EFFECT OF EMBRYONIC ORGAN-SPECIFIC RNA ON HYPERTROPHY OF THE MYOCARDIUM

N. D. Skuba and N. A. Levkova

UDC 616.127-007.61-092
.9-085.31:547.963.32

The relative weight of the heart and the thickness of the muscle fibers in the myocardium of the left ventricle were determined in rats 70-91 and 120-227 days after the formation of stenosis of the abdominal aorta and in intact animals. Half of the animals with stenosis received an injection of cardiac species-specific embryonic RNA, the other half received physiological saline. The results were subjected to variance, dispersion, and mathematical analysis. The degree of development of myocardial hypertrophy was reduced after injection of RNA.

KEY WORDS: myocardium; hypertrophy; exogenous RNA.

Hypertrophy of heart muscle cells is based on hyperplasia of specific ultrastructures [3]. The increase in volume of the ultrastructures and their constant self-renewal in this increased volume require appropriate metabolic provision. It was shown previously, using a model of experimental necrosis of the myocardium [4, 5], that RNA from embryonic myocardium has a stimulating effect on both parenchymatous and stromal cells of heart muscle. The object of the present investigation was to study the effect of organ-specific embryonic RNA on hypertrophy of the myocardium.

METHODS

Experiments were carried out on 29 adult male albino rats. An increased load on the myocardium was created by the formation of artificial stenosis of the abdominal aorta above the point of origin of the renal arteries. The lumen of the aorta was narrowed by about 30%. A preparation of total RNA from embryonic rat hearts was obtained by the phenolic method of Kirby and Georgiev [6]. The stage of precipitation of RNA with alcohol was omitted: The RNA solution, purified to remove phenol, was used at once for injection or was kept for several days in a frozen state. All animals undergoing the operation were divided into four groups: two experimental and two control. The experimental rats of group 1 (five animals) started to receive intravenous injections of the RNA preparation 2 months after the operation, animals of group 2 (five rats) 4.5 months after the formation of aortic stenosis. The two groups of control animals (groups 3 and 4, five animals in each) received intravenous injections of physiological saline at the same time. A group of intact animals, undergoing no operations (group 5, nine animals altogether), served as an additional control. RNA was injected 12 or 13 times in group 1, usually on alternate days, in a mean sessional dose of 0.65 mg in a volume of 1 ml, in group 2 it was injected 12 times at intervals of 1-8 days between injections, in a mean sessional dose of 0.44 mg. The animals of groups 1 and 3 were killed 70-91 days after the formation of aortic stenosis and 7-13 days after the last injection of RNA. The rats of groups 2 and 4 were killed 120-227 days after the formation of aortic stenosis and 11-38 days after the last injection of RNA. The method of controlled pairs was used.

The absolute and relative weight of the heart was determined. By means of an ocular micrometer the thickness of the muscle fibers was measured in the left ventricle in paraffin sections stained with hematoxylin—eosin. The mean thickness of the muscle fiber was determined by measuring the thickness of 50 unselected muscle fibers. All numerical data were subjected to statistical analysis by Student's t-test, to two-factor dispersion analysis with

Department of Pathomorphology, A. V. Vishnevskii Institute of Surgery, Moscow. Department of Pathological Anatomy, North-Ossetian Medical Institute, Ordzhonikidze. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 11, pp. 621-623, November, 1979. Original article submitted April 19, 1979.

 0007-4888/79/8811-1372$07.50

TABLE 1. Basic Indices of Dispersion and Mathematical Analysis

Feature studied	Factor studied	Index of factor analysis			Index of mathematical analysis			
		degree of effect, %	correlation ratio	probability of error, P	*I, bits	†Eq, %	‡K_{cor}	**P_{err}
Cardiac index	RNA	3,8	0,19	>0,2				
	Time	5,8	0,24	>0,2	1,49	16,5	0,82	0,04
	RNA and time	11,0	0,33	>0,05 <0,2				
Diameter of muscle fiber, μ	RNA	2,5	0,16	<0,05				
	Time	5,1	0,23	<0,01	10,41	10,9	0,96	0,81
	RNA and time	0,03	0,02	>0,2				

*Informativeness
†Equivocation
‡Coefficient of correlation (Rozova and Meshalkin [2])
**Error of plausibility of inferences

calculation of Fisher's criterion (the effect of the RNA preparation and of the time factor was analyzed), and to mathematical analysis [1, 2]: The effect of the RNA preparation was analyzed on the cardiac index and the thickness of the muscle fiber. Histograms of distribution of muscle fibers by diameter were drawn and analyzed.

RESULTS

An increase in the cardiac index (the relative weight of the hearts) compared with the group of intact animals took place 3 months after the formation of aortic stenosis (groups 1 and 3). For instance, in experimental animals receiving embryonic RNA (group 1) the mean cardiac index was 3.06 ± 0.26 (the same as in intact animals). In the control animals with aortic stenosis (not receiving RNA, group 3) the cardiac index was 2.92 ± 0.07 ($P < 0.05$), and in the intact rats (no operation, no RNA given, group 5) it was 2.66 ± 0.06. It must be emphasized that the difference between the group of animals receiving RNA and the intact animals was not statistically significant, whereas in the group of animals not receiving RNA there was a statistically significant increase in the relative weight of the heart compared with the group of intact rats.

