As a manuscript

Khabibrakhmanov Insaf Ilkhamovich

The role of α1-adrenoreceptors in the regulation of heart function in early postnatal ontogenesis rats

03.03.01 - physiology

Abstract of the thesis
on competition of a scientific degree
of candidate of biological sciences.

Kazan – 2017

The work was performed at the Department of Human Health Protection of the Institute of Fundamental Medicine and Biology of the "Kazan (Volga region) Federal University", Kazan

Scientific adviser: **Ziyatdinova Nafisa Ilgizovna** Doctor of Biological Sciences, Associate Professor

Official opponents: **Maslyukov Pyotr Mikhailovich** Doctor of Medicine, Professor, Head of the Department of Normal Physiology with Biophysics at the "Yaroslavl State Medical University" of the Ministry of Healthcare of the Russian Federation, Yaroslavl

Karimova Rufia Gabdelhaevna Doctor of Biological Sciences, Associate Professor, Head of the Department of Physiology and Pathological Physiology at the "Kazan State Academy of Veterinary Medicine named after NE Bauman", Kazan

Leading organization: Federal State Budgetary Educational Institution of Higher Education «Samara State Medical University» of the Ministry of Healthcare of the Russian Federation, Samara

Dissertation defense will be held on 26 January 2018 in ____ hours at the meeting of the dissertation Council D 212.081.36 of the "Kazan (Volga region) Federal University" address: 420015, Kazan, Karl Marx st., 74, Institute of fundamental medicine and biology, aud. 205.

With the thesis can be found in the Scientific library named after N. Lobachevsky at Kazan (Volga region) Federal University, 420008, Kazan, Kremlyovskaya st., 35.

Abstract of thesis sent out «___» _____ 2017.

Scientific Secretary of the dissertation council
Doctor of Biological Sciences, Professor  Z.I. Abramova

GENERAL DESCRIPTION OF WORK

Relevance of the topic. Extracardiac nerve influences are carried out by the sympathetic and parasympathetic divisions of the autonomic nervous system, through adrenergic and cholinergic heart receptors [Sitdikov, FG, 1974; Zefirov, T.L., 1999; Anikina T.A. et al., 2013; Chinkin A.S., 1988; Abramochkin D.V., Sukhova G.S., 2009; Nigmatullina R.R. et al., 1999a; Tarasova, O.S., 2005; Barbuti A. et al., 2009; Jensen, V.C. et. al., 2011; Robinins R.B. et al., 2003; Wedawen N. et al., 2014]. Currently, it is known that there are ten subtypes of adrenoreceptors (AR): α1A-, α1B-, α1D-, α2A-, α2B-, α2C-, β1-, β2- and β3-AR [Brodde O.E. et al., 2006]. The main regulators in the heart are β1-AR and M2-cholinergic receptors.

α1-AR in the heart make up about 10 % of the total number of adrenergic receptors. In rats, all three α1-AP subtypes are found in cardiomyocytes [Bylund D.B. et al., 1998; Luther, H. et al, 2001; Yasutake M, Avkiran M., 1995], and in human and mouse cardiomyocytes only α1A- and α1B-AR are localized [O'Connell T.D. et al., 2003; Jensen B.C. et al., 2009c]. α1D-AR are present in endotheliocytes and smooth muscle cells of the coronary arteries and are involved in the regulation of the tone of these vessels [Jensen B.C. et al., 2009b]. In the heart of rats, during the first 2 weeks after birth, α1-AR increases significantly, while α2-AR decreases during the first week, and then remain at a low level [Metz L.D., et al., 1996]. Despite their low level in the heart, α1-AR are involved in a wide range of functions. According to the results of research by scientists, stimulation of α1-AR causes both positive and negative effects on the chronotropic and inotropic functions of the mammalian heart [Endoh M., 1996; El Amrani A. et al., 1990; Chu C. et al., 2013; O'Connell T.D. et al., 2014; Turnbull L. et al., 2003; Skomedal T. et al., 1997]. In heart failure, as a rule, an increased activity of the sympathetic system is observed, the effects of β1-AR are suppressed, the effects of α1-AR do not change or slightly increase. This leads to an increase in the coefficient α1-AR / β1-AR. α1-AR may assume the role of a secondary inotropic system. Selective α1A-AR activation protects against heart failure in a mouse model [Jensen B.C. et al., 2014]. In addition, a selective agonist - A61603, through stimulation of α1A-AR, prevents cardiomyopathy in male mice, supporting the theory that α1A-AR agonists have the potential to be new types of treatment for heart failure [Montgomery M.D. et al., 2017]. The existing inconsistency of data on the time of formation and features of extracardiac nerve influences on the heart, as well as the lack of complete information about the involvement of α1-AR regulatory processes in these processes predetermine the relevance of research in this direction.

Aim of the study. The aim of our work is to study the role of different α1-adrenoreceptor subtypes in the regulation of rat heart at different stages of postnatal development.

