Changes in the Rhythmoinotropic Dependence of the Myocardium in Rats with Postinfarction Cardiosclerosis after β_1-Adrenoreceptor Blocking

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The rhythmoinotropic dependence of the papillary muscles was studied in rats with postinfarction cardiosclerosis after blocking of β_1-adrenoreceptors by concor (7 mg/kg daily). The development of postinfarction cardiosclerosis led to a reduction of the postextrasystolic potentiation and of potentiation induced by periods of rest. Preliminary blocking of β_1-adrenoreceptors stimulated the postextrasystolic contractions and contractions after periods of rest in the myocardium of rats with postinfarction cardiosclerosis. These results suggest that blocking of β_1-adrenoreceptors promotes an improvement of calcium-accumulating function of the cardiomyocyte sarcoplasmatic reticulum in the myocardium of rats with postinfarction cardiosclerosis.

Key Words: *sarcoplasmatic reticulum; cardiosclerosis; rats; β_1-adrenoblocker*

Inotropic potentialities of the myocardium are largely determined by the capacity of intracellular structures to maintain the balance of calcium ions. It is particularly important in contractile myocardial dysfunctions. Cardiac failure is associated with disorders in intracellular homeostasis of calcium ions [5,15]. The imbalance of Ca^{2+} in the cells of a pathological myocardium is associated with changed function of the sarcoplasmatic reticulum (SR), providing release and return capture of calcium ions during cardiomyocyte contraction/relaxation processes [3]. Dysfunctions of cardiomyocyte SR during the formation of heart failure largely tells on the rhythmoinotropic reactions of the myocardium and manifests by inversion of the rate—force relationship [9].

The treatment of patients with chronic cardiac insufficiency by β-adrenoreceptor blockers leads to reduction of mortality and improvement of left-ventricular contractile function [4,8]. It is assumed that the efficiency of these drugs is explained by not only limitation of the adrenergic effects on the myocardium, but also by their capacity to modulate the number and activity of the SR Ca^{2+}-regulating proteins, which, in turn, can promote restoration of intracellular homeostasis of calcium ions [6,10,12]. On the other hand, the changes in cardiomyocyte SR function in the postinfarction myocardium under conditions of β_1-adrenoreceptor blocking remain little studied.

We studied the changes in the myocardial rhythmoinotropic reaction in rats with postinfarction cardiosclerosis (PICS) during selective blocking of β_1-adrenoreceptors.

MATERIALS AND METHODS

The study was carried out on 37 adult male Wistar rats (250-300 g), divided into 4 groups: 1) intact animals (n=12), 2) rats with PICS (n=11), 3) rats

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with PICS orally receiving selective β_1-adrenoreceptor blocker concor (bisoprolol) in a daily dose of 7 mg/kg from day 8 after coronary occlusion during 14 days (n=10), and 4) sham-operated animals (n=4). Myocardial infarction was induced by coronary occlusion. The thoracic cavity of ether narcotized animals was opened and the upper third of the left descending coronary artery was ligated [12]. The thoracic cavity was then sutured and the animals were left under standard vivarium conditions. After 6 weeks PICS was verified morphologically (Fig. 1). The development of cardiac and left-ventricular hypertrophy was evaluated by the body/heart and left ventricle/heart weight proportions (Table 1).

