The increase in the concentration of glutamine – the principal ammonia carrier in the myocardium – during perfusion with ammonium acetate may be due to activation of glutamine synthetase under the influence of ammonia (but to a negligible degree), by an increase in proteolysis, inhibition of protein synthesis [8], inhibition of glutaminase, and a decrease in the liberation of glutamine into the perfusion fluid, although this effect of ammonia was not confirmed by the results obtained in [4]. Further experiments are required to assess the contribution of the above-mentioned factors more definitely.

The results with respect to synthesis of amino acids from ammonia-^{15}N are evidence that ammonia is neutralized in the myocardium in the same ways as the corresponding processes in the liver, brain, and muscles, although changes in the concentration of these compounds in the heart during perfusion with ammonium acetate are determined to a greater degree by other causes. Ammonia metabolism in necrosis of heart muscle is substantially modified, and this calls for further study.

LITERATURE CITED

1. E. I. Chazov, V. N. Smirnov, A. V. Mazaev, et al., Kardiologiya, No. 2, 11 (1972).
2. V. N. Smirnov, G. B. Asafov, N. M. Cherpachenko, et al., Circulat. Res., 34-35, Suppl. III, 58 (1974).
3. V. Brodan, I. Fabian, I. Pechar, et al., Cas. Lek. Ces., 114, 988 (1975).
4. S. Davidson and E. H. Sonnenblick, Cardiovasc. Res., 9, 295 (1975).
5. T. Kato, Jpn. Circulat. J., 32, 1401 (1968).
6. A. Takahashi, Jpn. Circulat. J., 31, 581 (1967).
7. W. Torn and I. Heimann, J. Neurochem., 2, 166 (1958).
8. T. Watanabe, Jpn. Circulat. J., 32, 1811 (1968).

CALCIUM TRANSPORT AND ATPase ACTIVITY IN MITOCHONDRIA AND FRAGMENTS OF SARCOPLASMIC RETICULUM OF THE HEART AND MUSCLES OF RABBITS WITH HYPERCHOLESTEREMIA

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Keeping rabbits on a high-cholesterol diet (1 g/kg) for 3-7 months led to an increase in cholesterol concentration in the mitochondrial membranes and fragments of the sarcoplasmic reticulum (SPR) of the myocardium and skeletal muscles. Saturation of the membranes with cholesterol led to a decrease in efficiency of the Ca-pump of the SPR, as reflected in lowering of the Ca/ATP ratio and an increase in the outflow of Ca^{++} from the SPR. Under these conditions the rate of accumulation of Ca^{++} was higher in SPR than in the mitochondria. Activity of mitochondrial Mg^{++}-activated 2,4-DNP-ATPase was reduced in hypercholesteremia.

KEY WORDS: calcium transport; Ca-ATPase; mitochondria; fragments of sarcoplasmic reticulum; heart; skeletal muscle; hypercholesteremia.

Investigations have shown the role of calcium ions in reactions of carbohydrate, lipid, and protein metabolism and in the processes of muscular contraction and oxidative phosphorylation. Accordingly the study of Ca^{++} transport through subcellular membranes is of special interest. One function of Ca^{++} is to regulate muscle contraction and relaxation. The sarcoplasmic reticulum (SPR) of muscles plays a leading role in this process [7, 8]. However,

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TABLE 1. Cholesterol Concentration in Heart, Skeletal Muscles, and Blood of Rabbits on High-Cholesterol Diet (M±m)

Experimental conditions	Time of investigation, months	Heart		Skeletal muscle	Blood, mg %
		mitochondria	microsomes	SPR	
		mg cholesterol/mg protein			
Normal	—	0,048±0,008	0,041±0,007	0,03	71±11
Hypercholesteremia	3	0,24±0,01*	0,16±0,005*		1250±77*
	7	0,17±0,012*	0,14±0,004*	0,15±0,006*	485±15*

Legend. Here and in Tables 2 and 5, asterisk denotes significance of differences between values at $P<0.01$ level.

TABLE 2. Microsomal ATPase Activity (in μmoles p_{inorg}/mg protein/min) of Rabbit Heart (M±m)

Experimental conditions	Microsomal fraction	Ca^{2+}-ATPase		Mg^{2+}-ATPase
Normal	Total	0,51±0,04		0,18±0,03
	Light	0,33±0,02		0,38±0,02
	Heavy	0,24±0,04		0,28±0,03
		3 months	7 months	7 months
Hypercholesteremia	Total	0,62±0,03	0,64±0,02*	0,34±0,01*
	Light	0,40±0,02*	0,48±0,03*	0,35±0,02
	Heavy	0,30±0,02	0,23±0,03	0,45±0,02*

evidence has recently been obtained that, besides SPR, mitochondria, with high calcium capacity, are also concerned in regulation of the Ca^{++} concentration in the cytoplasm [5, 6, 10, 12].

