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Langendorff-Isolated Rat Heart after Acute Experimental Myocardial Infarction

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A comparative analysis of functioning of Langendorff-isolated heart from intact rats, sham-operated rats, and rats with a model of myocardial infarction one day after ligation of the left coronary artery was carried out. No significant differences in inotropic and chronotropic functions of the heart were found in the groups of intact and sham-operated animals. In the group of rats with the myocardial infarction model, an increase in HR and a decrease in the maximum contraction and relaxation rates and left-ventricular developed pressure were shown in comparison with the groups of intact and sham-operated animals. At the same time, in the group of sham-operated animals, a decrease in coronary flow and temporal parameters of contraction was observed.

Key Words: *myocardial infarction model; isolated heart; rat*

Coronary heart disease is the most frequent cause of death in people worldwide, and the incidence of this pathology is steadily increasing. Myocardial infarction (MI) is an acute condition, a clinical form of coronary heart disease, in which necrosis develops as a result of complete or partial disruption of the blood supply to the myocardial area.

One of the common experimental methods to study MI is its modeling in laboratory animals. Many experimental models for reproducing MI are described, for example, diathermocoagulation of the interventricular artery, occlusion models, models with reversible occlusion, induction of MI by hormonal changes typical of type 2 diabetes mellitus and stress conditions (the Panin technique), MI modeling in isolated hearts (retrograde perfusion according to Langendorff) [1]. Pharmacological experimental models of MI induced by β-agonists are also described. Histological analysis of these models confirms the presence of changes in MI [2]. However, the most common model of experimental MI reproduction is the occlusion model: ligation of the left coronary artery (LCA). This model effectively reproduces changes typical of human heart.

After LCA ligation at the stage of acute myocardial ischemia (up to 7 h after LCA ligation), a pathological *Q* wave is detected on rat ECG in lead II. During the first day (acute MI), it is impossible to distinguish the ischemic zone from the adjacent intact compartments. It is the stage of acute ischemic myocardial degeneration lasting from 7 to 48 h after LCA ligation [3].

According to histological data, during the most acute stage of MI (30 min after LCA ligation), coagulation necrosis of cardiomyocytes, edema and hemorrhages without inflammatory reaction are formed. In 12 h, necrotic changes in cells are intensified. In 24 h, well-defined necrosis of cardiomyocytes and interstitial neutrophilic infiltration were seen. On day 4, phagocytosis of dead cardiomyocytes begins, and by day 10, granulation tissue appears in the edges of the MI zone. In 2 weeks, the replacement of the MI zone with granulation tissue is completed, collagen appears, and scar tissue is formed during the next 6 weeks [4].

Experimental modeling of acute MI in combination with classical and modern research methods is indispensable in the study of the pathological mechanisms underlying the development of this disease and new treatment methods.

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Our aim was a comparative analysis of the parameters of Langendorff-isolated rat heart 1 day after MI and in sham-operated and healthy animals.

MATERIALS AND METHODS

The experiments were performed in accordance with the ethical norms and regulations on the use of laboratory animals. White outbred rats (n=38, body weight 200-250 g) were kept under standard vivarium conditions. The animals were divided into 3 groups: intact (n=14), sham-operated (control; n=10), and rats one day after MI modeling (n=14). The control group of sham-operated animals was formed in order to exclude the possible effect of surgical intervention on the myocardium (in this group, the thorax was opened and the heart was dislocated without LCA ligation).

For MI modeling in rats, LCA ligation at the level of the lower edge of the left atrial auricle was performed under ether anesthesia. The animal was fixed to the operating table. A longitudinal skin incision was made on the left side of the rat chest. Using wound spreaders, the pectoral muscles were pulled apart, and an incision about 10-15 mm long was made between ribs V and VI. Then the heart was removed from the thoracic cavity by pressing fingers on both sides of the thorax. The heart was held by the ventricles and a ligature (Prolene 6/0, Ethicon) was applied to the anterior branch of the left descending coronary artery 0.5-1 mm below its branching below the heart auricle and ligated three times without further reperfusion; the heart was returned to the chest cavity. The muscles were apposed, the skin was sutured and treated with an antiseptic agent. Immediately after the muscles and skin were pulled together, the animal began to breathe. Further experimental studies were performed in 1 day after MI simulation.

