EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Functional Activity of 5-HT_4 Receptors in Children with Congenital Heart Disease

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The effect of 5-methoxytryptamine (5-HT_4 receptor agonist) on the inotropic function of atrial myocardium was studied in children aged 2 months to 17 years, operated on for congenital heart disease. Functional activity of 5-HT_4 receptors was 8.4 times higher in dysfunction of the atrial septum in comparison with other congenital heart diseases. The positive inotropic effects of 5-HT_4 receptor agonist can promote compensation of myocardial work in children with pathological circulation.

Key Words: *5-HT_4 receptors; congenital heart diseases; atrial septum defect; inotropic function of atrial myocardium; children*

Neurohormone serotonin (5-hydroxytryptamine; 5-HT), a representative of biogenic amines is synthesized by serotoninergic neurons in the CNS and by intestinal APUD cells at the periphery. Plasma content of 5-HT is negligible; its main depot are platelets, in which 5-HT concentration is several-fold higher [5]. 5-HT is released under the effect of platelet activators: ADP, thrombin, collagen, *etc.* 5-HT exhibits a morphogenetic effect during the embryogenesis [13] and a positive inotropic effect on the myocardium during the postnatal development of mammals [1,2].

Plasma concentration of 5-HT in patients with pulmonary hypertension is 34.4 nmol/liter, which is 3.8 times higher than in health [7]. The serotoninergic system is involved in the pathogenesis of atherosclerosis, arterial and pulmonary hypertension, coronary heart disease, atrial fibrillation, and cardiac failure [4,5,14]. Presumably, the dilatational and hypertrophic cardiomyopathies result from genetic aberrations of this component of the neurohormonal system [8,9].

A wide spectrum of 5-HT effects is explained by a great variety of 5-HT receptors. 5-HT_2 and 5-HT_4 receptors (5-HT_4 activates the intracellular adenylate cyclase cascade mechanism, 5-HT_2 the phosphatidylinositol pathway) were detected in mammalian heart [6,12]. 5-HT elevates cAMP content and regulates the concentration of Ca^{2+} through 5-HT_4 receptors in human atrial cardiomyocytes [6]. 5-HT_4 receptors were detected in human sinoatrial node and atrial cardiomyocytes; activation of these receptors causes a positive inotropic, chronotropic, lusitropic effects, tachicardia and atrial fibrillation [10,11,15].

Hence, the serotoninergic system is involved in the functioning of the cardiovascular system and in the pathogenetic mechanisms of its diseases. However, the role of 5-HT in the regulation of cardiac inotropic function during human postnatal ontogenesis in various congenital heart diseases remains poorly studied.

We studied the functional role of 5-HT_4 receptors in the regulation of inotropic function of atrial myocardium in children with congenital heart diseases.

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MATERIALS AND METHODS

Patients (n=19) were divided into 2 groups. Group 1 consisted of 8 patients aged 2 months to 15 years (mean age 8.3±2.2 years) with atrial septum defect (ASD) and blood shunt at the level of ASD causing pronounced volume overload of the right atrium. Group 2 consisted of 11 patients aged 2 months to 17 years (mean age 6.3±1.8 years) with other heart diseases associated with negligible volume overload of the right atrium (8 cases with ventricular septum defect, 1 with patent ductus arteriosus, 1 with aortic valve insufficiency, and 1 with Fallot's tetralogy). The degree of right atrium overload was evaluated by echocardiography.

Myocardial contractility was evaluated on a Power Lab device (ADInstruments) and MLT 050/D power pickup (ADInstruments). Muscle fibers 2-3 mm long and 0.8-1.0 mm in diameter were prepared at the site of atrial incision during aortocoronary bypass surgery for congenital heart disease in children aged 2 months to 17 years. The preparations were collected at the stage of myocardial reperfusion by the moment of adequate heart rhythm recovery.

The myocardium was plunged into carbogen-oxygenated working solution at 20-22°C. The working solution contained (mmol/liter): 119.8 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 0.42 $NaHPO_4$, 0.28 ascorbic acid, 5.05 glucose at pH 7.3-7.4 (all reagents were from Sigma). 5-HT_4 receptor agonist 5-methoxytryptamine (5-MT; ICN Biomedicals) was used in concentrations of 0.1, 1.0, and 10.0 μmol/liter.

The results were registered and processed using Chart 5 software. The differences were evaluated using Student's test (p<0.05).