With these data in mind it was decided to study Ca^{++} transport and activity of transport ATPases in mitochondria and in fragments of SPR isolated from the heart and skeletal muscles of rabbits during the development of hypercholesteremia, i.e., under conditions when the introduction of exogenous cholesterol into membranes of subcellular fractions modifies their lipid composition and density [15].

EXPERIMENTAL METHOD

Experiments were carried out on 28 rabbits. Hypercholesteremia was induced by adding cholesterol (1 g/kg) to the diet and its development was assessed by determining the blood cholesterol concentration. The microsomal fraction of the heart, consisting mainly of fragments of SPR, was obtained as described in [3] and divided into subfractions by the known method [11]. Mitochondria were isolated by differential centrifugation in 0.25 M sucrose and 0.01 M EDTA. SPR fragments were obtained from rabbit hind limb muscles [2]. The calcium-accumulating capacity and Ca^{++}-ATPase activity were measured by a pH method [2]. Mg^{++}-activated 2,4-DNP-ATPase was determined by two methods [3, 4]. The phosphorus [13] and protein [14] concentrations were measured. Lipids of the subcellular membranes were extracted [9] and then fractionated by thin-layer chromatography [1], and the cholesterol content was determined. The ATP, albumin, and histidine used were from Reanal, sodium deoxycholate from Palfa, and the imidazole and sodium oxalate from Merck.

EXPERIMENTAL RESULTS

The highest blood cholesterol level was found in rabbits 3 months after the beginning of cholesterol feeding. By 7 months the cholesterol concentration had fallen slightly. The same trend of cholesterol incorporation also was observed in the mitochondrial and microsomal fractions of the heart. The cholesterol concentration after 3 months on the high-cholesterol diet was increased fivefold in the mitochondria and fourfold in the microsomes (Table 1).

Determination of Ca^{++}- and Mg^{++}-dependent ATPase in the cardiac microsomes showed that activity in the control animals was concentrated mainly in the light microsomal fraction.

TABLE 3. Changes in ATPase Activity and Ca^{++} Transport of Heart SPR in Hypercholesteremia (M±m)

Parameters at 26 °C	Normal	Hypercholesteremia
V_{ATP}, μmoles P_{inorg}/mg·min	0,59±0,02	3,80±0,11*
V_{Ca}, μmoles P_{inorg}/mg·min	0,707±0,03	2,80±0,08*
Ca/ATP	1,29±0,05	0,74±0,03*

Legend. Here and in Table 4: Medium contained: 100 mM KCl, 2 mM ATP, 2 mM $MgCl_2$, 4 mM imidazole, 1.5-6 mM sodium oxalate; pH 7.2-7.4. Asterisk indicates significance of differences between values at the $P<0.05$ level.

TABLE 4. Changes in ATPase Activity and Ca^{++} Transport of Skeletal Muscle SPR of Rabbits with Hypercholesteremia (M±m)

Parameters at 20°C	Normal	Hypercholesteremia, % of normal	
		3 months	7 months
V_{ATP}, μmoles P_{inorg}/mg·min	0,34±0,01	+74,4±3,8	+211±16
V_{Ca}, μmoles P_{inorg}/mg·min	0,65±0,02	—	+53,8±3,4
Ca/ATP	1,90±0,09	—	−52,6±4,2
V_{in}, μmoles P_{inorg}/mg·min	0,63±0,04	+63,4±4,4	+211,7±7,2
V_{out}, μmoles P_{inorg}/mg·min	0,20±0,01	+115±8,1	+150,0±12

TABLE 5. Changes in Ca^{++} Transport and Activity of 2,4-DNP-Stimulated Mg^{++}-ATPase of Heart Mitochondria (M±m) in Hypercholesteremia (7 months)

Experimental conditions	Rate of accumulation of Ca^{++}, μ moles Ca/mg/min	H^+/Ca^{2+}	Mg^{++}-ATPase	2,4-DNP-ATPase
		μmoles pinorg/mg protein/min		
Normal	0,57±0,02	0,88±0,03	0,95±0,05	1,62±0,08
Hypercholesteremia	0,76±0,03	0,70±0,04*	0,56±0,03*	0,63±0,04*

Legend. Medium for determining Ca^{++} transport contained: 150 mM sucrose, 75 mM KCl, 5 mM KH_2PO_4, 2.5 mM $MgCl_2$, 10 mM succinate, 5 mM rotenone; pH 7.4; protein 3-6 mg.

