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Stimulation Of A_{1A}-Adrenergic Receptors Has A Different Effect On The Rat Myocardial Inotropy.

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ABSTRACT

α_1-adrenergic receptors (AR) are found with the help of modern research methods in myocardiocytes, endotheliocytes, smooth muscle cells of coronary arteries of human and animals. Based on the results of studies of some scientists, stimulation of myocardial α_1-AR has a positive effect, while in the opinion of others - negative, according to the third - two-phase inotropic effects. Perhaps, multidirectional effects can be obtained not only by different signaling pathways that arise when both individual α_1-AP subtypes and different links of the same signal chain are activated. The study was conducted on outbred rats at the age of 20 weeks. To stimulate α1A-adrenoreceptors, the pharmacological drug A-61603 was used in concentrations of 10^{-9}-10^{-6}M. The reaction of the contraction force of isolated myocardial strips in response to the action of the selective agonist was recorded. It was found that low concentrations of the agonist α_{1A}-AP – A-61603 (10^{-9}, 10^{-8} M) cause a decrease, while higher concentrations (10^{-7}, 10^{-6} M) increase the strength of the atrial myocardial strip contractions of 20-week rats. At the same time, A-61603, in all the concentrations studied by us, had only a negative inotropic effect on the contractility of ventricular myocardial strips. This study showed a multidirectional inotropic myocardial response in rats to stimulation of α_{1A}-adrenergic receptors. Probably, α1-adrenergic receptors, along with the main regulators - β-adrenergic receptors, perform a finer tuning of the heart.

Keywords: rat, heart, inotropy, α_1-adrenergic receptors.

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INTRODUCTION

α_1-adrenergic receptors (AR) are found with the help of modern research methods in myocardiocytes, endotheliocytes, smooth muscle cells of coronary arteries of human and animals. It is known about three subtypes of these receptors: α_{1A}, α_{1B}, and α_{1D} [1]. Rat cardiomyocytes hae all these three subtypes of the receptor [2]. α_1-AP increases significantly in the heart of rats during the first 2 weeks after birth [3]. Despite the fact that α_1-AP is 10% of the total AP, they participate in the regulation of inotropic and chronotropic functions of the heart. According to the results of studies of some scientists, stimulation of myocardial α_1-AR has a positive, in the opinion of others - negative, and according to the third - two-phase inotropic effects [4]. Positive inotropic effect of α_1-AP activity was obtained in the studies of the cardiac ventricles of the rabbit, the papillary muscle of the left ventricle of the rat, and the left ventricular myocardial strips of the mouse [5]. Negative inotropic effect with stimulation of α_1-AP was shown in studies of the specimens of the right and left ventricles of the mouse, papillary muscles of the right ventricle of the rat, and the ventricular myocardium of the dog [6].

Catecholamine interaction with α_1-AP in the myocardium triggers the splitting of G_q protein into α- and βγ-subunits and activates phospholipase C [7; 8]. A positive inotropic effect occurs through protein kinase (PC) C due to an increase in the entry of Ca^{2+} ions into the cardiomyocyte via L-type Ca channels [9], the TRPC channels [10], and the $STIM_1$ channels [11]. In addition, Ca^{2+} transit from the sarcoplasmic reticulum (CPR) increases due to IF_3-receptors [12] and the intracellular calcium concentration during systole increases, which strengthens the inotropic effect of cardiomyocytes. In turn, diacylglycerol may alter the activity of PCC and thereby modulate the action of ion channels, as well as inhibit G_i proteins associated with M-cholinergic receptors [13]. Scientists have shown that PCC activates PCD, which is involved in the regulation of inotropy of the myocardium and most often reduces it [7; 8]. Reducing myocardial contractility by means of PCD occurs by phosphorylation of troponin I, and the time of relaxation of cardiomyocytes is also accelerated [7; 8]. In addition, PCD phosphorylates myosin-binding protein C (MyBP-C) and, thus, reduces the sensitivity of myofillations to Ca^{2+} [14], hence the rate of myocardial relaxation increases [15]. Activation of MyBP-C can also occur via PCA. According to different authors, the activation of MyBP-C is capable of both increasing myocardial contractility [16] and reducing it [17], and the effect is most likely associated with the myosin isoform [18].

Based on the foregoing, the objective of the research was to study the effect of selective stimulation of α_{1A}-AP on the inotropic function of the myocardium of the atria and ventricles of adult rats.

RESEARCH METHODS

The experiment was conducted on 20-week-old white outbred rats. The animals were anesthetized with a 25% urethane solution at a dose of 800 mg/kg. Strips of the myocardium from the right atrium and right ventricle were placed in a bath with a working solution and fixed vertically to the mechanical sensor at the top and to the glass hook of the holder at the bottom. The specimens were stimulated with an electrical signal with a frequency of 6 stimuli per minute, a duration of 5 ms, an amplitude of 10 mV. The immersion of the specimen into the working solution for 40-60 minutes was followed by a period of study. Further, the original contraction parameters were recorded for 5 minutes. For pharmacological stimulation of α_{1A}-AP, the drug A-61603 was used in concentrations of 10^{-9}-10^{-6} M. The reaction of the contraction force in response to the action of the agonist was recorded. The contraction force (F) was expressed in grams (g). The processing of the results was carried out with the help of the program Acceloaded 4.1 on the MP-150 device (BIOPAC Systems, USA) using Staggraphs software package. Statistical processing of the results was carried out using Student's t-test.

