Research Article

The effect of α_1-adrenergic receptor subtypes blockade on the rat myocardium inotropy

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ABSTRACT

Aim: It is believed that catecholamines (epinephrine and norepinephrine) more activate β_1-adrenergic receptor (AR), β_2-AR, and α_1-AR. Although the density of α_1-AR compared to β-AR is lower, they play an important role in the regulation of heart function. **Materials and Method:** The literature presents various data on the role of α_1-AR in the regulation of blood circulation. Different authors, with the activation of α_1-AR, obtained both positive and negative inotropic effects on the heart. The effect of blockade of α_1-AR subtypes on the cardiac chronotropy in rats of different ages is shown in vivo. In connection with the lack of selective agonists α_{1B}-and α_{1D}-AR, researchers increasingly use blockers of these receptor subtypes. In the literature, there are no convincing descriptions of the effects of blockers of α_1-AR on the inotropic function of myocardium in rats. In connection with this, we studied the effect of blockade of different α_1-AR subtypes on the contractility of isolated strias of the myocardium atrium and ventricles of adult rats. **Result and Discussion:** The results of this study showed that all subtypes of α_1-AR are involved in the regulation of myocardial contractility at both the auricles and ventricles in rats. The blocker α_{1A}-AR WB4101 reduces the contractility of the atrial myocardium but leads to multidirectional ventricular contractility effects depending on the concentration. **Conclusion:** Summing up the studies of the effect of selective blockade of different α_1-AR subtypes, it can be concluded that all subtypes of α_1-AR are involved in the regulation of myocardial contractility of both the atria and ventricles of rats. The blockade of α_{1B}-and α_{1D}-AR has only a negative inotropic effect on the myocardium of the atria and ventricles.

KEY WORDS: Heart, Inotropy, Rat, α1-adrenergic receptors

INTRODUCTION

Adrenergic control of the heart is implemented through receptor structures, which differ in the variety of physiological responses during their activation. There are six subtypes of α- and three subtypes of β-adrenergic receptor (AR) known.[1] It is believed that catecholamines (epinephrine and norepinephrine) more activate β_1-AR, β_2-AR, and α_1-AR. The mRNA analysis showed that the α_1-AR density in the heart is about 30 times higher than that of α_2-AR.[2] All known α_1-AR subtypes were detected at mRNA level in the heart of mouse, rat, and human. However, binding sites are present only for α_{1A}- and α_{1B}-AR.[3] The α_{1A}-AR mRNA level in cardiomyocytes is 65% of the total α-AR mRNA. Despite the presence of the α_{1D} mRNA, the receptor is not identified in the rodent cardiomyocytes.[4] In humans, the α_{1D}-AR is present in the smooth muscle cells of the coronary arteries. The α_{1D} subtype level is 75% of the total α_1-AR protein.[5] α_{1B}-AR predominates in human coronary endothelial cells, which regulate vasodilation and angiogenesis.[6,7] α_1-AR send their signals through Gq/FLSβ1-IF3/PKC system, but further signaling pathways are diverse.[8,9] To date, it is known that there are 15 isoforms of PKS.[10] α_1-AR can activate both phospholipase C and phospholipase D. α_{1B}-AR can also activate the Gi protein system.[11,12] Although the density of α_1-AR compared to β-AR is lower, they play an important role in the regulation of heart function. The literature has various data on the role of α_1-AR in the regulation of blood circulation. Different authors, with the activation of α_1-AR, obtained both positive and negative inotropic effects. The scientists of our team showed the effect of α_1-AR blockade on the cardiac chronotropy in rats during ontogenesis. Non-selective blockade with α_1-AR prazosin resulted in a decrease in rat heart rate since the age of 3 weeks and did not

