Rhythmoinotropic Myocardial Reactions in Rats with Postinfarction Cardiosclerosis against the Background of Streptozotocin-Induced Diabetes

D. S. Kondtratieva, S. A. Afanasiev, T. Yu. Rebrova, L. P. Tsapko, and R. S. Karpov

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We studied rhythmoinotropic reactions of the myocardium in rats with postinfarction cardiosclerosis and in rats with postinfarction cardiosclerosis against the background of streptozotocin-induced diabetes. Inotropic myocardial response in rats with postinfarction cardiosclerosis was significantly inhibited after rest periods, while in streptozotocin diabetic rats the rhythmoinotropic myocardial reaction was comparable with the reaction of intact myocardium. The combination of postinfarction cardiosclerosis and diabetes paradoxically contributed to preservation of contractile function of the myocardium in rats.

Key Words: *rhythmoinotropic reaction; diabetes; postinfarction cardiosclerosis; sarcoplasmic reticulum*

Diabetes mellitus (DM) aggravates the severity of CHD and increases probability of vascular catastrophes. Among major factors disturbing cardiac function in diabetes mellitus is remodeling of cardiomyocyte membranes by glycosylation and free-radical oxidation end-products [14]. This, in turn, serves as an additional factor of calcium ion imbalance in cardiomyocytes. Sarcoplasmic reticulum (SR) is the major structure responsible for intracellular Ca^{2+} oscillations. Disturbed function of SR in cardiomyocytes in heart failure considerably impairs rhythmoinotropic reactions of the myocardium and manifests in inverted force—frequency relationship [1,4,9]. This reflects a relationship between rhythmoinotropic myocardial reactions and functional state of SR, activity of its Ca^{2+}-systems and energy supply. Cardiomyocyte remodeling during heart failure leads to inhibition of SR Ca^{2+}-ATPase providing calcium ion reuptake and ryanodine receptors responsible for calcium release into the myoplasm [13]. The mechanisms underlying changes in functional activity of SR in CHD associated with DM are little studied.

Here we evaluated the rhythmoinotropic reaction of the myocardium in rats with postinfarction cardiosclerosis (PICS) against the background of streptozotocin-induced DM.

MATERIALS AND METHODS

The study was carried out using 44 mature male Wistar rats. The animals were divided into 5 groups: group 1 comprised intact animals (n=12), group 2 included rats with PICS (n=8), group 3 consisted of rats with PICS, in whom DM was modeled 2 weeks after coronary occlusion (n=8), group 4 comprised rats with induced DM (n=8), and group 5 comprised intact rats receiving vehicle (citrate buffer) instead of streptozotocin (n=8) according to the same scheme. DM was induced by single intraperitoneal injection of 60 mg/kg streptozotocin (Sigma) dissolved *ex tempore* in 0.01 mol/liter citrate buffer pH 4.5. Myocardial infarction was modeled by occlusion of the left descending coronary

Institute of Cardiology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences, Russia. ***Address for correspondence:*** dina@cardio.tsu.ru. D. S. Kondtratieva

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artery [12]. After surgery, the animals were kept under standard vivarium conditions. PICS was morphologically verified after 6 weeks. The development of heart and left ventricular hypertrophy was assessed by the ratio of animal body weight to heart weight and by ratio of the left ventricle weight to heart weight (Table 1). Group 4 rats were taken into the experiment 4 weeks after DM induction. Serum glucose concentration was measured using enzyme colorimetric test (Biocon Diagnostic)

The rhythmoinotropic relationship was studied on papillary muscles. To this end, the animals were immobilized by cervical dislocation under ether anesthesia, the thorax was opened, and the heart was isolated. The heart was washed on a flow chamber with Krebs—Henseleit solution containing (in 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 glucose (Sigma). The papillary muscles were isolated. In hearts of rats subjected to coronary occlusion, the size of postinfarction scar was measured by planimetry and the percent of the scar area from the free left ventricle wall area was calculated [2]. The isolated papillary muscles were placed into temperature-controlled flow chamber: one end was fixed to the chamber wall and another to an isometric transducer (6MX1C dynamoelectric converter). The muscles were perfused with oxygenated (95% O_2, 5% CO_2) Krebs—Henseleit solution at 36.5°C. The muscles were stimulated with 5-msec rectangular pulses (0.5 Hz frequency). The curves of isometric contraction of the papillary muscle were recorded. Functional activity of SR was assessed by inotropic reaction of the papillary muscles to resting periods [6]. The exposure to resting periods was provided against the background of regular contractions. For this purpose, stimulation was once discontinued for 4-60 sec and then regular stimulation was resumed [6]. The characteristics of the first contraction after rest period and regular contraction were compared. The dynamics of the relationship between contraction amplitude and duration of the rest period (mechanical restitution) [6] was assessed. The amplitude of the first contraction after rest period was expressed in percents of regular contraction amplitude. The rhythmoinotropic reactions of the myocardium in intact rats and in rats receiving vehicle did not significantly differ, therefore group 5 was not taken into account in final analysis of experimental data.

