



ALPHA(1A)-ADRENORECEPTOR PARTICIPATION IN MYOCARDIAL CONTRACTILITY REGULATION AMONG NEWBORN RATS

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Abstract

The study was conducted on outbred newborn 6-7 day old rats. To stimulate α_{1A} -adrenergic receptors, the pharmacological preparation A-61603 was used in concentrations of 10^{-9} - 10^{-6} mol. They studied the reaction of contraction force of isolated atrial and ventricular myocardial strips of the heart in response to the action of a selective agonist. Stimulation of α_{1A} -adrenergic receptors depending on the concentration of agonist A-61603 caused multidirectional inotropic effects. A-61603 at concentrations of 10^{-9} and 10^{-8} mol leads to the decrease, and at the concentrations of 10^{-7} and 10^{-6} mol, to the increase of the atrial myocardial strips and ventricle contraction strength among newborn rat. In addition, the duration of a positive inotropic effect also depends on the concentration of the agonist. Our previous studies have shown that in the ventricular myocardium of adult rats, A-61603 in the concentration range of 10^{-9} - 10^{-6} mol causes only a negative inotropic effect. It should be noted that the nature of the inotropic effects of α_{1A} -adrenergic receptors may depend not only on the activity of different signaling pathways triggered by both different subtypes and a separate subtype of receptors, but may also be associated with the features of activation and maturity of individual elements of one signaling system at different stages of postnatal ontogenesis.

Key words : Rat, heart, myocardium, inotropy, A-61603, α_{1A} -adrenergic receptors.

Introduction

The studies using the radioligand binding method in the mid-1980-ies confirmed the existence of subtypes of α_1 -adrenergic receptors (α_1 -AR) (Morrow & Creese, 1986). Three molecular genes of α_1 -adrenergic receptors (α_{1A} , α_{1B} , α_{1D}) were finally identified by molecular biological methods (Docherty, 2019). In rat cardiomyocytes, all three receptor subtypes are present and their density increases significantly during the first week after birth (Luther *et al.*, 2001; Metz *et al.*, 1996). α_{1A} - and α_{1B} -AR prevail in the heart of a person and rodents. mRNA expression of all three α_1 -AP subtypes was detected in the heart of the mouse. Moreover, the expression of α_{1B} -AR was higher than the total level of α_{1A} - and α_{1D} -AR in all parts of the heart. However, the level of α_{1A} -adrenergic receptors was slightly higher in the ventricles than in the atria (Zhang *et al.*, 2018).

α_1 -adrenergic receptors are associated with the

variety of secondary messengers through G-proteins, mainly through the proteins of the Gq/11 family, which activate phospholipase C to form inositol triphosphate (IP3) and diacylglycerol. The latter stimulates protein kinase C, and IP3 acts on the IP3 receptors of the endoplasmic reticulum and releases accumulated calcium (Tsirkin & Korotaeva, 2015). The positive inotropic effect of α_1 -adrenergic receptor agonists in the rat heart is realized through the Gs-system and increased production of cAMP, which inhibits the outflow of potassium ions (Gallego *et al.*, 2005). The sensitization of contractile elements by α_1 -AR is mainly conditioned by the activation of Rho kinase (Docherty, 2010).

α_1 -adrenergic receptors cause smooth muscle contraction, and play a major role in the vascular system controlling blood pressure, due to α_{1D} and α_{1A} -adrenergic receptors. In addition to smooth muscle contraction, α_1 -adrenergic receptors can induce endothelium-dependent relaxation of blood vessels (Filippi *et al.*, 2001). It was proved that selective stimulation of

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α 1A-AR in cardiomyocytes potentiates Ca^{2+} current through L-type Ca^{2+} channels. The subtype α 1A-AR mediates positive inotropic responses, while α 1B-AR seems to be associated with contractile function decrease of the heart (Micucci *et al.*, 2019).

According to the scientific literature, alpha-adrenergic receptors in the heart perform regulatory and cardioprotective effects (Zefirov *et al.*, 2018; Zefirov *et al.*, 2016; Chinkin, 2014; Ziyatdinova *et al.*, 2011; O'Connell *et al.*, 2014; Nozdrachev *et al.*, 2016). In contrast to α -AR, the amount of α 1A-AR is maintained or increased slightly in people with heart failure (Beak *et al.*, 2017). At that, the responses of the α 1A receptor subtype remain stable in the myocardium. Thus, α 1A-subtype-mediated inotropy can represent a significant source of inotropic support in the human heart (Janssen *et al.*, 2018).

According to the results of research by some scientists, stimulation of the myocardial α 1-AR has a positive effect, according to others it is negative, and according to the data of others, it has two-phase inotropic effects (Nozdrachev *et al.*, 2016). According to scientists, inotropic responses of α 1-AR are not required for basal contractile function, but can prevent cardiac contractile function decrease during a pathological condition and mediate important protective and adaptive effects (Micucci *et al.*, 2019). Positive inotropic effects of alpha1-adrenergic receptor stimulation by phenylephrine in mice are associated with the α 1A subtype. α 1B-adrenergic receptors can play the trophic role and participate in the expression of other receptor subtypes on the cell surface (Hague *et al.*, 2004). Overexpression of α 1B-AR decreases, and overexpression of α 1A-AR enhances α -AR-mediated myocardial contractility and improves the effects of myocardial infarction (Docherty, 2019).

