LONG-TERM EFFICACY OF PROPRANOLOL IN EXPERIMENTAL MYOCARDIAL INFARCTION

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The direct correlation which exists between the size of the pathological focus and severity of the clinical course in myocardial infarction (MI) and the degree of circulatory failure accounts for the urgency of the problem of limiting and reducing the size of the infarct [6, 7]. The patient's subsequent fate also depends on the state of zones of heart muscle outside the infarct, for they are responsible for compensating the functions of the heart [5]. The more rapid normalization of the metabolism, structure, and function of the peri-infarct zones may lead to an improvement of the prognosis in MI. β-Adrenoblockers have justified themselves as one of the principal therapeutic measures in chronic ischemic heart disease [1, 8]. Meanwhile the question of the indications for their use in acute MI remains undecided. There are reports in the literature that mortality among patients with acute MI can be reduced by long-term treatment with β-adrenoblockers [10, 13]. A course of treatment with these drugs led to the improvement of myocardial contractility.

The aim of the present investigation was to study some of the subcellular mechanisms which lie at the basis of the improvement of cardiac function during long-term experimental treatment with β-adrenoblockers.

EXPERIMENTAL METHOD

Experiments were carried out on 126 noninbred albino rats weighing 150-200 g. MI was produced in the animals by ligation of the descending branch of the left coronary artery under hexobarbital anesthesia with artificial respiration. The animals were divided into three groups: 1) intact rats, 2) control animals with ligation of the coronary artery but receiving no drugs, 3) experimental animals, treated after ligation of the coronary artery by intraperitoneal injection of propranolol in a dose of 0.5 mg/kg twice a day at intervals of 12 h. The animals were killed 1, 3, 7, 15, and 30 days after ligation, i.e., at times corresponding most closely to definite stages of development of MI [4].

The zone of necrosis, the perinecrotic zone, and also the posterior wall of the left ventricle were investigated. The volume of the zone of necrosis, as a percentage of the total weight of the heart, was determined on the 7th day after ligation of the coronary artery by gravimetric planimetry [12]. To study the general morphological picture, sections of myocardium 4-5 μ thick were stained with hematoxylin and eosin. The ultrastructure was studied in ultrathin sections (50-60 nm) obtained from the samples of myocardium from the above-mentioned zones, embedded in Epon 812 by the usual method. The sections were stained with uranyl acetate and lead citrate by Reynolds' method. The degree of hypertrophy of the cardiomyocytes was determined on the 15th and 30th days of the experiment by planimetry of the area of cross section of the cardiomyocytes (100 in each case) at the level of the nucleus. The state of energy metabolism was assessed by polarography, using the LP-7 polarograph with rotating bare platinum electrode. Parameters of respiration were estimated by the method in [9]. The intensity of protein biosynthesis was judged from incorporation of the tritiated amino acid methionine into total protein. Radioactivity was measured on a β-Mate-II Liquid Scintillation Counter (Beckman, USA).

