DOI 10.1007/s10517-022-05640-x

Response of Isolated Rat Heart to α_2-Adrenergic Receptor Stimulation after I_f Current Blockade in the Late Postinfarction Period

R. K. Bugrov, A. M. Kuptsova, N. I. Ziyatdinova, and T. L. Zefrov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 174, No. 7, pp. 28-32, July, 2022
Original article submitted March 23, 2022

We studied the effect of α_2-adrenoreceptor activation after preliminary I_f-current blockade on the performance of the Langendorff-isolated rat heart 54 days after modeling myocardial infarction. Stimulation of α_2-adrenoreceptors against the background of application of I_f blocker ZD7288 in concentrations of 10^{-9} and 10^{-5} M decreased myocardial inotropy in isolated rat hearts by 50 and 39% ($p<0.05$) and increased HR by 20 and 15% ($p<0.05$), respectively. Activation of α_2-adrenoreceptors against the background of application of ZD7288 in a concentration of 10^{-9} and 10^{-5} M led to a decrease in the coronary flow in the isolated rat heart with the model of myocardial infarction by 21% ($p<0.05$) and 32% ($p<0.05$), respectively.

Key Words: *α_2-adrenergic receptors; I_f-currents; myocardial infarction model; isolated heart; rat*

Myocardial infarction (MI) is caused by ischemia following coronary occlusion. Acute and chronic MI are classified as two different phases of injury, depending on the time of myocardial necrosis relative to the time of coronary vessel occlusion. The acute phase occurring within the first 7 days is associated with cardiomyocyte death, extensive inflammation, and fibroblast recruitment. The subacute phase characterized by healing of cardiac tissue and scar formation occurs between the 1st and 4-6th weeks. Expansion of the infarct zone, thinning of the ventricular wall and dilatation of the ventricle characterize the chronic phase that occurs after 8-9 weeks. Extensive remodeling of the left ventricle (LV) gradually leads to severe heart failure [1].

The most important role in the pathogenesis of heart failure is played by hyperactivation of the sympathetic nervous system, as a result of which catecholamines cause dysfunction of the heart, arrhythmias, myocardial hypertrophy, and apoptosis. Another consequence of increased level of circulating catecholamines is a decrease of myocardial adrenoreactivity related to a decrease in the density of β-adrenergic receptors and their desensitization, which leads to disruption of cAMP-dependent mechanisms of Ca^{2+}-channel activation and inhibition of the inotropic response to agonists [2].

We have previously demonstrated the role of α_2-adrenergic receptors (α_2-AR) in the regulation of cardiac activity in healthy animals [3-6]. According to published reports, rats with spontaneous hypertension demonstrate enhanced expression of α_2-AR subtypes, dysfunction of α_2-AR, and inefficiency of signaling pathways associated with α_2-AR in a model of the cardiovascular system pathology [7]. In rats with ventricular tachycardia/fibrillation induced by ischemia/reperfusion, stimulation of α_2-AR with selective agonist UK-14304 produced an antiarrhythmic effect. Another α_2-AR agonist, dexmedetomidine, had a pronounced cardioprotective effect in LV dysfunction caused by hypoxia/oxygenation. In addition, α_2-AR antagonist yohimbine aggravated ventricular tachycardia during acute ischemia in dogs [7].

I_f is a non-selective cationic current activated by hyperpolarization and entering through hyperpolarization-activated cyclic nucleotide-gated chan-

Department of Human Health Protection, Kazan (Volga region) State University, Kazan, Republic of Tatarstan, Russia. ***Address for correspondence:*** zefirovtl@mail.ru. T. L. Zefrov

nels (HCN channels) [8]. Initially, I_f was considered characteristic of the heart conduction system cells and neurons, but later it was identified in atrial and ventricular myocytes in animals and humans [8]. I_f currents are modulated by the level of cAMP, sympathetic and parasympathetic divisions of the autonomic nervous system [9]. Experiments on rats have shown that I_f density is significantly higher in LV myocytes isolated from hypertrophied myocardium. Functional overexpression of I_f in cardiomyopathy can be explained by transcription mechanisms; this was associated with a significant increase in mRNA isoforms of HCN2 and HCN4 channels in rat LV tissue after MI [10]. Since the presence of HCN channels and α_2-AR has been shown in cardiomyocytes in the pathology of the cardiovascular system, HCN channels can be an important effector of adrenergic regulation of the heart.

The aim of this work is to investigate the effect of α_2-AR activation against the background of I_f currents blockade on the performance of Langendorff-isolated rat heart 54 days after experimental MI.