The rhythmoinotropic dependence was studied on papillary muscles as follows. Animals under light ether narcosis were sacrificed by cervical dislocation, the thoracic cavity was opened, and the heart was removed. The heart was then washed in a special flow chamber through the aorta by the Krebs—Henselight's solution of the following composition (mM): 120 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20.0 $NaHCO_3$, and 10.0 glucose. The papillary muscles were then isolated. The size of postinfarction cicatrices in the hearts of rats subjected to coronary occlusion was measured by the planimetric method and calculated in percent of the area of left-ventricular free wall [2]. Isolated papillary muscles were placed into a flow thermostat chamber. One end of the muscle was fixed to the chamber wall, the other to the isometric pickup (6MX1C mechanoelectrical transformer) stock. The muscles were perfused at 36.5°C by oxygenated (95% O_2, 5% CO_2) Krebs—Henselight's solution and stimulated by rectangular electrical pulses (5 msec) at 0.5 Hz frequency. The curves of papillary muscle isometric contractions were recorded. The functional activity of SR was evaluated by the inotropic reaction of papillary muscles to extrasystolic exposure and to periods of rest [7,13]. Extrasystolic exposure was realized by a single extra electrical pulse 0.2, 0.225, and 0.25 sec after the beginning of the regular cycle. Exposure to periods of rest was carried out during regular contractions: electrical stimulation of the muscles was ceased for 4-60 sec (periods of rest), after which regular stimulation was renewed [7]. The results were analyzed by comparing the parameters of the postextrasystolic contraction and first contraction after a period of rest with the regular contraction. The time course of relationship between the contraction amplitude and duration of periods of rest (mechanical restitution) [7] was evaluated. The amplitude of postextrasystolic and first after period of rest contractions was expressed in percent of the regular contraction in each case.

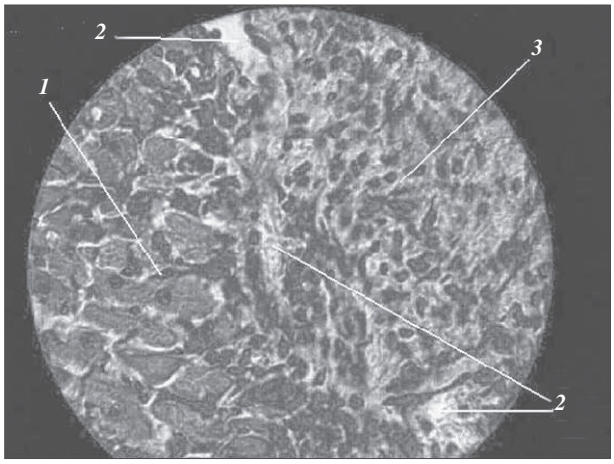

Fig. 1. Transverse section of the rat left ventricle 6 weeks after experimental coronary occlusion, ×200. *1*) structurally intact myocardium; *2*) cicatricial tissue; *3*) myocardial site with modified structure.

The data were statistically processed ($M\pm SEM$), the significance of differences was evaluated using Mann—Whitney's nonparametrical U test.

RESULTS

Simulation of experimental PICS in rats led to cardiac hypertrophy (Table 1). Heart to body weight proportion in these animals was 6.27±0.33 *vs.* 3.29±0.21 and 3.48±0.18 in intact and sham-operated animals, respectively (p<0.01). Left ventricle to heart weight proportion in rats with PICS also increased significantly in comparison with intact and sham-operated animals (Table 1). The area of cicatricial tissue zone was 48.3±6.9% of left-ventricular area in rats with PICS. Selective blocking of β_1-adrenoreceptors by concor virtually did not change the heart and left ventricle weights or the size of cicatricial zone (Table 1).

Application of an extra electrical pulse 0.2 sec after the beginning of the regular cycle led to a 37% increase in the amplitude of postextrasystolic contractions (PEC) of muscle strips of intact rat myocardium (p<0.05; Fig. 2). The amplitude of PEC increased with prolongation of the interval between the regular and extra stimuli. After an extrasystolic exposure 0.225 sec after the cycle beginning the PEC amplitude increased by 51% (p<0.01) *vs.* the regular contraction, while after an extra electrical pulse 0.25 sec after the cycle beginning it was 156% of the regular cycle values (p<0.01). This inotropic reaction is explained by the fact that an extra electrical pulse initiates additional entry of external calcium ions into cardiomyocyte myo-

TABLE 1. Changes in Body Weight and Heart Weight of Rats after Coronary Occlusion (*M*±*SEM*)

