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SERT MEMBRANE TRANSPORTER AND 5HT 2B RECEPTORS INVOLVED IN MYOCARDIUM AND AORTA REMODELING OF IMMATURE SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract

It is well-know that the serotonin (5-HT) regulates cardiovascular functions during embryogenesis and postnatal period. The action of 5-HT is realized by different types of receptors, especially by 5-HT_{2B} subtype, and the SERT membrane transporter. The role of 5-HT in arterial hypertension development remains still unclear. We have studied the role of SERT membrane transporter and 5-HT_{2B} receptors in myocardium and aorta remodeling of immature spontaneously hypertensive rats (SHR) at the age of 5-7 weeks.

Methods: A simple weighing of left and right ventricles (LV, RV), intraventricular septum (IVS) and aorta for myocardial hypertrophy evaluation, immunohistochemical (IHC) staining for 5-HT2B receptors and SERT expression revealing, and Van Gieson staining for fibrosis revealing have been performed. Even-aged Wistar-Kyoto rats were used as controls.

Results: Five-week-old male SHRs had myocardial hypertrophy, significant fibrosis and positive IHC reaction on 5-HT_{2B} receptors and SERT. Six-week-old WKRs had a tendency to a decrease in HT2B-immunopositive receptors and SERT cells count, and the SHRs, otherwise, showed increase ($p \leq 0.05$).

Conclusion: SERT membrane transporter and $5HT_{2B}$ receptors are involved in compensatory processes due to increased blood pressure of SHR associated with the development of morpho functional heart defects.

Keywords: SERT membrane transporter, $5HT_{2B}$ receptors, immature spontaneously hypertensive rats, myocardium remodeling

Introduction

It is known that serotonin is involved in the proliferation and differentiation of myocardium cells during embryogenesis [12]. Its effect is realized through the SERT transporter membrane and G-protein-mediated HT_{2B} receptors [7].SERT – is the main element of the mechanism that regulates the serotonin blood level [2]. The membrane transporter is expressed by platelets [2], cardiomyocytes and cardiac valve cells during embryogenesis, and takes part in cardiac embryogenesis [10].

Spontaneously hypertensive rats under the age of 10 weeks are immature and used to study the mechanisms of arterial hypertension (AH) [11].

Objective: to study the role of SERT membrane transporter and 5-HT_{2B} receptors in myocardium and aorta remodeling of immature spontaneously hypertensive rats (SHR) at the age of 5-7 weeks has.

Materials and Methods

The study was conducted on 46 5-7-week-old male rats. The experimental group consisted of 25 spontaneously hypertensive rats, and the control group - 21 Wistar-Kyoto rats. The rats were anesthetized with 30% urethane solution (dose 800 mg/kg). After opening the chest, hearts were isolated and weighed, divided into sections, and weighed successively the left ventricle (LV), the right ventricle (RV), the interventricular septum (IVS) and a part of the ascending aorta 0.5 cm from its beginning. The material was fixed in buffered formalin for 6-24 hours, with further paraffin embedding of specimens according to standard scheme. The preparation of specimens was carried out on the electroadhesive glass.

To assess the development and restructuring of the connective tissue of the heart, the specimen Van Gieson staining was performed. This method of staining results in collagen and elastic fibers becoming red / bright pink color, which allows quantifying their size in the specimen. The micrographs were used to quantify the areas painted in red / bright pink color with the use of image analyzing system ImageJ.

Some sections were used for immunohistochemical detection of active $5HT_{2B}$ receptors and SERT membrane transporter. This analysis was performed with the use of antibodies antiHTR_{2B} (Sigma-Aldrich) / anti-SERT (Sigma-Aldrich) and Novolink visualization system (Leica). Positive immunohistochemical reaction was detected with the use of diaminobenzidine (DAB).

The sections were counterstained with hematoxylin. The results assessment was carried out based on the photomicrographs in multiple fields of view by counting the number of immunopositive cells.