In the experimental animals (group 2) 6 months after the creation of aortic stenosis, as before no statistically significant increase in the cardiac index was found compared either with the previous period ($P > 0.1$) or with the intact animals of group 5 ($0.05 < P < 0.1$); the cardiac index of the experimental animals was 2.97 ± 0.12. In control group 4, on the other hand, a further increase in the cardiac index was observed, to 3.55 ± 0.32, i.e., it considerably exceeded the cardiac index of the intact animals ($P < 0.05$).

According to the results of two-factor dispersion analysis (Table 1) the greatest degree of influence (11%) was observed from combined action of RNA and the time factor, i.e., longer administration of RNA could have a more marked effect.

The mean thickness of the muscle fiber in control group 3, 6 months after the formation of aortic stenosis, was 9.31 ± 0.5 μ, compared with 9.15 ± 0.63 μ in the intact animals of group 5. In experimental group 1, 3 months after the formation of aortic stenosis the mean thickness of the muscle fiber was 8.51 ± 0.4 μ. Six months after the operation in control group 4 some increase was observed in the mean thickness of the muscle fibers (9.94 ± 0.69 μ). The mean thickness of the muscle fiber in experimental group 2 was 9.32 ± 0.66 μ. Although comparison of the mean values by Student's test showed no significant differences between the mean diameter of the muscle fiber in the different groups of animals, according to the results of two-factor dispersion analysis, for which a large sample was used (50 measurements in each group), RNA and the time factor had a significant effect on the thickness of the muscle fiber; the degree of influence of the time factor (5.1%; $P < 0.01$) was twice as great as the degree of influence of the RNA preparation (2.5%; $P < 0.05$). The combined effect of the time factor and RNA on the diameter of the muscle fiber was only 0.03% ($P > 0.2$), evidence that the influence of these two factors on hypertrophy of the myocardium was opposite in direction: Unlike the time factor, RNA reduced the degree of hypertrophy.

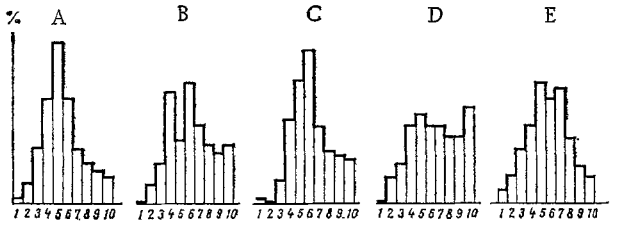

Fig. 1. Histograms of distribution of muscle fibers from myocardium of left ventricle in different groups of animals by diameter. Groups of animals: A) 1, B) 2, C) 3, D) 4, E) 5. Classes of muscle fibers: 1) up to 4.33 μ, 2) 4.33-5.19 μ, 3) 5.41-6.28 μ, 4) 6.49-7.36 μ, 5) 7.57-8.44 μ, 6) 8.66-9.52 μ, 7) 9.74-10.6 μ, 8) 10.82-11.47 μ, 9) 11.69-12.77 μ, 10) 12.98 μ and over.

Histograms of distribution of muscle fibers by thickness (Fig. 1) showed that the number of fibers with larger diameters increased in the control animals with an increase in the duration of aortic stenosis. In the experimental animals, with a shorter period of observation, in general no increase was found in the number of muscle fibers of increased diameter, whereas after a longer period of observation there was only a small increase in the size of the classes of thicker muscle fibers.

The main results of mathematical analysis are given in Table 1. They show that the features studied (relative weight of the heart, diameter of the muscle fiber) are highly informative. Changes in the relative weight of the heart of the experimental animals correlated by 93.5%, and changes in the thickness of the muscle fibers by 89.1% with the action of the RNA preparation.

Injection of a preparation of embryonic cardiac RNA into animals with aortic stenosis thus led to a decrease in the degree of hypertrophy of the myocardium. There is reason to suppose that exogenous organ-specific RNA promotes the more rapid renewal of specific ultrastructures of the myocardial cell, thus enabling the myocardium to perform increased function with a less marked increase in their volume.

LITERATURE CITED

1. V. A. Bondarin, in: Information Theory in Medicine [in Russian], Minsk (1974), p. 6.
2. N. K. Rozova and L. D. Meshalkin, Kardiologiya, No. 9, 77 (1975).
3. D. S. Sarkisov and B. V. Vtyurin, Electron-Microscopic Analysis of Increased Tolerance of the Heart [in Russian], Moscow (1969).
4. N. D. Skuba, in: Proceedings of the First Congress of Pathological Anatomists of the Ukrainian SSR [in Russian], Kiev (1971), p. 257.
5. N. D. Skuba, Arkh. Patol., No. 12, 55 (1974).
6. V. S. Tongur and N. S. Vladychenskaya, in: Modern Methods in Biochemistry [in Russian], Vol. 1, Moscow (1964), p. 222.