoro, J. Downs, et al., J. Biol. Chem., 252, 5262 (1977).
13. H. Huang, J. W. Kuan, and G. G. Guilbault, Clin. Chem., 21, 1605 (1975).