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Involvement of alpha-adrenergic receptors in the regulation of inotropy in the isolated rat heart

I.I. Khabibrakhmanov^{1*}, A.L. Zefirov², N.I. Ziyatdinova¹,
M.A. Mukhamedyarov², T.L. Zefirov¹

¹Kazan (Volga Region) Federal University, Kazan, Russia;

²Kazan State Medical University, Kazan, Russia

Abstract

Background. Although activation of α_1 -adrenergic receptors is not required for myocardial contractility, α_1 -adrenergic receptors can provide significant support for myocardial inotropy in various heart diseases.

Aim. To study the participation of α_{1A} - and α_{1B} -adrenergic receptors in the force contraction regulation of the left ventricle of an isolated rat heart.

Material and methods. Isolated hearts of 20-week-old rats were perfused with Krebs–Henseleit solution according to the Langendorff method. The most important indicator of the inotropic function of the heart, the pressure developed by the left ventricle, was recorded using a latex balloon placed in the cavity of the left ventricle. At the 1st stage of the work, the effects of blockers of α_1 -adrenergic receptor subtypes on the force of contractions of an isolated heart were evaluated. At the 2nd stage, the effect of non-selective stimulation of α_1 -adrenergic receptors by methoxamine was studied. At the 3rd stage, the effect of methoxamine was evaluated against the background of selective blockade of α_{1A} - or α_{1B} -adrenergic receptors. Statistical analysis of data was performed using Statistica 6.0 software. The significance of changes between dependent data was assessed using the Wilcoxon test. To assess the differences between two sets of independent data, the Mann–Whitney U-test was used. Changes were considered statistically significant at $p < 0.05$.

Results. Infusion of the α_{1A} -adrenergic blocker WB4101 at a concentration of 10^{-6} mol/l led to an increase in the force of contraction of the isolated heart by 5.6% ($p=0.048$). Blocker of α_{1B} -adrenergic receptors chloroethylclonidine dihydrochloride at a concentration of 10^{-8} mol/l caused a decrease in the force of contraction of the heart by 15% ($p=0.046$). Stimulation of α_{1A} -adrenergic receptors with methoxamine (10^{-8} mol/l) led to a decrease in pressure developed by the left ventricle of an isolated heart by 47% ($p=0.002$). Preliminary blockade of α_{1A} -adrenergic receptors significantly reduced the severity of the negative inotropic effect of methoxamine on the left ventricular myocardium. Preliminary blockade of α_{1B} -adrenergic receptors changed the direction of the inotropic effect of methoxamine on the myocardium of the left ventricle of an isolated heart.

Conclusion. Non-selective stimulation of α_1 -adrenergic receptors by methoxamine leads to a significant decrease in cardiac inotropy, while preliminary blockade of α_{1A} - or α_{1B} -adrenergic receptors reduces the severity of this effect.

Keywords: α_1 -adrenergic receptors, isolated heart, left ventricular pressure, rat.

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Background

Currently, three subtypes of α_1 -adrenergic receptors (α_1 -ARs) are recognized: α_{1A} , α_{1B} , and α_{1D} . However, the functional significance of each subtype in regulating cardiac function remains largely unknown [1]. In the heart, α_1 -ARs comprise 10% of the total number of ARs [2].

The rodent myocardium contains all α_1 -AR subtypes [2]. The density of α_1 -ARs significantly

increases during the early stages of postnatal ontogenesis [3]. In mice, α_{1B} -ARs are present in all examined cardiomyocytes, whereas α_{1A} -ARs are present in only 60% of the isolated cardiomyocytes [1]. In rabbit ventricular myocardium, α_{1B} -AR mRNAs¹ account for 99% of the total number of α_{1B} -ARs, whereas α_{1A} - and α_{1D} -AR mRNAs account for <1% [4].

Literature data suggest the involvement of α_{1A} - and α_{1D} -AR in the adaptive and cardioprotec-

*For correspondence: nafisaz@mail.ru

¹ mRNA, matrix ribonucleic acid

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tive processes in the heart. In coronary endothelial cells, α_{1D} -ARs cause their vasodilation through nitric oxide (NO) synthase activation. Conversely, α_{1A} -ARs may mediate the endothelium-dependent constriction of the coronary lumen [5]. Owing to their high sensitivity to norepinephrine, α_{1D} -ARs demonstrate the fastest response to low-frequency stimulation, whereas the functional role of α_{1A} -ARs is more important at high-frequency stimulation because they have a lower affinity for noradrenaline [5, 6].