MATERIALS AND METHODS

Outbred rats (n=20, age 100-120 days, body weight 200-250 g) were used in the experiment. The experiments were carried out in compliance with the European Convention for the Protection of Animals Used in Experiments or for Other Scientific Purposes (Strasbourg, 1986).

The effect of clonidine hydrochloride against the background of I_f-currents blockade was studied in rats (n=14) on day 54 after MI modeling (period of scar formation) [11]. In control experiments (n=6), the effect of clonidine hydrochloride without prior I_f-currents blockade was assessed.

MI was modeled under ether anesthesia. The animal was fixed on the operating table, an incision was made on the left side of the chest, the pectoral muscles were pulled apart, and a 10-15-mm incision was made between ribs V and VI. The heart was taken out of the chest cavity by pressing fingers on the chest from both sides. The heart was held by the ventricles and a ligature was applied to the anterior branch of the left coronary artery (Prolene 6/0, Ethicon) 0.5-1.0 mm below its orifice under the auricle, and tied 3 times; then, the heart was returned to the chest cavity, the muscles were brought together, and the skin was sutured. During the postoperative period, the animals were kept under standard conditions.

Ex vivo experiments were carried out using a Langendorff apparatus (ADInstruments). The animals (n=20) were intraperitoneally injected with a 25% solution of urethane (800 mg/kg body weight). The heart was isolated, washed, and placed in cold Krebs–Henseleit solution (2-5°C). The aorta was cannulated and retrograde perfusion of the isolated heart with oxygenated (95% O_2, 5% CO_2) solution was performed under a constant hydrostatic pressure of 60-65 mm Hg at 37°C. The contractile activity of the myocardium was studied in the isovolumic mode using an MLT844 pressure sensor (ADInstruments) and a latex balloon filled with water and inserted into the LV cavity. HR (bpm), left-ventricular developed pressure (LVDP, mm Hg), and coronary flow (CF, ml/min) were determined. The curve was recorded using on PowerLab 8/35 system (ADInstruments) and LabChart Pro software. α_2-AR were stimulated with clonidine hydrochloride (Sigma) in a concentration of 10^{-6} M; for blockade of I_f currents, ZD7288 (Tocris) was used in concentrations of 10^{-9} and 10^{-5} M.

Statistical processing of the obtained results was carried out using Microsoft Excel program by one-way ANOVA followed by a posteriori tests (T test) for related and unrelated groups; paired and unpaired Student's t tests were also applied. The data are presented as $M \pm m$. The differences were significant at $p<0.05$.

RESULTS

Addition of the α_2-AR agonist clonidine (10^{-6} M) against the background of preliminary I_f-current blockade with ZD7288 (10^{-9} M) decreased LVDP from 44.6±8.1 to 35.1±7.8 mm Hg by the 5th minute. By the 10th minute of observation, LVDP decreased to 26.5±4.6 mm Hg ($p<0.05$) and at the 20th minute it was 22.5±3.1 mm Hg ($p<0.05$) (Fig. 1, *a*; Fig. 2, *b*). Thus, LVDP decreased to 50% of the initial value. Activation with clonidine against the background of I_f current blockade reduced HR at the 1st minute of the study from 140.0±8.3 to 134.0±9.2 bpm. By the 5th minute, the HR increased to 150.0±12.5 bpm and by the 20th minute to 168.0±11.4 bpm ($p<0.05$), *i.e.*, increased by 20% (Fig. 2, *b*). Stimulation of α_2-AR against the background of I_f-current blockade reduced CF values from 5.7±0.7 to 5.1±0.7 ml/min ($p\leqslant0.05$) at the 5th minute of observation, to 4.6±0.6 ml/min ($p\leqslant0.05$) at the 15th minute, and to 4.5±0.6 ml/min ($p\leqslant0.05$) at the 20th minute (Fig. 2, *b*); thus, CF decreased by 21%.

Clonidine against the background of administration of ZD7288 (10^{-5} M) reduced LVDP by the 5th minute of the experiment from 79.3±14.4 to 58.5±11.5 mm Hg, by the 15th minute this parameter decreased to 51.9±8.2 mm Hg ($p<0.05$) and by the 20th minute of observation, LVDP decreased to 48.3±7.8 mm Hg ($p<0.05$) (Fig. 1, *b*; Fig. 2, *c*). Clonidine against the background of I_f-current blockade increased HR from 178.0±15.5 to 191.7±14.5 bpm ($p<0.05$) at the 5th

