SOME INDICES OF METABOLISM OF THE HYPERTROPHIED MYOCARDIUM UNDER THE INFLUENCE OF EMBRYONIC ORGAN-SPECIFIC RNA

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Cardiac failure in clinical practice usually develops against the background of hypertrophy of the myocardium. The latter has been shown to be a reversible process [5]. According to a previous report, the degree of hypertrophy can be reduced under the influence of embryonic myocardial RNA [6]. In order to shed light on the mechanism of this phenomenon, some indices of metabolism of the hypertrophied myocardium were studied.

EXPERIMENTAL METHOD

Stenosis of the abdominal aorta was induced in 20 adult male rats. Half of the animals, after induction of stenosis, were given a preparation of total RNA from rat embryonic myocardium, isolated by the phenolic method of Kirby and Georgiev [7], intravenously. The animals of group 1 (five rats) received 12 or 13 injections of 0.65 mg RNA on alternate days 2 months after stenosis of the aorta. They were then killed 70-91 days after the formation of stenosis (7-13 days after the last injection of RNA). Animals of group 2 (five rats) received RNA in a mean sessional dose of 0.44 mg 12 times 4.5 months after the beginning of aortic stenosis. The animals of this group were killed 120-227 days after the beginning of aortic stenosis (11-38 days after the last injection of RNA). The method of matched pairs was used for the control: The third (control) group of five animals corresponded in times of observation to the first experimental group, and the fourth (control) group of five animals corresponded to the second experimental group. The control rats with aortic stenosis were given intravenous injections of physiological saline. The fifth group consisted of nine intact animals, without aortic stenosis.

After decapitation of the rats their heart was removed and the tissue of the left ventricle divided into three parts. One part served as weighed sample for determination of the actomyosin concentration. Actomyosin was extracted by the method of Ivanov and Yur'ev [2] and the protein concentration in the solution was determined by a biuret micromethod [8] on the SF-4 spectrophotometer. The second part of the heart tissue was used for preparations of frozen sections and subsequent testing for succinate dehydrogenase (SDH) by the method described in [9]. The third part was fixed on Carnoy's fluid. The quantitative cytochemical reaction of Barnett and Seligman [10] with DDD reagent was carried out on paraffin sections. The concentration of formazan granules, reflecting SDH activity, and also the concentration of SH groups in single muscle fibers were determined on the MUF-5 cytophotometer by a scanning method.

All the numerical data were subjected to statistical analysis by Student's t-test, by two-factor dispersion analysis using Fisher's criterion (the effect of RNA and of the time factor was analyzed), and by information analysis [1, 4]: The effect of the RNA preparation was studied on the actomyosin concentration, SDH activity, and the number of SH groups. Sampling of measurements for dispersion and information analysis was done by the use of random number tables. Histograms of distribution of muscle fibers by SDH activity and by number of sulfhydryl groups in them also were drawn and analyzed.

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TABLE 1. Actomyosin Concentration, SDH Activity, and Number of SH Groups in Myocardium of Left Ventricle of Rats (M ± m)

Index of metabolism	Group of animals				
	1	2	3	4	5
Actomyosin concentration (extinction)	0,25±0,02	0,96±0,27	0,36±0,04	0,34±0,05	0,35±0.06
P	>0,1	>0,9	<0,1 >0,05	>0,9	
SDH activity (optical density)	0,206±0,02	0,178±0,026	0,146±0,034	0,139±0,004	0,188±0.014
P	>0,5	>0,8	>0,4	<0,2 >0,1	
Number of SH-groups (optical density)	0,101±0,009	0,120±0,03	0,137±0,041	0,106±0,015	0,119±0.026
P	>0,8	>0,9	>0,8	>0,9	

TABLE 2. Basic Indices of Dispersion and Information Analysis

Feature studied	Factor	Index of factor analysis			Index of information analysis			
		degree of effect, %	correlation, η	probability of error, P	I, bits	Eq, %	K_{ass}	P_{error}
Actomyosin concentration	RNA	9,5	0,31	<0,2 >0.05				
	Time	17,8	0,42	<0,05	1,56	19.4	0,80	0.19
	RNA and time	19,6	0,44	<0,05				
SDH activity	RNA	73,5	0,86	<0,001				
	Time	71,6	0,85	<0,001	12,7	12.2	0,96	0.88
	RNA and time	71,3	0,84	<0,001				
Number of SH groups	RNA	28,2	0,53	<0,001				
	Time	28,2	0,53	<0,001	6,42	12,2	0,93	0.76
	RNA and time	28,2	0,53	<0,001				

Legend. I) Informativeness, Eq) equivocation, K_{ass}) association factor [4], P_{error}) error of probability.

