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Dose-Dependent Influence Of Clonidine Hydrochloride To The 6 Week Old Rats Isolated Heart Activity.

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ABSTRACT

Adrenoreceptors (AR) are the most common receptors in the human body. AR regulate blood pressure, secretion, metabolism, muscle contraction and, undoubtedly, are attractive targets for research. α_2-AR are located in the smooth muscle cells of the vessels, on the presynaptic membrane of adrenergic fibers, on the postsynaptic membrane of myocardiocytes, but the value of α_2-AR in the heart of man and animals is the subject of numerous discussions. According to the literature, stimulation of α_2-AR causes multidirectional inotropic and chronotropic effects. Clonidine hydrochloride is an agonist of all α_2-AR subtypes. The aim of this work was to study the effect of clonidine hydrochloride (10^{-8}-10^{-6} M) on inotropy, chronotropy, and coronary flow of the isolated Langendorf heart of 6 week old rats. Ex vivo experiments were performed on an isolated heart at the Langendorf plant. During the study, heart rate, pressure developed in the left ventricle and coronary duct were counted. It was found that the α_2-AR agonist increased the pressure developed by the left ventricle at a concentration of 10^{-9} M and decreased in concentrations of 10^{-8}-10^{-6} M. The stimulation of α_2-AR caused a decrease in the heart rate and reduced the coronary duct of the isolated heart of 6 week animals.

Keywords: alpha$_2$-adrenergic receptors, isolated heart, pressure developed by the left ventricle, heart rate, coronary duct, rat.

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INTRODUCTION

The activity of the cardiovascular system is under the control of catechol amines acting on the adrenoreceptors (AR) of cardiomyocytes and ensuring the optimal functioning of the human heart and animals [1, 2]. AR are most often found in the body, and when they are activated, multiple functional responses are observed [3]. AR regulate blood pressure, secretion, metabolism, muscle contraction and, undoubtedly, are attractive targets for research [4] and, as a result, attractive objects of therapeutic value for the treatment of a large number of diseases.

It is known about the presence of nine subtypes of AR: α1A-, α1B-, α1D-, α2A-, α2B-, α2C-, β1-, β2- and β3-AR [4].

Regulatory effects on the heart are carried out with the participation of presynaptic α_2-AR, modulating the release of noradrenalin [5]. α2-AR are located in the smooth muscle cells of the vessels, on the presynaptic membrane of adrenergic fibers, on the postsynaptic membrane of cardiomyocytes [6]. However, the subject of a large number of studies remains the question of the presence and significance of these receptors in the heart of humans and mammals.

According to the literature, the change in the heart rate (HR) with the activation of α_2-AR may be different. According to one data, the isolation of norepinephrine from the endings of sympathetic neurons activates α_2-AR, which lead to bradycardia [7]. Guth B. et al. found tachycardia in dogs in response to the action of idazoxan, an α_2-AR blocker [8]. It has been shown that, the antagonist α_2-AR yohimbine reduces the heart rate in 1- and 3-week rats, and does not change it in 6- and 20-week-old rats [9]. Mariappan R. et al. reported that in patients taking agonists α_2-AR clonidine and dexmedetomidine heart rate did not change [10]. Perhaps the absence of changes in the dynamics of heart rate is associated with the involvement of different α_2-AR subtypes or the level of norepinephrine release from presynapse.

In studies on the effect of stimulation of α_2-AR, a decrease in myocardial contraction force was shown [11].

A group of researchers led by Parker J. and co-authors suggested that a negative inotropic effect may be associated with a decrease in the amount of isolated noradrenaline from presynapse upon activation of presynaptic α_2-AR [12]. In clinical studies, a nonselective α_2-AR agonist clonidine hydrochloride has been shown to lower blood pressure in hypertension [13]. However, the mechanisms of lowering blood pressure, a group of scientists led by Gilsbach R. associated with an increase in the influence of the parasympathetic nervous system on the myocardium [11]. In experiments on isolated hearts of rat fetuses, Porter A. and co-authors showed that the positive inotropic effect caused by the activation of β1- and β_2-AR isoproterenol does not develop with the action of the α_2-AR dexmedetomidine agonist [12]. The agonist α_2-AR medetomidine decreases the minute volume of the blood circulation and, consequently, reduces the contractility of the myocardium of the dog's heart [12]. Only Westby J. and co-authors report an increase in myocardial contractility in the activation of α_2-AR [12].

