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ALPHA(1)-ADRENOCEPTORS ACTIVATION DECREASES MYOCARDIAL CONTRACTILITY IN NEWBORN RATS

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ABSTRACT

Alpha(1)-adrenergic receptors (α_1-AR) are found in cardiomyocytes, endothelial cells, and smooth muscle cells of humans and animals. Despite the fact that α_1-AR make up 10% of the total number of adrenergic receptors, these receptors also involved in the regulation of inotropic and chronotropic functions of the heart. According to some scientists, the effects of α_1-AR activation are not required for the basal contractile function of the heart while other group of researchers believe that α_1-AR can be considered as cardioprotective targets; in particular, it is postulated that the α_{1A}-subtype of adrenergic receptors can provide significant inotropic support in cardiac pathologies. This study was carried out on 6-7-day-old outbred newborn rat pups to evaluate the effect of alpha(1)-adrenoceptors activation on the myocardial contractility in newborn rats. For this, Alpha$_1$-adrenergic receptors were stimulated by the pharmacological drug methoxamine at concentrations of 10^{-9}-10^{-6} mol and the reaction of the contractile force of the strips of myocardium ventricles and heart atria in response to the agonist was investigated. Results of study revealed that stimulation of alpha$_1$-adrenergic receptors, regardless of the methoxamine concentration, led to a negative inotropic reaction of the myocardium of atria and ventricles of newborn rat pups. This study showed unidirectional inotropic responses on rat atrial and ventricular myocardium in response to α_1-adrenergic receptors stimulation. Methoxamine smoothly reduces the contractile force of the strips of myocardium atria and ventricles. At the same time, the concentration dependence on the inotropic reaction of the myocardium was observed. Results of study suggested that probably α_1-adrenergic receptors along with the main regulators β-adrenergic receptors carry out fine tuning of the heart activity.

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1 Introduction

Alpha(1) - adrenergic receptors (α_1-AR) were identified in the mid-1980s. Using modern research methods, α_1-AR were found in myocardiocytes, endotheliocytes, smooth muscle cells of coronary arteries in humans and animals (Luther et al., 2001). All three receptor subtypes (α_{1A}, α_{1B}, α_{1D}) are found in rat cardiomyocytes, and their density significantly increases in the first week after birth (Nozdrachev et al., 2016). The mRNA of all three subtypes of α_1-AR was found in the mouse heart: the expression of α_{1B}-AR was higher than the total level of α_{1A} and α_{1D}-AR. However, the level of α_{1A}-adrenergic receptors was slightly higher in the ventricles than in the atria (Zhang et al., 2018). In mice, the β_1- and α_{1B}-subtypes are dominant receptors in heart myocytes and are present in all cells, where α_{1B}-AR can have an inhibitory effect on myocardial inotropy. α_{1A}-AR were found in 60% of isolated cardiomyocytes, only 20% of them had a high receptor expression (Myagmar et al., 2017).

In the rabbit myocardium, α_{1A}-AR are functionally active, mediating a negative inotropic effect in vitro, despite the low level; and α_{1B}-subtype of AR prevails quantitatively and mediates a positive inotropic effect at the level of mRNA and protein (Thomas et al., 2016).

It is generally accepted that α_1-adrenergic receptors play a major role in the blood pressure control in the cardiovascular system. In addition to smooth muscle contraction, α_1-adrenergic receptors can induce endothelium-dependent vascular relaxation (Filippi et al., 2001). It has been proven that selective stimulation of α_{1A}-AR in the cardiomyocytes potentiates Ca^{2+}-current through Ca^{2+} - L-type channels. The α_{1A}-AR subtype mediates positive inotropic responses, while α_{1B}-AR appears to be associated with a decrease in cardiac contractile function (Micucci et al., 2019). Also, α_{1B}-adrenergic receptors can play trophic role and participate in the expression of other receptor subtypes on the cell surface.

α_1-adrenergic receptors mediate their effects through proteins of the Gq/11 family. In this case, the Gq-protein is decomposed into α- and βγ-subunits and phospholipase C is activated with the formation of inositol triphosphate (IF_3) and diacylglycerol. The latter stimulates protein kinase C, while IF_3 affects the IF_3-receptors of the endoplasmic reticulum and releases the accumulated calcium. The positive inotropic effect of alpha$_1$-adrenergic receptor agonists in the rat heart is implemented through the Gs-system and an increase in cAMP production, which inhibits the outflow of potassium ions (Tsirkin & Korotaeva, 2015).

According to some scientists, the effects of α_1-AR activation are not required for basal cardiac contractile function. Recently, α_1-AR are considered as cardioprotective targets. In particular, it is postulated that the α_{1A}-adrenergic receptor subtype can provide significant inotropic support in different heart diseases (Nozdrachev et al., 2016).

