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PROLIFERATION OF MYOCYTES OF THE LEFT ATRIUM AND VENTRICLE AFTER VARIOUS TYPES OF MYOCARDIAL INJURY IN ADULT RATS

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An infarct of the myocardium of the left ventricle was produced in adult rats weighing 120-160 g by ligating the left coronary artery at different levels: in the atrial region, at the level of the first third of the left ventricle, and in its middle. In other series of experiments the left atrium was damaged by applying a ligature to its anterior wall or to the auricle. Animals undergoing a mock operation during which the pericardium was removed acted as the control. The left half of the heart was investigated on the 5th day after the operation. Mitotically dividing myocytes were found in the atrium or auricle of the animals in those series of experiments in which these parts of the heart had been directly injured and had a thickened epicardium (in 35 of 49 cases); the mitotic index varied from 0.9 to 10%. After litigation of the coronary artery in the middle of the ventricle mitoses were not found in the myocytes of the atrium and auricle. In all series of experiments mitoses were rare in the myocytes of the ventricle (in seven of 49 cases) and were located at a distance from the infarct, in subepicardial zone; the mitotic index there varied from 1 to 2%.

KEY WORDS: *Myocardial infarct; division of cardiomyocytes; mitotic index.*

The problem of the degree of proliferation of the muscle cells of the myocardium during its repair after injury in adult mammals has not yet been solved. Experimental data both confirming [3, 4, 10, 13] and refuting [9, 11, 12] the possibility of repeated divisions of the cardiomyocytes after various types of myocardial injuries have been published. In some reports well-marked proliferative activity of the atrial myocytes has been observed by comparison with the myocytes of the ventricular musculature [5-7]. However, these observations have recently been questioned [2]. It has accordingly been postulated that differences in the response of myocytes in different parts of the myocardium to trauma may depend on the size and location of the defect arising in the organ.

The object of this investigation was to study the level of proliferation of myocytes of the left half of the heart (left ventricle, atrium, and auricle) after infliction of injuries differing in intensity and location.

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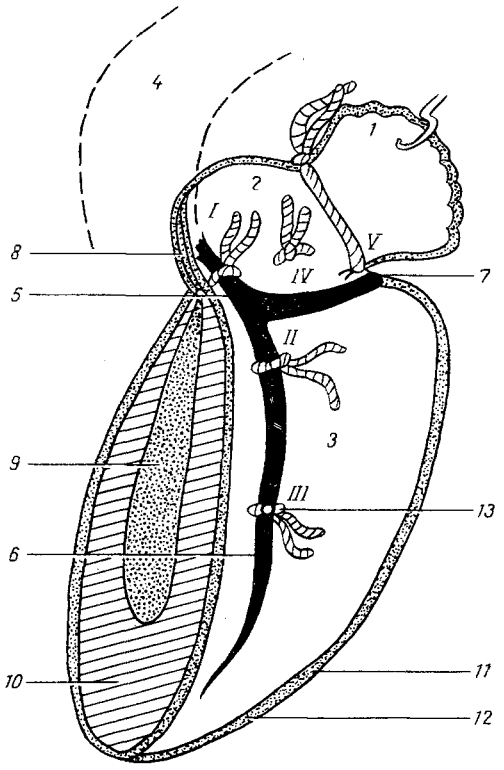

Fig. 1. Scheme of operations in different series of experiments: I-V) sites of application of ligature. 1) auricle; 2) atrium; 3) ventricle; 4) aorta; 5) left coronary artery; 6) anterior descending branch; 7) circumflex branch; 8) atrial cavity; 9) ventricular cavity; 10) myocardium; 11) outer layer of pericardium; 12) epicardium; 13) ligature.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 120-160 g were used. Six series of experiments were carried out. In the first three series, to produce an infarct of the left ventricular myocardium, after thoracotomy and removal of the outer layer of the pericardium a ligature was applied to the anterior descending branch of the left coronary artery. These series of experiments differed in the site of application of the ligature (Fig. 1): In series I the ligature was applied at the level of origin of the left coronary artery from the aorta, i.e., the operation was performed in the region of the atrium, in series II the ligature was applied to the coronary artery at the level of the first third of the left ventricle, close to the atrium, whereas in series III the left coronary artery was ligated at the level of the middle third of the left ventricle, i.e., at a distance from the atrium. In the animals of series IV and V only the left atrium was damaged: In series IV the ligature was applied to the muscular tissue of the atrium in the region of its anterior wall, and in series V the left auricle was ligated, approximately at its middle. In the experiments of series VI a mock operation was performed: After thoracotomy the pericardium was removed from the heart, after which the heart was replaced. Altogether 120 animals underwent operations. In all series of experiments the rats were killed on the 5th day after the operation at 10 a.m.