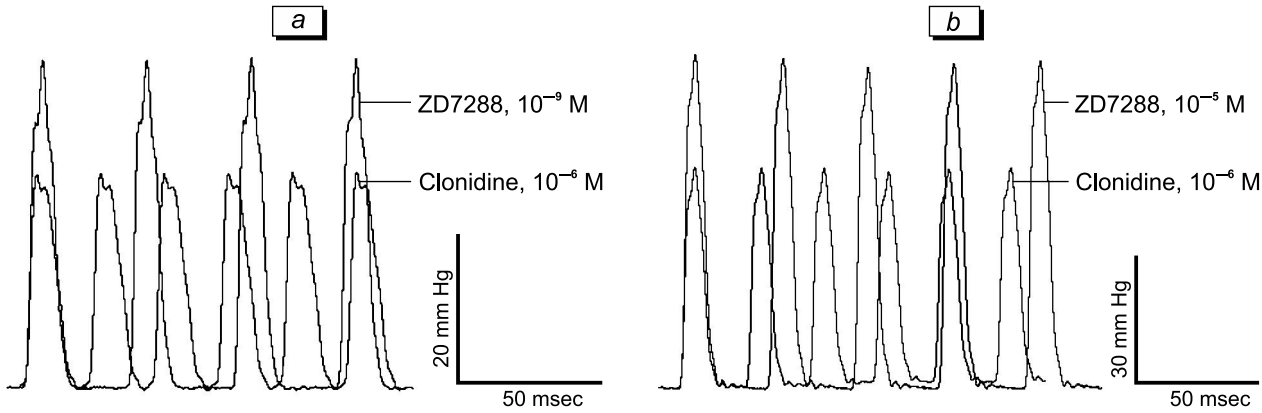

Fig. 1. Effect of α_2-AR agonist clonidine hydrochloride (10^{-6} M) against the background of I_f blocker ZD7288 in concentrations of 10^{-9} M (*a*) and 10^{-5} M (*b*) on LVDP and HR of Langendorff-isolated adult rat heart on day 54 after MI modeling. Original record.