RESULTS

A positive inotropic effect of 5-MT on the myocardium was detected in 17 preparations. The maximum increment of atrial myocardium contraction strength (by 443.0±140.1%) was observed in patients with ASD (Fig. 1). In group 2 this increase was less pronounced (27-94%).

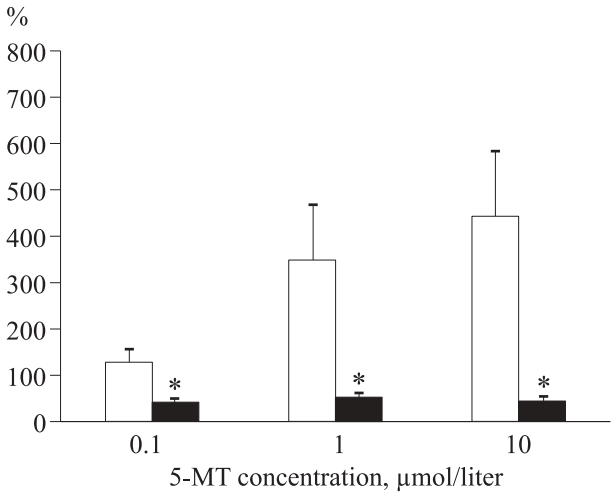

Fig. 1. Reaction of atrial myocardial contraction strength to 5-MT in patients with ASD (light bars) and other congenital heart diseases (dark bars). *p<0.01 compared to ASD.

In children with ASD, the increase in the strength of contractions in response to 5-MT was dose-dependent: 127.8±28.6% in response to 0.1 μmol/liter, 348.3±119.7% in response to 1.0 μmol/liter. The maximum positive inotropic effect (443.0±140.1%) was produced by 5-MT in a concentration of 10.0 μmol/liter. In group 2 no changes in the strength of atrial myocardium contractions were observed with increasing 5-MT dose (Fig. 1).

Comparison of myocardial reaction to 5-MT agonist injection showed a significant increase in the rate of contraction, relaxation, and shortening of the period of myocardial contraction in ASD patients in comparison with patients with other congenital heart diseases (Table 1).

No reaction to 5-MT injection was observed in 2 cases: in a patient aged 17 years with Fallot's

TABLE 1. Effect of 5-MT on Inotropic Function of the Heart (M±m)

Parameter	Group	Without 5-MT	Concentration of 5-MT, μM		
			0.1	1	10
Contraction rate, g/sec	1	0.248±0.095	0.433±0.097	0.656±0.104*	0.852±0.116**
	2	0.289±0.065	0.355±0.076	0.380±0.089	0.366±0.091
Relaxation rate, g/sec	1	0.138±0.029	0.201±0.048	0.268±0.049*	0.305±0.063*
	2	0.148±0.034	0.188±0.032	0.198±0.049	0.186±0.059
Total duration of contraction, sec	1	1.171±0.112	0.852±0.075*	0.882±0.064*	0.923±0.057
	2	0.841±0.106	0.859±0.150	0.790±0.162	0.863±0.151

Note. *p<0.05, **p<0.01 compared to initial values.

tetralogy and severe cardiac failure and in a patient aged 6 years with large ASD (of the common atrium type) and decompensation of the right heart compartments (dilatation of the right atrium and ventricle).

Comparative analysis of the group of patients with right atrium overload (ASD) and group without echocardiographic signs of right atrium overload showed that the maximum increase in the strength of contractions in the myocardium exposed to overload in response to 5-MT was 8.4 times higher than in normally working myocardium. Increasing 5-MT concentration led to a proportional increase in its inotropic effect only for the myocardium with volume overload.

It was previously found that activation of 5-HT_4 receptors can be a potential cause of arrhythmias and fibrillations [6]. We singled out a group of ASD patients with significantly elevated 5-HT_4 receptor activity in comparison with other patients. Atrial fibrillation and flutter are observed in about 20% of adult patients with not corrected ASD [3], but virtually never in patients with other congenital heart diseases.

Hence, functional activity of 5-HT_4 receptors is increased in children with ASD characterized by myocardial overload. A positive inotropic effect of 5-MT seems to promote compensation of myocardial work during abnormal circulation. The increase of 5-HT_4 receptor activity in atrial myocardium can be involved in the pathogenesis of arrhythmias in children with ASD.

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