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Research Article

Effect of Ca^{2+} channels blockers dantrolene, methoxyiverapamil on inotropic function of rat myocardium with altered serotonin level

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ABSTRACT

The effect of calcium channel blockers dantrolen and metoxyverapamil on the amplitude of the contraction of the left ventricle cardiomyocytes of 7- and 10-day rat s, in which embryonic period a deficit of serotonin or *5-HT*(produced by chronic administration of a *PCPA* neurotoxin) was revealed. It was found, that the initial value of the left ventricle contraction force of rats with altered serotonin metabolism wasот 33% lower than in the control group of rats. Dantrolen in different concentrations and metoxyverapamil reduced the force of contraction of the left ventricle,reaction in control heart was 51-80% and 13-25% in experimental group. Subsequent administration of serotonin had a positive effect on the contractility of the left ventricle on 37,5-72% in control and 55-66% in experimental group. On the background of serotonin administration, the calcium channel blockers, dantrolen and metoxyverapamil, also reduced the force of contraction of the left ventricle. We have found that the reducing power of the left ventricular contraction in rats with serotonin deficiency were less pronounced.

Keywords:blockade of the serotonin synthesis, p-chlorphenylalanine, calcium channels, dantrolen, metoxyverapamil, serotonin, embryonic ontogenesis, rat.

INTRODUCTION

Serotonin (5-HT), being a neurotransmitter, is synthesized on the periphery as a hormone and has an effect on cells and target organs [15]. Particular attention is given to the effect of 5-HT on the functioning of the cardiovascular system. It was proved the influenceof 5-HT on the formation of the cardiovascular system in the embryonic period [1, 6, 10, 11, 14], and on the function of the heart in different periods of postnatal ontogenesis [13, 3, 6, 10, 11]. The action of 5-HT is mediated via serotonin receptors. Metabotropic 5-HT2 and 5-HT4 receptors are involved in the regulation of rat myocardial contractility [6, 12]. The most sensitive to serotonin are 5-HT4 receptors. Their activation causes positive inotropic, chronotropic, lyuzitropic effects, as well as tachycardia and atrial fibrillation through the movement of calcium ions through calcium channels in cardiomyocytes[6]. In the heart the most widely distributed calcium channels are L-type [9, 1]. In the sinoatrial node, they contribute to pacemaker activity, and in the atrioventricular node to conduction of impulses through the node [9, 1, 17]. Calcium channels of T type are expressed in embryonic cells, as well as in the embryonic heart [5], which indicates their participation in cell growth and proliferation [9]. Serotonin, interacting with 5-HT4 receptors, causes a cascade of intracellular reactions via the Gs-protein [14,6,8], as a result of which stimulation of cAMP-dependent kinase occurs. The latter phosphorylates the β2-subunit of L-type calcium channels of the myocardium, which leads to the opening of channels and the entry of calcium ions into the cell, increasing myocardial contractility [16].

On the membrane of the sarcoplasmic reticulum (SR) are intracellular calcium channels [9, 4,7]. Their activation leads to the release of calcium ions from the SR, which in turn increases the strength of myocardial contraction. These include ryanodine receptors and inositol-1,4,5-triphosphate receptors (IP3 receptors) [2, 16]. The existing relationship between the serotonin system and the calcium channels provides a normal heart development in the embryonic period and further functioning in postnatal ontogenesis [7, 10]. The change in one of the systems leads to aalteration in the functioning of cardiomyocytes and to the disruption of the heart. One of the blockers of ryanodine receptors and L-type calcium channels, used both in experimental studies and in clinical practice as a muscle relaxant and antiarrhythmic drug, are dantrolene and methoxyverapamil, respectively. Despite the wide use of these drugs, the study of their effect on the inotropic function of the myocardium of rats with altered metabolism of serotonin has not been previously carried out.

Purpose: to study the effect of calcium channel blockade on the inotropic function of rat myocardium with altered metabolism of serotonin.