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affect the heart rate of newborn rats.[13] It has been shown that selective blockade of α_{1A}-ARs leads to an increase in cardiac activity in newborn rats, and in the remaining age groups of animals, it causes bradycardia. In addition, the severity of the chronotropic response increased with age.[14] The studies of the effect of α_{1B}-AR blockade revealed a significant decrease in heart rate in rats of 6 and 20 weeks of age,[15] and selective blockade of α_{1D}-AR led to bradycardia of the rat heart at all stages of postnatal ontogenesis.[16] Based on the results obtained, the researchers note that different subtypes of α_1-ARs have the opposite functional significance in the development of mechanisms for the regulation of cardiac activity.[13] In our recent studies, we showed that non-selective stimulation of α_1-ARs has a negative chronotropic effect on the heart of adult rats.[17] In connection with the lack of selective agonists α_{1B}- and α_{1D}-AR, researchers increasingly use blockers of these receptor subtypes. The literature presents no convincing descriptions of the effects of blockers of α_1-AR subtypes on the inotropic function of myocardium in rats. In connection with the foregoing, the objective of the research was to study the effect of blockade of different subtypes of α_1-AR on the inotropic function of the atrial and ventricular myocardium of rats.

METHODS

The rats were anesthetized with intraperitoneal injection of urethane at a dose of 800 mg/kgw. The heart was quickly isolated and placed in a tray with a process solution with two stimulating electrode connected, and the myocardium striae of 4.5 mm long and 0.8-1.0 mm in diameter were cut out from the right atrium and right ventricle. Isolated striae of the atrial and ventricular myocardium were placed in a tray (V = 20 ml) with a process solution. The process solution pre-oxygenated with carbogen had the following composition: NaCl - 7.6 g, KCl - 0.42 g, NaH_2PO_4 - 0.07 g, $MgCl_2$ - 0.104 g, $CaCl_2$ - 0.2 g, $NaHCO_3$ - 1.7 g, glucose - 2 g, Trizma base - 0.25 g/l, pH = 7.3-7.4, and T = 37°C. The striae of the myocardium were fixed vertically, with their top to the mechanical sensor, and the bottom to the hook of the holder. The preparations were stimulated by an electrical signal through 2 silver electrodes with a frequency of 6 stimuli per minute, amplitude of 10 mV, and a duration of 5 ms. After immersing the preparation in the tray, an optimal tension was gradually applied to the muscle fibers, and for 40-60 min, the stabilization of the contractions proceeded. At the end of the study, the initial values of the contractions were recorded for 3 min, and then, for 20 min with the addition of pharmacological preparations in the process solution. Subsequent concentrations of drugs were added only after the final washing with the working solution of the substance and stabilization of the contractions of the myocardial striae. The pharmacological agents used were α_{1A}-AR WB4101-blocker, α_{1B}-AR chloroethyl-clonidine blocker, and α_{1D}-AR BMY7378-blocker (all drugs manufactured by Sigma). The reaction of the contractile force was calculated in response to the action of the pharmacological agents in relation to the initial values. The reduction force (F) was expressed in grams (g). The work was carried out on the MP-150 installation (BIOPAC Systems, USA). The data were recorded and processed on a personal computer using Acknowledge 4.1 software.

RESULTS

Effect of WB4101 on Myocardial Contractility of 20-week-old Rats

The introduction of the α_{1A}-AR WB4101 blocker into the process solution at a concentration of 10^{-9} M ($n = 7$) did not cause significant changes in the contractility of the atrial myocardium striae. The contractility of ventricular myocardium striae at introduction of WB4101 at a concentration of 10^{-9} M ($n = 8$) tended to increase; however, these changes in contractility were not reliable (Figure 1).

At the 10th min, after the administration of WB4101 at a concentration of 10^{-8} M ($n = 7$), the contractile force of the atrial myocardium striae decreased from 0.094 ± 0.0184 g to 0.0837 ± 0.01667 g ($P < 0.05$), at the 19th min - to 0.074 ± 0.0133 g ($P < 0.05$). At the 20th min, the contractility of the atrial striae was 0.0744 ± 0.0135 g ($P < 0.05$). The contractile force of the ventricular myocardium striae 5 min after the administration of WB4101 at a concentration of 10^{-8} M ($n = 7$) increased from 0.2032 ± 0.0414 g to 0.2139 ± 0.0422 g ($P < 0.01$) (Figure 1). By the 10th min of the experiment, the ventricular contractile force was 0.2003 ± 0.0411 g, and by the 20th min, 0.1879 ± 0.0384 g.

