Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Role of A_2-adrenoceptors in Rat Heart with the Model of Myocardial Infarction

Anna Mihailovna Kuptsova[*1], Roman Kutdusovich Bugrov[2], Insaf Ilkhamovich Khabibrakhmanov[3], Roman Sergeevich Kobzarev[2], Nafisa Ilgizovna Ziyatdinova[4], Timur Lvovich Zefirov[4]

[1]*Associate Professor (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan,* Russia

[2] *Postgraduate (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan,* Russia

[3] *Senior Lecturer (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan,* Russia

[4] *Professor (Department of Human Health Protection) Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan,* Russia

(Received: 25 October 2020 Accepted: 28 December 2020)

KEYWORDS

Model;
Myocardial infarction;
α_2-adrenoreceptors;
Isolated heart;
Rat

ABSTRACT: Chronic adrenergic and angiotensin-energic stimulation of the heart muscle is one of the main causes of the onset and development of heart failure. α_2-AR is widespread in the cardiovascular system and can be further applied and introduced into the heart's regenerative cell therapy. This study's objective was to study the effect of α_2-AR stimulation on the performance of an isolated heart in rats with a model of myocardial infarction. To study the mechanisms of MI, experiments were carried out according to the classical technique of H. Selye - ligation of the anterior branch of the left coronary artery and perfusion of a Langendorff heart with a model of myocardial infarction. We studied the parameters of myocardial activity by changes in heart rate, as well as the left ventricle pressure (LVP), and the CF $(dp/dt_{max})/(dp/dt_{min})$ of the left ventricle. Coronary dilator activity was assessed by measuring the outflow of the perfusion solution through the coronary arteries. The amplitude of the left ventricle's pressure wave after application of the α_2-AR agonist clonidine hydrochloride at a concentration of 10^{-6} M to the perfused solution increased by 44% ($p \leq 0.05$) from the initial values. The rate of contraction and the rate of relaxation of the left ventricular myocardium also increased by 20% ($p \leq 0.05$) and 37% ($p \leq 0.05$), respectively. The activation of α2-AR in adult rats' isolated hearts with a model of myocardial infarction caused a decrease in heart rate by 18% ($p \leq 0.05$) and CF by 2% ($p \leq 0.05$).

INTRODUCTION

Chronic adrenergic and angiotensin-energic stimulation of the heart muscle is one of the main causes of the onset and development of heart failure [1-5].

The β-AR and α_1-AR activation mechanisms and their participation in myocardial contractility have been studied in detail. Early studies did not reveal the participation of α_2-AR in the regulation of myocardial contractility, isolated papillary muscle, and the whole heart. Indeed, the presence of α_2-AR (α_{2A}, α_{2B} и α_{2C}) in the cardiac tissue of experimental animals was small

*Corresponding author: anuta0285@mail.ru (A. Mihailovna Kuptsova)
DOI: 10.22034/jchr.2021.682031

compared to the amount of these receptors in endothelial tissues, kidneys, liver, and neurons [6]. In the last decade, all types of α_2-AR have been identified in the myocardium [7, 8]. Domestic researchers using DNA microarrays in embryonic stem cell cardiomyocytes showed the presence of three subtypes of α_2-AR at different stages of embryonic development [9]. The results obtained can be further applied and introduced into the regenerative cell therapy of the heart.

Fundamental studies on the effect of various pharmacological agents indicate multidirectional effects of heart rate.

A decrease in heart rate in response to stimulation of α_2-AR is caused by inhibition of norepinephrine release from sympathetic nerves [10]. In other studies, stimulation of α_2-AR with clonidine either increases heart rate [11] or does not change it [8].

Experiments on an isolated heart showed that activation of α_2-AR of different concentrations caused a decrease in heart rate, and only 10^{-6} M had multidirectional effects [12]. The age-related features of α_2-AR blockade on the heart rate of rats were shown [13]. Studies on the inotropic function of the heart of animals are also ambiguous [14, 15]. The previous Vitro studies recorded multidirectional effects on inotropy of rat atrial and ventricular myocardial strips upon activation of α_2-AR [16], revealed a biphasic change in inotropy in rats without sympathetic innervation of the heart [13]; while ex vitro studies showed a decrease in the amplitude of left ventricular pressure [12].