Statistical processing was performed with the determination of the arithmetic mean (M), standard error (m) and standard square deviation σ, and the significance of differences was calculated by Origin8-based ANOVA method. Differences were considered statistically significant at $p \leq 0.05$.

Results

I. The masses of myocardium, its compartments and the aorta in SHRs and Wistar-Kyoto rats at the age of 5-7 weeks.

By week 5, the mass of myocardium and its compartments in the SHRs was significantly higher than that in Wistar-Kyoto rats by 26%, LV - 125%, IVS - 75% RV - by more than 3 times ($p \leq 0.05$).

By week 7, the mass of all myocardium compartments in the control group increased significantly and reached values of the experimental group that have the same values as at the age of 5 weeks. However, the RV mass in the SHRs exceeds significantly the same in Wistar-Kyoto rats by 75% ($p \leq 0.05$). Aortic mass in rats of the experimental group was significantly higher than that in the control rats only at the age of 6 weeks ($p \leq 0.05$) (Figure 1).

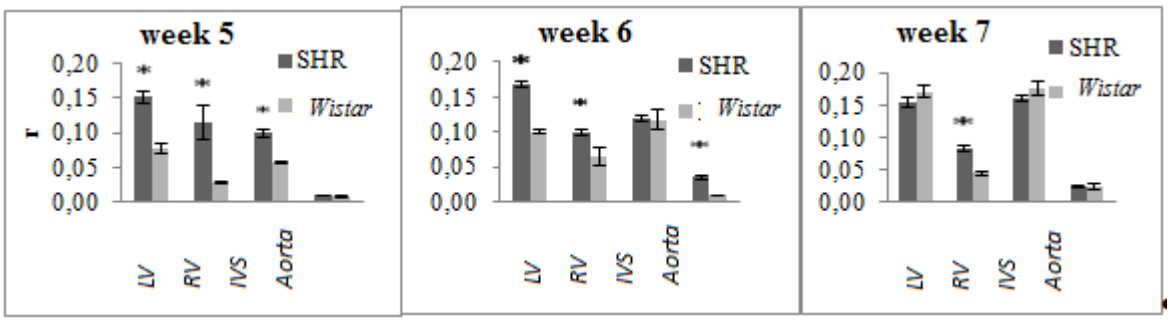

Fig.1. The masses of myocardium compartments and the aorta in SHRs and Wistar-Kyoto rats

Note: horizontal direction - the myocardium compartments: LV - left ventricle, RV - right ventricle, IVS - interventricular septum and aorta; vertical direction - the mass in grams; SHR - SHR-line rats; Wistar - Wistar-Kyoto-line rats;

* - significance of differences $p \leq 0.05$; 5, 6, 7 weeks - weeks of rats' postnatal development.

II. Immunohistochemical reaction to $5HT_{2B}$ receptors and SERT membrane transporter in SHRs and Wistar-Kyoto rats at the age of 5-7 weeks.

Positive immunohistochemical reaction to $5HT_{2B}$ receptors and SERT membrane transporter is determined in the working cardiomyocytes of rats of both groups and detected by the presence of brown staining on the myocardium microslides. Counting is carried out in multiple fields of view.

The number of cardiomyocytes expressing both receptors and SERT membrane transporter in a group of control animals do not differ significantly during week 5 and 7 ($p \leq 0.05$). In the experimental group, the number of cells

expressing SERT and $5HT_{2B}$ receptors at week 7, is higher than at week 5 in the LV - by 3.4 and 1.36 times, in the RV - by 2.6 and 3.7 times, IVS - by 3.7 and 2.5 times, respectively ($p \leq 0.05$).

Up to week 6, the process is unidirectional in both the experimental and control groups, but starting from week 6, the number of cardiomyocytes in Wistar-Kyoto rats having positive immunohistochemical staining for $5HT_{2B}$ receptors decreases, while the same in the SHRs continues increasing (Fig. 2).