10 min after the administration of WB4101 at a concentration of 10^{-7} M ($n = 7$), the contractile force of the atrial myocardium striae decreased from 0.0732 ± 0.0173 g to 0.0617 ± 0.0153 g ($P < 0.05$), and by the 20th min, to 0.056 ± 0.0133 g ($P < 0.05$). After the administration of WB4101 at a concentration of 10^{-7} M ($n = 6$), the contractile force of the ventricular myocardium striae of 20-week-old rats decreased from 0.11664 ± 0.0186 g to 0.1385 ± 0.0179 g; however, these changes in the contractility were not reliable (Figure 1).

At the 10th min after the administration of WB4101 at a concentration of 10^{-6} M ($n = 5$), the contractile force of the atrial myocardium striae of 20-week-old rats slightly decreased from 0.072 ± 0.019 g to 0.0614 ± 0.0158 g. The maximum decrease in the contractility of the atrial striae to 0.0524 ± 0.0141 g ($P < 0.05$)

was observed at the 20th min of the experiment and was 27.2%. By the 20th min after the administration of WB4101 at a concentration of 10^{-6} M (n = 6), the contractile force of the ventricular myocardium striae decreased from 0.1328 ± 0.021 g to 0.116 ± 0.0185 g ($P < 0.01$), and the change was 12% of the initial value (Figure 1).

The Effect of Chloroethyl-clonidine on Myocardial Contractility of 20-week-old Rats

10 min after the administration of chloroethyl-clonidine at a concentration of 10^{-9} M (n = 8), the contractile force of the atrial myocardium striae decreased from 0.0768 ± 0.0112 g to 0.0698 ± 0.011 g ($P < 0.01$), and by the 20th min, to 0.0633 ± 0.0117 g ($P < 0.001$). By the 11th min after the administration of chloroethyl-clonidine at a concentration of 10^{-9} M (n = 10), the contractility of the ventricular myocardium striae decreased from 0.1757 ± 0.034 g to 0.1684 ± 0.0329 g ($P < 0.05$), and by the 20th min, to 0.1584 ± 0.0309 g ($P < 0.01$) (Figure 2).

At the 10th min after the administration of chloroethyl-clonidine at a concentration of 10^{-8} M (n = 8), the contractile force of the atrial myocardium striae decreased from 0.082 ± 0.0125 g to 0.0721 ± 0.0123 g ($P < 0.001$). At the 20th min of the experiment, the maximum decrease in myocardial contractility was reduced to 0.0641 ± 0.0118 g ($P < 0.001$), the change was 22%. 10 min after the administration of chloroethyl-clonidine at a concentration of 10^{-8} M (n = 7), the contractile force of ventricular myocardium striae decreased from 0.1348 ± 0.0233 g to 0.1246 ± 0.024 g ($P < 0.01$), and by the 20th min of the experiment, it reached 0.113 ± 0.0234 g ($P < 0.001$) (Figure 2).

11 min after the administration of chloroethyl-clonidine at a concentration of 10^{-7} M (n = 7), the contractility of the atrial myocardium striae was reduced from 0.064343 ± 0.0135 g to 0.06021 ± 0.0132 g ($P < 0.01$), and after 19 min, to 0.05536 ± 0.0131 g ($P < 0.01$). At the 20th min of observation, the atrial contractility was 0.05567 ± 0.013 g ($P < 0.01$). 10 min after the administration of chloroethyl-clonidine in a concentration of 10^{-7} M (n = 7) into the process solution, the contractile force of the ventricular myocardium striae decreased from 0.1164 ± 0.0204 g to 0.1043 ± 0.0202 g ($P < 0.001$), and after 20 min, to 0.09554 ± 0.019 g ($P < 0.001$) (Figure 2).

At the 11th min after the administration of chloroethyl-clonidine at a concentration of 10^{-6} M (n = 7), the contractile force of the atrial myocardium striae decreased from 0.0589 ± 0.0146 g to 0.05574 ± 0.0145 g ($P < 0.05$), and by the 20th min of observation, the contractility decreased to 0.0513 ± 0.0136 g ($P < 0.01$). 10 min after the administration

