ACTIVATION OF DNA SYNTHESIS IN CELLS OF THE ATRIUM AND AURICLE IN RATS WITH INFARCTION OF THE LEFT VENTRICLE

V. R. Babaev and I. A. Kazantseva

UDC 616.124.2-005.8-092.9-07:616.125-008.939.633.2-074

From the first to the 15th day of experimental infarction of the left ventricle the number of DNA-synthesizing nuclei of connective-tissue cells was increased in the auricle and atrium of the rat heart. The number of labeled myocyte nuclei reached a maximum on the 5th day of the experiment and the number of mitoses in the muscle cells of the auricle on the 7th day. In the muscle cells of the atrium very few nuclei with incorporation of [^{3}H]thymidine and only solitary pathological mitoses were observed. After healing of the infarct an increased number of polyploid nuclei was observed in the cardiomyocytes of the auricle and atrium.

KEY WORDS: regeneration; myocardial infarction; mitoses of cardiomyocytes; auricle; atrium.

The morphological manifestation of ischemic heart disease can often be observed in the tissues of the auricle and atrium [5, 10]. The spread of the necrotic process from the ventricles to the atrium leads to clinical complications and is often the cause of sudden death [12]. The study of the character and intensity of repair processes in the cells of the auricle and atrium is thus of considerable interest. However, the possibility of the activation of DNA synthesis in the nuclei of the myocardial cells of the auricle and atrium and of their mitotic division under conditions of experimental myocardial infarction remains a matter for debate [8], and different workers hold opposite views on this matter [1-3, 9, 11].

In the investigation described below an attempt was made, on the basis of data of autoradiography, cytophotometry, and counting the number of mitoses in the myocyte nuclei, to determine whether DNA synthesis is activated in the muscle cells of the auricle and atrium of rats at various stages of infarction of the left ventricle.

EXPERIMENTAL METHOD

The descending branch of the coronary artery was transfixed and ligated immediately below the left auricle in 23 noninbred male albino rats weighing 90-100 g. The animals were killed 1, 2, 3, 5, 7, 15, 20, and

TABLE 1. Number of Mitoses of Myocytes and DNA-Synthesizing Nuclei in Auricle and Atrium at Different Times of Healing of Myocardial Infarcts in Rats (M ± m)

Time after formation of infarct, days	Auricle			Atrium		
	mitotic index of myocytes, %	labeled nuclei, %		mitotic index of myocytes, %	labeled nuclei, %	
		of muscle cells	of connective-tissue cells		of muscle cells	of connective-tissue cells
Control	0	0,42±0,21	1,01±0,17	0	0,26±0,18	0,98±0,20
1	0	0,21±0,15	4,11±0,31	0	0,13±0,13	3,58±0,37
2	0	0,32±0,18	4,08±0,33	0	0	2,41±0,28
3	2,12±1,50	0,31±0,18	3,04±0,28	0	0,12±0,12	1,94±0,24
5	2,32±1,64	9,88±1,01	4,21±0,33	1,33±1,33	0,27±0,19	3,00±0,30
7	11,66±4,18	3,23±0,61	2,63±0,26	0	0,52±0,26	3,30±0,31
15	4,76±2,37	1,30±0,40	1,19±0,18	0	0	0,81±0,15
20	3,94±2,27	0,61±0,27	0,60±0,13	1,33±1,33	0,13±0,13	0,51±0,12
90	3,70±2,13	0,37±0,21	0,44±0,11	2,12±1,50	0,14±0,14	0,56±0,13

Central Morbid Anatomical Laboratory, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 4, pp. 480-484, April, 1978. Original article submitted October 3, 1977.