Group	Heart/body weight (mg/g) proportion	Left ventricle/heart weight (mg) proportion	Area of cicatrix zone, %
Intact, *n*=12	3.29±0.21	0.645±0.013	—
Sham-operated, *n*=4	3.48±0.18	0.617±0.021	—
PICS, *n*=11	6.27±0.33*	0.687±0.016*	48.3±6.9
PICS+concor, *n*=10	5.57±0.29*	0.673±0.014*	45.1±6.6

Note. *$p<0.01$ *vs.* intact and sham-operated animals. The area of cicatrix zone was calculated in percent of left-ventricular area.

plasm; these ions accumulate in the SR and participate in PEC [11,13].

The papillary muscles isolated from the hearts of rats with PICS exhibited a different time course of PEC (Fig. 2). An extrasystole 0.2 sec after the cycle beginning stimulated the PEC amplitude only by 5%, the pulse after 0.225 sec increased PEC amplitude by 7%, and the extrasystolic exposure after 0.25 led to a 9% increase of PEC amplitude (Fig. 2). Our previous studies [1] showed that the rat cardiomyocyte excitability reduced in PICS, the PEC amplitude not depending on the degree of extrasystolic contraction. Our present findings indicate that muscle strips from postinfarction myocardium do not react to extrasystolic exposure by potentiation. This result most likely indicates a reduction of the calcium-accumulating capacity of the cardiomyocyte SR.

Blocking of β_1-adrenoreceptors did not change the inotropic reaction of the papillary muscles of rats with PICS to an extrasystole 0.2 sec after the cycle beginning (Fig. 2). However prolongation of the interval between the regular and extra stimuli led to an increase of the amplitude of the postinfarction myocardial PEC after blocking of β_1-adrenoreceptors. An extra exposure to an electrical pulse 0.225 sec after the beginning of the regular cycle led to a 23% increase in the PEC amplitude ($p<0.05$), while an exposure 0.25 sec after the cycle beginning potentiated the contractions by 32% ($p<0.05$) *vs.* the basal level. This reaction of muscle strips indicates a significant improvement of the functional activity of postinfarction cardiomyocyte SR after blocking of β_1-adrenoreceptors. Presumably, this reaction is due to the effect of selective blocker of β_1-adrenoreceptors, used in our study, on the activity of the SR Ca^{2+}-transporting proteins. This mechanism was demonstrated for some of β-adrenoreceptor blockers [4,6,10].

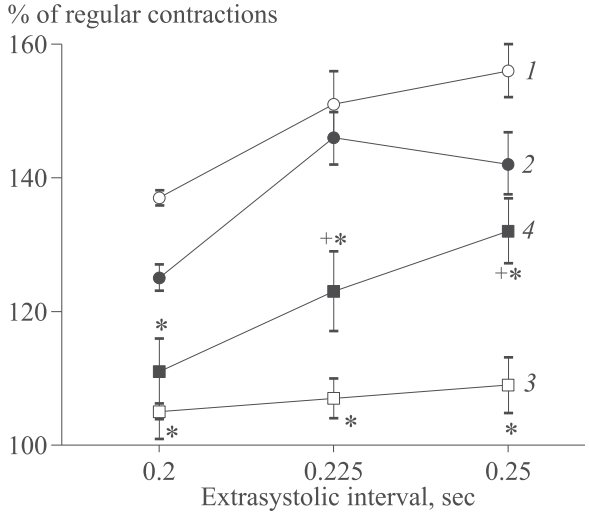

Fig. 2. Amplitude of papillary muscle PEC in intact (*1*), sham-operated rats (*2*), rats with PICS (*3*), and rats with PICS treated by β_1-adrenoreceptor blockers (*4*). Ordinate: amplitude of contractions. Here and in Fig. 3: $p<0.05$ *vs.* *intact animals, +rats with PICS.

