CHANGES IN THE CONNECTIVE-TISSUE STROMA OF THE MYOCARDIUM IN EXPERIMENTAL HYPERTROPHY OF THE HEART

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UDC 616.12-007.61-092.9-07:616.127-018.2-091.8

Histological changes were studied in the myocardium of mice and rats in which hypertrophy of the heart was induced by running on a treadmill, swimming, adaptation to hypoxia in a pressure chamber, administration of increasing doses of isopropylnoradrenalin, and constriction of the aorta. Changes in the stroma of the myocardium were connected with changes in the muscle cells. Hypertrophy of the muscle cells itself did not lead to sclerotic changes in the myocardium. The latter appeared as a reaction to degenerative, necrotic, and atrophic changes in the muscle cells. The most intensive hypertrophy of the connective tissue occurred following administration of cardiotoxic substances.

Experimental models of myocardial hypertrophy used in practice are equivalent from the point of view of the histological changes which take place. Differences in the reaction of the stroma, the predominant component of the myocardium in the number of cells, although not in mass, are particularly important yet they are not always taken into consideration in physiological and biochemical investigations. It was therefore felt that a comparative histological analysis of changes in the connective-tissue stroma in some of the models of myocardial hypertrophy most frequently used would be valuable.

EXPERIMENTAL METHOD

The following series of experiments were carried out on sexually mature laboratory mice and rats. 1) The mice were trained to swim for 25 days (20 animals). The duration of swimming was increased from 15 min on the 1st day to 2 h each day at the end of the period. 2) Mice and rats were trained to run on a treadmill. The animals used in the experiment (11 rats and four mice) were preselected for their running characteristics, and they were compelled to run at a speed of 2 m/min for 1 h daily for 30-40 days. 3) Mice were trained for exposure to "high-altitude" hypoxia by Repin's method [4]. Altogether 30 mice were used in the experiment and the animals were sacrificed between 2 days and 2 months after exposure. 4) Mice and rats received repeated injections of increasing doses of isopropylnoradrenalin sulfate (INAS) by the method of Rakuzan et al. [12]. The compound was injected subcutaneously by the following scheme: on the 1st day 1 mg/kg into rats and 0.5 mg/kg into mice; on the subsequent days the dose was increased daily by the amount of the initial dose. The animals (18 rats and 31 mice) were sacrificed from 1 to 15 days after the beginning of the experiment. 5) Constriction of the abdominal aorta was produced in rats by Beznak's method in Kogan's modification [2]. These experiments were carried out on 116 rats. The animals were sacrificed from 1 day to 10.5 months after the operation.

In each series of experiments intact animals of the same age were sacrificed at the same time as the experimental animals, for which they acted as the control.

Paraffin sections of the heart fixed with formalin were stained with hematoxylin-eosin, toluidine blue, by Van Gieson's method, by a combination of the PAS reaction and staining with iron-hematoxylin orange, and by silver impregnation by Gomori's method.

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Fig. 1.

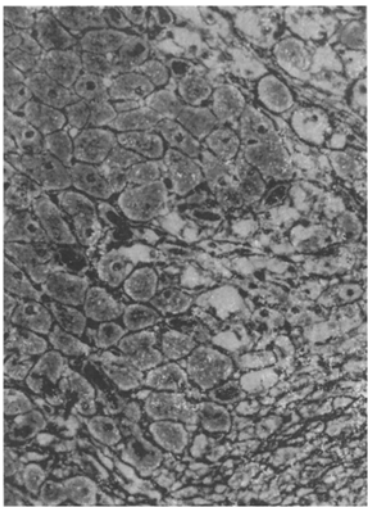

Fig. 2.

Fig. 1. Myocardium of rat sacrificed after six injections of INAS. Granulomas at site of necrotic muscle fibers in papillary muscle of left ventricle. Stained with colloidal iron-PAS-hematoxylin, 125 ×.

Fig. 2. Myocardium of a rat sacrificed after 15th injection of INAS. Stroma between hypertrophied muscle fibers consists of thin fibers. Coarsening of the stroma is seen in foci of necrosis of muscle fibers. Impregnation by Gomori's method, 320 ×.