A number of authors have shown the cardioprotective effect of myocardial α_2-AR [9,14]. Activation of α_2-AR was found to counteract the development of hypertrophy in transgenic rats with increased content of angiotensin II in the cardiac tissue [17]. We assumed that a study on the activation of α_2-AR in an isolated heart with a model of myocardial infarction (MI) would be of undoubted interest since it is known that myocardial infarction is one of the leading causes of death in the world (about 12% of the total number of deaths).

The search for new methods of disease modeling, diagnosis, and treatment is becoming a topical issue of modern research in the treatment of myocardial infarction (MI). The modern literature describes various methods of modeling acute myocardial infarction, which is used to study this disease. The most common models are diathermocoagulation of the interventricular artery, occlusive models, models with reversible occlusion, induction of MI by hormonal shifts characteristic of type 2 diabetes mellitus and stress conditions - Panin's technique, MI - Langendorff retrograde heart perfusion [18].

The most common and used for the reproduction of acute myocardial infarction is the occlusal model - the imposition of ligatures on various branches of the heart's coronary vessels. Thus, ligation of the descending branch of the left coronary artery in dogs is accompanied by the formation of a heart attack in the anterior wall of the left ventricle and the anterior part of the interventricular septum. A similar operation on rats also leads to the occurrence of MI, which reproduces human MI both by external signs and by ECG characteristics [19].

This research aimed to study the effect of α_2-AR stimulation on the performance of an isolated heart in rats with a model of myocardial infarction.

MATERIALS AND METHODS

The myocardial infarction model reproduction technique.

To study the mechanisms of myocardial infarction, we carried out experiments according to the classical method of H. Selye - ligation of the anterior branch of the left coronary artery, the formation of focal myocardial lesions with necrosis, infiltration of inflammatory cells, and subsequent fibrosis [20]. The ligation of a coronary vessel is a complex invasive procedure, while the zone of the spread of myocardial infarction may vary [9]. Surgical modeling of myocardial infarction is accompanied by high mortality in animals [21,22].

The experiments involved ten white outbred rats aged 100-120 days, with an average weight of 200-250 grams. The formation of the myocardial infarction model was performed under ether anesthesia. On the left side of the chest, the rats were shaved off, disinfected, and made the incision. With the help of retractors, the pectoral muscles were bred, and between the 5th and 6th ribs, an incision of about 15-20 mm was made, and the heart was removed from the chest cavity. The ventricles held the heart, and a ligature (Premiline 6/0, 2xDR12) was placed on the

anterior branch of the left coronary artery 0.5-1 mm below its exit from under the auricle of the heart and ligated, and the heart was returned to the chest cavity. The muscles were moved together; the skin was sutured and treated with an antiseptic. In the postoperative period, the animals were kept under standard conditions. Further studies to study the effect of an α2-AR agonist on an isolated heart with a model of myocardial infarction were carried out after 54 days, due to which heart failure was formed in rats [23].

Isolated heart specimen preparation

The methodology of the Langendorff heart developed by H. Selye remains fundamentally unchanged to this day. According to the original description of the method, the isolated heart is perfused by cannulating the aorta. The physiological Krebs-Henseleit buffer flows retrograde down the aorta, opposite to the normal physiological flow, at a constant pressure of 55-60 mm Hg and a constant temperature of 37^0C. A latex balloon filled with distilled water and connected by a catheter to a pressure transducer was placed in the left ventricle to determine contractile activity. An isolated heart's performance was recorded in the PowerLab system (ADInstruments, Australia) and processed in the LabChartPro 8V program.The ability of the considered α_2-AR agonist to change the studied parameters of myocardial activity was determined by changes in heart rate, as well as the pressure developed by the left ventricle (LVP), the rate of contraction (dp/dt_{max}) and relaxation (dp/dt_{min}) of the left ventricle. Coronary dilator activity was assessed by measuring the outflow of the perfusion solution through the coronary arteries.