A short-term cessation of electrical stimulation of papillary muscles of intact rats caused an increase of the amplitude of the first contraction after period of rest (Fig. 3). The amplitude of contractions increased by 18-88% of the basal contraction with prolongation of the period of rest; in other words, the time course of mechanical restitution was positive (Fig. 3). The mechanism of amplitude increment (potentiation of intact myocardium in response to period of rest) is attributed to SR capacity to accumulate and retain Ca ions during a short period of rest [7,9]. The time course of mechanical restitution of sham-operated animals virtually did not differ from mechanical restitution of the myocardium of intact rats, though after 60 sec of rest the amplitude of contractions was 17% less in comparison with the intact myocardium (Fig. 3). The inotropic reaction of the postinfarction myocardium to periods of rest differed significantly from the inotropic response of the myocardium of intact and sham-operated rats (Fig. 3). Short-term cessation of electrical stimulation did not lead to potentiation of contractions of papillary muscles of rats with PICS, the amplitude of contractions decreasing from 98 to 59% of the basal cycle

amplitude with prolongation of the period of rest. This inotropic reaction can indicate a reduction of calcium-modulating capacity of the cardiomyocyte SR in damaged myocardium. These data are in good agreement with the results of other studies [9], demonstrating a significant reduction of the functional activity of Ca^{2+}-ATPase and rianodine receptors in cardiac insufficiency. These changes can promote more active leakage of calcium ions from SR [3].

Blocking of β_1-adrenoreceptors promoted restoration of the positive time course of mechanical restitution of the myocardium of rats with PICS (Fig. 3). Blocking of β_1-adrenoreceptors led to potentiation of inotropic reaction of heart muscle strips from rats with PICS in response to periods of rest. The amplitude of contractions increased by 14-31% in comparison with the basal contraction with prolongation of the period of rest; in other words, a positive trend of mechanical restitution was observed, similarly as in intact and sham-operated rats (Fig. 3). Blocking of β_1-adrenoreceptors stimulated the inotropic response to periods of rest (16-66% increase; $p<0.01$) in comparison with the inotropic reaction of the postinfarction myocardium without β_1-adrenoreceptor blocking. These results indicate that selective blocking of β_1-adrenoreceptors promotes a significant improvement of the SR function in the postinfarction myocardium. Presumably, the positive effect of β_1-adrenoreceptor blocking on the functional activity of SR is explained by restoration of the quantity and/or activity of the SR calcium-regulating proteins [6,12,14].

Hence, heart remodeling after experimental myocardial infarction modifies significantly the rhythmoinotropic dependence, which is explained by deterioration of the SR functional characteristics. Blocking of β_1-adrenoreceptors under these conditions promotes normalization of the rhythmoinotropic dependence of the myocardium.

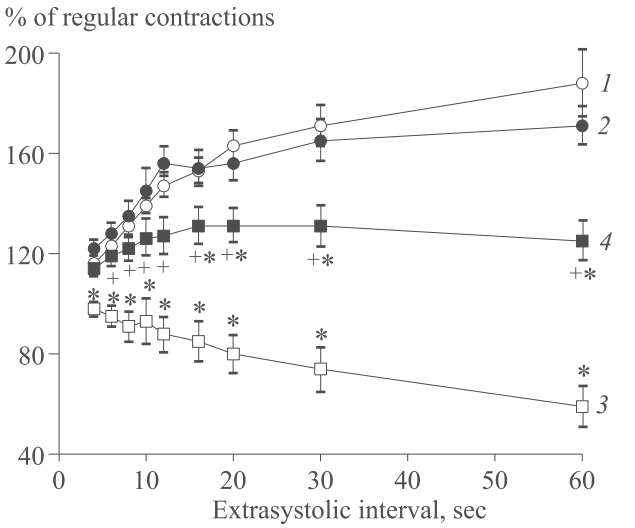

Fig. 3. Time course of mechanical restitution of papillary muscles of intact (*1*), sham-operated rats (*2*), rats with PICS (*3*), and rats with PICS treated by β_1-adrenoreceptor blockers (*4*). Ordinate: amplitude of contractions.

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