Since there is a large amount of ambiguous data on the effect of α_2-AR agonists on chronotropy and inotropy of the heart, the relevance of research in this area is increasing. The aim of this study was to study the dose-dependent effect of a non-selective agonist α_2-AR clonidine hydrochloride on contractility, heart rate and blood supply to the isolated heart of 6 week old rats.

METHODS

Experiments ex vivo were performed on white non-native rats of 6 weeks of age. The age of 6 weeks in animals is characterized by a period of completion of the onset of sympathetic innervation of the heart [19] and the period of the onset of puberty when the hormonal status changes.

Experiments to study the effect of the α_2-AR agonist have been performed on an isolated heart in a Power Lab 8/35 (ADinstruments) setup. The rats were intraperitoneally injected with a 25% urethane solution at a dose of 800 mg/kg of animal weight. The anesthetized animal was placed on the operating table and the thorax was opened, the heart was quickly removed and placed in a cold working solution.

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Then the heart was fixed on the cannula and perfused on the Langendorf apparatus with the Krebza-Henseleit solution with a constant hydrostatic pressure of 55-60 mm Hg. The solution was oxygenated with carbogenes and a constant temperature of 37 °C was maintained. As an α_2-AR agonist, clonidine hydrochloride (Sigma) was used in concentrations of 10^{-9}-10^{-6} mol. The contractile activity of the left ventricular myocardium was measured with a latex balloon injected through the hole behind the left eye, using the MLT844 pressure sensor (ADinstruments, Australia).

With the help of the program LabChart Pro the heart rate (HR), pressure developed by the left ventricle (PDLV) and coronary duct (CD) were calculated. Statistical processing of the results was carried out (Student's t-test).

RESULTS

By the 10-th minute of the experiment, after the addition of the α_2 -adrenoceptor agonist at a concentration of 10^{-9} M PDLV, it increased. At the end of the 15-th minute, PDLV increased from 16.9 ± 2.2 mm Hg. 19.9 ± 2.4 mm Hg (p≤0.05) (Fig. 1), the increase was 18% of the initial. The heart rate after perfusion of the isolated heart with clonidine hydrochloride decreased from 178.9 ± 25.3 bpm to 138.2 ± 10.7 bpm (p≤0.05) for 6 minutes of observation. At the last minute of the experiment the heart rate decreased to 119.1 ± 8.1 bpm (p≤0.01) (Fig. 2). Decrease was 34%. The coronary duct of the isolated heart of 6 week old animals decreased from 9.5 ± 1.1 ml/min to 8.7 ± 1.0 ml/min (p≤0.01) by the 10-th minute of the experiment. The maximum decrease was 9% - 8.6 ± 1.0 ml/min (p≤0.01) (Fig. 3) at the last minute of observation.

