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STIMULATION OF α1-ADRENORECEPTORS INHIBITS MYOCARDIAL CONTRACTILITY IN RATS

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Abstract

The exact role of α1-adrenoreceptors (α1-AR) as myocardial contractility modulators is still not clear. We studied the effects of α1-AR stimulation with methoxamine hydrochloride (Sigma) on the myocardial contractility ex vivo and in vitro: on the isolated heart (Langendorff's model) and on the atrium and ventricular myocardium strips in rats, respectively. The experiments were performed on random-bred albino 20-week-old rats ($n = 28$) with the average weight of 200–250 g. The contractility force (F) of myocardium strips was measured in grams (g). The contractility of isolated heart was measured as pressure in the left ventricle (mm Hg). All the studied concentrations of methoxamine (10^{-9}–10^{-6} M) inhibited the contractility of the myocardium strips of rats' atria and ventricles. The stimulation of α1-AR with methoxamine (10^{-9} and 10^{-8} M) led to a decrease in the left ventricular pressure of the isolated heart in rats. The intensity of the negative inotropic effects was proportionate to the agonist concentration.

Keywords: α1-adrenoreceptors, heart, myocardial contractility, rats

Introduction

Adrenoreceptors (AR) play a key role in regulation of the cardiovascular system. There are nine known subtypes of AR: α1A-, α1B-, α1D-, α2A-, α2B-, α2C-, β1-, β2-, and β3-AR [1]. β-AR prevail in the mammalian heart, while α1-AR constitute 10% of all AR. In newborn rats' myocardium, the concentration of α1-AR increases over the first two weeks of postnatal period, while that of α2-AR decreases within first week after birth and then remains low [2]. Three subtypes of α1-AR have been described: α1A-, α1B-, and α1D-AP [3]. α1-AR participate in numerous physiological processes, such as increasing inotropic effects, genes transcription, protein synthesis, glucose metabolism, and inhibition of apoptosis [4].

α1D-AR mediate the sympathetic regulation of blood pressure through vasoconstriction [5]. α1-AR interact with Gq/11 proteins and activate phospholipase C. The latter induces the hydrolysis of phosphatidylinositol triphosphate to produce diacylglycerol (DAG) and inositoltriphosphate (IP3). DAG stimulates protein kinase C (PKC), which phosphorylates intracellular proteins, and inositoltriphosphate stimulates the release of Ca^{2+} from the sarcoplasmic reticulum [6]. PKC and IP3 can affect other intracellular reactions as well. Stimulation of α1-AR expression in the heart can activate both phospholipase C and phospholipase D [7]. PKC has been recently found to activate

protein kinase D (PKD) [8, 9]. PKD decreases the myocardial contractility [9, 10] by phosphorylation of troponin I and also accelerates the relaxation of cardiomyocytes [9]. In cardiomyocytes, PKD phosphorylates myosin-binding protein C, thereby decreasing the sensitivity of monofilaments to Ca^{2+} [11]. PKD activation leads to myocardial hypertrophy and possibly heart failure [8]. The regulation of L-type calcium channels (LTCC) through G proteins, as well as PKC activation, is a complex process which can lead to both stimulation and inhibition of I (Ca) [12]. Activation of α1-AR can also mediate negative inotropic effect though decreased Ca^{2+} current (ICa) though PKC [13]. PKC activation can also increase the permeability of L-type calcium channels (LTCC) [12]. PKC also mediates α1-AR stimulation in myocytes of the blood vessels; it also regulates immune reactions, gene transcription, cell cycle, and cell growth [14]. α1-AR stimulation with methoxamine induced a negative chronotropic effect on rats' myocardium during both in vivo and in vitro experiments [15]. Age-related changes in rats' myocardial contractility with α1-AR blockage have been described as well. The non-selective blockade of α1-AR with prazosin led to bradycardia in pups aged 3 weeks and older, but not in newborn rats [16].

In this study, we evaluated the effects of α1-AR stimulation on the inotropic function of rats' myocardium.