The heart was fixed in Carnoy's fluid and its left half was then embedded in paraffin wax. Histological and morphometric investigations were made of eight to 10 hearts from each series of experiments. Sections 5-7μ thick were cut in the longitudinal direction, with the object of obtaining a middle section including the left auricle, atrium, and left ventricle at the same time. Some sections from different parts of the heart were cut in the transverse direction. The sections were stained with hematoxylin-eosin and by Mallory's method;

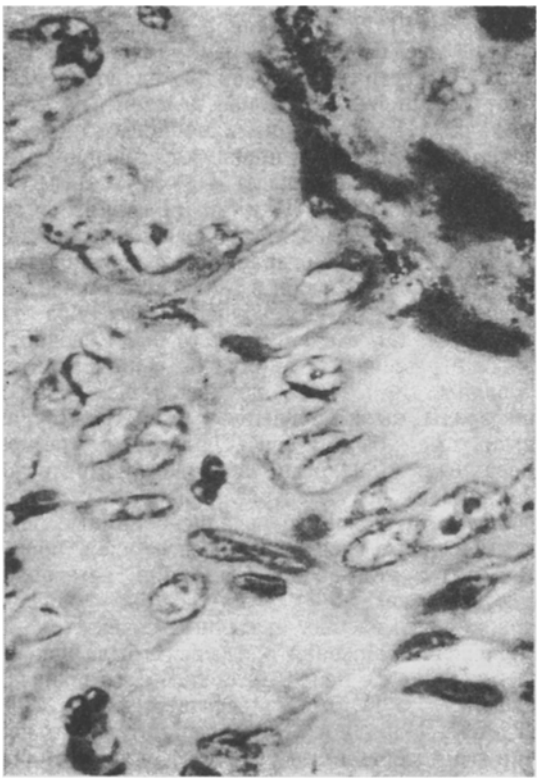

Fig. 2. Accumulation of nuclei in muscle fibers close to infarct. PAS-reaction with amylase treatment, 1100×.

the PAS-reaction was carried out and the sections treated with amylase to reveal muscle fibers, for in that case they appeared pale, having lost their glycogen. The mitotic index (MI) was determined for the muscle nuclei of the atrium, the auricle, and the subepicardial zone of the ventricle (in 3000-4000 nuclei). The dimensions of the infarct and the width of the epicardium were measured with a linear ocular micrometer. The severity of the myocardial injury was expressed as a percentage by Avtandilov's method [1]. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Infarcts of the ventricle in the experiments of series I and II were located mainly in the upper two thirds of the left ventricle. The rate of survival of the animals in series I was 30%, compared with 80-100% in series II. The combination of injury to the left atrium (during application of the ligature) and infarct of the ventricular myocardium proved particularly lethal to the animals. In series III none of the rats died: The zone of injury in these animals as a rule extended only to the lower third of the ventricle. Practically none of the animals died likewise in series IV, V, and VI of the experiments.

The size of the infarct, the intensity of the inflammatory reaction, the proliferation of fibrous tissue around the zone of the infarct, and also the reactive thickening of the epicardium both near the site of injury to the myocardium and also at a distance from it, differed in the different series of experiments. In cases in which the infarcts were large (transmural) the necrotic muscle fibers were not yet completely resorbed. Sometimes long chains or whole bunches of muscle nuclei, similar to those observed during regeneration of skeletal muscle fibers, when so-called muscle buds are formed, were sometimes observed close to the infarcts at the ends of grossly hypertrophied ventricular muscle fibers (Fig. 2).

