ULTRASTRUCTURE AND METABOLIC HETEROGENEITY OF CARDIOMYOCYTES IN ACUTE MYOCARDIAL LESIONS IN AGING RATS

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UDC 616.127-091.8+616.127-008.91-053.9-092.9-02:615.357.452

KEY WORDS: aging; heterogeneity of cardiomyocytes; ultrastructure; electron-microscopic cytochemistry; histoautoradiography.

The action of many extremal factors on the myocardium is mediated through catecholamines [12] which, in large doses, have a damaging action and play an important role in the onset of cardiomyopathies [13]. Exposure to catecholamines enables the structural and metabolic reserves of the heart tissue to be assessed and sheds light on the protective mechanisms which are exhibited during the first minutes after administration of the drug, thereby significantly affecting the whole course of the subsequent morphological changes.

The object of this investigation was to study structural and metabolic characteristics of the myocardium during acute injury by catecholamines in rats, depending on the age of the animal; this is an important step toward elucidation of the mechanisms determining the course and reliability of compensatory and adaptive responses to various extremal factors in the course of aging.

EXPERIMENTAL METHOD

Experiments were carried out on 11 male Wistar rats aged 3.5, 26, and 37 months. [^{3}H]Uridine (specific activity 41 Ci/mmole) was injected intraperitoneally in a dose of 5 μCi/g body weight into the animals 1.5 h before sacrifice, and this was followed by an injection of isoproterenol in a dose of 0.1 mg/g body weight 20 min before sacrifice. Ten intact rats of the same age served as the control.

The autoradiographic investigation was conducted on semithin (1 μ) Epon-Araldite sections, coated on slides with type M emulsion. After exposure for 10 days the autoradiographs were developed and stained with azure II. Paraffin sections stained with hematoxylin and eosin, by Van Gieson's method, and the PAS reaction were examined in polarized light by means of a "Docuval" microscope (Carl Zeiss, East Germany).

Pieces of myocardium from all the animals for electron-microscopic investigation were fixed in cold 4% buffered formaldehyde solution, postfixed with 1% OsO_4 solution, dehydrated in alcohols and propylene oxide, and embedded in a mixture of Epon and Araldite. Ultrathin sections, stained by the usual method, were examined in the JEM 100B electron microscope. Electron-cytochemical demonstration of succinate dehydrogenase (SDH) activity with copper ferrocyanide was carried out on specimens of the wall of the left ventricle from rats aged 3.5 and 37 months. The bulk density of granules of the SDH reaction product in mitochondria of the cardiomyocytes was determined by morphometric methods [3].

EXPERIMENTAL RESULTS

Lesions developed in the myocardium of the rats 20 ml after injection of isoproterenol (Fig. 1), mainly of the contracture type [3, 7, 8]. As a result of disturbances of capillary permeability, edema of the myocardial tissue developed, with inhibition of plasma by the walls of the blood vessels and disturbance of the hemodynamics. These changes were most marked in rats aged 37 months. Electron-microscopic examination (Fig. 2) revealed cardiomyocytes most frequently with definite ultrastructural disorganization in these same animals, reflecting a disturbance of integrity of the sarcolemma and commencing necrosis of the cells. The number of granules of glycogen was sharply reduced in the sarcoplasm, especially in the old animals.

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 0007-4888/82/9410-1458$07.50