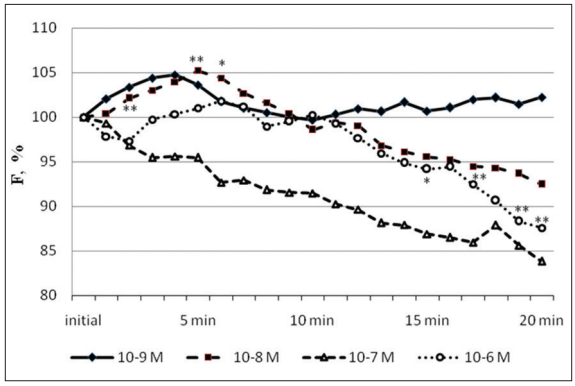

Figure 1: Changes in the contractile force of the ventricular myocardium after the blockade of α_{1A}-adrenergic receptors. Y-axis - myocardium striae contractile force (F, %), X-axis - experiment recording time (minutes). Note: The significance of differences as compared with initial values: *$P < 0.05$; **$P < 0.01$

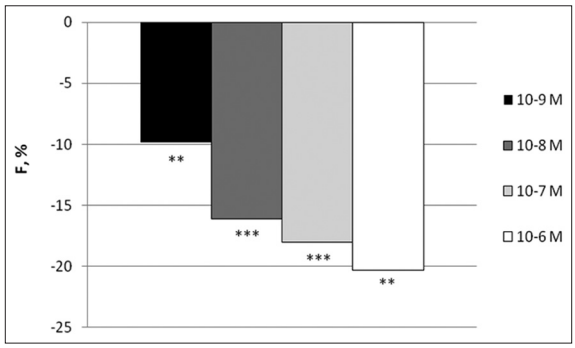

Figure 2: Changes in the contractile force of the ventricular myocardium after the blockade of α_{1B}-adrenergic receptors. Y-axis - myocardium striae contractile force (F, %), X-axis - experiment recording time (minutes). Note: Data reliability as compared with initial values: **$P < 0.01$; ***$P < 0.001$

of chloroethyl-clonidine at a concentration of 10^{-6} M (n = 7), the contractile force of the ventricular myocardium striae of 20-week-old rats reduced from 0.10504 ± 0.0175 g to 0.099687 ± 0.0172 g ($P < 0.05$). The maximum decrease in the contractile force up to 0.0837 ± 0.0168 g ($P < 0.01$) was recorded at the 20th min and was 20.7% (Figure 2).

The Effect of BMY7378 on Myocardial Contractility of 20-week-old Rats

10 min after the administration of BMY7378 at a concentration of 10^{-9} M (n = 8), the contractility of the atrial myocardium striae decreased from 0.0842 ± 0.01577 g to 0.0752 ± 0.01401 g ($P < 0.01$). The maximum decrease in contractility up to 0.071 ± 0.01285 g ($P < 0.01$) was observed at the 19th min of the experiment. At the 20th min of the experiment, the atrial contractility was 0.0712 ± 0.0129 g ($P < 0.01$). 12 min after the administration of BMY7378 at a concentration of 10^{-9} M (n = 8), the contractility of ventricular myocardium striae decreased from 0.1385 ± 0.0353 g

to 0.1344 ± 0.0356 g ($P < 0.05$), and by the 20th min, decreased to 0.127 ± 0.034 g ($P < 0.01$) (Figure 3).

10 min after the administration of BMY7378 at a concentration of 10^{-8} M ($n = 8$), the contractility of the atrial myocardium striae decreased from 0.0854 ± 0.0129 g to 0.0788 ± 0.0128 g ($P < 0.001$), and after 20 min, decreased to 0.071 ± 0.0125 g ($P < 0.001$). 10 min after the administration of BMY7378 at a concentration of 10^{-8} M ($n = 8$), the contractility of ventricular myocardium striae in rats decreased from 0.1348 ± 0.0365 g to 0.1278 ± 0.0357 g ($P < 0.01$). At the 20th min of the experiment, the maximum reduction in contractility was recorded at the point of 0.1183 ± 0.0345 g ($P < 0.01$), and the change was 12% of the initial value (Figure 3).

10 min after the administration of BMY7378 at a concentration of 10^{-7} M ($n = 7$), the contractility of the atrial myocardium striae decreased from 0.073 ± 0.0167 g to 0.0629 ± 0.0153 g ($P < 0.01$). At the 19th min of the experiment, the contractility decreased up to 0.0587 ± 0.0133 g ($P < 0.05$). At the 20th min, the contractility was 0.059 ± 0.0131 g ($P < 0.05$). The contractility of ventricular myocardium striae at the 9th min after the administration of BMY7378 at a concentration of 10^{-7} M ($n = 8$) decreased from 0.1086 ± 0.031 g to 0.1055 ± 0.0307 g ($P < 0.05$) (Figure 3). At the final 20th min of the experiment, the contractility was 0.10198 ± 0.0314 g.