Research objectives

1. To investigate the effect of non-selective stimulation of α1-adrenoreceptors with methoxamine on the parameters of the heart work of 1, 3, 6, 20 week old rats.
2. To study the effects of stimulation of α1A-adrenergic receptors by selective agonist A-61603 on the cardiac performance of rats of 1, 3, 6, 20 weeks of age.
3. To identify the effects of selective blockade of different α1-adrenoreceptor subtypes on the cardiac performance of rats of 1, 3, 6, 20 weeks of age.
4. Determine the effect of methoxamine after the selective blockade of α1A-AR with WB 4101 on the performance of the heart of rats 1, 3, 6, 20 weeks of age.
5. Investigate the effect of methoxamine after selective blockade of α1B-AR with chloroethyl clonidine on the cardiac performance of rats of 1, 3, 6, 20 weeks of age.
6. To study the effect of methoxamine after selective blockade of α1D-AR with BMY7378 on the performance of the heart of 1, 3, 6, 20 week old rats.

Scientific novelty. In this paper, for the first time using various research methods, it was shown that non-selective stimulation of α1-AR by methoxamine leads to a change in the cardiac activity of rats of 1, 3, 6, and 20 weeks of age. In all the animal age groups studied by us, methoxamine caused a decrease in the strength of the contraction of the Langendorf-isolated heart and right atrial myocardium strips and the right ventricle. It was revealed that methoxamine (10^{-8}M) reduces the frequency of contractions of an isolated heart in 20-, 6-, 3-week-old rats and does not cause changes in heart rate in newborn rats. Stimulation of α1-AR reduces the coronary duct of the heart in 20-week-olds and increases in 1-week-old animals. For the first time, it has been shown that selective stimulation of α1A-AR with A-61603 does not affect the chronotropy of an isolated heart of adults and newborns in rats, but it significantly alters the heart rate under conditions of the whole organism. The α1A-AR agonist A-61603 does not lead to significant changes in the coronary duct of an isolated rat heart. The results showed that high concentrations of the selective α1A-AR-A-61603 agonist increase contractility of atrial myocardial strips in all studied age groups of rats and ventricular myocardial strips of newborn rat pups, and low concentrations of the drug cause a decrease in their contractility. Studies have shown that the preliminary blockade of each of the three α1-AR subtypes does not relieve the negative inotropic effect of methoxamine in the atrial and ventricular myocardium of all the rat age groups studied. However, pre-blockade of one of the α1-adrenoreceptor subtypes leads to changes in the severity of the negative effect of methoxamine on the contractility of atrial and ventricular strips.

Scientific and practical significance of the work. The data obtained complement the modern understanding of the mechanisms of regulation of the heart. Experiments, with the use of various methodological approaches, expand the understanding of the effect of adrenergic blockers and agonists on the strength and frequency of contractions, as well as on the coronary circulation of the heart of rats during different periods of postnatal ontogenesis. The obtained data should be considered when prescribing α1-adrenoreceptor blockers and agonists as cardioprotective drugs.

The obtained research results are of practical interest for pharmacologists, physiologists, and biochemists who study the effect on the cardiovascular system of various agonists and adrenoreceptor blockers using rats as experimental animals. Research material also deserves attention from specialists in age and normal physiology, cardiology and pediatrics.

Provisions for protection

1. Selective blockade of different α1 – AR subtypes inhibits the frequency and force of contraction of the heart of rats at all stages of early postnatal ontogenesis.
2. Stimulation of α1 – adrenoreceptors does not affect the chronotropy of the isolated heart of newborn rats.
3. α1-adrenergic regulation of the coronary flow of the hearts of the rats has a pronounced age-related features. Non-selective stimulation of α1-AR leads to narrowing in 20-week rats, and in 1-week rats to dilation of coronary vessels of the isolated heart.

Personal contribution of the author to the research. Experimental data obtained with the personal participation of the author at all stages of work including the organization and conduct of experiments, analysis of experimental data and theoretical generalization of the results of the study. The work was carried out in a complex, on a sufficient quantitative statistical material of research.

Approbation of work. The dissertation materials are presented at the All-Russian Scientific Conference "Theory and Practice of Physical Culture and Sports" (Kazan, 2014); XII International School-Conference "Adaptation of a Growing Body" Kazan-Yalchik, 2014; The final scientific and educational conference of students of the Kazan Federal University (Kazan, 2015); The VI All-Russian International School Conference on the Physiology of Blood Circulation (Moscow, 2016); XIII International School-Conference "Adaptation of the developing organism" (Kazan, 2016); All-Russian scientific-practical conference with international participation dedicated to the 150th anniversary of A.F. Samoilova: “Fundamental and clinical heart electrophysiology. Topical issues of arrhythmology”(Kazan, 2017); XXIII Congress of the Physiological Society. I.P. Pavlova (Voronezh, 2017).

Publications. The author has published 23 printed scientific papers, including 6 articles in scientific peer-reviewed journals.

The structure and scope of the thesis. The dissertation is presented on 196 pages of typewritten text and consists of an introduction, review of literature, description of materials and research methods, research results and discussion, conclusions, conclusions and references, including 297 titles, including 48 domestic and 249 foreign literary sources. The work is illustrated with 17 tables and 34 figures.