In the myocardium, the interaction of catecholamines with α_1 -AR leads to the cleavage of Gq protein, activation of phospholipase C, and the formation of inositol triphosphate (IP_3) as well as diacylglycerol. With IP_3 involvement, calcium is released from the sarcoplasmic reticulum, and diacylglycerol activates protein kinase C [7].

Researchers believe that the effects of α_1 -AR activation are unnecessary for basal cardiac contractile function. Recently, α_1 -ARs have been considered cardioprotective targets; particularly, α_1 -AR may provide significant inotropic support in cardiac diseases [8, 9].

Recently, the nonselective stimulation of α_1 -AR with methoxamine was found to inhibit the contraction rate and coronary flow of the isolated heart in adult rats [10]. By contrast, the selective stimulation of α_1A -ARs by the agonist A-61603 caused negative ventricular inotropy in adult rats; however, it increased ventricular myocardial contractility in neonatal animals. The magnitude and duration of the myocardial inotropic responses were dependent on the agonist concentration [11].

Many studies have indicated the heterogeneity of myocardial inotropic responses involving α_1 -AR in different species and age groups of animals [8, 9]. Studies have also demonstrated the quantitative predominance of α_{1A} - and α_{1B} -ARs in both human and rodent hearts [1, 4, 9, 12].

Aim

Given the above, this study aimed to investigate the involvement of α_{1A} - and α_{1B} -ARs in the regulation of left ventricular (LV) contractile force in the isolated rat heart.

Material and methods

Adult white crossbred rats weighing 250–300 g were used in the experiments. The animals were drugged by intraperitoneal injection of 25% urethane solution at a dose of 800 mg/kg of rat body weight.

The isolated heart was washed with cooled (4–8°C) Krebs–Henseleit solution (NaCl 118 mM, KCl 4.7 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.2 mM, $CaCl_2$

2.5 mM, KH_2PO_4 1.2 mM, and glucose 5.5 mM, pH = 7.3–7.4), then suspended behind the aorta on a cannula, and perfused with the same solution at a constant pressure of 60–65 mm Hg and temperature of 37°C on the Langendorff system (ADInstruments, Australia).

The study was approved by the local ethics committee of Kazan Federal University (Protocol #8 dated May 5, 2015).

The left ventricular developed pressure (LVDP), an indicator of inotropic cardiac function, was recorded using an MLT844 pressure sensor (ADInstruments). A latex balloon connected to the pressure sensor by a Teflon tube was placed in the LV cavity, and filled with distilled water to generate an end-diastolic pressure of 10–12 mmHg. Data were recorded and analyzed using LabChart Pro software version v8 (Australia).

The following Sigma pharmacological preparations were used in the study: methoxamine hydrochloride (10^{-8} mol/L), a nonselective α_1 -AR agonist; WB4101 (10^{-6} mol/L), a selective α_{1A} -AR blocker; and chloroethylclonidine dihydrochloride (10^{-8} mol/L), a selective α_{1B} -AR blocker. Drug concentrations were selected based on literature data and previous studies [10, 13].

In the first stage, the effects of blockers of α_1 -AR subtypes on the LVDP of the isolated heart were evaluated in separate experimental groups relative to baseline values. In the second stage, the effects of nonselective α_1 -AR stimulation with methoxamine were evaluated. In the third stage, the effect of methoxamine together with selective blockade of α_{1A} - or α_{1B} -AR was assessed.

Data are presented as mean $\pm$ error of the mean ($M \pm m$) and expressed in millimeters of mercury (mmHg). Statistical analysis of data was performed using Statistica 6.0. The normality of the sample distribution was assessed by the Shapiro–Wilk W test. The reliability of the effects of α_1 -AR subtype blockers on cardiac inotropic function was tested using the Wilcoxon test for pairwise comparisons. The Mann–Whitney U criterion was used to assess differences between two samples of independent data (between experimental and control groups). Changes at $p < 0.05$ were considered statistically significant.

