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AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN RATS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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Tritium-labeled thymidine was injected into rats with an experimental myocardial infarct and the number of DNA-synthesizing nuclei was determined in various parts of the heart. Myocardial infarction activated DNA synthesis to some extent in the nuclei of monocytes lying at the periphery of the focus of injury. However, there was no doubt about the extremely low density of labeling in the muscle nucleus. Increasing the dose and giving three injections of thymidine-^{3}H did not increase the number of labeled muscle cell nuclei. Activation of proliferation of the connective tissue cells was observed in all parts of the heart. The number of connective-tissue nuclei synthesizing DNA was increased after 24 h, reached a maximum on the second day, and remained above the control level until the end of the experiment.

KEY WORDS: myocardial infarct; DNA synthesis; cardiomyocytes.

The most debatable problem in the subject of regeneration of the myocardium is the possibility of DNA synthesis in the nuclei of the muscle cells of the ventricle of the adult mammalian heart. Although Grove et al. [10] and Sasari et al. [15] found an increase in the number of polyploid cardiomyocyte nuclei and in the number of muscle cells in the hypertrophied rat heart, autoradiographic investigations with tritium-labeled thymidine (thymidine-^{3}H) have yielded the opposite results. For instance, in addition to data pointing to the absence of DNA-synthesizing myocyte nuclei in the hypertrophied [4, 9] and infarcted [1] myocardium of adult animals, in other investigations activation of DNA synthesis has been reported in some muscle cells of the ventricles [3], especially cells near the focus of injury [11, 12].

Meanwhile, during the electron-autoradiographic study of DNA synthesis in cardiomyocytes after physical exertion, nuclei with a small number of grains were found, and their appearance could be attributed to slow synthesis of nuclear DNA [5]. This is in agreement with the view that an increase in the level of differentiation of the myocardial myocytes is accompanied by a marked increase in the duration of the main periods of the cell cycle [2, 13].

It thus appeared interesting to use autoradiographic techniques to study the topography of DNA-synthesizing nuclei of the ventricles of the rat heart in relation to a focus of infarction.

EXPERIMENTAL METHOD

An infarct was produced in the myocardium of 20 noninbred male albino rats weighing 90-100 g by suture and ligation of the left coronary artery. The animals were killed 1, 2, 3, 5, 7, 15, and 20 days after the begin-

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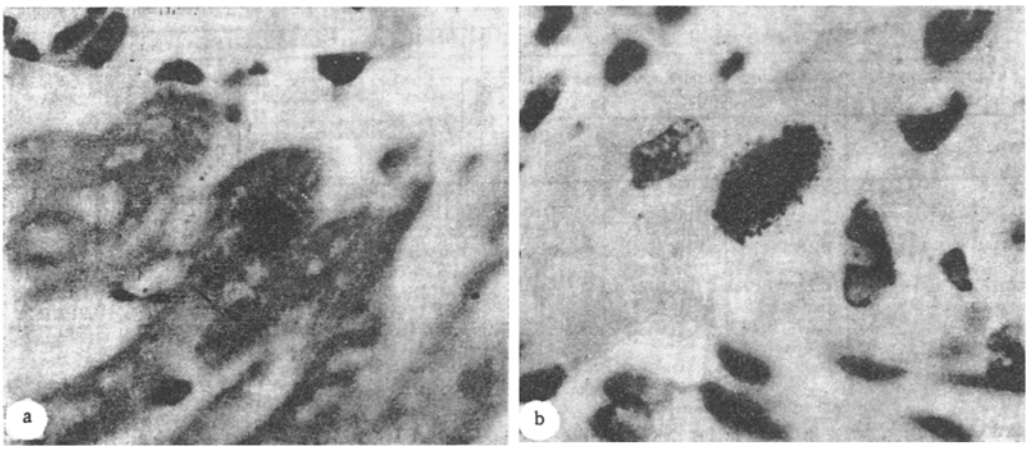

Fig. 1. Labeled nuclei of rat cardiomyocytes 15 (a) and 20 (b) days after onset of myocardial infarct. 1100×.