We have previously shown that non-selective stimulation of α 1-adrenergic receptors by methoxamine inhibited the contractility of the left ventricle of the isolated heart among adult rats (Zefirov *et al.*, 2018). Whereas, selective stimulation of the α 1A receptor subtype by the A-61603 agonist caused negative ventricular inotropy, it positively induced atrial myocardial contractility among 20-week-old rats. Moreover, the severity and duration of inotropic myocardial responses depended on the concentration of the agonist (Khabibrakhmanov *et al.*, 2018).

Against the background of scattered data on the quantitative composition and activity of α 1-adrenergic receptors, as well as the heterogeneity of inotropic

myocardial reactions in response to the activation of these receptors in different species and age groups of animals, we formulated the hypothesis about the presence of age-related features in inotropic myocardial reactions among newborn rats. In relation to the foregoing, the aim of this work was to study the inotropic effect of alpha1A-adrenergic receptor stimulation on the myocardium of the atria and ventricles among newborn rats.

Materials and Methods

The work was carried out on white outbred 6-7 days old rats. For anesthesia, they used 25% solution of urethane at the dose of 800 mg/kg of animal weight. The study was carried out according to the previously described method (Khabibrakhmanov *et al.*, 2018). We studied the inotropic reaction of the myocardium of the heart atria and ventricles in response to the selective stimulation of α 1A-adrenergic receptors. To stimulate α 1A-AR, the selective agonist A-61603 was used at the concentrations of 10⁻⁹–10⁻⁶ mol. The force of contraction was expressed in grams (g). The processing of the data was carried out using the program Acknowledge 4.1. The statistical processing of the results was carried out using t-student test.

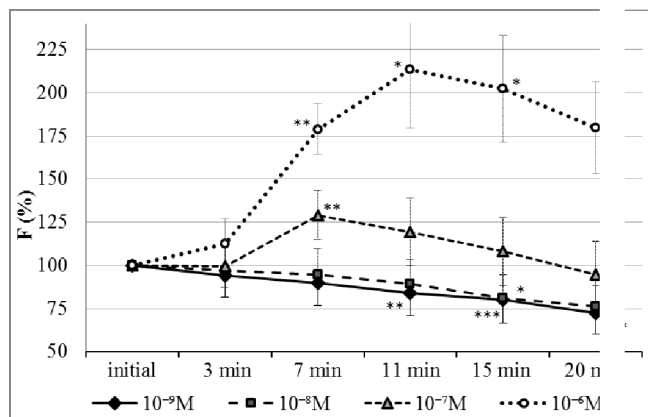
Results and Discussion

The initial values of the atrial contraction strength among newborn rats were in the range of 0.0245 - 0.0396 g.

The stimulation of α 1A-adrenergic receptors by the selective agonist A-61603 with the concentration of 10⁻⁹ mol caused the decrease of contraction force for atrial myocardial strips (n = 6) among newborn rats from 0.0396 ± 0.005 g to 0.0286 ± 0.005 g (p < 0.001), the negative inotropic effect was 28% (Fig. 1). In response to the action of A-61603 at the concentration of 10⁻⁸ mol, the force of the atrial myocardial strip (n = 6) contraction gradually decreased from 0.0348 ± 0.005 g to 0.0265 ± 0.004 g (p < 0.05), the change was 24%.

Higher concentrations of the alpha-1A-adrenergic receptor agonist, A-61603, caused a positive inotropic atrial myocardial response. The introduction of A-61603 at the concentration of 10⁻⁷ mol had a positive inotropic effect on the atrial myocardial strips (n = 6) among 1-week-old rats, which reached the maximum by the 7th minute of the agonist action. At the same time, the values of the contraction force changed from 0.0268 ± 0.003 g to 0.0346 ± 0.004 g (p < 0.01), the effect was 29%. After the positive component of the inotropic effect, there was the tendency to restore the strength of the atrial myocardial contractions (Fig. 1).

A similar dynamics of atrial contractility was also observed with A-61603 at the concentration of 10^{-6} mol ($n = 6$): the force of contraction increased by 113% from 0.0245 ± 0.00393 g to 0.0523 ± 0.0083 g ($p < 0.05$). However, the peak of the positive inotropic effect of $\alpha 1A$ -adrenergic receptor stimulation was reached at the 11th minute of the experimental recording, after which the



(*- $p < 0.05$; **- $p < 0.01$; ***- $p < 0.001$)

Fig. 1: The effect of A-61603 on the strength of atrial myocardial strip contractions among 1-week-old rats.