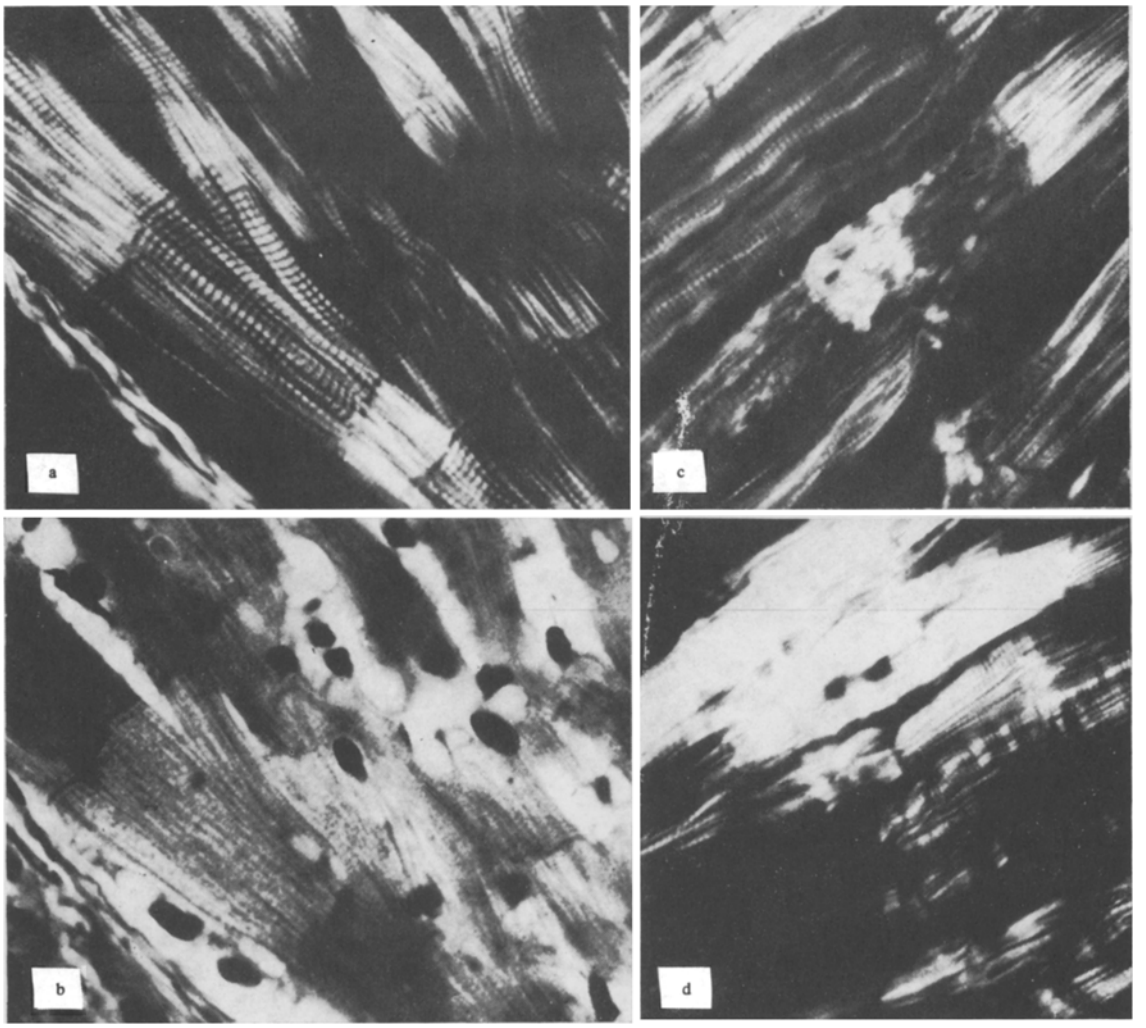

Fig. 1. Changes in myofibrils and cardiomyocytes of old rats 20 min after injection of isoproterenol. a) First degree contracture in myocardium of rat aged 26 months. Hematoxylin and eosin, photographed in polarized light, 1000×; b) the same, in ordinary light. Focal eosinophilia of sarcoplasm; c) second degree contracture and myocytolysis; d) third degree contracture; c, d) rats aged 37 months, photographed in polarized light, 1250×.

Structural changes in the mitochondria were not discovered in the early stages of the lesions, but the functional state of these organelles could be judged by the activity of their respiratory cycle enzymes.