Results

Effects of α_{1A} - and α_{1B} -adrenergic receptors blockade on the pressure developed by the left ventricle of the isolated rat heart. At 30 s after the perfusion of WB4101 in the concentration of 10^{-6} mol/L ($n = 7$), the LVDP of the isolated rat heart increased from 54.5 ± 4.6 mmHg to 57.5 ± 4.6 mmHg ($p = 0.048$), and the effect was

5.6% (Fig. 1). Thereafter, the index tended to decrease. By the third minute of observation, the LVDP returned to baseline levels. At the seventh minute after the addition of α_{1A} -AR blocker, the mean LVDP in the experimental group decreased to 51.9 ± 3.9 mmHg ($p = 0.426$).

At the third minute of cardiac perfusion with the addition of chloroethylclonidine dihydrochloride (10^{-8} mol/L, $n = 9$) to the solution, the LVDP decreased from 58.8 ± 5.2 mmHg to 53.4 ± 2.8 mmHg ($p = 0.159$). At the seventh minute of the experiment, it decreased to 50 ± 2.8 mmHg ($p = 0.046$), with the maximum negative inotropic effect reaching 15% (Fig. 1).

Effect of methoxamine on LVDP of the isolated rat heart. In the next series of experiments, the infusion of methoxamine (10^{-8} mol/L, $n = 9$) resulted in a significant decrease in the LVDP of the isolated rat heart, with a maximum negative effect of 47% ($p = 0.002$; Fig. 2).

Effect of methoxamine after blockade of α_{1A} - and α_{1B} -adrenergic receptors on the left ventricular developed pressure of the isolated rat heart. The perfusion of methoxamine (10^{-8} mol/L) and WB4101 (10^{-6} mol/L) resulted in a smooth decrease in the LVDP from 51.9 ± 3.9 to 48.2 ± 3.8 mmHg ($p = 0.044$; $n = 9$), with a 7.3% reduction in LVDP. Notably, preliminary α_{1A} -AR blockade significantly reduced the severity of the negative inotropic effect of methoxamine on the LV myocardium of the isolated heart (Fig. 2).

The administration of methoxamine at a concentration of 10^{-8} mol/L during the action of chloroethylclonidine dihydrochloride (10^{-8} mol/L) caused a 5.2% increase in the LVDP from 50 ± 2.8 to 52.6 ± 3.7 mmHg by the second minute of observation ($p = 0.001$; $n = 9$) compared with control (Fig. 2). The preliminary blockade of α_{1B} -AR resulted in a positive inotropic effect of methoxamine on the isolated rat heart. After the third minute of agonist action, the positive dynamics of the LVDP became negative. At the seventh minute of the experiment, the LVDP decreased by 6% of the initial value ($p = 0.049$); however, the severity of this effect was significantly less than that of the control group ($p = 0.002$; Fig. 2).

Discussion

This study demonstrated that the multidirectional inotropic effects on the ventricular myocardium of the isolated rat heart can be realized with the participation of α_1 -AR. During α_{1A} - and α_{1B} -AR blockade, methoxamine caused an insignificant inotropic response of the left ventricle of the isolated rat heart and an insignificant increase in the LVDP after α_{1B} -AR blockade and a decrease after

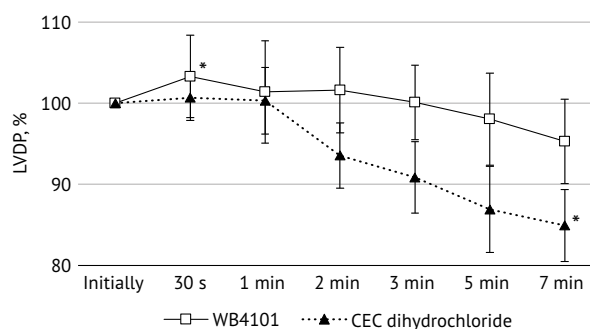


Fig. 1. Dynamics of the left ventricular developed pressure (LVDP) in the isolated rat heart during perfusion with WB4101 and chloroethylclonidine (CEC) dihydrochloride. *Significance of changes compared with baseline.