1. Materials and Methods

The heart was isolated from random-bred albino 20-week-old rats ($n = 28$) with the average weight of 200–250 g. The rats were anesthetized intraperitoneally with 25% urethane (800 mg/kg body weight). Myocardial strips (2–3 mm in length and 0.8–1.0 mm in diameter) were cut from the right atrium and right ventricle. The preparation was immersed vertically into a chamber (20 mL) perfused with oxygenated carbogen (97% O_2, 3% CO_2) at 37 °C. The upper end of the strip was fixed to a stainless rod connected to the strain gage, while its lower end was attached to a rubber plate. The strip was stimulated using an ESL-2 electric stimulator via two silver electrodes. The stimulation parameters were as follows: pulse amplitude – 10 mV, duration – 5 msec, and frequency – 6–10 min^{-1}. Initially, the immersed strips were let sit for 40–60 min to optimize the tension. The optimal tension corresponded to the critical stretch beyond which a decrease in the contractility force (F, measured in g) was observed. After the conditioning period, the initial contraction parameters were recorded over 5 min. Methoxamine (MX, an agonist affecting all subtypes of α1-ARs, Sigma) was applied in concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M. The data were processed using the Chart 5 and Statgraphics software (Power Lab platform; AD Instruments). Statistical analysis and evaluation of the significance of differences were performed using Student's *t*-test and the Wilcoxon test (Microsoft Excel). The results were processed using the AcKnowledge 4.1 program on the MP-150 unit (BIOPAC Systems, USA) with the help of the Statgraphics software.

In the ex vivo series of experiments, the heart was rapidly isolated and completely arrested by placing it into cold physiological saline (2–5 °C). The aorta was carefully cannulated to prevent touching and injuring the aortic valve and penetration of the test solution into the left ventricle lumen. The heart was perfused in a Langendorff System (ADInstruments) with the carbogen-oxygenated Krebs–Henseleit solution (containing, mM: 118.0 NaCl, 4.7 KCl, 25.0 $NaHCO_3$, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2

PO_4, and 5.5 glucose; pH 7.3–7.4) at 37 °C. The retrograde perfusion was driven by the constant hydrostatic pressure of 60–65 mm Hg. To stimulate α1-ARs, MX was used at the concentrations of 10^{-9} and 10^{-8} M. To measure the intraventricular pressure, a latex water-filled balloon (V=0.03 mL) was placed into the lumen of the left ventricle via an orifice made posterior to the left atrial auricle. The end-systolic pressure was set at the level of 10–20 mm Hg. The left ventricular pressure was recorded with a MLT844 pressure transducer (ADInstruments). The left ventricular pressure (LVP) was measured in mm Hg. The signals were recorded in a PowerLab 8/35 system (ADInstruments) with the help of the LabChart Pro 8.0 software. The data were processed statistically using Microsoft Excel software and Student's *t*-test

2. Results and Discussion

We started with assessing the contractility (F) of the myocardium strips of atria and ventricles of adult rats' heart in response to the α1-AR-agonist methoxamine applied at the concentrations of 10^{-9}–10^{-6} M.

Ten minutes after adding methoxamine at the concentration of 10^{-9} M ($n = 10$), F of the atria decreased from 0.1212 ± 0.0166 to 0.1138 ± 0.0173 g ($p < 0.05$). The maximal drop in the contractility of the atria by 13.47% was registered 19 min after adding the chemical and equaled 0.10492 ± 0.0166 g ($p < 0.01$). The maximal decrease (by 5.54%) in the F value of the myocardium strips of the ventricles ($n = 12$) from 0.1477 ± 0.0167 g to 0.1395 ± 0.0142 g was registered 15 min after adding the agonist (Fig. 1).

Adding methoxamine at the concentration of 10^{-8} M ($n = 10$) also decreased F of the myocardial strips in 20-week-old rats. The maximal change in the contractility of the atria by 19.3%, from 0.126 ± 0.0171 to 0.1017 ± 0.0162 g ($p < 0.001$), was registered 20 min later. Nineteen minutes after adding methoxamine at the concentration of 10^{-8} M ($n = 12$), F of the ventricular myocardium decreased from 0.2045 ± 0.0164 to 0.1723 ± 0.0124 g ($p < 0.001$).

Methoxamine at the concentration of 10^{-7} M ($n = 8$) caused a decrease in F of the atria from 0.1058 ± 0.015 to 0.0841 ± 0.0137 g ($p < 0.01$). The contractility of ventricular myocardium strips after adding methoxamine at the same concentration (10^{-7} M ($n = 8$)) decreased from 0.1856 ± 0.0157 to 0.1457 ± 0.0116 g ($p < 0.001$), i.e., by 21.5% in total.

