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Effect Of α_{1A}-Adrenergic Receptors Stimulation To The Isolated Rat Hearts Chronotropy.

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ABSTRACT

The adrenergic receptor (AR) family is the most common group of receptors in the body. The most important effect of stimulation of α_1-AR is believed to be the narrowing of the lumen of the blood vessels, which leads to an increase in blood pressure. In the heart, of α_{1A} and α_{1B} subtypes of adrenergic receptors are widely found in the myocardium, while α_{1D}-AR are located in smooth muscle cells and epicardial coronary arteries. The presence of α_1-AR is shown in the cells of the sinoatrial and atrioventricular nodes of the rat heart, which confirms their participation in the regulation of heart rate. The observed contradictions in experimental observations point to the need for further research to identify the role of the α-adrenergic system in the regulation of heart functions. The influence of α_{1A}-adrenergic receptor stimulation on the isolated heart contraction rate according to Langendorff and chronotropy of the heart in vivo was studied. Bradycardia of the heart in response to the selective activation of α_{1A}-AR in in vivo experiments was short-term and was replaced by an increase in frequency, which may be due to the activation of the reflex compensatory mechanisms of the organism. However, the chronotropic effects of selective stimulation of α_{1A}-adrenergic receptors directly on the isolated heart in ex vivo experiments have not been revealed. The results obtained may indicate that this subtype of α_1-adrenergic receptors is not involved in the regulation of heart rate. The change in heart rate in in vivo experiments is most likely due to the reflex mechanisms of regulation of the functioning of the rat heart, as well as changes in the vascular tone.

Keywords: α_1-adrenergic receptors, chronotropy, heart, rat

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INTRODUCTION

The adrenoreceptor (AR) family is the most common group of receptors in the body. Currently, there are nine subtypes of adrenergic receptors (AR), known as: α_{1A}- , α_{1B}- , α_{1D}- , α_{2A}- , α_{2B}- , α_{2C}- , β_1- , β_2- and β_3-AR [1]. Stimulation of α_1-adrenergic receptors increases the concentration of Ca^{2+} within the cell, which causes muscle contraction. Agonists of α_1-adrenergic receptors cause a decrease in the radial muscle of the iris [2], narrowing of the bronchi [3] and vessels [4], contraction of the uterus, sphincters of the urinary and gastrointestinal tract [5]. The activation of α_1-AR also reduces the secretion of insulin, growth hormone, stimulates glycogenolysis and gluconeogenesis in the human body [6]. α_1-adrenergic heart receptors take part in many adaptive processes: changes in inotropy, transcription of genes, protein synthesis, glucose metabolism, inhibition of apoptosis [7]. The most important effect of stimulation of α_1-AR is believed to be narrowing of blood vessels and an increase in arterial blood pressure [8]. α_1-adrenoreceptors are able to bind ligands on the cell surface and each subtype can exist separately in the absence of other subtypes [9]. Different subtypes of adrenergic receptors may adversely affect the tone of mesenteric vessels in rats. In particular, stimulation of the α_{1D} subtype of receptors causes a vasodilating effect [10], while α_{1A}-AR activation causes a vasoconstriction effect [11].

The location and quantity of α_1-adrenergic receptors in the heart are similar in different animals, except for rats [12]. α_{1A} and α_{1B} subtypes of adrenergic receptors are densely represented in the myocardium, while α_{1D}-AR are found in smooth muscle cells and epicardial coronary arteries [13]. In vitro and in vivo studies have found that chronic stimulation of α_{1A} and α_{1B}-AR rat cardiomyocytes can cause a different regulatory effect [13]. Blocking of various subtypes of α_1-adrenergic receptors causes opposite chronotropic effects on the heart of 1-week-old rats [14]. t is shown that blockade of α_1-AP prazosin results in a negative chronotropic heart reaction in 20- and 6-week-old rats, and a similar effect is not observed in 3- and 1-week-old rats [15]. Stimulation of α_1-adrenergic receptors causes unidirectional changes in the chronotropy (bradycardia) of the heart, which are associated with age-related features [16]. A positive chronotropic effect of the selective blockade of α_{1A}-AR on the heart of newborn rats and inhibition of heart chronotropy in other age groups of animals is shown. In addition, there was an increase in the severity of bradycardia with aging in response to the action of the α_{1A}-adrenoreceptor blocker - WB 4101 [17].