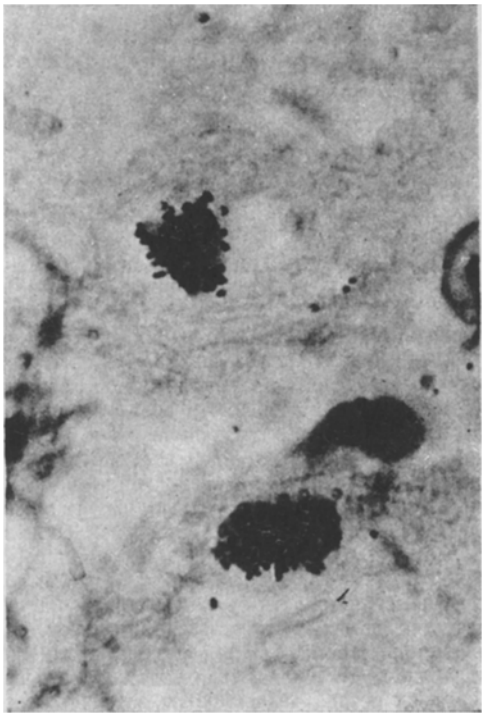

Fig. 1. Labeled nuclei of cardiomyocytes in rat auricle on 5th day of infarction (1600×).

90 days after the beginning of the experiment. Pairs of intact rats were decapitated at the beginning and end of the experiment. All the animals received an intraperitoneal injection of [^{3}H]thymidine in a dose of 1 μCi/g (specific activity 12.5 Ci/mmole) 2 h before sacrifice. The hearts were fixed in Carnoy's fluid. Sections 5 μ thick were coated with 5 M emulsion, developed, and treated in the usual way to obtain autoradiographs. The number of labeled nuclei of muscle and connective-tissue cells per 100 fields of vision under the magnification of 1350× was counted in the left auricle and atria at each period. In sections of the same series stained with hematoxylin–eosin the mitotic index was calculated in the cardiomyocytes of the auricle and atrium. Pieces of atria and auricles of rats decapitated 90 days after ligation of the arteries and of control canimals of the same age were homogenized, films were prepared, and these were then stained by the Feulgen method (hydrolysis with 5N HCl for 45 min at 20°C, staining with Schiff's reagent for 1 h). At each time the DNA content was determined microspectrophotometrically in 100 nuclei of muscle cells of the auricle and atrium [6, 7]. The mean DNA content per lymphocyte nucleus of the same rats was taken as the standard of ploidy. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During experimental infarction of the left ventricle ischemic foci of necrosis were observed in the atrium and auricle of all the rats; the size and number of the foci differed in different animals, and this explains the variability of the numerical indices of proliferative activity of the stromal cells, especially in the early period of infarction (Table 1). Starting with the 1st day of the experiment, the number of nuclei of connective-tissue cells in the atrium to have incorporated [^{3}H]thymidine was twice or three times greater than in the control. After the second week of the experiment their number decreased, and by the 20th day it was significantly less than in the control ($P < 0.001$).

In the auricle as early as 24 h after the beginning of the experiment the number of labeled connective-tissue nuclei was increased by more than 4 times compared with the control. During the first 5 days of infarction their number still remained high, then began to fall, and by the 15th day of the experiment it has regained the control level. The number of nuclei to have incorporated [^{3}H]thymidine 90 days after the beginning of the experiment was actually smaller than in the control animals ($P < 0.001$).

Although the muscle fibers of the auricle were thin and sometimes chaotically arranged, the presence of incorporation of labeled thymidine in the nuclei of the cardiomyocytes of the auricle is in no doubt, whereas in the control rats and in rats killed during the first 3 days of infarction only solitary labeled myocyte nuclei were found, on the 5th day of the experiment their number had increased by more than 20 times and it remained