ning of the experiments. Two rats were taken each time. As the control, four intact rats of the same weight were used and were killed at the beginning and end of the experiments. All the animals were given an intraperitoneal injection of thymidine-^{3}H (specific activity 2.8 Ci/mmole) in a dose of 1 μCi/g 2 h before sacrifice. Four rats received thymidine-^{3}H in the same dose as three injections with an interval of 24 h after each injection. The animals of this group were killed 5 and 7 days after development of the infarct and 2 h after the last injection of thymidine-^{3}H. Thymidine-^{3}H was injected into two rats in a dose of 3 μCi/g 1 week after development of the myocardial infarct and these animals were killed 3 h after the injection. The hearts were cut transversely across the ventricles, fixed in Carnoy's fluid, and embedded in paraffin wax. An undercoating was applied to sections 5 μ thick [8], which were covered with type M emulsion. The sections were exposed for 15, 30, and 45 days. The following zones of the heart were distinguished in histoautoradiographs stained with hematoxylin–eosin and azure II–eosin: the focus of injury, the boundary zone, intact regions of the left and right ventricles. In all these zones of the myocardium the number of labeled nuclei of muscle and connective-tissue cells of the heart was counted in 100 fields of vision under a magnification of 1350×. Labeled nuclei were not counted in the group of animals receiving three injections of an increased dose of thumidine-^{3}H. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Nuclei of muscle cells synthesizing DNA were found extremely rarely in both the intact and the infarcted rat heart. Increasing the dose and the number of injections of thymidine-^{3}H caused no significant increase in the number of labeled cardiomyocytes. Of the 16,000 myocyte nuclei counted in the histoautoradiographs 23 were labeled, but only in three of them was there no doubt that a label was located above the nucleus of the muscle cell, for it was found in the transverse section of a muscle fiber (Fig. 1a). Otherwise, despite the very large size of the nuclei and their location strictly in the center of the muscle fiber (Fig. 1b), differentiation between labeled nuclei of myocytes and fibroblasts was difficult. By contrast with the ventricles, labeled nuclei of muscle cells were found relatively frequently in the myocardium of the atria and auricles.

The number of DNA-synthesizing nuclei in the hearts of the intact rats killed at the beginning and end of the experiments did not differ significantly, and for that reason the control data are combined into a single group (Table 1).

The myocardial infarct stimulated the synthetic activity of the myocyte nuclei a little. The number of labeled muscle nuclei was increased 48 h after the beginning of the experiment, especially in the peripheral zone of the infarct, when it amounted to 0.54 ± 0.19% compared with 0.12 ± 0.08% in the wall of the left ventricle of the myocardium of the control rats ($P < 0.05$). In the later stages of infarction the number of nuclei incorporating thymidine-^{3}H also was somewhat greater than in the control, especially in muscle cells bounding the focus of injury; however, differences from the myocardium of the intact animals were not statistically significant.

In myocardial infarction proliferation of the connective-tissue cells took place in all parts of the heart. Labeled nuclei of stromal cells were particularly numerous at the periphery of the focus of injury, where the

TABLE 1. Topography of Changes in Number of DNA-Synthesizing Nuclei of Rat Myocardium at Different Stages of Experimental Infarction

Time after appearance of infarct, days	Labeled muscle cell nuclei, %			Labeled connective-tissue cell nuclei, %			
	peripheral zone of infarct	left ventricle	right ventricle	focus of injury	peripheral zone of infarct	left ventricle	right ventricle
Control		0,12±0,08	0,07±0,06			0,49±0,12	0,46±0,12
1	0	0	0	4,10±0,48	6,32±0,39	0,93±0,22	1,12±0,24
2	0,54±0,18	0,06±0,06	0,14±0,01	9,69±0,35	10,78±0,45	1,39±0,19	1,80±0,23
3	0,18±0,18	0,13±0,13	0,13±0,13	7,70±0,37	5,04±0,43	1,15±0,24	6,74±0,18
5	0	0	0	2,76±0,21	3,08±0,31	1,28±0,24	0,69±0,18
7	0,21±0,21	0	0	2,68±0,25	2,16±0,30	1,02±0,20	0,68±0,16
15	0,20±0,20	0	0	0,79±0,12	0,71±0,17	0,40±0,13	0,21±0,09
20	0,54±0,38	0	0	1,61±0,23	1,19±0,20	0,29±0,11	0,28±0,12

