

Age peculiarities of the blockade of α_2 -adrenoceptors in the formation of the heart sympathetic innervations

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ABSTRACT

Aim: α_2 -adrenoceptors (ARs) in the heart of mammals modulate various regulatory effects. Located on the presynaptic membrane, α_2 -ARs regulate the release of noradrenaline and epinephrine from the nerve terminals. Wide expression of this type of ARs in various tissues and organs mediates various physiological and pharmacological effects in the cardiovascular system. α_2 -AR subtypes have been identified in the human myocardium based on pharmacological analysis and molecular cloning. **Material and Methods:** However, there are insufficient data on the study of the influence of this AR subtype in the cardiac regulation. The study involved a series of experiments with a α_2 -AR blockade by a specific blocker yohimbine. The experiments were performed on the striae of the myocardium of the right atrium and ventricles of rats of 3, 6, and 20 weeks of age. **Results:** The results obtained indicate that the α_2 -AR specific blockade reduces the contractile force of the atrial and ventricular myocardium striae of rats of 20 weeks of age. The 3-week-old rats had a reduced contractile force in the atria and a steady contractile force in the ventricles. In 6-week-old rats, the contractile force in the atria did not change but decreased in the ventricles. **Conclusion:** It is possible that during the formation of the sympathetic cardiac innervation, α_2 -ARs regulate the contractile function of the heart muscle only in some of its departments.

KEY WORDS: α_2 -adrenoceptors, Inotropy, Ontogenesis, Sympathetic cardiac innervation

INTRODUCTION

Sympathetic nerves affect the heart by releasing mediators - norepinephrine and epinephrine and interacting with adrenoceptors (ARs) located on the outer membrane of cardiomyocytes. In the adrenergic system, the biological signals of norepinephrine and epinephrine affect cardiomyocytes through three different receptor families: α_1 -AR, α_2 -AR, and β -AR. Currently, there are nine confirmed subtypes of ARs: α_{1A} -, α_{1B} -, α_{1D} -, α_{2A} -, α_{2B} -, α_{2C} -, β_1 -, β_2 -, and β_3 -ARs.^[1,2]

It is believed that α_2 -ARs in the heart of mammals modulate regulatory effects. α_2 -ARs are located mainly on the presynaptic membrane and regulate the release of norepinephrine and epinephrine from nerve terminals.^[3] All subtypes of α_2 -ARs are interacting with G_i/G_o proteins decrease the activity of adenylate cyclase, which reduces the amount of intracellular cyclic adenosine monophosphate.^[3,4]

α_2 -ARs are one of the first G-protein receptors identified in the human and mouse genome.^[5] All α_2 -AR subtypes have been identified based on pharmacological analysis and molecular cloning.^[6] Immunoblotting assay revealed α_2 -ARs in cardiac tissue of rats.^[7] The method of real-time polymerase chain reaction (PCR, real-time PCR) used in the studies at the mRNA level, immunoblotting, and cytochemical assays with isolated cardiomyocytes confirmed the presence of all three α_2 -AR subtypes. The mRNA of all α_2 -AR subtypes was also detected in the human heart by PCR.^[8] These three subtypes are widely expressed in various tissues and organs, and mediate various physiological and pharmacological effects in the cardiovascular system.^[2,9] During the stimulation of α_2 -AR, a wide range of the effects of agonists of these receptors - hypotension and bradycardia, anesthetic effect, hypothermia, and sedation.^[10] Studies in mice with blockade of separate α_2 -AR subtypes have shown that only a few functions proceed with the participation of one subtype. Most of the physiological and pharmacological functions of α_2 -ARs were assigned to the α_{2A} -AR subtype. It is known that α_{2A} subtype of AR mediates hypotension, bradycardia, and modulates

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baroreflex sensitivity.^[11,12] The participation of α_2 -AR subtypes was also shown in the regulation of chronotropic and inotropic functions of the heart in rats of different ages.^[13-18]

The modern literature has a number of works on the importance of α_2 -ARs in the treatment of hypertension, obesity, depression, anxiety, and pain. Since the importance of α_2 -ARs in the human heart has been studied little, the relevance of this paper is of unconditional interest. Objective of this research was to study the effect of α_2 -ARs blockade on inotropy of the right atrium and ventricle of the myocardium during the period of sympathetic innervation of the heart.