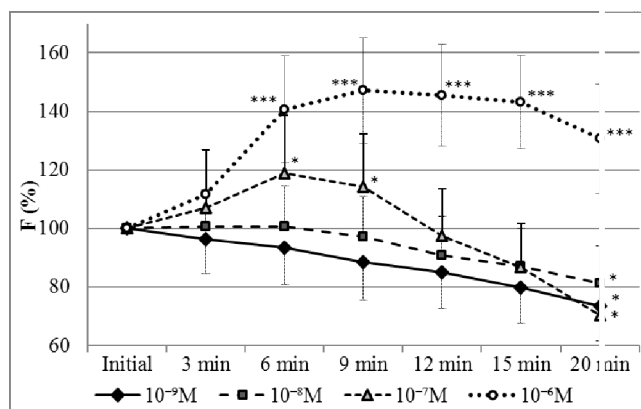
reverse dynamics of atrial contractility was observed.

The initial values of the ventricular strip contraction force were in the range of 0.041 - 0.052 g.

The force of the ventricular myocardial strip contraction ($n = 6$) among newborn rats in response to the action of A-61603 at the concentration of 10^{-9} mol gradually decreased from 0.052 ± 0.006 g to 0.0382 ± 0.006 g ($p < 0.05$), the change was 26%. After administration of A-61603 at a concentration of 10^{-8} mol, the contraction force of the ventricular myocardial strips ($n = 6$) decreased from 0.0505 ± 0.006 g to 0.0411 ± 0.006 g by 19% ($p < 0.05$) (Fig. 2).

The stimulation of $\alpha 1A$ -AP with the application of A-61603 at the concentrations of 10^{-7} and 10^{-6} mol had a positive inotropic effect on the myocardium of the ventricles among rats. The increase of ventricular myocardial contraction force from 0.049 ± 0.009 g to 0.0583 ± 0.01 g ($p < 0.05$) ($n = 6$) continued until the 6th minute of A-61603 introduction at the concentration of 10^{-7} mol, after which the opposite dynamics of indicators was observed. The maximum positive effect was 18%. By the 20th minute of the experiment, the force of ventricular contraction decreased by 30% as compared with the initial data ($p < 0.05$).

The introduction of A-61603 at the concentration of 10^{-6} mol increased the force of the ventricular myocardial strip contraction from 0.041 ± 0.007 g to 0.0607 ± 0.0075



(*- $p < 0.05$; ***- $p < 0.001$)

Fig. 2: The effect of A-61603 on the contraction force of the ventricular strips among 1-week-old rats.

g ($p < 0.001$), the maximum effect was 47% of the initial values. It should be noted that the positive effect was slowly replaced by reverse dynamics, in contrast to the previous concentration of the agonist. By the end of the experimental observation, the force of the ventricular myocardial strip contraction was 30% higher than the initial level ($p < 0.001$) (Fig. 2).

Summary

A-61603 at low concentrations reduces the force of contractions of the atrial and ventricular myocardial strip smoothly. With the concentration increase of the selective agonist, a positive inotropy reaction of both the atria and the ventricles of the rat heart occurs.

Conclusions

The stimulation of alpha ($1A$)-adrenergic receptors depending on the concentration of agonist - A-61603 causes multidirectional inotropic effects on the myocardium of newborn rats. At low concentrations A-61603 smoothly reduces the force of the atrial and ventricular myocardial strip contraction. With the selective agonist concentration increase, a positive inotropy reaction of both the atria and the ventricles of the heart occurs. In addition, the duration of the positive inotropic effect also depended on the concentration of the agonist (Fig. 1.2). The negative inotropic effect of $\alpha 1A$ -AR activation may be based on NO synthesis increase, the activation of the Na^+/Ca^{2+} exchange process and/or the activation of Na^+/H^+ exchanger, the enhancement of the outgoing K^+ current, the inhibition of L-type Ca^{2+} channels, the decrease of myofilament sensitivity to Ca^{2+} . Negative inotropy may also depend on the activity of PKD, which reduces the force of contraction by the phosphorylation of troponin I. A positive inotropic effect can be obtained by the means of PKC by increasing the concentration of

Ca²⁺ inside the cell through L-type Ca channels and IF3 receptors or through PKC-independent activation of myosin light chain kinases (Nozdrachev *et al.*, 2016).

In our previous studies, it was shown that in the ventricular myocardium of adult rats, A-61603 in the concentration range of 10⁻⁹–10⁻⁶ mol causes only a negative inotropic effect (Khabibrakhmanov *et al.*, 2018). Differences in the inotropic responses of the ventricular myocardium to the stimulation of alpha (1A) -adrenoreceptors in adult and newborn rats may depend on the density and characteristics of the signaling mechanisms of these receptors at different stages of postnatal development. The features of the contractile effects of α 1-adrenergic receptors can also be associated with the differences in the structure of contractile proteins and/or involved secondary messengers, such as PKC, which has 15 different isoforms (Hirano *et al.*, 2006). It is possible that if a positive inotropic effect is realized by the interaction of α 1-AP with a Gq protein, then a negative inotropic effect can be obtained by Gi protein activation (O'Connell *et al.*, 2014).

Thus, it should be noted that the nature of the inotropic effects of alpha1-adrenergic receptors may depend not only on the activity of different signaling pathways triggered by both different subtypes and a separate subtype of α 1-adrenergic receptors, but may be associated with the activation and maturity of individual elements of one signal system on different stages of postnatal ontogenesis.

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