METHODS

The permission of the Ethics Committee under the Ministry of Health of the Republic of Tatarstan was obtained for the study. The study was carried out on pregnant female Wistar rats and their offspring at the age of 10 days. In the first group (serotonin deficiency) in pregnant female from 11 days of gestation for 10 days, a serotonin synthesis blocker PCPA (p-chlorphenylalanine, SigmaChemical Co., StLouis, MO, USA) was injected intraperitoneally at a dose of 100 mg / kg. In the second group (control) of pregnant females, starting from the 11th day of pregnancy within 10 days, intraperitoneal administration of saline. In the offspring of each group at the age of 10 days, an inotropic function of the myocardium was investigated.

Study of myocardial contractility. The experiments were carried out on the PowerLab (ADInstruments), MLT 050 / D force sensor (ADInstruments). Anesthetized by urethane (800 mg / kg, Sigma, USA) rats of 10-day-old age were removed from the heart, muscle myocardium strips 2-3 mm in length and 0.8-1.0 mm in diameter were prepared and immersed in oxygenated saline. To maintain the pH in the range 7.3-7.4, Trizma base and / or acid buffers (all substances Sigma, USA) were added to the solution. The preparations were stimulated through platinum electrodes with the above characteristics. The amplitudecharacteristics of the contraction were calculated by the method of Laeretal., (1998).

Statistical method. The results of the research were statistically processed on a personal computer using the programs MicrosoftOfficeExcel 2003 and Statistics 6.0. All results are presented in the form M ± m. The reliability of the differences was estimated using the methods of Mann-Whitney and Wilcoxon. Differences were considered statistically significant at $p < 0.05$.

At the born rats were examined:

I series. The experiment was recorded on a computer using the Chart 4.0 software. After immersing the muscle strips in the reservoirs, the run-in period lasted for 40-60 minutes, during which the optimal tension was gradually given to the muscle strips. Optimal tension was considered such a point of stretching the drug, after overcoming which began to reduce the strength of the contraction. At the end of the run-in, the initial contraction parameters were recorded for 5 minutes, then the influence of dantrolene at a concentration of 10-4 mM / l was recorded in the first group of working solutions and 10-3 mM / l in the second group of working solutions. At the end of stimulation, the preparations were washed with a working solution for 5 minutes, then serotonin was administered at a concentration of 1.0 μM / L and data was recorded for 20 minutes.

II series. At the end of run-in for 5 minutes, the initial parameters of contraction of muscle bands were recorded, then serotonin was added to the working solution at a concentration of 1 μM / l with data registration for 20 minutes. Subsequently, dantrolene was introduced into the first group of working solutions at a

concentration of 10-4 mM / l, and in the second - 10-3 mM / l.

III series. A series of experiments was performed on 7 day-old rats: a serotonin synthesis blocker of PCPA (p-chlorphenylalanine, SigmaChemical Co., StLouis, MO, USA) was administered at a dose of 100 mg / kg to female rats from 10 days of gestation for 10 days. At 7 day old pups, the inotropic function of the heart and their responce to methoxyaveramil at a concentration of 2.5 μM / L against the background of serotonin introduced at a concentration of 1.0 μM / l were investigated and vice versa.

RESULTS OF RESEARCH AND DISCUSSION

1. The effect of the ryanodin receptor blocker dantrolene on the inotropic function of rat myocardium with altered metabolism of serotonin.

We found that the initial value of the left ventricular contraction force in rats with altered serotonin metabolism is 33% lower than in the control group of rats (Table 1).

Table 1. Effect of dantrolene and subsequent administration of serotonin on indices of inotropic function of left ventricular myocardium in 10 day old rats with chronic injection of PCPA in the embryonic period

F, din	Initial	Dantrolene 10^{-4}mM/l	5-HT 1,0 μM /l
Control	0.024±0.001**	0.015±0.001	0.027±0.002*
Pcpa	0.016±0.002**	0.012±0.002	0.020±0.002*
F, din	Initial	Dantrolene 10^{-3}mM/l	5-HT 1,0 μM /l
Control	0.037±0.004*	0.018±0.006	0.049±0.006
Pcpa	0.023±0.005*	0.020±0.005	0.031±0.007

Note: 5-HT-serotonin, P-p-chlorophenylalanine, F-contraction force. * - statistically significant differences in comparison with baseline values (* - P <0.05, ** - P <0.01, *** - P <0.001).