The results of numerous biochemical and molecular studies showed the presence of α_1-adrenergic receptors in the human heart [18]. The quantitative ratio of α_1-AR in the human heart is 10%-15% of the total number of adrenergic receptors. α_1-AR induce Gq-protein activation, which results in the formation of inositol triphosphate [19] and the development of positive inotropy of the heart [18]. α_1-AR can participate in the development of myocardial hypertrophy in rats [20]. However, the involvement of α_1-AR in hypertrophy of the human heart remains unclear [1]. A group of researchers notes that in adult rats, myocardial hypertrophy occurs with the participation of α_{1A}-adrenergic cardiac receptors [21]. Contradictory results of clinical observations emphasize scientific problems and indicate the need for further studies in this area, for the development of new approaches to treatment through the α-adrenergic system [12].

The objective of this research was to study the effect of stimulation of α_{1A}-adrenergic receptors on the chronotropy of the isolated heart and the heart of the whole organism.

METHODS

The experiment was conducted on 20-week-old white outbred rats of 200-250 g (n=15). The animals were anesthetized with a 25% urethane solution at a dose of 0,8 g/kg of animal weight, administered intraperitoneally. In the in vivo experiment, A-61603 (α_{1A}-AR agonist, Sigma) was administered into the right femoral vein of the rat at a dose of 1 μg/kg. In the course of the experiment, ECG was constantly recorded and electronically processed.

In the ex vivo experiment, the heart was isolated from the body and placed in a cold working solution (2-5°C) to completely stop the contractions. The isolated heart was mounted through the aorta on the cannula of the Langendorff device (ADinstruments, Australia) and perfused with Krebs-Henselite solution (NaCl-118 мМ, KCl-4,7 мМ, $NaHCO_3$-25 мМ, $MgSO_4$-1,2 мМ, $CaCl_2$-2,5 мМ, KH_2PO_4-1,2 мМ, glucose-5.5 мМ, pH=7.3-7.4) at 370°C and constant pressure of 60-65 mm Hg. To stimulate α_{1A}-AR, A-61603 (Sigma) was used at a concentration of 10^{-9} M. To record the heart activity in the left ventricle, a latex cylinder was placed through

the atrioventricular valve. The cylinder was filled with water and a pressure of 10-15 mm Hg was set in the recording system. Changes in pressure inside the cylinder with a contraction of the left ventricle were recorded using the ML T844 sensor. The contraction rate of an isolated heart was counted from the curve of pressure changes. Experimental materials were recorded on the PowerLab 8/35 device (ADinstruments, Australia) with the help of the original LabChartPr program (version v8). Statistical analysis and determination of the reliability of the differences in the results of the study were carried out using Student's t-test.

RESULTS

The in vivo studies showed a decrease in the heart beat frequency by 24% from 342±11 bpm to 261±10 bpm ($p<0.01$) 15 seconds after intravenous administration of the α_{1A}-AR-A-61603 agonist at a dose of 1 μg/kg (N=5). 30 seconds after administration of the agonist, heart rate changed to 295±12.4 bpm ($p<0.01$). At the 2nd minute of the experiment the contraction rate of the heart was 263±33 bpm ($p<0.05$), at the 3rd minute - 282±26 bpm ($p<0.05$). Thus, the bradycardia was maintained up to the 3rd minute of the experiment, then there was a tendency to the heart rate recovery (Fig.). By the 30th minute of observation, the heart rate of the 20-week-old rats increased to 431±21.5 bpm ($p<0.05$), an increase was 26% of the initial value.

