STRESS-INDUCED CHANGES IN THE RELATIVE AREA AND NUMBER OF MITOCHONDRIA AND MYOFIBRILS IN THE MYOCARDIUM AND THEIR CORRECTION BY THYROID HORMONES

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Participation of hormones in the response of the body to stress is not disputed [12, 13, 15]. However, the role of thyroid hormones in this process has received very little study [9]. We know that, of the many different effects of thyroid hormones, control of the synthesis of nuclear and mitochondrial proteins is the most important. A particular feature of this control is activation of synthesis of a highly specific protein that is connected with the structural organization of the cell and, in particular, of mitochondrial protein [2, 8, 11, 14].

The heart is the principal target of stress. During stress the metabolism, structure, and function of the myocardium have been shown to be distrubed [5, 7]. Successful adaptation of the heart to environmental factors, including stress, is determined by a selective increase in weight and power of the structures responsible for muscular contraction and the supply of energy for it [4]. A decrease in the ratio of the energy-forming structures of the cell (mitochondria) to the energy-using structures (myofibrils), it can be tentatively suggested, may lead to a disturbance of myocardial functions during stress.

The aim of this investigation was an electron-microscopic study of relations between energy-forming and contractile structures of the myocardium during immobilization of stress and to examine the effect of thyroid hormones on these relations.

EXPERIMENTAL METHOD

The myocardium of noninbred rats, in which stress was induced by immobilization with or without preliminary administration of dried thyroid was studied in the electron microscope. Immobilization stress was induced by fixing the animals in the supine position for 6 h. Dried thyroid (from Minsk Endocrine Preparations Factory) was given in increasing concentrations (from 1.5 to 3 mg/100 g) per os in starch mucilage daily for 35 days.

Altogether 30 male rats weighing 220-300 g were used. Ten intact rats receiving starch mucilage alone for 53 days served as the control. There were three series of experiments: I)

TABLE 1. Effect of Dried Thyroid on Body Weight, HR, and Serum T_4 Concentration of Rats

Group of animals	Conditions for determination of parameter	Parameter		
		body weight, g	HR, beats/min	T_4 concentration, nmoles/liter
1. Control	Before administration of starch mucilage	262	419	
	After administration of starch mucilage	260	425	138±41
	Mean difference	3±0,7	6±0,3	
2. Experimental	Before administration of hormone	230	415	
	After administration of hormone	224	431	173±32
	Mean difference	7±0,5	14±3	
	P_{1-2}	>0,05	>0,05	>0,05

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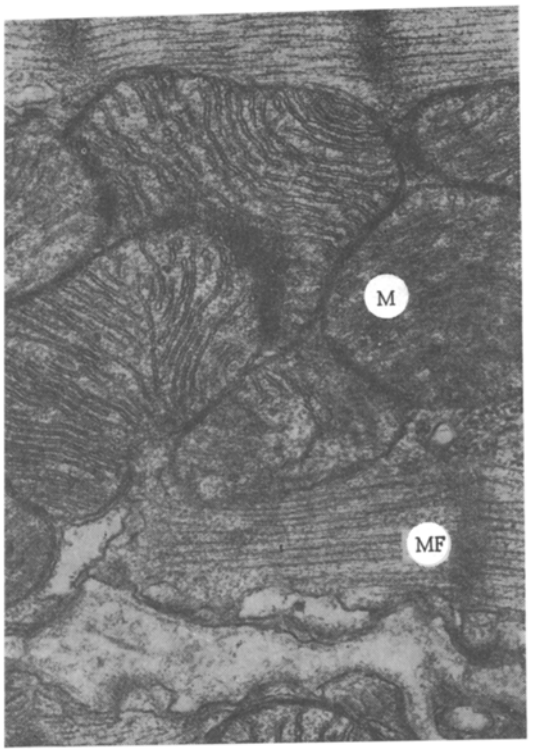