In *ex vivo* experiments, the parameters of Langendorff-isolated rat heart were analyzed using a Langendorff unit (ADInstruments). The animals were intraperitoneally injected with 25% urethane solution (in a dose of 800 mg/kg weight). The heart was extracted, washed, and placed in cold Krebs–Henseleit solution (2-5°C). The isolated heart was fixed by the aorta on a cannula and retrograded with oxygenated (95% O_2, 5% CO_2) solution, at a constant hydrostatic pressure of 75 mm Hg and 37°C. Myocardial contractive activity was studied in isovolumic mode using a MLT844 pressure sensor (ADInstruments) and a latex balloon filled with water and inserted into left ventricular cavity. HR (bpm), left-ventricular pressure (LVP, mm Hg), left ventricular contraction (dp/dt_{max}, mm Hg/sec) and relaxation rates (dp/dt_{min}, mm Hg/sec), duration of LV contraction and relaxation (mm Hg/sec), coronary flow (CF, ml/min) were calculated from the curve. Power Lab 8/35 system and LabChart Pro software were used.

Statistical processing of the results was performed using Microsoft Excel; one-way ANOVA followed by a post-hoc test (T-test) for related groups; paired and unpaired Student's t tests were also applied. The data are presented as $M \pm m$.

RESULTS

In the group of intact and sham-operated animals, no significant differences between the initial values of LVP were revealed (82.0±6.8 and 80±7 mm Hg, respectively). One day after MI modeling in the experimental group, LVP was 57.1±8.0 mm Hg and was significantly lower than in intact (p<0.05) and sham-operated (p<0.01) rats (Figs. 1 and 2).

The maximum rate of LV myocardial contraction (dp/dt_{max}) was 2859.4±188.0 mm Hg/sec in intact rats and 3027.8±214.7 mm Hg/sec in sham-operated rats. In the group with acute MI, dp/dt_{max} (2085.3±994.0 mm Hg/sec) was significantly lower than in the groups of intact (p<0.05) and sham-operated (p<0.01) animals (Fig. 2).

The maximum relaxation rate of the LV myocardium (dp/dt_{min}) was -1743.1±200.0 mm Hg/sec in intact rats and -1645.4±167.7 mm Hg/sec in sham-operated animals; in rats with a model of acute MI, this indicator (-1183.1±181.8 mm Hg/sec) was significantly lower than that in sham-operated rats (p<0.05) (Fig. 2).

The duration of LV myocardial contraction in intact animals and rats with acute MI was 0.116±0.004 and 0.110±0.004 mm Hg/sec, respectively, in the group of sham-operated animals this parameter was slightly lower (0.104±0.002 mm Hg/sec).

The duration of relaxation of the LV myocardium in the group of intact rats and with the model of acute MI was 0.218±0.010 and 0.206±0.020 mm Hg/sec,

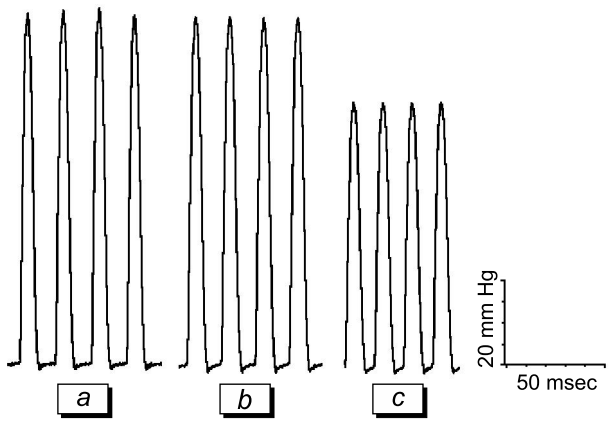

Fig. 1. Initial values of LVP and HR of the isolated heart of intact (*a*), sham-operated (*b*) rats and on the first day of MI modeling (*c*). Original record.