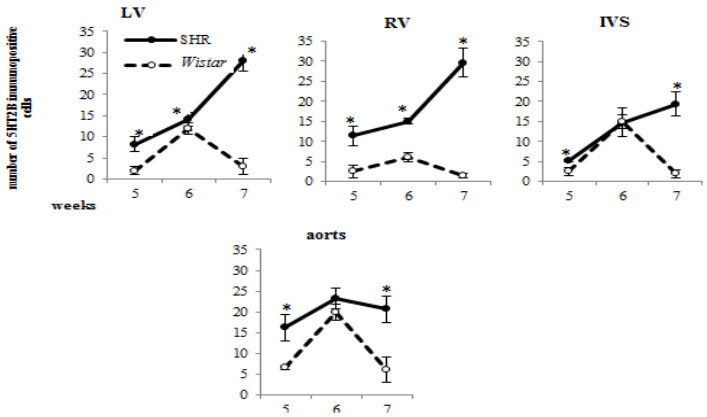

Fig. 2 The number of cardiomyocytes,immunopositively-stained for $5HT_{2B}$ receptor in different regions of the aorta and myocardium in the experimental and control groups of 5-7-week-old rats.

Note: horizontal direction - week 5, 6, 7 of rats' postnatal development; vertical direction - number of immunopositively-stained cells for $5HT_{2B}$ receptor; SHR - SHR-line rats; Wistar - Wistar-Kyoto-line rats; * - significance of differences $p \leq 0.05$.

Expression of SERT membrane transporter in different compartments of the myocardium and aorta at the age of up to 6 weeks is unidirectional in both groups of rats. Starting from week 6, the number of cardiomyocytes with positive immunohistochemical staining in Wistar-Kyoto rats decreases significantly or tends to insignificant growth, for example in LV, while the same in the SHRs continues increasing everywhere (Fig. 3).

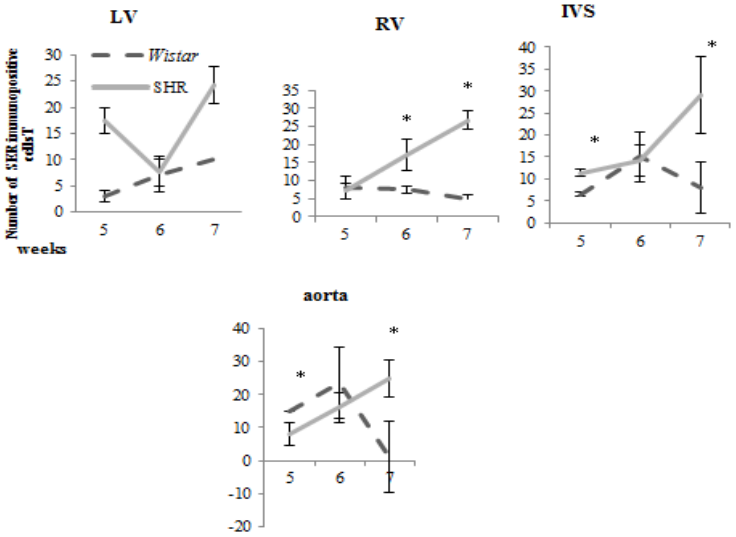

Fig. 3. The number of cardiomyocytes,immunopositively-stained for SERT membrane transporter in different compartments of the aorta and myocardium in the experimental and control groups of 5-7-week-old rats.

Note: horizontal direction - week 5, 6, 7 of rats' postnatal development; vertical direction - number of immunopositively-stained cells for SERT membrane transporter; SHR - SHR-line rats; Wistar - Wistar-Kyoto-line rats; * - significance of differences $p \leq 0.05$.

III. Identification of the connective tissue by Van Gieson method in SHRs and Wistar-Kyoto rats at the age of 5-7 weeks.

The microslides of different myocardic and aortic compartments have a significant growth of fibrous tissue in SHRs as compared to Wistar-Kyoto rats. The process is mostly expressed in the aorta, IVS and RV, respectively.