The degree of injury to the atrium was greatest in the series in which it was injured directly: 71±8 and 77±8% in series I and IV respectively. Meanwhile the inflammatory changes in the atrium or auricle (especially on the side of the epicardium) could arise even when these parts of the heart were not affected by the operation. The thickness of the epi-

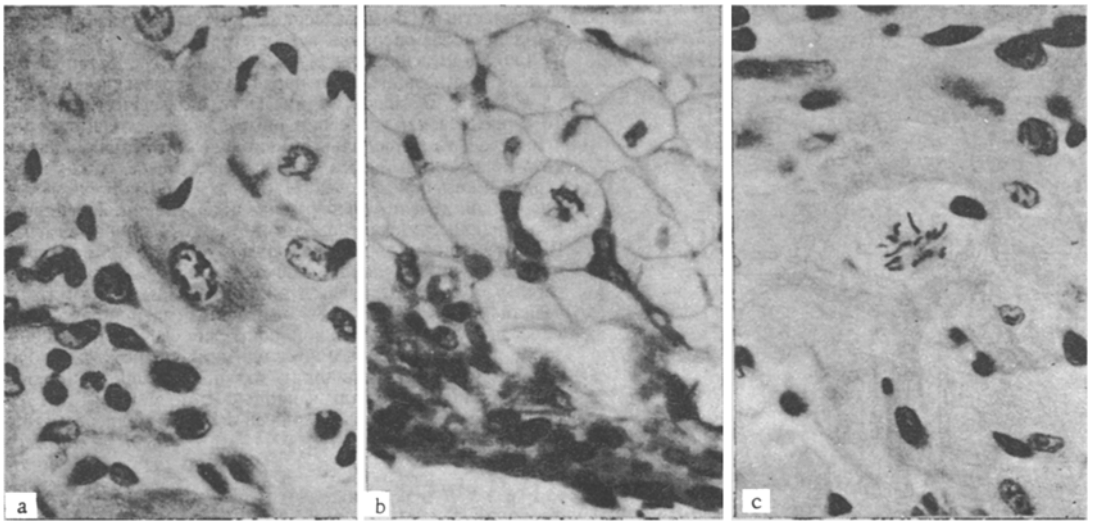

Fig. 3. Mitotically dividing myocytes: a) in atrium (hematoxylin-eosin, 1100×); b and c) in subepicardial zone of ventricular myocardium (PAS reaction and treatment with amylase, 1100×).

TABLE 1. MI of Myocytes in Different Parts of the Myocardium after Injury to It

Series of experiments	No. of rat	MI (‰) auricle	atrium†	ventricle*
I. Ligature to coronary artery in region of left atrium	1	10	0,9	0
	2	3,7	1,3	0
	3	2,6	1,04	0
	4	1,4	0	0
	5	6,1	0,4	0
	6	2,6	—	0
	7	3,6	—	0
	8	3,5	0,9	0
	9	3,3	—	0
	10	4,4	—	11,5
	$M \pm m$	4,1±0,8	0,83±0,21	—
II. Ligature to coronary artery in region of upper third of left ventricle	1	7,5	6,3	0
	2	4,7	3,1	0
	3	0	0	0
	4	2,6	0	0
	5	1,6	0	0
	6	1,8	1,3	0
	7	3,4	1,5	0
	8	0,9	0	0
	$M \pm m$	2,8±0,6	1,5±0,8	—
III. Ligature to coronary artery in middle third of left ventricle	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	$M \pm m$	0	0	0
IV. Ligature on anterior wall of left atrium	1	5,0	—	2,4
	2	1,2	0	0
	3	1,4	—	1,3
	4	3,5	—	0
	5	0,6	—	0
	6	0,5	—	0
	$M \pm m$	2±0,7	—	—
V. Ligature in region of left auricle	1	1,2	—	0
	2	0,9	1,4	0
	3	2,3	—	1,3
	4	1,6	0,5	0
	5	7,4	—	0
	6	2,5	—	0
	7	5,5	—	0
	8	0	0	0
	9	3,6	1,5	0
	10	1,7	0	1,0
	$M \pm m$	2,6±0,6	0,34±0,3	—
VI. Mock operation	1	7,0	7,0	2,0
	2	0,3	0	0
	3	0	0	1,3
	4	7,5	0	0
	5	0	0	0
	6	0	0	0
	7	0	0	0
	8	0	0	0
	9	0	0	0
	10	0	0	0
	$M \pm m$	1,5±0,3	—	—

*Mitoses counted only in myocytes of subepicardial zone of ventricular myocardium.