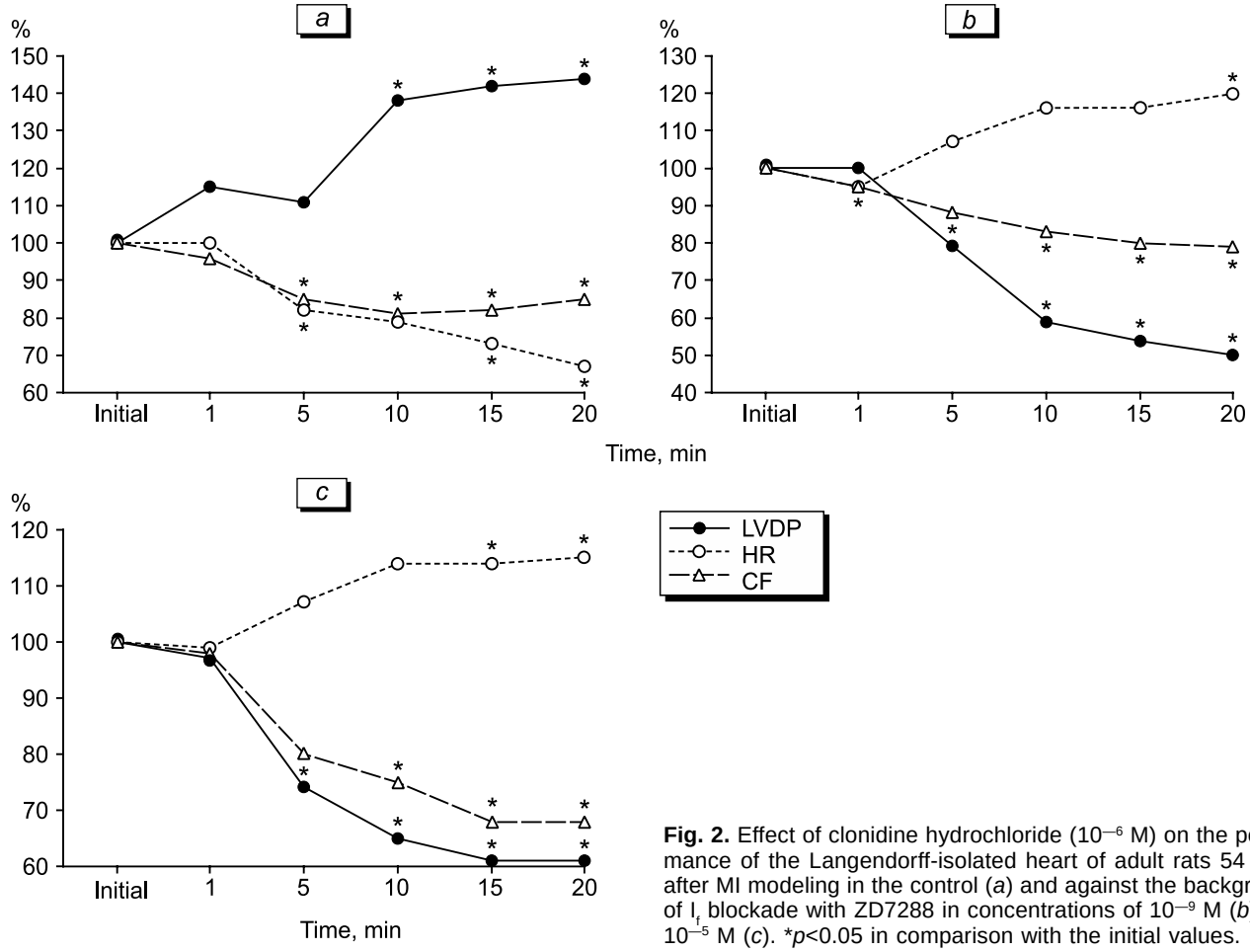

Fig. 2. Effect of clonidine hydrochloride (10^{-6} M) on the performance of the Langendorff-isolated heart of adult rats 54 days after MI modeling in the control (*a*) and against the background of I_f blockade with ZD7288 in concentrations of 10^{-9} M (*b*) and 10^{-5} M (*c*). **p*<0.05 in comparison with the initial values.

minute and up to 202.8±14.3 bpm at the 15th minute of the experiment. By the 20th minute, the HR increased to 205.0±12.4 bpm ($p<0.05$). Administration of an α_2-AR agonist against the background of blockade decreased CF from 7.7±1.1 to 5.7±1.1 ml/min ($p<0.05$) at the 10th minute of observation and to 5.2±1.2 ml/min ($p<0.05$) by the 15th minute (Fig. 2, *c*).

We also performed a comparative analysis of activation of α_2-AR with clonidine (control) and clonidine against the background of I_f-current blockade with ZD7288 (10^{-9} and 10^{-5} M) in the isolated heart of adult rats on day 54 after MI modeling. Preliminary I_f blockade changed the direction of the dynamics of myocardial inotropy in the rat isolated heart upon stimulation

with α_2-AR from positive to negative. Stimulation of α_2-AR in the control increased LVDP by 44% ($p<0.05$) (Fig. 2, *a*), while stimulation of α_2-AR against the background of ZD7288 in concentrations of 10^{-9} and 10^{-5} M decreased this parameter by 50% ($p<0.05$) and 39% ($p<0.05$). In the control, activation of α_2-AR caused a decrease in HR by 33% ($p<0.05$) (Fig. 2, *a*). Administration of α_2-AR agonist against the background of ZD7288 blocker in concentrations of 10^{-9} and 10^{-5} M, on the contrary, led to an increase in HR by 20 and 15% ($p<0.05$ in both cases), respectively. It should be noted that CF decreased in the control (Fig. 2, *a*) and in the experiment. At the same time, the maximum decrease (by 32%, $p<0.05$) was observed after administration of α_2-AR agonist against the background of ZD7288 blocker in a concentration of 10^{-5} M.

Thus, pre-blockade of I_f changed the chronotropic and inotropic responses of rat heart with an MI model to stimulation of α_2-AR, but did not affect the direction of CF dynamics.

Based on published data, we can assume the existence of several mechanisms of joint influence of α_2-AR and I_f currents on the work of the heart. Activation of α_2-AR with clonidine directly inhibits the I_f current [12]. Activation of α_2-AR reduces the level of adenylate cyclase, inhibits activity of L-type Ca^{2+} channels, and stimulates K^+ channels. α_2-AR agonists also stimulate NO synthase and the NO-cGMP pathway [13]. There is evidence that, despite the overexpression of α_2-AR subtypes, their agonists act on cardiomyocytes of spontaneously hypertensive rats much weaker than on cardiomyocytes of normotensive rats [7]. The observed increase in the number of α_2-AR in spontaneously hypertensive rats may play an important role in the effective adaptation of the heart in other models of cardiovascular pathology [7]. It has been shown that stimulation of α_2-AR protects the heart from damage during ischemia. It is assumed that this mechanism is associated with inhibition of catecholamine release in sympathetic synapses and a decrease in the oxygen demand of cardiomyocytes during ischemia/reperfusion [14]. Functional overexpression of HCN channels has also been shown in cardiac pathologies [10]. Thus, an increase in α_2-AR-mediated signals in cardiomyocytes with an increase of α_2-AR in cardiovascular pathologies [7] can be determined, among other things, by more complex interaction between I_f and α_2-AR of the heart after MI.