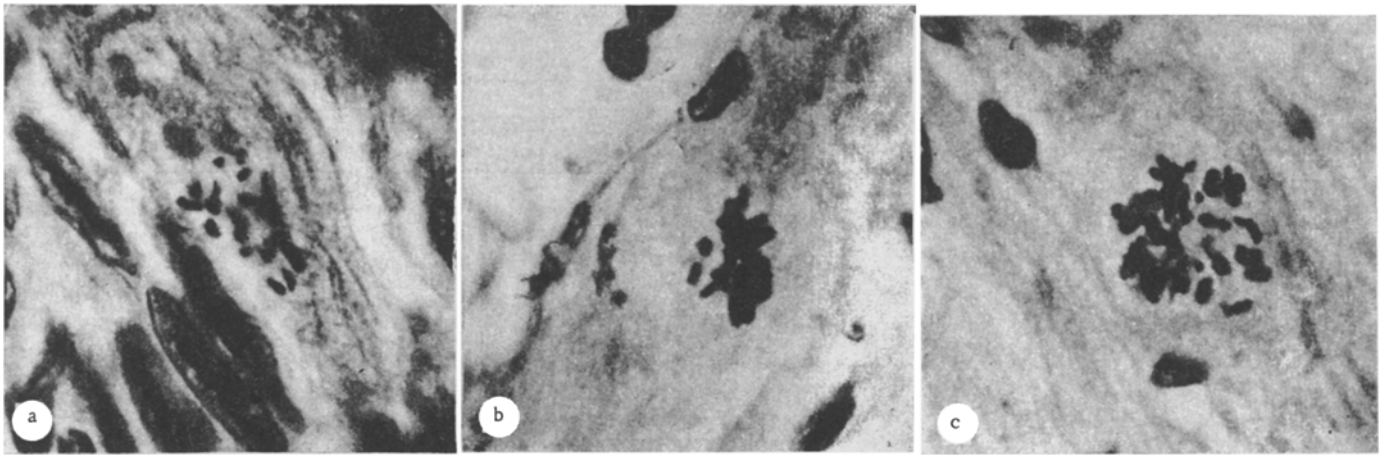

Fig. 2. Pathological mitoses of cardiomyocytes in auricle (a) and atrium (b and c) 20 and 90 days after the beginning of infarction (1600×).

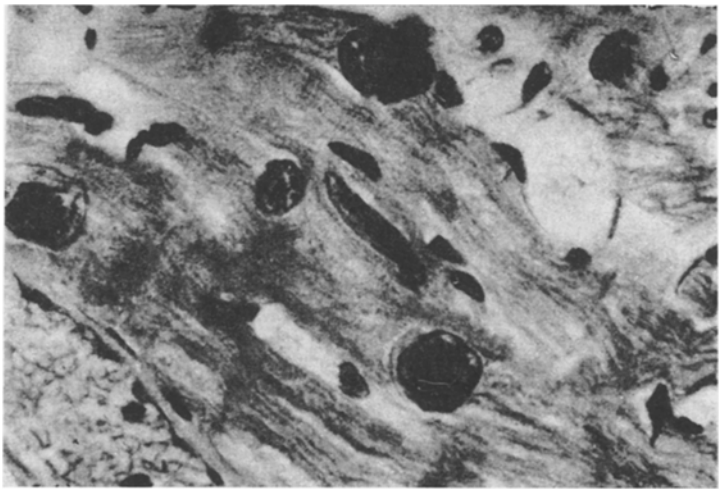

Fig. 3. Polyploid nuclei of cardiomyocytes of auricle of rat myocardium 90 days after beginning of infarction (650×).

high until the 20th day of the experiment. Myocyte nuclei with incorporation of [^{3}H]thymidine were found in muscle fibers of the auricle cut both longitudinally and transversely (Fig. 1). After 3 days and until the end of the experiment figures of mitotic division were observed in the myocytes of the auricle; mitoses were particularly numerous in myocytes on the 7th day of infarction. Among mitoses of auricular cardiomyocytes there were some pathological forms (mainly C mitoses), especially in the late stages of the experiment (Fig. 2a).