Clonidine hydrochloride (Sigma) at a concentration of 10^{-6} M was used to stimulate α_2-AR. This concentration of the agonist was selected on the basis of previously conducted experiments, which showed significant changes in the performance of an isolated heart of rats in the control group [7]. The results were statistically processed in Excel using paired and unpaired Student's t-test. Values at * - $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

The initial pressure developed by the left ventricle in rats with a myocardial infarction model was 19.9 ± 5.3 mm Hg. The application of an α_2-AR agonist into the working solution caused an increase in LVP to 22.9 ± 6.4 mm Hg. By the 5th minute of the experiment, LVP was 22 ± 5.8 mm Hg. Then, by 10 minutes, LVP began to increase to 27.5 ± 6.7 mm Hg ($p \leq 0.05$), and at 15 minutes, it was 28.3 ± 7.2 mm Hg ($p \leq 0.05$). At the final minute of the experiment, LVP increased to 28.7 ± 7.1 mm Hg ($p \leq 0.05$) (Figure 1). The control group of healthy animals had the opposite effect of α_2-AR activation on LVP - a 26% decrease from the initial value (Figure 2).

The maximum rate of increase in the pressure of the left ventricular myocardium after introducing clonidine hydrochloride into the perfused solution during the first minute of observation increased from 594.8 ± 138.8 mm Hg/sec to 713.2 ± 175.1 mm Hg/sec. Further, by the 5th minute, the rate of pressure rise decreased to 653.5 ± 147.8 mm Hg/sec. Then dP/dt_{max} increased to 784.4 ± 159.1 mm Hg/sec ($p \leq 0.05$) by the 15th minute. During the 20th minute of the experiment, the maximum rate of increase in left ventricular myocardial pressure was 782.4 ± 151.4 mm Hg/sec ($p \leq 0.05$).

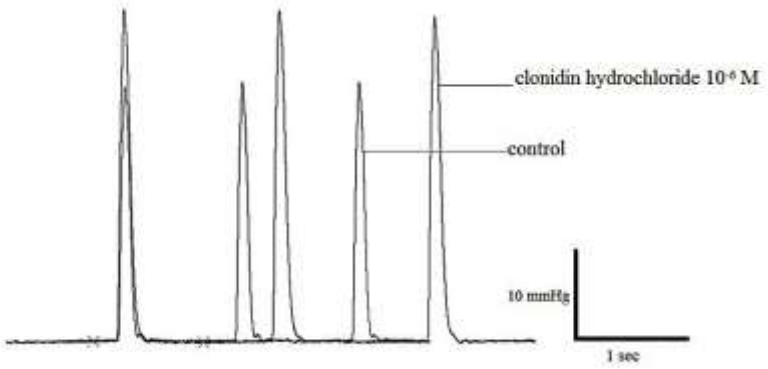

Figure 1. The effect of a_2-AR agonist, clonidine hydrochloride, (10_{-6} M) on LVP and heart rate of the Langendorff heart of adult rats with a model of myocardial infarction (original entry). Control - recording prior to administration of a_2-AR agonist.

The minimum relaxation rate of the left ventricular myocardium after the addition of a2-AR agonist increased from 340 ± 101.7 mm Hg/sec to 489.9 ± 124.5 mm Hg/sec during the first minute of the experiment. During the 5th minute of observation, there was a slight decrease in the left ventricular myocardium's minimum relaxation rate to 429.6 ± 102.2 mm Hg/sec. Then the minimum rate of myocardial relaxation began to increase to 559.8 ± 140 mm Hg/sec (p≤0.05) during the 10th minute of the experiment, to 556.5 ± 149.4 mm Hg/sec (p≤0.05) during the 14th minute, up to 589.2 ± 154.2 mm Hg/sec (p≤0.05) during the final 20th minute of the experiment.