Previously, it was shown that nonselective stimulation of α_1-adrenergic receptors with metoxamine (10^{-9} and 10^{-8} M) inhibits the frequency (Zefirov et al., 2016) and the strength of isolated heart contractions (Zefirov et al., 2018a), and it was also revealed that methoxamine causes a decrease in the contractile response within the experiments performed on the strips of myocardium ventricles and atria of adult rats (Zefirov et al., 2018b). At the same time, selective stimulation of the α_{1A} receptor subtype by the A-61603 agonist (regardless of concentration) caused negative inotropy of the ventricles in 20-week-old rats, but positively induced the ventricular myocardium contractility in newborn animals. Moreover, the severity and duration of myocardial inotropic responses depended on the agonist concentration (Khabibrakhmanov et al., 2019).

The results of numerous researchers indicated that the heterogeneity of myocardial inotropic responses to the activation of alpha$_1$-adrenergic receptors in different species and age groups of animals. Further, Nozdrachev et al. (2016) also specified an increase in the density of the studied heart receptors at the early stages of postnatal development. Based on the foregoing, current study formulated a hypothesis about the presence of age-related features in the inotropic responses of the myocardium to stimulation of alpha$_1$-adrenergic receptors in newborn rat pups. The aim of this study was to evaluate the inotropic effect of stimulation of alpha$_1$-adrenergic receptors in the myocardium ventricles and atria of 1-week-old rat pups.

2 Materials and Methods

The study was carried out on 6-7-day-old outbred newborn rat pups. For anaesthetize the rate 25% urethane solution (dose: 800 mg/kg, intraperitoneally) was used. Myocardial strips were excised from the right atrium and right ventricle of the heart and placed in a bath with a process solution (composition, in g/l: NaC1 – 7.6; KC1 – 0.42; NaH_2P0_4 – 0.07; $MgC1_2$ – 0.104; $CaC1_2$ – 0.198; $NaHC0_3$ – 1.69; $C_6H_{12}0_6$ – 1.98; Trizmabase – 0.25) by fixing them vertically from above to the contractile force registration sensor, and below to the holder. The heart myocardium strips were stimulated by an electrical signal through silver ring electrodes (frequency - 6 stimuli/min, amplitude - 10 mV, duration - 5 ms). After this myocardium strips were immersed in the solution and the thread tension was adjusted, their fixation was followed by an elaboration period (30-40 minutes). After 5 minute of elaboration, the initial values of the contractile force of the strips were recorded. Further, we recorded the inotropic reaction of the atrial and ventricular myocardium in response to non-selective

stimulation of α_1-adrenergic receptors. For non-selective stimulation of α1-adrenergic receptors, methoxamine hydrochloride (MH) (Sigma) drug was used in the concentration range of 10^{-9}-10^{-6} mol/L. Changes in the contractile force (F, expressed in grams (g)) of myocardial strips after substance addition were evaluated as inotropic effects of methoxamine and analyzed in relation to baseline values. The statistical analysis of the current study was carried out using the original software "AcKnowledge 4.1", MB-150 experimental installation (BIOPAC Systems, USA). The reliability of changes was determined by the Student's test at * - $p<0.05$; ** - $p<0.01$, *** - $p<0.001$.

3 Results and Discussion

3.1. Effects of methoxamine on contractility of atrial myocardium strips

The initial values of the atrial contractile force in newborn rat pups were in the range of 0.0213 - 0.0299 g. While the stimulation of α1-adrenergic receptors with a non-selective agonist metoxamine with a concentration of 10^{-9} mol caused a decrease in the contractile force of atrial myocardial strips (n = 6) in newborn rat pups from 0.0299±0.007 g to 0.0225±0.005 g ($p<0.05$), and the negative inotropic effect was 25% (Figure 1). While in response to the metoxamine effect at a concentration of 10^{-8} mol, the contractile force of atrial myocardial stripes (n = 6) gradually decreased from 0.0276±0.006 g to 0.0222±0.004 g ($p<0.05$), the change was 20%.

Further, MH introduction at a concentration of 10^{-7} mol had a negative inotropic effect on the atrial myocardium stripes (n = 6) of 1-week-old rat pups, which reached a maximum effect by the 20th minute of the agonist action. At the same time, the contractile force values changed from 0.0237±0.002 g to 0.0171±0.0009 g ($p<0.01$) and the effect was 29% (Figure 1). A similar dynamics of atrial contractility was also observed under the effect of methoxamine at a concentration of 10^{-6} mol (n = 6) and the contractile force decreased from 0.021±0.001 g to 0.015±0.0008 g which is 31% ($p<0.001$). This series of experiments showed that an increase in the negative inotropic reaction of the atrial myocardium of 1-week-old rat pups was observed with an increase in the agonist concentration.

3.2. Effects of methoxamine on contractility of ventricular myocardium strips

The initial values of the contractile force of the ventricular myocardium strips was in the range of 0.0280-0.0393 g. The contractile force of the ventricular myocardium stripes (n = 7) on newborn rat pups in response to the metoxamine effects at a concentration of 10^{-9} mol gradually decreased from 0.0352±0.0044 g to 0.0301±0.004 g ($p<0.05$) with a change of 15% (Figure 2). While addition of 10^{-8} mol methoxamine, the contractile force of the ventricular myocardium of newborn rat pups decreased by 20% ($p<0.01$). The indicators of myocardial inotropy changed from 0.0393±0.0034 g to 0.0314±0.0036 g.