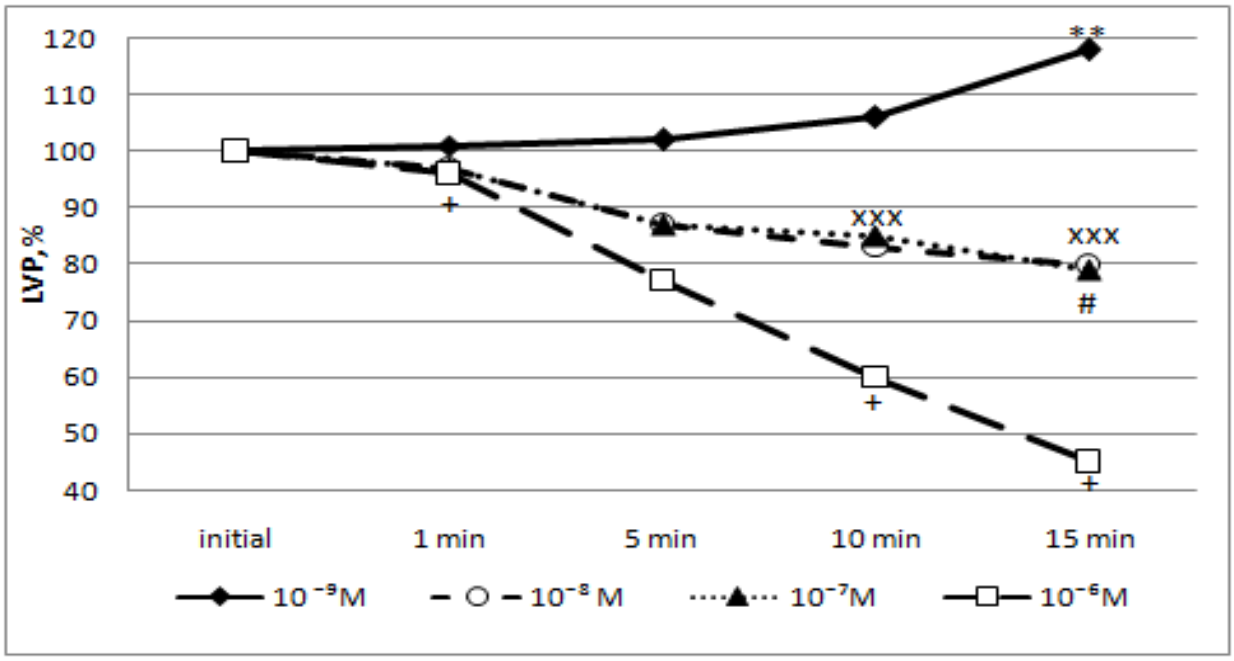

Fig 1: Effect of clonidine hydrochloride on PDLV in the isolated heart of 6 week old rats.

The ordinate axis is the pressure developed by the left ventricle (PDLV) (%), the abscissa axis is the time of recording the experiment (minutes).

Note: the reliability is indicated in comparison with the initial values: # + - p <0,05; ** - p <0.01; xxx - p <0.001.

* - for a concentration of 10^{-9} M; x - for a concentration of 10^{-8} M; # - for a concentration of 10^{-7} M; + - for a concentration of 10^{-6} M.

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The introduction of clonidine hydrochloride in the perfusion solution at a concentration of 10^{-8} M to 10 minutes of the experiment reduced the pressure developed by the left ventricle from 51.54 ± 7.59 mm Hg. up to 42.35 ± 7.08 mm Hg. (p≤0.001). Further, PDLV decreased to 41.3 ± 7.1 mm Hg. (p≤0,001) (Fig. 1). Reduction of PDLV was 20%. The maximum decrease in heart rate of an isolated heart was observed at 10 minutes of the experiment from 285.7 ± 36.7 bpm to 241.7 ± 26.8 bpm (p≤0.01) (Fig. 2), which was 16%. The coronary duct of an isolated heart with agonist perfusion decreased from 3.4 ± 1.1 ml/min to 2.6 ± 0.9 ml/min (p≤0.05) (Fig. 3) by the 15th minute. The decrease was 24% of the initial value.

When a clonidine hydrochloride agonist was added to the working solution at a concentration of 10-7 M to 15 minutes, PDLV decreased from 47.1 ± 9.1 mm Hg. up to 37.4 ± 7.3 mm Hg. (p≤0,05) (рис.1), this decrease was 21%.

The heart rate of an isolated heart after the addition of an α_2-AR agonist to the working solution decreased from 233.7 ± 15.8 bpm to 219.7 ± 12.7 bpm (p≤0.05) at 5 minutes of the experiment. By the 15-th minute, the heart rate was reduced to 199.4 ± 17.7 bpm (p≤0.01) (Fig. 2). Bradycardia was 15%. In the course of the experiment, the coronary duct decreased from 2.6 ± 0.4 ml/min to 1.91 ± 0.3 ml/min (p≤0.05) by 5 minutes of observation. By the final minute of observation, the coronary duct decreased to 1.7 ± 0.3 ml/min (p≤0.05) (Fig. 3). This decrease was 35%.