F of the isolated myocardium strips of the right atria after adding methoxamine at the concentration of 10^{-6} M ($n = 8$) decreased from 0.0883 ± 0.0137 to 0.0744 ± 0.0138 g ($p < 0.05$). The ventricular myocardium contractility after adding methoxamine at the same concentration decreased by 20.8%, from 0.1544 ± 0.0135 to 0.1221 ± 0.0118 g ($p < 0.001$).

A considerable limitation for accurate assessment of the contractility of the myocardium strip is its fixed position and predetermined parameters of stimulation.

In the second part of the study, the perfusion with methoxamine (10^{-9} and 10^{-8} M) without electric stimulation was used to reveal the effect of α1-AR stimulation on the isolated heart of adult rats. Here again, the Langendorff's technique was applied.

One minute after adding methoxamine at the concentration of 10^{-9} M ($n = 7$), LVP decreased from 56.2 ± 3.8 to 46.2 ± 3.2 mm Hg ($p < 0.01$). In the second minute, LVP decreased to 44.2 ± 2.8 mm Hg ($p < 0.01$). Seven minutes after the methoxamine

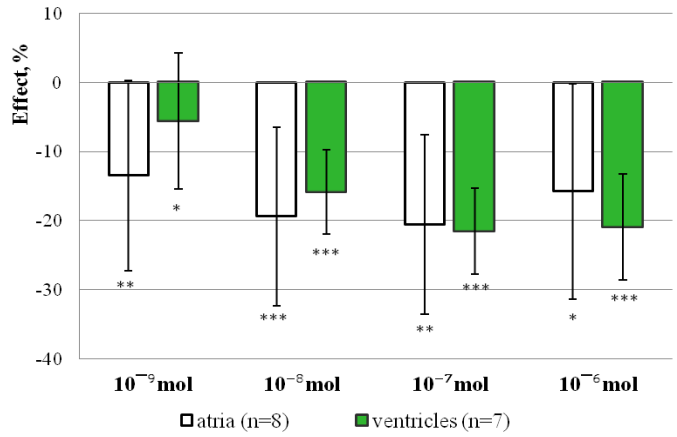

Fig. 1. The influence of α1-AR methoxamine stimulation on the myocardial contractility in 20-week-old rats. *Y*-axis is the effect, % of the original values; *X*-axis is the concentration (mol). Note: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in comparison with the initial level. Original values are the values of myocardial contractility before methoxamine administration

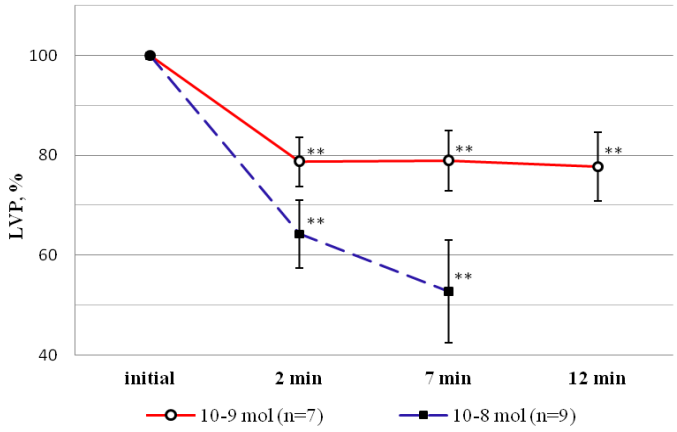

Fig. 2. The effect of methoxamine on LVP of the hearts isolated from 20-week-old rats. *Y*-axis is LVP (%); *X*-axis is time (min). Note: ** is the reliability in comparison with the original values: $p < 0.01$. Original values are the values of LVP before methoxamine administration. ** – $p < 0.01$

administration, LVP equaled 44.4 ± 3.38 mm Hg ($p < 0.01$). The maximal decrease in the left ventricular contractility (43.7 ± 3.88 mm Hg ($p < 0.01$) was registered after 12 min; this value was 22.2% lower than the initial pressure (Fig. 2).