Summary

1. Cardiomyocyte hypertrophy was detected in 5-week-old SHRs as compared to Wistar-Kyoto rats.
2. A statistically significant difference in the level of expression of 5-HT_{2B} receptors and SERT membrane transporter in the aorta and myocardium between normo- and hypertensive rats can be already observed at week 5. Expression of both 5-HT_{2B} receptors and SERT membrane transporter in the experimental group of 5-week-old rats is more pronounced than in the control group. At week 6, the number of 5-HT_{2B} receptors continues increasing in SHRs, while the same in Wistar-Kyoto rats increases and reaches the values of experimental groups of rats. At week 7, the experimental group of rats has further increase in the number of 5-HT_{2B}-immunopositive cells, while the control group of rats shows the reverse process. Expression of SERT membrane transporter during week 6 is multidirectional. However, at week 7 there is an increase in the number of cells positively stained for SERT in all studied sections of the myocardium and aorta in SHR rats, and decrease in Wistar-Kyoto rats in all sections, except for LV.
3. We detected a considerable growth of fibrous tissue in all studied sections of the myocardium and aorta in 5-7week-old experimental rats as compared to control group.

Discussion

This study shows the morphological changes of different sections of the myocardium in 5-7-week-old spontaneously hypertensive rats.

AH is the major cause of the left ventricular hypertrophy and myocardial fibrosis [3]. Decompensation of hypertrophy processes, manifested in the reduction of the final systolic and diastolic volume and the dereased ejection fraction

during the ultrasound examination of the heart, leads to left ventricular systolic and diastolic dysfunction and heart failure [1].

The study of the development mechanism of pathological processes observed in hypertension is performed in spontaneously hypertensive rats [5].

The process LVH starts in spontaneously hypertensive rats at the age of 1 month [4]. Stable hypertrophy at the age of 18-25 months is decompensated and turns into heart failure [1]. At the age of 16-25 months, the SHRs have an actively progressing fibrosis [3]. We have found that the mass of the myocardium and its compartments (LV, RV, IVS) in 5-week-old SHRs significantly exceeds the same in Wistar-Kyoto rats. Starting from week 6, the weight ratio is aligned, but the RV mass in hypertensive rats is greater than the RV mass of the normotensive group. Such data may indicate the development of hypertrophy in 5-week-old hypertensive rats.

Using Van Gieson method, we identified the active growth of fibrous tissue in all studied sections of the myocardium and aorta in 5-7-week-old SHRs, mostly in the aorta, IVS, and RV, respectively.

It is known that the free serotonin blood level in adult increases, while the mechanism of serotonin capture by platelets via SERT is damaged in such a way that the density of the membrane transporter on the membrane decreases [2]. Using the immunohistochemical method, we have shown the change in density of the SERT membrane transporter on the membrane of cardiomyocytes. By counting the number of cells we have found that the number of SERT-containing cardiomyocytes was significantly higher in the SHRs at the age of 5-7 weeks. This may reflect the compensation development aimed at depositing of free serotonin, increased under hypertension, into cardiomyocytes Patients with chronic heart failure showed a significant increase in the concentration of plasma and platelet serotonin depending on the presence of diastolic dysfunction, diastolic dysfunction with left ventricular hypertrophy, and combined diastolic and systolic dysfunction [9].

It is known that serotonin regulates morphogenesis of the myocardium and function of the cardiovascular system in the mature myocardium through 5-HT_{2B} receptors [8]. Using the immunohistochemical method, we have shown the change in density of the 5-HT_{2B} receptors. We have found that starting from week 9 of development of the spontaneously hypertensive rats, the number of positively stained cardiomyocytes was significantly higher than the same of Wistar-Kyoto rats.An increased expression of 5-HT_{2B} receptors we revealed can cause development of fibrosis and hypertrophy in immature SHRs, which may be confirmed by literature data on the expression of these receptors on cardiac fibroblasts [7], and cardiomyocytes[6] animals. The data obtained allow us to conclude that the $5HT_{2B}$

receptors and SERT membrane transporter are involved in compensatory processes due to increased blood pressure of SHR associated with the development of morphofunctional heart defects.

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