Stimulation of α_1-AR by the methoxamine application at a concentration of 10^{-7} mol caused the maximum negative inotropic reaction of the ventricular myocardium of newborn rat pups in comparison with other concentrations. The decrease in the contractile force of the ventricular myocardium from 0.0342±0.0034 g to 0.0251±0.0031 g ($p<0.001$) (n = 7) lasted until the 20th minute of the methoxamine affect (10^{-7} mol) and the effect was 27%.

MH administration at 10^{-6} mol reduced the contractile force of the ventricular myocardium in rat pups from 0.0282±0.0018 g to

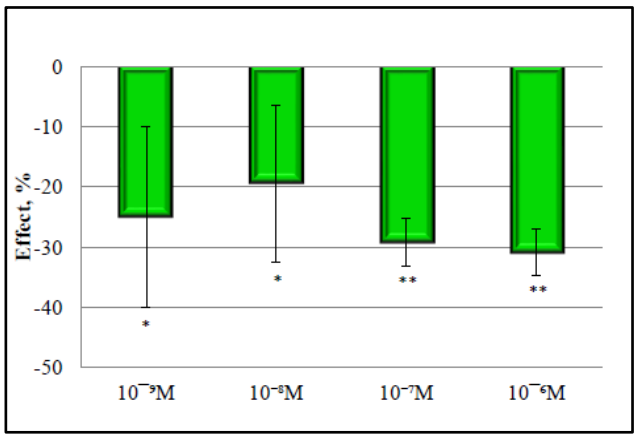

Figure 1 Effects of metoxamine on the contractile force of atrial myocardial strips in 1-week-old rats (Note:* represent significance level at $p<0.05$; ** represent significance level at $p<0.01$)

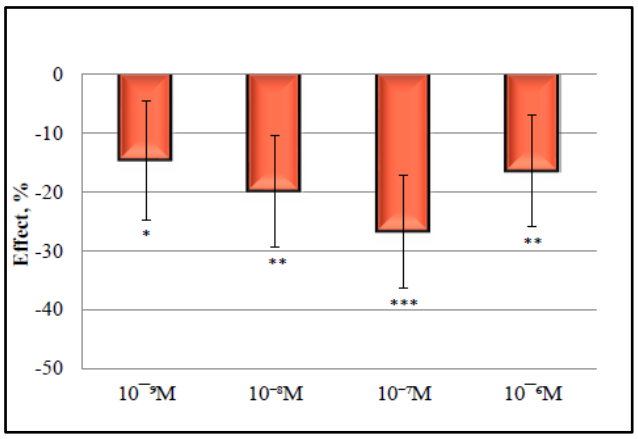

Figure 2 Effects of methoxamine on the contractile force of the ventricular myocardium strips of 1-week-old rats.
Note: * - $p<0.05$; ** - $p<0.01$; *** -$p<0.001$.

0.0233±0.0025 g ($p<0.01$), the change in contractility was 17% (Figure 2).

Results of study suggested that the stimulation of alpha(1)-adrenergic receptors, regardless of the concentration of the agonist metoxamine, causes unidirectional inotropic effects on the myocardium of newborn rat pups. Methoxamine smoothly reduces the contractile force of the strips of myocardium atria and ventricles. At the same time, the concentration dependent inotropic reaction of the myocardium was also obesrved. Methoxamine caused the strongest decrease in the contractile force of the atrial myocardium. These results are in agreement with the findings of Zefirov et al. (2018a) those who reported that activation of alpha(1)-adrenergic receptors by methoxamine has a negative inotropic effect on the heart of 20-week-old rats. Further, this study did not revealed age-related features in the inotropic response of the myocardium in newborn rat pups on stimulation of alpha 1-adrenergic receptors.

The negative inotropic effect on the heart can be implemented mainly through the α_{1B}-adrenergic receptor subtype. The mechanisms of the negative inotropic effect on α_1-AR activation might be because of an increase in the synthesis of nitric oxide (II), activation of the $Na^+/Ca2^+$ metabolic process, an increase in the outbound K^+ current, inhibition of Ca^{2+} channels of L-type, a decrease in the sensitivity of myofilaments to Ca^{2+} etc. Negative inotropy may also depend on the activity of protein kinase D, which reduces the contractile force by phosphorylation of troponin I (Tsirkin & Korotaeva, 2015). Probably, α1-adrenergic receptors, along with the main regulators - β-adrenergic receptors, carry out more fine tuning of the heart activity.

Conclusion

Methoxamine, an agonist of $alpha_1$-adrenergic receptors led only to a unidirectional negative inotropic reaction of the myocardial strips of both the heart atria and the heart ventricles of newborn rat pups at the concentrations studied by us. At the same time, we observed the concentration dependence of the inotropic reaction of the myocardium.

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Conflict Of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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