Inotropic Response of the Myocardium in Rats with Postinfarction Cardiosclerosis Exposed to Extrasystolic Treatment

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The inotropic response of the myocardium to extrasystolic treatment was studied on isolated perfused papillary muscles from rats with postinfarction cardiosclerosis. The development of postinfarction cardiosclerosis was accompanied by a decrease in myocardial excitability. The amplitude of extrasystolic contractions in the remodeled myocardium far surpassed the control. However, the amplitude of postextrasystolic contraction did not surpass that in normal contraction—relaxation cycle. Our results suggest that the ability of the sarcoplasmic reticulum in cardiomyocytes to accumulate Ca^{2+} is impaired during postinfarction remodeling.

Key Words: *sarcoplasmic reticulum; myocardium; rats; postinfarction cardiosclerosis*

Myocardial ischemia is accompanied by impairment of electromechanical coupling, which results in a decrease or loss of contractile activity in cardiomyocytes [1,4]. Contraction and relaxation of the myocardium are closely related to energy metabolism and calcium pump of the sarcoplasmic reticulum (SPR) is the main consumer of ATP [1]. Ischemia impairs one of the main functions of SPR (*i.e.*, Ca^{2+} reuptake from the cytoplasm), which can contribute to persistent disturbances in intracellular Ca^{2+} homeostasis in the ischemic myocardium [3]. These changes determine Ca^{2+} overload in the cardiomyocyte cytoplasm, which has a role in the pathogenesis of contractile dysfunction and decrease in electrical stability of the myocardium during coronary heart disease [1]. The role of SPR in contractile dysfunction of the myocardium during postinfarction remodeling is poorly understood. SPR plays an important role in electromechanical coupling [3]. A correlation was found between the strength and frequency of contractions. The strength—frequency dependence is often evaluated during studying of SPR function under physiological conditions. It reflects the ability of cardiomyocyte SPR to release and accumulate Ca^{2+} during a single contraction—relaxation cycle of isolated cardiac muscles.

Here we studied the inotropic response of the myocardium in rats with postinfarction cardiosclerosis to modulation of intracellular Ca^{2+} homeostasis upon extrasystolic (ES) treatment.

MATERIALS AND METHODS

Experiments were performed on 22 adult male Wistar rats weighing 200-250 g. The control group included 12 animals. Myocardial ischemia was produced by coronary artery occlusion. The thorax was opened under ether anesthesia. The upper third of the left descending coronary artery was ligated [5]. The chest wall was sutured, and the animals were maintained in a vivarium under standard conditions. Postinfarction cardiosclerosis developed 45 days after surgery.

Morphological changes in the myocardial tissue were visualized by histological examination (Fig. 1). Myocardial hypertrophy was observed in treated ani-

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mals. The hearts of operated rats were larger than in intact animals (by 80.30±3.61%). The zone of myocardial necrosis in the left ventricle was 55.22±2.16%.

The animals of both groups were immobilized by cervical dislocation under light ether anesthesia. The hearts were removed after thoracotomy. The heart and coronary vessels were washed in a special flow chamber. Krebs—Henseleit solution containing 120 mM NaCl, 4.8 mM KCl, 2.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20.0 mM $NaHCO_3$, and 10.0 mM glucose was delivered through the aorta. The zone of cardiac necrosis in treated rats was studied after isolation of the papillary muscles. The papillary muscles (<1 mm in diameter) were placed in a thermostatic flow chamber. One end of the muscle was fixed to the chamber wall and another was fastened to the rod of an isometric transducer (mechanoelectrical transducer 6MKh1C). The muscles were perfused with Krebs—Henseleit solution at 36.5°C. The solution was oxygenated with carbogen (95% O_2 and 5% CO_2). The muscles were stimulated with rectangular electrical impulses (5 msec) delivered through 2 massive silver electrodes in the perfusion chamber. The frequency of stimulation was 0.5 Hz. We examined muscle preparations capable of producing a force of not less than $^1/_2$ calibration signal by the end of an adaptation period (1 V, 60 min).