Two minutes after the perfusion of the isolated heart with 10^{-8} M ($n = 9$) of methoxamine, a decrease in the ventricular pressure from 64 ± 5.2 to 41.16 ± 4.4 mm Hg ($p < 0.01$) was observed. In the fifth minute, the pressure went down to 34.6 ± 5.8 mm Hg ($p < 0.01$). The maximal decrease of the LVP value by 37%, 33.8 ± 6.6 mm Hg ($p < 0.01$), was registered in the seventh minute. Further observations were not possible due to the absence of contractile activity.

Conclusions

The exact role of α1-AR as modulators of the myocardium contractility is not fully understood. One of the reasons may be that chatecholamines activate numerous intracellular targets which can affect the myocardium in opposite ways [12, 13].

Although α1-AR contribute to merely 10% of all adrenergic receptors in the mammalian heart, they still play an important role in its regulation. It is widely believed that α1-AR normally enhance the myocardium contractility, but the opposite effect – a decrease in the contractility force – is possible [17]. In our experiments, all the studied concentrations of methoxamine induced a negative effect on the atria and ventricular contractility in 20-week-old rats. The non-selective stimulation of α1-AR with methoxamine caused a negative inotropic reaction of the isolated left ventricle in adult rats. The intensity of the negative inotropic effect was determined by the agonist concentration. In our prior experiments, we discovered that α1-AR stimulation with methoxamine decreases the contractility in the isolated heart [5]. α1-AR in the heart are stimulated by postganglionic sympathetic neurons and further mediate the signaling pathways activated by Gq/11 proteins. A decrease in the myocardium contractility with α1-AR activation may be secondary to that of the Ca^{2+} current (ICa) due the activation of PKC [13]. It is possible that α1-AR participate in a more delicate regulation of the cardiac function and most likely effects of the stimulation depend on the activity of other receptors and various intracellular systems.

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References

1. Brodde O.E., Bruck H., Leineweber K. Cardiac adrenoceptors: Physiological and pathophysiological relevance. *J. Pharmacol. Sci.*, 2006, vol. 100, no. 5, pp. 323–337. doi: 10.1254/jphs.CRJ06001X.
2. Metz L.D., Seidler F.J., McCook E.C., Slotkin T.A. Cardiac alpha-adrenergic receptor expression is regulated by thyroid hormone during a critical developmental period. *J. Mol. Cell. Cardiol.*, 1996, vol. 28, no. 5, pp. 1033–1044. doi: 10.1006/jmcc.1996.0096.
3. Jensen B.C., O'Connell T.D., Simpson P.C. Alpha-1-adrenergic receptors: Targets for agonist drugs to treat heart failure. *J. Mol. Cell. Cardiol.*, 2011, vol. 51, no. 4, pp. 518–528. doi: 10.1016/j.yjmcc.2010.11.014.
4. Simpson P. Lessons from knockouts: The alpha1-ARs. In: Perez D.M. (Ed.) *The Adrenergic Receptors in the 21st Century*. Totowa, N. J., Hum, Press. 2006, pp. 207–240.
5. Tanoue A., Nasa Y., Koshimizu T., Shinoura H., Oshikawa S., Kawai T., Sunada S., Takeo S., Tsujimoto G. The α_{1D}-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J. Clin. Invest.*, 2002, vol. 109, no. 6, pp. 765–775. doi: 10.1172/JCI14001.
6. Hirano S., Kusakari Y., O-Uchi J., Morimoto S., Kawai M., Hongo K., Kurihara S. Intracellular mechanism of the negative inotropic effect induced by alpha1-adrenoceptor stimulation in mouse myocardium. *J. Physiol. Sci.*, 2006, vol. 56, no. 4, pp. 297−304. doi: 10.2170/physiolsci.RP007306.