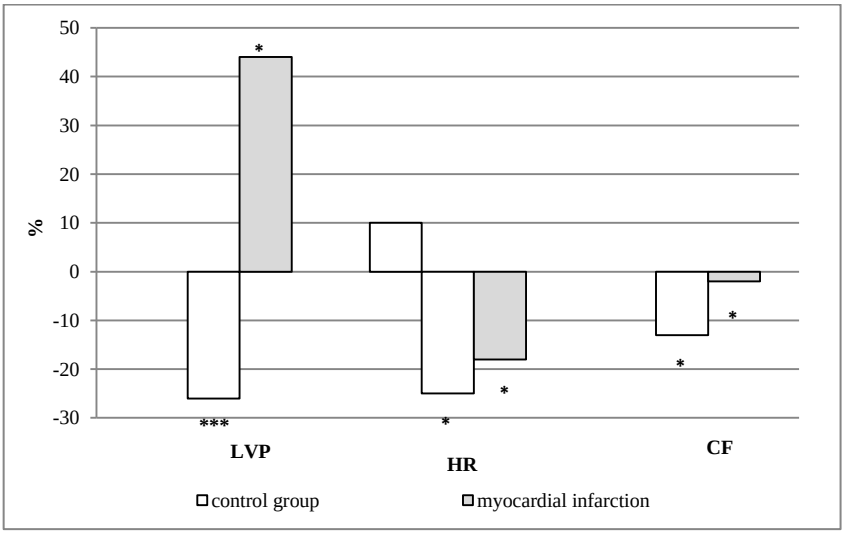

Figure 2. The effect of clonidine hydrochloride (10^{-6} M) on the Langendorff heart's performance in adult rats (control group) and with a model of myocardial infarction. The ordinate axis is the change in values (in%), the abscissa is the performance of an isolated heart - the pressure developed by the left ventricle (LVP), heart rate (HR), CF. Note: the reliability is indicated relative to the initial values: * -p <0.05, *** - p <0.001.

The application of an α_2-AR agonist to the perfused solution caused a decrease in heart rate in a model of myocardial infarction. During the first minute, the heart rate did not change and amounted to 141.6 ± 15.3 bpm. By the 5th minute of the experiment, the heart rate began to slow down to 116.7 ± 11.4 bpm (p≤0.05). During the 10th minute, the heart rate decreased to 112.1 ± 17.5 bpm and continued to decline to 103.5 ± 14.4 bpm (p≤0.05) at the 15th minute of the experiment and to 95.6 ± 12.5 bpm (p≤0.05) at the final minute of observation (Figure 1). The control group of healthy animals showed multidirectional effects of clonidine hydrochloride on heart rate, namely, a 10% increase in one group and a 25% decrease in the other group (Figure 2).

The coronary flow of an isolated heart with a model of myocardial infarction after adding clonidine hydrochloride to the working solution decreased in the first minute of the experiment from 6.2 ± 1 ml/min to 5.9 ± 1 ml/min. By the 5th minute, the CF decreased to 5.2 ± 1.1 ml/min (p≤0.05). Then, by the 10th minute, the CF was 5 ± 1.1 ml/min (p≤0.05). At the 15th minute, the CF did not change and amounted to 5.1 ± 1.1 ml/min (p≤0.05); during the 18th minute, the CF was 5.1 ± 1.1 ml/min (p≤0.05). In the control group, the addition of an α2-AR agonist to the perfusion solution caused a decrease in CF by 13% from the initial values (Figure 2).

After analyzing the data obtained from the isolated heart of rats with a model of myocardial infarction, the amplitude of the pressure wave of the left ventricle after application of the α_2-AR agonist clonidine hydrochloride at a concentration of 10^{-6} M to the perfused solution showed to increase by 44% (p≤0.05) from the initial values. The rate of contraction and the rate of relaxation of the left ventricular myocardium also increased by 20% (p≤0.05) and 37% (p≤0.05), respectively. The activation of α_2-AR in adult rats' isolated heart with a model of myocardial infarction caused a decrease in heart rate by 18% (p≤0.05), and CF by 2% (p≤0.05).

Summary

The activation of α_2-AR causes the opposite effects of LVP in the control group of animals and in rats with

experimental myocardial infarction. The addition of an α_2-AR agonist to the perfused solution resulted in a decrease in heart rate in healthy rats and increased heart rate in the other group. Bradycardia was observed in the heart of rats with a myocardial infarction model. Perfusion with clonidine hydrochloride reduced the blood supply to the isolated heart of healthy animals and had no effect on CF of the heart of rats with a model of myocardial infarction (Figure 2).