METHODS

The *in vitro* experiments were performed in white, non-native rats of 6 and 3 weeks of age. A control was a group of adult rats of 20 weeks of age. The selected age groups of animals are associated with the stages of innervation of the cardiovascular system. The age of 3 weeks in animals is characterized by the onset of the development of the sympathetic cardiac innervation, 6 weeks - by its completion, and the 20-week-old animals have mature innervation of the heart.^[19]

The inotropic function of the heart was studied using the striae of the atrial and ventricular myocardium of rats. The myocardium striae of 2-3 mm in length and 0.8-1.0 mm in diameter were cut out from the right atrium and right ventricle in accordance with the anatomical structure. The striae were placed vertically in a reservoir of $V = 20$ ml, filled with a standard solution at 37°C, and oxygenated with carbogen (97% O_2 and 3% CO_2). The upper end of the preparation was attached to a stainless steel rod connected to a voltage meter on the PowerLab unit, and the lower end was attached to the rubber block. The preparation was stimulated with an electrical signal through two silver electrodes using ESL-2 stimulator (Russia) and with signal amplitude of 10 mV, with stimulus duration of 5 ms. After dipping into the reservoir, the slice was left for 40-60 min, during which an optimal voltage was gradually applied to the muscle fibers. Optimal stress was such a stretch point of the preparation after which overcoming the contractile force began to decrease. At the end, the initial parameters of contraction were recorded for 5 min, then, for 21 min with the addition of the specific blocker to the standard solution. The specific α_2 -AR antagonist yohimbine was added at a concentration of 10^{-6} M. Contractile force (F) was stated in grams (g). The obtained results were processed in Chart 5 program, using the PowerLab unit (AD Instruments, Australia), with Statgraphics software package. Statistical analysis and identification of the

reliability of the research results by Student t-test were performed in Microsoft Excel editor.

RESULTS

First of all, a series of experiments was conducted to study the effect of blockade of α_2 -AR yohimbine in 20-week-old animals selected as control changes in the contractile force. 5 min after the administration of yohimbine into the standard solution, the contractile force of the right atrium of the adult rats decreased from 0.042 ± 0.01 g to 0.0409 ± 0.01 g ($P < 0.05$). Further, the contractile force continued to decline. Yohimbine reduced the contractile force of the atrial myocardium striae at the 10th and 15th min to 0.0397 ± 0.01 g ($P < 0.01$) and to 0.0381 ± 0.01 g ($P < 0.01$), respectively. At the final minute of the experiment, we observed the maximum decrease in the contractile force of the right atrial myocardium striae of the 20-week-old rats to 0.0372 ± 0.01 g ($P < 0.01$) (Figure 1). During the 1st min, after the addition of yohimbine, the contractile force of the right ventricular myocardium striae slightly decreased from 0.0702 ± 0.01 g to 0.0685 ± 0.01 g. During the 5th min of the experiment, the decrease in the contractile force of the right ventricular myocardial striae reached 0.0673 ± 0.01 g, and during the 10th min – 0.0645 ± 0.01 g. 15 min after the addition of the blocker, we registered a significant decrease in the ventricular myocardial contractile force up to 0.0621 ± 0.01 g ($P < 0.05$). By the 20th min of the experimental recording, the ventricular contractile force in adult animals decreased to 0.0576 ± 0.009 g ($P < 0.05$) (Figure 2).

The addition of a specific blocker yohimbine to the standard solution during the 1st min of the experiment did not change the value of the contractile force of the right atrial myocardium striae of 3-week-old rats. By the 5th min of observation, the contractile force decreased from 0.0328 ± 0.002 g to 0.031 ± 0.002 g ($P < 0.05$). During the 10th min of the experiment,

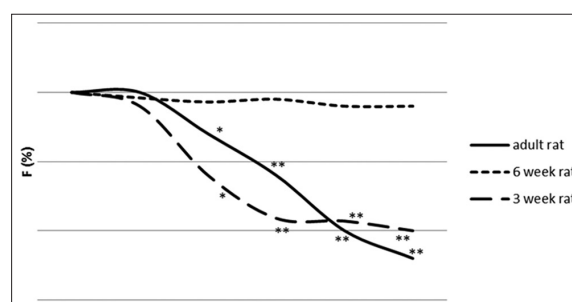


Figure 1: Changes in the contractile force of the atrial myocardium after the blockade of α_2 -adrenoreceptors. Y-axis – myocardium striae contractile force (F, %), X-axis – experiment recording time (minutes). *Data reliability as compared with initial values: $P < 0.05$, **data reliability as compared with initial values: $P < 0.01$