In all animals granules of the reaction product (copper ferrocyanide) were found in the mitochondria, where they were located on the cristae – corresponding to the site of SDH. Activity of this enzyme in the control animals in the cardiomyocyte mitochondria was reduced almost by half during aging, as could be judged from the relative volume of granules of the chelate (0.076 ± 0.00003 in the young and 0.047 ± 0.02 in the old rats; $P < 0.01$). After injection of isoproterenol SDH activity was considerably increased in both young and old rats, as shown by the following values of the bulk density of the chelate granules: 0.105 ± 0.011 and 0.062 ± 0.02 for animals aged 3.5 and 37 months respectively ($P < 0.05$). Despite the parallel increase in SDH activity in the two groups of animals, activity of this important enzyme in catecholamine lesions in old rats nevertheless remained lower than in young animals.

Autoradiographic analysis of RNA synthesis in the cardiomyocyte nuclei showed (Fig. 3) most silver grains to be located above nuclei of heart muscle cells in the young animals. The number of grains of silver above cardiomyocyte nuclei of rats aged 26 and 37 months was 1.6 and 1.7 times less respectively than in young rats. The intensity of incorporation of the labeled RNA precursor into the cardiomyocytes demonstrated heterogeneity of the level of RNA synthesis in cells of the intact myocardium. Heterogeneity of the cardiomyocytes in the animals of the three different ages, incidentally, differed in its character. For instance, the popu-