EXPERIMENTAL RESULTS

The experimental results showed (Table 1) that, by contrast with the control, injections of organ-specific embryonic RNA led to a close to statistically significant (t = 2.2) increase in the actomyosin concentration in the myocardium of the rats with aortic stenosis during the later stage of observation. Two-factor dispersion analysis showed (Table 2) that the time factor significantly affects the increase in actomyosin concentration in the myocardium of the left ventricle ($P < 0.05$). No significant effect of RNA on the actomyosin concentration was observed ($P < 0.2 > 0.05$), evidently because of the small number of observations, but the combined effect of RNA and the time factor was significant ($P < 0.05$).

Histograms of distribution of muscle fibers by degree of their SDH activity in animals of the control groups (groups 3 and 4, Fig. 1) show an increase in classes of muscle fibers with lowered SDH activity. In the first experimental group, on the other hand, there was a marked increase in the number of classes of muscle fibers with higher SDH activity. In the second experimental group, although the histogram shows a shift to the left, this was only on account of a small increase in the number of muscle fibers with lowered SDH activity. Student's t-test (Table 1) showed no significant differences between control and experiment as regards the level of SDH activity in single muscle fibers, but as two-factor dispersion analysis showed (Table 2), close correlation exists between exposure to RNA and SDH activity, and also between the duration of previous aortic stenosis (the time factor) and SDH activity. The degree of the influence of both factors tested on SDH activity was very high (RNA 73.5%, time 71.6%), and the significance of this effect was correspondingly high ($P < 0.001$). Consequently, it can be concluded from different methods of analysis of the results of cytophotometry that SDH activity in the myocardium exposed to prolonged and increased functional stress increases or remains at a high level.

It will be clear from the histograms of distribution of muscle fibers by content of SH groups (Fig. 2) that the trend of fluctuations in the content of SH groups in single muscle

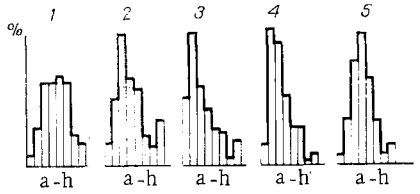

Fig. 1

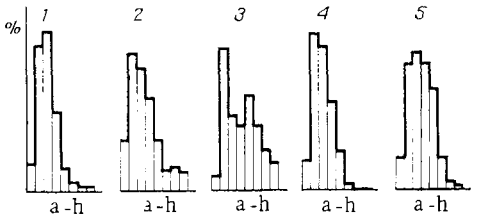

Fig. 2

Fig. 1. Histograms of distribution of myocardial muscle fibers from left ventricle depending on SDH activity in animals of different groups. Classes of muscle fibers according to SDH activity (in optical units): a) 0.032-0.05, b) 0.051-0.1, c) 0.101-0.15, d) 0.151-0.2, e) 0.201-0.25, f) 0.251-0.3, g) 0.301-0.35, h) 0.351 and over. 1-5) 1st-5th groups of animals respectively.

Fig. 2. Histograms of distribution of myocardial muscle fibers from left ventricle depending on concentration of SH groups. Classes of muscle fibers by activity of SH groups (in optical units): a) up to 0.06, b) 0.061-0.09, c) 0.091-0.12, d) 0.121-0.15, e) 0.151-0.18, f) 0.181-0.21, g) 0.211-0.24, h) over 0.24. 1-5) 1st-5th groups of animals respectively.

fibers from the myocardium of the left ventricle coincides exactly with the trend of fluctuations in actomyosin concentration. This is not by accident, for actomyosin is the main carrier of SH groups in heart muscle tissue [3]. The increase in the content of SH groups under the influence of RNA, like the increase in the actomyosin concentration, was observed only at late times of observation. The small decrease in the level of SH groups and actomyosin concentration in the experimental animals in the early periods of investigation was evidently due to a redistribution and was associated with a relative rise in the content of mitochondrial proteins in the cytoplasm of the myocardial cells. Despite the fact that comparison of the mean values (Table 1) revealed no significant differences in the number of SH groups in the myocardial muscle fibers of different groups of animals, according to the results of two-factor dispersion analysis (Table 2) RNA, the time factor, and these two factors combined have a significant effect on the content of SH groups (the degree of the effect is the same, namely 28.2%; $P < 0.001$).