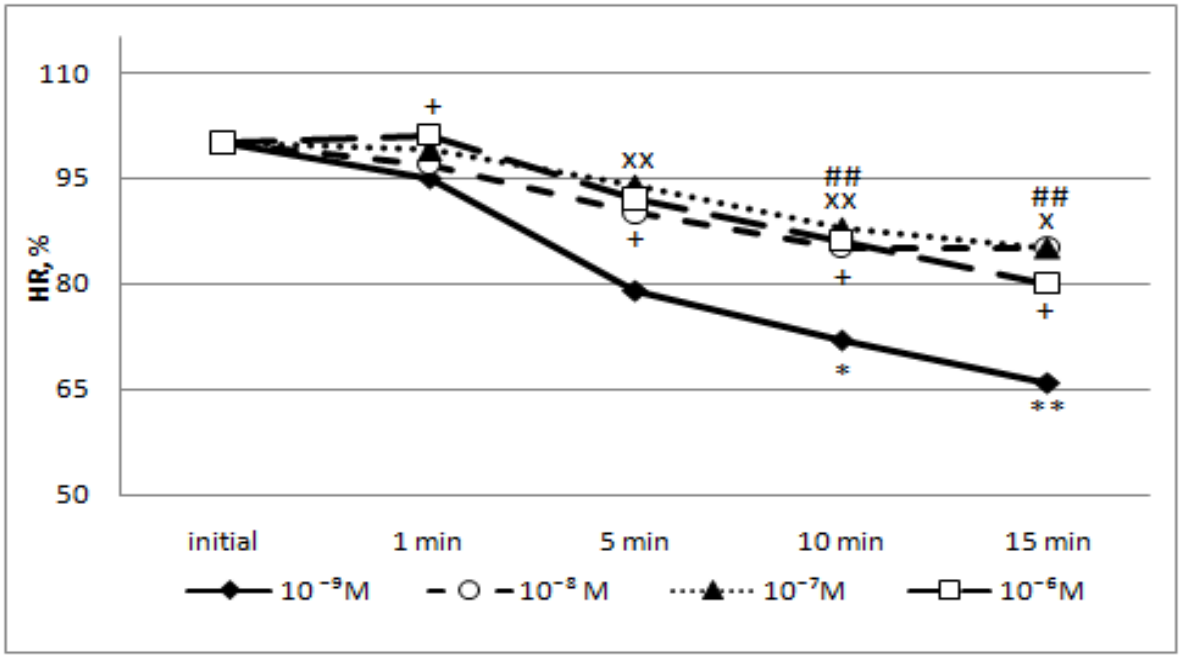

Fig 2: Effect of clonidine hydrochloride on the heart rate in the isolated heart of 6 week old rats.

The ordinate axis is HR (%), the abscissa axis is the recording time of the experiment (minutes).

Note: the reliability is indicated in comparison with the initial values: * + - p <0,05; ** ## xx - p <0.01

* - for a concentration of 10^{-9} M; x - for a concentration of 10^{-8} M; # - for a concentration of 10^{-7} M; + - for a concentration of 10^{-6} M.

Perfusion with an isolated cardiac agonist at a concentration of 10^{-6} M reduced the pressure developed by the left ventricle by 53% (p≤0.05) (Fig. 1). The addition of the α_2-AR agonist resulted in a decrease in the heart rate of 6 week-old rats from 210.2 ± 16.9 bpm to 184.5 ± 12.7 bpm (p≤0.05) by the 8th minute of the experiment.

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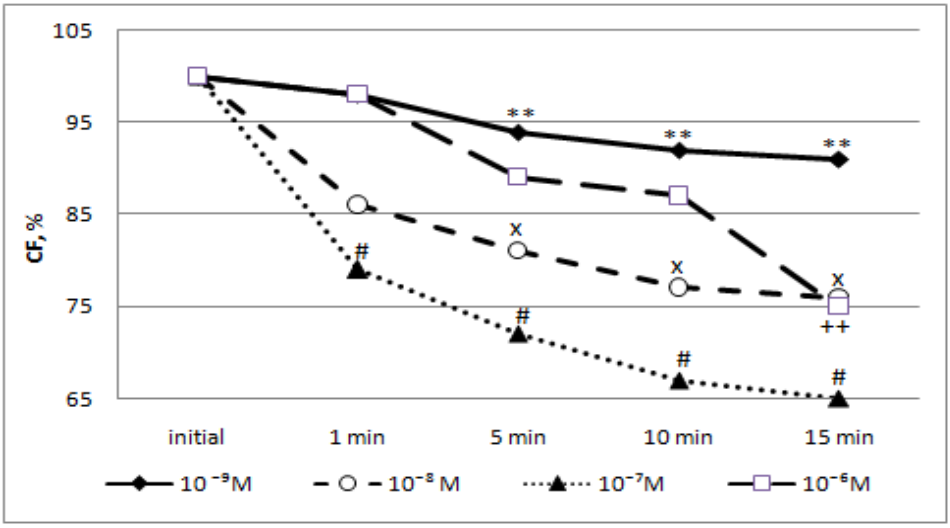