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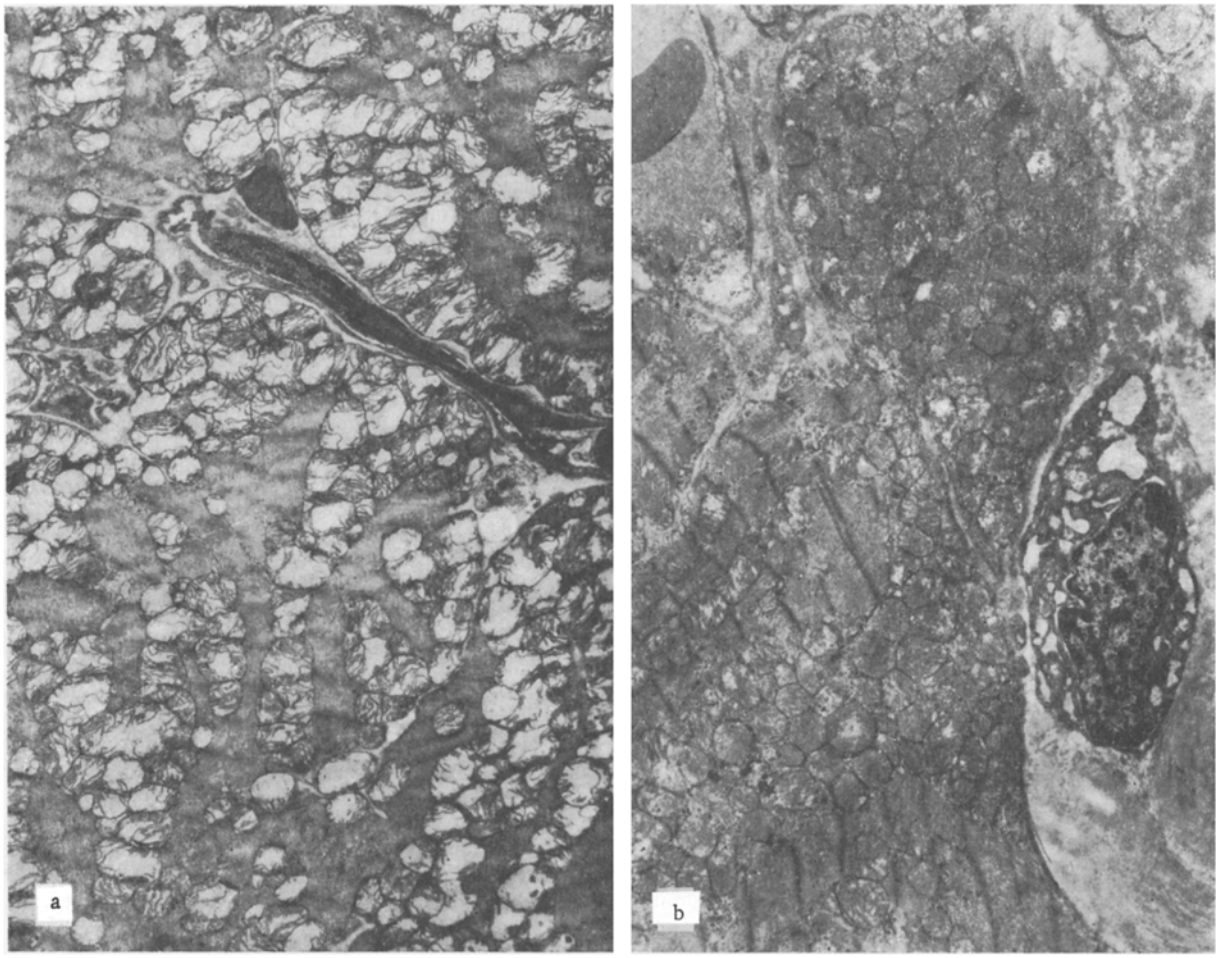

Fig. 1. Cardiomyocyte ultrastructure in experimental MI (10,000 ×): a) swelling of mitochondria with translucency of matrix and destruction of cristae; b) hyperplasia of mitochondria and increased number of cytogranules after treatment with propranolol.

EXPERIMENTAL RESULTS

The volume of the zone of necrosis in the treated animals was reduced by 2.5 times (10.6 ± 1.4% compared with 26.8 ± 3.9% in the control, $P < 0.01$). This was reflected in the dynamics of the ECG: The degree of elevation of S–T in lead I after 24 h in the treated animals was significantly reduced compared with the control (0.86 ± 0.09 mV compared with 1.85 ± 0.15 mV, $P < 0.001$).