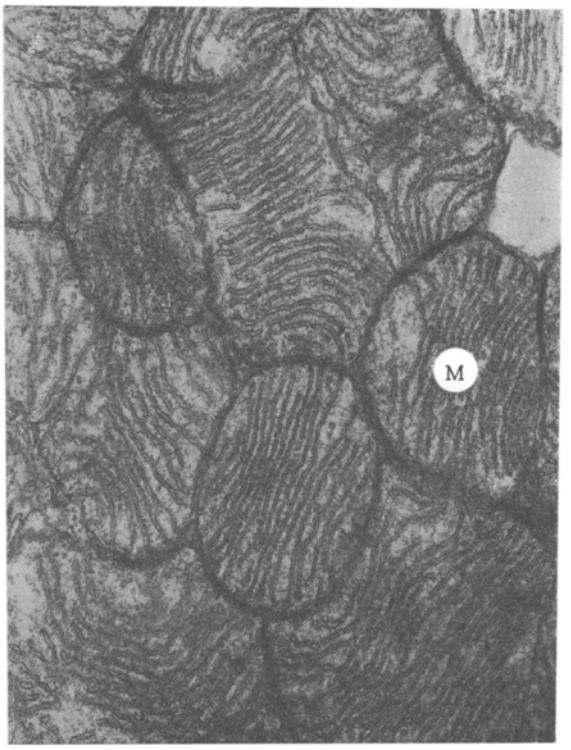

Fig. 1 Fig. 2

Fig. 1. Electron micrograph of LV of intact rats. Here and in Fig. 2: M) mitochondria; 38,000×. MF) Myofibrils.

Fig. 2. Electron micrograph of LV of rats receiving physiological dose of thyroid.

five rats received starch mucilage and were then immobilized to induce stress; II) five rats receiving dried thyroid were then immobilized to induce stress; III) the 10 control rats received dried thyroid. The serum thyroxine (T_4) level in the animals was determined by radioimmunoassay, using the RIO-T_4-PG kit (Institute of Bioorganic Chemistry, Academy of Sciences of the Belorussian SSR); the body weight was measured and the heart rate (HR) calculated from the EKG. Immediately after exposure to stress the animals were decapitated under superficial ether anesthesia, the heart was removed and, under a binocular loupe, pieces of tissue were cut with a razor from the middle third of the anterior wall of the left ventricle (LV) and the ventricular septum (VS), and fixed with glutaraldehyde and OsO_4. The material was then treated by the usual methods and embedded in Araldite. Sections were cut on the LKB 8802A ultramicrorome, stained with uranyl acetate and lead citrate, and examined in the JEM-100 CS and JEM-100 B electron microscopes, under a magnification of between 9800 and 38,000. In each series of experiments, the area of the mitochondria (S_{mc}) and of the myofibrils (S_{mf}) was measured in 30 electron micrographs, using the Leitz A.S.M. Image Analysis System, and expressed as a percentage of the total area of the electron micrograph. The ratio between the area of the mitochondria and the area of the myofibrils S_{mc}/S_{mf} was calculated. The results were subjected to statistical analysis by Student's method and by Oivin's difference method. Differences between means were considered to be significant at the $P \leqslant 0.05$ level.

EXPERIMENTAL RESULTS

Data on the effect of the administered doses of dried thyroid on body weight, HR, and serum T_4 concentration are given in Table 1.

Dried thyroid had no significant effect on body weight or HR. The serum T_4 concentration did not differ significantly from the control. The doses of thyroid used in the experiments could therefore be taken as physiological.

The electron-microscope investigation revealed definite heterogeneity of the cellular ultrastructures of the contractile myocardium. Myofibrils of intact rats had the typical

TABLE 2. Effect of Thyroid on Electron Microscopic Parameters of the Rat Heart in Immobilization Stress (M ± m)

Parameter	Control	Expt. I	Expt. II	III) Control + thyroid
S_{mc}, %				
LV	30,2±2,8	25,1±0,5	44,6±4,7	49,5±2,4
VS	25,3±3,5	16,4±0,2	32,5±1,9	44,5±7,4
S_{mf}, %				
LV	36,6±3,6	44,5±2,0	35,0±4,9	32,0±3,1
VS	51,5±4,5	56,9±6,9	40,5±1,4	40,9±2,5
S_{mf}				
LV	0,869±0,130	0,568±0,013 P_{1-2} 0,05	1,466±0,210 P_{1-3} 0,05 P_{2-3} 0,05	1,547±0,050 P_{1-4} 0,05
VS	0,550±0,110*	0,292±0,015 P_{1-2} 0,05	0,802±0,048 P_{1-3} 0,05 P_{2-3} 0,05	1,080±0,055 P_{1-4} 0,05

Legend. Asterisk denotes statistically significant difference between values of LV and VS.

structure and mitochondria were arranged in the form of chains among the myofibrils (Fig. 1). Most mitochondria were oval in shape and contained many parallel cristae. As a rule some of the mitochondria were moderately swollen. These mitochondria showed fragmentation and homogenization of the cristae. Focal homogenization or patchy translucency of the matrix was noted. Electron-microscopic investigation of the myocardium of the rats receiving physiological doses of thyroid showed an increase in the number of cristae in the mitochondria, the appearance of giant mitochondria, and an increased quantity of chromatin in the nuclei (Fig. 2).

