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REGULATION OF RIGHT VENTRICULAR MYOCARDIAL CONTRACTILITY IN INFANT RATS WITH ALTERED SEROTONIN CONCENTRATION IN THE EMBRYONIC PERIOD OF ONTOGENESIS

of dissertation for the degree of Doctor of Philosophy (PhD) in specialty 6D060700-« Biology»

General description. The given thesis is dedicated to the study of the inotropic function of the right ventricle of pup rats with altered serotonin concentration during the embryonic period of ontogenesis.

Urgency of the research. Recently, significant attention has been directed towards studying the serotonergic system. Serotonin, or 5-hydroxytryptamine (5-HT), is a biogenic monoamine, which is widely distributed in the body. The role of the serotonergic system in the development of cardiovascular diseases is extensively discussed in the global literature. In the central mechanisms of cardiovascular regulatory activity, key roles are played by receptor subtypes: activation of 5-HT_{1A} receptors leads to central inhibition of sympathetic influences and subsequently bradycardia, while 5-HT_2 receptors lead to excitation of the sympathetic division, elevation of arterial pressure, tachycardia, and the development of atrial fibrillations.

The heart development is constantly under the control of serotonin. Heart cells actively accumulate 5-HT throughout the entire developmental process. Serotonin can also be actively transported through the placenta during pregnancy and is later synthesized in the embryonic gut and brain while heart is actively forming. In the culture of rats' embryos, a high level of 5-HT has been found in the myocardium of the developing heart. The serotonin membrane transporter protein (SERT) has been detected in the myocardium of mammals during the early embryonic stage.

Serotonin is a key signaling molecule in cardiac precursor cells, which is involved in the development and differentiation of myocardial cells as well as in the division of cardiac chambers. It has been shown that the serotonin receptor 5-HT_{2B} is critically important during embryogenesis, as knockout of this gene in mice leads to cardiac defects, alterations in the contractile apparatus of the myocardium, reduced ventricular mass, and embryonic lethality. The mechanisms leading to the impaired contractile capacity may involve changes in myofibrillar organization, the structure of the intercalated disc, abnormal arrangement of subepicardial sarcomeres, and absence of trabeculae in the myocardium. Therefore, it has been suggested that intervening in the serotonergic system in the womb could disrupt the normal development of the cardiovascular system in the fetus.

As evidenced by the published studies, some significant attention has been lately paid to the assessment of right ventricular function. It has been demonstrated that the impaired right ventricular function holds independent significance in the context of pulmonary hypertension. The prognosis remains unfavorable in a

considerable number of infants with congenital heart defects complicated by pulmonary arterial hypertension. Therefore, addressing this issue necessitates further research using animal models.

Cardiovascular dysfunction might possibly be influenced by the level of circulating serotonin. It is also a key factor in maintaining normal cardiovascular activity during the embryonic period of development. This opens up new perspectives on utilizing serotonin in the therapy of cardiovascular diseases. The cardiovascular effects of serotonin are complex, and its contribution to myocardial physiological processes remains insufficiently understood, necessitating further research. Consequently, the impact of altering serotonin concentrations on cardiac inotropic function in early postnatal ontogenesis needed to be studied during prenatal development. The study design involves some experimental research using animal models.

Research aim: To study the impact of serotonin synthesis blockade and serotonin membrane transporter inhibition during the embryonic period of ontogenesis on the inotropic function of the right ventricular myocardium in neonatal rats.

Research objectives:

1. To study the impact of serotonin synthesis blockade and serotonin membrane transporter inhibition during the embryonic period of ontogenesis on the inotropic function of the right ventricular myocardium in 7- and 14-day-old pup rats exposed to increasing serotonin concentrations.
2. To examine the impact of serotonin synthesis blockade and serotonin membrane transporter inhibition during the embryonic period of ontogenesis on the inotropic function of the right ventricular myocardium in 7- and 14-day-old pup rats subjected to increasing adrenaline concentrations.
3. To determine the effect of serotonin synthesis blockade and serotonin membrane transporter inhibition during the embryonic period of ontogenesis on the morphology of the right ventricular myocardium in 14-day-old pup rats.
4. To assess the expression of SERT (serotonin transporter) in the platelets of 14-day-old pup rats with serotonin synthesis blockade and serotonin membrane transporter inhibition during the embryonic period of ontogenesis.