RESULTS

The contraction force of the atrial myocardial strips (n=7) of 20-week-old rats after the administration of A-61603 at a concentration of **10^{-9} M** decreased by 19% from 0.0281±0.0025 g to 0.0226±0.0022 g ($p<0.01$) (Figure 1), the same of ventricular myocardial strips (n=8) - by 12% from 0.0839±0.0169 g to 0.0739±0.0121 g.

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After the administration of A-61603 at a concentration of **10^{-8} M**, the contraction force of the atrial myocardial strips (n=8) decreased by 20% from 0.0376±0.0053 g to 0.0299±0.0047 g ($p<0.001$), the same of ventricular myocardial strips (n=8) – by 19% from 0.1161±0.0163 g to 0.0942±0.0122 g ($p<0.05$) (Fig. 2).

By the 7the minute after the administration of A-61603 at a concentration of **10^{-7} M**, the contraction force of the atrial myocardial strips (n=8) of 20-week-old rats increased by 93% from 0.0348±0.0045 g to 0.0673±0.0115 g ($p<0.01$), followed by a trend towards recovery. After restoring to the original level, the contraction force of the atrial myocardium decreased up to 0.0305±0.0052 g ($p<0.05$); this change was 12% of the initial value (Fig. 1).

The contraction force of ventricular myocardial strips (n=7) under the influence of A-61603 (10-7 M) decreased from 0.1152±0.0162 g to 0.089±0.0112 g ($p<0.01$); the change was 23% (Fig. 2.).

A-61603 at a concentration of **10^{-6} M** (n=8) resulted in an increase in the contraction strength of the atrial myocardial strips of 20-week-old rats by 164% from 0.0288±0.0048 g to 0.0761±0.0113 g ($p<0.001$). Further, there was a tendency to recovery of contractility. The maximum recovery of atrial myocardial contraction force of 20-week-old rats to 0.0501±0.0072 g ($p<0.01$) was observed at the 20th minutes after adding the selective agonist (Fig. 1).

The contractility force of ventricular myocardial strips (n=7) after the addition of A-61603 at a concentration of **10^{-6} M** decreased from 0.1009±0.0128 g to 0.0821±0.0129 g ($p<0.05$). The negative inotropic effect was 18% (Fig. 2).

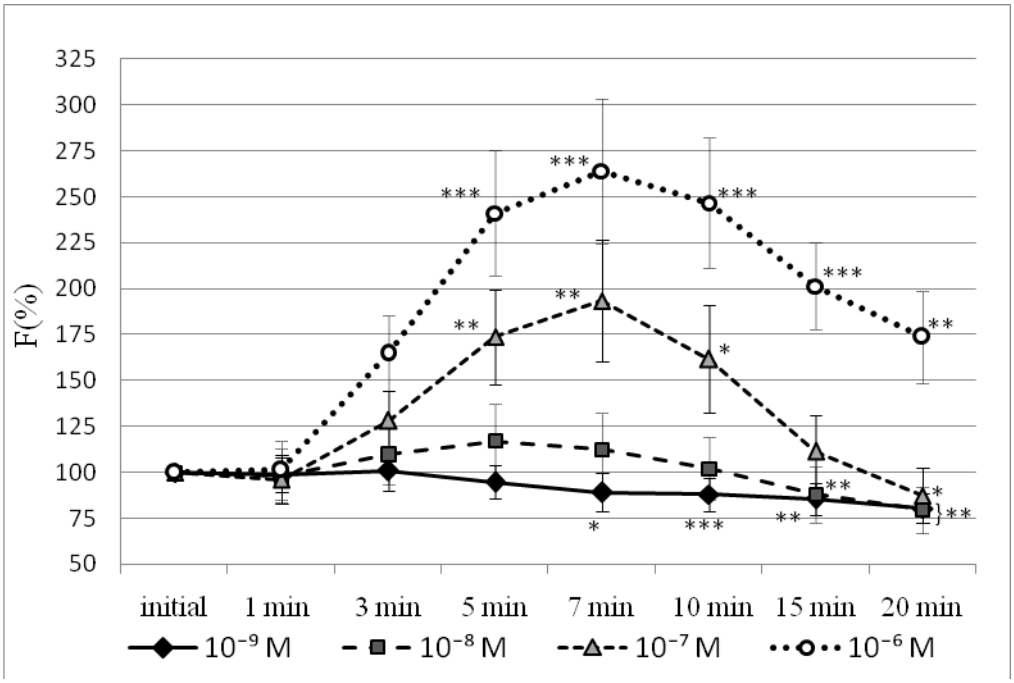

Figure 1: Effect of A-61603 on the force of contractions of atrial myocardial strips of 20-week-old rats. Note: the reliability of the changes as compared with the initial values - *- P <0.05; **- P <0.01; *- P <0.001.**