CONCLUSIONS

A comparative analysis of the left ventricle pressure's initial values showed this parameter in rats with a model of myocardial infarction to be lower than in the control group ($p \leq 0.05$). Analysis of the heart rate of rats revealed significantly significant differences between the initial values. In the group with a myocardial infarction model, the heart rate is significantly lower ($p \leq 0.05$) compared to the control group (Figure 3). No significant differences were found in the baseline parameters and the effect of clonidine hydrochloride on the coronary flow of an isolated rat heart in the study groups. At the same time, we recorded a significant difference in the effects of activation of the second subtype of α_2-adrenergic receptors in the control group and in the group of animals with experimental myocardial infarction. As the results of our studies show, myocardial infarction practically neutralized the decrease in the coronary duct and reduced the severity of changes in the frequency of contractions of an isolated heart. However, most significantly, myocardial infarction affected the isolated heart's contractile response when stimulated with α_2-AR. The control group had a decrease in the left ventricle pressure, while the study group showed a significant increase in this parameter. The results obtained once again confirm the facts of the ability of α_2-AR to contribute to the development of cardiac pathologies. It is possible that this occurs as a result of gene modifications in the α_2-AR isoforms, which can lead to a change in the interaction of receptors with G-proteins [12].

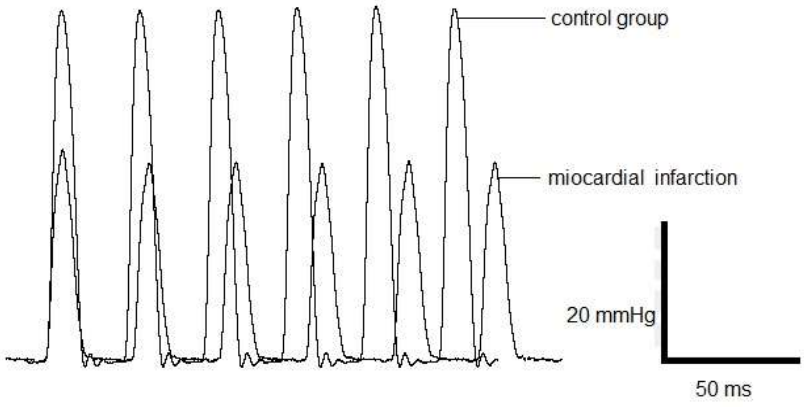

Figure 3. Initial values of left ventricular pressure and heart rate in rats of the control group and with the model of myocardial infarction (original entry).

ACKNOWLEDGEMENTS

This study was prepared in accordance with the Russian state program of competitive growth of Kazan Federal University and supported by the RFBR and Government of the Republic of № 18-44-160022.

Conflict of interests

The author declares that the provided information has no conflicts of interest.

REFERENCES

1. sangy S., Bahaoddini A., Tavassoli A., 2020. Evaluation of Histopathological Changes of Osteoarthritis on the Cardiovascular System by Gavage of Hydro Alcoholic Extract of Wild Anemone (Papaver Rhoeas L.) in Male Rats. Progress in Chemical and Biochemical Research. 3(4), 402-411.

2. Ukwubile C.A., Ikpefan E.O., Bingari M.S., Tam L., 2019. Acute and subchronic toxicity profiles of Melastomastrum capitatum (Vahl) Fern. (Melastomataceae) root aqueous extract in Swiss albino

mice. Progress in Chemical and Biochemical Research. 2(2), 74-83.

3. Khan Muluh E., Odokpe Ugbede A., Tor-Anyin T.A., 2019. Screening of Cassia Sieberiana (Fabaceae) Leaf Extract for In-Vitro Anti-Microbial and Anti-Ulcer Activities. Progress in Chemical and Biochemical Research. 2(3), 143-149.