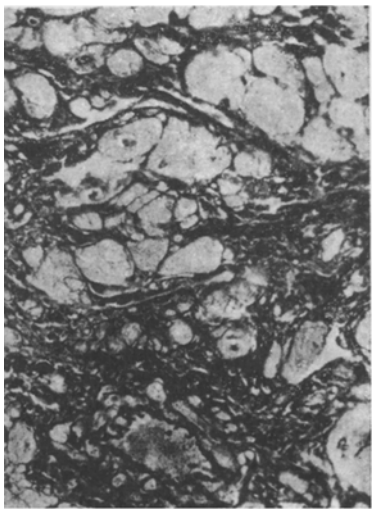

Fig. 3. Myocardium of rat killed 6 months after operation of constriction of abdominal aorta. Coarsening of stroma in zone of atrophic changes in muscle fibers. Stained with colloidal iron-PAS-hematoxylin, 500 ×.

The ratio between the number of nuclei of muscle and connective-tissue cells was determined by counting the number of muscle nuclei per thousand connective-tissue nuclei in zones of the myocardium with the most marked hypertrophy of the muscle fibers, far away from muscle cells undergoing degeneration and necrosis.

The proportion by volume of the various components of the myocardium was determined by Glagolev's dot method [1].

EXPERIMENTAL RESULTS

In all series of experiments there was an increase in the absolute weight of the heart, which by the end of the experiment showed an average increase of 16% in the mice trained to swim, 25.4% in mice running on the treadmill, by 23.1% in rats on the treadmill, and by 44.1 and 71.8% respectively in mice and rats receiving INAS compared with initially. In the rats with constriction of the abdominal aorta the weight of the heart was increased on the 10th day by 24.1%, after 2 months by 75.7%, and after 5 months by 86.8% compared with initially. The mice trained by exposure to hypoxia showed hypertrophy mainly of the right ventricle and the total weight of the heart was increased by only 7.3%.

Microscopic investigation of the myocardium of the animals trained in the pressure chamber, by swimming, or by running on a treadmill, toward the end of the experiment revealed a more or less irregular increase in thickness of the muscle fibers and an increase in the volume of the nuclei of the muscle cells. Degenerative and necrobiotic changes were not detected in the muscle cells and, correspondingly, there was no activation of the stromal elements. The ratio between the number of nuclei of the muscle and connective-tissue cells in these animals was not significantly different from the control. In animals trained by physical excretion it was 1: (1.73 ± 0.03), in the control group 1: (2.01 ± 0.13); in the mice trained by exposure to hypoxia 1: (1.14 ± 0.03), and in the control 1: (1.20 ± 0.05).

TABLE 1. Relative Volumes (in percent) of Different Components in Tissue of Capillary Muscles of Left Ventricle in Rats Receiving Repeated Injections of INAS

Animal No.	Time of expt. (in days)	Muscle fibers				Connective tissue	
		intact	myocytolysis	contractures	necrosis	cells and fibers	edema fluid
KN-21	1	70,2	0	8,3	0	5,4	16,1
KN-22	1	63,0	0,6	9,5	0,2	15,2	11,5
KN-23	2	64,1	2,1	6,5	6,1	13,4	7,8
KN-24	2	62,2	4,8	8,4	5,2	12,4	7,0
KN-25	3	37,6	4,7	6,7	17,2	27,8	6,0
KN-26	3	49,7	1,5	4,5	20,3	13,8	10,7
KN-27	6	40,1	3,1	5,4	12,3	24,5	14,6
KN-28	6	27.9	0,5	6,6	13,5	41,3	10,2
KN-31	10	56,3	0,8	3,0	5,3	27,4	7,2
KN-32	10	75,2	2,9	5,0	0,9	10,8	5,2
KN-4	15	66,2	0	0,7	1,7	23,8	7,6
KN-5	15	50,5	0	0,8	4,2	41,0	3,5
KN-6	15	45,6	0	0,8	6,7	41,3	5,6
KN-7	15	55,7	0	1,8	5,0	30,0	7,5
KN-8	15	51,7	0	1,3	3,2	40,8	3,0
KN-9	15	52,3	0	1,5	5,3	37,4	3,5
KN-10	15	55,2	0	1,1	4,7	35,8	3,2

Note. Determination made by counting 1000 dots, giving a probability of error of about 1%.

The connective-tissue fibers were not thickened, while in zones with more marked hypertrophy of the muscle fibers the connective-tissue framework sometimes appeared to have even thinner fibers than normally.