Fig 3: Effect of clonidine hydrochloride on the coronary duct in the isolated heart of 6 week old rats.

The ordinate axis is the coronary duct (CD) (%), the abscissa axis is the recording time of the experiment (minutes).

Note: the reliability is indicated in comparison with the initial values: # + - p <0,05; ** ++ - p <0.01.
* - p <0.05 for a concentration of 10^{-9} M; x - for a concentration of 10^{-8} M; # - for a concentration of 10^{-7} M; + - for a concentration of 10^{-6} M.

The heart rate of the isolated heart decreased to 167.1 ± 13.8 bpm (p≤0.05) by 15 minutes (Fig. 2) and was 21%. The coronary duct of the isolated heart of 6 week old animals decreased from 1.9 ± 0.2 ml/min to 1.6 ± 0.3 ml/min (p≤0.05) by the 13-th minute of the experiment. At the final minute of observation, the CD decreased to 1.5 ± 0.3 ml/min (p≤0.05) (Fig. 3) - 25%.

SUMMARY

Based on the results of the study, it can be concluded that stimulation of α_2-AR by clonidine hydrochloride in all the concentrations studied by us caused a decrease in cardiac activity and reduced the coronary duct of the isolated heart of 6 week old animals.

The α_2-adrenoreceptor agonist clonidine hydrochloride increased the pressure developed by the left ventricle at a concentration of 10^{-9} M, and decreased in concentrations of 10^{-8}-10^{-6} M.

CONCLUSION

After analyzing the results obtained with stimulation of α_2-AR on the parameters of the isolated heart, during the period when the sympathetic innervation of the heart is complete and the initial period of pubertal development there is a change in all the functions of the heart studied. Previously, we showed that a nonselective α_2-AR agonist clonidine hydrochloride lowered the heart rate in in vivo experiments [15], and reduced inotropy of isolated myocardium strips in various parts of the heart of adult rats in in vitro studies [15]. In experiments on an isolated heart, we received multidirectional inotropic effects. Clonidine in a concentration of 10^{-9} M increased the pressure developed by the left ventricle, the remaining concentrations of the agonist reduced the pressure developed by the left ventricle. The maximum decrease in pressure in the cavity of the left ventricle was observed with a maximum perfusion of the heart (10^{-6} M). In experiments with adult animals having a system of regulation of the cardiovascular system, the α_2-AR agonist reduced contractility in all concentrations studied [16]. Stimulation of α_2-AR led to a decrease in the heart rate and a decrease in the coronary duct of the isolated heart of 6 week-old rats. It is possible that stimulation of this subtype of adrenoreceptors leads to the activation of inhibitory G-proteins and, as a consequence, to

bradycardia and a negative inotropic effect. It is possible that this age group has features of regulation of the cardiovascular system in connection with the onset of puberty development of the body.

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REFERENCES

[1] Protas L. Neuropeptide Y is an essential in vivo developmental regulator of cardiac ICa,L / L. Protas, A. Barbuti, J. Qu et al. // Circ Res. – 2003. – V. 93. – P. 972 – 979.

[2] Hongo M. Age-related effects of dexmedetomidine on myocardial contraction and coronary circulation in isolated guinea pig hearts / M. Hongo, S. Fujisawa, T. Adachi et al. // J Pharmacol Sci. -2016. - V. 131(2). - P. 118-125.

[3] Mannelli L. α2 Adrenoceptor: a Target for Neuropathic Pain Treatment / L. Mannelli, L. Micheli, L. Crocetti, MP. Giovannoni, C. Vergelli, C. Ghelardini // Mini Rev Med Chem. – 2017. – V. 17 (2). – P. 95-107.