7. Tsirkin V.I., Korotaeva Yu.V. The role of protein kinase A, B, C and D in the regulation of cardiomyocyte contractility (Review). Report I. *Vestn. Sev. (Arkt.) Fed Univ. Ser. Med.-Biol. Nauki*, 2015, no. 2, pp. 53-61. doi: 10.1042 / BJ20021626.
8. Fu Y., Rubin C.S. Protein kinase D: Coupling extracellular stimuli to the regulation of cell physiology. *EMBO Rep.*, 2011, vol. 12, no. 8, pp. 785−796. doi: 10.1038/embor.2011.139.
9. Haworth R.S., Cuello F., Avkiran M. Regulation by phosphodiesterase isoforms of protein kinase A-mediated attenuation of myocardial protein kinase D activation. *Basic Res. Cardiol.*, 2011, vol. 106, no. 1, pp. 51−63. doi: 10.1007/s00395-010-0116-1.
10. Stathopoulou K., Cuello F., Candasamy A.J., Kemp E.M., Ehler E., Haworth R.S., Avkiran M. Four-and-a-half LIM domains proteins are novel regulators of the protein kinase D pathway in cardiac myocytes. *Biochem. J.*, 2014, vol. 457, no. 3, pp. 451−461. doi: 10.1042/BJ20131026.
11. Bardswell S.C., Cuello F., Rowland A.J., Sadayappan S., Robbins J., Gautel M., Walker J.W., Kentish J.C., Avkiran M. Distinct sarcomeric substrates are responsible for protein kinase D-mediated regulation of cardiac myofilament Ca^{2+} sensitivity and cross-bridge cycling. *J. Biol. Chem.*, 2010, vol. 285, no. 8, pp. 5674−5682. doi: 10.1074/jbc.M109.066456.
12. Kamp T.J., Hell J.W. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. *Circ. Res.*, 2000, vol. 87, no. 12, pp. 1095−1102. doi: 10.1161/01.RES.87.12.1095.
13. Nishimaru K., Tanaka Y., Tanaka H., Shigenobu K. Pharmacological evidence for involvement of phospholipase D, protein kinase C, and sodium-calcium exchanger in alpha-adrenoceptor-mediated negative inotropy in adult mouse ventricle. *J. Pharmacol. Sci.*, 2003, vol. 92, no. 3, pp. 196–202. doi: 10.1254/jphs.92.196.
14. Rang H. *Pharmacology*. Edinburgh, Churchill Livingstone, 2003. xii, 796 p.
15. Zefirov T.L., Khabibrakhmanov I.I., Ziyatdinova N.I., Zefirov A.L. Peculiar aspects in influence of α_1-adrenoceptor stimulation on isolated rat heart. *Bull. Exp. Biol. Med.*, 2016, vol. 162, no. 1, pp. 4–6. doi: 10.1007/s10517-016-3530-z.
16. Ziatdinova N.I., Zefirov A.L., Zefirov T.L. Opposite changes in cardiac chronotropy induced by selective blockade of α_{1A}-adrenoceptors in rats of different age. *Bull. Exp. Biol. Med.*, 2011, vol. 152, no. 1, pp. 19-21. doi: 10.1007/s10517-011-1442-5.
17. Myslivecek J, Nováková M, Klein M. Receptor subtype abundance as a tool for effective intracellular signaling. *Cardiovasc. Hematol. Disord.: Drug Targets*, 2008, vol. 8, no. 1, pp. 66–79. doi: 10.2174/187152908783884939.

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Стимуляция α1-адренорецепторов ингибирует сократимость миокарда у крыс

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Аннотация

Влияние стимуляции α1-адренорецепторов на сократимость миокарда до сих пор не изучено в полной мере. В данной работе обсуждаются результаты исследования воздействия метоксамина гидрохлорида (Sigma) на сократимость миокарда у крыс, полученные в ходе экспериментов ex vivo (изолированное сердце, модель Лангендорфа) и in vitro (изолированные полоски миокарда предсердий и желудочков). Эксперименты проводились на белых беспородных крысах 20-недельного возраста (n = 28), средняя масса тела которых составляла 200–250 г. Силу сокращения (F) полосок миокарда выражали в граммах (г), а сократимость изолированного сердца понимали как кровяное давление в левом желудочке (мм рт. ст.). Метоксамин во всех концентрациях (10^{-9}–10^{-6} М) ингибировал сократимость полосок миокарда предсердий и желудочков. Стимуляция α1-АР метоксамином (10^{-9} и 10^{-8} М) приводила к снижению кровяного давления в левом желудочке изолированного сердца. Интенсивность негативных инотропных реакций миокарда была пропорциональна концентрации метоксамина.

Ключевые слова: α1-адренорецепторы, сердце, сократимость миокарда, крысы

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