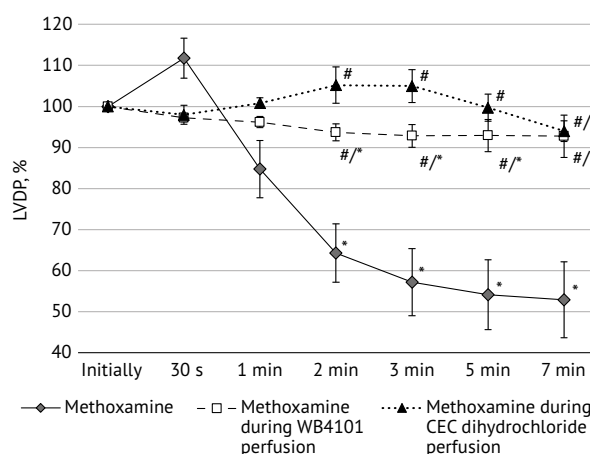


Fig. 2. Dynamics of the left ventricular developed pressure (LVDP) of the isolated rat heart during sequential perfusion with methoxamine after the administration of WB4101 and chloroethylclonidine (CEC) dihydrochloride. *Significance of changes compared with baseline. #Significance of changes compared with the control group (methoxamine).

α_{1A} -AR blockade. The results confirm the hypothesis about the modulating effect of α_1 -AR on cardiac contractile function.

The results of the present study agreed with our previous data [11], and confirmed the thoughts of other researchers that during postnatal ontogenesis in mammals, the suppression of cardiac activity occurs with the participation of α_1 -AR. Based on these data, we can assume that the negative inotropic response of the LV myocardium in adult rats is realized in response to the stimulation of α_{1B} -AR.

This finding is supported by numerous literature data on the quantitative predominance of α_{1B} -ARs over other subtypes in the rodent heart. The positive inotropic response of the LV myocardium is probably realized with the participation of α_{1A} -ARs. However, a similar inotropic response of the ventricular myocardium occurs in response to the stimulation and blockade of the same AR subtype, which requires a more detailed study of these phenomena.

According to literature data, during α_1 -AR stimulation, the positive inotropic effect is realized by the activation of protein kinase C, an increase in Ca^{2+} entry into the cell through L-type membrane calcium channels, and Ca^{2+} transit from the sarcoplasmic reticulum through IF_3 receptors [7]. In turn, diacylglycerol can inhibit Gi proteins associated with m-cholinergic receptors [9]. The negative inotropic effect during α_1 -AR activation can be achieved by increasing NO synthesis, activating the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, activating the Na^+/H^+ exchange mechanism, increasing the K^+ outward current, inhibiting L-type calcium channels, decreasing myofilament sensitivity to Ca^{2+} , etc. [9, 14, 15].

Conclusions

The nonselective stimulation of α_1 -ARs with methoxamine leads to a significant reduction in cardiac inotropy, whereas prior blockade of α_{1A} or α_{1B} -ARs reduces the severity of this effect.

Author contributions. I.I.H. conducted the experiments, collected as well as processed the data, and wrote the text. A.L.Z. and M.A.M. analyzed the obtained data and discussed the results. N.I.Z. reviewed the literature and analyzed the obtained data. T.L.Z. conceived and designed the study as well as discussed the results.

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Conflict of interest. The authors declare no conflict of interest on the submitted article.

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Author details

Insaf I. Khabibrakhmanov, Cand. Sci. (Biol.), Assoc. Prof., Depart. of Human Health Protection, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia; insaf1201@mail.ru; ORCID: <http://orcid.org/0000-0001-5494-6553>

Andrey L. Zefirov, M.D., D. Sci. (Med.), Academician of Russian Academy of Sciences, Prof., Depart. of Physiology, Kazan State Medical University, Kazan, Russia; zefiroval@rambler.ru; ORCID: <http://orcid.org/0000-0001-7436-7815>

Nafisa I. Ziyatdinova, Doct. Sci. (Biol.), Prof., Depart. of Human Health Protection, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia; NIZiyatdinova@kpfu.ru; ORCID: <http://orcid.org/0000-0002-4503-7451>

Marat A. Mukhamedyarov, M.D., D. Sci. (Med.), Assoc. Prof., Head of Depart., Depart. of Physiology, Kazan State Medical University, Kazan, Russia; marat.muhamedyarov@kazangmu.ru; ORCID: <http://orcid.org/0000-0002-0397-9002>

Timur L. Zefirov, M.D., D. Sci. (Med.), Prof., Head of Depart., Depart. of Human Health Protection, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia; Timur.Zefirov@kpfu.ru; ORCID: <http://orcid.org/0000-0001-9557-1639>