Research subjects: Pregnant Wistar rats and their offspring at 7 and 14 days of age. The research is focused on the right ventricle only.

Research methods: The following methods were employed for data collection and analysis: in vitro assessment of myocardial contractility in rats; determination of morphometric changes in the myocardium; determination of serotonin membrane transporter expression using Western-blotting. Statistical analysis was completed by using Microsoft Excel 2019 and Statistica 7.0 with significance differences determined using the t-test. All animal experiments were approved by the local Ethics Committee of the Kazan State Medical University.

Scientific novelty of the obtained results:

This novel research demonstrates the impact of both excess and deficiency of serotonin during the embryonic period of ontogenesis on the inotropic function of

the right ventricular myocardium in early postnatal ontogenesis in 7- and 14-day-old pup rats.

The given research is the first one to study the influence of serotonin and adrenaline on the contractility of the right ventricular myocardium in rats with altered serotonin concentrations during the embryonic period of ontogenesis.

It has been established that alterations in serotonergic regulation during the embryonic period of ontogenesis lead to morphological changes in cardiomyocytes during postnatal ontogenesis.

This novel study also identifies the expression of the serotonin membrane transporter in platelets of rats with both excess and deficiency of serotonin during the embryonic period of ontogenesis.

Theoretical and practical significance:

The theoretical significance of this work lies in the obtained data to confirm that intervention in the serotonergic system during maternal pregnancy results in disturbances in the inotropic function of the fetus. The research results contribute to our knowledge regarding the cardiovascular effects of serotonin and its contribution to the physiological processes of the myocardium.

The studies aimed to identify new aspects of cardiovascular diseases through animal modeling make a valuable contribution to our understanding of the physiology of the cardiovascular system. Therefore, the practical significance of these findings is their ability to develop some deeper understanding of the crucial role of the serotonergic system in the development of conditions such as pulmonary hypertension, heart failure, and myocardial hypertrophy.

The effects of serotonin on the indicators of cardiac inotropic function, such as contraction strength and duration of contraction and relaxation, change with age and significantly vary as sexual maturity is reached. The pharmacological agents capable of altering serotonin metabolism or its receptor’s impacts are used as psychotropic agents; their indiscriminate usage during pregnancy poses a risk of disrupting fetal heart development. Those substances that block the activity of the key enzyme in serotonin synthesis, tryptophan hydroxylase, include para-chloro-phenyl-alanine (pCPA). A substance that inhibits the membrane transporter of serotonin is fluoxetine, which belongs to the group of selective serotonin reuptake inhibitors (SSRIs).

The obtained results expand our existing notions about the role of serotonin in regulating the contractile function of the right ventricular myocardium and its contractility in the early postnatal ontogenesis of rats. The research findings indicate a significant influence of the decreased and increased levels of serotonin during the embryonic period on the functioning of the right ventricular myocardium in the early postnatal ontogeny. The experimental results on the 7 and 14-day-old animals reveal some specific mechanisms in the regulation of the contractility of the right ventricular myocardium by adrenaline and serotonin, mediated by various adrenergic and serotonin receptors, in pup rats with deficits and excesses of serotonin during the embryonic developmental period.

The obtained results are recommended for interpreting the outcomes of physiological and pharmacological studies on the cardiovascular functions of rats

in relation to their age. The research materials can be utilized in educational settings for teaching courses on animal physiology, developmental and normal physiology, pharmacology, and cardiology.

The main dissertation propositions presented for defense are as follows:

1. Altering serotonin concentration during prenatal development diminishes the contractile function of the right ventricular myocardium in neonatal rats.

2. Modifying serotonin concentration during the embryonic period of ontogeny results in adverse morphological changes in the right ventricular myocardium of neonatal rats.

3. Decreasing and increasing serotonin concentration during the embryonic period of ontogeny reduces the expression of the active phosphorylated form of the membrane serotonin transporter in neonatal rats.