The data was analyzed statistically ($M \pm SEM$), significance of results was assessed using nonparametric Mann—Whitney U test.

RESULTS

Modeling of experimental PICS in rats led to myocardium hypertrophy (Table 1). Thus, the heart weight to body weight ratio in these animals was 6.27±0.33 mg/g *vs.* 3.29±0.21 mg/g in intact animals ($p<0.01$). The left ventricle to heart weight ratio in rats with PICS also significantly increased in comparison with that in intact animals (Table 1). Scar area in animals with PICS constituted 51.3±8.9% from the area of the left ventricle.

Injection of streptozotocin to rats 4.5-fold increased glucose concentration (Table 1). Moreover, body weight in rats with DM after 4 weeks decreased by 56% ($p<0.01$) in comparison with rats injected with vehicle (Table 1). These results attests to the development of DM after streptozotocin injection. PICS modeling before DM induction led to less pronounced body weight loss. Thus, body weight in rats with PICS and DM decreased by 30% compared to animals injected with vehicle. At the same time, the heart weight to body weight ratio and left ventricle weight to heart weight ratio in groups of animals with DM did not significantly differ (Table 1).

Evaluation of the inotropic myocardial reaction to rest periods showed that the dynamics of mechanical restitution of the myocardium in control (intact) group did not significantly differ by the amplitude and was

TABLE 1. Changes in Body Weight and Heart Weight in Rats after Coronary Occlusion and DM Induction

Group	*n*	Body weight, g	Glucose, mol/liter	Heart weight/body weight, mg/g	Left ventricle weight/heart weight, mg/mg	Scar area, %
Intact	12	298.0±23.7	6.00±0.37	3.29±0.21	0.645±0.013	—
Intact+citrate buffer	6	287.00±7.65	7.00±0.42	3.21±0.21	0.675±0.018	—
DM	10	160.0±14.8*	27.00±2.75*	3.77±0.31	0.676±0.014	—
PICS+DM	14	221.00±4.51*	18.00±1.79*	3.37±0.11	0.673±0.019	46.1±9.7
PICS	11	242.00±11.17**	7.00±0.13	6.27±0.33**	0.687±0.016**	51.3±8.9

Note. *$p<0.01$, **$p<0.05$ compared to intact animals (receiving vehicle — citrate buffer). Scar area — % from area of left ventricle.

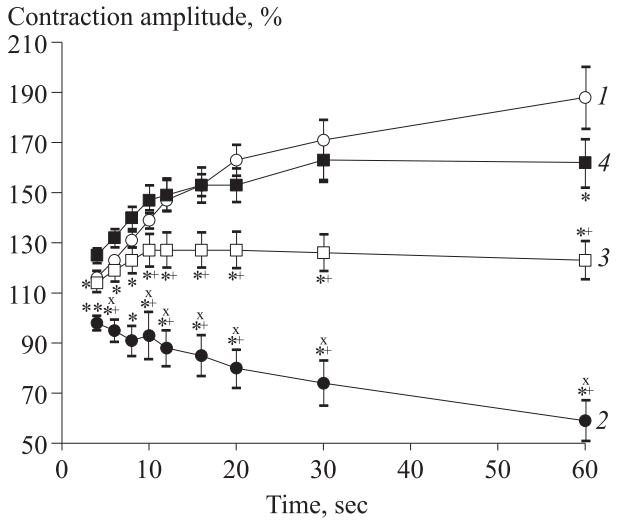

Fig. 1. Dynamics of mechanical restitution of the myocardium in rats with PICS and DM. *1*) intact rats; *2*) rats with PICS; *3*) rats with PICS+DM; *4*) rats with DM. $p<0.001$ compared to: *intact rats, +rats with DM, xrats with PICS+DM.

similarly directed (Fig. 1). Thus, the first contraction after rest period surpassed the regular contraction by amplitude by 16-88% depending on the duration of the rest period (Fig. 1). The inotropic myocardial response to rest periods characterizes SR capacity to accumulate and store calcium ions [6,10]. The increase in contraction amplitude (potentiation) in response to rest period can be explained by accumulation of calcium ions in SR during the pause. That is why the amplitude of the first contraction after resumption of papillary muscle stimulation in intact mice exceeds the amplitude of regular contraction [10].