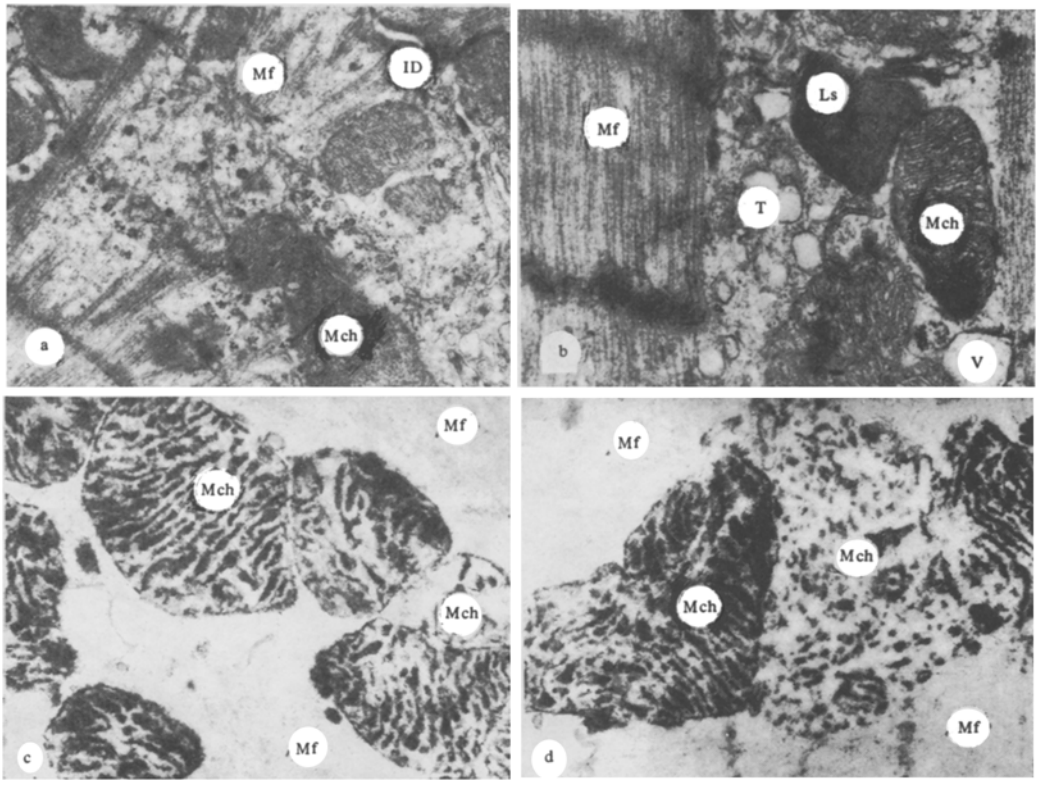

Fig. 2. Ultrastructural and electron-cytochemical changes in cardiomyocytes of rats under the influence of isoproterenol. a) Rat aged 26 months. Lysis of myofibrils (Mf), widening of intercalated disk (ID), no changes in mitochondria (Mch). 9000×; b) rat aged 37 months. Dilatation of T system (T) with formation of vacuoles (V) against the background of increased volume of myofibrils and accumulation of lysosomes (Ls). 10,000×; c) changes in SDH activity in mitochondria of cardiomyocyte of rat aged 3.5 months; d) marked heterogeneity of SDH activity in mitochondria of cardiomyocyte of rat aged 37 months. c, d) Reaction of Kerpel–Fronius and Hajos, 12,000×.

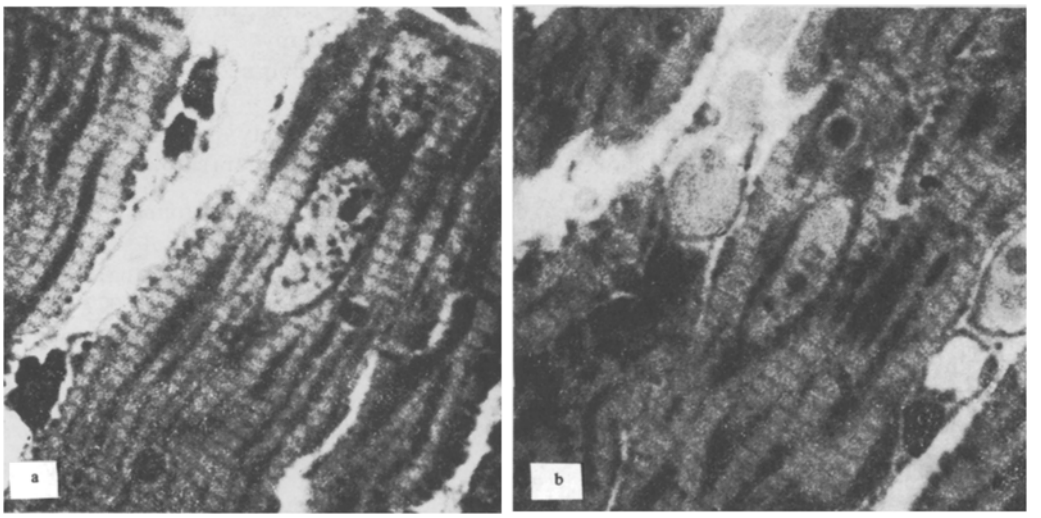

Fig. 3. Histoautoradiographic changes in cardiomyocytes of rats aged 37 months receiving isoproterenol. a) High level of RNA synthesis in nucleus of uninjured cardiomyocyte; b) low level of RNA synthesis in nucleus of injured cardiomyocyte. Semithin (1 μ) Epon-Araldite sections, azure-II stain. 1600×.

TABLE 1. Histoautoradiographic Investigation of Level of RNA Synthesis in Cardiomyocytes of Rats Receiving Isoproterenol

Age of rats, months	No. of animal	Intact cells		Injured cells	
		No. of nuclei	total No. of grains above nuclei	No. of nuclei	total No. of grains above nuclei
3[illegible]	80	30	321	30	187
	81	30	289	30	165
	82	30	308	30	188
	83	30	320	30	187
26	64	30	256	30	133
	65	30	258	30	143
	66	30	258	30	126
37	21	30	241	30	126
	22	30	249	30	112
	23	30	268	30	112
	24	30	220	30	102

Legend. Differences significant by Wilcoxon's test.

lation of heart muscle cells of the young rats contained 26% of cells with RNA synthesis at a low level (below average). In animals aged 26 and 37 months the number of these cells was 75 and 79% respectively.

Investigation of the problem whether cells which have suffered acute metabolic injury differ from cells remaining intact in the intensity of their RNA synthesis [1] revealed that there were far fewer grains of silver above the nuclei of injured cardiomyocytes in rats of all age groups than above intact cells (Table 1). The difference as regards incorporation of the labeled RNA precursor into injured and intact cells reflects the metabolic heterogeneity of the cardiac myocyte population.