Such a considerable decrease in the volume of affected tissue was evidently responsible for the improvement in the state of the myocardium in the perinecrotic zone. For instance, in our material, a more favorable state of the muscle tissue could be detected under the light microscope after 1 and 3 days in the treated animals. Whereas in the control animals gross structural changes were found in the zone described, in the form of homogenization and lysis of of cardiomyocytes, and large areas of their total destruction with the formation of finely granular debris, destructively changed cardiomyocytes were much less frequently seen in treated animals.

Ultrastructural changes in the cardiomyocytes of rats of the control group had the form of swelling of the mitochondria with translucency of the matrix and considerable destruction of cristae (Fig. 1a). At this stage treatment with propranolol led to considerable preservation of the ultrastructural organization of the mitochondria. It can be postulated that the protective effect of propranolol is mediated through preservation of mitochondrial structure and function [3, 11]. This view is supported by the results of the polarographic studies. After 24 h all the parameters of mitochondrial respiration studied were inhibited in the control animals, so that the respiratory control (RC) fell to 42% of its initial value ($P < 0.001$). Treatment with propranolol led to an even greater decrease in respiratory activity of the mitochondria. However, because of comparatively greater inhibition of state 4 (down to 44.9% of the initial value, $P < 0.001$) RC increased by 8.3% ($P < 0.05$), and three days later its value was

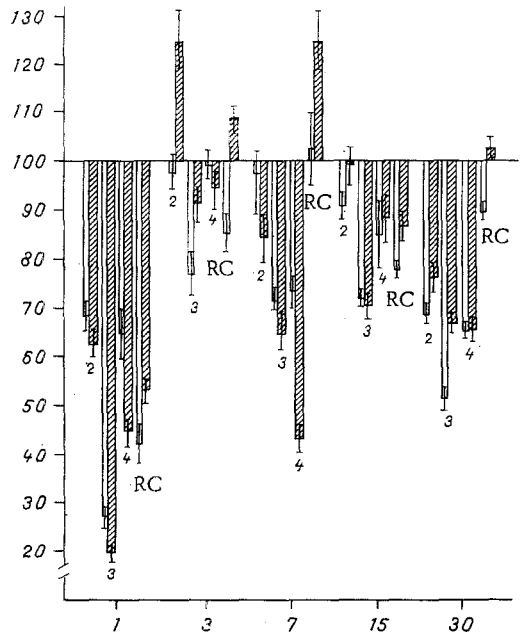

Fig. 2

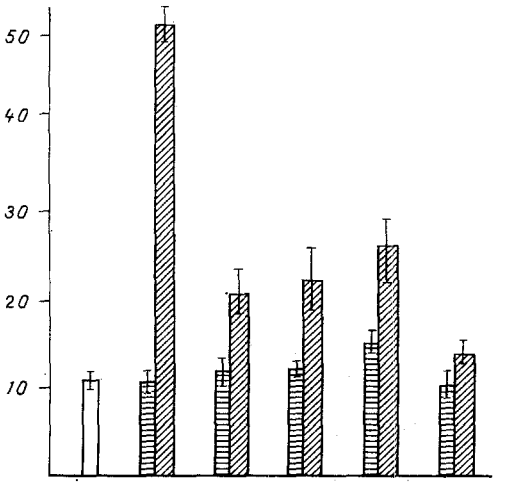

Fig. 3

Fig. 2. Changes in parameters of oxidative phosphorylation in left ventricular myocardium in experimental MI under treatment with propranolol. Abscissa, time (in days); ordinate, changes in parameters (in percent). Unshaded columns — control, shaded — treatment with propranolol, 0.5 mg/kg. Numbers indicate corresponding metabolic states. RC) respiratory control.

Fig. 3. Intensity of incorporation of [^{3}H]methionine into total protein of left ventricular myocardium in experimental MI under treatment with propranolol. Abscissa, time (in days); ordinate, intensity of incorporation of label (in thousands of cpm/mg protein). Unshaded column — intact animals, horizontal shading — control, oblique shading — treatment with propranolol, 0.5 mg/kg.