Intracellular Ca^{2+} homeostasis was modulated by delivering an extra electrical impulse (ES-treatment) whose characteristics were similar to those of baseline stimulation. ES-treatment was applied 0.2-1.5 sec after the regulatory excitation impulse. In our experiments the ES-interval is the time to deliver an extra electrical impulse. Excitability of the sarcolemma was estimated by the ability of muscle to respond to extra stimulation. The ability of SPR to accumulate excess Ca^{2+} released into the myoplasm during stimulation was determined by a change in postextrasystolic (PES) contraction. The amplitude of ES- and PES-contractions was expressed in percents of a normal cycle. ES-treatment was applied after adaptation of the papillary muscles to perfusion conditions. Electrical impulses were delivered with a 10-min interval. Over this period the initial characteristics of muscle contraction returned to normal upon exposure to regular excitation impulses.

Isometric contraction was recorded on an analogue-digital converter and personal computer. The maximum muscle power was calculated using special software. The results were analyzed by Student's *t* test.

RESULTS

Stimulation of muscle strips from normal myocardium with an extra electrical impulse delivered 0.2 sec after the start of normal contraction—relaxation cycle increased duration of this cycle (Fig. 2, *a*). Therefore,

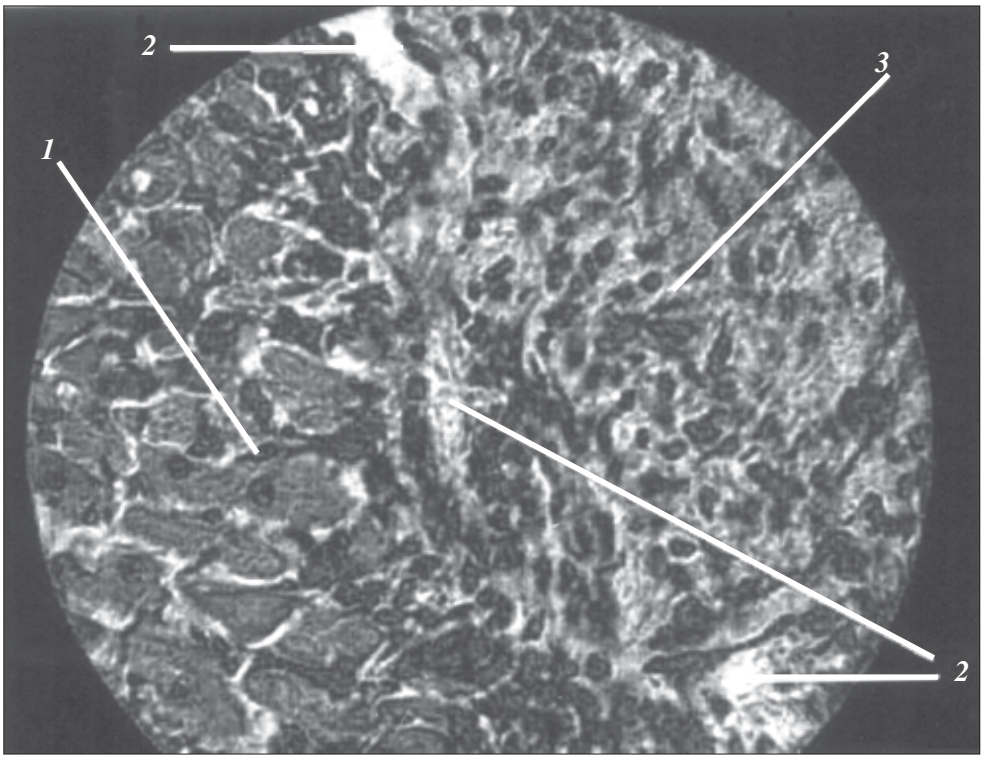

Fig. 1. Transverse section of rat left ventricle 6 weeks after experimental coronary artery occlusion, ×200. Normal structure of the myocardium (*1*); cicatrix tissue (*2*); myocardium with structural changes (*3*).