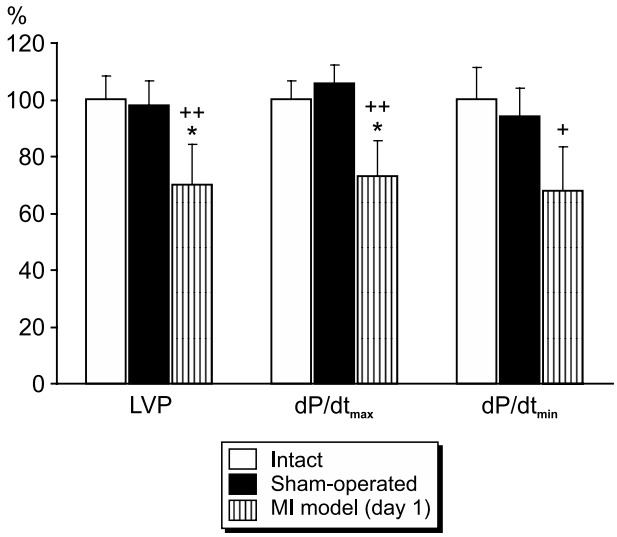

Fig. 2. Comparative analysis of contractility of Langendorff-isolated heart in intact, sham-operated rats, and animals with modeled acute MI. *p<0.05 in comparison with intact rats, ^{+}p<0.05, ^{++}p<0.01 in comparison with sham-operated rats.

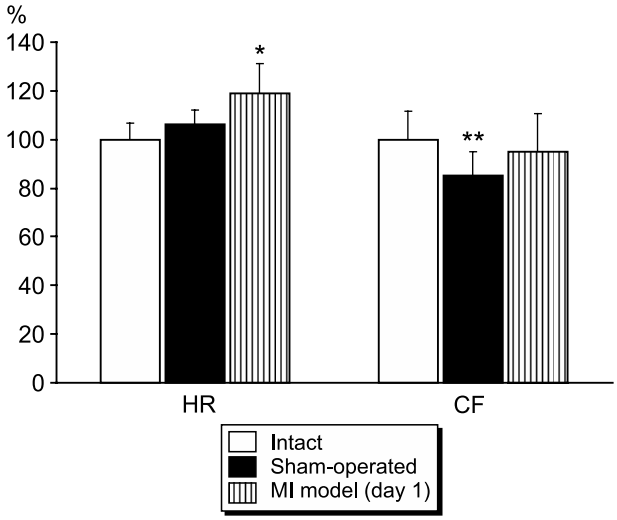

Fig. 3. Comparative analysis of HR and CF of Langendorff-isolated heart of intact, sham-operated rats, and animals with modeled acute MI. *p<0.05, **p<0.01 in comparison with intact rats.

respectively. In the group of sham-operated animals, it was significantly lower (0.149±0.010 mm Hg/sec, p<0.05) than in intact animals.

HR in intact and sham-operated animals did not differ significantly (187.1±5.6 and 194±13 bpm, respectively). In the group of rats with acute MI, the HR was higher (218±12 bpm, p<0.05) than in intact rats (Fig. 2).

CF in the heart isolated from intact and sham-operated animals was 9.1±0.6 and 7.9±0.4 ml/min (p<0.05), respectively. In rats with acute MI, this parameter was 9.2±0.9 ml/min (Fig. 3).

According to published reports, the contractile function of the heart considerably decreases one day after MI modeling, which can be associated with acute violation of the coronary blood supply and a decrease in oxygen and nutrient supply to the ischemic area of the myocardium, as well as increasing changes in myocardium in the form of small scattered foci of coagulation necrosis, cell disorganization, hemorrhages. In our study, LVP, the rate of contraction and relaxation of LV one day after MI modeling was lower than in intact and sham-operated rats, which is consistent with published data [5,6]. A pronounced chronotropic reaction to experimental MI was shown: one week after surgery, HR was higher than in intact animals [5,6]. Increased HR was also recorded one day after MI modeling. It is known that in the stage of acute MI, the content of NO, endothelin-1, and VEGF increases and reaches a maximum value by the end of the first day of the experiment. It is assumed that the vasodilatory effect of NO is directed to counterbalance the vasoconstrictor effect of endothelin [7]. It is possible that vasodilatory effect NO prevented significant changes in CF in animals with modeled acute MI. It should be noted that in the group of sham-operated rats, a decrease in CF and contraction time parameters was found, which are most likely associated with activation of adaptive mechanisms in response to surgical interventions. Thus, despite the absence of the changes in the inotropic and chronotropic functions of the heart in the group of sham-operated rats, these interventions can affect energy resources, the sensitivity of the heart muscle to Ca^{2+} ions, changes in the rate of formation of actomyosin bridges, and the rate of myocyte relaxation [8].

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