The heterogeneous reactivity of the myocardial cells is evidently based on cyclic renewal of intracellular structures; certain phases of the cell cycle, moreover, are more sensitive or, conversely, resistant to harmful influences [6]. The increase in the number of injured muscle cells in the hypertrophied myocardium of the old animals [4] in response to isoproterenol and the decrease in RNA synthesis in the cardiomyocytes thereby reflect the reduced powers of regeneration of the heart tissue as a whole, although in individual cells, intracellular regeneration processes can take place at a high level. In animals of different ages, a mosaic pattern of injury and of intracellular regeneration of the myocardium thus depends on the metabolic heterogeneity of the cardiomyocytes connected with their different levels of RNA synthesis.

Isoproterenol has a marked necrogenic effect, based on uncontrollable inflow of external Ca^{++} ions into the cardiomyocytes [10]. According to one view, short-term exposure to this factor does not affect nucleic acid biosynthesis in the cells [14], which means that isoproterenol can be used as "probe" for the study of structural and functional bases of development of adaptive reactions in experimental animals during aging. Meanwhile injection of isoproterenol into animals causes an increase in the oxygen consumption of the heart muscle and an increase in its energy consumption [11]. This effect is explained by stimulation of myocardial metabolism, which begins immediately after the harmful action. These properties of isoproterenol make it possible to assess the potential of myocardial tissue as regards its powers of regeneration and the role of metabolic changes in the cardiomyocytes in the process of structural and functional heterogeneity, a fundamental stage in the development of general pathological processes in the heart [2, 3, 5], to be revealed in one and the same experimental model.

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FUNCTIONAL MORPHOLOGY OF GASTRIC FUNDAL GLANDULOCYTES PERMANENTLY EXPOSED TO BILE

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UDC 616.33-018.72-02:616.36-008.8-008.17-032:611.33

KEY WORDS: bile; mitochondrial enzymes; mucous neck cells, parietal cells, and chief cells.

Many workers regard regurgitation of bile into the stomach as a factor which is conducive to disturbance of the functional state of the mucosal barrier, to increased reversed diffusion of hydrogen ions, and as a result of this, to the development of ulceration of the mucosa [2, 8, 11].

Meanwhile other workers have obtained evidence of a positive regulatory effect of bile on the motor, secretory, and endocrine functions of the gastroduodenal complex [1, 5, 6, 12, 13] and they consider that bile has a protective action on the gastric mucosa in the presence of hyperchlorhydria [6, 9, 10].

The aim of this investigation was a differential study of the dynamics of mitochondrial enzyme activity in the mucous neck cells and parietal cells and also of the RNA content in the chief cells and vascular permeability in the mucosa of the gastric fundus in rats, in order to assess the functional state of the gastric mucosal barrier and of the morphological substrate of the secretion of acid and digestive enzymes after diversion of all the bile into the stomach.

EXPERIMENTAL METHOD

Experiments were carried out on 47 mature male rats weighing 240-250 g. An anastomosis was formed in the animals between the bile duct and the body of the stomach. The experimental and control (mock operation) rats were decapitated 10, 30, 60, and 90 days after the beginning of permanent diversion of bile into the stomach. Material for investigation was taken after starvation of the animals for 24 h, but with unrestricted fluid intake. After autopsy the gastric mucosa was carefully inspected for macroscopic defects. The subsequent histochemical investigation and preservation of the material were undertaken in accordance with recommendations in [3].

Frozen sections of the mucosa of the body of the stomach were stained for activity of NADH-dependent dehydrogenase (NADH-DH), succinate (SDH), isocitrate (ICDH), and malate dehydrogenases (MDH), and alkaline phosphatase (AlP) and for quantitative determination of RNA and neutral and acid mucopolysaccharides (MPS) by the usual histochemical methods.

Central Research Laboratory, Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR I. V. Toroptsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 123-125, October, 1982. Original article submitted February 18, 1982.

 0007-4888/82/9410-1462$07.50