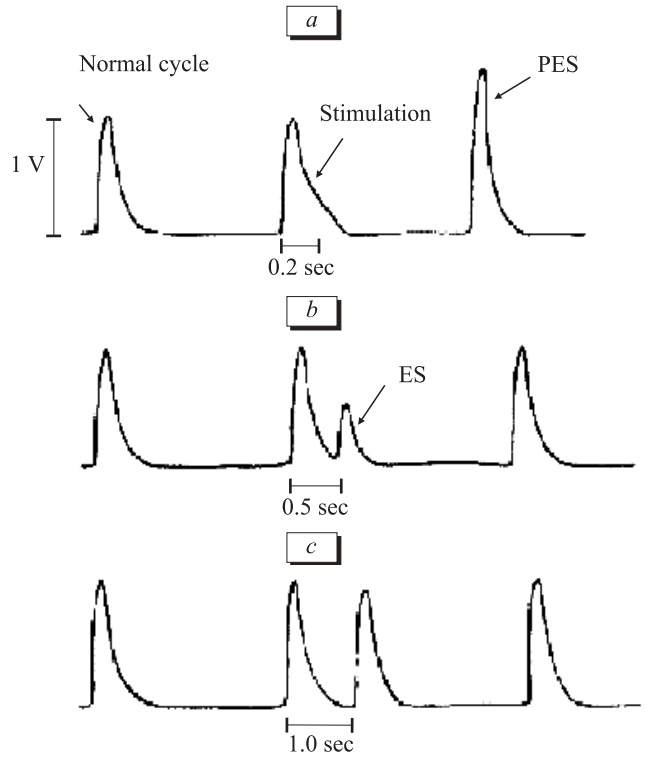

Fig. 2. Typical curves for isometric contraction of papillary muscles from normal rat myocardium after extrasystolic treatment. Extrasystolic interval: 0.2 (*a*), 0.5 (*b*), and 1.0 sec (*c*). ES, extrasystolic contraction; PES, postextrasystolic contraction.

the extra electrical stimulation coincided with the period of absolute refractoriness. The delivery of electrical impulses after 0.225 sec induced extrasystoles. In this case, the amplitude of contraction was 27.00± 1.42% of the normal cycle (Fig. 3, *a*). Lengthening the time of extra stimulation was accompanied by an increase in the amplitude of extrasystole (Fig. 2, *a*).

Electrical stimulation in phase 3 of the action potential (AP) produces no contractile response. However, this treatment initiates influx of extracellular Ca^{2+} into the myoplasm of cardiomyocytes. Calcium ions are accumulated in SPR and participate in the first PES-cycle of contraction—relaxation [2]. In our experiments it manifested during stimulation over the shortest ES-interval (0.2 sec). The amplitude of PES-contraction exceeded that of normal contraction by 23.00±1.78% (Fig. 3, *b*). The degree of PES-potentiation decreased with the appearance of non-stimulated ES and increase in its amplitude. PES-potentiation was least pronounced over the longest ES-interval (Fig. 3, *a*, *b*).

The ES-induced inotropic response of papillary muscles from the remodeled myocardium significantly differed from that in normal myocardium. These muscles exhibited ES-contraction when the ES-interval was not less than 0.25 sec, and amplitude of the inotropic response corresponded to 39.00±2.96% of the normal cycle (Fig. 3, *a*). ES of papillary muscles from rats with postinfarction cardiosclerosis was produced by stimulation in a later phase of AP (compared to muscle preparations from normal myocardium). Our findings demonstrate a decrease in excitability of remodeled myocardium. This effect is probably associated with lengthening of AP in cardiomyocyte membranes from the postinfarction myocardium. The decrease in excitability of muscle preparations was accompanied by a 7-11% increase in the amplitude of ES over an ES-interval of 0.75-1.50 sec (Fig. 2, *a*). Changes in the density of ion transport structures in the membrane of cardiomyocytes from postinfarction rats was followed by a decrease in an outward and rectifier K^+ current. Lengthening of AP increased in

