

Research Article**Yohimbine influence on myocardium contractile activity among newborn rats**

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ABSTRACT:

Alpha2-adrenergic receptors (α_2 -AP) are widespread in a man's body, including the cardiovascular system. As G-protein coupled receptors, they perform different functions in the intracellular mechanism regulation. Despite the fact that the α_2 -adrenoceptors were discovered in a man's heart, it was not possible to detect receptor within protein level, and the study of α_2 -adrenoceptors in a man's heart was insufficient. The study of α_2 -adrenoceptor yohimbine blockade influence on the inotropy of right atrium and right ventricle myocardium among newborn and adult rats was carried out in vitro using the device POWER LAB (AD Instruments, Australia) by the force sensor MLT 050/D (AD Instruments, Australia). The obtained results indicate the decrease of contraction force of the atrium and ventricle myocardial strips at α_2 -adrenergic receptor blockade with yohimbine among adult rats. A slight short increase of contraction power in atrium myocardium and the reduction of contraction power in ventricular myocardium were observed among newborn rats at the introduction of yohimbine. It is possible that the changes in the regulation of the heart inotropic function occur among newborn rats at the absence of sympathetic innervation.

Keywords: heart, inotropy, α_2 -adrenergic receptors, postnatal ontogenesis.

1. INTRODUCTION

The autonomic nervous system innervates a myocardium and regulates its operation. The sympathetic and parasympathetic parts of the autonomic nervous system influence on cardiomyocytes through the interaction of noradrenaline with the adrenergic receptors and the interaction of acetylcholine with muscarinic cholinergic receptors [1, 2, 3]. According to classical ideas β -adrenergic receptors (β -AR) and muscarinic cholinergic receptors (MCHR) are involved in autonomic regulation of a heart [2]. Nowadays it is accepted to determine nine subtypes of adrenergic receptors (AR): α_1a -, α_1b -, α_1d -, α_2A -, α_2b -, α_2c -, β_1 -, β_2 - and β_3 - AR [4]. The activation of α_1 -adrenergic and β -adrenergic receptors leads to the functional changes in the operation of myocardium and cardiomyocytes [5]. Mostly the presynaptic location of α_2 -adrenergic receptors influences

the release of catecholamine's from nerve endings [5]. According to mRNA analysis their number is 30 times less than the number of α_1 -AR [6; 2]. There are three subtypes of α_2 -AR in modern literature: α_2A -, α_2B - and α_2C -AR [7]. They are connected by G-protein coupled receptors (GPCR). Binding to Gi/Go proteins they reduce adenylate cyclase activity, which reduces the amount of intracellular cAMP, mediates Na^+ channel receptor activity, the inhibition of stress during Ca^{2+} channel closure [2; 14]. α_2 -AR were detected in a man's heart using molecular biology techniques. However, we didn't manage to detect the presence of α_2 -AR in a man's heart at the protein level. The presence of α_2 -AR was studied not enough in a man's heart [5; 4]. But the groups of researchers showed the presynaptic regulation of noradrenaline release through α_2 -AR using the

preparations of the isolated right atrium [8]. In vivo experiments in the system and intracoronary administration of phentolamine showed the increase of norepinephrine level in the blood plasma [9; 10]. α_2 -adrenergic receptors are widely distributed in a man's body, they are involved in the regulation of various functions, including the cardiovascular system [11]. They are present on the presynaptic membranes of adrenergic fibers, on the postsynaptic membranes of myocardiocytes in vascular smooth muscle, in the peripheral and the central nervous system, in kidney and intestinal epithelium [12; 14]. The stimulation of α_2 -AR leads to the manifestation of α_2 -agonists AR classical effects - hypotension, sedation, analgesia and blood pressure decrease [13]. AR distributed in peripheral tissues mediate some important physiological responses, such as hypertension and vasoconstrictor effect. They are involved in the development of reproductive processes [14, 15]. In the central nervous system (CNS) They control the release of presynaptic norepinephrine from nerve-endings [13, 5].

At the moment, the issue about the presence and the functional significance of α_2 -AP in a man's and animals heart remains a relevant one [16; 5; 4]. Alpha2-AR are the attractive targets for the treatment of certain modern diseases of mankind, such as hypertension, pain, depression, anxiety, and obesity. Thus, the study of the influence of α_2 -AR blockade influence is very important. The aim of this study was the comparative analysis of α_2 -AR blockade influence with yohimbine on the inotropy of atrium and ventricle myocardium among newborn and 20-week-old rats.

2. METHODS

Experimental work was carried out on white mongrel newborn (1 week old) rats. The group of adult rats (20 weeks of age) acted as the control one. The main stages of the cardiovascular system innervation development were in the basis of ages selected by us. The hearts of 20-week-old animals have a developed heart innervation. There is no sympathetic innervation in the hearts of newborn rats [17].

