EFFECT OF STIMULATION OF THE HYPOTHALAMIC NUCLEI ON LIPID CONCENTRATION AND ATPase ACTIVITY IN MITOCHONDRIAL AND MICROSOMAL FRACTIONS OF THE RABBIT HEART

G. V. Chernysheva, L. N. Lebedeva,
G. G. Amarantova, and N. M. Dmitrieva

UDC 612.173.1.015.1-06:612.826.4

Stimulation of the ventromedial nuclei of the hypothalamus led to a decrease in the concentration of phospholipids and cholesterol in the mitochondria of the rabbit heart. Cholesterol feeding combined with stimulation increased the cholesterol concentration but reduced the phospholipid concentration still more. Activity of Ca^{2+}-ATPase was reduced in mitochondria and microsomes isolated from the heart. A high cholesterol diet without stimulation of the hypothalamic nuclei caused different changes. These results indicate that the action of exogenous cholesterol depends on the state of the mechanisms regulating myocardial metabolism.

KEY WORDS: heart; hypothalamic nuclei; experimental atherosclerosis; mitochondrial lipids; ATPase activity.

The study of the influence of hypothalamic structures of the brain on the lipid metabolism of the myocardium is essential to the understanding of the mechanisms of disturbance of cardiac activity in coronary atherosclerosis. Research in this field has dealt mainly with the effect of coagulation [5] or stimulation [4, 13] of the hypothalamic nuclei on the blood cholesterol level. However, in hypercholesteremia the ratio between the lipid components changes not only in the blood, but also in subcellular fractions of organs and tissues [10, 19]; this leads to changes in the structure, the enzyme activity, and the transport function of the membranes of intracellular organelles [9, 11] and it may be the cause of disturbance of the function of the organ as a whole.

The object of this investigation was to study the effect of stimulation of the ventromedial (VM) nuclei of the hypothalamus and stimulation of the hypothalamic nuclei combined with cholesterol feeding on the mitochondrial lipids of the heart. The ATPase activity of mitochondria and microsomes isolated from the heart was used as the index of the state of the transport systems.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits weighing 2-2.5 kg, divided into four groups: eight control rabbits, five with stimulation of the hypothalamic nuclei, five with stimulation of the hypothalamus combined with cholesterol feeding (1 g cholesterol/kg body weight daily), and seven with cholesterol feeding only. The VM hypothalamic nuclei were stimulated through previously implanted electrodes inserted stereotaxically. Square pulses (90 Hz, 1 msec, 2.5-5 V) were used. The duration of the experiments in all series was 30 days. The rabbits were decapitated and mitochondria isolated from the heart [14] in 0.25 M sucrose, 0.01 M EDTA, pH 7.4. Lipids were extracted [12] and fractionated by thin-layer chromatography [2]. ATPase activity was measured from the increase in the inorganic phosphorus concentration [15]. The incubation medium contained (in mM): ATP-Na 5, KCl 15.0, $MgCl_2 \cdot 7H_2O$ 1.0, sucrose 120, in 0.05 M Tris-HCl buffer, pH 7.4. The protein concentration in the sample was 0.2-0.5 mg. The microsomal fraction was isolated from the eluates after removal of the mitochondria by centrifugation for 1 h at 105,000 g (the MSE Superspeed-60 centrifuge). Contractile proteins were removed from the microsomal fraction with 0.6 M KCl in 0.005 M histidine buffer, pH 7.2, at 2°C. The residue washed to remove KCl was used for determination of ATPase activity. The composition of the incubation medium (volume 1 ml) was: 50-100 μg microsomal protein, 4 mM ATP-Na, 5 mM $MgCl_2$,

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 23-25, January, 1978. Original article submitted April 7, 1977.

 0007-4888/78/8501-0026$07.50

TABLE 1. Lipid Concentration in Mitochondria of Rabbit Heart (M ± m)

Experimental conditions	Phospholipids, μg P/mg protein	Cholesterol, μg/mg protein		Cholesterol/ phospholipids	Blood cholesterol, mg%
		free	esterified		
Control	109,0±4,3	10,5±0,44	6,3±0,21	0,096±0,008	45,0±1,5
Stimulation of VM hypothalamic nuclei	88,1±5,8	7,2±0,18	2,5±0,1	0,082±0,01	52,8±3,3
P	<0,02	<0,02	<0,01		
Stimulation of VM nuclei + cholesterol feeding	69,5±3,3	16,4±2,3	15,5±2,6	0,23±0,007	238±5,2
P	<0,01	<0,01	<0,01	<0,001	<0,001
Cholesterol feeding	92,7±4,0	24,0±3,0	18,1±2,6	0,28±0,01	430±14
P		<0,01	<0,01	<0,01	<0,01

TABLE 2. ATPase Activity (in μmoles P/mg protein/h) of Mitochondria and Microsomes of Rabbit Heart (M ± m)

Experimental conditions	Mitochondria		Microsomes	
	Ca^{2+}-ATPase	Mg^{2+}-ATPase	Ca^{2+}-ATPase	Mg^{2+}-ATPase
Control	29,2±1,1	33,9±0,8	22,6±1,4	23,5±1,1
Stimulation of VM hypothalamic nuclei	25,1±0,77	33,5±0,45	20,0±0,4	21,0±0,22
Stimulation of VM nuclei + cholesterol feeding	19,6±0,8	28,7±1,2	18,5±0,5	25,0±0,4
P	<0,01		<0,02	
Cholesterol feeding	26,2±0,79	37,5±0,93	29,3±0,31	24,7±0,3
P			<0,01	

5 mM $CaCl_2$, 50 mM Tris-HCl buffer, pH 7.4. The protein concentration was determined by the method of Lowry et al. [16]. The severity of the atherosclerosis was judged from changes in the intramural vessels and by the atherosclerotic index of damage to the aorta [1]. The results were subjected to statistical analysis [8].