In the animals receiving increasing doses of INAS the increase in the weight of the heart took place particularly rapidly and intensively. However, in these experiments gross pathological changes were observed in the myocardium, in the form of edema of the stroma, multiple foci of necrosis of muscle cells, and the formation of extensive areas of cicatrizing granulomas (Fig. 1). These changes correspond to those described in adrenalin injuries to the myocardium [9] but they were much more severe. The results of a dynamic investigation of the changes showed that new foci of necrosis appeared after each injection, so that as a result a variegated picture of necrotic and sclerotic changes of many different degrees could be seen. The results of measurement of the volume of the individual components in the myocardium of the rats from this series of experiments are given in Table 1. They show that, first, considerable individual variations existed in the intensity of the pathological changes and, second, a considerable part (sometimes more than half) of the mass of the myocardium was accounted for by connective-tissue elements and edema fluid. The degenerative and necrotic changes were focal in character, and outside the foci of injury zones of intact myocardium remained, in which groups of greatly thickened muscle fibers could be seen. In these zones the stroma appeared to have thin fibers (Fig. 2), and in some places the thinnest crosswise connective-tissue fibrils were even reduced in size, and the ratio between the number of nuclei of muscle and connective-tissue cells was 1: (1.37 ± 0.06) compared with the normal value of 1: (2.33 ± 0.12). Since muscle cells in the ventricles of adult animals virtually do not multiply [3, 5-7], a decrease in the number of connective-tissue cells must be assumed to have taken place in these zones, possibly on account of their migration toward the foci of necrosis.

In the early stage of myocardial hyperfunction in rats with constriction of the abdominal aorta degenerative changes appeared in the muscle cells which, in their character and dynamics of development, were similar to lesions produced by catecholamines. Granulomas formed in the foci of micronecrosis, subsequently to be converted into scars. In the zones of intact myocardium the muscle fibers showed hypertrophy which, as in other cases, was uneven in distribution. The hypertrophied muscle cells at this stage were rich in sarcoplasm, and the myofibrils were loosely arranged. In the later stages (after the 2nd month) the number of myofibrils in the hypertrophied cells increased and they were more compactly arranged. The connective-tissue framework in these zones did not appear coarse in structure, and in places it actually appeared slightly reduced. The ratio between the nuclei in the muscle and connective-tissue cells on the 10th day of the experiment was 1: (1.94 ± 0.04), after 2 months it was 1: (2.04 ± 0.03), and after 5 months 1: (2.33 ± 0.03), and did not differ significantly from the normal value for that age [for the control 1: (1.83 ± 0.02), 1: (1.94 ± 0.04), and 1: (2.25 ± 0.03) respectively]. The small scars appearing on the 1st day of the experiment underwent resolution and disappeared in the same way as those described in adrenalin lesions [10]. Later still atrophic changes appeared in some muscle fibers. In these areas the stroma showed a progressive thickening of its fibers (Fig. 3). Later the combination of fibrosis with atrophic changes of some muscle cells led in some animals to the formation of extensive areas of sclerosis and of aneurysms in the region of the apex of the left ventricle. In this experimental model also the sclerotic changes were thus due not to hypertrophy but to regression in the muscle fibers. The fact that collagenization of the stroma is not a direct result of hypertrophy of the muscle fibers was observed by Krymskii [8] in a study of autopsy material.

The results of these experiments agree closely with those of biochemical investigations [11] which showed that during cardio-megaly produced in rats by physical exertion (running) the collagen content in

the myocardium is virtually not increased, whereas after repeated injection of isopropylnoradrenalin the collagen content reaches 228%, and after coarctation of the aorta it reaches 160% of the normal level.

The results of the present investigation confirm that the character of the stromal response differs significantly in different experimental models of hypertrophy of the heart. The fact will be noted that hypertrophy of the muscle cells does not itself induce coarsening of the stroma, and indeed, a tendency toward slight reduction of the fibrous framework is seen. In myocardial hypertrophy induced by physical exertion (running, swimming) or by exposure to hypoxia, changes in the stroma were minimal. In hypertrophy of the heart induced by coarctation of the aorta or by repeated injection of isopropylnoradrenalin, considerable hypertrophy of the connective tissue was observed: this hypertrophy was connected with degenerative and necrotic changes in the muscle cells, and in the late stages of myocardial hyperfunction it was connected with atrophic changes in some of the muscle cells.

When an experimental model of myocardial hypertrophy is chosen for physiological or biochemical investigations, the histological picture of the changes taking place must be taken into account. If the aim of the investigation is to study the properties of the hypertrophied muscle cells of the heart, models based on the use of cardiotoxic agents will evidently be least suitable.

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