At the 10th min of the action of BMY7378 at a concentration of 10^{-6} M ($n = 6$), the contractility of the atrial myocardium striae decreased from 0.0621 ± 0.0158 g to 0.0534 ± 0.0145 g ($P < 0.01$). The maximum decrease in the contractility up to 0.0497 ± 0.0148 g ($P < 0.01$) was recorded at the 18th min of administration and was 20% of the initial value. 10 min after the administration of BMY7378 at a concentration of 10^{-6} M ($n = 6$), the contractility of ventricular myocardium striae decreased from 0.0957 ± 0.0301 g to 0.088 ± 0.0274 g ($P < 0.05$). The maximum decrease in the contractility up to 0.0844 ± 0.0273 g ($P < 0.05$) was recorded at the 20th min of the experiment and was 12% of the initial value (Figure 3).

SUMMARY

Thus, high concentrations of WB4101 reduced the contractile force of the atrial myocardium. Different concentrations of WB4101 had multidirectional effects on the contractility of the ventricular striae of the 20-week-old rats. In response to lower concentrations of the blocker, a slight positive response of myocardial contractility was observed, which was then replaced by the opposite effect. WB4101 at a concentration

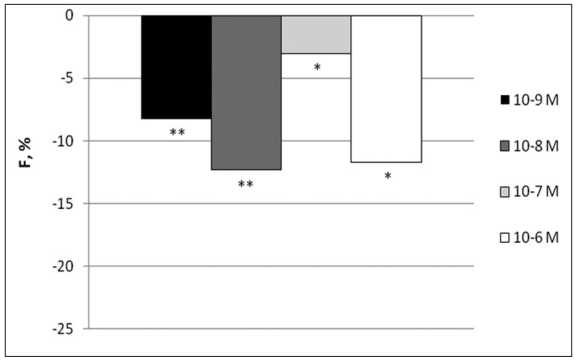

Figure 3: Changes in the contractile force of the ventricular myocardium after the blockade of α_{1D}-adrenergic receptors. Y-axis - myocardium striae contractile force (F, %), X-axis - experiment recording time (minutes). Note: The significance of differences as compared with initial values: *$P < 0.05$; **$P < 0.01$

of 10^{-6} M reduced the contractility of the ventricular myocardium. Chloroethyl-clonidine and BMY7378 at all the concentrations studied led to a decrease in the contractile force of the atrial and ventricular myocardium of the rats.

CONCLUSION

Summing up the studies of the effect of selective blockade of different α_1-AR subtypes, it can be concluded that all subtypes of α_1-AR are involved in the regulation of myocardial contractility of both the atria and ventricles of rats. At the same time, it should be noted that blockade of all three subtypes of α_1-ARs in the atria led to a negative inotropic effect. The blockade of α_{1B}- and α_{1D}-ARs in the ventricles also caused a negative inotropic effect. Blockade of α_{1A}-ARs in the ventricles caused both negative and positive inotropic effect, depending on the concentrations administered. Earlier, we showed opposite chronotropic effects after blocking α_{1A}-ARs in rats of different ages.[14] There are several possible mechanisms of the opposite effect of α_{1A}-AR blockade on rat myocardial contractility. First, membrane receptors can be linked to several different G-proteins. α_1-AR signal can be linked through Gq proteins, and can also activate the Gi protein system.[8,9,11,12] Selective interaction of the receptor with any of G-proteins may have the opposite effect on the systems of secondary mediators and/or different ion channels. Second, the presence of various intracellular enzymes should be considered. It is known that the long-term effect of stimulation of a_1-AR is the activation of protein kinase C, numerous isoforms of which are revealed in the heart. To date, it is known that there are 15 isoforms of PKC.[8-10] In addition, α_1-AR can activate both phospholipase C and phospholipase D. All these factors can modulate the activity of various effectors, ultimately determining the contractile effect.

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