AUTORADIOGRAPHIC INVESTIGATION OF DNA SYNTHESIS IN MUSCLE AND CONNECTIVE-TISSUE CELLS OF THE HEART AFTER INJURY BY ISOPROPYLNORADRENALIN

O. A. Kostyrev and T. A. Leont'eva

UDC 616.12-007.61-092.9-07:616.12-008.939.633.2-074

The ventricles of adult mice contain relatively few (hundredths of 1 percent) muscle cells which can still synthesize DNA. On the second week of injections of increasing doses of isopropylnoradrenalin the number of muscle cells synthesizing DNA is increased by 2-3 times. The localization of the muscle cells synthesizing DNA is unconnected with the localization of necrosis. The labeling index in the connective-tissue cells is some hundreds of times higher than in the muscle cells. The relative number of connective-tissue cells in the intact zones of the myocardium is reduced, probably as a result of their migration into foci of injury.

DNA synthesis and the mitotic index in the muscle cells of the ventricles of the mammalian heart are sharply reduced in the early postnatal period. In adult animals the myocardium of the ventricles is regarded as a tissue which virtually does not renew its cell composition [3, 6, 10, 11, 13, 14]. So far as the possibility of proliferation of the myocardial cells during regeneration and hypertrophy is concerned, data in the literature on this question are contradictory [4, 5, 7, 8].

The investigation described below was undertaken to study the ability of the muscle and connective-tissue cells of the heart to synthesize DNA.

EXPERIMENTAL METHOD

Male albino mice weighing 20-25 g received daily injections of isopropylnoradrenalin by the scheme described earlier [1]. Injection of increasing doses of this compound is regarded as the most effective method of reproducing rapid hypertrophy of the myocardium together with focal damage [1, 12]. Thymidine-H^3 with a specific activity of 3.5 Ci/mmole was injected intraperitoneally in a dose of 1μCi/g body weight twice a day 6 and 12 h after the injection of isopropylnoradrenalin. The control animals were injected with thymidine-H^3 only. The mice of group 1 (five experimental and five control) received isopropylnoradrenalin and thymidine-H^3 for six days and then were decapitated one day after the last injection of thymidine-H^3. The animals of group 2 (eight experimental and five control) received injections of isopropylnoradrenalin and thymidine-H^3 for 14 days; these mice were sacrificed 1 h after the last injection of thymidine-H^3.

Autoradiographs were prepared by Khrushchev's method [9] using type M (NIKFI) liquid emulsion. After exposure and development the sections were stained with Mayer's hematoxylin. To determine the relative number of labeled nuclei, 40,000 nuclei of muscle cells and 1,000 to 2,000 nuclei of connective-tissue cells (outside the zone of injury) were examined in the sections through the cardiac ventricles. The decision whether labeled nuclei belonged to muscle cells or not was verified by the use of polarized light (Fig. 1).

Laboratory of Pathomorphology, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 7, pp. 108-111, July, 1973. Original article submitted November 21, 1972.

TABLE 1. Increase in Relative Weight of the Heart (weight of heart/body weight) and Labeling Index of Muscle and Connective-Tissue Cells in Myocardium of Mice during Injury by Isopropylnoradrenalin

Group of animals	Time of injection of isopropylnoradrenaline and thymidine H^3 (days)	Increase in relative weight of heart (in %)	Labeling index (in %)		
			muscle cells	connective-tissue cells	
				left ventricle	right ventricle
1	6	12	$0,6 \cdot 10^{-2} \pm 0,16 \cdot 10^{-2}$	3,5±0,5	2,8±1,5
Control 1 . . .	6	—	$1,0 \cdot 10^{-2} \pm 0,17 \cdot 10^{-2}$	10,0±1,8	3,3±0,5
2	14	42	$3,5 \cdot 10^{-2} \pm 0,30 \cdot 10^{-2}$	24,7±2,9	8,9±0,6
Control 2 . . .	14	—	$1,2 \cdot 10^{-2} \pm 0,34 \cdot 10^{-2}$	13,0±1,0	6,3±1,1

EXPERIMENTAL RESULTS

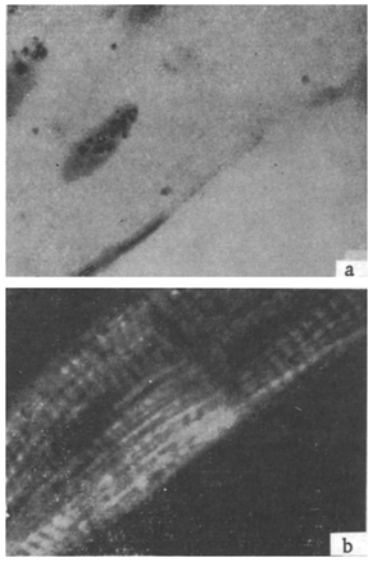

Fig. 1. Incorporation of thymidine-H^3 into nucleus of a muscle cell: a) stained with Mayer's hematoxylin, 2000×; b) polarized light.