The inotropic response of rat hearts was determined using POWER LAB device (AD Instruments, Australia), by the force meter MLT 050/D (AD Instruments, Australia). The myocardium strips were cut out from the isolated heart right atrium and right ventricle in accordance with the anatomical structure of the heart. The strips were 2.3 mm long and their diameter made 0.8-1.0 mm. The device was placed vertically in 20 ml, in the working solution oxygenated with carbogen (97% of O₂ and 3% of CO₂) at the temperature of 37 °C. The upper end of the device was attached to a stainless rod connected to the voltage meter, the lower end was attached to the rubber block. The device was stimulated by an electrical signal via 2 silver electrodes (using ECL stimulator - 2 (Russia), with the signal amplitude of 10 mV, the stimulus duration made 5 ms. After the dipping of the device into the reservoir the processing period followed for 40-60 minutes, during which an optimum voltage was given gradually to muscle fibers. The optimal voltage was such stretch point of the device, after which the decrease of contractile force started. At the end of the processing the baseline contraction parameters were recorded for 5 minutes, then these parameters were recorded for 21 minutes with the addition of a specific blocker in the working solution. The specific α_2 -AR antagonist yohimbine was added at the concentration of 10⁻⁶ M. The contraction force (F) was expressed in grams (g). The processing of obtained results was performed using Chart 5 software and the device POWER LAB, using Stat graphics software package. The statistical processing and the determination of research result reliability was performed in Microsoft Excel editor according to the Student t-criterion.

3. RESULTS

During the first minute the addition of specific α_2 -adrenoceptor blocker yohimbine at the concentration of 10⁻⁶ M did not change myocardial contractile force of the right atrium among 20-week-old rats. During the 5-th minute of the experiment the introduction of yohimbine into the working solution reduced the contraction force of atrial myocardium strips

from $0,042 \pm 0,01$ g to $0,04 \pm 0,01$ g ($p < 0,05$). By the 10-th minute of observation the contraction force of atrial myocardium strips decreased to $0,039 \pm 0,01$ g ($p < 0,01$). During the 15-th minute yohimbine reduced the values of atrial myocardium strip contraction force to

$0,038 \pm 0,01$ g ($p < 0,01$). During the final minute of the experiment, we observed a significant decrease to $0,037 \pm 0,01$ g ($p < 0,01$) in myocardium strip contraction force of the right atrium among 20-week rats (Figure 1).

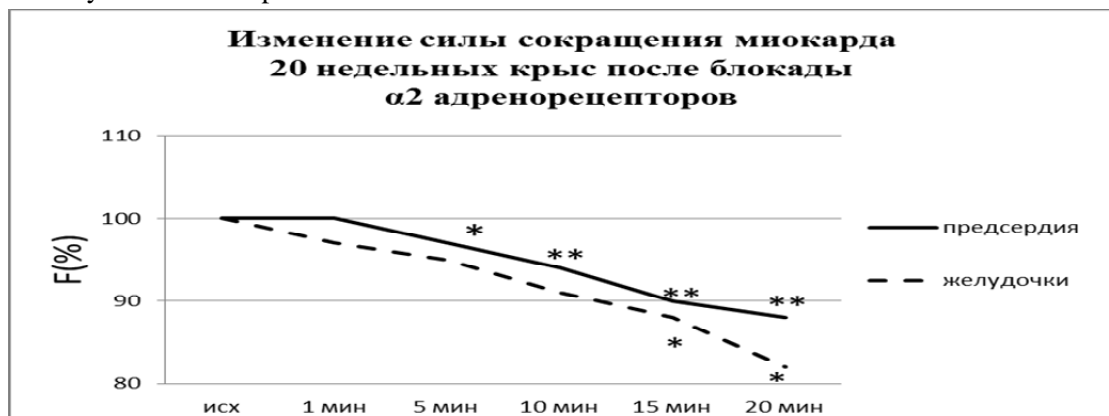


Fig. (1). The change of myocardium contraction force among 20-week old rats after the blockade of α_2 adrenergic receptors. The y-axis is the force of myocardial strip contraction (F, %), abscissa axis - the experiment record time (minutes). Note: * - the reliability in comparison with baseline values: $p < 0,05$, ** - reliability in comparison with the baseline values: $p < 0,01$.

The introduction of the specific blocker yohimbine to the working solution reduced the myocardium strip contraction force of the right ventricle from $0,07 \pm 0,01$ g to $0,068 \pm 0,01$ g during the first minute of the experiment. By the 5-th minute, we continued to observe a gradual reduction of myocardium strip contraction to $0,067 \pm 0,01$ g within the right ventricle. By the 10-th minute they recorded the contractile force of the ventricle myocardium to $0,065 \pm 0,01$ g among 20-week old rats. By the 15-th minute the specific blockade of α_2 -adrenergic receptors reduced the contraction force of ventricular myocardium strips to $0,062 \pm 0,01$ g ($p < 0,05$). By the 20-th minute of the experiment the strength of ventricular myocardial strips among adult rats decreased to $0,057 \pm 0,009$ g ($p < 0,05$) (Figure 1).