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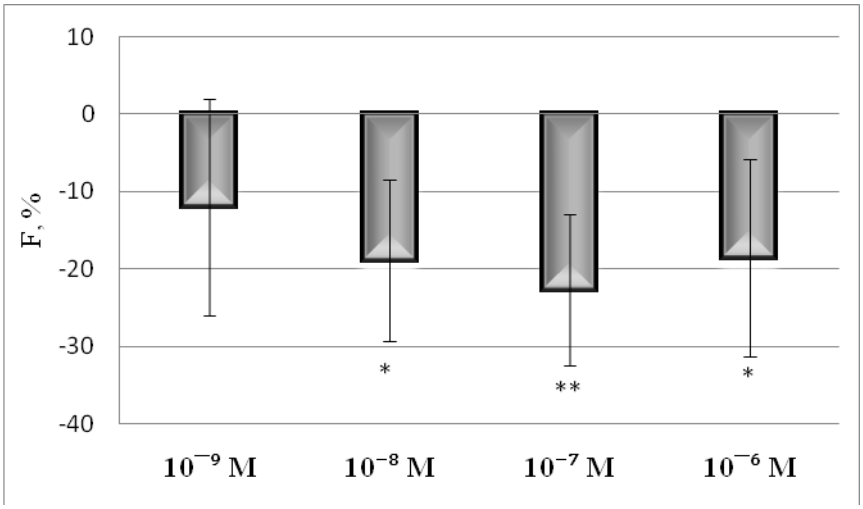

Figure 2: Maximum effects of A-61603 on the contraction force of ventricular myocardial strips of 20-week-old rats. Note: the reliability of the changes as compared with the reference values - *- P <0.05; **- P <0.01.

Thus, low concentrations (10^{-9}, 10^{-8} M) of the α_{1A}-AP agonist – A-61603 caused a decrease, and higher concentrations (10^{-7}, 10^{-6} M) increased the contraction force of the atrial myocardial strips in adult rats. At the same time, A-61603, in all the concentrations studied, resulted only in a unidirectional negative inotropic response of the ventricular myocardial strips.

DISCUSSION

The studies have shown the possible multidirectional inotropic myocardial responses in rats to stimulation of α_{1A}-adrenergic receptors. Probably, α_1-adrenergic receptors, along with the main β-adrenoreceptor regulators, perform a finer tuning of the heart activity. This is also confirmed by a change in their activity in the pathological processes in the heart.

Tanaka H. et al. showed that α_{1A}-AP reduced cardiac muscle contractility in adult mice by reducing the sensitivity of contractile elements to Ca^{2+} ions [19]. According to the results of other authors, α_{1A}-AR causes a negative inotropic effect of the right ventricular myocardium in the mouse, but positive of the left ventricular myocardium. The a_1-AR mediated decrease in contractility of the ventricular myocardium was associated with an increased isolation of Ca^{2+} from the cell and a decrease in Ca^{2+} content in the SPR. An increase in Ca^{2+} in the myocytes was otherwise accompanied not by transit through the SPR, but by other mechanisms that promote an increase in the Ca^{2+} current into the cell [20]. The peculiarities of the contractile effects of α_1-adrenergic receptors may be due to differences in contractile proteins and/or secondary messengers involved, such as PCC, which has 15 different isoforms [21].

A positive component of the inotropic effect observed in the atria can result from PCC due to increase in Ca^{2+} concentration inside the cell via L-type Ca channels, [9] and IF_3 receptors [7]. Also, PCC-independent activation of myosin light chain kinases (MLCKs) can serve as the main mechanism in the α_1-AP-induced positive inotropic reaction [22].

A selective stimulation of α_{1A}-adrenergic receptors caused a decrease in the contraction force of the ventricular myocardium, as well as of the atrial myocardium at lower concentrations of the agonist. The negative inotropic effect of α_1-AP activation can be based on an increase in NO synthesis [6], activation of the Na^+ / Ca^{2+} exchange [23], activation of the Na^+/H^+ exchange mechanism, enhancement of the emerging K^+ current, inhibition of the L-type of Ca_{2+} channels [20; 5], a decrease in the sensitivity of myofilament to Ca^{2+} by phosphorylation of the light chain of myosin and/or troponin I [5], as well as MyBP-C [14]. D. Phan et al. refer negative inotropy to the activity of PCD, which phosphorylates troponin I and reduces the contraction force [8]. It is possible that if a positive inotropic effect is realized by the interaction of α_1-AP with G_q-protein, the negative inotropic effect may be a consequence of activation of the G_i-protein [5].

The study of the mechanisms underlying the inotropic effects of α_1-AP activation suggests that the direction of the effects can be associated not only with the different signaling pathways, in particular involving different G proteins being triggered by different subtypes, as well as by a separate subtype of α_1-adrenergic receptors, but also with the activity of individual elements of a single signaling system.

Conflict of interests: The author declares that the provided information has no conflicts of interest.

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