When the rabbits were put on a high-cholesterol diet the increase in Ca^{++}-ATPase activity took place on account of the light microsomal fraction, in which SPR is mainly concentrated. Mg^{++}-ATPase activity was increased only in the heavy fraction (Table 2).

The results of the pH-metric investigation showed that the rate of hydrolysis of ATP by Ca^{++}-ATPase (V_{ATP}) was increased by a greater degree in hypercholesteremia than the rate of accumulation of Ca^{++} (V_{Ca}). As a result of this the efficiency of work of the SPR calcium pump, calculated (Table 3) as the ratio between the rate of Ca^{++} accumulation and the rate of ATP hydrolysis (Ca/ATP), was reduced.

Comparison of these results with those obtained for SPR isolated from skeletal muscles showed the same general rule (Table 4). To analyze the data, the rate of inflow and outflow of calcium from the vesicles was determined by the equations:

$$V_{in} = V_{hydr} \times Ca/ATP;$$
$$V_{out} = V_{hydr} \times Ca/ATP_{max} - Ca/ATP_{ex}$$

As Table 4 shows, not only the rate of inflow of calcium increased, but also the rate of its outflow, and by an even greater degree. This caused a decrease in efficiency of work of the SPR calcium pump during hypercholesteremia.

Determination of active Ca^{++} transport in mitochondria isolated from heart muscle after 7 months on a high-cholesterol diet showed an increase of 33% in the rate of calcium accumulation and a decrease in the stoichiometric H^+/Ca^{++} ratio. Activity of Mg^{++}-ATPase and the degree of its stimulation by 2,4-DNP were reduced under these circumstances (Table 5).

Introduction of exogenous cholesterol into membranes and mitochondria and SPR of the heart and muscles thus activates Ca^{++} transport into the organelles and raises the SPR Ca^{++}-ATPase level. However, the intensity of work of the SPR calcium pump was reduced because of a marked increase in outflow of Ca^{++} from the vesicles. Comparison of the rate of calcium accumulation by SPR and mitochondria showed higher values for SPR.

Increased accumulation of Ca^{++} by the mitochondria of the heart creates a situation linked with a decrease in the intensity of oxidative phosphorylation [4] and, as a result, with a decrease in provision for contractile activity of the heart in the presence of a marked degree of hypercholesteremia. This conclusion is supported by the results showing a decrease in the stimulating effect of 2,4-DNP on Mg^{++}-ATPase, the action of which is directed toward mitochondria with a high level of respiration, not coupled with phosphorylation.

The results are evidence that marked degrees of hypercholesteremia, by modifying the structure of subcellular membranes, lead to disturbances of permeability and of the energy supply for heart and muscle cells.

LITERATURE CITED

1. A. A. Akhrem and A. I. Kuznetsova, Thin-Layer Chromatography [in Russian], Moscow (1964).
2. A. A. Boldyrev, in: Transport Adenosinetriphosphatases [in Russian], Moscow (1977), pp. 21 and 69.
3. D. O. Levittskii, in: Transport Adenosinetriphosphatases [in Russian], Moscow (1977), p. 36.
4. Yu. N. Leikin, "Transport of calcium ions in liver mitochondria," Author's Abstract of Candidate's Dissertation, Moscow (1972).
5. A. L. Lehninger, in: Metabolism of the Myocardium [Russian translation], Moscow (1975), p. 178.
6. E. Carafoli, K. Dabrowska, and F. Grovetti, Biochem. Biophys. Res. Commun., 62, 980 (1975).
7. S. Ebashi and M. Endo, Prog. Biophys. Molec. Biol., 18, 123 (1968).
8. F. Fuchs, Annu. Rev. Physiol., 36, 461 (1974).
9. J. Folch and M. Lees, J. Biol. Chem., 226, 497 (1957).
10. T. Kitazawa, J. Biochem. (Tokyo), 80, 1129 (1976).
11. A. M. Katz and D. I. Repke, Circulat. Res., 21, 153 (1967).
12. A. M. Lehninger and E. Carafoli, Arch. Biochem., 143, 506 (1971).
13. O. H. Lowry and J. A. Lopez, J. Biol. Chem., 162, 427 (1946).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
15. D. Papahadyopoulos, J. Theor. Biol., 43, 329 (1974).