The study was supported by the Russian Science Foundation (grant No. 21-15-00121; https://rscf.ru/project/21-15-00121/).

REFERENCES

1. Konstam MA, Kramer DG, Patel AR, Maron MS, Udelson JE. Left ventricular remodeling in heart failure: current concepts in clinical significance and assessment. JACC Cardiovasc. Imaging. 2011;4(1):98-108. doi: 10.1016/j.jcmg.2010.10.008
2. Mikhailichenko VYu. Contractile function of rat heart 1 and 3 months after experimental myocardial infarction and mesenchymal stem cell transplantation. Vestn. Neotl. Vosstanov. Med. 2011;12(4):435-438. Ukranian.
3. Kuptsova AM, Ziyatdinova NI, Zefirov TL. Age peculiarities of the blockade of α2-adrenoceptors in the formation of the heart sympathetic innervations. J. Pharm. Res. 2017;11(10):1201-1204.
4. Kuptsova AM, Bugrov RK, Khabibrakhmanov II, Kobzarev RS, Ziyatdinova NI, Zefirov TL. Role of α2-adrenoceptors in rat heart with the model of myocardial infarction. J. Chem. Health Risks. 2021;11(2):129-134. doi: 10.22034/jchr.2021.682031
5. Ziyatdinova NI, Kuptsova AM, Faskhutdinov LI, Zefirov AL, Zefirov TL. Effect of α2-adrenoceptor stimulation on functional parameters of Langendorff-isolated rat heart. Bull. Exp. Biol. Med. 2018;165(5):593-596. doi: 10.1007/s10517-018-4220-9
6. Ziyatdinova NI, Kuptsova AM, Sungatullina MI, Zefirov TL. Effect of α2-adrenergic receptor stimulation on the isolated rat heart against the background of I_f blockade. Bull. Exp. Biol. Med. 2020;169(5):635-638. doi: 10.1007/s10517-020-04943-1
7. Kokoz YM, Evdokimovskii EV, Maltsev AV. Upregulation of α2-adrenoceptor synthesis in SHR cardiomyocytes: Recompense without sense — Increased amounts, impaired commands. Arch. Biochem. Biophys. 2019;674:108109. doi: 10.1016/j.abb.2019.108109
8. Stillitano F, Lonardo G, Zicha S, Varro A, Cerbai E, Mugelli A, Nattel S. Molecular basis of funny current (I_f) in normal and failing human heart. J. Mol. Cell. Cardiol. 2008;45(2):289-299. doi: 10.1016/j.yjmcc.2008.04.013
9. DiFrancesco JC, DiFrancesco D. Dysfunctional HCN ion channels in neurological diseases. Front. Cell Neurosci. 2015;6:174. doi: 10.3389/fncel.2015.00071
10. Suffredini S, Stillitano F, Comini L, Bouly M, Brogioni S, Ceconi C, Ferrari R, Mugelli A, Cerbai E. Long-term treatment with ivabradine in post-myocardial infarcted rats counteracts f-channel overexpression. Br. J. Pharmacol. 2012;165(5):1457-1466. doi: 10.1111/j.1476-5381.2011.01627.x
11. Ovsepyan AA, Panchenkov DN, Prokhortchouk EB, Telegin GB, Zhigalova NA, Golubev EP, Sviridova TE, Matskeplishvili ST, Skryabin KG, Buziashvili UI. Modeling myocardial infarction in mice: methodology, monitoring, pathomorphology. Acta Naturae. 2011;3(1):107-115. doi: 10.32607/20758251-2011-3-1-107-115
12. Ji F, Li Z, Nguyen H, Young N, Shi P, Fleming N, Liu H. Perioperative dexmedetomidine improves outcomes of cardiac surgery. Circulation. 2013;127(15):1576-1584. doi: 10.1161/CIRCULATIONAHA.112.000936
13. Yoshikawa Y, Hirata N, Kawaguchi R, Tokinaga Y, Yamakage M. Dexmedetomidine maintains its direct cardioprotective effect against ischemia/reperfusion injury in hypertensive hypertrophied myocardium. Anesth. Analg. 2018;126(2):443-452. doi: 10.1213/ANE.0000000000002452
14. Song J, J Du J, Tan X, Wu Z, Yuan J, Cong B. Dexmedetomidine protects the heart against ischemia reperfusion injury via regulation of the bradykinin receptors. Eur. J. Pharmacol. 2021;911:174493. doi: 10.1016/j.ejphar.2021.174493