Comparison of inotropic myocardial reaction to rest period revealed significant differences between experimental groups (Fig. 1). The amplitude of myocardial contraction in rats with PICS did not exceed the amplitude of regular contraction. Moreover, increasing the duration of the rest period led to progressive decrease in contraction amplitude from 98% to 59% from amplitude of regular cycle (Fig. 1). This inotropic reaction indicates impaired Ca^{2+} accumulating capacity of SR in cardiomyocytes of pathologically changed myocardium. These findings agree with published reports [8,11] on reduced functional activity of Ca^{2+}-ATPase and ryanodine receptors in heart failure. These changes can contribute to intensification of calcium leakage current from SR [5].

The dynamics of rhythmoinotropic myocardial reactions in rats with streptozotocin-induced diabetes did not significantly differ from the reactions of intact myocardium after rest periods of various durations, except 60-sec rest. The latter significantly decreased contraction amplitude by 26% ($p<0.05$) compared to the inotropic response of myocardium in intact animals (Fig. 1). It can be hypothesized that in this case Ca^{2+}-accumulating capacity of SR was slightly reduced, but these changes manifested only after long rest period, when calcium leakage current started to prevail in SR.

The amplitude of myocardial contraction in rats with combined pathologies (PICS and DM) after rest periods was significantly lower than in rats with DM and intact mice. However, the dynamics of mechanical restitution this group, unlike animals with PICS, had ascending direction (Fig. 1). Hence, induction of DM against the background of PICS paradoxically preserves the contractile properties of the myocardium. We can hypothesize that glycosylation products increase rigidity of cardiomyocyte membranes; these changes not only decrease activity of enzymes counteracting ischemic damage, but also prevents excessive Ca^{2+} entry into the myoplasm [14]. Our results agree with previous reports demonstrating high ischemic resistance of the myocardium (*in vivo* and *in vitro*) at early stages of streptozotocin-induced diabetes [3,7].

Thus, experimental combination of DM and PICS prevents impairment of the rhythmoinotropic reactions of the myocardium. At the same time, the mechanisms of this resistance require further investigations.

REFERENCES

1. D. S. Kondtratieva, S. A. Afanasev, A. V. Evtushenko, *et al.*, *Kardiologia*, No. 10, 45-50 (2008).
2. M. A. Usacheva, E. V. Popkova, E. A. Smirnova, *et al.*, *Bull. Exp. Biol. Med.*, **144**, No. 12, 624-628 (2007).
3. H. Chen, W. L. Shen, X. H. Wang, *et al.*, *Clin. Exp. Pharmacol. Physiol.*, **33**, No. 10, 910-916 (2006).
4. R. R. Lamberts, N. Hamdani, T. W. Soekhoe, *et al.*, *J. Physiol.*, **582**, Pt. 2, 695-709 (2007).
5. S. E. Lehnart, X. H. Wehrens, A. Kushnir, and A. R. Marks, *Ann. N. Y. Acad. Sci.*, **1015**, 144-159 (2004).
6. G. H. Lin, H. L. Ru, P. L. Wu, *et al.*, *Conf. Proc. IEEE Eng. Med. Biol. Soc*., **5**, 3628-3631 (2004).
7. T. Nawata, N. Takahashi, T. Ooie, *et al.*, *J. Cardiovasc. Pharmacol.*, **40**, No. 4, 491-500 (2002).
8. K. Ono, M. Yano, T. Ohkusa, *et al.*, *Cardiovasc. Res.*, **48**, No. 2, 323-331 (2000).
9. J. Palomeque, M. V. Petroff, L. Sapia, *et al.*, *J. Card. Fail.*, **13**, No. 2, 143-154 (2007).
10. B. Pieske, L. S. Maier, and S. Schmidt-Schweda, *Basic Res. Cardiol.*, **97**, No. 1, 163-171 (2002).
11. L. Sen, G. Cui, G. C. Fonarow, H. Laks, *Am. J. Physiol. Heart Circ. Physiol.*, **279**, No. 2, H709-H718 (2000).
12. Y. L. Sun, S. J. Hu, L. H. Wang, *et al.*, *Chest*, **128**, No. 3, 1812-1821 (2005).
13. M. Yano, Y. Ikeda, and M. Matsuzaki, *J. Clin. Invest.*, **115**, No. 3, 556-564 (2005).
14. B. Ziegelhöffer-Mihalovicová, I. Waczuliková, L. Sikurová, *et al.*, *Mol. Cell. Biochem.*, **249**, Nos. 1-2, 175-182 (2003).