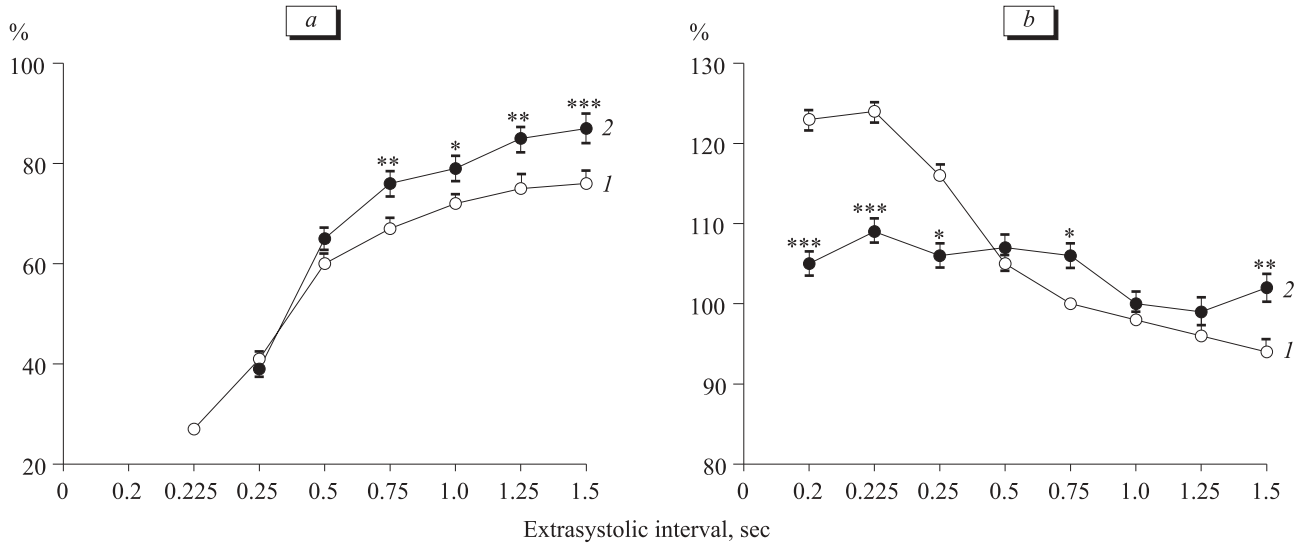

Fig. 3. Amplitude of extrasystolic (*a*) and postextrasystolic contraction (*b*) of papillary muscles from intact rats (*1*) and animals with postinfarction cardiosclerosis (*2*). **p*<0.001, ***p*<0.01, and ****p*<0.05 compared to *1*.

Ca^{2+} influx into the cytoplasm. It can be suggested that ES-stimulation is accompanied by accumulation of a considerable amount of Ca^{2+} in postinfarction cardiomyocytes. The amplitude of ES-contraction was higher compared to normal myocardium.

Intracellular changes resulting from postinfarction cardiosclerosis also modulated PES-contraction. PES-potentiation was not revealed in papillary muscles from remodeled myocardium (Fig. 2, *b*). The amplitude of PES in these muscles slightly surpassed the baseline level (independently on the duration of ES-stimulation). We believe that excess calcium ions released during stimulation were not accumulated in SPR. Postinfarction remodeling of rat myocardium is probably accompanied by dysfunction of cardiomyocyte SPR responsible for Ca^{2+} accumulation and release. These changes may be associated with inactivation of Ca^{2+}-ATPase in SPR and significant decrease in the density of ryanodine receptors.

Our results show that postinfarction remodeling in rats is accompanied by a decrease in excitability of the myocardium and impairment of Ca^{2+} accumulation in SPR. These changes are the most probable cause of heart failure and arrhythmia during postinfarction remodeling of the myocardium in humans.

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