The addition of α_2 -adrenoceptor blocker in the working solution did not cause the reliable changes of the right atrium myocardium contractions among 1-week-old animals. by the 5-th minute of the experiment, we observed a short unreliable increase of atrial myocardium contraction force from $0,0119 \pm 0,005$ g up to $0,0122 \pm 0,005$ g. By the 10-th minute of observation the myocardium strip contraction force of the right atrium among newborn rats decreased to $0,0117 \pm 0,005$ g. During the subsequent 15-th and 20-th minute of the experiment the contraction force was equal to $0,0112 \pm 0,005$ g (Figure 2).

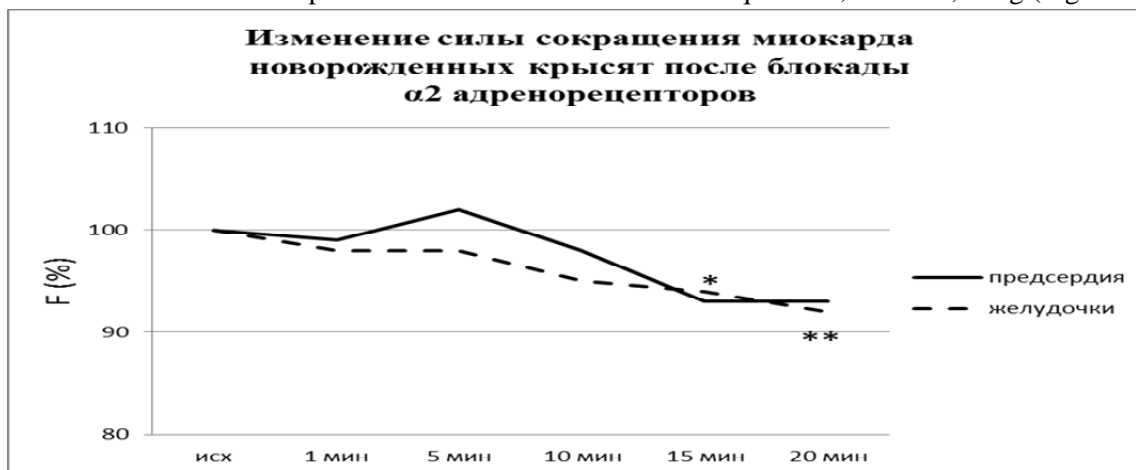


Fig. (2). The change of myocardial contractile force among newborn rats after the blockade of α_2 -adrenoceptors. The y-axis is the force of myocardial strip contraction (F, %), abscissa axis - the experiment record time

(minutes). Note: * - the reliability in comparison with baseline values: $p < 0.05$, ** - the reliability in comparison with the baseline values: $p < 0.01$.

The specific blockade of α_2 -adrenoceptors did not cause the significant changes in the contractile force of ventricular myocardium strips among newborn rats during the first five minutes of the experiment. By the 10-th minute yohimbine reduced the contraction force of the right ventricle myocardium strips from $0,0200 \pm 0,005$ g to $0,0190 \pm 0,005$ g. By the 15-th minute of observation the contraction force of right ventricular myocardium strips reduced to $0,0188 \pm 0,005$ g ($p < 0.05$). During the last minute of the experiment the contraction force after the α_2 -adrenoceptor blockade among newborn rats made $0,0185 \pm 0,005$ g ($p < 0.01$) (Figure 2).

4. CONCLUSIONS

The specific α_2 -adrenoceptor blockade reduces the myocardium strip contractile force of the atria and ventricles among rats 20 week old rats. The myocardium of the right atrium among newborn rats has the tendency to a slight short-term increase of contraction force with its subsequent decrease. The contractile force decrease is observed in ventricular myocardium.

5. SUMMARY

The performed studies showed that the blockade with α_2 -AR yohimbine reduces the contraction force of the myocardium strips among newborn and adult animals. The most significant decrease of atrium and ventricle myocardium contractility was observed in the control group of 20 week old animals. The reliable changes in contractility at α_2 -AR blockade with yohimbine were observed only in ventricles among newborn animals. The atria of newborn rats showed at first the tendency to a slight short term increase of contractile force with its subsequent reduction. Previously, we carried out the research of α_2 -adrenoceptor blockade concerning the heart chronotropic action among the rats of all ages [9]. It was shown that blockade of α_2 -adrenoceptors with yohimbine causes the heartbeat frequency changes among 6- and 20-week-old rats. The introduction of yohimbine for 1- and 3-week-old rats resulted in

heart work decrease. Thus, it was revealed that the blockade of α_2 -AR with yohimbine makes a significant influence on heart chronotropic action [9], rather than on the myocardial inotropy among newborn animals. It is possible that the regulation of the frequency component via this type of α -AR is more necessary at this age. However, it should be noted that there is evidence of different α_2 -AR subtype localization: α_2A - and α_2C -AR are localized presynaptically, α_2B -AR are localized mostly post-synaptically [8]. Therefore, we can not exclude the possible opposite effects of each of α_2 -AR subtypes. Thus, it led to the myocardium contractility reaction reduction among newborn animals at the blocker administration of yohimbine α -adrenoceptor second subtype.

CONFLICT OF INTEREST

The author confirms that the presented data do not contain any conflict of interest.

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