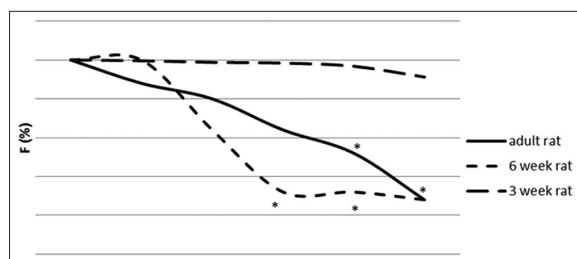


Figure 2: Changes in the contractile force of the ventricular myocardium after the blockade of α_2 -adrenoreceptors. Y-axis – myocardium striae contractile force (F, %), X-axis – experiment recording time (minutes). *Data reliability as compared with initial values: $P < 0.05$

the contractile force of the atrial myocardium striae changed to 0.0298 ± 0.002 g ($P < 0.01$). Blockade of α_2 -ARs at the 15 min of the experiment significantly reduced the contractile force of the atrial myocardium to 0.0297 ± 0.002 g ($P < 0.01$). The minimum force of contraction of the atrial myocardium strips of 3-week-old rats was recorded at the final minute of the experiment – 0.0295 ± 0.002 g ($P < 0.01$) (Figure 1). During the first 10 min, after the addition of the α_2 -AR specific blocker, the contraction strength of the ventricular myocardium striae of 3-week-old rats did not change. During the 15th min of the experiment, yohimbine slightly reduced the contractile force of the ventricular myocardium from 0.0924 ± 0.014 g to 0.0918 ± 0.013 g. At the 20th min of the experiment, the value of the contractile force of ventricular myocardial striae was 0.0904 ± 0.013 g (Figure 2).

The addition of the α_2 -AR blocker yohimbine to the standard solution did not lead to any substantial and significant changes in the contractile force of the right atrial myocardium striae of the 6-week-old rats (Figure 1). 10 min after the administration into the standard solution, yohimbine reduced the strength of contraction of ventricular myocardium striae of the 6-week-old animals from 0.0383 ± 0.009 g to 0.0322 ± 0.007 g ($P < 0.05$). Then, at the 15th min of the experiment, we observed a decrease in the contractile force of the myocardium strips up to 0.0321 ± 0.007 g ($P < 0.05$) (Figure 2). By the 20th min of recording, the contractile force of ventricular myocardium striae of the 6-week-old rats was 0.0316 ± 0.007 g.

SUMMARY

The specific blockade of α_2 -ARs reduces the contractile force of the atrial and ventricular myocardium striae of rats of 20 weeks of age. The 3-week-old rats had a reduced contractile force in the atria and a steady contractile force in the ventricles. In 6-week-old rats, the contractile force in the atria did not change but decreased in the ventricles.

CONCLUSION

Having analyzed the results of the effects of specific blockade of α_2 -ARs by yohimbine on myocardial inoculation of the right atrium and right ventricle of rats during the development of sympathetic innervation of the heart, a decrease in the myocardial contractile force of the right atrium was revealed in 3-week-old rats with the onset of the development of sympathetic innervation of the heart and the absence of a response to the blocker in the ventricles. The opposite results were obtained in the age group of 6-week-old animals, during the completion of the formation of sympathetic myocardial innervation such as the absence of inotropic response in the atria and a decrease in the contractile force of the atrial and ventricular myocardium. In the control group of adult animals, a significant decrease in the contractile force of the atrial and ventricular myocardium was revealed. The earlier results of the effects of α_2 -AR blockade on myocardial inotropy of newborn rats also showed multidirectional effects: In atria of newborn rats, there initially was a tendency to an insignificant short-term increase in the contractile force, with its subsequent reduction, while in the ventricles the contractile force decreased.^[20] The previous results of the effects of α_2 -AR blockade on chronotropy of rat hearts also revealed the peculiarities of blockade effect on the heart rate in different age groups; bradycardia was detected in 1- and 3-week-old rats and lack of chronotropic effect in 6- and 20-week-old animals.^[18] It is known that during the formation of sympathetic innervation of the heart, there occur quantitative and qualitative transformations of receptors in the myocardium. Probably, this subtype of α -ARs is functionally significant in the regulation of heart chronotropy without sympathetic innervation. After completion of the formation of sympathetic innervation, α_2 -ARs regulate inotropy of the heart muscle. It was previously thought that α_2 -ARs are located on the presynaptic membrane, but we may assume that the expression, and then, the synaptic localization of α -ARs on the cardiomyocyte membrane changes with age. Undoubtedly, the age-related changes in the activity of the sympathetic part of the autonomic nervous system affect the functional role of the ARs of the heart.

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