4. Sangy S., Bahaoddini A., Alsadat Miryousefiata F., 2020. Therapeutic Effects of Hydro-alcoholic Extract of Papaver Rhoeas on Cardiovascular side effect of MIA Induced Osteoarthritis in male Rat. Progress in Chemical and Biochemical Research. 3(4), 340-349.

5. Hassan Zahraee A., 2019. Manufacturing High Quality Medical Catheters: A Technology Mastered by Few. Advanced Journal of Chemistry-Section B. 1(1), 1-2.

6. Lorenz W.,1990. Expression of three alpha 2-adrenergic receptor subtypes in rat tissues: implications for alpha 2 receptor classification. Mol Pharmacol. 38, 599-603.

7. Berkowitz D.E.,1994. Localization of messenger RNA for three distinct alpha 2-adrenergic receptor subtypes in human tissues: Evidence for species heterogeneity and implications for human pharmacology. Anesthesiology. 81, 1235–44.

8. Mariappan R., 2014.Comparing the effects of oral clonidine premedication with intraoperative dexmedetomidine infusion on anesthetic requirement and recovery from anesthesia in patients undergoing major spine surgery. Neurosurg Anesthesiol. 26(3), 192–197.

9. Nahrendorf M.,2001. Serial cine-magnetic resonance imaging of left ventricular remodeling after myocardial infarction in rats. J Magn Reson Imaging. 14(5), 547-555.

10. Gilsbach R., 2010. Sympathetic alpha2-adrenoceptors prevent cardiac hypertrophy and fibrosis in mice at baseline but not after chronic pressure overload. Cardiovasc Res. 86(3), 432–442.

11. Zefirov T.L., 2011. Comparative Analysis of the Impact of α1- and α2-Adrenoreceptor Blockade on Cardiac Function in Rats during Postnatal Ontogeny. Bul Exp Biol Med. 151(6), 664-666.

12. Ziyatdinova N.I., 2018. Effect of α2-Adrenoceptor Stimulation on Functional Parameters of Langendorff-Isolated Rat Heart. Bul Exp Biol Med. 165(5), 593-596.

13. Kuptsova A.M., 2016. Yohimbine influence on myocardium contractile activity among newborn rats. Int J of Adv Biotech Res. 7(4), 1305-1309.

14. Gilsbach R., 2012. Are the pharmacology and physiology of α2-adrenoceptors determined by α2-heteroreceptors and autoreceptors respectively? Br J Pharmacol. 165(1), 90-102.

15. Arekhi M., & Jamshidi M. 2018. Influences of inorganic binder on photocatalytic oxidation (PCO) and degradation of nano/micro TiO_2 containing acrylic composites. Progress in Organic Coatings. 115, 1-8.

16. Korotaeva Iu V., 2015. Alpha2-adrenergic receptors of the myocardium (Literature review). News of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences. 2(22), 57-64.

17. Zefirov T.L.,2014. Effect of α2-Adrenoceptor Stimulation on Cardiac Activity in Rats. BulExp Biol Med. 157(2), 194-197.

18. Pimenov O.I., 2019. Myocardial α2-adrenergic receptors prevent cardiac hypertrophy under increased angiotensin II. Collection: Receptors and intracellular signaling, ed. V.P. Zinchenko, A.V. Berezhnova. 268-273.

19. Arekhi M., Terry L. G., John G.F., Al-Khayat J. A., Castillo A.B., Vethamony P., & Clement T.P. 2020. Field and laboratory investigation of tarmat deposits found on Ras Rakan Island and northern beaches of Qatar. Science of The Total Environment. 735, 139516.

20. Dzidzhikhiia K.M.,2012. Obtaining a model of large-focal myocardial infarction in an acute experiment on rats. Fundamental Research. 4, 270-273.

21. Ershov K.I.,2014. Modification of the myocardial ischemia/reperfusion method. Medicine and Education in Siberia. 3, 62.

22. Selyt H.,1960. Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology. 11, 398-408.

23. Hosseinpour J., & Mahdavy-Moghaddam H., 2020. Computational study of magnetic field effects on the nozzle of hydrogen micro flame. Combustion and Flame. 220, 247-256.