Morphometry of LV and VS showed that the mitochondria of intact animals occupied a rather larger area in LV than in VS, whereas the opposite relationship applied to the area of the myofibrils, with the result that S_{mc}/S_{mf} in LV was 58% higher than in VS (Table 2). These results were in agreement with previous data [14]. Under the influence of immobilization stress S_{mc} decreased somewhat whereas S_{mf} increased, although these changes were not statistically significant. Meanwhile the ratio between these structures fell significantly: by 34.6% in LV and by 46.9% in VS. Immobilization stress in rats receiving thyroid hormones beforehand not only did not reduce S_{mc}/S_{mf} but, on the contrary, increased it: by 68.7% in LV and by 45.8% in VS. This ratio in rats exposed to stress after administration of thyroid hormones was thus greater than in rats exposed to stress without thyroid: by 2.5 times in LV and by 2.7 times in VS.

Administration of thyroid to the control animals likewise was followed by an increase of S_{mc}/S_{mf}: by 1.8 times in LV and by 1.9 times in VS.

When the results are analyzed the first point to note is that quite prolonged stresses, induced in rats by immobilization for 6 h, leads to considerable reduction of S_{mc} relative to S_{mf}. This means a significant decrease in size of the structures supplying energy to the cardiomyocytes, and this may be one cause of depression of cardiac contractility during prolonged stress.

Preliminary administration of small doses of thyroid hormones not only prevented this change in relations between the myocardial ultrastructures, but actually led to accumulation of intracellular structures responsible for adaptation. The doses of thyroid administered caused no significant change in the serum thyroid hormone concentrations and had no significant effect on the animals' body weight or HR. Consequently, the ultrastructural effect observed was due to a near-physiological concentration of hormones, which may be in harmony with existing views on thyroid hormones as powerful inducers of the nuclear and mitochondrial cell genomes [1, 10]. The course of the stress syndrome is known to be phasic: the initial catabolic phase is followed by post-stressor activation of RNA and protein synthesis [6], in which thyroid hormones may perhaps participate. Thyroid hormones also increase the secretion of somatotrophic hormone, a powerful anabolic agent which, together with thyroid hormones, ensures the most prolonged responses to stress [3].

Thyroid hormones thus induce anabolic mechanisms and limit the harmful action of stress on the myocardium significantly.

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IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL STUDY OF α_2-GLYCOPROTEIN FROM THE ATHEROSCLEROTIC AORTIC WALL

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The principal changes in the cardiovascular system and other organs in atherosclerosis are due, it is generally agreed, to morphological and functional changes in the vascular wall. The intimate mechanism of the genesis of the atherosclerotic plaque in the wall of the aorta and other arteries remains a crucial problem in the pathogenesis of atherosclerosis that still awaits solution [1, 3, 6].

The study of metabolism in the atherosclerotically changed vascular wall and, in particular, the study of its protein composition, is particularly interesting on account of the discovery of the so-called "atherocalcin" [4], a specific protein contained in the atherosclerotic plaque.

The aim of this investigation was an immunochemical study of the antigenic composition of the atherosclerotic aortic wall and to isolate specific components contained mainly in the affected zone of the aortic wall.

EXEPRIMENTAL METHOD

Human aortic tissue with well-marked atherosclerotic plaques was taken for investigation. The plaques (weighing about 50 g) were excised inside their visible boundaries, washed with physiological saline to remove blood, homogenized in a "Biomix" homogenizer (Hungary) at 10,000 rpm, and delipidized with a mixture of chloroform and methanol (3:1) for 24 h at 0-4°C. The residue was freeze-dried and dissolved in the minimal volume of distilled water. The resulting preparation was used for primary immunization of chinchilla rabbits by the usual method. The antiserum obtained was adsorbed with freeze-dried extracts of human liver and spleen and also with dried human blood plasma.

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