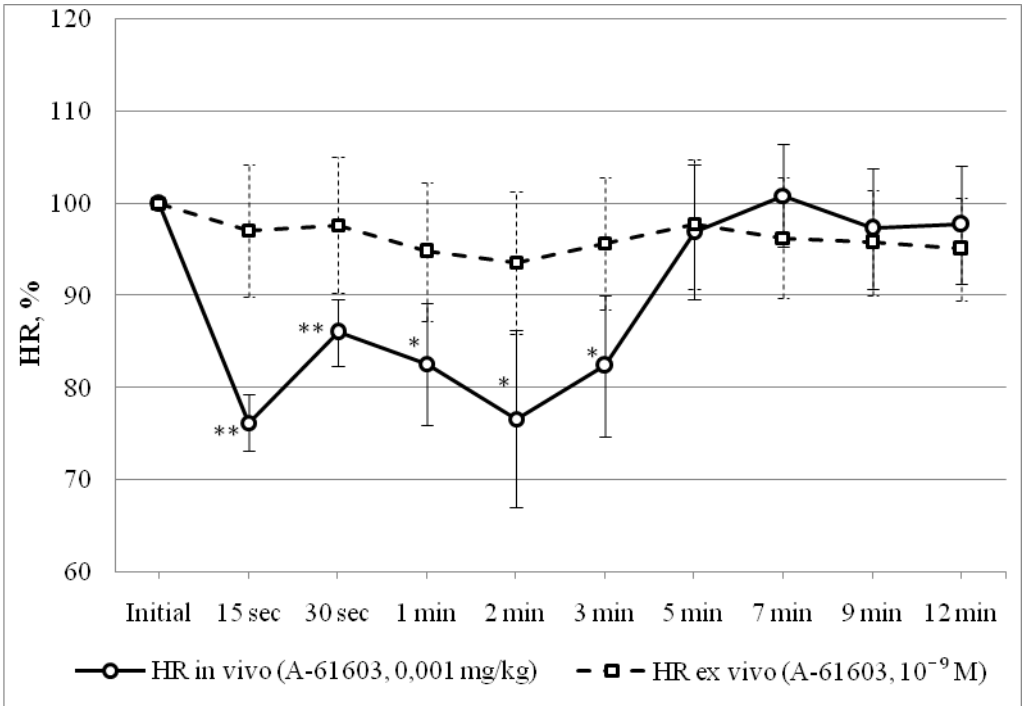

Figure. Effect of A-61603 on the contraction rate of the isolated heart and heart rate of the whole animal. The ordinate axis is the heart rate (HR,%), the abscissa axis is the time (sec, min). Note: * - data reliability as compared with initial values: $p<0.05$, ** - data reliability as compared with initial values: $p<0.01$.

To identify the effects of A-61603 directly on the heart in ex-vivo experiments on an isolated heart, the effect of α_{1A}-adrenergic receptor stimulation on chronotropic heart function of rats at 10^{-9} M substance concentration (n=10) was studied.

15 seconds after the administration of the α_{1A}-adrenoreceptor agonist A-61603 (10^{-9} M), the contraction rate of the isolated heart decreased from 207.4±12.6 bpm to 201.3±15 bpm. After 2 minutes of A-61603 perfusion, heart rate decreased to 194±16 bpm. At the 5th minute after the administration of the agonist, the rate of cardiac contractions was 203±15 bpm. At the final minute, the heart rate was 197 bpm.

SUMMARY

Stimulation of α_{1A}-AR with A-61603 did not lead to a significant change in the chronotropy of the isolated heart of rats. At the same time, in in vivo experiments, the α_{1A}-adrenoreceptor agonist A-61603 at a

dose of 1 μg/kg caused a bradycardia that occurred immediately after the injection of the selective agonist, and then, at the end of the experimental observation, an increase in heart rate.

CONCLUSION

Stimulation of α_{1A}-adrenergic receptors with A-61603 caused a decrease in heart rate in a whole organism. It should be noted that a bradycardia observed almost immediately after the injection of a selective agonist was maintained for 3 minutes, followed by a trend towards the heart rate recovery. Then, by the end of the experimental observation, the heart rate increased. Experiments ex vivo on the isolated heart showed no chronotropic effects after the administration of the selective α_{1A}-adrenoreceptor agonist A-61603. The presence of α_1-AR is shown in the cells of the sinoatrial and atrioventricular nodes of the rat heart, which confirms their participation in the regulation of heart rate [22]. The participation of α_1-adrenergic receptors in the regulation of the heart rhythm is confirmed by previous studies, which showed that the activity of α_1-AR with methoxamine leads to a decrease in the part of the heart, both in the whole organism and in the isolated heart. Effects differed only in time indicators. The bradycardia of the isolated heart in response to the activity of α_1-adrenergic receptors developed within a few minutes. The decrease in the incidence of heart abnormalities observed in the in vivo study was short-term, which may be related to the response of vessels and the activity of the reflex compensatory mechanisms of the whole organism [16]. However, this research did not reveal any chronotropic effects of selective stimulation of α_{1A}-adrenergic receptors directly on the isolated heart in ex vivo experiments. The results obtained may indicate that this subtype of α_1-adrenergic receptors is not involved in the regulation of heart rate. The effects of methoxamine apparently occur through other α_1-adrenoreceptor subtypes (α_{1B}- and α_{1D}-AR). The change in heart rate in in vivo experiments is most likely due to the reflex mechanisms of regulation of the functioning of the rat heart, as well as changes in the vascular tone.

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