It was found that dantrolene at a concentration of 10-4 mM / L reduces the force of contraction of the left ventricle in the experimental group of rats by 25%. Further introduction of serotonin at a working concentration of 1 μM / l against the background of dantrolene introduced causes an increase in the left ventricular contraction force by 66% (p <0.05). In the rats of the control group, decrease of the contractile force by 80% was registered for dantrolene and a subsequent increase in serotonin by 37.5%. An increase in the concentration of dantrolene to 10-3 mM / l also reduces the ventricular contraction of the experimental groups of rats by 13%. The subsequent enjection on serotonin causes an increase in the contractile force by 55%. Corresponding changes are also observed in the rats of the control group: a decrease in the contraction force by 51% for dantrolene and an increase of 72% in serotonin.

Serotonin at a working concentration of 1 μM / L causes a positive inotropic effect in both the group of rats with a serotonin deficiency and in the rats of the control group by 50% and 67%, respectively (p <0.05). Against the backdrop of a pronounced positive effect of serotonin, dantrolene at a concentration of 10-4 mM / L causes a significant decrease in the contraction force by 50% (p <0.05) in the experimental group and by 55% (p <0.05) in the control group. Decrease in the force of contraction of the ventricles is also observed at a dantrolene concentration of 10-3 mM / l (Table 2).

Table 2. Effect of dantrolene on the background of serotonin administered on parameters of inotropic function of left ventricular myocardium in 10 day old rats with chronic administration of PCPA in the embryonic period

F, din	Initial	5-HT 1,0 μM /l	Dantrolene 10^{-4} mM/l
Control	0.024±0.003	0.040±0.003*	0.022±0.003*
PCPA	0.016±0.003	0.024±0.005*	0.012±0.003*
F, din	Initial	5-HT 1,0 μM /l	Dantrolene 10^{-3} mM/l
Control	0.036±0.003**	0.060±0.003***	0.026±0.005
PCPA	0.021±0.003**	0.027±0.003***	0.015±0.002

Note: 5-HT-serotonin, P-p-chlorophenylalanine, F-reduction force. * - the reliability of the differences in comparison with the initial indicators (* - P <0,05, ** - P <0,01, *** - P <0,001).

2. The effect of the L-type calcium channel blocker methoxyvapamil on the inotropic function of rat myocardium with altered metabolism of serotonin.

We found that methoxyvertamil at a concentration of 2.5 mM / L causes a decrease in the left ventricular contraction force in the rats of the control group by 48%, in rats in the experimental group by 54%. Further introduction of serotonin has a positive inotropic effect and an increase in the

contraction force occurs both in the control and in the experimental group by 113% and 29%, respectively. Methoxyvertamil, against the injected serotonin, which has a positive inotropic effect, causes a decrease in the contraction force in the control group of rats at 48%.Thus, the conducted studies indicate that in 7- and 10-day-old rats, in the embryonic period of development of which there was a 5-HT deficiency created by the chronic administration of the PCPA neurotoxin, significant changes occur in the response to serotonin and calcium channel blockers. Perhaps the lack of serotonin in the embryonic period causes structural rearrangements of the calcium channels, insufficient formation of them, a decrease in receptor sensitivity or a decrease in their number both on the cardiomyocyte membrane and on the surface of the SR, which is expressed by a significantly low response of cardiomyocytes to the introduction of calcium channel blockers. In addition, in the rats of the experimental group, in which serotonin deficiency is observed in the body, caused by chronic administration of neurotoxin PCPA, the initial contraction force is significantly reduced compared to the control group rats. Therefore, it is necessary to minimize the effect on the metabolism of serotonin in the embryonic period of development in connection with the effect on the inotropic function of the heart and its regulation through metabotropic receptors in postnatal ontogenesis.

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