[4] Brodde O.E. Cardiac adrenoceptors: physiological and pathophysiological relevance/ O.E. Brodde, H. Bruck, K. Leineweber // J Pharmacol Sci. - 2006. - V. 100(5). - P. 323-337.

[5] Dudek M.A comparison of the anorectic effect and safety of the alpha2-adrenoceptor ligands guanfacine and yohimbine in rats with diet-induced obesity / M.A. Dudek, J. Knutelska, B. Mednarski, L. Nowinski, M. Zygmunt, B. Mordyl et al. // PLOS ONE. - 2015. - V. 10. - P. 1327-1371.

[6] Maltsev A.V. Alpha-2 adrenoceptors and imidazoline receptors in cardiomyocytes mediate counterbalancing effect of agmatine on NO synthesis and intracellular calcium handling / A.V. Maltsev, Y.M. Kokoz , E.V. Evdokimovskii, O.Y. Pimenov, S. Reyes, A.E. Alekseev // J. Mol. Cell Cardiol. – 2014. - V. 68. - P. 66-74.

[7] Gilsbach R. Sympathetic alpha2-adrenoceptors prevent cardiac hypertrophy and fibrosis in mice at baseline but not after chronic pressure overload / R. Gilsbach, J. Schneider, A. Lother et al. // Cardiovasc Res. - 2010. - Vol. 86(3). - P. 432–442.

[8] Guth B. Alpha-adrenergic regulation of myocardial performance in the exercising dog: evidence for both presynaptic alpha1- and alpha2-adrenoceptors / B. Guth, E. Thaulow, G. Heusch et al. // Basic. Res Cardiol. - 1990. - Vol. 85(1). - P. 131–141.

[9] Zefirov T.L. Comparative Analysis of the Impact of α1- and α2-Adrenoreceptor Blockade on Cardiac Function in Rats during Postnatal Ontogeny / T.L. Zefirov, N.I. Ziatdinova, L. I. Khisamieva, and A.L. Zefirov // Bul. Exp. Biol. Med. – 2011. - Vol. 151(6). P. 664-666.

[10] Mariappan R. Comparing the effects of oral clonidine premedication with intraoperative dexmedetomidine infusion on anesthetic requirement and recovery from anesthesia in patients undergoing major spine surgery / R. Mariappan, H. Ashokkumar, B. Kuppuswamy // Neurosurg Anesthesiol. - 2014. - Vol. 26(3). - P. 192–197.

[11] Gilsbach R. Are the pharmacology and physiology of α2- adrenoceptors determined by α2-heteroreceptors and autoreceptors respectively? / R. Gilsbach, L. Hein // Br. J. Pharmacol. – 2012. - Vol. 165(1). - P. 90-102.

[12] Korotaeva Yu.V. α_2-adrenoreceptors of the myocardium (Review of the literature) / Yu.V. Korotaeva, V.I. Tsirkin // Proceedings of the Komi Science Center, Ural Branch of the Russian Academy of Sciences. Issue 2 (22). Syktyvkar. - 2015.-p. 57-64.

[13] Shishkina G.T. Subtype of specific clinically important effects of alpha2-adrenergic receptors / G.T.Shishkina, N.N. Gypsy // Successes phyziol. sciences -2002. - T. 33. № 2.-C.30-40.

[14] Robinson R.B. Autonomic receptor–effector coupling during postnatal development / R.B. Robinson // Cardiovasc Res. – 1996. – V.31. – P. 68 – 76.

[15] Zefirov T.L. Effect of α2-Adrenoceptor Stimulation on Cardiac Activity in Rats / Zefirov T.L., Ziyatdinova N.I., Khisamieva L.I., Zefirov A.L. // Bul. Exp. Biol. Med., - 2014. - Vol. 157(2). - p. 194-197.

[16] Ziyatdinova N.I. Effect of stimulation of α_2-adrenoreceptors on the performance of a rat isolated from Langendorf / N.I. Ziyatdinova, A.M. Kuptsova, L.I. Fashutdinov, A.L. Zefirov, T.L. Zefirov // Bul. expert. Biol. med. - 2018. - Volume 165. - № 5. - P. 532-535.