List of abbreviations. AR - adrenoreceptors, HR - heart rate, LVP - left ventricular pressure, CF - coronary flow, ChEC - chloroethylclonidine, M - mol, F - force of contraction, g - gram, n - number of animals.

THE CONTENT OF THE WORK

Materials and methods of research

For the experiments, white mongrel outbred rats aged 1, 3, 6, 20 weeks were used. The paper presents data obtained on 370 animals. As anesthesia, a 25% urethane solution was used, which was administered intraperitoneally in the amount of 800 mg / kg of animal weight.

The contractile activity of the myocardium in an in vitro experiment was studied on atrial and ventricular strips on an MP-150 unit (BIOPAC Systems, USA). After stabilization of the isometric contraction of the myocardial strips for 3 minutes, the initial contractility values were recorded, then 20 minutes with the addition of pharmacological preparations to the working solution. Expected reaction force reduction in response to the action of pharmacological substances relative to the original values. The force of contraction (F) was expressed in grams (g). The data were recorded and processed on a personal computer using software: AcKnowledge 4.1 and OriginPro 8.0.

The study of the performance of an isolated rat heart was carried out on a Langendorf installation (ADinstruments, Australia). The heart was quickly removed and, in order to completely stop the contractions, they were placed in a cold solution (0–2°C) of Krebs – Henseleit (KH). The perfusion was carried out according to the standard Langendorff method [Langendorff O., 1895; Lopukhin Yu.M., 1971], oxygenated by carbogen solution of KH at 37 ° C and constant pressure of 60-65 mm Hg. To study the pharmacological effects, the frequency of isolated heart rate (HR), left ventricular pressure (LVP) and the coronary flow (CF) were recorded. LVP was expressed in mm Hg., HR in beats / min, CF in ml / min. Due to the lack of registration of the contractile activity of the heart using a balloon in 3 and 1-week-old rats, an electrogram of the heart was recorded during the experiment. Signals were recorded on a computer via PowerLab 8/35 (ADinstruments, Australia) using the LabChartPro program (version v8, Australia).

In in vivo experiments throughout the experiment, an electrocardiogram was continuously recorded in rats. ECG registration was performed using the PowerLab 8/35 installation

(ADinstruments, Australia). Pharmacological substances using insulin syringes were smoothly injected into the right femoral vein and changes in heart rate were recorded for 30 minutes. Data from the installation was recorded on a computer and analyzed using the software "LabChartProv8".

The average of the measured value and standard error of mean M+SEM were obtained upon statistical processing. The significance of differences of average values was tested using Student's t-test. Differences were considered statistically significant at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

CONCLUSIONS

1. Non-selective stimulation of α1–AR by methoxamine reduces the force of contraction of atrial and ventricular myocardium in all age groups of rats in a dose-dependent manner.
2. Methoxamine (10^{-8}M) reduces the frequency of contractions of an isolated heart in 20-, 6-, 3-week-old rats and does not cause changes in heart rate in newborn rats. Stimulation of α1-AR reduces the coronary duct of the heart in 20-week-olds and increases in 1, 3, and 6-week-old animals.
3. In vivo, methoxamine (0,1 mg / kg) briefly reduces heart rate in all age groups of animals.
4. In vitro, A-61603 at a concentration of 10^{-6}M increases, and at a concentration of 10^{-9}M, decreases atrial myocardial contractility in all age groups of rats.
5. A-61603 (10^{-9}M) significantly reduces the work of an isolated heart of 6 and 3 week old rats, but does not affect the frequency of contractions of an isolated heart in 1 and 20 week old animals. At the same time, the coronary duct of the isolated heart increases in 20- and 6-week-old rats.
6. In vivo A-61603 (0,001 mg / kg) leads to cardiac bradycardia in all age groups of animals.
7. Selective blockade of all α1–AR subtypes in ex vivo and in vitro experiments leads to a decrease in myocardial inotropy and does not prevent the negative effect of methoxamine on the contractility of rat myocardium in postnatal ontogenesis.
8. Selective blockade of all α1–AR subtypes does not prevent the inhibitory effect of methoxamine on the frequency and force of contraction of an isolated rat heart in postnatal ontogenesis.
9. Selective blockade of all α1–AR subtypes changes the directionality of the effect of methoxamine on the coronary duct in 1, 3, and 6-week-old animals, but not 20-week-old animals.
10. The blockade of α1A-AR causes a decrease in the frequency of contraction of an isolated heart of 20, 6, 3-week-old rats and does not affect the chronotropy of the heart of newborn animals.

11. The α1B-AR blockade leads to the bradycardia of an isolated heart of 6-, 3-, 1-week-old rats and does not affect the heart rate of 20-week-old animals. At the same time, a decrease in the coronary duct was observed only in 1-week-old rats.

12. The blockade of α1D-AR causes a decrease in the work of an isolated heart in 6-, 3-, 1-week-old rats and does not affect the chronotropy of the heart of rats of 20 weeks of age. The blockade of α1D-AR in 20-week-old rats increases the coronary duct of an isolated heart and reduces it in 1-week-old rats.