Key findings:

- In neonatal rats of 7- and 14-day-old age, which have been developed under conditions of embryonic serotonin deficiency, a decrease in the contractile ability of the right ventricular myocardium was observed. Their responses to increasing serotonin concentrations were reduced if compared to other groups.
- It was found that in 14-day-old rats, serotonin exerts a positive inotropic effect on the right ventricular myocardium across all groups.
- In the experimental group of 7- and 14-day-old rats with serotonin transporter blockade during embryonic ontogeny, compared to the control group and the experimental group of rats with serotonin synthesis blockade during embryonic ontogeny, the least reduction in the total duration of right ventricular myocardial contraction occurred due to a decrease in the time of contraction and relaxation.
- In postnatal ontogenesis of 7- and 14-day-old rats, the myocardial contraction response to increasing concentrations of adrenaline is reduced in the experimental groups compared to the control group.
- In the experimental group of 7-day-old rats exposed to the influence of SSRI fluoxetine (a serotonin excess model) during the early embryonic period of ontogenesis, a decrease in the total contraction time in response to adrenaline action was observed compared to other groups of rats.
- It has been established that in 14-day-old rats, which were exposed to the impact of the tryptophan hydroxylase enzyme blocker para-chloro-phenyl-alanine (a serotonin deficiency model) during the embryonic period of ontogenesis, a shortening of the overall duration of contraction of the right ventricular myocardium was identified compared to other groups.
- Morphological changes in the right ventricular myocardium have been detected in the experimental groups of 14-day-old rats. The primary changes involve alterations in the blood microcirculatory channel, marked interstitial edema with disarray of muscle fibers. Micronecroses of cardiomyocytes are identified in some isolated observations.
- It has been revealed that in 14-day-old rats with altered serotonin concentration during the embryonic period of ontogenesis, the active

phosphorylated form of SERT (serotonin transporter), which is directly involved in the transportation of serotonin from plasma to platelets, is significantly reduced.

Implementation.

The results of the research have been implemented into the educational process at the Department of Physiology of the Biological-Geographical Faculty of NCJSC «Karaganda Buketov University» as methodological recommendations in the following manner: the experimental data are used for conducting laboratory work in the *Cardiovascular System* section. They have also been integrated into the educational process at the Department of Physiology at the NCJSC «Karaganda Medical University» in the following manner: the experimental data are utilized for practical sessions in the following disciplines: *Cardiovascular and Lymphatic System*, *Fluids and Transport module* for the General Medicine specialty covering topics such as *Physiological Properties of Cardiac Muscle* for the Pharmacy specialty and *Physiology of Circulation. Neurohumoral Regulation of Circulation* for the Biomedicine specialty.

Thesis connection to the plans of study.

The work was conducted within the framework of the scientific project titled as *Clinical-physiological Substantiation of an Early Diagnosis Method for Pulmonary Hypertension in Infants* under the project number AP05136034.

Author's contribution.

The author completed the literature review, defined the objectives of the study, conducted research, performed statistical processing and analysis of the findings. The experimental work was conducted in the research laboratories of the Departments of Normal Physiology and General Pathology at the Kazan State Medical University (the city of Kazan, Russia) with the author's personal involvement.

Structure and scope.

The work consists of an introduction, literature review, description of the materials and research methods, three sections containing the results of the author's research, conclusions, and a list of references. The total volume is 133 pages. The references include 288 sources. The work is illustrated with 10 tables and 53 figures. There are 3 appendices.

Presentation and publications.

The main content of the given work is reflected in 14 published works, including 2 articles in the peer-reviewed scientific journals indexed in the Scopus database, and 3 articles recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

A number of works were presented and published in the proceedings of international conferences held in Russia. The primary results were discussed at the *X All-Russian School of Young Scientists* dedicated to the memory of Academician Evgeny Evgenievich Nikolsky, the Satellite Symposium *From Neuron to Brain* (the city of Kazan, 2019), and the conference for young scientists *Current Issues in Developmental Biology* (the city of Moscow, 2021). As a result of the research, a monograph has been published.