As Table 2 shows, all the factors studied (actomyosin concentration, SDH activity, number of SH groups) were highly informative. The most informative features in the study of the biological activity of the RNA preparation were SDH activity and the number of SH groups. Changes in the actomyosin concentration of the experimental animals by 80.6%, in SDH activity by 87.8%, and in the number of SH groups by 87.8% were associated with the action of the exogenous RNA. The association factor for all features studied was over 0.5 and close to 1, evidence of close correlation between changes in the magnitude of these parameters and the administration of exogenous organ-specific RNA to the experimental animals. The most important factors confirming the biological efficacy of the RNA preparation used, according to the results of information analysis, are changes in the actomyosin concentration, for they were significant ($P_{error} < 0.5$, actual value $P = 0.19$).

All the changes in these indices described above are evidence of the beneficial effect of RNA on intracellular repair processes. The metabolic changes observed can prolong the period of stable compensation should an increased and prolonged load be placed on the myocardium.

LITERATURE CITED

1. V. A. Bandarin, in: Information Theory in Medicine [in Russian], Minsk (1974), pp. 6-77.
2. I. I. Ivanov and V. A. Yur'ev, Biochemistry and Pathobiochemistry of Muscles [in Russian], Leningrad (1961).
3. N. A. Levkova, Morphological Bases of Cardiac Failure in Old Age [in Russian], Moscow (1974).
4. N. K. Rozova and L. D. Meshalkin, Kardiologiya, No. 9, 77 (1975).
5. D. S. Sarkisov, V. D. Arutyunov, L. D. Krymskii, et al., Hypertrophy of the Myocardium and Its Reversibility [in Russian], Leningrad (1966).

6. N. D. Skuba and N. A. Levkova, Byull. Éksp. Biol. Med., 89 (1980).
7. V. S. Tongur and N. S. Vladychenskaya, in: Modern Methods in Biochemistry [in Russian], Vol. 1, Moscow (1964), p. 222.
8. J. L. Bailey, Techniques in Protein Chemistry, Elsevier, Amsterdam (1962).
9. D. Kiszely, Practical Microtechniques and Histochemistry [Russian translation], Budapest (1962), p. 301.
10. A. G. E. Pearse, Histochemistry, Theoretical and Applied, Williams and Wilkins (1969).

HISTOMETRIC CHARACTERISTICS OF CAPILLARY–GLIA–NEURON RELATIONS OF THE CERVICO-THORACIC (STELLATE) GANGLIA OF CATS WITH EXPERIMENTAL ISOPROTERENOL NECROSIS OF THE MYOCARDIUM

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KEY WORDS: capillary–glia–neuron complex; cervico-thoracic ganglia; myocardium; experimental necrosis of the myocardium; electrocardiography; histometric analysis.

Investigations [4, 6] have shown that close morphological and functional correlation exists between the heart and the cervico-thoracic ganglia of the sympathetic trunk, which are the source of its postganglionic innervation. Morphological equivalents of irritation, in the form of coarsening of sensory nerve endings, hyperargyrophilia of the cytoplasma of the neurons, and pycnosis of their nuclei have been found in the cervico-thoracic (stellate) ganglia of persons dying from myocardial infarction [3, 9]. In previous investigations [5] the writer gave details of the architectonics of capillary–glia–neuron complexes in the normal cervico-thoracic ganglia. Since changes in the components of these complexes during the development of myocardial infarction have not been adequately studied, it was decided to examine this problem with the aim of shedding further light on the role of the neurovascular factor in the development of myocardial infarction.

EXPERIMENTAL METHOD

Experiments were carried out on 52 adult cats *(Felis domestica)*. Altogether 60 cervico-thoracic ganglia were studied under normal conditions and 44 at different times (3 h, 3 and 7 days, 2 weeks) after induction of experimental myocardial necrosis by three subcutaneous injections of isoproterenol (at intervals of 24 h) in a dose of 10 mg/kg body weight. The tests used to confirm the presence of foci of necrosis in the myocardium included electrocardiography and examination of sections from histological preparations of heart muscle stained with hematoxylin and eosin. The lower thoracic ganglia of the sympathetic trunk served as control objects. Six stellate ganglia taken at autopsy from persons dying from myocardial infarction also were investigated. Capillary–glia–neuron relations were investigated in microtome sections through the cervico-thoracic ganglia after staining with hematoxylin and eosin, by Van Gieson and Nissl's methods, and by gallocyanin and chrome alum. A suspension of Paris green in chloroform with ether was injected into their microcirculation. Histometric data on capillary–glia–neuron relations were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Under normal conditions cardiomyocytes stained with hematoxylin and eosin are cylindrical in shape, with a large, oval, weakly basophilic nucleus in the center of the sarcoplasm. Signs of hemostasis in the blood vessels and of myocytolysis [7], manifested as disappearance of cross striation, pallor of the sarcoplasm, and pycnosis of the nucleus and its displacement toward the periphery, were observed in the myocardium 3 h after the last injection of isopro-

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