EXPERIMENTAL RESULTS AND DISCUSSION

Stimulation of the VM hypothalamic nuclei led to a decrease in the concentration of phospholipids and of free and esterified cholesterol in the mitochondria of the rabbit heart. The cholesterol/phospholipids ratio was reduced a little. The blood cholesterol level remained normal (Table 1). Morphologically, dystrophic changes reflecting disturbances of carbohydrate and protein metabolism were observed in the heart. Other evidence of such disturbances was given by dystrophic changes in the ECG, irrespective of which hypothalamic structures were injured [3]. Dystrophic changes evidently developed also in other organs and tissues, for the body weight of the experimental animals decreased considerably [5].

Feeding cholesterol to the rabbits undergoing stimulation of the hypothalamic nuclei lowered the phospholipid concentration still further. The free cholesterol level rose by 61% and the esterified cholesterol level was more than doubled. The cholesterol/phospholipids ratio in the mitochondria increased correspondingly. The blood cholesterol concentration rose (Table 1).

Cholesterol feeding of the animals by itself for 30 days led to only an insignificant decrease in the phospholipid concentration in the mitochondria of the heart. However, the level of free and esterified cholesterol was doubled or trebled (Table 1), in agreement with results obtained by other workers [19].

Exogenous cholesterol, administered in large doses, thus differed in its effect on the quantity and relative proportions of the lipids in the mitochondria of the heart depending on the functional state of the organ.

With a combination of stimulation of the hypothalamic nuclei and cholesterol feeding, activity of Ca^{2+}-ATPase in the mitochondria and microsomes of the heart fell. The action of a high cholesterol diet alone increased Ca^{2+}-ATPase activity. Considering that the ATPase of the sarcoplasmic reticulum, which is activated by calcium ions, is a calcium-transporting enzyme [17, 18], the changes observed in the Ca^{2+}-ATPase of the microsomes, which consist chiefly of proteins of the sarcoplasmic reticulum [17, 18], could indicate a reduction in the work of the calcium pump during combined stimulation of the hypothalamic nuclei and cholesterol feeding and an increase in the work of that pump in the early stages of development of atherosclerosis. This

conclusion is supported by the results of investigations which demonstrated activation of protein synthesis and an increase in the activity of several enzymes in atherosclerosis [6, 7].

The ATPase of the mitochondrial and microsomal fractions, which is activated by magnesium ions, showed no significant changes in all series of experiments (Table 2).

The results of the histological investigations showed that lipoidosis of the walls of the small and medium-sized arteries of the myocardium was observed more often than in rabbits kept on an atherogenic diet during prolonged stimulation of the VM hypothalamic nuclei. The severity of the dystrophic changes in the muscle fibers of the myocardium varied sharply in the animals of the above-mentioned groups but they were more marked in rabbits on an atherogenic diet during prolonged stimulation of the hypothalamic nuclei. The atherosclerotic index of damage to the aorta was 19% in the group receiving the atherogenic diet and 4% in the second group.

Prolonged stimulation of the VM hypothalamic nuclei thus leads to dystrophic changes in the tissues of the heart. Administration of exogenous cholesterol against this background, while reducing the phospholipid concentration in the mitochondria of the heart, increases the cholesterol concentration in them. However, the degree of this increase is smaller than in animals receiving a high cholesterol diet only.

Taken as a whole, the results described above confirm that the effect of exogenous cholesterol differs depending on the functional state of the heart and on the mechanisms regulating its metabolism.

LITERATURE CITED

1. G. G. Avtandilov, Dynamics of the Atherosclerotic Process in Man [in Russian], Moscow (1970).
2. A. A. Akhrem and A. I. Kuznetsova, Thin-Layer Chromatography [in Russian], Moscow (1964).
3. E. A. Gromova, L. N. Lebedeva, K. N. Tkachenko, et al., Kardiologiya, No. 7, 83 (1972).
4. A. F. Kosenko and L. Kh. Finagin, Byull. Éksp. Biol. Med., No. 4, 34 (1964).
5. M. S. Kokhana and É. P. Pulbere, Byull. Eksp. Biol. Med., No. 12, 12 (1972).
6. M. G. Kritsman, in: Current Problems in Cardiovascular Pathology [in Russian], Moscow (1967), pp. 273-282.
7. N. V. Petrova, Kardiologiya, No. 2, 54 (1968).
8. P. F. Rokitskii, Fundamentals of Analysis of Variance for Biologists [in Russian], Minsk (1961).
9. I. V. Sidorenko, S. N. Gil'miyarova, V. M. Radomskaya, et al., Byull. Éksp. Biol. Med., No. 11, 52 (1974).
10. L. Kh. Finagin, I. M. Kozhura, and T. N. Pechenkova, Byull. Éksp. Biol. Med., No. 8, 56 (1973).
11. D. Bojanovski, E. Schlimme, and K. W. Stahl, in: 9th International Congress of Biochemistry, Stockholm (1973), p. 277.
12. J. Folch and M. Lees, J. Biol. Chem., 226, 497 (1957).
13. W. H. Gutstein and O. Farrel, Proc. Soc. Exp. Biol. (New York), 141, 137 (1972).
14. M. H. Holton and W. Hulsman, Biochem. J., 67, 501 (1957).
15. O. H. Lowry and J. A. Lopez, J. Biol. Chem., 162, 421 (1946).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
17. A. Martonosi and R. Feretos, J. Biol. Chem., 239, 659 (1964).
18. B. Meissner, Biochim. Biophys. Acta, 389, 51 (1975).
19. M. S. Morrison, H. F. Scott, H. Imain, et al., Atherosclerosis, 12, 139 (1970).