108.7% of the initial level, 23.6% higher than in the control ($P < 0.001$; Fig. 2). Consequently, in the early period of MI propranolol diminishes uncoupling of oxidation and phosphorylation.

The morphological and ultrastructural changes in the cardiomyocytes of the control animals described above correspond to changes discovered in the "emergency stage" of compensatory hyperfunction of the myocardium [2]. Consequently, preservation of the ultrastructural organization of the mitochondria in treated animals is linked with reduction of their respiratory activity under the influence of propranolol, whereas the increase in the values of RC points to an increase in the energy efficiency of oxidation. This increase in the energy supply evidently is responsible for the intensification of protein biosynthesis in the treated animals: After 1 day the intensity of incorporation of the labeled amino acids into total protein in the zone of necrosis was increased by 296.1% ($P < 0.001$) compared with the control, and by the 3rd day it still remained 29.2% higher than in the control. These figures in the "intact" zones of the left ventricle were 310.4% ($P < 0.001$) and 78.4% ($P < 0.05$; Fig. 3) respectively.

In the later stages the number and size of foci of total destruction of cardiomyocytes were significantly reduced compared with the control both in the perinecrotic zone and in the posterior wall of the left ventricle. Meanwhile, in the zone of necrosis, the macrophagal-fibroblastic reaction was intensified.

The cardiomyocyte ultrastructure showed considerable preservation of both the contractile and the energy providing apparatus. Transformation of the perinuclear zone also was noteworthy in the treated animals, where numerous small mitochondria with an electron-dense matrix, a marked increase in the number of ribosomes and polysomes, and also an increase in the number of glycogen granules compared with the control could be observed. The mitochondria differed in size and shape, giant mitochondria could be seen, and many of them had signs of constriction rings and division. The number of mitochondria in these cardiomyocytes was increased,

and they formed large accumulations (Fig. 1b). Near the nucleus the rough endoplasmic reticulum, cross-sections of which were widened, could be seen. The changes described reflect considerable activation of intracellular regeneration of cardiomyocyte organelles [4].

In stages of organization (7 and 15 days) and postinfarction changes (30 days) the RC parameters remained higher than the control values by 24.5% ($P < 0.001$), 8.5% ($P < 0.01$), and 12.9% ($P < 0.001$) respectively. This increase in the energy efficiency of oxidation led to intensification of structural metabolism: The intensity of utilization of [^{3}H]methionine was increased after 7 days by 90.3% and after 15 days by 97.3% ($P < 0.05$). Increased incorporation of [^{3}H]methionine into total protein of the peri-infarct zones of the left ventricle were still present until the 30th day (122.4% of the initial value). This increase in protein biosynthesis under the influence of propranolol is thus evidently the structural basis for intensification of intracellular regeneration.

The planimetric studies showed that the area of cross section of the cardiomyocytes at the level of the nucleus after 15 days did not differ significantly from the control, whereas by the 30th day it was reduced by 22.1% ($P < 0.001$) compared with the control. The increase in the intensity of protein biosynthesis with a decrease in the degree of hypertrophy may characterize acceleration of the cycle of renewal of functional structures of the cardiomyocytes, i.e., intensification of intracellular physiological regeneration, leading to a decrease in the degree of hypertrophy of the cardiomyocytes.

In the acute stage of experimental MI propranolol thus contributes to reduction in size of the zone of necrosis through improvement of metabolism and structure of the myocardium in the perinecrotic zone. During long-term administration propranolol leads to intensification of intracellular reparative regeneration, accelerates the cycle of renewal of functional structures of the cardiomyocytes, and thereby reduces the degree of hypertrophy of the cardiomyocytes. These processes, taken together, provide the basis for exercising of the compensatory function of the myocardium and increase endurance of the heart as a whole. It can be tentatively suggested that the long-term administration of propranolol in MI can lead to considerable lengthening of the period of relatively stable myocardial hyperfunction and to the less frequent development of heart failure.

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