percentage of nuclei synthesizing DNA 24 h after the beginning of the experiments was 1.5 times the percentage of labeled nuclei in the focus of injury ($P < 0.001$) and 12 times greater than the number in the myocardium of the control rats. The percentage of DNA-synthesizing nuclei of connective-tissue cells after 2 days was the same in the zone around the infarct and in the focus of injury, but on the third day of the experiment the number of labeled connective-tissue cell nuclei in the focus of injury was greater than their number in the boundary zone ($P < 0.001$). Not until the 15th day of the experiment was equality reached between the number of labeled stromal cell nuclei in the focus of injury and at its periphery, but it exceeded the control level until the end of the experiment. This is evidence of growth of granulation tissue mainly from the peripheral zones and partly from areas of stroma remaining intact in the focus of the infarct. The small increase in the number of labeled stromal cell nuclei in the heart on the 20th day of the experiment compared with the 15th day can evidently be attributed to differences in the rates of maturation of the connective tissue [7] and to recurrence of the infarct.

During the first day of development of the infarct, in areas of myocardium of the left and right ventricles remote from the focus of injury the number of stromal cell nuclei of the heart incorporating the label was already increased. The number of labeled nuclei in these zones of the heart 48 h after the beginning of the experiment was three times greater than in the control ($P < 0.001$). Not until the third day in the wall of the right ventricle and until the 15th day in the wall of the left ventricle was the percentage of labeled nuclei the same as the percentage of labeled stromal cell nuclei in the intact heart.

Consequently, in the boundary zone around the infarct in the rat heart solitary muscle cell nuclei incorporate thymidine-^{3}H. The appearance of labeled nuclei of myocardial cells in this zone around the infarct can evidently be attributed to the fact that ischemia and tissue breakdown products stimulate their entry into the mitotic cycle. That is why in the subepicardial part of the myocardium, where the muscle cells remain capable of mitotic division [6], there were relatively many labeled muscle nuclei whereas in the remaining part of the ventricles their number was small, despite the repeated injections of thymidine-^{3}H [14].

Myocardial infarction stimulates proliferation chiefly of connective tissue in all parts of the heart. The largest number of labeled connective-tissue cell nuclei in the heart was found on the second day of the experiment, and this index remained higher than the control level throughout the experiment.

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HISTOCHEMICAL AND MORPHOMETRIC CHANGES IN THE ADRENAL CORTEX DURING ACUTE VASCULAR INSUFFICIENCY

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Artificial hypotension for 5 h produced in cats by intravenous injection of Arfonad is accompanied by depression of adrenal function. Insufficiency of the adrenals, especially of their glucocorticoid function, in established acute vascular insufficiency can be interpreted as adaptation aimed specifically at maintaining homeostasis.

KEY WORDS: adrenals; artificial hypotension; tissue enzyme profile.

The regulation of vascular tone is largely predetermined by the function of the adrenal glands [2, 4]. Hence, there is a need for studying the morphological and functional state of the adrenal cortex in acute vascular insufficiency accompanied by hypoxia during the first few hours [1, 6].

EXPERIMENTAL METHOD

Experiments were carried out on male cats weighing 3.5-4 kg. For morphological study the adrenals were removed immediately and again 24 h after the end of a 5-h period of artificial hypotension produced by injection of a 0.1% solution of Arfonad. For comparison the adrenals of intact and anesthetized animals were studied. The dimensions of the cells in all zones of the cortex were determined by drawing and weighing.

The material was fixed in 10% neutral formalin and paraffin sections were stained with hematoxylin–eosin and with gallocyanin and chrome alum by Einarson's method. The content of lipids and activity of various enzymes (glucose-6-phosphate dehydrogenase, G6PD; β-hydroxybutyrate dehydrogenase, β-HBD; succinate dehydrogenase, SD; lactate dehydrogenase, LD; NADH and NADPH dehydrogenases, NADH-D and NADPH-D; 3β-hydroxysteroid dehydrogenase, 3β-HSD) were determined in sections from unfixed tissue. The RNA content and activity of the enzymes were estimated cytophotometrically. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Unlike in previous investigations of the adrenals of intact cats [7-9], their histochemical profile was studied. Predominance of direct oxidation of glucose in the hexose monophosphate shunt was found, as shown by the relatively high activity of G6PD, which participates in the initial stages of oxidation by this pathway (Fig. 1a).

Another no less important pathway for carbohydrate oxidation is anaerobic glycolysis, the importance of which in the adrenal cortex of cats can be assessed by the relatively high LD activity (Fig. 1c). The importance of other pathways of carbohydrate oxidation, especially the Krebs cycle, for the cat adrenals is evidently

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