†Mitoses were not counted in these cases because of intensive destruction of atrial muscle fibers.

cardium sometimes reached 140μ compared with a normal value of 5-7μ (series II, III, and VI). No abnormalities were found in the ventricular or atrial myocardium of animals undergoing the mock operation, except reactive thickening of the epicardium, to not more than 40μ in most cases.

Investigation of the myocardium of the atrium, auricle, and ventricle of the animals undergoing the mock operation revealed mitotically dividing nuclei in the myocytes in all series of experiments except III. Mitoses were seen particularly clearly after treatment of the sections with amylase followed by the PAS reaction. In these cases the smaller dividing connective-tissue cells gave a positive PAS reaction, whereas myofibrils and a large dividing nucleus could be clearly detected in myocytes already freed from glycogen. Mitotically dividing myocytes were observed in both longitudinally and transversely sectioned muscle fibers (Fig. 3 a, b).

Mitotically dividing myocytes were seen much more frequently in the auricle or atrium, especially if these parts of the heart were directly involved in the trauma (series I, IV, V) or if injury to the ventricular myocardium occurred close to the atrium (series II). At a point remote from the atrium or auricle, the latter did not respond to this injury by proliferation of myocytes (series III). In series I, for instance, myocytes were found in the auricle in all 10 cases (MI=4.1±0.8‰), in the atrium in five cases (MI=0.83±0.2‰), and in the subepicardial zone of the ventricular myocardium in only one case (MI=11.5‰). In the experiments of series II no mitoses were found in the ventricular myocytes in any of the eight cases; in the atrium and auricle, on the other hand, MI was 1.5±0.8 and 2.8±0.6‰ respectively. MI in the auricle in series I differed significantly from the same index in the other series ($P = 0.05$).

In all 16 animals with local injury to the atrium or auricle (experiments of series IV and V) mitoses were found in the myocytes of the auricle and in some in the atrium (if the latter was not severely damaged), where MI was 2±0.7 and 2.6±0.6‰, respectively.

The existence of mitotic division of myocytes in the subepicardial zone of the ventricular myocardium in two of the animals undergoing the mock operation (series VI) was a striking fact; MI was 2 and 1.3‰ respectively. In three rats of this series mitoses were found in the myocytes of the auricle. Individual variations in MI in the different series of experiments are given in Table 1.

Hence, regardless of the type of injury to the heart, mitotically dividing ventricular myocytes were found only in the subepicardial zone of the myocardium with a width of 60-80 μ. The myocytes were generally more loosely distributed there, and their cytoplasm and nucleus were more juicy than in other parts of the rat myocardium. The phenomenon of mitotically dividing myocytes in the subepicardial zone of the ventricular myocardium of adult rats requires special study and explanation, more especially because attention has previously been drawn to this fact in the literature [8].

Another fact must also be emphasized: Mitotically dividing myocytes were found in the ventricle, auricle, or atrium in most cases when the epicardium was reactively greatly thickened and inflamed. In these cases the epicardium could be 10-15 times wider than in the control, sometimes up to 150 μ.

It can be concluded on the whole from these results that different parts of the myocardium in adult mammals respond differently to trauma. Differences observed in the response of the different parts of the myocardium to injury are evidently attributable to morphological and functional differences. The contradictions found in the literature on the question of proliferative power of the myocytes after injury to the myocardium can evidently be attributed to differences in the experimental conditions used by different workers [2, 5-7]. In order to excite the atrial myocytes to mitotic division, it is important where and how the injury is inflicted. Application of a ligature to the coronary artery in the region of the atrium or slightly below it evidently induces proliferation of the myocytes in the atrium [5-7]; meanwhile, if the ligature is applied to the left coronary artery more distally, the atrium and auricle do not necessarily respond by division of the myocytes [2].

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