In the animals sacrificed after injections of isopropylnoradrenalin for six days, multiple small foci of necrosis of muscle cells appeared in the wall of the left ventricle, in its papillary muscle, and in the ventricular septum, surrounded by zones of infiltration with leukocytes and histiocytes. In the animals sacrificed after administration of isopropylnoradrenalin for 14 days the zone of injury to the muscle cells was considerably widened. Together with foci of necrosis of the muscle cells, extensive cicatrizing granulomas and scars appeared [1]. The thymidine label was found very rarely (in hundredths of 1 percent) in the nuclei of the muscle cells of the heart in both the control and the experimental animals, despite the repeated injection of the DNA precursor (Table 1). Nevertheless, the presence of this label in some nuclei, which undoubtedly belonged to muscle cells, indicates that the myocardium of adult animals contains a cell population capable of synthesizing DNA, and also, evidently, of reproducing [7].

After death of some of the muscle cells and hypertrophy of the rest the number of muscle nuclei synthesizing DNA increased. The number of cells synthesizing DNA was clearly less than the number of dying cells. It must be emphasized that the labeled muscle cells were scattered among the others in no visible order, and their localization was unconnected with that of the foci of injury. This probably indicates that the observed intensification of DNA synthesis in the nuclei is not the result of a local reaction of the myocardium to necrosis and that the process is not of the character of ordinary reparative regeneration.

The labeling indices of the nuclei of the myocardial cells obtained in these experiments were lower than those given by other investigators [3, 6, 15]. This may have been due to the stricter identification of the muscle cells in the present experiments.

After six days of the experiment some decrease was observed in the labeling index of the connective-tissue cells in the left ventricle outside the zones of injury, while after 14 days the labeling index was increased (Table 1). Meanwhile nearly all the connective-tissue cells of the granulomas incorporated thymidine-H^3. The decrease in the labeling index of the connective-tissue cells after injections of isopropylnoradrenalin for six days was probably associated with the commencing migration of connective-tissue cells into the zones of injury. During this period the ratio between the number of nuclei of muscle cells and the number of nuclei of connective-tissue cells in the intact zones of the myocardium was 1:(2.14 ± 0.15) compared with 1: (2.63 ± 0.28) in the control; by the 14th day of the experiment this ratio was 1:(1.37 ± 0.06 compared with 1: (2.33 ± 0.12) in the control. The increase in proliferative activity of the connective-

tissue cells outside the zones of injury at this period of the experiment may be connected with the making good of the loss of cells in these areas.

The increase in labeling index in the cells of the stroma cannot be regarded as a manifestation of "companion hypertrophy" aimed at strengthening the connective-tissue framework of the heart [2], for in zones in which the muscle fibers were hypertrophied but no foci of necrosis were present thickening of the argyrophilic skeleton and the formation of new collagen fibers were not observed [1] while the number of connective-tissue cells in these zones, as was pointed out above, was actually reduced.

LITERATURE CITED

1. T. A. Leont'eva and L. A. Semenova, Cor et Vasa (Prague), 13, 265 (1971).
2. F. Z. Meerson and G. M. Alekhina, Acta Anat. (Basel), 70, 559 (1968).
3. V. O. Mirakyan and P. P. Rumyantsev, Tsitologiya, No. 8, 964 (1968).
4. L. V. Polezhaev, A. V. Akhabadze, N. A. Muzlaeva, et al., Simulation of Regeneration of the Heart Muscle [in Russian], Moscow (1965).
5. P. P. Rumyantsev, Dokl. Akad. Nauk SSSR, 100, 601 (1955).
6. P. P. Rumyantsev, Folia Histochem. Cytochem., 4, 397 (1966).
7. P. P. Rumyantsev, in: Symposium on Regeneration of the Myocardium [in Russian], Erevan (1970), p. 10.
8. N. P. Sinitsin, in: Mechanisms of Regeneration and Cell Division [in Russian], Moscow (1971), p. 157.
9. N. G. Khrushchev, Functional Cytochemistry of Connective Tissue [in Russian], Moscow (1969).
10. I. L. Cameron, Texas Rep. Biol. Med., 28, 203 (1970).
11. O. Kling and E. Stocher, Experientia, 24, 167 (1968).
12. K. Rakusan, H. Tietzova, Z. Turek, et al., Physiol. Bohemoslov., 14, 456 (1965).
13. R. Sasaki, G. Watanabe, T. Morishita, et al., Tohoku J. Exp. Med., 95, 177 (1968).
14. R. Sasaki, T. Morishita, and S. Jamagata, Tohoku J. Exp. Med., 96, 405 (1968).
15. R. Sasaki, T. Morishita, and S. Jamagata, Tohoku J. Exp. Med., 101, 153 (1970).