Compared with the auricle, labeled nuclei in muscle cells of the atrium were rare: the probability of incorporation of [^{3}H]thymidine was 1.8 and 0.17% respectively. Since it was doubtful whether some of the labeled nuclei belonged to muscle cells, and since the individual variability of the number of labeled nuclei was high, it must be assumed that the incorporation of [^{3}H]thymidine into myocyte nuclei in the atrium of rats of this age is random in character. The exception was the seventh day of infarction, when the number of labeled myocyte nuclei was significantly greater than in the control ($P < 0.05$). The above hypothesis was confirmed by the discovery of single mitoses in the muscle cells of the atrium (Fig. 2b, c). A special feature of the mitoses was their pathological character (C mitoses with scattering of the chromosomes). Furthermore, at the same periods of the experiment micronuclei were observed to appear in the myocytes, evidently on account of pathological mitoses [4]. Both in the auricles and in the atria, besides "paired" (lying side by side) nuclei, myocyte nuclei of large size were found (Fig. 3). This probably points to the existence of additional processes aimed at compensating for the sharply increased function of the remaining cells. Amitotic division and polyploidization of the cardiomyocyte nuclei could be processes of this type.

Microspectrophotometric investigations of the DNA content in the nuclei of the muscle cells of the auricle and atrium confirmed this suggestion. For instance, the number of tetraploid and octaploid myocyte nuclei in rats killed 90 days after the beginning of the experiments amounted to 4 and 1%, and in the auricle 2 and 2%. The number of tetraploid myocyte nuclei in intact rats of the same age was 2% in both the auricle and the atrium. The "paired" myocyte nuclei in the animals of the experimental group in this case were diploid, indirect evidence that amitosis of myocyte nuclei is connected with DNA synthesis.

In infarction of the left ventricle the temporal and quantitative characteristics of connective-tissue cell proliferation are thus approximately identical in the auricle and atrium. Activation of DNA synthesis in the nuclei of the muscle cells of the auricle, in the writers' view, is the result of the increased hemodynamic function of the organ associated with left-ventricular failure, leading to an increase in the size of the auricle in the late stages of infarction. This does not rule out the possibility of proliferation of some myocytes in response to injury [9]. Whereas incorporation of [^{3}H]thymidine in the auricle of the ischemic heart followed by mitosis of the myocyte nuclei is combined with other methods of increasing the quantity of genetic material, in the atrium incorporation of [^{3}H]thymidine like mitotic division of the myocyte nuclei, is a rare phenomenon. The pathological character of all the mitoses found in myocyte nuclei in the atrium emphasizes the atypical and random character of this type of regenerative response. The increase in the total quantity of nuclear DNA in the muscle cells of the rat atrium takes place as a result of polyploidization and also, possibly, of amitotic division of the cardiomyocyte nuclei, and the role played by each of these two processes may differ in importance.

LITERATURE CITED

1. G. B. Bol'shakova, Byull. Éksp. Biol. Med., No. 5, 610 (1977).
2. V. N. Galankin, "Some problems in regeneration and hypertrophy of the myocardium," Author's Abstract of Doctoral Dissertation, Moscow (1974).
3. V. N. Galankin and A. A. Pal'tsyn, Trudy Gor'kovsk. Med. Inst., No. 66, 247 (1975).
4. I. A. Kazantseva and V. R. Babaev, Arkh. Pat., No. 5, 64 (1977).
5. E. E. Matova, D. Yu. Khodzhaeva, and N. A. Mazur, Arkh. Patol., No. 4, 30 (1975).
6. Yu. E. Morozov, Tsitologiya, No. 5, 690 (1970).
7. B. L. Pereverzev, V. M. Andreev, S. I. Konovalov, et al., Tsitologiya, No. 8, 1050 (1974).
8. L. V. Polezhaeva, Arkh. Anat., No. 4, 115 (1977).
9. V. F. Sidorova and G. B. Bol'shakova, Byull, Éksp. Biol. Med., No. 8, 998 (1976).
10. D. R. Alonso, J. G. Hayes, L. G. Eisenhauer, et al., Circulation, 18, 39 (1973).
11. P. P. Rumyantsev, Arch. Pathol. Anat. Abt. B. Zellpath, 15, 357 (1974).
12. W. B. Wartman and J. C. Sonders, Arch. Pathol., 50, 329 (1950).